Low Force Decelerates L-selectin Dissociation from P-selectin Glycoprotein Ligand-1 and Endoglycan*

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Selectin-ligand interactions mediate the tethering and rolling of circulating leukocytes on vascular surfaces during inflammation and immune surveillance. To support rolling, these interactions are thought to have rapid off-rates that increase as wall shear stress increases. However, the increase of off-rate with force, an intuitive characteristic named slip bonds, is at odds with a shear threshold requirement for selectin-mediated cell rolling. As shear drops below the threshold, fewer cells roll and those that do roll less stably and with higher velocity. We recently demonstrated a low force regime where the off-rate of P-selectin interacting with P-selectin glycoprotein ligand-1 (PSGL-1) decreased with increasing force. This counter-intuitive characteristic, named catch bonds, might partially explain the shear threshold phenomenon. Because L-selectin-mediated cell rolling exhibits a much more pronounced shear threshold, we used atomic force microscopy and flow chamber experiments to determine off-rates of L-selectin interacting with their physiological ligands and with an antibody. Catch bonds were observed at low forces for L-selectin-PSGL-1 interactions coinciding with the shear threshold range, whereas slip bonds were observed at higher forces. These catch-slip transitional bonds were also observed for L-selectin interacting with endoglycan, a newly identified PSGL-1-like ligand. By contrast, only slip bonds were observed for L-selectin-antibody interactions. These findings suggest that catch bonds contribute to the shear threshold for rolling and are a common characteristic of selectin-ligand interactions.

Selectins are a family of adhesion molecules that contribute to leukocyte trafficking during inflammation and tissue injury and to lymphocyte homing (1–3). E-selectin is expressed on activated endothelial cells; L-selectin is expressed on leukocytes, and P-selectin is expressed on activated endothelial cells and platelets. L- and P-selectins interact in a related manner with P-selectin glycoprotein ligand-1 (PSGL-1); a mucin expressed on leukocytes. These interactions mediate tethering and rolling of circulating leukocytes on endothelial cells, platelets, and adherent leukocytes under flow. Selectin-ligand interactions are rapid and transient. In addition, the mechanically stressful environment in the circulation imposes forces on selectin-ligand bonds, which affect their dissociation rates.

Bell suggested that applied force could accelerate bond dissociation, because work done by the force could lower the energy barrier between the bound and free states (4). Dembo et al. (5, 6) conversely envisioned that force could also decelerate bond dissociation by deforming the molecules such that they locked more tightly. These two types of behavior are named slip and catch bonds, respectively. Bonds whose off-rates are independent of force are called ideal bonds (5, 6). Since the first experimental determination of the relationship between force and off-rate (7), many studies found slip bond behavior (7–18). Counter-intuitive catch bonds were only recently observed for interactions of P-selectin with PSGL-1 in a force range below those measured previously (19).

Both P- and L-selectin bind to an N-terminal region of PSGL-1 that must be modified with tyrosine sulfates and an appropriately oriented, sialylated, and fucosylated O-glycan (3, 13, 20–23). Monoclonal antibodies (mAbs) to this region block binding of PSGL-1 to both P- and L-selectin (24, 25). Compared with P-selectin, L-selectin binds to PSGL-1 with lower affinity and more rapid dissociation kinetics (9, 13, 15). However, the general binding similarities suggest that the L-selectin-PSGL-1 interaction, like the P-selectin-PSGL-1 interaction, might also behave as catch-slip transitional bonds.

Catch bonds have been suggested to partly explain the shear threshold requirement for selectin-mediated adhesion (19). Below the threshold, fewer cells roll, and those that do roll less stably and with higher velocity (26, 27). Bond lifetimes at low wall shear stresses might be too brief to support stable rolling. As the wall shear stress is increased, catch bonds might retard dissociation at the trailing edge, thereby stabilizing rolling.

