Adhesive Dynamics Simulations of Sialyl-Lewis^x/E-selectin-Mediated Rolling in a Cell-Free System

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ABSTRACT Selectin-mediated leukocyte rolling is crucial for the proper function of the immune response. Recently, selectin-mediated rolling was recreated in a cell-free system (*Biophysical Journal* 71:2902–2907 (1996)); it was shown that sialyl Lewis^x (sLe^x)-coated microspheres roll over E-selectin-coated surfaces under hydrodynamic flow. The cell-free system removes many confounding cellular features, such as cell deformability and signaling, allowing us to focus on the role of carbohydrate/selectin physical chemistry in mediating rolling. In this paper, we use adhesive dynamics, a computational method that allows us to simulate adhesion, to analyze the experimental data produced in the cell-free system. We simulate the effects of shear rate, ligand density, and number of receptors per particle on rolling velocity and compare them with experimental results obtained with the cell-free system. If we assume the population of particles is homogeneous in receptor density, we predict that particle rolling velocity calculated in simulations is more sensitive to shear rate than found in experiments. Also, the calculated rolling velocity is more sensitive to the number of receptors on the microspheres than to the ligand density on the surface, again in contrast to experiment. We argue that heterogeneity in the distribution of receptors throughout the particle population causes these discrepancies. We improve the agreement between experiment and simulation by calculating the average rolling velocity of a popoulation whose receptors follow a normal distribution, suggesting heterogeneity among particles significantly affects the experimental results. Further comparison between theory and experiment yields an estimate of the reactive compliance of sLe^x/E-selectin interactions of 0.25 Å, close to that reported in the literature for E-selectin and its natural ligand (0.3 Å). We also provide an estimate of the value of the intrinsic association rate (between 10^4 and 10^5 s^{-1}) for the formation of sLe^x/E-selectin bonds.

INTRODUCTION

During the inflammatory response, circulating leukocytes roll over stimulated endothelial cells; rolling is a prerequisite for firm adhesion (Ley et al., 1998) and diapedesis (Springer, 1994). Rolling, or dynamic friction, occurs when a cell's velocity is reduced by adhesive interactions to a level significantly below that displayed by non-interacting cells adjacent to the wall. The molecular mechanisms that control leukocyte rolling are being elucidated (Lawrence and Springer, 1991; Springer, 1994; Pouyani and Seed, 1995; Alon et al., 1997, 1998; Chen et al., 1997; Liu et al., 1998). Several chemistries have been shown to support rolling, including interactions between selectins and their glycosylated ligands (Phillips et al., 1990; Polley et al., 1991; Lawrence and Springer, 1991, 1993; Varki, 1994; Brunk et al., 1996; Brunk and Hammer, 1997), VLA-4 and VCAM-1 (Jones et al., 1994; Alon et al., 1995b), laminin and an integrin (Tozeren et al., 1994), and tenascin and an unknown receptor (Clark et al., 1997). However, it is not clear what functional properties of recognition between receptor and ligand (i.e., between selectin and carbohydrates) are required for rolling. Meanwhile, other receptors with presumably different functional properties, such as LFA-1 and ICAM-1 (Lawrence et al., 1990; Sheikh and Nash, 1996; Campbell et al., 1998), or antigens and antibodies (Tempelman and Hammer, 1994; Chen et al., 1997; Swift et al., 1998), mediate firm adhesion. A goal of our laboratory is to understand how these different dynamic states are controlled by receptor functional properties.

A tool that can be used to relate observed dynamic states of adhesion to molecular properties is theory, and specifically computer simulation. Starting with the seminal work of Dembo and colleagues (1988), who used a tape-peeling model of cell adhesion to model cell rolling, and continuing through our laboratory's development of adhesive dynamics (Hammer and Apte, 1992), the properties of adhesion molecules needed to support rolling have been suggested. These properties appear to be fast rates of dissociation and a weak coupling between the rate of dissociation and the force applied to the molecule. In this article, we refer to the parameter that gives the coupling between force and dissociation as "reactive compliance." Thus, the reactive compliance must be small but nonzero to give rolling, according to current theoretical predictions.

In this paper, we choose to use the simplest postulated model for the coupling between dissociation rate and force (Bell, 1978). More complex relationships have recently been proposed, with supporting experimental evidence (Evans and Ritchie, 1997; Merkel et al., 1999); the main distinction of recent models is the idea that the loading rate can influence the dynamics of failure. Variation of the

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Abbreviations used: VLA, very late antigen; VCAM, vascular cell adhesion molecule; LFA, lymphocyte function associated; ICAM, intercellular adhesion molecule.

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loading rate can expose many different transition states in the energy landscape of an associated receptor-ligand pair, whereas the Bell model postulates that a single dominant transition state governs dissociation. Under fast loading rates as typically seen in leukocyte adhesion (100 to 1000 pN/s), these more complex models yield a single transition state and reduce to the Bell model. Although detailed calculations with more complex laws will be the subject of future work, the basic idea that force modulates the rate of dissociation is captured in the Bell model, and, under fast loading rates, the assumption of a single dominant transition state seems reasonable.

In adhesive dynamics, to make the calculations feasible, the "cell" must be somewhat idealized. For example, in Hammer and Apte (1992), we modeled the cell as a hard sphere coated with spring-like adhesion molecules. Clearly, cells are rough and deformable, with heterogeneous distributions of cell-surface receptors; failure to account for these factors in the theory can lead to erroneous conclusions about molecular properties. To bridge the gap between theory and experiment, either the "cells" had to be simplified in the experiments, or the theory had to be made more robust to account for complex cellular features. Our laboratory pursued the former route by attaching selectin ligands to microspheres and measuring adhesion to selectin-coated surfaces under flow (Brunk et al., 1996; Brunk and Hammer, 1997; Greenberg et al., 2000). This route has certain appealing features. It allows us to focus on the properties of the molecules that are responsible for rolling, eliminating confounding cellular features, and provides a platform for direct comparison between theory and experiment.

To make a cell-free system to mimic leukocyte rolling over endothelium (Brunk et al., 1996; Brunk and Hammer, 1997), we attached a carbohydrate selectin ligand, sialyl-Lewis^x (sLe^x), to the surface of polystyrene microspheres 10 μm in diameter. To model an endothelial cell, we attached an E-selectin-IgG chimera to a glass slide. We then measured the adhesion and rolling of sLe^x-coated beads on selectin-coated surfaces under hydrodynamic flow. We found that we could recreate rolling with this system, that the rolling could be as slow as several microns per second or as fast as tens of microns per second (depending on the density of adhesion molecules), and that the rolling velocity varied with time, as is also seen in leukocyte/endothelial systems (Goetz et al., 1994). These experiments provide direct proof that rolling is controlled by the physical chemistry of selectin-carbohydrate interactions, and not by cellular features such as morphology, signaling, or deformability.

