ON THE NATURE OF THE HYDROGEN BOND AND BIOMOLECULAR SOLVATION

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A DISSERTATION

IN

BIOCHEMISTRY AND MOLECULAR BIOPHYSICS

Presented to the Faculties of the University of Pennsylvania

in

Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy

2010

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DEDICATION

This work is dedicated to Beth Scott and the Memory of James H. Scott, and with Love to Stacey and Jack
AKNOWLEDGEMENT

I wish to acknowledge the invaluable help of a number of students, faculty, and staff who have made this work possible. Thank you to Ms. Ruth Keris for answering a plethora of questions on just about everything to do with being a graduate student at Penn. Thank you to Dr. Bogumil Zelent for help with materials and experimental procedures, as well as discussions involving this work. Thank you to Dr. Ryan Coleman for assistance with a variety of computational issues over the course of several years and several computers. Thank you to Bill Pennie and Mike Carman of the Research Instrumentation Shop for their incredibly skillful help with instrumentation, as well as thoughtful discussions on instrumentation issues over the years. Thank you to the Physics Department of Louisiana Tech University, for providing me with a thorough and strong background in basics physics, which has made the present work possible. Thank you to Tammer Farid, for providing a wonderful home for four years.

Finally, thank you to Drs. Jane M. Vanderkooi and Nathaniel V. Nucci, for their boundless and infectious enthusiasm regarding all aspects of water and biophysical solvation, and for the countless hours of discussion which helped in shaping the ideas and research presented in this Dissertation.
ABSTRACT

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James N Scott

Dr. Jane M. Vanderkooi, Ph.D.

Water is the most studied molecule in the history of scientific research, and yet much remains unknown about this structurally simple but functionally complex molecule. There is ample evidence that understanding the fundamental nature of the hydrogen bond and its response to changing chemical and thermodynamic conditions is the key to understanding water’s many properties and functions. The goal of this work was to examine the H-bond in the context of its bond angle and bond distance, and furthermore to investigate the impact that alteration of water’s bulk structure can have on biomolecules and vice versa. A two-dimensional potential energy surface was constructed for the gaseous water dimer that showed changes in the O-O distance between two H-bonded water molecules have a negligible effect on bond energy as compared to H-bond angle. This surface supports the idea that in liquid water, where O separation is known to be spatially constrained, H-bond angle plays the primary role in bond energetics. Since the decoupled OH stretching vibration is known to be a sensitive reporter of the local electronic environment of the oscillator, the OH stretch absorption of liquid water can be analyzed in terms of H-bond angles in the solution. High concentrations of the guanidinium ion were found to have a profound effect on water structure, causing a higher proportion of more linear H-bonds in solution than is found in bulk water. This finding raises the possibility that alteration of water structure may contribute to the ability of guanidinium to denature proteins in solution. Water structure under high hydrostatic pressures was also examined, and it was found that water’s ice VII form undergoes a structural rearrangement at pressures right above the ice VI to VII transition.
transition, from two H-bond populations to a single H-bond population. In addition, this work shows that hydrostatic pressures within the liquid water regime have effects on the structure of small peptides that are dependent on the identity of the side chain. This result has implications for determining the conditions under which amino acid based life can form the macromolecules and perform the chemistry necessary to remain viable.
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CHAPTER 1

INTRODUCTION AND OBJECTIVE

Water: An Overview

Water is the stuff of life. Perhaps exceeded in its importance only by carbon, water seems to be the foundation upon which all life is built. Though scientists have speculated that life has evolved on other planets to take advantage of alternate solvents, such as liquid ammonia, (Molton, 1974) the presence of water is still one of the de facto tests for the presence and viability of life as we know it. Some microorganisms can tolerate severe desiccation, (Beblo et al., 2009, Potts, 1994) but water appears to be completely obligate, and all life as we currently know it requires at least some water to remain viable and perform the chemistry necessary for life.

Water is almost certainly the single most studied molecule on Earth, and with good reason. Aside from its critical importance to life, ours is a wet planet, with over 70% of its total surface area covered by water. Water is also a very mobile molecule, constantly circulating through the Earth’s life forms, ecosystems, and atmosphere. This mobility and ubiquity means that water has important roles in nearly all of the Earth’s processes.

But what makes water necessary for life? If it were simply a liquid medium in which chemistry is possible then we should be able to replace the water in an organism with another solvent and have that organism remain viable. Water it seems is more than simply a liquid medium. Its simple combination of three atoms and ten electrons gives water the properties that make it unique amongst all known solvents and give it the solvation properties critical for biomolecular solvation.
As of this writing much remains unknown about water and its structure and functions. The structural simplicity of the single water molecule is misleading, and despite tens of thousands of scientific studies on nearly every aspect of water structure and solvation, both biochemical and otherwise, water has thus far refused to give up all of its secrets. Along with the experimental studies of water structure and function, nearly every aspect of water has been incessantly modeled. Though models, by definition, do not accurately describe every aspect of water many models have served to deepen our understanding of water’s intricacies.

This Chapter contains a brief introduction to what is currently known of water. To describe the water literature as voluminous and contentious is an understatement, so this introduction only touches on key points and examples and is generally broken down by investigative technique.

Ways of Thinking About Water and H-bonds

Before discussing experimental and theoretical evidence related to water structure, dynamics, and H-bonding it is important to at least briefly consider the historical schools of thought on water. Water has been studied for a very long time, and literature on the subject from the early twentieth century highlights the disagreement and contrasting theories that still abound today. A very early review examines the then current theoretical views on water and highlights the ongoing debate.(Chadwell, 1928)

One class of the early water models came to be known as the mixture model, since it generally refers to water as a mixture of ice-like, strongly H-bonded water molecules of varying sizes and geometrical arrangements and weakly bound or unbound water molecules. Nearly all of the early work in describing this model was performed by Bernal and Fowler,(Bernal and Fowler, 1933) but the mixture model has been updated and
revised by numerous theoreticians, including Henry Frank, who along with coworkers published nearly a dozen papers on water structure between 1945 and 1970. (Frank and Evans, 1945, Frank and Wen, 1957, Frank and Quist, 1961, Frank, 1965, Frank, 1970) At the same time Harold Scheraga was developing his own version of the mixture model, which shared some features with Frank’s. (Hagler et al., 1972, Nemethy and Scheraga, 1962a, Nemethy and Scheraga, 1962b) Scheraga also did a great deal of the early work in characterizing hydrophobic solvation and showed the thermodynamic advantage of ice-like water structuring near hydrophobic surfaces. The characteristic shared by all mixture models is clustering and ordering of some fraction of water molecules within an otherwise weakly interacting solvent. The principal distinction amongst mixture models is the extent of the clustering that they prescribe, from the very minimal clusters that Scheraga proposed to much higher order, and consequently longer lived, structures.

While mixture models have had some success in describing water’s known thermodynamic properties, they are basically wrong. There is to date no experimental evidence for the sort of ordering of water that is required by mixture models, and any number of experimental techniques would have discovered the long range heterogeneity they prescribe.

The other main class of water models can broadly be called continuum models. These models are the antithesis of mixture models in that they describe liquid water as containing a continuum of fluctuating H-bond geometries rather than the long lived clusters described by mixture models. Much of the early work on a continuum model of water was done in the 1950’s by Pople and Lennard-Jones, (Lennard-Jones and Pople, 1951, Pople, 1951) and for the purposes of this dissertation it is worth noting that Pople was careful to highlight the importance of H-bond angle to the H-bond strength in liquid water while numerous other researcher before and since have thought about H-bonds
only in terms of their length. Pople’s continuum model was able to reproduce the X-ray diffraction O-O radial distribution function of water better than any other model of its day but failed in its ability to account for numerous other properties of water.

The continuum description of water languished until the early 1980’s when Sceats and Rice published their work on a new continuum model. (Rice and Sceats, 1981, Sceats and Rice, 1980a, Sceats and Rice, 1980b, Sceats and Rice, 1982) The Random Network Model describes the water H-bond network exactly as the name implies, as a random network of intermolecular H-bond geometries. The Random Network Model is basically a simple potential function with two key terms, one of which depends on the length of an H-bond (in this case defined as the O-O distance) and the other of which depends on the H-bond angle. Despite its overall simplicity the Random Network Model achieved a number of successes which had eluded other water models, including its prediction of the two OH stretch relaxation timescales later discovered using ultrafast infrared spectroscopy. The model was also able to reproduce quite well the by then well known water radial distribution functions and was even able to approximate the infrared spectra of water. Though incomplete and imperfect, the Random Network Model was still a huge step forward in the collective thinking on water structure, and it forms the basis for many treatments of water used to this day.

**Structure and Dynamics of Liquid Water**

The water monomer is structurally simple, consisting of a single oxygen atom covalently bonded to two hydrogen atoms. Due to its relative simplicity, the single water molecule has been thoroughly studied using a variety of techniques, both experimental (Bernath, 2002, Bondarenko and Gorbatiy, 1991, Dyke and Muenter, 1974, Odutola and Dyke, 1980) and computational. (Lee et al., 2000, Xantheas and Dunning,
The water dimer is a considerably more interesting system since it contains the simplest example of a single intermolecular H-bond. The water dimer has also been studied using many techniques, from the microwave spectroscopy of Dyke et al., (Dyke and Muenter, 1974, Odutola and Dyke, 1980) which has proven to be the definitive experimental structural work on the water dimer, to the multidimensional potential energy surfaces of Bowman and coworkers (Huang et al., 2008, Shank et al., 2009, Wang et al., 2008) and others (Leforestier et al., 2009) that have principally focused on calculating the dimer’s transition structures and vibrational rotational tunneling splittings with a very high degree of precision. In fact, the water dimer’s interaction energy is so well experimentally known now that quantum mechanical calculations of the water dimer are routinely used in the testing and validation of new quantum mechanical density functionals. (Gonzalez et al., 1997, Inada and Orita, 2007, Santra et al., 2007, Xu and Goddard, 2004)

Of greater general interest are condensed phase water clusters, like those found in the liquid or solid forms. Small clusters of typically less than twenty water molecules have been studied extensively using vibrational spectroscopic methods (Brudermann et al., 1999, Buck et al., 1998, Devlin et al., 2000, Devlin et al., 2001, Starzak and Mathlouthi, 2003) and quantum mechanical calculations (Buck et al., 1998, Gregory et al., 1997, Herndon and Radhakrishnan, 1988, Weinhold et al., 2005, Xantheas and Dunning, 1993) of varying sophistication. However, small clusters are just that, small clusters, and only approach the complexity of real water as cluster size grows large. Xantheas and Dunning found (Xantheas and Dunning, 1993) that O-O separation computed for water clusters using \textit{ab initio} methods exponentially approaches the experimentally well known 2.8 Å first peak (Soper and Phillips, 1986, Soper et al., 1997, Soper, 2000) in the O-O radial distribution function of liquid water as cluster size is increased.
Though studies of small clusters of water molecules are interesting from a theoretical standpoint and some have also added to our understanding of H-bonding in water (see Chapter 2 for an example of such work, where the water dimer is explored in terms of the distance and angular dependence of the single water-water H-bond), the condensed phases of water remain its most widely studied forms.

**Scattering Techniques**

Much of what is known or believed today of liquid water structure is still actively disputed, and this is particularly true of the information derived from scattering studies. Diffraction studies, both neutron and X-ray, are theoretically capable of determining the average interatomic distances in a simple liquid. However, the existence and continual publication of numerous scattering studies on the exact same experimental subject points to the complexity of the data and its analysis. On the subject of error and uncertainty in the data collected in X-ray and neutron diffraction studies Alan Soper said

There are two primary sources of error in the diffraction data on water. Firstly, especially with neutrons, and also with X-rays if energy analysis is used to remove the Compton scattering, the data can only be obtained with finite statistical uncertainty. In general, the statistical uncertainty is worse at large values of the wave vector transfer, $Q$, which has the effect of making it difficult to determine at precisely which value of $Q$ the structural oscillations disappear. In addition for the OH and HH structure factors, the diffraction pattern has oscillations due to the intramolecular interferences which proceed to large $Q$. Measuring these oscillations precisely is an essential prerequisite to determine the intramolecular structure. Thus, finite counting statistics give rise to an effective truncation of the diffraction data, resulting in a loss of resolution in real space. The second main source of error comes from the need to estimate the single atom scattering for neutrons or the single electron (Compton) scattering for X-rays. This scattering does not contain useful information on the relative arrangement of atoms or electrons, but it does constitute a significant part of the diffraction pattern. (Soper, 2000)

Soper also mentions a third source of error, “that the nuclear recoil distortion is neutron energy dependent, rather than $Q$ dependent," which leads to blurring “when the different detector arrays are combined at a given $Q$ value.” (Soper, 2000) The errors highlighted by Soper in the preceding passages do not address the considerable impact that
structural refinement techniques have on the final radial distribution function, or how molecular dynamics simulations are often used to explore atomistic and molecular quantities that were not directly accessible by the experiment.

Even what should be the most easily determinable quantity in liquid water diffraction studies, the O-O radial distribution function (due to its scattering cross section), is proving to be contentious. Though the majority of the diffraction studies point to a predominantly tetrahedral structure in liquid water, with a very sharp O-O radial distribution function peak at 2.8 Å and a much broader, lower area peak at 4.5 Å, (Bosio et al., 1983, Narten and Levy, 1971, Soper, 2000) other recent studies (Fu et al., 2009) report a significant density at 3.4 Å, which would point to the existence of a population of interstitial water molecules disrupting the tetrahedral water structure. Opinions of the validity of the reported 3.4 Å peak in the radial distribution function are decidedly mixed, though even ardent supporters admit that the observed peak is extraordinarily small when compared to the more agreed upon features in the radial distribution functions.

It must be noted that insofar as scattering techniques may be useful for determining radial distribution and pairwise correlation functions, even infinitely precise radial distribution information for liquid water does not unambiguously determine structure, since any number of H-bond angles between water molecules can yield an identical radial distribution function profile. (Kusalik and Svishtchev, 1994) Since all of the radii in the functions are spherically averaged and liquid water is constantly fluctuating, scattering studies alone never give a precise view of the intermolecular geometry between liquid water molecules. Diffraction studies of ice Ih (so-called “normal” ice) show perfectly coordinated water, (Soper, 1984, Soper, 2000) with each water molecule having exactly four closest neighbors, two of which are H-bond donors and two of which are H-bond acceptors. Integration of the 2.8 Å radial distribution peak for liquid water
typically yields a coordination number of ~5 (depending somewhat on the radial limits of integration), which indicates that liquid water maintains much of the tetrahedral structure of ice while relaxing enough to form another weak H-bond. (Sharp and Vanderkooi, 2009)

Diffraction studies have also been of some use in determining the effect of various solutes on liquid water structure. Soper, (Mancinelli et al., 2007) Bowron, (Bowron, 2004) Finney, (Finney, 2004) and others have produced work that shows a minimal effect, if any, of monatomic ions within the first shell of liquid water but perhaps some effect on longer range structure. (Mancinelli et al., 2007) More complex ions have been found to have an impact on water structure dependent on the size and chemical makeup of the ion in question. For instance, Mason et al. found (Mason et al., 2004) that the planar guanidinium cation strongly coordinates water molecules around its perimeter and has a deficiency of water molecules at its planar surfaces. Noble gases have also been dissolved in water and their effects studied. Boradbent et al. discovered (Broadbent and Neilson, 1994) that gaseous argon dissolved in water has a strong first coordination shell like that of the potassium ion, but Sullivan et al. found that increased temperature weakens the first shell structuring Broadbent et al. found at ambient temperatures. (Sullivan et al., 2001)

Scattering techniques have also been brought to bear on the solvation of more biologically relevant molecules, though these have typically been model systems and the quantity of interest has been water and solute dynamics, which has then been used to make arguments concerning structure. It has been shown that biomolecular dynamics depend on the hydration state of the molecule, and both the exact nature of the water binding sites and the hydration water network influence the intrinsic motions of solvated biomolecules. Pérez et al. found for myoglobin and lysozyme that even one water layer enables protein surface side chains to locally diffuse, but full hydration increased the
rates of diffusive motions, increased the amplitude of the motions, and reduced the relaxation time by over half. (Pérez et al., 1999) Zaccai and coworkers found similar hydration dependence for human hemoglobin (Stadler et al., 2009) and the various macromolecules in *Escherichia coli* as well. (Jasnin et al., 2008)

Specifically of interest in biomolecular solvation is the dependence of local water behavior on the electrostatics of different parts of a macromolecule. Proteins especially have complex hydrophobic and hydrophilic patches of varying size and shape scattered across their surfaces, leading to a complex electrostatic environment. The classical view of hydrophobic solvation, put forth by Frank and Evans 1945, is frequently referred to as the “iceberg” model. (Frank and Evans, 1945) In this model nonpolar solutes interact unfavorably with water, essentially causing water in the first and second solvation shells to interact very strongly with itself by forming very strong, linear H-bonds. In recent years this view has been challenged. Head-Gordon and coworkers have argued for instance that, based on the study of model peptides, amphiphilicity rather than hydrophobicity causes heterogeneity in local water structure compared with bulk water, (Johnson et al., 2008, Murarka and Head-Gordon, 2007) though Russo et al. attribute the water dynamic differences seen in their data on the same model peptides to destructuring of the local water network. This use of localized water dynamics information to interpret structure has been a recent, common feature in the water literature and is not unique to scattering studies. Hydrophobic solvation is discussed in more detail in later sections of Chapter 1.