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The abbreviations used are: PSGL-1, P-selectin glycoprotein ligand-1; AFM, atomic force microscopy; BSA, bovine serum albumin; DFS, dynamic force spectroscopy; HBSS, Hanks’ balanced salt solution; HSA, human serum albumin; mAb, monoclonal antibody; PEL, polyethylenimine; PZT, piezoelectric transistor; pN, piconewton.
increasing the number of rolling cells, and lowering their velocities. Compared with that for P-selectin, the shear threshold of L-selectin-mediated leukocyte rolling is much more pronounced and occurs over a much wider range of wall shear stresses (26, 27). If catch bonds contribute to the shear threshold, the L-selectin-PSGL-1 interaction should behave as catch-slip transitional bonds with transition over a much wider force range.

Endoglycan is a recently identified PSGL-1-like member of the CD34 family of sialomucins expressed on endothelial cells (28). Interactions of endoglycan with L-selectin also support cell rolling under flow (29). If catch bonds are a general feature of selectin-ligand interactions, the L-selectin-endoglycan interaction should also exhibit catch-slip transitional bonds.

We tested these hypotheses by using atomic force microscopy (AFM) and flow chamber experiments to measure off-rates of L-selectin dissociating from two forms of PSGL-1, from endoglycan, and from an antibody. Catch-slip transitional bonds were documented in interactions of L-selectin with ligands but not with antibody, which prompts renewed interest in how to model these bonds.

**MATERIALS AND METHODS**

**Proteins and Antibodies—**A cDNA encoding the lectin and epidermal growth factor domains and the two consensus repeats of human L-selectin plus a donor splicing sequence was amplified by the PCR and ligated into the pG1 vector (30), which encodes the heavy chain CH2-CH3 and hinge region of human IgG1. The vector was transiently transfected into COS7 cells using FuGENE 6 (Roche Applied Science). Cultures were incubated in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum. The L-selectin-Ig was purified from supernatants by protein G affinity chromatography.

Membrane L-selectin was purified from human tonsils (27), and native dimeric PSGL-1 was purified from human neutrophils (31). Recombinant monomeric soluble PSGL-1 (sPSGL-1) was purified from supernatants of CHO cell transfectants (18). The endoglycan-Ig, which consists of the extracellular region of the molecule fused to a human Fc domain, was collected from transfected COS7 cell supernatants (28). The blocking anti-L-selectin mAb DREG56 (32) and the blocking (PL2) and capture (PL2) anti-PSGL-1 mAbs (24) have been described. Anti-human IgG Fc polyclonal antibody was purchased from Chemicon (Temecula, CA).

**The AFM System—**The AFM system was built and calibrated in-house (design adapted from Vincent Moy, University of Miami) (Fig. 1A) (19). It consists of a piezoelectric translator (PZT) (Polytec Physik Instrumente, Boston, MA) on which a cantilever (Thermomicrometers, Sunnyvale, CA) is directly mounted. A laser (Oz Optics, Ontario, Canada) focused on the end of the cantilever is deflected onto a photodiode (Hamamatsu, Bridgewater, NJ) to measure cantilever deflection, which is converted to force using the cantilever spring constant. Each cantilever spring constant (4–30 pN/nm) was calibrated during the experiment using thermal fluctuation analysis (33). A personal computer with a data acquisition board (National Instruments, Austin, TX) was used to control the movement of the PZT and to collect the signal from the photodiode. LabView (National Instruments) was used as the interface between the user and the data acquisition board.

**Functionalizing the AFM Cantilever Tip—**Cantilever tips were incubated overnight at 4 °C in 10 μg/ml PL2, protein G, or DREG56 or 1% bovine serum albumin (BSA). After rinsing, the coated cantilevers were incubated for 30 min at room temperature in Hanks’ balanced salt solution (HBSS) containing 1% BSA to block nonspecific adhesion. PL2-coated (or protein G-coated) cantilevers were further functionalized by incubation in 10 μl of 100 ng/ml sPSGL-1 (or endoglycan supernatant) for 20 min at room temperature. DREG56-, BSA-, and some PL2- and protein G-coated cantilevers were used without further modification (Fig. 1B).