The cell-free rolling experiments demonstrate that rolling can be recreated experimentally with rigid spheres. The cell-free system allows us to understand how rolling velocity depends quantitatively upon parameters such as receptor number, ligand density, and shear rate. In addition to the expected parameter dependencies of rolling, the cell-free system elucidated some surprising results. First, no rolling was measured at a wall shear stress greater than 2.2 dyn/cm^2, although robust rolling (at a velocity of 5% of the unencumbered velocity) was observed at 2.2 dyn/cm^2 for the particles that did adhere. This abrupt transition from rolling to no adhesion as wall shear stress is increased past 2.2 dyn/cm^2 was surprising, but is also seen in leukocyte/endothelial systems (Lawrence et al., 1990). Second, it was determined experimentally that rolling velocity is more sensitive to E-selectin density than to the sLe^x density on the microsphere. Because the density of E-selectin on the substrate is at least 10 times larger than the sLe^x density on the bead, it is not clear (in fact, it is counterintuitive) that changes in E-selectin density have greater effect on rolling velocity.

To interpret these experimental results and understand the molecular basis of rolling, we simulate cell-free rolling using adhesive dynamics. We compare simulation results with the experimental results presented by Brunk and Hammer (1997). The aim is to recreate the rolling dynamics reported in experiments and to extract numerical values for the molecular parameters that describe how receptor-ligand interactions work. The comparison between theory and experiment suggests that receptor heterogeneity across the population of particles plays a significant role in determining the experimental results, in particular the average rolling velocity. By averaging velocity over a heterogeneous population of particles, we are able to improve the agreement between experiment and simulation. In addition, we find the binding parameters of the E-selectin-sLe^x bond needed to explain the experimental data. We find they are close to the values of these parameters reported for E-selectin binding to its physiological ligand (Alon et al., 1997). We can extract the value of the association rate between sLe^x and E-selectin, giving a first estimate of this constant. Also, we recreate the disappearance of the rolling velocity at shear stresses greater than 2.2 dyn/cm^2 and explain why it occurs. The simulation results provide guidance for future work on elucidating the mechanisms of rolling, in particular the need to study the influence of cell heterogeneity on cell rolling.

METHOD

In this paper we applied adhesive dynamics (Hammer and Apte, 1992; Chang and Hammer, 1996, 1998) to simulate rolling observed in the cell-free system of Brunk and Hammer (1997). A detailed description of adhesive dynamics has been published in several places (Hammer and Apte, 1992; Chang and Hammer, 1996; Kuo et al., 1997; Vijayendran et al., 1998); thus, we only outline the method here.

sLe^x-coated microspheres are modeled as hard spheres with receptors distributed randomly over their surface. The planar surface coated with E-selectin is assumed to be uniformly reactive, since the E-selectin density is always larger than sLe^x density on the particles. Our idealization of the cell-free system is illustrated in Fig. 1. The model for receptor-ligand interaction is as previously described (Chang and Hammer, 1996). Receptors and ligand are modeled as adhesive springs with spring constant σ and equilibrium length λ. Each adhesive molecule reacts with the substrate with an overall association rate k_f and a dissociation rate k_r. The association rate

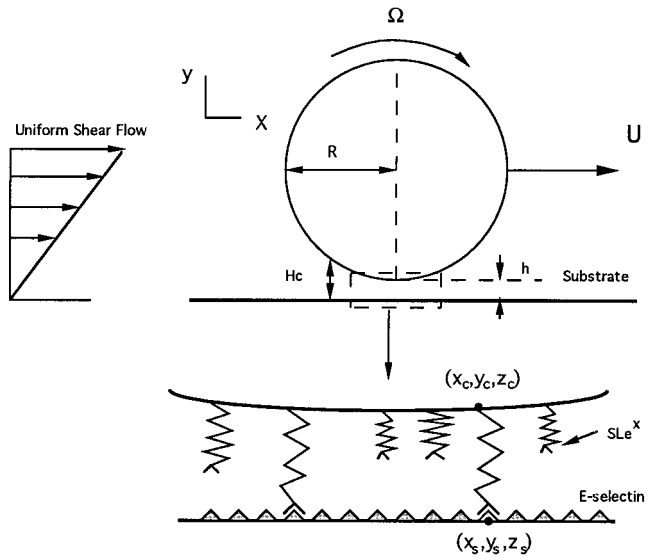

FIGURE 1 Schematic diagram of the cell-free system. The sLe^x coated microsphere interacts with a E-selectin substrate surface under a shear flow. The distance h is the separation distance between cell and surface. Receptors at a distance less than or equal to H_c are reactive. H_c is chosen to be 2 times the bond length.

includes the effect of particle translation on the rate of reaction (Chang and Hammer, 1998). k_f is a function of ligand density, relative velocity between the particle and substrate surface, and the intrinsic forward rate constant k_{in} (Chang and Hammer, 1998)

We adopt the model originally proposed by Bell to account for the effect of applied force, F, on dissociation rate (Bell, 1978),

$$k_r = k_r^o \exp(\gamma F/k_b T), \quad (1)$$

where k_r^o is the unstressed dissociation rate constant, k_b is Boltzmann's constant, T is temperature, and γ is the reactive compliance. The origin of the reactive compliance is in the molecular structure of the receptor-ligand complex. Phenomenologically, this parameter represents the sensitivity of dissociation to applied force, and it is equivalent to the length scale of the transition state along the reaction coordinate (Evans and Ritchie, 1997). Using a detailed solution of the Smoluchowski equation, Evans and Ritchie (1997) showed that this model is an appropriate way to describe the dissociation of receptor-ligand bonds in the fast loading regime, when the rate at which forces are applied to molecules is between 10 to 1000 pN/s. In Evans' formalism, the parameter that captures the force sensitivity of the receptor-ligand pair is $F_\nu = k_b T/\gamma$, which is inversely proportional to the reactive compliance.

Each free receptor inside the reactive region can become tethered in the time interval dt with a probability

$$P_f = 1 - \exp(-k_f\, dt), \quad (2)$$

and each tethered receptor can become free in a time interval dt with a probability

$$P_r = 1 - \exp(-k_r\, dt). \quad (3)$$

During each time step, bond formation and breakage are simulated by a Monte Carlo sampling of these probability distributions.

At any instant, the forces exerted by the tethers on the particle can be calculated, because the endpoint positions of all bonds are recorded. The tether force is added to the hydrodynamic and interfacial forces to obtain the net force acting on the particle. The motion of the particle can be fully described by the following relation:

$$\underline{U} = \mathbf{M}\underline{F}, \quad (4)$$

where **M** is the mobility matrix, $\underline{U}$ is a vector with 3 components of linear velocities and 3 components of angular velocities, and $\underline{F}$ is a vector comprised of 3 components of the total force and 3 components of the net torque acting on the sphere. The mobility matrix for a sphere near a plane wall in a viscous fluid is known (Jeffrey, 1915; Brenner, 1961; Goldman et al., 1967a,b; Chang and Hammer, 1996). Thus, once the net force and torque are known, the velocity of the particle can be calculated.

