Rolling Adhesion of Yeast Engineered to Express Cell Adhesion Molecules

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Abstract- **Selectins are cell adhesion molecules that mediate capture of leukocytes on vascular endothelium as an essential component of the inflammatory response. Here we describe a method for yeast surface display of selectins, together with a functional assay that measures rolling adhesion of selectin-expressing yeast on a ligand-coated surface. E-selectin-expressing yeast roll specifically on surfaces bearing sialyl-Lewis^x ligands. Observation of yeast rolling dynamics at various stages of their life cycle indicates that the kinematics of yeast motion depends on the ratio of the bud radius to the parent radius (B/P). Large-budded yeast "walk" across the surface, alternately pivoting about bud and parent. Small-budded yeast "wobble" across the surface, with bud pivoting about parent. Tracking the bud location of budding yeast allows measurement of the angular velocity of the yeast particle. Comparison of translational and angular velocities of budding yeast demonstrates that selectin-expressing cells are rolling rather than slipping across ligand-coated surfaces.**

I. INTRODUCTION

The selectin family of cell adhesion molecules has been implicated in numerous inflammatory diseases, including atherosclerosis [1], vasculitis [2], allograft dysfunction [3, 4], rheumatoid arthritis [5], and ischemia/reperfusion injury [6]. Selectin expression has also been suggested as a mechanism governing tumor cell metastasis [7], as well as lymphocyte depletion during HIV infection [8]. Selectins initiate cell recruitment to a site of inflammation, mediating capture and dynamic adhesion (referred to as "rolling") of blood borne leukocytes onto activated vascular endothelium [9]. E- and P-selectin expressed on endothelium interact with carbohydrate-presenting ligands on leukocytes; L-selectin expressed on leukocytes binds carbohydrate-presenting ligands on endothelium [10, 11]. The carbohydrate sialyl-Lewis^x (sLe^x) has been shown to bind all selectins in static and dynamic studies [12-15], and more complex selectin ligands, such as P-selectin-glycoprotein-ligand-1 (PSGL-1) bear an sLe^x-related carbohydrate that is essential for function [16]. Selectin-sLe^x binding and force-driven unbinding generates transient adhesion (rolling) of leukocytes to endothelium, which is an essential pre-step for β_2-mediated firm adhesion and diapedesis [17]. With the recent elucidation of the structure of E- and P-selectin in co-crystals containing sLe^x and sLe^x-bearing PSGL-1-derived peptides

[18], and critical importance of selectin-mediated rolling in inflammation and disease, systems in which selectins can be mutated, expressed and screened for adhesive function will be essential for identifying the structural determinants of selectins' mechanical function.

II. METHODS

Yeast surface display is a means of performing directed evolution of molecules for therapeutic applications and molecular biophysics [19]. E-selectin display on the surface of yeast is achieved by expressing the molecule as a fusion protein to yeast cell wall proteins. We have constructed the pCT302 plasmid for expression of protein fusions to the Aga2p mating adhesion receptor of *Saccharomyces cerevisiae* [20]. This construct places Aga2p fusion protein expression under the control of the GAL1,10 galactose-inducible promoter. As the Aga2p protein is attached to the yeast cell wall via disulfide linkage to Aga1p, we have constructed yeast strain EBY100 in which Aga1p expression is also galactose-inducible. We subcloned the E-selectin gene in-frame with Aga2p in pCT302, and transformed the expression plasmid into yeast strain EBY100. E-selectin expression on the yeast surface was detected by immunofluorescence labeling with anti E-selectin monoclonal antibody.

To assay the ligand-binding properties of selectin-expressing yeast under hydrodynamic flow, we use sLe^x-coated substrate and a flow chamber. A microscope slide is coated first with avidin and then biotinylated sLe^x, to give a surface site density of sLe^x of 15,000 sites/μm^2. The slide is placed in the well of a parallel-plate, straight channel flow chamber. Yeast cells are infused into the chamber and drawn over the surface by a withdrawal syringe pump. Capture of yeast onto and motion of yeast across the surface are visualized by video light microscopy.