**Formation of L-selectin Bilayer—**Membrane L-selectin was reconstituted into glass-supported, polyethyleneimine (PEI)-cushioned lipid bilayers (Fig. 1B) by using vesicle fusion (19, 34–36). Our AFM experiments attempted to measure specific bond lifetimes at forces as low as thermally driven force fluctuations, which have a standard deviation of ~4–11 pN at room temperature, depending on the stiffness of the cantilever used (19). These are much smaller than the adhesion forces between coverslips and SiN3 cantilever tips, yielding ~100% nonspecific binding because all nonspecific interactions can be detected. Covering the glass with an intact lipid bilayer reduced the nonspecific binding to nearly zero. Nonspecific binding was increased when L-selectin was incorporated in the bilayer, presumably because it was partly disrupted by the inversely inserted molecules that have extracellular domains much larger than the normal gap distance between the bilayer and the coverglass. It was crucial to add a PEI cushion to accommodate the inversely oriented molecules between the glass and the bilayer (Fig. 1B), which reduced the nonspecific binding to ~5% (Fig. 2, A-D).

Coverslips of 40 mm in diameter (Biotecips, Butler, PA) were cleaned by using a mixture of 70% 12 N sulfuric acid and 30% hydrogen peroxide by volume at a temperature of 100 °C for 45 min, rinsed extensively with deionized water, and dried completely under an argon stream. The cleaned coverslips were stored in a desiccator and used within a 2-week period.
The coverlip was first immersed in a 100-µm PEI (Mr = 1800 g/mol, 95% purity; Polysciences, Inc., Warrington, PA) solution of 0.5 mM KNO₃ (Fisher) in deionized water, pH 7.0, for 20 min. After rinsing, excess water was removed from the coverlip surface by a gentle stream of argon. The coverlip was then placed in a desiccator for 10 min to dry the PEI layer (34) before forming an L-selectin bilayer on it.

An L-selectin-incorporated lipid vesicle solution was prepared following the method of McConnell et al. (35) and Dustin et al. (36). Briefly, vesicles were formed by hydrating a dried lipid film of egg phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) with 2% octyl β-D-glucopyranoside (Fisher), Tris saline solution creating 250 µl of 0.8 mM lipid solution. The 2% octyl β-D-glucopyranoside, egg phosphatidylcholine solution was combined with 250 µl of 1% octyl β-D-glucopyranoside solution with a 28 γ/ml concentration of membrane L-selectin. The resulting 0.4 mM lipid solution was dialyzed with three 1-liter changes of Tris saline buffer (25 mM Tris-HCl, 150 mM NaCl, pH 7.4) in 12-h increments. The resulting lipid vesicle solution was stored under argon at 4 °C and used within several months.

Bilayers were formed using the method of vesicle fusion (35, 36). A PEI-coated coverlip was placed in a Petri dish, and a 4-µl drop of L-selectin-incorporated lipid vesicle solution was placed on the surface of the coverlip. After 20 min of incubation under a damp paper towel, the Petri dish was filled with 10 ml of HBSS with 1% BSA. The L-selectin bilayers so formed had low molecular densities, which ensured their infrequent binding to the sPSGL-1, endoglycan-, or DREG56-coated cantilever tips, as required for measuring single bond interactions. Bilayers were immediately used in AFM experiments.

AFM Adhesion Event and Lifetime Measurements—Binding was enabled by driving the cantilever tip with the PZT to contact the bilayer for 2 s with an ~30-pN compressive force. The cantilever was then retracted at a speed of 250 nm/s. The presence or absence of adhesion was detected from the force-scan curves. The curves in Fig. 1, C and D, illustrate how the cantilever was bent (Fig. 1D, insets) when a compressive or a tensile force was respectively applied to the tip. The approach curves were horizontal (zero mean force) initially but were bent downward when the tip was pressed onto the bilayer. The retraction curve mirrored the approach curve until the cantilever was no longer bent. The upper curves in Fig. 1, C and D, illustrate a contact cycle resulting in a tensile force that signified binding, as illustrated by the lower curves in Fig. 1, C and D. Adhesion frequency was measured as the number of binding events per 100 repeated approach-retraction cycles using the same tip contacting the same L-selectin bilayer spot. Once the cantilever retracted a predetermined distance and stopped, a constant force was applied to the bond, if a bond was present, and it survived the ramping to the desired force. The lifetime was measured from the instant when the PZT stopped to the instant of bond failure.