**Vibrational Spectroscopy**

Vibrational spectroscopy of water (see references (Smith, 1996) and (Mukamel, 1995) for thorough references on the theory of linear FTIR and nonlinear spectroscopy respectively) is often used as a tool for studying water’s structure and dynamics and has
added immensely to the understanding of liquid water. Unlike other experimental
techniques, vibrational spectroscopy has the ability to directly probe the strength and
dynamics of hydrogen bond network of water under different conditions.

In particular, the OH or OD stretching vibration of water has been successfully used to
interpret water structure. Very dilute solutions of HOD in D₂O (H₂O) have been shown to
have their OH (OD) stretching vibrations almost entirely decoupled from one another,
meaning that an individual OH (OD) oscillator is sensitive only to its own local electronic
environment and does not transfer energy to or from other OH (OD) groups.(Graener et
al., 1991) The decoupled OH stretch therefore reports directly on the H-bond strength of
oscillators in the sample. Often used in conjunction with variable temperature(Dashnau
et al., 2006, Dashnau et al., 2008a, Fujita and Ikawa, 1989, Furutaka and Ikawa, 2001,
2005, Zelent et al., 2004) or pressure (see Chapter 4), OH stretch spectra can tell us in
an independent, model-free way about H-bond strength, though models are often also
used when interpreting OH stretch spectra.

Beginning in the early 1990’s, spurred by the advent of ultrafast pulse infrared lasers
which had not been available before, a wealth of picosecond and femtosecond infrared
studies began to emerge on water. One of the first ultrafast studies of water was
performed by Laubereau and coworkers, who used tunable picosecond laser pulses and
hole burning techniques to examine the substructure of the OH stretching absorption in a
mixture of dilute HOD in D₂O.(Graener et al., 1991) They found that there were three
distinct bands that composed the broad OH absorption and assigned these bands to
different populations of H-bonds. Later work by Dlott and coworkers,(Wang et al., 2003,
Wang et al., 2004) Bakker and coworkers,(Woutersen et al., 1997, Woutersen et al.,
1998) and others,(Asbury et al., 2003, Piletic et al., 2006a, Steinel et al., 2004a, Steinel
et al., 2004b) using more advanced optical hardware revised the number of substructure bands underlying the OH stretch spectrum down to two, which were assigned to distinct populations of H-bonds and had dramatically different lifetimes. The high frequency band, which was found to be centered at $\sim 3450 \text{ cm}^{-1}$, was assigned by all of the aforementioned groups to bent or broken H-bonds and has a vibrational lifetime on the order of 1-2 ps. The low frequency band, which the various groups assigned to strong, linear H-bonds, is centered at $\sim 3350 \text{ cm}^{-1}$ and has a much shorter vibrational relaxation lifetime, only a few hundred femtoseconds. These two very different vibrational lifetimes point not only to fundamentally different H-bonding structures of the OH oscillators, but to very different relaxation mechanisms. In addition to discovering the different substructures and the lifetimes of the bands composing the OH stretch spectrum the work discussed above was critical in measuring the large anharmonicity of the water-water H-bond.(Asbury et al., 2004, Corcelli et al., 2004, Golonzka et al., 2001, Graener et al., 1991, Wang et al., 2004) It must also be noted that the sum total of the ultrafast work aimed at uncovering the OH absorption substructure directly supports the research on the linear Raman OH absorption done decades earlier by Walrafen and coworkers.(Walrafen, 1964, Walrafen, 1967, Walrafen, 1968, Walrafen, 1969) Though Walrafen’s findings have not proven to be precisely correct with regard to band position and disparate lifetimes, his work was clearly pioneering.

The power of vibrational spectroscopy has also been brought to bear on the structure and dynamics of solutions. Bakker and coworkers have argued that ions have little to no effect on water structure(Bakker et al., 2005, Omta et al., 2003a, Omta et al., 2003b) (this result was based on the analysis of orientational correlation times), while Vanderkooi and Nucci showed clear, network wide reordering of the water in salt solutions that was dependent on the ion’s position in the Hofmeister series.(Nucci and
Also, groups using both ultrafast nonlinear (Cringus et al., 2005, Patzlaff et al., 2000, Piletic et al., 2005, Piletic et al., 2006b, Seifert et al., 2002, Tan et al., 2005) and linear (Nucci and Vanderkooi, 2005) infrared experiments have shown the profound effect on water dynamics and structure that size of the water pool can have, which has important implications for many high concentration solutions, including the cytosol of living cells.

The major shortcoming of vibrational spectroscopy of aqueous solutions is the issue of scale/concentration. Though vibrational spectroscopy has been immensely useful in studying macromolecules themselves, (see for example (Ackels et al., 2009, Andrei Piryatinski, 2000, Bredenbeck and Hamm, 2003, Goossens et al., 1996, Hamm et al., 1998, Nucci and Vanderkooi, 2007)) changes imposed on water structure are often relatively small and are only discernible when the relative concentration of the structurally altered water molecules is high. Such experiments are fairly straightforward for very soluble ions and small molecules but decidedly more difficult for large biological macromolecules. In addition, infrared spectroscopy of the OH band does not directly point to what part of a molecule, if any, the water is solvating. New work from several groups is overcoming these challenges to the site specific infrared monitoring of water behavior by the judicial use of site labeling in peptides and proteins. (Kim et al., 2009, Lin et al., 2008) Hochstrasser and coworkers, for instance, recently found that isotopically labeled β-amyloid fibrils contain mobile, structurally important water molecules. (Kim et al., 2009)

**Nuclear Magnetic Resonance**

Nuclear Magnetic Resonance (NMR) has yet to add much to the collective knowledge of bulk water structure, though there has been at least one important study focused on
biomolecular solvation. Much like what has been done in scattering studies, NMR can be used to obtain information on the magnetic shielding anisotropy, which can then be used to derive intermolecular water geometries by fitting to quantum mechanically calculated shielding tensors.(Modig and Halle, 2002, Modig et al., 2003a) Bertil Halle has coauthored much of this work, and it remains controversial due to its heavy use of simulation and parameterization, as well as its principle finding that H-bonds form a continuum of angles in liquid water rather than the distinct populations other experimental and theoretical techniques have found.

A great deal of the recent work on biomolecular solvation has also been performed by Halle and coworkers and uses a technique known as magnetic relaxation dispersion (MRD) of the $^{17}$O relaxation in isotopically labeled water. In one of their early studies using this technique Halle and coworkers found water dynamics in the binding cavities of three different lipid-binding proteins were slowed by two orders of magnitude compared with bulk water,(Modig et al., 2003b) and also recently reported that the 315 Å$^3$ binding cavity of bovine β-lactoglobulin is completely devoid of water when the free protein is in solution and waiting to bind its ligand.(Qvist et al., 2008) Halle’s group has also used the MRD technique to look at live cells, though this work is hotly debated.(Persson and Halle, 2008, Sunde et al., 2009) Halle certainly is not alone in his interest in biomolecular solvation. For instance, Böckmann et al. recently showed that in solid state NMR protein samples, supernatant water gives a completely different signal than the protein solvation water, and that the supernatant water does not interact with the protein on experimental timescales.(Böckmann et al., 2009)

Though NMR has only made modest contributions to our collective understanding of water structure and solvation dynamics, ongoing studies using isotopic labeling of protein surface residues and encapsulation in reverse micelles (which greatly slows
water dynamics) are extremely promising and may one day very soon yield site specific information on the solvation of proteins.

**Quantum Mechanical and Molecular Mechanical Calculations**

Though calculations and simulations related to water behavior have certainly increased in recent years due to greater, cheaper, and more accessible computing power, these sorts of studies have been performed for quite some time. Early molecular dynamics (MD) simulations by Rahman and Stillinger (Rahman and Stillinger, 1971) and Monte Carlo simulations by Barker and Watts (Barker and Watts, 1969) have paved the way for the large scale and complex atomistic simulations routinely performed on aqueous systems today. Likewise, in the late 1960’s growing computer power finally made possible electronic structure calculations based on existing quantum theory. Though a handful of earlier studies were performed on other small H-bonded complexes, Morokuma and Pedersen published the first result on the structure of the H-bonded water dimer in 1968. (Morokuma and Pedersen, 1968) Though these calculations were rudimentary and the results, among them a 12.6 kcal/mol H-bond energy, were quickly shown to be wrong, they made possible more advanced quantum mechanical studies that quickly followed. (Kollman and Allen, 1972) As discussed at the beginning of this Chapter, pure quantum mechanical calculations have been of great utility in studying small clusters of water molecules, but because quantum mechanical formalisms scale very poorly with the number of electrons included they have been of little use thus far in studying liquid water. It is certainly possible that advances in computing power will enable future researchers to examine water pools of sufficient size to approach condensed phase water behavior.
Much of our recent understanding of atomistic water structure and solvation dynamics comes from the use of simplified models for water which are then employed in MD simulations. Though a review of water models is beyond the scope of this Chapter, they tend to fall into a few categories with common features. The first group of models ignore the atomistic nature of water completely (and therefore tell us nothing about the water itself) and instead place molecules of interest in a solvation reaction field. The principal quantity of interest in these sorts of simulations is calculation of the polarization energy of the solute/solvent system. Although variations exist, two main models are often used, the Generalized Born (Qui et al., 1997, Still et al., 1990) method and the Finite Difference Poisson-Boltzmann method. (Sharp and Honig, 1990)

While the implicit solvation models have proven useful for studying the structure and dynamics of biomolecules and other solutes in aqueous solutions (see for example (Khalili et al., 2005, Prabhu et al., 2004)), to examine the behavior of water itself explicit models must be used. The four models used most often are SPC,(Berendsen et al., 1981) SPC/E,(Berendsen et al., 1987) TIP3P,(Jorgensen et al., 1983) and TIP4P.(Jorgensen et al., 1983) These models and others like them are based on the fixed, geometrical assignment of centers of mass and some number of static partial charges, usually three or four. Some newer water models allow for flexible water molecule geometry and charges that can change magnitude and position within the molecule to account for the well known polarization effects in real liquid water.(Rick et al., 1994) Because they are incomplete, simplified descriptions of water, each of the water models has a limited range of applicability. In addition, though the newer, more complex models may better reproduce some aspects of water behavior, each additional interaction term in the Hamiltonian in MD simulations adds to the total computational cost. For this reason, the older, less complex models are still immensely useful in
studying aqueous systems. It must be mentioned that one of the failings of classical MD simulations is that because they essentially simply iteratively solve equations of motion they are not capable of modeling bond breaking/formation. This missing ability is especially troublesome in modeling many biological systems, where bond making/breaking ability is essential for accurate modeling.

Simulations have produced a great deal of data on water structure and solvation effects. Of particular relevance to the research presented in this dissertation is the work of Sharp and Vanderkooi and coworkers in analysis of H-bonding angles in bulk and solvation water. Sharp and Vanderkooi and coworkers used a combination of Monte Carlo simulations and infrared spectroscopy to investigate the differences imposed on water structure by a number of biologically interesting solutes. In their seminal work on the subject,(Sharp et al., 2001) Sharp and coworkers used MD simulations to show that bulk water contains a bimodal distribution of H-bond angles (which was the first solid, theoretical support for the ultrafast infrared spectroscopy work on the substructure of the OH stretching vibration discussed earlier) and that trimethylamine-N-oxide methyl groups were solvated by water with more linear H-bonds as compared to bulk solvent. In the same study they found that potassium and chloride ions have the opposite effect, strongly biasing solvation water to weaker, bent H-bonds. Gallagher and Sharp also demonstrated that the methyl and hydroxyl groups of ethanol have different solvation characteristics (see Figure 1) because of their relative hydrophobicity and hydrophilicity.(Gallagher and Sharp, 2003) The Monte Carlo generated bimodal H-bond distributions by Sharp and coworkers were observed using both TIP3P and TIP4P water models, though later work by others confirmed the finding extended to both the F3C(Smolin and Daggett, 2008) and SPC/E(Kumar et al., 2007) models as well. In addition to finding solvation differences between polar and apolar chemical groups, the
work by Sharp and coworkers also quantitatively explained the well known observation that hydration of polar molecules causes a decrease in water’s heat capacity while hydration of hydrophobic molecules causes an increase in water’s heat capacity. (Gallagher and Sharp, 2003, Madan and Sharp, 1996, Madan and Sharp, 1997, Madan and Sharp, 1999, Prabhu and Sharp, 2005, Sharp et al., 2001)

Figure 1: (a) Distribution of H-bond angles and distances for pairs of water molecules in the first hydration shell of the methyl group of ethanol. (b) Distribution of H-bond angles and distances for pairs of water molecules in the first hydration shell of the hydroxyl group of ethanol. Figure taken from Gallagher and Sharp. (Gallagher and Sharp, 2003)

Though pure quantum mechanical calculations have thus far been of limited use in examining liquid water properties, in recent years mixed quantum mechanical/molecular mechanical (QM/MM) simulations have provided great insight into the role of water in chemistry. These methods break down the system of interest into two or three parts. One of the parts is treated using a quantum mechanical formalism, another of the parts is treated using a classical MD approach, and sometimes both of these parts are embedded in a dielectric field or a more advanced implicit solvation field. Simulations of this sort are much more computationally expensive than traditional MD simulations, but they are capable of describing a great deal of molecular and atomistic chemistry.
including enzymatic processes (Friesner and Guallar, 2004) and electronic excited state chemistry (Sinicropi et al., 2008).

Recent work in this field has pointed to the critical role of water in biochemistry not as simple solvent but as a reactive chemical constituent in its own right. Water has also recently been shown to be a necessary and constitutively bound structural component in several systems. Sproviero et al. showed that water molecules are bound to calcium and manganese in the oxygen evolving center of photosystem II and act as substrates for dioxygen formation (Sproviero et al., 2008). Suresh et al. found that a single, strongly bound and conserved water molecule at the interface of six different HIV protease-inhibitor complexes is critical to stabilization of the complex, and thus has a direct role in the mechanism of drug action (Suresh et al., 2008). Along the same lines, recently Ke et al. used a QM/MM method to show the hydrolysis step in protein arginine deiminase 4’s catalytic action (Ke et al., 2009).

As computational power continues to both grow and become cheaper, we can reasonably expect that the power of QM/MM simulations will continue shed light on new and perhaps unexpected roles of water in biochemistry, probing interactions that were once thought to be the sole purview of experimental techniques.

**Water’s Ice Forms**

Diffraction studies have been considerably more successful in their characterization of water’s various crystalline ice forms than they have been of liquid water, and as such the vast majority of structural ice data has been generated by scattering techniques. As can be seen in Figure 2, water has a complex and rich phase diagram, with more than a dozen solid forms so far discovered.
The Bridgman (Bridgman, 1935, Bridgman, 1937, McMillan, 2005) nomenclature, named for Percy Bridgman, a Nobel Prize winning physicist who did pioneering high pressure research, is usually used in reference to the different ice forms. Hexagonal ice, or ice Ih in the Bridgman nomenclature, can be thought of as normal, everyday ice and is the form of all naturally occurring ice on Earth. It is formed over a large range of pressures when the temperature of water is lowered to 0 °C. Ice Ih is characterized by perfect tetrahedral ordering with disordered and thermally fluctuating proton positions. Vibrational spectroscopy of ice Ih has been very useful in understanding water H-bonding in that it has pointed to the impact of H-bonding geometry on the OH stretch spectrum of water. The OH stretch spectrum of ice Ih, as can be seen later in this work in Figure 40B, is very sharp and red shifted as compared to the liquid water OH stretch spectrum. Given that the structure of ice Ih is known to a very high degree of precision over a wide range of temperature, and that it is composed of a single type of H-bond,
one may reasonably infer a direct relationship between H-bond geometry and the OH stretch spectrum. This inference is further supported by the spectra of higher pressure ices containing multiple distinct types of H-bonds.

