Measuring the Lifetime of Bonds Made between Surface-linked Molecules*

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It is not well known how the kinetic constants of association between soluble receptors and ligands may be used to predict the behavior of these molecules when they are bound to cell surfaces. Spherical beads were coated with varying densities of anti-rabbit immunoglobulin monoclonal antibodies and driven along glass surfaces derivatized with rabbit anti-dinitrophenol. Particle motion was analyzed. The velocity, attachment frequency, and duration of binding events were determined on individual particles. It was found that i) beads exhibited frequent arrests lasting between a few tenths of a second and more than one minute; ii) when antibodies were diluted, the median arrest duration remained fairly constant (~ 1 s) whereas binding frequency varied as the first power of the antibody concentration, suggesting that most particle arrests were due to the formation of a single bond; iii) when the shear rate was increased 7-fold, the duration of transient binding events remained constant. The disruptive force exerted on attachment points was estimated to range between about 6 and 37 piconewtons; and iv) the distribution of arrest durations suggested that binding was not a monophasic reaction but involved at least one intermediate step. Therefore, transient binding events reflected the formation of unstable associations that are not detected with standard techniques.

An obvious requirement for a molecular understanding of cell adhesion would be to obtain a precise knowledge of the rates of bond formation and dissociation between membrane- associated receptors and ligands. Indeed, it was recently emphasized that the outcome of an intercellular contact might be associated receptors and ligands. Indeed, it was recently emphasized that the outcome of an intercellular contact might be associated receptors and ligands. Indeed, it was recently emphasized that the outcome of an intercellular contact might be associated receptors and ligands. Indeed, it was recently emphasized that the outcome of an intercellular contact might be associated receptors and ligands. Indeed, it was recently emphasized that the outcome of an intercellular contact might be associated receptors and ligands.

However, to our knowledge, no previously reported methodology allowed a direct measurement of the lifetime of interactions between particle-bound molecules. Tha et al. (6) used a travelling microtube to study the time and force dependence of rupture of antibody-mediated erythrocyte doublets. However, they did not study very transient attachments. Wattenbarger et al. (7) studied the adhesion of glycoporphin-containing liposomes to a lectin-coated surface in shear flow. Although they studied the motion of individual particles, they did not present quantitative data on short-term arrests. Other experiments done with the parallel plate flow chamber yielded direct information on binding efficiency and binding strength rather than binding kinetics (8, 9). Also, Evans et al. (10) performed micromanipulation to determine the mechanical resistance of molecular point attachments between erythrocytes. However, the contact time preceding separation was kept constant, which prevented the authors from obtaining any information on the natural lifetime of stable bonds. Recently, several authors used atomic force microscopy to study the interaction between individual surface-bound molecules (11, 12). They reported information on binding strength rather than kinetics.

This emphasizes the importance of the theoretical framework elaborated by Bell (13) to relate the behavior of surface-bound molecules to well known kinetic and thermodynamic constants of association between soluble receptors and ligands (see also Ref. 14 for additional information). The basic idea was to represent the interaction between molecules A and B as a two-step process. The first step is a purely diffusive encounter between molecules A and B, which approach into sufficiently close proximity to allow bond formation. Kinetic parameters can be estimated with standard diffusion theory. The second step, i.e. molecular association, is assumed to be described with the same constants when molecules A and B are free or bound to surfaces. The numerical values of these parameters may thus be derived from experimental data obtained on soluble forms of receptors and ligands. The limitation of this approach is that i) the reaction is assumed to be monophasic; ii) accurate information is required on the mobility of reacting molecules; iii) drastic assumptions are required to account for the dependence of bond formation on the distance between interacting surfaces; and iv) Bell’s theory could only be checked through theoretical models involving adjustable parameters (15, 16).

It was therefore felt useful to develop an experimental methodology allowing direct measurement of the lifetime of individual ligand–receptor bonds involving surface-bound molecules. The basic idea was to study the motion of receptor-bearing cells or particles along ligand-coated surfaces under laminar shear flow. The hydrodynamic force was less than the reported value of the mechanical resistance of associations between biological molecules (i.e. several tens of piconewtons [5, 10, 11, 12]). This approach was applied to human neutrophils interacting with endothelial cell monolayers (17) and murine lymphoma cells moving along antibody-coated surfaces (4). The wall shear rate was a few seconds−1, corresponding to an hydrodynamic drag of a few piconewtons. It was indeed possible to detect transient cell arrests that were probably due to the formation and dissociation of a low number of molecular bonds. However, two