Force-scan data were acquired at 5 kHz. Since most of the lifetimes were longer than 100 ms, tens of events were averaged to allow lifetime measurement at forces comparable with the level of thermal fluctuations (19). Fig. 1D exemplifies how the amplitude of force fluctuations in Fig. 1C was reduced by a sliding average of 10 points, such that the levels of force-scan traces before and after the retraction event no longer overlap. This is equivalent to trading average of 10 points, such that the levels of force-scan traces before and after the rupture event no longer overlap. This is equivalent to trading

where $R$, $L$, and $B$ denote receptor, ligand, and bond, respectively. For lifetimes measured at constant force $f$, $k_{off}$ is assumed to be a constant at each force and independent of time $t$. This model predicts that lifetimes of single bonds are exponentially distributed, $\tau \sim \exp(-\lambda t)$, where $\lambda = \left( \frac{R}{R+L} \right) k_{on}$ is the probability at a bond observed at a time $t$ that remained intact at time $t$. Taking the semi-log linearizes the exponential distribution; thus the slope of the ln(number of events with a lifetime $>t$) versus $t$ plot equals $-k_{off}$. It should be noted that the scattering of the individual lifetime data is not a reflection of the lack of measurement accuracy, which is better than 4%, but rather due to the natural individual molecular bond dissociation, which is stochastic by nature. Thus, the mean $\langle \tau \rangle$ should not be used as error bars because it measures a statistical property of the probability distribution rather than the uncertainty in the data. Indeed, it can be easily shown that $\sigma(\langle \tau \rangle)$ of an exponential distribution is equal to the mean $\langle \tau \rangle$ (37), both of which equal the reciprocal of off-rate, $1/k_{off}$.

Several methods were employed to assess the statistical significance of the differences in the $k_{off}$ values estimated at different forces, especially in the force range where catch bonds were observed. First, individual AFM experiments were performed to measure a few lifetimes at each of several force levels that covered the entire force range. The off-rate at each force level was estimated from the reciprocal of the mean and of the S.D. of these measurements. It was found that the $k_{off}$ versus $f$ curves so obtained were stable as soon as the number of measurements reached ~50. To ensure statistical stability, the data shown in Fig. 4 include 350–400 measurements for L-selectin interacting with either form of PSGL-1 and with endoglycan.

The larger data set allowed us to examine the distributions of lifetimes using the ln(number of events with a lifetime $>t$) versus $t$ plots (Fig. 3), which used the negative slope of the linear fit as estimates for $k_{off}$. The 95% confidence intervals of the slopes were used to measure the uncertainties of the $k_{off}$ values (shown as error bars in Fig. 4). The statistical significance of differences between any two neighboring points in the catch bond range (where the majority of the data reside) was determined by the F test that compared the slopes of the two lines (number of events with a lifetime $>t$) versus $t$ plots (Fig. 3), which used the negative slope of the linear fit as estimates for $k_{off}$.

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**RESULTS**

**Binding Specificity in AFM Experiments**—Non-specific binding was minimized by incorporating L-selectin in a lipid bilayer that was cushioned by a PEI layer, which accommodated inversely oriented molecules (Fig. 2, A–C, open bars). A substantial increase in binding was seen after functionalizing the tip with sPSGL-1 or endoglycan (solid bars). The binding was specific, because it was abolished by addition of a blocking mAb to L-selectin (DREG56) or to (s)PSGL-1 (PL1) or by addition of the divalent cation chelator EDTA (hatched bars); finally, the EDTA-containing medium was changed back to normal Ca²⁺-containing medium (stippled bars). D, the L-selectin bilayer was contacted with a tip adsorbed with PL2, BSA, protein G, or DREG56. Adhesion frequency for each condition in A–D was measured by making 100 repeated contacts to a single spot on a single bilayer with a single tip and presented as mean ± S.E. of at least three separate bilayer-tip pairs. E, mean ± S.E. of indicated numbers of unbinding events with a lifetime longer than that force (indicated). The 95% confidence interval of each linear fit is shown as paired, color-matched lines. The goodness-of-fit for each data set is indicated by an $R^2$ value.