The algorithm of the simulation is as follows. At any time t, the positions of the receptors on the microsphere and the ends of tethers, the velocity of the bead, and the net force acting on it are known. Depending on the positions of free receptors at time t, tethers are formed according to the probability of Eq. 2 at t + dt, as probed with a Monte Carlo sampling. Simulation of bond breakage is executed by using a similar technique, using the force on the tether at time t to calculate the dissociation rate constant. Existing tethers are broken according to the probability given by Eq. 3 at t + dt, as probed with Monte Carlo sampling. Then, the positions of free receptors and tethers at t + dt are calculated from the kinematics of the cell (velocity and position at t). From the updated positions of tethers, the total force at time t + dt can be calculated. Using Eq. 4, the velocity of the sphere at t + dt is obtained. The process is repeated until the required time is reached or the particle travels across the field of view. We have set 10 s as the observation time and 0.1 mm as the length of the field.

The initial condition for each simulation is that of a free-flowing particle with an initial separation distance of 40 nm to the substrate surface. After an initial startup time during which the velocity and the separation distance become constant, the specific binding between receptors and substrate surface is initiated. After each simulation, the trajectory of the particle in the direction of flow is recorded. The average rolling velocity is calculated by dividing the total displacement by the duration of interaction. We also calculate instantaneous velocity using the observation interval used in the cell-free experiments (Brunk and Hammer, 1997), which was normally a fraction of a second.

RESULTS

Homogeneous populations

We perform simulations to mimic the effect of changes in ligand (E-selectin) density, receptor (sLe^x) density, and shear rate in the experiments by Brunk and Hammer (1997), and search for a set of molecular parameters that would produce simulation results matching results from these experiments. In the first simulations, particles are assumed to be homogeneous in receptor number (i.e., all spheres have identical numbers of receptors). There are four molecular parameters that are not strictly known: on rate (k_f), unstressed off rate (k_r^o), reactive compliance (γ), and spring constant (σ). Data showing the effect of shear rate, and the effect of changes in either E-selectin site density or sLe^x site density at 1.0 dyn/cm^2 wall shear stress are analyzed. In this analysis, we also neglect populations that roll with velocities smaller than 1 μm/s or larger than 20% of the hydrodynamic velocity, because in the experiment these particles are regarded as the firmly adherent and fast rolling particles, respectively, and were not taken into account in the measurement of the average rolling velocity. The parameters

used in these calculations are listed in Table 1 unless otherwise noted.

Effect of shear rate on rolling velocity

Using the reported values of mean sLe^x density (25,000 #/bead) and E-selectin density (3600 #/μm^2), we performed simulations at several different shear rates (100, 140, 180, and 220 s^{-1}) and calculated the rolling velocity. In Fig. 2 we plot the rolling velocity as a function of shear rate. Again, population homogeneity has been assumed. The experimental data are also plotted for comparison. Our simulations indicate a more sensitive response of rolling velocity to shear rate than was observed in the experiments. For example, at a shear rate of 140 s^{-1}, the simulated rolling velocity for particles with 25,000 sLe^x/bead is 6 times higher than the velocity reported in experiment; this discrepancy between actual and simulated rolling velocity will increase as shear rate increases at constant sLe^x density. As indicated in Table 1, the values of k_r^o and γ used in this set of simulations are 1 s^{-1} and 0.25 Å, respectively, which are close to the values reported for the interaction of E-selectin bond with its physiological ligand (Alon et al., 1997).

It is possible to match the experimentally observed velocity as a function of shear rate over the entire range of shear rate for a homogeneous population of particles expressing 25,000 copies of sLe^x per bead if different values of off rate and reactive compliance are assumed (k_r^o = 30 s^{-1}, γ = 0.05 Å). However, various major discrepancies emerge if these parameters are used for all our calculations. For example, these parameters would predict that particles roll at 20 μm/s at shear rates beyond 220 s^{-1}; however, no rolling is observed beyond this shear rate. Furthermore, using these parameters, we cannot recreate the effect of E-selectin density or sLe^x density on rolling velocity. Thus, we find there is no set of parameters for which we can describe experimental observations if particles are assumed to be homogeneous in receptor density.

Clearly, as reported by Brunk et al. (1996), the particles are heterogeneous in the expression of sLe^x. To understand whether this heterogeneity can lead to a difference in the effect of shear rate on rolling velocity, we simulated the rolling of two other homogeneous populations with two additional receptor densities, namely 35,000 and 40,000/bead. The results are illustrated in Fig. 2. Particles with higher receptor density roll more slowly at any given shear rate. Also, the shear rate at which slow rolling is not sustained (at which the rolling velocity increases dramatically) increases with increasing sLe^x density. The ability of different subpopulations expressing different numbers of receptors to respond differently to shear rate suggests that a population heterogeneous in receptor number may explain the complete response of the population to shear rate. As the shear rate increases, fewer particles have enough receptors to support rolling on the surface and thus the total number of rolling particles decreases. At a shear rate of 220 s^{-1}, there may be no particles with enough receptors to roll.

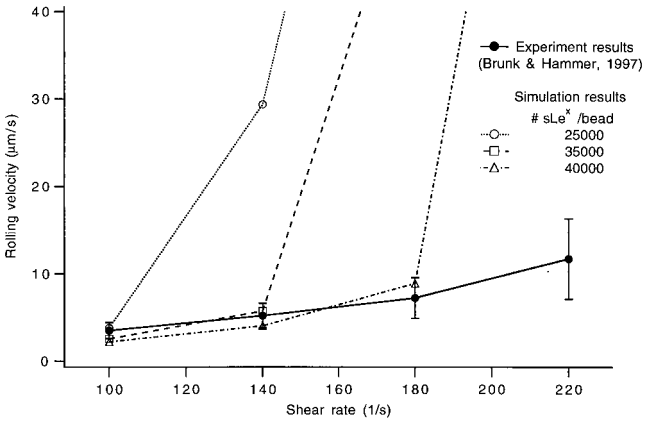

FIGURE 2 Rolling velocity as a function of shear rate. The simulation results give the behavior of homogeneous populations of spheres with 25,000, 35,000, and 40,000 molecules/bead. Parameters are as given in Table 1. Experimental data from Fig. 3 of Brunk and Hammer (1997) are plotted with *solid circles*.