Infrared and Raman spectra of numerous ices collected by Bertie and Whalley (Bertie et al., 1963, Bertie and Whalley, 1964a, Bertie and Whalley, 1964b, Bertie et al., 1968, Engelhardt and Whalley, 1979, Klug and Whalley, 1984, Marckmann and Whalley, 1964, Taylor and Whalley, 1964, Whalley, 1976, Wong and Whalley, 1976) differ in a predictable way based upon the H-bond geometries in the ice, which have all been measured using scattering techniques. Ices Ih and Ic (cubic ice) for instance are both known to have nearly linear H-bonds, and each water molecule in the lattice is known to experience a nearly identical electronic environment. Consequently, each of the ice spectra is very sharp and red shifted as compared to the liquid state. Generally speaking the high pressure ices have been found to be composed of at most two distinct H-bond angle geometries, a nearly linear population of H-bonds and a slightly distorted (~15 degrees) population of H-bonds, though a number of different O-O distances are found in some ices. The high pressure ices appear to pack into their decreased volumes by longer range ordering (a number of ices form interpenetrating networks) and by mixing stabilizing linear H-bonds in one part of the crystal with space saving bent H-bonds in another, rather than by continuously varying their H-bond angles.

Unlike ices Ih and Ic, most ices contain multiple possible H-bonding environments. However, much of the diffraction data on high pressure ices, though of generally higher quality than that from liquid water, comes from a relatively small handful of points on the pressure-temperature phase diagram. (See for example (Jorgensen and Worlton, 1985, Kamb and Davis, 1964, Kamb, 1965, Kamb, 1969, Knight and Singer, 2009, Kolesnikov et al., 1997, Kuhs et al., 1984, Nelmes et al., 1998a, Nelmes et al., 1998b, Polian and
Grimsditch, 1983, Somayazulu et al., 2008) for a number of diffraction studies that examine only a very small part of the pressure-temperature space in their characterization of high pressure ices. For this reason much about high pressure ice structure, and how it changes with pressure within a particular phase, is still open to study and debate. In addition, the problems inherent in using neutron diffraction for structural determination addressed earlier still hold for solid phases. These crystal structure determinations have the additional problem of often imposing too rigid of a structure on oxygen atoms within the ice lattice, which then has the unintended side effect of yielding very imprecise intra- and intermolecular distances. For this reason structural refinements of several high pressure ice forms are ongoing with no consensus yet reached on the true structure. Ice VII for instance has been reported with several quantitatively different structures due to differences in refinement method. (Jorgensen and Worlton, 1985, Kamb and Davis, 1964, Knight and Singer, 2009, Kolesnikov et al., 1997, Kuhs et al., 1984, Nelmes et al., 1998a, Nelmes et al., 1998b, Somayazulu et al., 2008, Walrafen et al., 1982) One of the agreed upon features of ice VII, which is composed of interpenetrating but unconnected ice Ic lattices, is that each H-bond in the lattice experiences an identical molecular environment, however the work presented here in Chapter 4 clearly shows multiple, distinct H-bonded species within the ice VII crystal at pressures right above the ice VI to VII transition. Figure 3 shows the ice VI crystal lattice while Figure 4 depicts the ice VII lattice.
Figure 3: Crystal lattice of ice VI, with yellow and white hydrogen atoms each indicating one of the two interpenetrating but unconnected H-bond networks. Protons are shown as ordered while in reality they are disordered. Image taken from Martin Chaplin’s website. (Chaplin, 2009b)

Figure 4: Portion of the crystal lattice of ice VI, showing the two cubic sublattices of interpenetrating but unconnected H-bond networks. Protons are shown as ordered while in reality they are disordered. Image taken from Martin Chaplin’s website. (Chaplin, 2009c)

Water ice polymorphs of all forms have also been studied using theoretical methods, including quantum mechanical calculations and MD simulations. These computational
approaches to ice formation have been used as a validation test for various MD water models, to determine the conditions under which, or even if, they form the appropriate crystal structure. (Kumagai et al., 1994, Svishchev and Kusalik, 1994, Vega et al., 2009) Simulations and calculations have also been used to predict the structure of new ices before they have been experimentally observed. (Knight and Singer, 2005, Kuo and Kuhs, 2006, Lee et al., 1993) This particular use of theoretical methods is particularly powerful since the development of new experimental methods for reaching increasingly higher pressures is usually technically difficult and time consuming. In contrast, computational studies have the luxury of exploring and modifying chemical interactions with relative ease.

Simulation work has also been invaluable in the study of ice formation and structure in situations difficult to study experimentally. Ice nucleation, for instance, is very difficult to study using conventional experimental techniques but is readily amenable to Monte Carlo methods (Radhakrishnan and Trout, 2003b) and MD simulations, (Matsumoto et al., 2002, Quigley and Rodger, 2008) while the structure and freezing of ice in nanoscale environments can be modeled computationally with relative ease compared to examining such systems experimentally. (Koga et al., 1997, Radhakrishnan et al., 2000, Radhakrishnan and Trout, 2003a) Finally, computational efforts have been invaluable in the quantification of static distortions in the crystal lattice of ice polymorphs. Static distortions in the ice lattice are often difficult to quantify when ice structures are refined from diffraction data, but ab initio and QM/MM techniques can make predictions on both the magnitudes and directions of static distortions. Kuo et. al (Kuo and Kuhs, 2006) were able to use Car-Parrinello molecular dynamics to rationalize several anomalous parameters found for the ice VI lattice using neutron diffraction. (Kuhs et al., 1984)
Summary

Water has been studied using nearly every technique imaginable, and though our understanding of water’s behavior today is perhaps greater than it has ever before been, there are still countless unanswered questions regarding water H-bonding and the particulars of biomolecular solvation. The huge volume of ongoing research into water and solvation promises to continue unabated for the foreseeable future. The water models and experiments discussed above should provide an adequate framework through which to understand the motivation behind and consequences of the work presented in this dissertation.

The goals of the work presented here in Chapters 2, 3, and 4 are perhaps modest, though it is hoped that they add substantively to the body of research in the field. In short, Chapter 2 analyzes a model water-water H-bond in terms of only its H-bond angle and O-O distance. Though the water dimer has previously been studied in great detail, the quantum mechanical work presented here uncovers features of the water-water H-bond that may be of relevance to larger clusters of water molecules and even bulk water. In Chapter 3 a study done on the solvation of the guanidinium ion is presented. A combination of infrared spectroscopy and quantum mechanical calculations point to rather extreme structuring of the H-bond network by high concentrations of guanidinium, and this result is discussed in terms of its implications for the denaturing activity of guanidinium salts. In Chapter 4 a study is presented that examines the transition from ice VI to ice VII with increased pressure and shows a heretofore unknown H-bond rearrangement in ice VII just above the ice VI to VII transition pressure. This study also examines the effects of pressure on the conformational flexibility of three small model peptides and finds substantial side chain dependence. Finally, in Chapter 5 future
directions for the work presented here are discussed, along with the broader implications of the work to the body of understanding of water structure and solvation.
CHAPTER 2

THE POTENTIAL ENERGY SURFACE OF THE WATER DIMER’S SINGLE HYDROGEN BOND

Introduction

The H-bond is one of the most fundamental interactions in chemistry, and the role of H-bonds in the formation of liquid water is the context in which they have most often been studied. From the very early qualitative descriptions of water H-bonds by Latimer and Rodebush (Latimer and Rodebush, 1920) to the recent highly detailed multidimensional water dimer and trimer potential energy surfaces calculated at high levels of quantum theory by Huang et al. (Huang et al., 2008) and Wang et al.,(Wang et al., 2008) water H-bonding continues to be an extremely active area of research with many researchers attempting to better understand the H-bond both qualitatively and quantitatively.

H-bonding is found not only in water but also in a wide range of biologically interesting molecules. When contemplated within the framework of modern biochemistry H-bonds take on a new role. Beyond a mere theoretical curiosity, H-bonds have been shown to be critical determinants of biomolecular structure and function.(Creighton, 1991, Levy and Onuchic, 2006, Oleinikova et al., 2005, Riley and Hobza, 2007, Vanderkooi et al., 2005) Indeed, even intermolecular water H-bonding itself has been increasingly implicated in direct and indirect roles it may have on biologically relevant molecules.(Dashnau et al., 2006, Dashnau et al., 2008a, Levy and Onuchic, 2006, Nucci and Vanderkooi, 2005, Scott et al., 2008, Sharp et al., 2001, Sharp and Vanderkooi, 2009, Sorin et al., 2006, Vanderkooi et al., 2005)
Though there is widespread agreement that H-bonding is very important, both in its theoretical context and for its effect on the elements of life, there is still no consensus as to the precise nature of the H-bond or exactly what constitutes an H-bond. (Barbiellini and Shukla, 2002, Gallagher and Sharp, 2003, Isaacs et al., 1999, Isaacs et al., 2000, Kumar et al., 2007, Smith et al., 2004, Weinhold et al., 2005, Wernet et al., 2004) Insofar as water is concerned, the principle problem lies in the fact that there exists no experimental probe of water-water orientation. Therefore, though radial distribution functions can be obtained for bulk water’s individual atoms using scattering methods, (Brady et al., 2006, Hura et al., 2000, Narten and Levy, 1971, Soper and Phillips, 1986, Soper, 2000, Strassle et al., 2006, Wernet et al., 2004) it is difficult to determine with any degree of experimental certainty the average intermolecular geometry of condensed phase water molecules. It should also be noted that numerous intra- and intermolecular geometries can fall under the same radial distribution function, so even very precise radial distribution functions do not unambiguously determine structure.

Fortunately where direct experimental evidence is lacking theoretical methods still allow us to probe chemically interesting systems or interactions. The water dimer is one such system, constituting the simplest example of a water-water H-bond. This system is especially amenable to study using quantum mechanical methods due to its relatively small number of electrons. The water dimer has even become a de facto test for new quantum mechanical methods since there is experimental gas phase binding data against which their results can be compared. (Curtiss et al., 1979, Mas et al., 2000)

A great deal of the quantum mechanical work on the water dimer has aimed at describing local minima and stationary points in its multidimensional potential energy surface (see Ref. (Scheiner, 1994) for an excellent review of early work in the field). A
recent study by Shank et al.,(Shank et al., 2009) for instance, fit a coupled cluster calculated 30,000 point full-dimensional global PES, encompassing 10 stationary points, for a water dimer. The present study differs from this and other previous studies of the water dimer PES in that we chose to not only simplify the two water monomers by fixing their internal geometries but that we also purposefully restricted the intermolecular orientation to examine very specific variables. As such, the vast majority of our calculated PES is far away from any sort of minimum structure or transition state. Our reasoning for adopting this approach was twofold. Since the dimer is used as a simplified mock up of liquid water, and liquid water itself contains constantly fluctuating H-bonds, we reasoned that areas of the PES far away from equilibrium might be of interest in their applicability to H-bond geometries in real water. This simplified approach also gave us the opportunity to specifically address the relative, independent effects of H-bond angle and H-bond length, which in this case were defined as the HOO angle and the O-O distance.

Materials and Methods

The internal molecular geometry for each water molecule constituting the water dimers studied was constructed such that its OH lengths were set to 0.0991 nm and its HOH angle was set to 105.5°.(Silvestrelli and Parrinello, 1999) This internal geometry was held rigid for both water monomers throughout all of the calculations described here. The dimers were then formed to test two general hydrogen bonding schemes. In the first formulation, the H-bond donor molecule’s oxygen atom and the H-bond donor hydrogen were placed in the plane with the H-bond acceptor water molecule (Figure 5A). This geometry is henceforth referred to as “planar” for the sake of brevity. In the second H-bonding arrangement, the H-bond donor molecule is placed relative to the H-bond acceptor molecule such that it donates its H-bond to lone pair electrons of the H-bond
acceptor’s oxygen atom (Figure 5B). (Odutola and Dyke, 1980) This geometry will hereafter be referred to as “tetrahedral.”

![Figure 5: Intra- and inter-molecular geometries of the “planar” and “tetrahedral” water dimers.](image)

All calculations were carried out using Gaussian 03 Revision D.01. (Frisch, 2004) All data manipulation and plotting was performed using MATLAB 7.6.0. Calculations for each geometry were performed using both MP2 (Head-Gordon et al., 1988) and B3LYP (Becke, 1993, Lee et al., 1988, Miehlich et al., 1989) chemistries with the aug-cc-pVTZ (Davidson, 1996, Kendall et al., 1992) and 6-311++G(d,p) (Krishnan et al., 1980) basis sets respectively. These chemistry/basis set combinations are not sufficient for high level calculations of H-bond interactions (Boese et al., 2007, Bukowski et al., 2008, Inada and Orita, 2007, Lee, 2007, Riley and Hobza, 2007, Santra et al., 2007, Schutz et al., 1997) (the coupled cluster methods currently give the most accurate H-bonding energies (Huang et al., 2008, Shank et al., 2009, Tschumper et al., 2002)) but were deemed to be acceptable for the sort of comparative analysis performed in this study. All vacuum energy calculations used the counterpoise method of basis set superposition error correction. (Boys and Bernardi, 1970, Simon et al., 1996)
In addition to standard gas phase vacuum calculations, the Polarizable Continuum Model (PCM) solvation model (Cossi et al., 2003) was also implemented in an effort to place the H-bonded dimer in the context of a simple liquid water reaction field. The PCM solvation method is rudimentary when compared with the effect of explicit first solvation shell water molecules, but the inclusion of first shell water molecules would have made it difficult, if not impossible, to unambiguously attribute changes in the PES to intermolecular orientation of the H-bond of interest. The combinations of dimer geometry, model chemistry and basis set, and solvation state yielded eight complete data sets.

For both H-bonding dimer geometries, the O-O distance was scanned at a fixed H-bond angle, defined herein as the angle between the H-bond donor molecule’s donor OH vector and the O-O vector. The H-bond angle was subsequently increased by one degree and the O-O distance was scanned again for the new angle. Angles from 0° to 90° in 1° increments were used, and O-O distances from 0.25 nm to 0.40 nm were scanned at every 0.01 nm. For each O-O distance and HOO angle system energy was calculated. Natural Population Analysis, a part of the Natural Bond Orbital formalism, (Rives and Weinhold, 1980) was used to calculate atomic charges. The calculated energy surface for the dimers was also examined by taking slices along the H-bond angle dimension. This subset of the data was analyzed by fitting the energy vs. O-O distance data for a particular H-bond angle to the Morse potential function,

\[ V(r) = D_e \left( 1 - e^{-a(r-r_e)} \right)^2. \]  

(1)
Results

Energies

Energies are given in kcal/mol, relative to the calculated minimum energy for a particular set of calculations. The true calculated minimum energy and O-O distance/H-bond angle position of that energy for each of the eight sets of calculations is given in Table 1. In Figures 6A and 7A the 3-dimensional energy landscapes for the MP2/aug-cc-pVTZ planar and tetrahedral vacuum cases are shown and Figures 6B and 7B contain the same information in 2-dimensional color mapped projections. Figures 8A and 9A show the 3-dimensional energy landscapes for the MP2/aug-cc-pVTZ planar and tetrahedral PCM cases, and Figures 8B and 9B contain the same information in 2-dimensional color mapped projections. Figures 10A and 11A show the 3-dimensional energy landscapes for the B3LYP/6-311++G(d,p) planar and tetrahedral vacuum cases, and Figures 10B and 11B contain the same information in 2-dimensional color mapped projections. Finally, Figures 12A and 13A show the 3-dimensional energy landscapes for the B3LYP/6-311++G(d,p) planar and tetrahedral PCM cases, and Figures 12B and 13B contain the same information in 2-dimensional color mapped projections.

Each of the calculated energy surfaces has the same essential characteristics, indicating that the trends we observe are not simply model or basis set dependent. Each of the surfaces has its global minimum at an H-bond angle of about 0° (the angle is somewhat distorted for both of the tetrahedral vacuum cases) and an O-O distance of about 0.29 nm. Increases in O-O distance beyond the global minimum for a given PES result in energy increases of 2.5 to 2.9 kcal/mol for the MP2/aug-cc-pVTZ calculations and 2.7 to 4.3 kcal/mol for the B3LYP/6-311++G(d,p) calculations.
<table>
<thead>
<tr>
<th>Method</th>
<th>Geometry</th>
<th>H-Bond Angle (°)</th>
<th>O-O Distance (nm)</th>
<th>Energy (Hartrees)</th>
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</thead>
<tbody>
<tr>
<td>B3LYP Planar</td>
<td>Vacuum</td>
<td>0</td>
<td>0.30</td>
<td>-152.921808332442</td>
</tr>
<tr>
<td></td>
<td>PCM</td>
<td>2</td>
<td>0.28</td>
<td>-152.947883176000</td>
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<tr>
<td>B3LYP Tetrahedral</td>
<td>Vacuum</td>
<td>6</td>
<td>0.29</td>
<td>-152.922480853247</td>
</tr>
<tr>
<td></td>
<td>PCM</td>
<td>3</td>
<td>0.28</td>
<td>-152.948069476000</td>
</tr>
<tr>
<td>MP2 Planar</td>
<td>Vacuum</td>
<td>2</td>
<td>0.29</td>
<td>-152.661892519226</td>
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<tr>
<td></td>
<td>PCM</td>
<td>2</td>
<td>0.29</td>
<td>-152.142119811000</td>
</tr>
<tr>
<td>MP2 Tetrahedral</td>
<td>Vacuum</td>
<td>6</td>
<td>0.29</td>
<td>-152.662601687797</td>
</tr>
<tr>
<td></td>
<td>PCM</td>
<td>3</td>
<td>0.29</td>
<td>-152.142102842000</td>
</tr>
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</table>
Figure 6: 3D (a) and 2D (b) views of the calculated energy landscape for the MP2/aug-cc-pVTZ planar vacuum dimer. Energies are relative to the calculated minimum for this particular PES.