In all lifetime measurements, the L-selectin bilayers were always tested first with yet-to-be-functionalized tips to confirm the low non-specific binding. After functionalizing with ligand or DREG56, the same tips were again tested to confirm the substantial increase in adhesion frequencies. This ensured that most lifetimes measured represented specific interactions of L-selectin with ligand or DREG56. However, the much less frequent non-specific binding might nevertheless contribute disproportionately long lifetimes and skew the distribution. To rule out this possibility, we compared the rupture forces of tips functionalized with or without ligand or DREG56 (Fig. 2E). Rupture forces between the L-selectin bilayers and tips coated only with PL2 or protein G were exponentially distributed (data not shown) with a mean of $-8$ or $4$ pN. By comparison, much higher mean forces (and hence much longer survival times during the ramping phase when force was increased) were required to detach tips coated with ligand or DREG56 from the L-selectin bilayers. The rupture force histograms were also qualitatively distinct, exhibiting a bell shape (data not shown). These data confirm that the measured lifetimes represented specific interactions.

**Catch-Slip Transitional Bonds Revealed by AFM Experiments**—To determine whether catch bonds existed at force ranges below those studied previously, we used AFM to measure lifetimes at forces as low as $5$ pN, using force averaging to circumvent thermally driven force fluctuations (Fig. 1D) (19). Lifetime measurements were sorted into force bins, and their distribution at each force bin was analyzed by the ln(number of events with a lifetime $> t$) versus $t$ plot, as exemplified in Fig. 3. The majority of the data (open symbols) followed a straight line, in agreement with the first-order dissociation kinetics of single bonds, which predicts exponential distribution of lifetimes that is linearized by the semi-log plot. A small fraction ($\leq 10\%$) of the longest lifetimes at some forces significantly deviated from the straight line (color-matched closed symbols),...
which were judged as outliers and excluded from further analysis.

The off-rate $k_{off}$ was estimated from the negative slope of the linear fit to the ln(number of events with a lifetime $\geq t$) versus $t$ data and plotted against force $f$ (Fig. 4). It was also estimated from the reciprocals of the mean ($t$) and S.D. $\sigma(t)$ of lifetimes, as the first-order dissociation kinetics model predicts that $k_{off} = 1/\langle t \rangle = 1/\langle \sigma(t) \rangle$. Compared with the lifetime distribution, the mean and S.D. require less data to become statistically stable. This allowed the force dependence of off-rate to be examined at finer force bins. The 1/$\langle t \rangle$ and 1/$\langle \sigma(t) \rangle$ data agree well with the $-\sigma$ slope data (Fig. 4), which imparts confidence in the $k_{off}$ estimates.

Similar to the P-selectin-(s)PSGL-1 interactions studied previously (19), the $k_{off}$ versus $f$ curves of the L-selectin-(s)PSGL-1 and -endoglycan interactions displayed biphasic catch-slip transitional bonds. At low forces, the off-rate decreased with increasing forces, whereas at high forces the off-rate increased with increasing force (Figs. 3 and 4, A–C). The error bars in Fig. 4 represent 95% confidence intervals of the $-\sigma$ slopes of the linear fits to the data (cf. Fig. 3). Except for a few cases where the differences in the forces were too small, the differences in the $k_{off}$ values in the catch bond regime were statistically significant ($p < 0.05$) as determined by the $F$ test and confirmed by the lack of overlaps in the confidence intervals.

We showed previously (19) that native dimeric PSGL-1 formed dimeric bonds with dimeric P-selectin, which doubled the lifetime and force relative to the interaction between monomeric sPSGL-1 and P-selectin. By comparison, the $k_{off}$ versus $f$ curve of the L-selectin-PSGL-1 interaction (Fig. 4B) was indistinguishable from that of the L-selectin-sPSGL-1 interaction (Fig. 4A). These data indicate that, although purified in a similar manner and incorporated in the lipid bilayer in the same way as membrane P-selectin, membrane L-selectin was unable to form dimeric bonds with PSGL-1 as P-selectin did, suggesting that L-selectin behaved as a monomer under the conditions tested. Although sharing a qualitatively similar catch-slip transitional bond, the L-selectin-endoglycan interaction exhibited quantitative differences. The catch bond occurred at 5–15 pN (Fig. 4C), a much narrower force range than the L-selectin-(s)PSGL-1 catch bond (Fig. 4, A and B). After transition to the slip bond, the off-rate of the L-selectin-endoglycan interaction rapidly increased with force, exceeding 200 s$^{-1}$ at 100 pN, much faster than the increase in off-rate of the L-selectin-(s)PSGL-1 slip bond in response to increasing forces.