TABLE 1 Parameters used in our simulations

Parameter	Definition	Value
R_c	Cell radius	5.0 μm
Rp	Receptor radius	1.0 nm
N_r	Receptor number	25,000
λ	Equilibrium bond length	20 nm
μ	Viscosity	0.01 g/cm-s
σ	Spring constant	100.0 dyn/cm
γ	Tensile strength	0.025 nm
H_c	Cutoff length	40 nm
T	Temperature	310 K
k_r^o	Unstressed off rate	1 1/s
k_{in}	Intrinsic on rate	5×10^4 1/s

Effect of E-selectin site density on rolling velocity

With the binding parameters given in Table 1 (same as used above), we calculated the effect of E-selectin (ligand) density on rolling velocity of homogeneous populations. Our results are illustrated in Fig. 3. The rolling velocities for ligand densities of 1400, 2100, 2500, and 3600/μm^2 at 1 dyn/cm^2 wall shear stress have been calculated for three different populations of spheres with a homogeneous distribution of receptors (from 25,000 to 40,000 sLe^x molecules per sphere). Experiments show that the rolling velocity increases most sharply as the E-selectin density decreases from 2100/μm^2 to 1400/μm^2; this sharp increase in rolling velocity with decreasing E-selectin density is seen in the simulation results for fewer than 30,000 sLe^x molecules per sphere. The rolling velocity of particles with 40,000 receptors does not change significantly as E-selectin density is varied. (The dynamics of rolling does change, as these

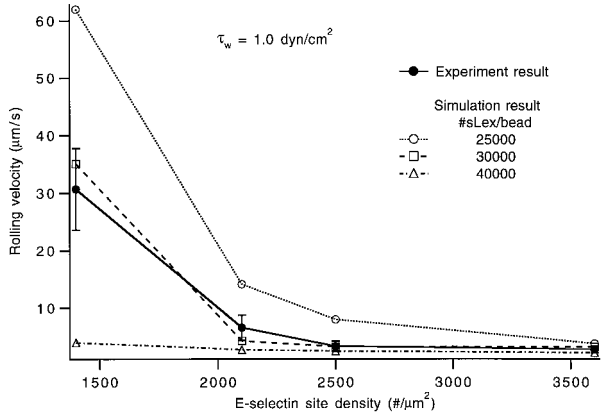

FIGURE 3 Rolling velocity as a function of E-selectin site density. Simulation data for three different receptor densities are shown. The wall shear stress τ_w is 1.0 dyn/cm^2. The experimental data are selected from Fig. 4 of Brunk and Hammer (1997).

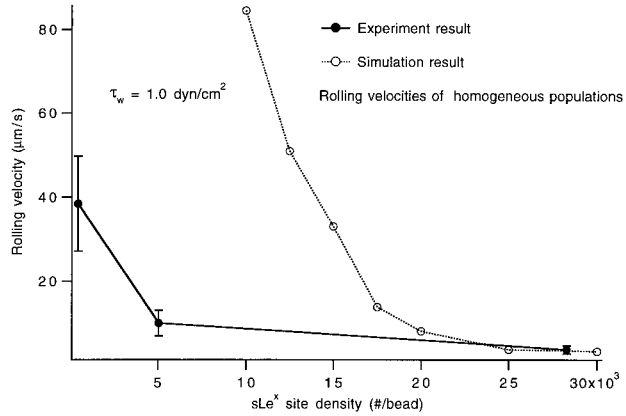

FIGURE 4 Rolling velocity as a function of sLe^x site density. The simulation data represent the rolling velocities of the particles with different receptor densities. The wall shear stress τ_w is 1.0 dyn/cm^2. The experimental data are taken from Fig. 5 of Brunk and Hammer (1997).

particles display more stops at higher ligand density.) There is overall good agreement between theory and experiment at an intermediate receptor density (30,000 sLe^x molecules per sphere) for the effect of ligand density on rolling. However, the mean reported number of sLe^x molecules per sphere is actually 25,000, which represents a discrepancy. In fact, calculations performed with this density of molecules per sphere clearly overestimate the rolling velocity at all E-selectin densities.

Effect of sLe^x site density on rolling velocity

Fixing the ligand density and shear rate to 3600/μm^2 and 100 s^{-1} (shear stress = 1 dyn/cm^2), respectively, we perform simulations with different sLe^x (receptor) densities. The rolling velocities at different sLe^x densities are plotted in Fig. 4. For both theory and experiment, the rolling velocity increases as the receptor density decreases. In the simulations, the rolling velocity for beads with 15,000 sLe^x/bead is as fast as that observed for beads with 1000 sLe^x/bead in experiments. In our simulations at 1000 sLe^x/bead, no adhesion is seen. At this average receptor density, if all the particles are homogeneous, there are too few receptors on any particle to support any adhesion. Thus, for homogeneous populations, our simulation results always overpredict the rolling velocity at any average sLe^x density. One possible explanation for this discrepancy is heterogeneity in receptor number. In the experiment, when the average number of receptors is 1000, the particles in the population that roll are likely those with a larger number of receptors than the average.

Heterogeneous populations

Based on the comparisons in the preceding section, it is not possible to explain the experimental data of Brunk and Hammer (1997) with any single homogeneous population. Our simulation results and the analysis of experimental data suggest that the ensemble-averaged rolling velocity obtained in the experiment may come from a heterogeneous population of particles with different number of receptors. Flow cytometry of the particles shows that the number density of receptors is heterogeneously distributed among particles (Brunk et al., 1996; Brunk, 1996). The standard deviations for the bead suspensions with average receptor number of 25,000, 5000, and 500 are 22,600, 8000, and 5000, respectively (Brunk, 1996). Thus, the existence of heterogeneity has been confirmed through flow cytometry.

To see if a heterogeneous populations can explain the apparent rolling behavior of the population, we performed simulations for an ensemble particles with different numbers of receptors, and averaged the observed rolling velocities. Since the full, exact distribution is not available and a qualitative illustration of the effect of the heterogeneity is sufficient for our purpose, we postulated a normal distribution of receptors as an approximation, and used the mean and standard deviation of the receptor number reported by Brunk (1996) to calculate the distribution of sLe^x across the population.

In Fig. 5, the ensemble-averaged rolling velocity from experiment and simulation are plotted as a function of shear rate. This figure is analogous to Fig. 2, calculated at the same E-selectin density (3600/μm^2), but with a heterogeneous population of beads with an average sLe^x density of 25,000/bead. After the process of averaging, the simulation results correspond remarkably well to the experimental data. In our calculations, we find the percentage of the particles perfused in the flow chamber that roll dropping from 40% to 11% as shear increases from 100 s^{-1} to 220 s^{-1}. As the shear rate increases from 110 s^{-1} to 220 s^{-1}, the lowest value of receptor number that can support rolling

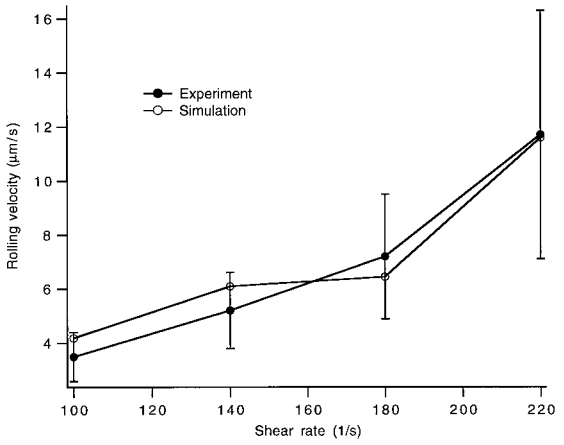

FIGURE 5 Comparison between simulation and experiment on the effect of shear rate on the ensemble-averaged rolling velocity for a normally distributed population. E-selectin density is 3600 molecules/μm^2, and the average sLe^x density per sphere is 2500.

increases from 19,000 to 48,000 sLe^x/bead. Thus, the particles that roll at higher shear rates are a subset of those that roll at low shear rate. In addition, at a shear rate of 250 s^{-1}, the fraction of particles that roll is about 3/100 of the entire bead population. Thus, as the shear rate increases, the probability that any particle will roll, if selected at random from the population, decreases.