Figure 7: 3D (a) and 2D (b) views of the calculated energy landscape for the MP2/aug-cc-pVTZ tetrahedral vacuum dimer. Energies are relative to the calculated minimum for this particular PES.
Figure 8: 3D (a) and 2D (b) views of the calculated energy landscape for the MP2/aug-cc-pVTZ planar PCM dimer. Energies are relative to the calculated minimum for this particular PES.

Figure 9: 3D (a) and 2D (b) views of the calculated energy landscape for the MP2/aug-cc-pVTZ tetrahedral PCM dimer. Energies are relative to the calculated minimum for this particular PES.
**Figure 10**: 3D (a) and 2D (b) views of the calculated energy landscape for the B3LYP/6-311++G(d,p) planar vacuum dimer. Energies are relative to the calculated minimum for this particular PES.

**Figure 11**: 3D (a) and 2D (b) views of the calculated energy landscape for the B3LYP/6-311++G(d,p) tetrahedral vacuum dimer. Energies are relative to the calculated minimum for this particular PES.
Figure 12: 3D (a) and 2D (b) views of the calculated energy landscape for the B3LYP/6-311++G(d,p) planar PCM dimer. Energies are relative to the calculated minimum for this particular PES.

Figure 13: 3D (a) and 2D (b) views of the calculated energy landscape for the B3LYP/6-311++G(d,p) tetrahedral PCM dimer. Energies are relative to the calculated minimum for this particular PES.
Increases in HOO angle beyond the calculated minimum energy for a given PES result in energy increases from 5.4 to 6.5 kcal/mol for the MP2/aug-cc-pVTZ calculations and 7.4 to 8.1 kcal/mol for the B3LYP/6-311++G(d,p) calculations. Table 2 catalogs the effect on dimer energy of O-O distance lengthening for a fixed H-bond angle and H-bond angle distortion for a fixed O-O separation for each chemistry/basis set, solvation state, and geometry combination used. The difference in the effects of O-O distance lengthening and H-bond angle distortion can also be seen in all of the 2-dimensional energy maps, where the “cold,” or low energy, portions of the energy surface are restricted to H-bond angles below approximately 50° while the higher energy regions of the surface are all found in the cases of more substantially bent H-bonds. In all cases, shortening of the O-O distance below 0.28 nm causes an abrupt increase in system energy due to repulsion.
Table 2. Change in System Energy, Relative to the Minimum Energy Geometry for a Given PES, with Increase in H-Bond Angle or O-O Distance

<table>
<thead>
<tr>
<th></th>
<th>ΔAngle(^a) (kcal/mol)</th>
<th>ΔDistance(^b) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B3LYP Planar Vacuum</td>
<td>7.6</td>
<td>2.7</td>
</tr>
<tr>
<td>B3LYP Planar PCM</td>
<td>7.9</td>
<td>4.3</td>
</tr>
<tr>
<td>B3LYP Tetrahedral Vacuum</td>
<td>7.4</td>
<td>3.0</td>
</tr>
<tr>
<td>B3LYP Tetrahedral PCM</td>
<td>8.1</td>
<td>4.3</td>
</tr>
<tr>
<td>MP2 Planar Vacuum</td>
<td>6.4</td>
<td>2.6</td>
</tr>
<tr>
<td>MP2 Planar PCM</td>
<td>5.4</td>
<td>2.5</td>
</tr>
<tr>
<td>MP2 Tetrahedral Vacuum</td>
<td>6.5</td>
<td>2.9</td>
</tr>
<tr>
<td>MP2 Tetrahedral PCM</td>
<td>5.6</td>
<td>2.5</td>
</tr>
</tbody>
</table>

\(^a\) ΔAngle energies were determined by subtracting the energy of the minimum energy geometry for a particular PES from the energy of the geometry with the same O-O distance but \(\angle \text{HOO} = 90^\circ\).

\(^b\) ΔDistance energies were determined by subtracting the energy of the minimum energy geometry for a particular PES from the energy of the geometry with the same H-bond angle but O-O = 0.40 nm.

**O-O Energy Fits**

In Figures 14A and 15A, 2-dimensional plots of the dimer system energy versus O-O distance for the MP2/aug-cc-pVTZ planar and tetrahedral vacuum cases are shown for H-bond angles of 0° to 90° at 5° intervals. Figures 14B and 15B show \(R^2\) values obtained from fitting a standard Morse potential (see Equation 1) to every calculated angle’s energy dependence on O-O distance. In Figures 16A and 17A, 2-dimensional plots of the dimer system energy versus O-O distance for the MP2/aug-cc-pVTZ planar
and tetrahedral PCM cases are shown for H-bond angles of 0° to 90° at 5° intervals, and figures 16B and 17B show $R^2$ values obtained from fitting a standard Morse potential to every calculated angle’s energy dependence on O-O distance for the same data sets. In Figures 18A and 19A, 2-dimensional plots of the dimer system energy versus O-O distance for the B3LYP/6-311++G(d,p) planar and tetrahedral vacuum cases are shown for H-bond angles of 0° to 90° at 5° intervals, and figures 18B and 19B show $R^2$ values obtained from fitting a standard Morse potential to every calculated angle’s energy dependence on O-O distance for the same data sets. In Figures 20A and 21A, 2-dimensional plots of the dimer system energy versus O-O distance for the B3LYP/6-311++G(d,p) planar and tetrahedral PCM cases are shown for H-bond angles of 0° to 90° at 5° intervals, and figures 20B and 21B show $R^2$ values obtained from fitting a standard Morse potential to every calculated angle’s energy dependence on O-O distance for the same data sets.

These slices, taken along the O-O distance dimension of the 3-dimensional energy surfaces, reveal energy profiles that fit extremely well to a Morse potential energy function for small H-bond angles. Though the exact point where “Morseness” breaks down is difficult to quantify, significant deviations in the $R^2$ value of the fits begin to occur at approximately 50-65° for the vacuum calculations (Figures 14B, 15B, 18B, and 19B) and 30-40° for the PCM calculations (Figures 16B, 17B, 20B, and 21B). These angles are also where errors increase greatly for the individual Morse fit parameters (not shown) and where the energy curves cease to have a local minimum. For example, the well depth parameter in the Morse function, $D_e$, fits with a 95% confidence interval of hundredths of a kcal/mol for all angles up to 60° for the MP2/aug-cc-pVTZ planar vacuum case, at which point the confidence intervals grow to several tenths of a kcal/mol. By the time the H-bond is distorted to 73° fits are exceptionally poor, yielding
confidence intervals of more than 1 kcal/mol. Similar trends were obtained for the other combinations of model chemistry/basis set, solvation state, and dimer geometry, with only the particular angle at which Morseness breaks down changing with the PES in question.
**Figure 14:** a) The change in system energy with O-O distance at fixed H-bond angle for the MP2/aug-cc-pVTZ planar vacuum case. Energy curves are shown for H-bond angles from 0° to 90° at 5° intervals, with the 0° curve being lowest in energy and 90° being highest in the figure. b) $R^2$ values obtained from fitting a standard Morse potential to every calculated angle’s energy dependence on O-O distance.

**Figure 15:** a) The change in system energy with O-O distance at fixed H-bond angle for the MP2/aug-cc-pVTZ tetrahedral vacuum case. Energy curves are shown for H-bond angles from 0° to 90° at 5° intervals, proceeding from bottom to top in the order 5°, 10°, 0°, 15°, 20°, etc. up to 90°. b) $R^2$ values obtained from fitting a standard Morse potential to every calculated angle’s energy dependence on O-O distance.
Figure 16: a) The change in system energy with O-O distance at fixed H-bond angle for the MP2/aug-cc-pVTZ planar PCM case. Energy curves are shown for H-bond angles from 0° to 90° at 5° intervals, with the 0° curve being lowest in energy and 90° being highest in the figure. b) $R^2$ values obtained from fitting a standard Morse potential to every calculated angle's energy dependence on O-O distance.

Figure 17: a) The change in system energy with O-O distance at fixed H-bond angle for the MP2/aug-cc-pVTZ tetrahedral PCM case. Energy curves are shown for H-bond angles from 0° to 90° at 5° intervals, with the 0° curve being lowest in energy and 90° being highest in the figure. b) $R^2$ values obtained from fitting a standard Morse potential to every calculated angle's energy dependence on O-O distance.
Figure 18: a) The change in system energy with O-O distance at fixed H-bond angle for the B3LYP/6-311++G(d,p) planar vacuum case. Energy curves are shown for H-bond angles from 0° to 90° at 5° intervals, with the 0° curve being lowest in energy and 90° being highest in the figure. b) $R^2$ values obtained from fitting a standard Morse potential to every calculated angle’s energy dependence on O-O distance.

Figure 19: a) The change in system energy with O-O distance at fixed H-bond angle for the B3LYP/6-311++G(d,p) tetrahedral vacuum case. Energy curves are shown for H-bond angles from 0° to 90° at 5° intervals, proceeding from bottom to top in the order 5°, 10°, 0°, 15°, 20°, etc. up to 90°. b) $R^2$ values obtained from fitting a standard Morse potential to every calculated angle’s energy dependence on O-O distance.
Figure 20: a) The change in system energy with O-O distance at fixed H-bond angle for the B3LYP/6-311++G(d,p) planar PCM case. Energy curves are shown for H-bond angles from 0° to 90° at 5° intervals, with the 0° curve being lowest in energy and 90° being highest in the figure. b) $R^2$ values obtained from fitting a standard Morse potential to every calculated angle’s energy dependence on O-O distance.

Figure 21: a) The change in system energy with O-O distance at fixed H-bond angle for the B3LYP/6-311++G(d,p) tetrahedral PCM case. Energy curves are shown for H-bond angles from 0° to 90° at 5° intervals, with the 0° curve being lowest in energy and 90° being highest in the figure. b) $R^2$ values obtained from fitting a standard Morse potential to every calculated angle’s energy dependence on O-O distance.
Charges

Figures 22A and 23A for the MP2/aug-cc-pVTZ planar and tetrahedral vacuum cases are 2-dimensional color maps depicting the magnitude of the charge on the oxygen atom of the H-bond acceptor water molecule and Figures 22B and 23B show similar maps for the charge on the H-bond donor hydrogen atom. Figures 24A and 25A are 2-dimensional color maps depicting the magnitude of the charge on the oxygen atom of the H-bond acceptor water molecule for the MP2/aug-cc-pVTZ planar and tetrahedral PCM cases and Figures 24B and 25B show similar maps for the charge on the H-bond donor hydrogen atom for the same data set. Figures 26A and 27A are 2-dimensional color maps depicting the magnitude of the charge on the oxygen atom of the H-bond acceptor water molecule for the B3LYP/6-311++G(d,p) planar and tetrahedral vacuum cases and Figures 26B and 27B show similar maps for the charge on the H-bond donor hydrogen atom for the same data set. Figures 28A and 29A are 2-dimensional color maps depicting the magnitude of the charge on the oxygen atom of the H-bond acceptor water molecule for the B3LYP/6-311++G(d,p) planar and tetrahedral PCM cases and Figures 28B and 29B show similar maps for the charge on the H-bond donor hydrogen atom for the same data set.

The overall similarity of the potential energy surfaces calculated for the different model chemistries and geometries extends to the charges on the H-bond acceptor water molecule’s oxygen and the hydrogen donor atoms. At the dimer’s minimum energy configuration the hydrogen atom has its largest positive charge, and the charge decreases slightly as the O-O distance grows and more significantly as the H-bond angle increases. The same trend is seen for the charge on the oxygen atom, with its being the most negative at the dimer's minimum energy configuration and becoming slightly less negative as the O-O distance grows and much less negative as the H-bond
angle increases. Exact values are given in Table 3 for the relative effects of O-O distance lengthening versus H-bond angle bending. In nearly every case H-bond angle distortion has at least twice the effect on charge as O-O lengthening does, though the PCM solvation method reverses the effect on the H-bond donor hydrogen atom.

**Figure 22:** 2D charge landscapes for the H-bond accepting oxygen atom (a) and H-bond donating hydrogen atom (b) for the MP2/aug-cc-pVTZ planar vacuum water dimer case.

**Figure 23:** 2D charge landscapes for the H-bond accepting oxygen atom (a) and H-bond donating hydrogen atom (b) for the MP2/aug-cc-pVTZ tetrahedral vacuum water dimer case.
Figure 24: 2D charge landscapes for the H-bond accepting oxygen atom (a) and H-bond donating hydrogen atom (b) for the MP2/aug-cc-pVTZ planar PCM water dimer case.

Figure 25: 2D charge landscapes for the H-bond accepting oxygen atom (a) and H-bond donating hydrogen atom (b) for the MP2/aug-cc-pVTZ tetrahedral PCM water dimer case.
Figure 26: 2D charge landscapes for the H-bond accepting oxygen atom (a) and H-bond donating hydrogen atom (b) for the B3LYP/6-311++G(d,p) planar vacuum water dimer case.

Figure 27: 2D charge landscapes for the H-bond accepting oxygen atom (a) and H-bond donating hydrogen atom (b) for the B3LYP/6-311++G(d,p) tetrahedral vacuum water dimer case.
Figure 28: 2D charge landscapes for the H-bond accepting oxygen atom (a) and H-bond donating hydrogen atom (b) for the B3LYP/6-311++G(d,p) planar PCM water dimer case.

Figure 29: 2D charge landscapes for the H-bond accepting oxygen atom (a) and H-bond donating hydrogen atom (b) for the B3LYP/6-311++G(d,p) tetrahedral PCM water dimer case.
Table 3. Change in Charge with Increase in O-O Distance and H-Bond Angle

<table>
<thead>
<tr>
<th>Method</th>
<th>Geometry</th>
<th>O/ΔAngle&lt;sup&gt;a&lt;/sup&gt; (e)</th>
<th>O/ΔDistance&lt;sup&gt;b&lt;/sup&gt; (e)</th>
<th>H/ΔAngle&lt;sup&gt;a&lt;/sup&gt; (e)</th>
<th>H/ΔDistance&lt;sup&gt;b&lt;/sup&gt; (e)</th>
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</thead>
<tbody>
<tr>
<td>MP2</td>
<td>Planar</td>
<td>0.032</td>
<td>0.013</td>
<td>-0.038</td>
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<td>PCM</td>
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<tr>
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<td>0.006</td>
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</tr>
<tr>
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<td>PCM</td>
<td>0.014</td>
<td>-0.003</td>
<td>-0.003</td>
<td>-0.012</td>
</tr>
</tbody>
</table>

<sup>a</sup> ΔAngle charges were determined by subtracting the charge of the minimum energy geometry for a particular PES from the charge of the geometry with the same O-O distance but ∠HOO = 90°.

<sup>b</sup> ΔDistance charge were determined by subtracting the charge of the minimum energy geometry for a particular PES from the charge of the geometry with the same H-bond angle but O-O = 0.40 nm.
Discussion

There has been a tremendous amount of research and discussion on the nature of H-bonds over the years. The H-bond itself is still so poorly understood at the fundamental level that it remains unclear exactly what geometry constitutes a “real” H-bond, with a variety of distance and angle cutoffs used to specify H-bonding interactions. This question is deeper than mere nomenclature and instead points to the underlying question, that being “what is the fundamental nature of the H-bond and how do we know when one exists?” In this study we chose to focus on one of the most commonly used theoretical instances of H-bonding in chemistry, the single H-bond between two water molecules in which one water molecule’s oxygen atom accepts a single H-bond from an H-bond donor water molecule. By standardizing the internal molecular geometries we were able to examine in detail the effect that intermolecular orientation, specifically the O-O separation and the H-bond angle, had on a number of quantum mechanically calculable quantities. Though internal fluctuations in OH bond length and HOH angle are certainly important in the energetics of real water H-bonds, it is only through the use of the rigid monomer approximation that we were able to elucidate the relative and independent effects of H-bond angle and O-O distance on the single intermolecular H-bond.

