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Adhesive Dynamics Simulations of the Mechanical Shedding of L-Selectin from the Neutrophil Surface

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Abstract

Here we accurately recreate the mechanical shedding of L-selectin and its effect on the rolling behavior of neutrophils in vitro using the Adhesive Dynamics simulation by incorporating the shear-dependent shedding of L-selectin. We have previously shown that constitutively expressed L-selectin is cleaved from the neutrophil surface during rolling on a sialyl Lewis x-coated planar surface at physiological shear rates without the addition of exogenous stimuli. Utilizing a Bell-like model to describe a shedding rate which presumably increases exponentially with force, we were able to reconstruct the characteristics of L-selectin-mediated neutrophil rolling observed in the experiments. First, the rolling velocity was found to increase during rolling due to the mechanical shedding of L-selectin. When most of the L-selectin concentrated on the tips of deformable microvilli was cleaved by force exerted on the L-selectin bonds, the cell detached from the reactive plane to join the free stream as observed in the experiments. In summary, we show through detailed computational modeling that the force-dependent shedding of L-selectin can explain the rolling behavior of neutrophils mediated by L-selectin in vitro.

Keywords

Bell model; L-selectin; adhesive dynamics; cell adhesion; mechanical shedding

1. Introduction

Much attention has been paid to the effects of biochemical and micro- to nano-scale physical factors on the dynamics of leukocyte adhesion under flow. Deformability of the cell membrane and microvilli affects the rolling of neutrophils under flow (Caputo and Hammer, 2005; Jadhav et al., 2005; King et al., 2005b). The mobility of L-selectin on neutrophils influences its adhesive behaviors and intracellular signaling (Green et al., 2003; Green et al., 2004; King et al., 2005a).

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In addition to the adhesive role of L-selectin during the initial recruitment of leukocytes, its surface expression has been widely studied. L-selectin is down-regulated rapidly by ectodomain shedding following neutrophil activation by chemical stimuli and physical cross-linking. Recently, we reported that L-selectin can also be shed mechanically during neutrophil rolling on a sLe^x-coated surface under shear flow without inflammatory stimuli (Lee et al., 2007). Interestingly, the mechanical shedding of L-selectin is dependent on the shear stress applied in flow chamber experiments. In this study, we investigated whether force-dependent L-selectin shedding is necessary for L-selectin-mediated neutrophil rolling behavior under shear, using an updated Adhesive Dynamics (AD) model that includes the shedding mechanism.

2. Model

AD is a computational modeling method to simulate cell adhesion on a ligand-bearing planar surface and has been used to explain distinctive leukocyte and platelet behaviors (Caputo and Hammer, 2005; Caputo et al., 2007; Hammer and Apte, 1992; King and Hammer, 2001; Mody and King, 2008a; Mody and King, 2008b). Based on an up-to-date AD model, which includes the binding kinetics of catch bonds in on- and off-rates (Caputo et al., 2007) and the physics of deformable microvilli (Caputo and Hammer, 2005; Liu et al., 2007), we introduced the shedding kinetics in which we assumed the average dissociation kinetics of the mechanical shedding could be described by a Bell-like model of dissociation:

$$k_s = k_s^0 \exp\left(\frac{\gamma_s \sigma_{\text{Lsel}} |z - \lambda_{\text{Lsel}}|}{k_B T}\right), \quad (1)$$

where k_s^0 is the unstressed L-selectin shedding rate and γ_s is the reactive compliance of shedding. The reactive compliance is a measure of the sensitivity of bonds to applied force with units of length that describes the degree to which force facilitates bond breakage - a smaller value of reactive compliance means that the bond is less susceptible to breakage by force. Thus, over each time step of the simulation, the probability for mechanical shedding of L-selectin was calculated as:

$$P_s = 1 - \exp(-k_s \Delta t), \quad (2)$$

and tested against a randomly generated number uniformly distributed over the interval [0,1]. Cell, microvilli and bond positions are updated according to the dynamics of cell motion at every time step of 10^{-7} s. All parameters used in the study are summarized in Table 1.