The ensemble-averaged rolling velocities at different ligand densities are illustrated in Fig. 6. The averaged rolling velocity predicted by our simulation agrees well with the experimental results except at E-selectin site densities of 1400/μm^2. To understand this discrepancy, we have closely examined the experimental data at this ligand density, we find the amount of data collected may have more statistical variation than suggested by the error bars presented by Brunk and Hammer (1997). This datum point is comprised of 16 particles reported to roll between 0 and 20 μm/s, 1 particle rolling between 20 and 40 μm/s, and 2 particles rolling at a velocity larger than 120 μm/s. The sparse and discontinuous distribution of these data gives rise to the large standard deviation associated with this datum point. The appearance of a few fast rolling particles (>120 μm/s) at this low E-selectin density also supports the argument that the heterogeneity among particles is the main cause for the widespread distribution of rolling velocities. Because the ensemble-averaged rolling velocity from our simulation lies between 0 and 20 μm/s, corresponding to the median values of rolling velocity, we feel the level of agreement between simulation and experiment is satisfactory.

In Fig. 7, we demonstrate how the averaged rolling velocity varies with receptor density. This figure corresponds to Fig. 4, which illustrated the effect of rolling velocity on sLe^x density for homogeneous populations. The wall shear stress is 1 dyn/cm^2 and the E-selectin density is 3600/μm^2. After considering the effect of particle heterogeneity, the improvement in the agreement between experiment and simulation is obvious. Now, the averaged rolling velocity predicted over the entire range of sLe^x density agrees well with the experimental data.

Determination of binding parameters

Despite the presence of heterogeneity, we can make an estimate of binding parameters of sLe^x-E-selectin bonds that are necessary for rolling. To perform this analysis, we use the effect of ligand density on rolling velocity because it is least affected by heterogeneity (compare Figs. 3 and 6). Our search strategy was to select a set of molecular parameters that matched the rolling velocity at a single datum point (shear rate = 100 s^{-1}, ligand density = 3600/μm^2,

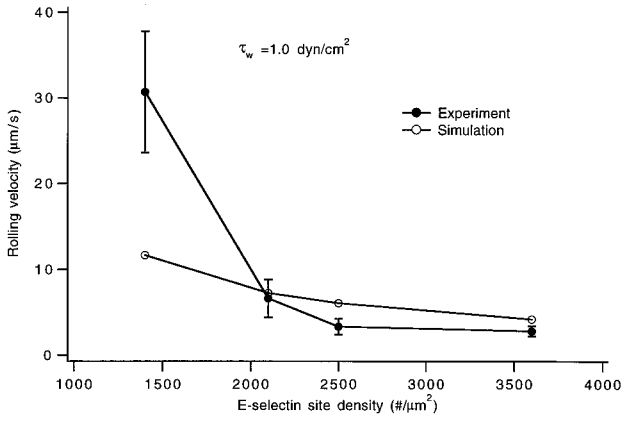

FIGURE 6 Comparison between simulation and the experimental data on the effect of E-selectin site density on the ensemble-averaged rolling velocity. The wall shear stress τ_w is at 1.0 dyn/cm^2 and the average sLe^x density per sphere is 2500.

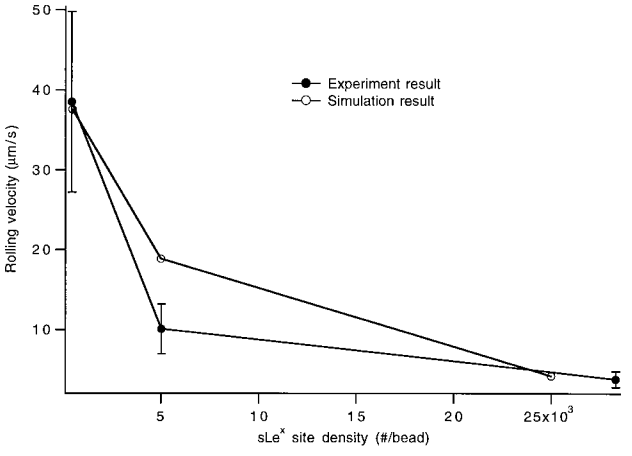

FIGURE 7 Comparison between simulation and the experimental data on the effect of sLe^x site density on the ensemble-averaged rolling velocity. The E-selectin density is 3600 molecules/μm2. The wall shear stress τ_w is at 1.0 dyn/cm^2.

and receptor number = 25,000). Since this one datum point is calculated for a single density of sLe^x (i.e., homogeneous population), we would expect this population to display a greater rolling velocity than seen in experiment at lower ligand densities (see the curve labeled 25,000 sLe^x/bead in Fig. 3). However, when averaged over the population, we expect the proper trend of rolling velocity with E-selectin density to re-emerge, which is confirmed by performing a full simulation over the entire population.

We compare our analysis with data presented in Fig. 4 by Brunk and Hammer (1997). We let the intrinsic on rate k_{in} and reactive compliance γ be our adjustable parameters and fix k_r^o at 1.0 s^{-1} (Alon et al., 1997). Also, we fix the spring constant at 100 dyn/cm, as it is known from previous calculations that rolling dynamics is not strongly dependent on the spring constant (Dembo et al., 1988; Hammer and Apte, 1992). The value of the spring constant we selected for our calculations has been estimated by Springer's laboratory (Chen et al., 1997; Alon et al., 1997). Our results are displayed in Fig. 8. For each k_{in}, we can determine a corresponding γ to match the rolling velocity 3600 E-selectin molecules/μm^2. For a homogeneous population, when k_{in} is larger than 10^5 s^{-1}, the predicted rolling velocity at E-selectin densities below 3600 E-selectin molecules/μm^2 is larger than seen in experiment. This suggests that k_{in} is less than or equal 10^5 s^{-1}, since as argued before, ensemble averaging over the population will lead to a decrease in rolling velocity. When k_{in} is smaller than 10^4 s^{-1}, the rolling velocity is too large to give an ensemble-averaged rolling velocity that matches the average rolling velocity reported in the experiment for ligand density = 1400/μm^2. Thus, $k_{in} > 10^4$ s^{-1}. From Fig. 8, we can determine an approximate range of combinations of k_{in} and γ in which the simulations match the data (i.e., we are fitting the values of k_{in} and γ to the data). The range of γ is from 0.019 nm to 0.026 nm, which is near the value of γ previously reported for E-selectin (γ = 0.03 nm; Alon et al. 1997). The estimated range of k_{in} (from 10^4 to 10^5) gives a range of association rate k_f from 10 to 50 s^{-1} for each receptor at condition where shear rate = 100 s^{-1} and ligand density = 3600/μm^2 (Chang and Hammer, 1998). As further vindication that these parameters are sufficiently robust to simulate the rolling behavior observed in the cell-free adhesion experiments, note that Figs. 5, 6 and 7 (the effects of shear rate, sLe^x density, and E-selectin density on rolling) were all calculated with $k_{in} = 5 \times 10^4$ s^{-1} and γ = 0.026 nm (as listed in Table 1). Thus, this one set of molecular parameters can be used to recreate all the parameter dependencies observed in the cell-free rolling experiments.