To examine whether the catch-slip bonds were specific to L-selectin-ligand interactions, we measured lifetimes of L-selectin dissociating from DREG56. The off-rate estimated from all three methods increased monotonically with increasing force (Fig. 4D). This slip bond behavior is typical of antibody-antigen interactions (19) and contrasts sharply with the catch-slip transitional bonds of L-selectin with its physiological ligands.

**Catch-Slip Transitional Bonds Revealed by Flow Chamber Experiments**—Previous flow chamber measurements of the force dependence of off-rate of the L-selectin-PSGL-1 interaction detected slip bonds at wall shears ranging from 0.35 to 2.0 dynes/cm$^2$ (9, 13, 15). To confirm that catch bonds operated at low forces, we performed flow chamber experiments in the 0.15–1.5 dynes/cm$^2$ range. Microspheres coupled with L-selectin-Ig were perfused over very low densities of sPSGL-1 or DREG56 that supported transient tethering but not rolling. The tethers represented specific interactions, because they did not occur on chamber floors coated only with HAS, and they were eliminated by anti-L-selectin mAb DREG56 or anti-PSGL-1 mAb PL1 (data not shown).

Measurements of transient tether lifetimes revealed catch bonds for L-selectin-sPSGL-1 interactions at low wall shear stresses, which transited to slip bonds at higher wall shear stresses in a similar biphasic curve as those observed in the AFM studies (Fig. 5A). Remarkable quantitative agreement between Figs. 5A and 4A became evident after converting wall shear stress (bottom abscissa) to force on the tether (top abscissa). Regardless of the methods used for their determination, most differences in the mean off-rates at any two neighboring

![Fig. 4. Off-rates measured by AFM. Off-rates were plotted against force for interactions of L-selectin with sPSGL-1 (A), PSGL-1 (B), endoglycan (C), or DREG56 (D) with different scales in different panels. The broken y axis in C was used to display both the catch bond at low forces and the extremely fast off-rates at high forces. In addition to plotting the negative slopes (\( -\sigma \)) for the \( k_{off} \) values, as exemplified in Fig. 3, off-rates were also estimated from the reciprocal mean, 1/$\langle t \rangle$ (\( \bigcirc \)), and the reciprocal S.D., 1/$\langle \sigma(t) \rangle$ (\( \bigtriangledown \)), of lifetimes. The 95% confidence intervals were used as error bars for the negative slope data.](image)

![Fig. 5. Off-rates measured by flow chamber. Off-rates were plotted against wall shear stress (bottom abscissa) and force on a tether (top abscissa) for interactions of L-selectin with sPSGL-1 (A) or DREG56 (B). The data are presented as mean ± S.D. of four independent sets of experiments for each of the three off-rate measures.](image)
Catch-Slip Bond Transition in \(L\)-selectin-Ligand Interaction

wall shear stresses below 0.5 dynes/cm\(^2\) were statistically significant (\(p\) values ranging from 0.008 to 0.08, Student’s \(t\) test). Unlike the \(L\)-selectin-sPSGL-1 interaction, the \(L\)-selectin-DREG56 interaction exhibited a force dependence of off-rate that was consistent only with slip bonds (Fig. 5B). The agreement between the AFM and flow chamber experiments strongly suggests that the catch-slip transitional bonds between \(L\)-selectin and its physiological ligands are not an experimental artifact but reflect an intrinsic feature of these interactions.