The first calculated quantity of interest in any quantum mechanical system is the energy, and the 3-dimensional and 2-dimensional energy landscapes shown in Figures 6 through 13 clearly show that H-bond angle has a far more pronounced effect on system energy than does the linear separation of the individual water molecules. At the known O-O distance for the largest fraction of liquid water pairs, approximately 0.28 nm, (Strassle et al., 2006) the MP2/aug-cc-pVTZ dimers show energy increases of between 5.4 and 6.4 kcal/mol when distorting the H-bond angle from 0° to 90°, while
lengthening the O-O distance to 0.40 nm with a fixed H-bond angle of 0° increases the energy by between 2.5 and 2.8 kcal/mol, or about half the effect seen upon increasing the H-bond angle. The B3LYP/6-311++G(d,p) dimers, though presenting a slightly more curved energy landscape, show the same behavior, with energy increases of 7.0 to 7.9 kcal/mol due to angular distortion for a dimer with the O-O distance fixed at 0.28 nm and only 2.7 to 4.2 kcal/mol for increase in O-O separation to 0.28 nm with a fixed H-bond angle of 0°. For both the MP2/aug-cc-pVTZ and the B3LYP/6-311++G(d,p) calculations, the PCM solvation method serves to flatten the PES at large H-bond angles, due to interaction between the H-bond donor hydrogen atom and the reaction field. In liquid water O-O separation of first shell water molecules is tightly confined, as can be seen in the narrow and well defined peak at 0.28 nm in the oxygen radial distribution. The computational results presented here seem to indicate that the small fluctuations in O-O separation, and therefore H-bond length, which are possible in condensed phase water are likely to have only very small effects on H-bond energy and that the principal factor in determining H-bond strength is in fact H-bond angle.

Examination of the slices along the H-bond distance dimension of the energy landscape also points to the critical role that H-bond angle plays in the H-bonding interaction of the water dimer, in addition to defining a range over which we may differentiate a strong H-bond from a bent or broken H-bond. The H-bond distance versus energy plots show Morse-like distance dependence, which is typically indicative of covalent interactions, up to about 50-65° for the vacuum calculations and 30-40° for the PCM calculations. The well depth of the energy profiles is at its deepest for small H-bond angles and gradually grows shallower as the H-bond angle distorts. At the point where there ceases to be a local minimum, the interaction is entirely repulsive, and a Morse fit becomes nonsensical. Though a water dimer with fixed bond lengths and angles is a
simplification of real water H-bonding it is not difficult to envision a similar, though likely more complex, H-bonding cutoff scheme for H-bonds in liquid water.

MP2/aug-cc-pVTZ and B3LYP/6-311++G(d,p) calculations, both for the vacuum and PCM cases, similarly point to H-bond angle having a much larger effect on the H-bond acceptor water molecule’s oxygen and H-bond donor hydrogen charges than does the H-bond distance. For the MP2/aug-cc-pVTZ vacuum calculations, for instance, the donor hydrogen charge decreases by 0.033 e to 0.038 e when the angle of the H-bond is increased from 0° to 90° with the O-O distance fixed at 0.28 nm, whereas the increase of H-bond distance from 0.28 nm to 0.40 nm at a 0° H-bond angle causes a decrease in charge of only 0.023 to 0.024 e (data read from Figures 22B and 23B). The same trend in the data is seen for all of the vacuum calculations, independent of dimer geometry or model chemistry/basis set. For the PCM calculations however, this finding is reversed, with O-O separation having the greater effect on the charge of the H-bond donor hydrogen atom due to strong interaction between it and the solvation reaction field.

The MP2/aug-cc-pVTZ vacuum dimer H-bond acceptor oxygen atoms show a change in charge similar to that seen for the H-bond donor hydrogen atom for both dimer geometries, with an increase of 0.021 to 0.033 e upon angular deviation at a fixed O-O distance of 0.28 nm and only 0.007 to 0.013 e when the O-O separation is lengthened to its maximum value while the H-bond angle is held at 0° (data read from Figures 22A and 23A). In fact, for all of the calculations, independent of model chemistry, solvation state, or dimer geometry, deviation of the H-bond angle to 90° with the O-O distance fixed at 0.28 nm has over twice the effect on oxygen charge that keeping the H-bond angle at 0° and separating the oxygen atoms to 0.40 nm does. Though the exact charge magnitudes change somewhat based on the geometry, model chemistry and basis set, and solvation state used the trend is consistent throughout the calculations, indicating
that H-bond angle has a much greater effect on the charge of the two H-bonding atoms than does their radial separation. The only exception to this finding is for the aforementioned H-bond donor hydrogen atom involved in PCM calculations.

The energy surfaces calculated show that there is a clear point beyond which the H-bond is no longer attractive, and though the exact angle at which this threshold is reached changes depending on the particular model chemistry/basis set, intermolecular geometry, or solvation state used, the finding is consistent for each of the eight calculations. The surfaces also show that O-O separation has a very small effect on the dimer energy as compared to distortion of the H-bond angle. The effect of the strong/broken H-bond dichotomy can also be observed in its effect on atomic charges.

We note that though we were mindful of liquid water in carrying out the calculations discussed here, even going so far as to use water monomer internal geometries calculated by Silvestrelli and Parrinello (Silvestrelli and Parrinello, 1999) as averages in liquid water, and discuss our results as they might be applied to liquid water, the approach we have chosen is simplified. Therefore, though we can speak with confidence about the trends in the calculated PES, an idealized OH-O H-bond, and a gas phase water dimer, the exact numbers we have calculated would not hold up to experimental scrutiny for real liquid water. Nevertheless, the strong consistency of the results we have presented here shows what we suggest are general trends in H-bond energetics.

Summary

This work presents a novel result on the influence of H-bond angle on the energetics and charges of a singly H-bonded water dimer. We have described what we believe to be new evidence and insight into what may well be a general feature of H-bond response to changes in basic donor/acceptor intermolecular geometry. By simplifying the
internal geometries of two water molecules we were able to examine in detail the dependency of energy and charge on H-bond angle and O-O separation. We found that H-bond angle appears to play the largest part in determining H-bond strength with O-O distance, and therefore H-bond length, accounting for a much smaller part. Though these results are not immediately applicable to the more complex case of multiple H-bond donation and acceptance found in liquid water, it may be that the sort of H-bond dependence found here for a single H-bond is still relevant at some level to a condensed phase H-bonding
CHAPTER 3

RESTRUCTURING OF THE HYDROGEN BOND NETWORK BY THE GUANIDINIUM CATION AND IMPLICATIONS FOR ITS DENATURANT ACTION

Introduction

Guanidine salts, such as guanidine-HCl, have been used for over 70 years to destabilize or completely denature proteins in solution.(Greenstein, 1938, Pace, 1986, Svedberg, 1937) Guanidine salts have proven to be particularly useful in the biochemical and biophysical study of protein folding, since a great number of proteins can be successfully refolded by dialyzing out the salt.(Neurath et al., 1942) Despite many avenues of investigation over the course of many years, the exact mechanism by which the guanidinium cation \((\text{Gdm}^+, \text{C(NH}_2)_3^+)\) destabilizes the folded structure of proteins is still a matter of debate and speculation.(Pace et al., 2005, Schellman, 2002, Tanford, 1970) The primary reason that has been proposed for \(\text{Gdm}^+\) denaturation of proteins is that \(\text{Gdm}^+\) directly interacts with the protein or some part of it,(Mason et al., 2003, O'Brien et al., 2007, Pace, 1986, Robinson and Jencks, 1965, Roseman and Jencks, 1975, Schellman, 1987, Timasheff, 1992) while alteration of the solution water structure has been studied much less than for the other common chemical denaturant, urea, and has been ignored or minimized as a possible denaturation mechanism.(Batchelor et al., 2004, Mason et al., 2003, Mason et al., 2004, Mountain and Thirumalai, 2004, Shimizu et al., 2000, Vanzi et al., 1998)

While there is certainly excellent evidence for the direct interaction model of guanidinium’s protein denaturation action, this particular model certainly does not rule out the possibility for other causes. Here, we offer support for an additional mechanism for the destabilization and unfolding of proteins whereby \(\text{Gdm}^+\) restructures the solution
water. We hypothesized, as have others, (Alonso and Dill, 1991, Breslow and Guo, 1990, Rupley, 1964, Schiffer and Dotsch, 1996, Tanford, 1970, Vanzi et al., 1998, Wetlaufer et al., 1964) that the Gdm⁺ cation might, in fact, destabilize folded proteins by altering the H-bonding network of water, such that the native, folded state of a given protein is no longer energetically favorable and net equilibrium is shifted towards the unfolded state.

To test this hypothesis, infrared spectroscopy was used to examine the OH stretching vibration of dilute HOD in D₂O solutions containing different concentrations of dissolved guanidine salts. Infrared spectroscopy is sensitive to changes in the environment and interactions local to vibrating molecules, and therefore the infrared absorption lineshapes of those vibrating molecules can be used as a probe of their environment. Also, for dilute solutions of HOD in D₂O an individual OH oscillator is almost completely decoupled from other OH oscillators, making the OH a selective and independent local probe. In contrast to other experimental methods previously used to examine the structure of water in a guanidinium-containing solution, such as neutron diffraction, calorimetry, and NMR, infrared spectroscopy is a uniquely sensitive probe of H-bond strength.

Since nearly all biological phenomena occur in an aqueous environment, solution FTIR spectroscopy can be a particularly useful technique for investigating biologically relevant interactions. Of particular interest in biological solution spectroscopy is how and to what degree biological solutes affect the structure of water. Because of its ability to form strong H-bonds with both itself and with solutes, water has the ability to solvate a tremendous variety of biologically relevant molecules. In addition, the ability to both donate and accept H-bonds allows water to adopt a flexible, three dimensional H-bond network. This H-bond network is responsive to both external and internal perturbations,
such as those caused by the addition or removal of thermal energy or by the addition of solutes.

Temperature excursion IR (TEIR) is very useful for studying H-bonded systems such as aqueous solutions. (Dashnau et al., 2006, Imamura et al., 2006, Nucci and Vanderkooi, 2005, Rozenberg et al., 2004, Vanderkooi et al., 2005) The structure of the H-bond network of water is sensitive to changes in temperature, and these temperature-dependent structural rearrangements of the H-bond network are reflected in the spectra. (Dashnau et al., 2006, Dashnau et al., 2008a, Dashnau et al., 2008b, Nucci and Vanderkooi, 2005, Vanderkooi et al., 2005) Since TEIR involves the collection of data at many different temperatures, the thermodynamic response of the OH stretch of water containing a solute of interest can be compared to the spectra of pure water to often yield both qualitative and quantitative structural details of the H-bond network.

In this paper, we examine the temperature dependence of water in the presence of guanidine-HCl and guanidine-HBr salts (Gdm⁺/Cl⁻ and Gdm⁺/Br⁻ in solution) using TEIR. We analyze the OH stretch spectrum of dilute HOD in D₂O samples using a straightforward ratio of absorbances on either side of the OH stretch peak maximum. Our results show that these guanidine salts drive the preferential formation of strong, linear H-bonds over weak, bent H-bonds. Quantum chemical calculations also indicate that a distinct portion of the OH stretch spectrum, a shoulder at ~3300 cm⁻¹, arises from water molecules directly accepting an amino H-bond from a guanidinium cation. We conclude that the H-bond network of water is substantially perturbed by guanidinium, and we discuss the relevance of this restructuring to Gdm⁺ denaturation of proteins and contrast it with previous findings for the other commonly used chemical denaturant, urea.
Materials and Methods

Materials

In our experiments, H$_2$O was deionized and then glass distilled. D$_2$O was purchased from Acros Organics (Geel, Belgium). H$_2$O and D$_2$O were degassed by aspiration before sample preparation. C$^{13}$/3N$^{15}$ labeled guanidine-HBr was purchased from Cambridge Isotope Laboratories Inc. (Andover, MA) and C$^{13}$/3N$^{15}$ labeled guanidine-HCl was purchased from Sigma-Aldrich (St. Louis, MO). Both guanidine salts were completely deuterated by successive rounds of dissolving in excess D$_2$O followed by lyophilization. C$^{13}$/3N$^{15}$ salts were used in order to shift the NH stretching vibration to a lower frequency such that it did not overlap with the OH stretching frequency.

Solutions at 7.27 molal (equivalent to ~5.2 M, or 6.9 water-to-Gdm$^+$/anion mole ratio) for both of the guanidine salts were obtained by dissolving them in a 5% H$_2$O / 95% D$_2$O (v/v) mixture, and then all lower concentrations were obtained by dilution of the 7.27 m stock solutions with this same H$_2$O / D$_2$O mixture.

Spectroscopy

Sample solution was placed between two 2 mm thick calcium fluoride windows, and spectra were collected using a dry nitrogen purged Bruker IFS 66 infrared spectrophotometer (Bruker Optics, Brookline, MA) fitted with an MCT detector. The spectra were collected in transmission mode with a 2 cm$^{-1}$ resolution.

Using a circulating water bath, temperature at the sample was held to +/-0.1 °C over the course of a spectrum acquisition. Spectra were collected from low temperature to high at 5 °C intervals. Spectra were corrected for H$_2$O atmospheric contribution, baseline corrected, and converted to absorbance using the OPUS software package (Bruker Optics, Brookline, MA) and imported into MATLAB 7.6 (Mathworks, Natick, MA) for
further processing. MATLAB was also used to produce Figures 30, 31, and 32. Cubic spline interpolation was used to increase the number of data points from 536 to 51501 and yield a frequency span of 3165 cm⁻¹ to 3680 cm⁻¹. Second degree Savitsky-Golay smoothing was applied to the interpolated data. Plot overlays of interpolated and smoothed data and data with just atmospheric and baseline correction confirmed that smoothing only removed noise and did not perturb the underlying lineshape in any significant way.

**Quantum Chemistry**

All quantum chemical calculations were carried out using Gaussian 03, Revision D.01,(Frisch, 2004) and the B3LYP model chemistry(Becke, 1992, Parr and Yang, 1989) and 6-311++G(d,p) basis set.(Krishnan et al., 1980, McLean and Chandler, 1980) We note that such a basis set is only suitable for the sort of qualitative comparison presented here and not for quantitative determinations of H-bond properties.(Boese et al., 2007, Inada and Orita, 2007, Lee, 2007, Riley and Hobza, 2007, Santra et al., 2007) In the context of the calculations performed, all H-bonds described as “linear” were actually set at 0.1° in order to avoid collinear atoms and the more complicated Z-matrices needed to specify truly linear H-bond angles in the water trimer and guanidinium/dimer cases. The standalone dimer case was tested and showed miniscule and negligible changes to atomic charges, vibrational frequencies, and ground state energy when a truly linear H-bond geometry was used in place of the 0.1° HOO H-bond angle. All calculations were checked for negative frequencies and none were found.

A linearly H-bonded HOD/D₂O water dimer was constructed as a Z-matrix. This water dimer was geometry optimized along the O-O distance, and the infrared frequencies and natural population charges were calculated. Natural Population Analysis, a part of the
Natural Bond Orbital formalism,(Rives and Weinhold, 1980) was used to calculate atomic charges.

Another D₂O water molecule was added to the starting dimer Z-matrix to build a chain of three linearly H-bonded water molecules. Two trimer geometries were used, a hydrogen “up” configuration and a hydrogen “down” configuration, differentiated by a 180° difference in the terminal hydrogen dihedral angle. The trimer systems were each geometry optimized along the O-O distances, and the infrared frequencies and natural population charges were calculated.