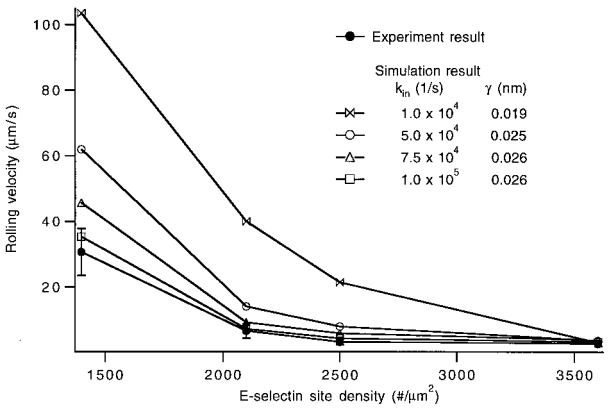

FIGURE 8 Simulations performed to extract k_{in} and γ from the experimental data. The experimental data from Fig. 4 of Brunk and Hammer (1997) are compared with simulation. Four sets of simulations with different pairs of intrinsic on rate k_{in} and reactive compliance γ are shown. Our results indicate that k_{in} is in the range from 10^4 to 10^5 s^{-1} and γ is between 0.019 and 0.026 nm.

Rolling velocity varies with time

In the previous sections, we focused on the average rolling velocity for a population of particles. To examine more closely the dynamics of particle rolling, we simulate the variation in particle velocity with time under different conditions. We calculate the instantaneous velocity by dividing the displacement of a rolling particle by a time interval of observation Δt. Depending on the rolling velocity, Δt is selected as the same value used in the experiment (a fraction of a second). In Fig. 9, we show the calculated instantaneous velocity as a function of time under different conditions, and, for comparison, the corresponding experimental results, taken from Fig. 8 of Brunk and Hammer (1997). Because of the discrepancy between theory and experiment described earlier, for the purpose of comparison we vary the receptor number to give an average velocity of the similar value reported in the experiment. (That is, we allow the receptor number to vary; since the population is heterogeneous, and we are observing an individual particle, it is quite likely the particle we are observing has a number of receptors much different than the average member of the population). Fig. 9 *A* shows the trajectories at two different wall shear stresses (τ_w). In order to give an average velocity of 4 μm/s at τ_w = 2 dyn/cm^2, the receptor number used in the simulation is 60,000, although the mean receptor density in the population is 25,000/bead in the experiment. Fig. 9 *B* shows the particle trajectories at two different ligand densities. The number of receptors used in the simulation is also larger than the average value reported in the experiment (see caption). The experimental data shown in Fig. 9 *C* are the typical trajectories for two populations of beads with different numbers of sLe^x molecules. By matching the average rolling velocity, our simulation results indicate that rolling particles are likely the ones with more receptors than the average would suggest. Although the mean receptor densities of these two populations prepared in the experiment differ by a factor of 10, according to the simulation, it is likely the receptor densities in these two realizations differ