III. RESULTS

E-selectin-expressing yeast exhibit dynamic friction and rolling over an sLe^x-coated surface under hydrodynamic flow. This interaction is specific: incubation of yeast with anti-E-selectin monoclonal antibody blocks rolling over sLe^x-coated surfaces. In addition, CD20-expressing yeast exhibit no adhesion on an sLe^x-coated surface, and E-selectin-expressing yeast display no adhesion to Le^x-coated or avidin-coated surfaces. The average rolling velocities of E-selectin-expressing yeast on sLe^x substrate are two orders of

magnitude lower than the freestream velocity near the surface of 200 μm/s. The average rolling velocity of yeast expressing E-selectin on the sLe^x-coated surface ranges from 0.5 to 4.0 μm/s at a wall shear stress of 1.0 dynes/cm^2. These values are of the same order of magnitude as values reported for bovine neutrophils over lipopolysaccharide-stimulated bovine aortic endothelium [21], and agree with results reported from cell-free assays utilizing sLe^x-coated microspheres of 10-μm diameter over E-selectin-IgG substrates [13].

Yeast are observed adhering to surfaces at all stages of their life cycle. Average yeast rolling velocity increases with increasing ratio of bud to parent radius, or B/P. Parent size of yeast in these measurements is not dependent on bud size; at all values of B/P, parent radius is 1.5-2.5 μm. Rolling yeast drift normal to the direction of flow. The lateral drift is not an experimental artifact, as it may occur to either side of the axis of flow. The absolute magnitude of lateral drift velocity ranges from 0.05 to 0.3 μm/s, approximately one order of magnitude lower than the yeast rolling velocity in the direction of flow. Average lateral drift velocity of yeast is also bud-size dependent and increases with increasing B/P.

The dependence of rolling velocity and lateral drift on bud /parent size ratio suggests that cell motion is affected by the presence of a bud. Large-budded yeast (B/P approaching 1) may rotate about either the bud or the parent. The yeast first anchors to the surface at one (parent) end of the body, while the other end (bud) pivots 180° about the parent in the plane of the surface. The yeast then anchors to the surface at the bud end of the body, while the parent pivots 180° about the bud in the plane of the surface. The resultant motion resembles a dumbbell "walking" across the surface, with bud and parent alternately pivoting about one another. At times, dumbbell yeast move laterally (along a vector normal to their line of centers) when the line of centers is oriented at a severe angle to the direction of flow. This phenomenon is observed for yeast with B/P > 0.4.

Motion tracking and visualization of small-budded yeast (B/P < 0.4) reveals that these cells wobble little and may rotate about the parent only. The yeast moves across the surface via dynamic adhesion of the parent end to the surface, with the bud only occasionally acting as a pivot. The bud pivots back and forth about the parent in the plane parallel to the surface. The resultant motion resembles a body "wobbling" across the surface.

Yeast cells with all bud sizes may experience rotation of the bud out of the plane of the surface. The parent end of the body remains in contact with the surface, while the bud completes a rotation up to 180° around the center of the parent. The motion of the bud allows us to determine whether selectin-expressing cells are rolling or slipping across the surface. If cells are rolling, their motion should obey $v = \omega r$ where v is the translational velocity of the parent, ω is the angular velocity of the parent, and r is the radius of the parent. Measurement of instantaneous translational and angular velocities of tumbling yeast demonstrates that their motion follows $v = \omega r$. The measured radius of a yeast parent agrees with calculated radii determined at each instant using $r = v/\omega$. This correspondence is observed throughout the angular rotation of the particle. There is agreement between the values of v and ωr for rotating yeast over the entire range of parent radii observed in our experiments. Dynamic adhesion of selectin-expressing cells on ligand-coated surfaces thus represents rolling and not slipping.

IV. DISCUSSION

Yeast surface display of selectin molecules is well-suited for directed evolution and studies of structure-function relationships of selectins. Measurement of yeast adhesion under flow provides a functional assay for the activity of selectin-ligand interactions. Structure-function relationships in selectins may be elucidated by determining the changes in adhesive properties that result from selectin mutation. Our system may be used to engineer cell adhesion molecules with desired stability, affinity, or specificity characteristics through the process of directed evolution. With each round of evolution, the adhesive properties of yeast can be screened for differences in adhesion, owing to functional differences in the selectins' properties of kinetics or strength. Furthermore, selection of high-affinity selectin mutants that block inflammatory cell recruitment is likely to find application in development of anti-inflammatory therapeutics.

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