We assumed in the simulations that L-selectin shedding occurs only when force is directly applied on an L-selectin bond. In other words, any signal transduction involved in the shedding to a neighboring L-selectin was not considered in the simulations because the kinetics of A Disintegrin and Metalloprotease 17 (ADAM17), which is known to mediate the L-selectin cleavage, has not been fully elucidated yet. Seventy-eight percent of the total number of L-selectin on a model cell was localized on the tip areas of microvilli based on published histology data and our previous work (Bruehl et al., 1996; Lee et al., 2007). In addition, it was assumed that the lateral mobility of unbound L-selectin concentrated on the tip of the microvilli in the contact area should not exceed 2×10^{-3} cm²/s to maintain the localization of L-selectin receptors on the tip of microvillus. The assumption is consistent with the value obtained from experimental measurements (Gaborski et al., 2008). Simulations were started at the given shear

rate with a model cell at a close position to the binding surface. At each set of conditions, five cells were simulated for 90 s each.

3. Results and discussion

To investigate the shear-dependent behaviors of the L-selectin-mediated cell rolling, the shear rates of 150 and 400 s^{-1} were applied in the simulations, respectively. As shown in Fig. 1A, the instantaneous velocity gradually increased at a shear rate of 400 s^{-1} , until 34 s when the velocity abruptly increased to the free stream velocity of the cell. This matched the neutrophil rolling behaviors which were observed in our previous experiments. Although we could not measure the velocity of a neutrophil after the cell detached from a sLe^x-coated surface in the experiments, the cell was assumed to join the free stream. It should be noted that this is caused directly by the cleavage of surface L-selectin induced by applied force as suggested by Fig. 1B. Around 1,100 of the L-selectin molecules at a shear rate of 400 s^{-1} still existed on the cell, but they were not involved in cell adhesion. Fig. 1C exemplifies cell trajectories at each condition. As described in the previous work (Caputo et al., 2007), the cell trajectory at a shear rate of 400 s^{-1} switched from rolling to no-adhesion at the same time interval as shown in Fig. 1A and B. This switch is sudden on the timescale of the simulation. The number of L-selectin bonds also decreased while cells rolled and then dropped to zero (Fig. 1D). However, the cells rolled for a longer time and lost their L-selectin slower at a shear rate 150 s^{-1} than at 400 s^{-1} , which agrees well with our experimental work.

Fig. 2A shows average rolling velocities at different wall shear rates in simulations. Our simulated rolling velocities which include the effect of mechanical shedding of L-selectin shows a shear threshold effect and matches our experimental data obtained from the flow chamber experiments with sLe^x-surface, and qualitatively matches similar experiments by Zhu, McEver and coworkers (Yago et al., 2004). Fig. 2B-F compare the instantaneous rolling velocities of cells simulations and experiments at a shear rate of 400 s^{-1} . The time at which the velocities of model cells and neutrophils consistently exceeded 60 $\mu\text{m/s}$ approximately agreed although model cells moved with larger oscillation in velocity as bonds broke and L-selectin receptors cleaved during rolling. The difference in rigidity between a rigid model cell and a deformable neutrophil could explain this discrepancy in mean rolling velocities particularly at high shear ($> 200 \text{ s}^{-1}$) as shown in Fig. 2A and in instantaneous velocities in Fig. 2B-F. As Konstantopoulos and coworkers reported recently (Pawar et al., 2008), leukocyte rolling behavior under shear flow is highly influenced by an interplay of two length-scales of deformation; mesoscopic cell deformation and microscopic microvillus deformation. Although we incorporated the effect of deformable microvilli in our model, as stated in previous work (Caputo and Hammer, 2005), the rolling velocities in simulations agree with experimental results of in vitro cell rolling at lower shear rates ($< 200 \text{ s}^{-1}$). Interestingly, Pawar *et al.* also showed that more compliant model cells roll slower than cells with a stiffer membrane and the difference in velocity increases as applied shear rate is increased.

We were able to determine the two parameters in the shedding kinetics of L-selectin – reactive compliance of shedding and unstressed shedding rate - based on the agreement with the experimental data, which were obtained from flow chamber assays using human neutrophils (Lee et al., 2007). Specifically, we visually inspected the cell rolling dynamics in simulations while varying the two values to determine whether the simulation data reproduced instantaneous rolling behaviors and the time at which a cell transited from a rolling state to a free-flowing state observed in previous experiments. The first was the reactive compliance of shedding, which was 0.005 nm. The estimated value is much smaller than that of an L-selectin bond, which means that the cleavage of L-selectin by force applied on the L-selectin bond would be less susceptible than the breakage of an L-selectin bond. In contrast, The unstressed rate of shedding, k_s^0 has been determined to be 85 s^{-1} . It is similar to the off-rate in the fast

pathway, but is greater than the off-rate in slow pathway. Therefore, this suggests that when a low magnitude of force is applied, L-selectin bond dissociation is more susceptible, but when a higher magnitude of force is applied, L-selectin shedding is more likely to occur. The further study of the detailed parameters involved in L-selectin shedding to formulate a state diagram of shedding remains to be examined.