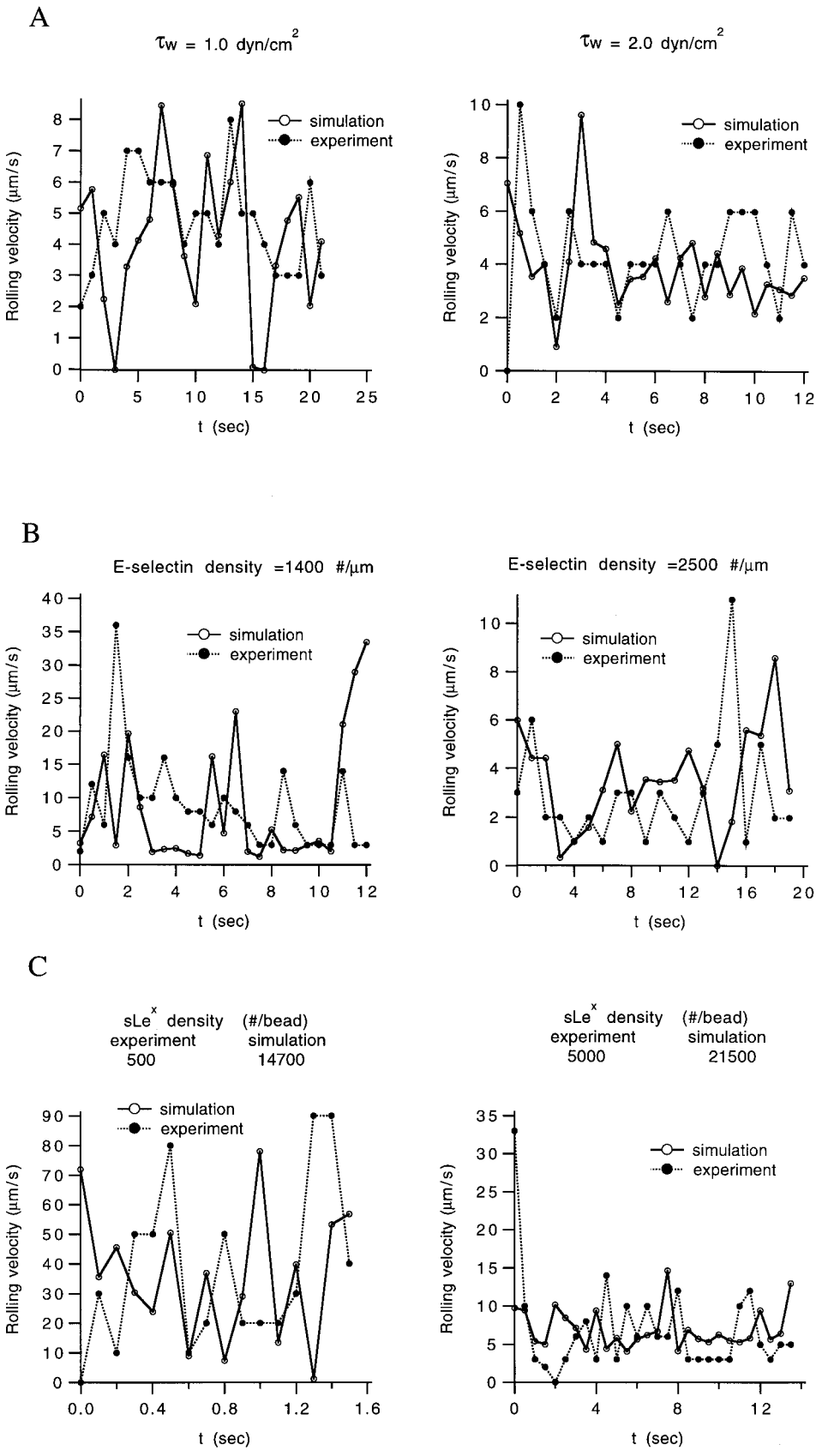

FIGURE 9 Typical particle trajectories at different conditions. Experimental data (Fig. 8 of Brunk and Hammer, 1997) are plotted for comparison. For each trajectory, the time step to determine instantaneous rolling velocities is as same as the reported in the experiment. (*A*) Effect of wall shear stress on rolling dynamics. Wall shear stress (τ_w) is either 1 or 2 dyn/cm^2 and ligand density = 3600/μm^2. The mean receptor density is 25,000/bead in the experiment. At τ_w = 1 dyn/cm^2, the receptor density in the simulation is 23,000/bead. At τ_w = 2 dyn/cm^2, the receptor density in the simulation is 60,000/bead. (*B*) Effect of E-selectin density on rolling dynamics. E-selectin density (N_L) is either 1400 or 2500/μm^2 (as indicated), and τ_w = 1 dyn/cm^2. The mean receptor density is 25000/bead in the experiment. At N_L = 1400/μm^2, the receptor density in the simulation is 28,000/bead. At N_L = 2500/μm^2, the receptor density in the simulation is 34000/bead. (*C*) Effect of sLe^x desnity on rolling dynamics. sLe^x density in the experiment is either 500/bead or 5000/bead, τ_w = 1 dyn/cm^2 and N_L = 3600/μm^2. The corresponding receptor densities used in the simulation are 14,700 and 21,500 molecules per bead.

by a factor less than 2. Generally, these plots show that the simulated rolling velocity of a particle fluctuates with time as described in the experiment; that the magnitude of the fluctuation predicted by theory matches the magnitude of the fluctuations seen in experiment; and that the rolling particles observed in the experiment may have a receptor number much higher than the average value number of receptors in the population.

DISCUSSION

To understand the molecular mechanisms of rolling, we have used adhesive dynamics to simulate sLe^x/E-selectin-mediated rolling in a cell-free system (Brunk and Hammer, 1997). We simulated the effects of shear rate, ligand density and receptor density on rolling velocity, and compared these predictions to experiment results. The comparison shows there are significant differences between the simulated behavior of a homogeneous population of particles and the data reported in the experiment. We argue that the heterogeneity in the number of receptors among particles is the main cause of the discrepancies between simulation results and experimental data. To support our argument, we have simulated the average behavior of a population of particles with a heterogeneous receptor distribution. The heterogeneity was confirmed by direct measurements of the receptor distribution on the particles by flow cytometry (Brunk, 1996). Note that we can only simulate the effect of shear rate, ligand density or receptor density on the average rolling behavior of particles when we invoke receptor heterogeneity. To see this, Figs. 2–4 document our failure to simulate the rolling velocity with homogeneous populations for shear rate, ligand density, or receptor density, respectively, and Figs. 5–7 document our success once particle heterogeneity is invoked.

We have documented the effect of heterogeneity of the behavior of a cell-free system, but it appears the effects of heterogeneity are also seen in cellular systems. In the data reported by Jones et al. (1993) on neutrophils rolling on histamine-stimulated endothelial cells, a wide distribution in rolling velocities was seen. At low wall shear stress, 25% of cells display firm adhesion. As shear stress is increased, the number of firmly adherent cells decreases and the distribution of rolling velocities throughout the population becomes wider. This trend can be explained by a heterogeneous population of cells. Since the time window of observation to determine the rolling velocities is only 4 s, the rolling velocity distribution might also be explained as the temporal fluctuation of the rolling velocity due to the stochasticity of the adhesion (Goetz et al., 1994; Zhao et al., 1995). A stochastic model developed by Zhao et al. (1995) was able to recreate the distribution of rolling velocities of individual cells from a homogeneous population. The model gives a trajectory of cell motion in a stop-and-go pattern. To match the experimental data of Jones et al. (1993), their model requires an average duration of pauses ranging from 0.92 to 0.53 s and an averaged displacement where particles travel at the unencumbered velocity between pauses ranging from 6 to 16 μm. In our view, an alternative explanation of the variability in observed behavior can be population heterogeneity.

The work of Zhao et al. (1995) also provides a method to determine the variance of rolling velocity contributed by the heterogeneity among cells. They applied the method to data obtained by Usami et al. (1993) and showed that the coefficient of variation contributed from the cell heterogeneity is about 30%. When the average velocity is determined with a time window of 4 s, the coefficient of variation contributed from both heterogeneity and temporal fluctuation is 50%. Thus, the heterogeneity has a dominant effect on the spread of the rolling velocity distribution, and this effect is greater when the time window increases. Following the arguments of Zhao et al. (1995), one may distinguish the effects of heterogeneity versus stochasticity by examining wider windows of time; the wider the window, the greater the influence of heterogeneity on any variation in the population average velocity.

Our work points out that heterogeneity plays a role in the adhesive behavior of cells, and that as conditions such as shear rate or ligand density vary, we may be selecting, or observing, various subpopulations which adhere favorably under the given experimental conditions. For example, as shear rate increases, fewer cells can interact with the substrate, and we select for those cells best able to bind. Under similar conditions, a homogeneous population of the same mean receptor number or size would show less adhesion (display a larger rolling velocity), since the homogeneous population does not have a well-equipped subset that can overcome selection pressures (high shear rates, low ligand densities) and support adhesion. The effects of heterogeneity in these types of experiments appear endemic. Saterbak and coworkers (1993) showed that heterogeneity played a dominant role over probabilistic binding effects in the detachment of rabbit anti-goat IgG-coated beads from goat IgG-coated surfaces. Similar to our study, the number of IgG molecules on the bead followed a normal distribution, with a standard deviation slightly smaller than the average number of molecules per bead. The detachment experiments could only be modeled theoretically if the knowledge of the heterogeneous decoration of beads with IgG was incorporated into the model (Saterbak et al., 1993).

It is well known from flow chamber adhesion experiments that neutrophils can only roll on stimulated endothelial cells in vitro at shear stresses less than 2 dyn/cm^2 (Lawrence et al., 1987, 1990). We have observed that sLe^x-coated spheres fail to roll on E-selectin surfaces at shear stresses less than 2.2 dyn/cm^2, which corresponds closely to the result seen with cells. A theoretical simulation of this threshold is provided in this paper. The reason for the threshold appears to be the inability of the particles to

sustain adhesion at these combinations of shear stress, receptor density, ligand density, and kinetic rates of binding, and not, however, a failure to form bonds. We performed a simple calculation to determine whether bonds do not form under these conditions, or whether bonds form but are sufficiently weak to sustain adhesion. Hammer and Lauffenburger (1987) showed that the mean time to form a bond is given $T_1 = (1/k_{in}N_L) \ln (1 - 1/A_cN_R)^{-1}$, where A_c is the contact area, N_R is the receptor density, and N_L is the ligand density. This time can be compared to the transit time, T_T, of receptors through the contact zone, $2(A_c)^{1/2}/3\pi^2a\gamma$, to identify the largest shear rate that can support adhesion. Using the parameters in Table 1, the critical shear rate appears to be order of 10^6 s^{-1}. Because shear rates in the experiments are order 10^2 s^{-1}, the shear rate seems to be amply small to support initial binding. Also, short transient binding is observed in the simulations for shear rates beyond 220 s^{-1}; thus, it appears that bonds can form under these conditions.