DISCUSSION

Comparison with Previous Results—Previous experiments with flow chambers have shown that the off-rates of \(L\)-selectin interactions with PSGL-1 or with mucin ligands derived from lymph node endothelial cells increased with increasing wall shear stress, behaving as slip bonds (8, 9, 13, 15–17). These experiments were conducted at wall shear stresses that correspond to tether forces >40 pN. The present work reveals that \(L\)-selectin-(s)PSGL-1 interactions behaved as catch bonds at forces <50 pN, extending previous results to a lower force range. At higher forces the \(L\)-selectin-(s)PSGL-1 interactions behaved as slip bonds, in agreement with previous results. These catch-slip transitional bonds are qualitatively similar to those observed for interactions of \(P\)-selectin with (s)PSGL-1 (19), consistent with the structural and functional similarities of \(L\)- and \(P\)-selectin. The force dependence of off-rate of endoglycan, a novel \(L\)-selectin ligand (29) whose \(k_{\text{off}}\) had not previously been measured, also behaved as catch-slip transitional bonds. These data suggest that catch-slip transitional bonds may be a common characteristic of selectin-ligand interactions.

By using a biointerface force probe, the dissociation of \(L\)-selectin from PSGL-1 has been studied by loading bonds with increasing forces until rupture. Off-rates derived from unbinding forces by dynamic force spectroscopy (DFS) analysis suggested only slip bonds (11). In a similar approach, we used AFM to study \(L\)-selectin-(s)PSGL-1 bonds that were loaded with increasing forces until rupture. Off-rates derived by DFS analysis also displayed only slip bond characteristics. We have resolved the discrepant results from these different methods by estimating off-rates with a new analysis, which shows that the off-rate is not a single-valued function of the instantaneous force level but depends on the entire history of force application.\(^2\) Catch-slip transitional bonds are detected under conditions in which force is rapidly increased and then becomes constant, as in the experiments reported here. These conditions resemble those that occur in the circulation and are thus likely to be physiologically relevant.

Evidence for Monomeric Interaction—\(P\)-selectin and PSGL-1 both form cell-surface dimers (37, 38), which enhances adhesion under flow (14, 19). They remain as stable dimers after purification in nonionic detergent (39, 40). By comparison, it is not known whether \(L\)-selectin exists as a monomer or oligomer in the cell membrane or in solution. Should our AFM data reflect oligomeric interactions, fitting them with a monomeric binding model would have estimated apparent rather than intrinsic off-rates.

We should emphasize that even oligomeric binding would not have negated the catch bond observation in our AFM experiments. To measure lifetimes at a higher force required a longer time to ramp the force to a higher level before clamping it constant to start lifetime measurements. Should some of the bonds in an oligomeric bond cluster break during ramping, more such rupture events would have occurred at higher forces, yielding oligomeric bonds of lower valency. This would have resulted in shorter lifetimes at higher forces than at lower forces, namely slip bonds. To the contrary, we observed longer lifetimes at higher forces than at lower forces, namely catch bonds.

In previous AFM and flow chamber experiments, we observed a rightward and upward shift of the lifetime versus force curve of \(P\)-selectin-PSGL-1 interaction relative to that of the \(P\)-selectin-sPSGL-1 interaction, approximately doubling the force and lifetime. This demonstrated that \(P\)-selectin formed dimeric bonds with PSGL-1 but monomeric bonds with sPSGL-1 (19). Thus, purified \(P\)-selectin remains dimeric after being reconstituted into glass-supported lipid bilayers, and purified PSGL-1 remains dimeric after being captured on the AFM tips by mAb PL2. The present AFM experiments were performed under identical conditions, except that purified \(L\)-selectin was in lieu of purified \(P\)-selectin. However, the lifetime versus force curves were indistinguishable for interactions of \(L\)-selectin with PSGL-1 or sPSGL-1, suggesting that monomeric bonds were measured in both cases. This conclusion was further supported by molecular elasticity measurements, which revealed the same spring constant for \(L\)-selectin complexes with either form of PSGL-1. By contrast, \(P\)-selectin complexed with PSGL-1 had a spring constant that was twice that of the \(P\)-selectin-sPSGL-1 complex.\(^3\)