*This work was supported by a grant from the Association pour la Recherche contre le Cancer. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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problems were raised by this approach. First, it was difficult to define cell arrests with high accuracy due to spontaneous velocity fluctuations and low velocity. Second, it was difficult to prove that observed arrests were due to single molecular bonds. The purpose of the present work was to overcome these difficulties with a better suited model. Particles were small spherical beads (2.8 μm diameter). This improved the accuracy of determination of arrest duration because the motion of spheres was more regular than that of actual cells, and, since the hydrodynamic drag is proportional to the square of particle radius, whereas the velocity is proportional to the first power of this radius (18), it was possible to achieve higher particle velocity without increasing the hydrodynamic force. Thus, improving the accuracy of time determinations (4). Spheres were coated with varying amounts of anti-rabbit immunoglobulin antibodies, and they moved along surfaces derivatized with rabbit immunoglobulin. Because molecules were not expected to exhibit free lateral diffusion on the sphere surface, the occurrence of multiple cell–substrate molecular bonds became more and more unlikely when dilution was increased. Analysis of experimental data strongly suggests that bond formation was not monophasic and that our method allowed to detect incomplete binding states that were not apparent with standard approaches.

MATERIALS AND METHODS

Particles and Surfaces

Particles were spheres of 2.8-μm diameter and 1.3 glit density (Dynabeads M280, Dynal, supplied by Biosys, Compiègne, France). These spheres were coated with streptavidin, a high affinity ligand for biotin. Before each experiment, 50-μl aliquots of biotin suspension (~7 × 10^10/ml) were incubated for 20 min at room temperature with an equal volume of a mixture of biotinylated mouse monoclonal antibodies at a final concentration of 0.2 mg/ml. These antibodies were an IgG2a specific for the Fc fragment of rabbit IgG (clone IgL, 17G, Immunotech, Marseille, France) and an IgG2a specific for CD14 antigen (clone UCHM1, Sigma) that was used as a control with irrelevant specificity to rabbit immunoglobulin. Because molecules were not expected to exhibit free lateral diffusion on the sphere surface, the occurrence of multiple cell–substrate molecular bonds became more and more unlikely when dilution was increased. Analysis of experimental data strongly suggests that bond formation was not monophasic and that our method allowed to detect incomplete binding states that were not apparent with standard approaches.

Analysis of Arrest Duration

The numerical values of the duration of typically 100 arrests observed under given experimental conditions were ordered in order to build a numerical plot of the variations of the number of particles remaining bound after a period of time following an initial arrest versus time. It was reasoned that a quantitative interpretation of experimental data was not possible at high binding site density, because the rate of formation of sequential bonds was probably dependent on the relative localization of binding sites in contact areas (in absence of lateral diffusion). Therefore, we only considered the low density limit. We assumed that bond formation between molecules A and B was a two-state process:

\[ A + B \rightleftharpoons (AB)_\alpha \rightleftharpoons (AB)_\beta \]

\[ k_1 \]

\[ k_2 \]

The probability \( P(t) \) for a particle bound in state \((AB)_\alpha \) at time 0 to remain bound at time t was estimated as described in the “Appendix.” An analytical formula allowed exact determination of \( P(t) \) when parameters \( k_1 \), \( k_2 \), and \( k_3 \) were varied. Comparison between Theoretical and Experimental Distributions of Arrest Duration

Theoretical curves were fitted to experimental data with \( \chi^2 \) test (23). Arrest durations were grouped in seven classes: 0–0.3 s, 0.3–0.6 s, 0.6–1.2 s, 1.2–2.4 s, 2.4–5 s, 5–60 s, and 60 s to ∞. Parameters \( k_1 \), \( k_2 \), and \( k_3 \) were systematically varied with a step of 0.1 in order to determine the values yielding minimal \( \chi^2 \). This procedure was repeated with a step of 0.01 or 0.001 to refine the minimization. Note that the threshold of \( \chi^2 \) for a 0.05 significance level is 12.9 when the number of degrees of freedom is 6.

Confocal Microscopy

Kinetics of Fluorescence Release by Labeled Beads—Dynabeads were coated with biotinylated anti-rabbit immunoglobulins and deposited on rabbit immunoglobulin-derivatized glass coverslips in a custom-made flow chamber. They were examined with a confocal laser scanning microscope (Leica, Heidelberg, Germany) connected to a desk computer bearing a PCVision++ imaging technology (Bedford, MA). Pixel size was 0.17 μm. A cursor driven by the computer mouse was superimposed on the microscopy image. Small (32 × 32 pixel) images pointed with the cursor in order to surround the analyzed bead were continuously transferred to the computer memory for delayed determination of the cell position. In this case, the resolution was limited by the accuracy of position determinations, because a particle might move by less than half a micrometer during a 0.08-s interval.