However, these bonds are not very long-lived. Chang and Hammer (1996) estimated the critical shear stress, τ_c, necessary to break a single bond on a time scale undetectable by the experiment, 1/30 s. They found that $\tau_c = (F_{bond}/7.8\pi a^2)(2(L\text{-}h)/a)^{1/2}$, where F_{bond} is the force to break a bond at a rate of 30 s^{-1}, L is the bond length, and h is the separation distance. Using L = 100 nm and h = 50 nm, and $F_{bond} = (k_bT/\gamma) \ln (30) = 680$ pN, we find $\tau_c = 1$ dyn/cm^2. Of course, at 2.2 dyn/cm^2 the bonds are even shorter lived. This rough estimate is of the same order as our observed critical shear stress of 2.2 dyn/cm^2; thus, the failure to sustain adhesion at 2.2 dyn/cm^2 is likely due to the fast failure of any bonds that form, along with an inability to quickly form additional bonds that sustain rolling.

One goal of our work was to determine the values of molecular parameters that give rise to rolling in the cell-free system. The four parameters we considered were the unstressed dissociation rate (k^o_r), the intrinsic reaction rate (k_{in}), the reactive compliance (γ), and the spring constant (σ). Theoretically, with these four degrees of freedom, a good model should be able to recreate all the experimentally determined parameter trends (effect of shear rate, ligand density, and receptor number). Indeed, we can match the trend with a restricted subset of these parameters once the spring constant and unstressed off rate were fixed. We found that the calculations were not very sensitive to the spring constant, confirming earlier work (Hammer and Apte, 1992). The unstressed off rate for most selectins is greater than or equal to 1 s^{-1}, so we set this parameter to 1 s^{-1}. We then found that values of k_{in} and γ were bounded in the following range: $10^4 < k_{in} < 10^5$ s^{-1} and 0.019 nm $< \gamma <$ 0.026 nm to match the experimental data. In general, γ may be measured using flow chambers (Alon et al., 1995a, 1997) or micropipette aspiration (Evans and Ritchie, 1997). The value of γ is very close to the value of 0.03 nm reported by Alon and coworkers (1997) for E-selectin interactions with its native ligand.

Our analysis also allows us to obtain an estimate for the forward reaction rate between receptor and ligand during rolling. Our best fit value for the intrinsic rate $k_{in} = 5 \times 10^4$ s^{-1} can be converted to the overall rate of reaction, k_f by the analysis of Chang and Hammer (1998) that accounts for the lateral motion of the sphere near the wall, and thus allows us to calculate the effect of transport on the collision between receptor and ligand. Combining this transport effect with the intrinsic reaction yields a value of k_f from 10 to 50 s^{-1} (depending on the shear rate). It is interesting to compare the overall on-rate k_f to that calculated by Kaplanski and coworkers (1993) for neutrophils rolling on endothelium. They deduced that $k_f = 0.038$ s^{-1} for a shear rate = 5.35 s^{-1}, and the on rate overall increased by a factor of 10 or more (to 0.75 s^{-1}) if the cell had paused briefly. These numbers are orders of magnitude lower than we have reported here, and there are several reasons for the discrepancies. First, the chemistry is different. Here, we have analyzed binding between sLe^x and E-selectin; it is not clear what ligands the granulocyte uses for binding E-selectin. Second, the cellular surface topography is different. The experiments of Brunk and Hammer (1997) involved hard spheres; alternatively, neutrophils are known to be rough with numerous microvilli (Kaplanski et al., 1993). Since receptors are concentrated on the tips of microvilli, the effective concentration of receptors is higher. Thus, a smaller intrinsic rate of reaction would be needed to give the same overall rate of reaction. Third, the shear rates used by Brunk and Hammer (1997) were as much as 40 times higher than those used by Kaplanski and coworkers; this difference itself can lead to a 40-fold difference in on-rate for transport-limited binding. Since 40 times 0.75 s^{-1} is 30 s^{-1}, within the range of 10 to 50 s^{-1} determined by our analysis, it may be possible that the results of these two studies concur.

There has been recent experimental and theoretical work on the origins of adhesion, static and dynamic friction in the physics and materials science literatures, and it is important to put our work in context. We employ phenomenological laws to describe the failure or adhesion bonds under an applied load. Although the constants in these laws may one day be derived from detailed knowledge of the molecular structure of the participants, such a connection to molecular structure does not now exist. In contrast, simple polymeric systems afford the luxury of elucidating the molecular origins of polymeric friction by performing molecular dynamics simulations using simple potentials (Baljon and Robbins, 1996). As more is learned about molecular structures involved in bioadhesion and as a computers become faster, it may ultimately be possible to perform similar calculations on biosystems on a reasonable time scale. However, we previously showed some uncanny similarities between rolling and polymeric dynamic friction (Chang and Hammer,

1996). For example, dynamic relaxation of polymer layers leading to greater adhesion will also lead to hysteresis in the dynamic friction (Yoshizawa et al., 1993). Likewise, hysteresis in rolling velocity is expected as a function of incubation time before the intitation of flow. However, insights obtained by Robbins and coworkers, such as the origin of energy dissipation and the relative balances of adhesive versus cohesive failure, are not yet possible in bioadhesion and cannot be gleaned from phenomenological models as presented here.

As pointed out by Goetz et al. (1994), the dynamics of rolling cannot be completely described by the average velocity. The adhesion dynamics of particles may differ greatly even if the average rolling velocity is the same. For example, some particle trajectories may have more stops than the others. Thus, it is necessary to examine the variation of rolling velocity with time as well in order to make sure that our simulated rolling motion resembles that observed in the experiment. The simulated rolling velocity fluctuation agrees well with the rolling dynamics reported in the experiment. The simulated trajectories display few durable stops just as the reported in the experiment. To compare with experimental data, we have adjusted the number of receptors on our simulated particle to match the time-averaged rolling velocity of the experimental particle. The receptor densities in our simulations all are different than the mean value reported in the experiment, which reflects the obvious fact that when we observe the motion of an individual particle or cell, it may have different characteristics than the mean particle or cell.

It should be obvious that the best way to test the role of heterogeneity in adhesion is to eliminate it through the use of sorted populations that have well defined, narrow distributions of receptors. Comparison between such well defined systems and heterogeneous parent systems should conclusively demonstrate the role of heterogeneity in cell adhesion.

Finally, we point out that adhesive dynamics can successfully recreate the adhesive behavior of populations of cells, including both the mean population behavior and the time-dependent behavior of individual particles. Thus, it continues to be a useful tool that can be used to elucidate the molecular mechanisms of cell adhesion.

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