Finally, a deuterated guanidinium cation was added to the water dimer Z-matrix such that it donated a single linear amino H-bond to the oxygen of the HOD molecule. The internal geometry of the cation was fixed, based on a separate previous geometry optimization using the same model chemistry and basis set, and geometry optimization was carried out along the O-O distance of the two water molecules and the guanidinium deuterium/HOD oxygen distance. Infrared frequencies and natural population charges were calculated for the optimized system. Translational frequencies were all very close to zero and rotational frequencies were less than ~30 cm⁻¹ for each of the systems, indicating close proximity to the global energy minimum

All OH/OD lengths were fixed at 0.991 Å and the HOD/DOD angles were fixed at 105.5°, as per the findings of Silvestrelli and Parrinello concerning condensed phase water.(Silvestrelli and Parrinello, 1999) Atomic models of the systems studied in the linear HOO angle case are shown in Figure 33, and were visualized using Gaussview 3.07. Electrostatic potential maps (Figures 34 and 35) were generated using Molekel 5.3.0.6. The surfaces shown are the solvent accessible surfaces, probed with a 1.4 Å solvent radius and colored by molecular electrostatic potential. Electron density maps
(Figures 36 and 37) were generated using VMD 1.8.6 (Humphrey et al., 1996) and rendered using Tachyon 0.97 (Stone, 1998)

Results

IR Spectroscopy

OH stretch spectra for all Gdm\(^+/-\)Cl\(^-\) concentrations measured are shown in Figure 30, along with a solute free sample containing only 5% H\(_2\)O / 95% D\(_2\)O. Similar spectra were obtained at several of the same concentrations for Gdm\(^+/-\)Br\(^-\) solutions (not shown). Two features of note are immediately visible in the Gdm\(^+/-\)Cl\(^-\) OH stretch spectra that are not seen in the 5% H\(_2\)O / 95% D\(_2\)O spectra. The first is a strong decrease in temperature dependence. The characteristic shifts of both the low and high frequency parts of the OH stretch spectrum are diminished as the concentration of Gdm\(^+/-\)Cl\(^-\) is increased. This effect is most pronounced at low frequency (~3200-3400 cm\(^{-1}\)), where for the more concentrated samples there is little change in lineshape with increasing temperature, culminating in nearly complete temperature-independence below 3450 cm\(^{-1}\) for the highest concentration sample studied.

The second feature of interest is an increase in absorbance at ~3300 cm\(^{-1}\). Like the change in lineshape temperature dependence, this localized increase in absorbance can be seen even at low Gdm\(^+/-\)Cl\(^-\) concentrations, but it is most apparent at the highest concentrations, where a shoulder is easily visible. Though both the infrared and Raman OH stretch spectra of pure water have long been known to possess a pronounced asymmetry, as well as a characteristic temperature dependence,(Iwata et al., 1997, Walrafen, 1967) this data shows a profound change in the lower frequency water OH stretch absorption with the addition of guanidinium.
The shoulder that grows in at ~3300 cm$^{-1}$ shows a minimal decrease in absorbance with temperature, and therefore the solvation environment that produces this feature must be retained over the whole temperature range for high guanidinium concentrations. To emphasize this feature, in Figure 31, the spectra of a solution of 7.27 m Gdm$^+$/Br$^-$ and that of 5% H$_2$O / 95% D$_2$O water at 90°C are overlaid. The figure also shows the spectrum of 5% H$_2$O / 95% D$_2$O ice at -10°C. A quantitative measure of this shoulder’s appearance with an increase in guanidinium concentration can be seen in Figure 32.
Figure 30. OH stretch TEIR spectra for 5% H₂O / 95% D₂O solutions containing increasing concentrations of guanidinium chloride.
Computation

In order to rationalize the experimental results, quantum calculations of water – water interactions and water – guanidinium ion were undertaken. The graphical representations of the systems studied are shown in Figure 33. This figure shows geometries in which the HOO angle (H5-O4-O1) was set at 0.1°, as well as the water monomer. Calculations were also performed for a variation on these H-bonded systems in which the HOO angle (H5-O4-O1) was set at 45°.

The calculated atomic charges are given in Table 4 for the water monomer, in Table 5 for the water dimer, in Table 6 for the water trimers, and in Table 7 for guanidinium plus water dimer. Images of the electrostatic potentials are given in Figures 34 and 35.

In solution, water will be H-bonded to other molecules, so for the discussion of charges, the atoms of most interest are O4 and H5, since this OH oscillator is the one that is H-bonded to a neighboring water molecule. The results show that the donation of...
an H-bond makes the donor water molecule’s oxygen take on a more negative charge while the donor hydrogen becomes more positive. When that H-bond donating water molecule accepts an H-bond itself from a third water molecule its oxygen becomes even more negative and its hydrogen becomes more positive. When the third water molecule is replaced by a guanidinium cation, this effect is amplified, making the donor water molecule’s oxygen its most negative and the donor hydrogen its most positive.
Figure 33: Shown here are graphical representations of the systems for which the OH stretch frequency and natural population charges were calculated. Calculations were also performed for a variation on these systems in which the HOO angle (H5-O4-O1) was set at 45°. From top to bottom, the monomer, dimer, trimer (H up orientation), trimer (H down orientation), and guanidinium – water dimer geometries are shown, along with a numerical key to referencing tables 4 – 7.
**Table 4.** Calculated Charges for the Water Monomer Systems

<table>
<thead>
<tr>
<th>atom type</th>
<th>atom number</th>
<th>charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>1</td>
<td>-0.92048</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>0.46024</td>
</tr>
<tr>
<td>H</td>
<td>3</td>
<td>0.46024</td>
</tr>
</tbody>
</table>

Atom numbers reference Model 1 in Figure 33.

**Table 5.** Calculated Charges for the Water Dimer Systems

<table>
<thead>
<tr>
<th>atom type</th>
<th>atom number</th>
<th>charge ((0.1° H5-O4-O1))</th>
<th>charge ((45° H5-O4-O1))</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>1</td>
<td>-0.94514</td>
<td>-0.92934</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>0.47788</td>
<td>0.46579</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>0.47788</td>
<td>0.46579</td>
</tr>
<tr>
<td>O</td>
<td>4</td>
<td>-0.94971</td>
<td>-0.92511</td>
</tr>
<tr>
<td>H</td>
<td>5</td>
<td>0.48857</td>
<td>0.47427</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>0.45053</td>
<td>0.44859</td>
</tr>
</tbody>
</table>

Atom numbers reference Model 2 in Figure 33.
Table 6. Calculated Charges for the Water Trimer Systems

<table>
<thead>
<tr>
<th>atom type</th>
<th>atom number</th>
<th>H-up orientation charge (0.1° H5-O4-O1)</th>
<th>H-up orientation charge (45° H5-O4-O1)</th>
<th>H-down orientation charge (0.1° H5-O4-O1)</th>
<th>H-down orientation charge (45° H5-O4-O1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
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<td>-0.94915</td>
<td>-0.93924</td>
<td>-0.94846</td>
<td>-0.93648</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>0.48211</td>
<td>0.47072</td>
<td>0.48224</td>
<td>0.47024</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>0.48211</td>
<td>0.47072</td>
<td>0.48224</td>
<td>0.47024</td>
</tr>
<tr>
<td>O</td>
<td>4</td>
<td>-0.97304</td>
<td>-0.94904</td>
<td>-0.97323</td>
<td>-0.95070</td>
</tr>
<tr>
<td>H</td>
<td>5</td>
<td>0.50282</td>
<td>0.49129</td>
<td>0.50572</td>
<td>0.49504</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>0.47001</td>
<td>0.46823</td>
<td>0.46624</td>
<td>0.46349</td>
</tr>
<tr>
<td>O</td>
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<td>-0.95792</td>
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<td>-0.95300</td>
</tr>
<tr>
<td>D</td>
<td>8</td>
<td>0.49414</td>
<td>0.49484</td>
<td>0.49422</td>
<td>0.49519</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>0.44737</td>
<td>0.45039</td>
<td>0.44725</td>
<td>0.44596</td>
</tr>
</tbody>
</table>

Atom numbers for “H-up orientation” reference Model 3 in Figure 33 and atom numbers for “H-down orientation” reference Model 4 in Figure 33.
Table 7. Calculated Charges for the Guanidinium/Water Dimer Systems

<table>
<thead>
<tr>
<th>atom type</th>
<th>atom number</th>
<th>charge (0.1^\circ) H5-O4-O1</th>
<th>charge (45^\circ) H5-O4-O1</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>1</td>
<td>-0.96130</td>
<td>-0.95662</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>0.49595</td>
<td>0.48289</td>
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<tr>
<td>D</td>
<td>3</td>
<td>0.49256</td>
<td>0.47736</td>
</tr>
<tr>
<td>O</td>
<td>4</td>
<td>-0.99675</td>
<td>-0.97202</td>
</tr>
<tr>
<td>H</td>
<td>5</td>
<td>0.52080</td>
<td>0.51517</td>
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<tr>
<td>D</td>
<td>6</td>
<td>0.48922</td>
<td>0.48770</td>
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<tr>
<td>D</td>
<td>7</td>
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<td>0.45507</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>-0.75218</td>
<td>-0.74675</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
<td>0.40918</td>
<td>0.40890</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>0.67050</td>
<td>0.67021</td>
</tr>
<tr>
<td>N</td>
<td>11</td>
<td>-0.75132</td>
<td>-0.75149</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>-0.75143</td>
<td>-0.75185</td>
</tr>
<tr>
<td>D</td>
<td>13</td>
<td>0.43031</td>
<td>0.43020</td>
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<tr>
<td>D</td>
<td>14</td>
<td>0.41468</td>
<td>0.41459</td>
</tr>
<tr>
<td>D</td>
<td>15</td>
<td>0.42009</td>
<td>0.42003</td>
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<tr>
<td>D</td>
<td>16</td>
<td>0.41653</td>
<td>0.41658</td>
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Atom numbers reference Model 5 in Figure 33.
Table 8. Frequency of O4-H5 Stretching Vibration for All Quantum Chemical Models

<table>
<thead>
<tr>
<th>Model</th>
<th>O1 – H3 stretching vibration</th>
<th>O4 – H5 stretching vibration (HOO angle (H5-O4-O1) = 0.1°)</th>
<th>O4 – H5 stretching vibration (HOO angle (H5-O4-O1) = 45°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1, monomer</td>
<td>3499.7268 cm(^{-1})</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Model 2, dimer</td>
<td>-</td>
<td>3461.9444 cm(^{-1})</td>
<td>3500.5098 cm(^{-1})</td>
</tr>
<tr>
<td>Model 3, trimer (H up)</td>
<td>-</td>
<td>3446.7225 cm(^{-1})</td>
<td>3503.1583 cm(^{-1})</td>
</tr>
<tr>
<td>Model 4, trimer (H down)</td>
<td>-</td>
<td>3443.8779 cm(^{-1})</td>
<td>3501.9835 cm(^{-1})</td>
</tr>
<tr>
<td>Model 5, guanidinium – water dimer</td>
<td>-</td>
<td>3395.1482 cm(^{-1})</td>
<td>3502.1586 cm(^{-1})</td>
</tr>
</tbody>
</table>

With the addition of the guanidinium cation the charge difference between the water molecule’s hydrogen and oxygen grows to almost 1.52 e, a charge disparity nearly 0.17 e greater than that found in the isolated water monomer. For the case where the H-bond of the water molecule of interest is increased to 45° the O4-H5 charge disparity decreases by approximately 0.03 e. The atomic charges of the water molecule that is outside of the first sphere of interaction with guanidinium is likewise affected by interaction with guanidinium, though considerably less so than O4 and H5.

The frequencies of the stretching vibration of O4-H5 for the five model systems are given in Table 8. As is expected, the vibrational frequency drops considerably (3500 cm\(^{-1}\) to 3462 cm\(^{-1}\)) when the HOD monomer donates a linear H-bond to another water molecule. The frequency becomes even lower when the H-bond donating water accepts an H-bond from another water molecule (3462 cm\(^{-1}\) in the dimer to 3444 cm\(^{-1}\) or 3447 cm\(^{-1}\) in the trimer). However, the most dramatic change in frequency occurs when the H-bond donating water accepts an amino H-bond from a guanidinium cation. In this case,
the O4-H5 vibrational frequency drops to 3395 cm\(^{-1}\). This large shift is of the order of magnitude that is experimentally observed.

We note that the effect of the guanidinium cation’s amino H-bond donation on the OH stretch vibrational frequency is canceled out when the HOO (H5-O4-O1) angle is increased to 45\(^\circ\) (Table 8). This is true for the trimer cases as well, showing that a simple redistribution of charges cannot fully account for the large changes seen in the O4-H5 vibrational frequency. However, we do observe decreased electrostatic potential (Figures 34 and 35) in the region of the H5-O1 H-bond when the angle of that bond is increased, as well as a very large decrease in electron density overlap (Figures 36 and 37).

Finally, we need to point out caveats for these calculations. In a true liquid solution there is a distribution of H-bond angles, and each water molecule is further H-bonded to others to form the H-bond network, therefore these calculations represent a simplified picture of the chemistry. Although these calculations do not exactly mimic the experimental reality, they do offer strong support for the trends observed in the infrared spectra – that the H-bonding network in guanidinium-containing solutions favors linear H-bonds, which accounts for the overall shift to lower frequency absorption seen in the OH stretch band of guanidinium solutions, and that the acceptance of a guanidinium H-bond by an OH oscillator that is itself donating a linear H-bond causes a large decrease in that OH’s vibrational frequency.

**Discussion**

The results presented in this study demonstrate that the guanidinium cation has a substantial effect on water structure. These results are significant in that the techniques employed experimentally probe the H-bond network directly and correlate well with
model quantum chemistry calculations. Changes in the OH stretch spectra are indicative of changes in the local H-bonding environment of OH oscillators,(Falk and Ford, 1966, Murphy and Bernstein, 1972, Palamarev and Georgiev, 1994, Senior and Verrall, 1969, Wang et al., 2004) and as can be seen qualitatively in Figure 30, the OH stretch spectra are highly altered by the addition of guanidinium.

The fact that the OH stretching vibrations go to lower frequency, and therefore lower energy, with the addition of guanidinium is important. Low energy OH vibrations can be attributed to shorter, more linear H-bonds.(Dashnau et al., 2006, Laenen et al., 1998, Nucci and Vanderkooi, 2005, Sharp et al., 2001, Vanderkooi et al., 2005, Wang et al., 2003, Wang et al., 2004) The ultrafast spectroscopic research of Laenen et. al,(Laenen et al., 1998) Wang et. al.(Wang et al., 2003, Wang et al., 2004), Woutersen et. al.(Woutersen et al., 1997) and other groups indicate distinct structural subpopulations of the OH stretching vibrational spectrum that give rise to different OH stretch relaxation lifetimes, and the extensive computational studies of Sharp et. al.(Gallagher and Sharp, 2003, Sharp et al., 2001) and others(Kumar et al., 2007) confirm these findings for a number of different water models. Taken together, the experimental and theoretical evidence points to a water network consisting of distinct H-bond populations: strong, linear H-bonds and distorted, weak H-bonds.

Since the solutes studied are free from vibrational contributions that would overlap with the OH stretch of water, the shoulder at ~3300 cm\(^{-1}\) that grows in with Gdm\(^{+}\)/anion concentration can be attributed to a restructuring of the water H-bond network by the ions. The same reasoning holds for the differences observed in temperature dependence of the solutions. The decrease in temperature dependence of the OH stretch spectrum with the addition of guanidinium means that the rearrangement response of the water network to the addition of thermal energy is diminished. Our
spectra indicate that the presence of the guanidinium cation in high concentrations structures water into a network that is, on average, resistant to reordering when thermal energy is added.

The response of water to the presence of guanidinium is quite different from urea, which produces very little change in the OH stretch spectrum of water. (Sharp et al., 2001) Urea is a net uncharged molecule, and so it is unlikely to have a substantial effect on the natural charge distributions of the water molecules to which it H-bonds. Like guanidinium, there is a great deal of evidence for direct binding of urea to the folded protein or peptide backbone. (Caballero-Herrera et al., 2005, Das and Mukhopadhyay, 2008, O'Brien et al., 2007, Stumpe and Grubmueller, 2007) However, there is also a substantial amount of research, both computational (Kokubo et al., 2007, Kuharski and Rossky, 1984, Sharp et al., 2001) and experimental (Hayashi et al., 2007, Rezus and Bakker, 2006, Sharp et al., 2001) that urea is "floppy" (Hermida-Ramon et al., 2007) in solution and fits neatly into the bulk water H-bond network, leaving water structure mostly unperturbed.

Given the complex structure of the guanidinium cation and its potential for multiple types of interactions with water, anions, and other guanidinium cations, it seems most likely that guanidinium, and not the anions, plays the primary role in the network-wide restructuring of water. The OH stretch spectrum of a high concentration KCl solution supports this idea. (Sharp et al., 2001) The K⁺/Cl⁻ solution does not show the visible low frequency structuring of the OH stretch that a similar concentration of Gdm⁺/Cl⁻ does, indicating that the low frequency OH stretch component that grows in with Gdm⁺/Cl⁻ concentration is not simply due to an increase in strong OH–Cl interactions. To fully simulate the IR absorption spectra would require a more elaborate calculation than was carried out here, and would also need to include the counter ion, but our quantum
chemical calculations do qualitatively indicate a trend that accounts for the experimental spectral features; that both the charge distribution and OH stretch frequency of water are significantly altered by H-bonding to guanidinium, and that the effect on OH oscillator frequency is dependent on that oscillator’s donating its own linear H-bond.