Catch Bonds and the Shear Threshold Phenomenon—The counter-intuitive shear threshold requirement for selectin-mediated leukocyte adhesion has not been satisfactorily explained. Chen and Springer (41) showed that the average number of tether bonds calculated from pause times of \(L\)- and \(E\)-selectin-mediated neutrophil rolling decreased with decreasing wall shear stress, which extrapolated to a value <1 at wall shear stresses too low to support rolling. The authors hypothesized that a minimum shear was required to rotate the cells sufficiently fast to allow the interacting molecules to penetrate the repulsive barrier to enable binding. Chang and Hammer (42) modeled cell binding under flow as a two-step process: a transport step in which molecules are carried by the moving cell to the vicinity of counter-molecules immobilized on a surface, and a reaction step in which the interacting molecules dock. The authors suggested that a minimum shear was required for transport to enhance the overall on-rate to support rolling (see also Ref. 27). Cell deformation has also been suggested to contribute to the shear threshold. The hydrodynamic drag exerted on a rolling cell has to be balanced not only by the tensile force acting on the tethers at the cell rear but also by the compressive force acting on the cell front, which might flatten the cell membrane locally, thereby enlarging the contact area and enhancing new bond formation (11, 27, 43). These hypotheses attribute on-rate as responsible for the shear threshold. We hypothesize that catch bonds (an off-rate characteristic) also contribute to the shear threshold. At low shear, bonds dissociate too rapidly to support rolling; increasing shear decelerates bond dissociation, thereby stabilizing rolling (19).

The shear threshold requirement is much more pronounced for rolling mediated by \(L\)-selectin than by \(P\)- or \(E\)-selectin (8, 26, 27). \(L\)-selectin-(s)PSGL-1 interactions behave as catch bonds at low forces that coincide with subthreshold wall shear stresses (Figs. 3–5), where the number of \(L\)-selectin-mediated rolling cells increase and their velocities decrease with increasing shear. The force range for \(P\)-selectin catch bonds is much lower, consistent with the much more modest shear threshold for \(P\)-selectin-mediated rolling (19, 27). These combined data

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\(^2\) B. T. Marshall, K. K. Sarangapani, J. Lou, R. P. McEver, and C. Zhu, submitted for publication.

\(^3\) B. T. Marshall, K. K. Sarangapani, A. Leppänen, R. D. Cummings, R. P. McEver, and C. Zhu, unpublished data.
support the potential physiological relevance of catch-slip bond transitions for rolling adhesion mediated by each selectin.

Models for Catch-Slip Transitional Bonds—The concept of catch bonds originated as an offshoot of a mathematical expression for force dependence of off-rate (5, 6). To provide a physical picture for this theoretical possibility, the authors proposed a geometrical model, a finger prison in which applied force deformed the molecules such that they locked together more tightly. At least two physical mechanisms can be envisioned for the catch-slip transitional bond, which cannot be explained by the finger prison model.

The first mechanism stems from the chemical principle that off-rate is inversely proportional to the exponential of the height of the energy barrier to dissociation, i.e., the difference in binding energy between the transition state and the bound state, scaled by the thermal energy (Fig. 6A). Applied force tends to destabilize the bound state by raising its energy, which, for simplicity, is assumed to increase linearly with force (Fig. 6B). Suppose that the applied force also destabilizes the transition state (Fig. 6B) but raises its energy at a rate that is at first faster and then slower than the rate at which the bound state energy increases with increasing force. The resulting energy barrier height would first increase and then decrease with force, resulting in a catch-slip transitional bond (Fig. 6, A and B). This model is an extension of the previous model (5, 6). The different shapes of the energy versus force curves for the bound state and the transition state provide a mechanism for changing from catch to slip bonds in different force regimes. By comparison, the previous model assumes similarly shaped quadratic functions for the force dependence of both the bound state and the transition state energies, which are represented by two springs with either the same spring constant or the same resting length, which allows only slip, catch, or ideal bonds (5, 6).

The second mechanism assumes that a bond can dissociate along two force-accelerated pathways, one fast and the other slow, with different probabilities (Fig. 7A). Suppose that the probability of dissociation along the slow pathway is small at low force but increases with increasing force, and the probability of dissociation along the fast pathway is large at low force but decreases with increasing force. Increasing force would first slow dissociation because it is increasingly more likely to take place along the slow pathway (Fig. 7B). After the slow pathway becomes predominant, continued increase in force would then accelerate dissociation by suppressing the energy barrier on this pathway (Fig. 7C). These models provide a starting point for studying the mechanisms of catch-slip transitional bonds, which have now been demonstrated experimentally (Ref. 19 and this work).

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