In conclusion, we have verified by AD simulations that L-selectin is cleaved from the cell surface during rolling on a ligand-bearing surface under hydrodynamic shear flow, as previously observed experimentally. We have also shown that the mechanically facilitated L-selectin shedding depends on the magnitude of force per bond induced by shear force, in agreement with previous experimental work. As a result, rolling velocity increases during rolling and the cell joins the free stream when the cell down-regulates most of its L-selectin from the cell surface and cannot sustain rolling. Future work could incorporate the diffusivity of ADAM17 as shown in previous work (Schaff et al., 2008), to explore the physical interaction of L-selectin and ADAM17 and its functional role in cell recruitment.

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FIGURE 1.

The rolling behavior of model cells including the effect of mechanical shedding of L-selectin in simulations at different shear rates. A, The instantaneous velocities of cells at a wall shear rate of 150 and 400 s⁻¹, respectively. B, Number of L-selectin molecules remaining on model cells during rolling. C, X-direction displacement of cells during rolling. D, The number of bonds formed during rolling.

FIGURE 2.

A, The shear threshold effect in terms of rolling velocity in simulations and flow chamber experiments with a sLe^x-surface. The data with gray circles was from reference (Yago et al., 2004) and represent the more high affinity interaction with the complete PSGL-1 counterreceptor. B-F, Instantaneous rolling velocity of cells in simulations and experiments at a shear rate of 400 s^{-1} . B, The average instantaneous velocity of five rolling cells in simulations. C, A zoomed in graph of (B) to match the scale of y-axis with D-F. D-F, The instantaneous rolling velocity of individual neutrophils on a sLe^x-surface in flow chamber experiments (Lee et al., 2007).

Table 1
Parameters used in Adhesive Dynamics simulations

Parameter	Definition	Value	References
R	Cell radius	4μm	(Hammer and Apte, 1992)
μ	Fluid viscosity	1 cP	(Hammer and Apte, 1992)
G	Shear rate	150, 400 s ⁻¹	(Lee et al., 2007)
<i>L-selectin-mediated binding</i>			
σ_{Lsel}	Bond spring constant	4 pN/nm	(Marshall et al., 2006)
γ_{Lsel}	Reactive compliance (bond)	0.016 nm	(Ramachandran et al., 1999)
λ_{Lsel}	Equilibrium bond length	60 nm	
ρ_{Lsel}	Density of L-selectin on cell	35,000 – 45,000 sites/cell	(Lee et al., 2007)
<i>Mechanical shedding of L-selectin</i>			
k_s^0	Unstressed shedding rate	85-100 s ⁻¹	This work
γ_s	Reactive compliance (shedding)	0.005 nm	
<i>Deformable microvillus</i>			
σ_{mv}	Microvillus spring constant	56 pN/μm	(Liu et al., 2007)
λ_{mv}	Equilibrium microvillus length	0.266 μm	(Bruehl et al., 1996)
F_{trans}	Transition force	51 pN	(Liu et al., 2007)
F_0	Yield force	51 pN	(Liu et al., 2007)
μ_{eff}	Effective viscosity of tether	1.5 pN-s/μm	(Liu et al., 2007)
A_{mv}	Area of microvillus tip	0.02 μm ²	(Caputo and Hammer, 2005)
ρ_{mv}	Density of microvilli on cell	6 sites/μm ²	(Caputo and Hammer, 2005)
<i>Shear-controlled unstressed formation rate</i>			
D	Relative diffusion coefficient	0.15 μm ² /s	(Caputo et al., 2007)
a	Radius of reactivity circle around L-selectin	0.003 μm	(Caputo et al., 2007)
k_{in}	Intrinsic reaction rate	4.1×10 ⁵ s ⁻¹	(Caputo et al., 2007)
V	Inverse of timescale for exploring conformational space	1.5×10 ⁵ s ⁻¹	(Caputo et al., 2007)
<i>Two-pathway catch-slip dissociation rate</i>			
k_{1rup}	Dissociation rate (fast pathway)	84 s ⁻¹	(Yago et al., 2004)
k_2^0	Unstressed dissociation rate (slow pathway)	8.6 s ⁻¹	(Ramachandran et al., 1999)
ΔE_{21}	Difference in energy of two pathways	4 $k_{\text{B}}T$ pN-nm	(Evans et al., 2004)
f_{12}	Difference in force of two pathways	8 pN	(Evans et al., 2004)