Our results show that though the addition of a guanidinium amino H-bond to a water molecule does alter that water’s charge distribution, the effect on charge distribution is only minimally responsive to the angle at which that water molecule donates an H-bond of its own. In both the water trimer and guanidinium-dimer calculations, the angle of the H5-O4-O1 H-bond had very little effect on distribution of charges. Instead, the H5-O4-O1 H-bond angle had its most significant impact on the O4-H5 vibrational stretch frequency and the degree to which electron density was shared between H-bonded water molecules, along with a decrease in electrostatic potential at the H-bond site. Therefore, these results are consistent with the notion of a covalent component to the H-bond that is dependent primarily on H-bond angle.(Weinhold et al., 2005)

As guanidinium ions increase in concentration it can reasonably be expected that an increasing percentage of solution water is involved in first shell guanidinium solvation. Since it is known that guanidine salts do not partition out of solution at the concentrations used in these experiments, we can reason that it must either a) fit neatly into the bulk water H-bonding network, thereby not disrupting water structure at all, or b) rearrange the H-bonding network of water such that it can solvate the ions. Our data strongly indicates rearrangement of the H-bond network by high concentrations of guanidinium, and an increase, on average, in strong, linear H-bonds relative to weak, bent H-bonds. This relative deficit in weak H-bonds implies that guanidinium is forcing water to be over structured, as compared to pure water. This idea is also strongly
supported by the reduction in OH stretch temperature dependence of the concentrated guanidinium solutions.

The results we present here have very interesting implications for the chemical denaturation of proteins by dissolved guanidine salts. Direct interactions between guanidinium cations and proteins have typically been indicated as the mechanism through which guanidine salts destabilize and unfold proteins, and it seems clear that direct binding must play a primary role in protein denaturation. Though our experiments were not designed to test the direct binding mechanism directly, our results do show a substantial restructuring of the water network, and these denaturation mechanisms need not be mutually exclusive. The hydrophobic collapse model of protein folding states that

**Figure 34:** Electrostatic potential maps at the solvent accessible surface (1.4 Å solvent radius probe) generated for the water monomer and linear molecular configurations using Molekel 5.3.0.6 and colored by molecular electrostatic potential.

**Figure 35:** Electrostatic potential maps at the solvent accessible surface (1.4 Å solvent radius probe) generated for the 45° HOO (H5-O4-O1) H-bond angle molecular configurations using Molekel 5.3.0.6 and colored by molecular electrostatic potential.
the primary force driving protein folding and stability is the shedding of highly structured, entropically unfavorable, low-density solvation water from hydrophobic residues exposed to solvent. (Creighton, 1983, Levy and Onuchic, 2006, Sorin et al., 2006, Wiggins, 1997) The collapse of these hydrophobic residues into the protein interior releases the linearly H-bonded, low-density waters back to the bulk solvent, thereby allowing the protein/solvent system to attain a net lower energy state.

When the results presented here are contemplated in the context of hydrophobic collapse, it can be seen how the restructuring of the water network by guanidinium may play a role in the denaturation of proteins. Guanidinium in high concentrations, in addition to replacing much of the bulk water in solution, causes an overall structuring of
the water network, making its H-bonds much more linear than those found in pure water. The consequence of this solution-wide reordering for folded proteins is that their hydrophobic residues would no longer have as great an entropic drive to pack with one another in the interior of the protein as they do in a non-denaturing solution. With a large portion of the H-bonds in the high concentration guanidinium solution already very linear, there would be a reduced entropic penalty for their solvation of a hydrophobic residue. We believe that this reduction of the impetus for hydrophobic packing may be a factor in the denaturing action of guanidine salts. This rationale may also help in explaining the high relative solubility of molecules like benzene, (Breslow and Guo, 1990) hydrophobic amino acids, (Nozaki and Tanford, 1970) cyclic dipeptides, (Venkatesu et al., 2007) and hydrocarbons (Wetlaufer et al., 1964) in Gdm+/Cl− solutions. The data presented here does not provide for an estimate of the relative contribution of denaturation mechanisms, but given that solvation water structure plays such an important role in protein folding, it is difficult for us to envision guanidinium water structuring being a negligible component to the overall denaturation phenomenon.

Summary

We have presented here OH stretch spectra of the water present in several different concentrations of Gdm+/Cl− solutions. We showed that the spectra, and therefore the water H-bond network, are altered by Gdm+. The TEIR solution spectra show that the Gdm+ cation causes an increase in strong, linear H-bonds. We performed quantum chemical calculations that indicated a subpopulation of water OH oscillators in the presence of guanidinium, causing the peculiar spectral features we observed. Finally, we have indicated how these results could play a role in destabilization and denaturation of folded proteins by guanidine salts.
CHAPTER 4

THE EFFECTS OF HIGH HYDROSTATIC PRESSURE ON THE HYDROGEN BONDING NETWORK OF WATER AND THE STRUCTURE OF BIOLOGICAL SOLUTES

Introduction

The effect of pressure on liquids and solids has long been of interest to researchers. In the case of water, pressure is known to cause crystallization of the liquid into forms not observed in natural settings on Earth. These high pressure ice forms are compelling for the reason that no other single molecule is known to exist in so many different solid states. Also, some of the ice forms have recently come under renewed investigation for the reason that they may indeed exist naturally on icy moons or other planets. (Fu et al., 2010)

The effects of high pressures are also of great interest in the realm of biochemistry. While the majority of proteins irreversibly denature at pressures of a few kbar, there are numerous examples of proteins that maintain their structures at well over 10 kbar of pressure. (Goossens et al., 1996, Toelgyesi et al., 2004) Likewise, barophilic organisms have been collected in even the deepest parts of the ocean and below the ocean floor, and many microbes are capable of surviving and growing at pressures of over 10 kbar. (Sharma et al., 2002) As more research is done on deep sea environments it is becoming clear that barophilic organisms may be the rule rather than the exception, and that life may exist and even thrive far below even the deepest parts of the ocean. Even some organisms that have not evolved to thrive at high pressures have been found to survive exposure to very extreme pressures, (Sharma et al., 2002) and other organisms require high pressures to survive. (Kato et al., 1998, Straube et al., 1990)
Though the pressures at which living organisms have thus far been found are relatively small compared to those that can be achieved experimentally, the effects of extremely high pressures on biomolecular structure and function are still very interesting because they may help us in defining the theoretical boundaries within which amino acid and liquid water based life can exist. While most of the pressure/temperature phase diagram of water up to several hundred K is either gaseous or solid, the liquid water regime is still rather large, and as of now there are still no known regions of the liquid water phase where life is known to be chemically impossible.

As our laboratory has interests in both water structure and biomolecular structure and function, in the present study we have recorded high quality Fourier transform infrared (FTIR) spectra of the OH stretch of ices VI and VII in a dilute mixture of HOD in D$_2$O. Though spectra of these ices have appeared in the literature in the past,(Bertie et al., 1968, Klug and Whalley, 1984, Pruzan et al., 1990, Song et al., 2003, Walrafen et al., 1982) the modern FTIR instrument yields considerably more spectral detail than was previously available. In addition, we have examined the effect of increasing hydrostatic pressure on the amide I’ and II’ spectra of three simple, blocked peptide derivatives that have been studied extensively utilizing other theoretical and experimental methods, N-acetyl-glycine-methylamide (NAGMA), N-acetyl-L-leucine-N’-methylamide (NALMA), and N-acetyl-L-alanine-N’-methylamide (NAAMA),(Jalkanen and Suhai, 1996, Liu et al., 2009, Maksumov et al., 1975, Masman et al., 2007, Murarka and Head-Gordon, 2007, Russo et al., 2010) Though Measey et al. determined the effect of side chain on the amide I’ spectra of numerous dipeptides,(Measey et al., 2005) there does not appear to be any systematic examination of the pressure and side chain dependence of the amide I’ and II’ bands as is presented here.
Materials and Methods

**Materials** 99.98% D$_2$O was obtained from Sigma-Aldrich and used for preparing all samples. NAGMA and NALMA were also purchased from Sigma-Aldrich. NAAMA was purchased from Chem-Impex International, Inc. Before experimental use, each of the peptide derivatives was deuterated by being dissolved in a large excess of D$_2$O followed by rapid freezing and lyophilization. Barium sulfate (99.998% purity) was purchased from Sigma-Aldrich as well.

The Diacell LeverDAC-Mini diamond anvil cell (DAC) was purchased from EasyLab Technologies Ltd. The DAC housing was fitted to a 3x focusing apparatus by Bruker Optics, which was then installed and aligned in our Bruker IFS66 FTIR instrument (Bruker Optics, Brookline, MA). Fifty µm thick stainless steel sheets used for making gaskets for the DAC were purchased from Advent Research Materials Ltd. MATLAB 7.6 (Mathworks, Natick, MA) was used for plotting, and final figures were assembled using Adobe Illustrator CS4.

**Sample Preparation** Samples of the small peptide derivatives were prepared simply by dissolving the already deuterated and lyophilized powders in D$_2$O to yield final concentrations of 0.25 m. This particular concentration was chosen to yield strong absorption of both the amide I’ and amide II’ vibrational bands.

The DAC was assembled in an actively dried room (relative humidity < 30%) using a long working range microscope with 40x magnification. Gaskets for the DAC were made by punching 600 µm wide holes in 50 µm thick stainless steel sheets and then trimming the gaskets to a size convenient for placing in the DAC. OH stretch spectra were obtained at high pressure using the small amount of H contributed to the samples by atmospheric exchange in the time that it took to assemble the DAC after the sample was
pipetted into the sample hole of the gasket. OH stretch spectra were collected at atmospheric pressure using a 95% D₂O/5% H₂O solution and calcium fluoride windows. The sulfate stretch band of a fine barium sulfate powder placed in the sample hole before the sample was added was used to monitor pressure inside of the DAC. (Wong and Moffat, 1989)

**Spectra Collection and Processing.** All spectra were collected in a Bruker IFS66 FTIR instrument purged with dry nitrogen and equipped with a liquid nitrogen cooled MCT detector. All high pressure spectra were collected at ambient temperature, approximately 22 °C. The OPUS 5.0 software program (Bruker Optics, Brookline, MA) was used to collect and process spectra. All spectra were collected in transmission mode, at 2 cm⁻¹ resolution. A final, raw spectrum was generated by coadding the spectra of 512 scans. All spectra were corrected for H₂O atmospheric contribution, baseline corrected, and converted to absorbance. The liquid water and some of the amide I’ spectra were normalized to assist in visual comparison. Second-degree Savitsky-Golay smoothing was applied to some of the spectra. A 25 point span was used for the liquid OH stretch spectra, a 5 point span was used for the high pressure ice OH stretch spectra, and a 5 point span was used for the atmospheric pressure ice. Smoothing was not used at all for the amide I’ and II’ spectra.

**Results**

**OH Stretch Spectra.** The liquid water OH stretch spectra are shown in Figure 38. In Figure 38A we show the response of the OH stretch spectrum to the application of hydrostatic pressure, and in Figure 38B the influence of temperature on the same spectrum at atmospheric pressure. Unlike the application of increased temperature, which has the effect of broadening the OH stretch spectrum considerably and shifting it
to higher frequency, increased hydrostatic pressure broadens the spectrum only slightly
and instead shifts it to lower frequency. It can also be noted that increased pressure, like
some solutes, (Scott et al., 2008) has very little effect on the high frequency portion of the
spectrum. At the highest pressures obtained before the pressure-induced transition to
ice VI the OH stretch spectrum is shifted to an even lower frequency than that which can
be brought on by lowering the temperature of the liquid state.

OH stretch spectra of ice are shown in Figure 39. In Figure 39A we show the formation
of ice VI and its transition into ice VII, and in Figure 39B ice Ih at atmospheric pressure
and -15 °C is shown for comparison. Ice VI has a strong and broad absorption with
evidence of multiple H-bonding species, which overlaps but otherwise has little in
common with ice Ih at 3306 cm⁻¹. The ice VI spectrum does not appear to change much
qualitatively with increased pressure, aside from a slight decrease in absorbance and
red shift of the entire spectrum. The ice VII spectrum, however, alters considerably with
increased pressure. The ice VII OH stretch spectrum starts out with two separated
bands upon transition from ice VI, with one band being centered at ~3380 cm⁻¹ and the
other at ~3290 cm⁻¹. As pressure is increased above the transition point the ice VII band
at ~3290 cm⁻¹ decreases in absorbance until it disappears entirely from the spectrum, at
approximately 43.0 kbar. The band at ~3380 cm⁻¹ first increases in absorbance then
decreases in absorbance as pressure is increased, and the band shifts slightly to lower
frequency with increasing pressure.
In order to be certain that the barium sulfate pressure marker was not affecting the spectra we collected ice VI to VII transition spectra in the absence of barium sulfate. Though we could not monitor the pressure of the sample without an internal pressure marker present, the way the OH stretch spectra (not shown) evolved with increased pressure was identical to what we observed for the samples including barium sulfate.

**Figure 38**: Normalized OH stretch spectra of liquid water under the influence of a) hydrostatic pressure at ~22 °C and b) temperature, applied at atmospheric pressure.

**Figure 39**: OH stretch spectra of water ices under the influence of a) hydrostatic pressure at ~22 °C and b) at atmospheric pressure and -15 °C.
**Amide Spectra.** Amide I’ spectra are shown in Figures 40 and 41. The amide I’ spectra of all three peptide derivatives are similar, but with some important differences. We note that for both NAGMA and NAAMA amide I’ spectra, the peak frequency shifts very slightly (2 to 3 cm\(^{-1}\)) to higher frequency with increased pressure while the peak frequency remains static for NALMA. Also, though the amide I’ band of NAGMA and NAAMA both show a small decrease in absorbance with increased pressure, the same vibrational band in NALMA exhibits peak absorbance decreased by over a third at the highest pressure before the transition to ice VI.

Each of the molecules’ amide I’ bands broaden with increased pressure as well, with the low frequency side of the spectrum accounting for most of the broadening. The full width at half maximum (fwhm) of the NAGMA amide I’ band goes from 39.6 cm\(^{-1}\) at atmospheric pressure to 43.1 cm\(^{-1}\) at 15.4 kbar, the highest pressure at which a spectrum was taken before the ice VI phase transition was reached. For NAAMA, the fwhm goes from 41.7 cm\(^{-1}\) at atmospheric pressure to 44.7 cm\(^{-1}\) at the highest liquid-water pressure reached, 15.5 kbar. NALMA exhibits the largest broadening of the three molecules, with its amide I’ fwhm going from 40.6 cm\(^{-1}\) at atmospheric pressure to 49.8 cm\(^{-1}\) at 15.3 kbar, the highest pressure at which the solution was liquid. These increases with pressure in the amide I’ fwhm of NAGMA, NAAMA, and NALMA are 8.9%, 7.2%, and 22.7% respectively.

As can be seen in Figure 42, which shows only the highest and lowest pressure amide I’ bands for each peptide, there is considerable splitting of the bands, owing to each peptide having two carbonyl groups. This splitting grows with pressure as the underlying bands shift relative to one another.
The amide II' spectra of the three peptide derivatives are shown in Figures 43 and 44. This band is somewhat contaminated by the absorption of the HOD bending vibration at approximately 1460 cm$^{-1}$, but this band only absorbs at the low frequency edge of the amide II' spectrum, is relatively narrow, and undergoes only a very small shift to higher frequency with increased pressure. Attempts to subtract the HOD band only served to obscure and distort the amide II' data, and since the band can be readily subtracted visually by the reader it was simply left alone and the spectrum is shown in an unmodified form.
Figure 40: Variation of the amide I' spectra of small peptide derivatives under the influence of increased hydrostatic pressure at ~22 °C. a) contains the pressure dependent shift for 0.25 m N-acetyl-glycine-methylamide, b) for 0.25 m N-acetyl-L-alanine-N'-methylamide, and c) for 0.25 m N-acetyl-L-leucine-N'-methylamide.

Figure 41: Changes in the normalized amide I' spectra of small peptide derivatives under the influence of hydrostatic pressure at ~22 °C. a) contains the pressure dependent shift for 0.25 m N-acetyl-glycine-N'-methylamide, b) for 0.25 m N-acetyl-L-alanine-N'-methylamide, and c) for 0.25 m N-acetyl-L-leucine-N'-methylamide.
Figure 42: Amide I' spectra taken at atmospheric pressure and \( \approx 22 \) °C (the blue spectrum) and the highest pressure spectrum taken before the water liquid to ice VI phase change and \( \approx 22 \) °C (the red spectrum) for a) 0.25 m N-acetyl-glycine-methylamide, b) 0.25 m N-acetyl-L-alanine-N'-methylamide, and c) 0.25 m N-acetyl-L-leucine-N'-methylamide.
**Figure 43:** Variation of the amide II’ spectra of small peptide derivatives under the influence of hydrostatic pressure at ~22 °C. a) contains the pressure dependent shift for 0.25 m N-acetyl-glycine-methylamide, b) for 0.25 m N-acetyl-L-alanine-N'-methylamide, and c) for 0.25 m N-acetyl-L-leucine-N'-methylamide.

**Figure 44:** Amide II’ spectra taken at atmospheric pressure at ~22 °C (the blue spectrum) and the highest pressure spectrum taken before the water liquid to ice VI phase change (the red spectrum) for a) 0.25 m N-acetyl-glycine-methylamide, b) 0.25 m N-acetyl-L-alanine-N'-methylamide, and c) 0.25 m N-acetyl-L-leucine-N'-methylamide.
The amide II' spectra show a great deal of difference amongst the three peptide derivatives. Each of the bands shifts to higher frequency with increased pressure, but the NAAMA spectra are red-shifted with respect to the NAGMA spectra by almost 10 cm⁻¹. Likewise, the peak frequency of NALMA is shifted to even lower frequency than that of NAAMA. All three of the peptide amide II’ bands are asymmetrical, and their broad absorption clearly results from the presence of multiple spectroscopic species. The distribution of those species shows substantial pressure dependence. In addition, the NAGMA and NAAMA bands narrow as pressure is increased while the NALMA band broadens.

Discussion

OH Stretch Spectra

The results presented in this study indicate that increased hydrostatic pressure can have interesting and somewhat unexpected effects on the structure of small peptides, dependent on the side chain, and that infrared spectroscopy is a useful tool for probing these structural changes. The results also show for the first time, to our knowledge, OH stretch infrared spectra that point to a change in the H-bonding pattern of ice VII as pressure is increased above the ice VI to VII transition point.

Ices VI (Figure 45) and VII (Figure 46), which are easily reached at ambient temperature using a DAC, are both known from scattering experiments and theoretical studies to be composed of two distinct, interpenetrating networks. These networks do not directly bond to one another and instead form so called “self clathrates.”(Kamb, 1965) For the case of ice VI, each of these interpenetrating networks is composed of hexamers, and the equatorial water molecules of the hexamer serve to connect the networks. This structural motif yields multiple different environments for the water
molecules in the crystal lattice, and gives rise to four different types of H-bond, with HOO angles of 12.30°, 12.12°, 2.27°, and 14.28°, and occupancy of 20%, 20%, 20%, and 40% respectively (calculated using Kuo et al’s data). (Kuo and Kuhs, 2006) For each of the ice VI H-bond types, the other water geometry parameters of interest are very consistent. The OH bond length for each type of ice VI H-bond is about 0.99 Å and the O-O separation for each type is approximately 2.8 Å. Incidentally, these values are very close to those found computationally by our laboratory to be the minimum energy configuration for the gaseous water dimer. (Scott and Vanderkooi, 2010)

**Figure 45.** Figure depicting the H-bonding geometries in the ice VI crystal lattice. Figure generated with data provided by Dr. Martin Chaplin. (Chaplin, 2009d)
Figure 46: The interpenetrating cubic lattices of the ice VI crystal lattice. This figure shows the protons as being ordered while in the ice VII crystal they are actually disordered. Figure generated with data provided by Dr. Martin Chaplin. (Chaplin, 2009e)

While ice VII shares some of the structural features of ice VI, such as interpenetrating water networks with no H-bonds between them, it also has some significant differences. For example, ice VII has a cubic unit cell (ice VI has a tetragonal unit cell), and it has only two water molecules per unit cell (ice VI has 10 water molecules per unit cell). Though the cubic crystal structure of ice VII should mean that each water molecule in the ice VII lattice experiences the same local environment, this turns out not to be the case.
In fact, there is ample evidence for site disorder in both the O and H/D positions of ice VII. (Knight and Singer, 2009, Nelmes et al., 1998b)

Though diffraction studies are well suited to studying crystalline structures such as ices, varying data quality and the uncertainty inherent in refinement techniques and models leave the door open to various structural interpretations. While FTIR as an experimental technique is wholly unsuited to the sort of structural determinations that are possible with diffraction studies, properly designed vibrational spectroscopy experiments can yield valuable information on the local environment of a given oscillator. Due to the very small amount of H present in the water samples we prepared (as indicated by the ratio of the DOD bending mode to the HOD bending mode, and the complete lack of an HOH bending absorption), the OH stretching vibration is almost completely decoupled from other OH oscillators, making it a sensitive probe to the local electronic environment. (Dashnau et al., 2006, Nucci and Vanderkooi, 2005, Scott et al., 2008, Sharp et al., 2001, Sharp and Vanderkooi, 2009, Vanderkooi et al., 2005, Wall and Hornig, 1965) Figure 39 indicates that for ice VI, and ice VII at pressures below about 43 kbar, there clearly exist multiple, distinct H-bonding species.

The OH stretch spectra of ice VI show an overall shift to lower frequency with increased pressure, which could be expected. As the crystal packs into a progressively smaller volume, individual H-bonds become stronger, and we observe a red shift much like that observed when the temperature of ice Ih is lowered. The stark heterogeneity in the H-bonding environment in the ice VI crystal is directly reflected in the infrared spectra shown in Figure 39A, and the evidence of multiple populations of H-bond oscillators is unsurprising given what is known about the types of H-bond in ice VI. (Kuo and Kuhs, 2006) At 24.8 kbar the spectra change significantly, indicating the transition to ice VII. As pressure is further increased, the band at ~3290 cm\(^{-1}\) in the ice VII spectra sharply
decreases in absorbance, finally disappearing entirely by the time a pressure of 43.0 kbar is reached.

The very narrow and symmetric 43.0 kbar ice spectrum is clearly indicative of a very homogeneous H-bonding structure, but the presence of the distinct band at 3290 cm\(^{-1}\) in the lower pressure ice VII spectra points to multiple H-bonding species existing within the ice VII crystal. The disappearance of the band at \(~3290\) cm\(^{-1}\) would appear to indicate some H-bond reorganization occurs as pressure increases above the ice VI to VII transition point, such that by the time 43.0 kbar is reached, the spectrum is dominated by the single band at \(~3380\) cm\(^{-1}\). The band at \(~3380\) cm\(^{-1}\) resembles the spectrum of ice Ih, shown in Figure 39B, except that the ice VII band is considerably narrower (fwhm 53.9 cm\(^{-1}\) for ice VII at 43.0 kbar, 66.1 cm\(^{-1}\) for ice Ih at \(-15^\circ\text{C}\)).

In addition to providing evidence for a heretofore unknown H-bond rearrangement in ice VII at low pressures, these results also reaffirm the utility of infrared spectroscopy of the OH stretch spectrum for studying H-bond structure, as the differing H-bond environments are unambiguously reflected in the spectra. We are uncertain at this point exactly what sorts of H-bond rearrangements take place at these relatively low pressures to affect such a change on the ice VII spectrum. Though proton ordering is known to increase in ice VII up to about 130 kbar,(Pruzan et al., 1990) it seems unlikely that proton ordering alone would account for the change from two such energetically separated H-bonding states to one. Another possibility is a change in the degree of disorder in the O lattice sites. There may be some portion of O displacements that is populated at relatively low ice VII pressures but vanishes quickly as pressure on the crystal is raised.
Amide Spectra of the NA*MA Peptide Derivatives

The amide I’ spectra of the peptide derivatives studied offer interesting clues as to the structural evolution that occurs as the molecules are exposed to increasing pressures via the surrounding solvent. One could reasonably expect that the three molecules’ amide I’ spectra would have very similar response to increased pressure, since this absorption is due almost entirely to localized carbonyl stretching, but instead we observe clear differences. In addition to shifting of the band depending on the particular side group present, the amount of broadening appears to depend on what groups are attached to the Cα carbon. Even the simple addition of a methyl group not only shifts the band to the red, but also changes the electronic environment of the carbonyl as pressure is increased, as evidenced by the differences in broadening between NAGMA and NAAMA. The effect is magnified when the leucine side group is included. Not only is the band shifted to even lower energy at all pressures for NALMA compared to the other two molecules, but it broadens by nearly a quarter of its starting width as the pressure is increased.

With respect to the carbonyl groups of the peptide derivatives, it appears that even at atmospheric pressure the carbonyl stretches are not degenerate. In Figure 42, the asymmetry and appearance of shoulders in the blue traces show that the two carbonyl groups in each of the peptides experience different environments, and the red traces indicate that the effect is greatly exacerbated by increased pressure in the solution. Despite the lack of a good theoretical model for the response of carbonyl stretching in these molecules to increased pressure we attempted fits of the amide I’ spectra to two Gaussian peaks, but all of the resulting fits were non-unique and had equally good “goodness of fit” parameters.
The broadening of amide I’ with increased pressure is asymmetric for all three peptide derivatives, but this effect is greatly exaggerated for NALMA. In the absence of the NAGMA and NAAMA data we might assume that this effect was simply due to the NALMA molecule accessing more configurations that allowed the two carbonyls to accept very strong H-bonds to water (thereby pushing their absorption to lower frequency), but if that were the case then we would expect to see a similar large, low frequency broadening for all of the molecules. Though ultimately we can only guess at the exact conformational changes that occur in any of these peptides with large increases in hydrostatic pressure, we do know, from the very large decrease in NALMA amide I’ absorbance, that inclusion of the large, hydrophobic side chain causes an extreme change in the electronic environment experienced by the two carbonyl groups. This change in the local electronic neighborhood around the two carbonyl groups results in a greatly reduced change in the dipole moment with stretching, leading to a significant decrease in absorbance. Though specific conformational information cannot be derived from the spectra shown here, it may be that the decrease in volume of the NALMA molecule at very high pressure causes the carbonyl oxygen atoms to reorient in close proximity to the leucine side chain, whose electrostatic field and hydrophobic solvation profile decreases the change in molecular dipole as the carbonyl stretches.

The amide II’ band of the three peptide derivatives shows even greater heterogeneity than does the amide I’ band. While the NAGMA and NAAMA amide II’ bands are similar in form, the NAAMA band is red-shifted nearly 10 cm\(^{-1}\) compared to the NAGMA band. This red shift is consistent with the difference seen in the amide I’ band, despite the fact that amide I’ is composed almost entirely of carbonyl stretching while amide II’ is an out of phase combination of C-N stretch and N-H bend. A portion of the NALMA amide II’ band is shifted to even lower frequency than that seen in NAGMA and NALMA, and in
addition, the NALMA spectra indicate that the presence of the large, hydrophobic, leucine side chain creates two very different electronic environments for the two sections of peptide backbone. The overall blue shift of this principally bending vibration with increased pressure in each of the molecules is consistent with what is known for the response of other bending modes to decreased temperature, such as the HOH bend of water. (Dashnau et al., 2006, Vanderkooi et al., 2005) As the constituent atoms of the bending mode become more fixed in position relative to another, or bond more strongly to solvent, it takes more energy to excite the vibration.

Unlike what was seen in the amide I' spectra, the amide II' spectra of NAGMA and NAAMA do not broaden or split as pressure is increased, but instead narrow to the point where any underlying band substructure that may be discernible at ambient pressure disappears by the time pressures right below the ice VI transition are reached. It is evident that the NAGMA and NALMA molecules are accessing fewer peptide bond configurations as pressure increases, relative to those that are populated at atmospheric pressure. This means that the peptide bonds in these molecules are probably becoming more rigid and occupying a more restricted conformational space as pressure goes up. This conclusion is not surprising when we keep in mind how tightly packed water must be around the peptide molecules at pressures close to the ice VI transition.

NALMA, on the other hand, exhibits only a small blue shift with increased pressure and broadens slightly rather than narrows. This difference may be due to the large, hydrophobic, leucine side chain. It is known that hydrophobic residues cause water molecules to form clathrate-like cages around their side chains, and this water structuring may prevent hydrophobic side-chain-containing peptides in certain conformations from having their peptide backbone atoms make strong H-bonds with surrounding solvent. (Gallagher and Sharp, 2003, Sharp and Madan, 1997, Sharp et al.,
Since a sizable portion of the NALMA molecule surrounding the Cα atom may be shielded by, but not bonded to, a strong, cage-like water structure, the reason why the NALMA amide II’ spectrum does not narrow with increased pressure may well be that the water-clathrate is much more translationally mobile in water than would be a polar side chain or a group containing some strong H-bonding group.

Summary

We have presented pressure dependent OH stretch spectra for a mixture of dilute HOD in D$_2$O liquid and ices VI and VII. Our analysis shows that just as it is for changes in temperature, the decoupled OH stretching vibration is sensitive to changes in pressure of the local environment of the OH oscillator. This is readily deduced from observation of the OH stretch ice spectra and knowledge of the structure of ices VI and VII. We have also shown the identity of the side chain in a simple peptide can have a large impact on the electronic environment of the backbone of that peptide. NAGMA, NAAMA, and NALMA exhibit very different changes in their amide I’ and II’ spectra with increased pressure despite their overall structural similarity, and these spectral differences are reflections of their dissimilar structural response to increased hydrostatic pressure.
CHAPTER 5

FUTURE DIRECTIONS FOR THIS WORK AND CONCLUDING THOUGHTS

The research and results presented here advance the collective knowledge of water in a few important ways. First, it is no longer possible to argue the relative importance of H-bond length and H-bond angle. As was shown in Chapter 2, H-bond angle plays the major role in determining the energetics of the H-bond dimer. Though liquid water is certainly far more complex, there is no reason to think that the relative importance of H-bond angle does not also apply to liquid water. The work in Chapter 2 also shows that H-bond angle has the greatest impact on the charge distribution within the water dimer, and that for small H-bond angles the dimer behaves like a classical Morse oscillator.

Second, the work presented in Chapter 3 raises the intriguing notion that the guanidinium cation itself may not play the only role in its protein denaturation ability. Chapter 3 presents very clear evidence that guanidinium at high concentrations dramatically and distinctively reorganizes the H-bond network of water, much more so than any other ion we have observed. This rearrangement forces water into a less dense, more ice-like structure, much like that that has been found to solvate hydrophobic groups. It may be that some part of guanidinium’s denaturing action is due to its structuring of the water, thereby removing the impetus for hydrophobic collapse. If true, this mechanism would be a radical departure from the traditional ways of thinking about protein chemical denaturation.

Third, the work presented in Chapter 4 demonstrates a rearrangement in the H-bonding structure of ice VII at pressures right above the ice VI to VII transition. This rearrangement had seemingly been missed in the nearly fifty years of research on ice VII, and forces us to rethink our understanding of ice polymorph phase transitions.
Chapter 4 also presents work that shows the considerable side chain dependence of the peptide backbone response to pressure. This work may have important implications for the mechanics of pressure induced protein denaturation, as well as the protein composition for barophilic organisms.

Much of the work presented in this Dissertation begs for future research. There is still a great deal of calculations that could be performed on the water dimer using the methodology outlined in Chapter 2, including a detailed examination of molecular orbital composition and how it varies with geometry. The angle/O-O distance dependence of the energy could also be performed with much finer distance and angle resolution. It could also be useful to perform the same basic experiment but to scan through the possible tetrahedral angles (as defined in Chapter 2) in addition to the H-bond angle and O-O separation. The proposal presented in Chapter 3 that guanidinium’s structuring of water may help drive denaturation could also be examined using various methodologies, including simulation work and perhaps ultrafast spectroscopy and NMR. Attempts to examine the solvation of model hydrophobic compounds in the presence of guanidinium may be able to shed more light on our proposed mechanism. The high pressure research presented in Chapter 4 begs for a great deal of further work. As has been shown repeatedly in this Dissertation, the decoupled OH oscillator is a sensitive probe of H-bond environment, and there may be other changes in ice structure that have been missed over the years using other experimental techniques. Also, examination of the other NA*MA peptides using the same methodology presented in Chapter 4 should complete the picture of the side chain dependence of backbone structural changes due to increased pressure.

In conclusion, the work presented in this Dissertation exposes new and previously unknown characteristics of H-bond structure under a variety of conditions and adds to
the body of knowledge on water structure in a substantive way. The quantum mechanical findings go directly to the heart of the ongoing debate over the relative importance of O-O separation and H-bond angle. The finding that the guanidinium cation substantially structures its solvation water provides an important piece of evidence in the decades long quest to determine the mechanism through which guanidine salts denature proteins. And finally, the high pressure research uncovered previously unknown structure in ice VII at pressures right above the ice VI to VII transition point and also showed the role that the side chain of small peptide derivatives can have on the response of the peptide backbone to increased pressure.


Hydrogen Bond Dynamics Probed with Ultrafast Infrared Heterodyne-Detected

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