Derivation of Particle-Substrate Separation

The distance between flowing beads and the chamber floor was estimated with theoretical data provided by Goldman et al. (18). The numerical results displayed in Table II of this paper were used to plot \( \cos(1 - 2\pi a) \) versus \( UaG \), where \( a \) is the sphere radius, \( G \) is the wall shear rate, \( z \) is the distance between the sphere center and the substrate, and \( U \) is the sphere translational velocity. This yielded a smooth plot, allowing fairly accurate regression with a second order polynomial (when \( UaG \) ranged between about 0.4 and 1.2), leading to the following formula:

\[ z = \cos(-0.1130(UaG)^2 + 1.1688(UaG) - 0.4308) \]
Bonds—

A major problem with our experimental approach is velocity range. The assumption was somewhat supported by the observation that corresponding to a gap width of 10% of the sphere radius. This and the particleradiustimes the shearrate was lower than 0.8, out of focus. The white bar in the lower left is 5 μm.

This point was made more quantitative by pooling results for at least one stop in the absence of specific antibody interaction. On 1/1,000, whereasonly about 10% of these particles displayed at least one transient arrest when specific antibody were diluted between 1/1 and 1/1,000, whereas only about 10% of these particles displayed at least one stop in the absence of specific antibody interaction.

Most recorded particle arrests are mediated by specific bonds. A major problem with our experimental approach is that ill-defined “nonspecific” interactions may be responsible for a significant proportion of particle arrests. It was important to assess the importance of these interactions in the present system. As shown in Table I, when the specific antibody dilution and shear rate were varied within a wide range of numerical values, more than 90% of beads exhibited at least one transient arrest when specific antibody were diluted between 1/1 and 1/1,000, whereas only about 10% of these particles displayed at least one stop in the absence of specific antibody interaction. This point was made more quantitative by pooling results obtained with different values of the wall shear rate and calculating the binding efficiency parameter (i.e. mean number of arrests/μm displacement). This parameter was 0.0335, 0.0404, 0.0256, 0.0231, and 0.0013 μm⁻¹ when the proportion of specific antibodies on spherical beads was 1, 1/10, 1/100, 1/1000, and 0, respectively. The binding probability was therefore between 17- and 30-fold higher in presence of specific antibody than when beads were coated with irrelevant antibodies. This finding is consistent with the hypothesis that specific bonds were responsible for most initial arrests.

The initial rate of cell detachment is independent of the shear rate. Beads were coated with different proportions of specific antibodies (between 1/1 and 1/1,000) and subjected to different flow rates for determination of the duration of transient arrests. Plots of the fraction of beads remaining bound at time t after arrest versus time are shown on Fig. 3. A total number of 1381 arrests (i.e. about 100 arrests/plot) were observed.

The initial rate of bead detachment was approximated as the slope of regression lines determined with arrests lasting 1 s or less. The correlation coefficient ranged between 0.813 and 0.992 (mean 0.947). Although experimental curves sometimes displayed significant curvature over this interval, it seemed difficult to consider a shorter period of time because the number of points might be too low and the accuracy of time determinations was too low to warrant such attempts. Results are shown in Table II. Two main conclusions were suggested: i) the rate of particle detachment was not markedly dependent on the shear rate within the studied range, and ii) the detachment rate was similar with the lowest two antibody concentrations used. An attractive interpretation of these findings would be

| Proportion of specific antibodies | Beads with at least one arrest for a wall shear rate |
|----------------------------------|-----------------------------------------------|
| 1                                | 11 s⁻¹  | 22 s⁻¹  | 44 s⁻¹  | 72 s⁻¹  |
| 1/10                            | 95 (39/41) | 94 (31/33) | 100 (21/21) | 90 (28/31) |
| 1/100                           | 97 (39/40) | 95 (104/109) | 96 (28/29) | 100 (45/45) |
| 1/1000                          | 85 (71/94) | 97 (33/34) | 79 (76/96) | 70 (28/40) |
| 1/10000                         | 86 (83/97) | 76 (48/63) | 100 (15/15) | 96 (49/51) |
| 0                               | 10 (40/414) | 17 (30/174) | 12 (19/163) | 8 (29/353) |
that arrests observed with 1/100 or 1/1,000 antibody dilutions involved isolated molecular bonds and that the duration of these bonds was not affected by shear forces within the studied range, which provided a minimal value of bond strength. The consistency of this hypothesis with experimental data was thus subjected to a quantitative test.

Experimental Values of Arrest Durations Corresponding to the Highest Antibody Dilutions May Be Fitted with a Quantitative Model of Bond Formation Involving a Single Bond and a Two-step Binding Process—In order to achieve a quantitative interpretation of experimental data, \( \chi^2 \) analysis was performed to compare theoretical and experimental distributions of arrest durations. In a first series of calculations (not shown), it was checked that experimental data displayed on Fig. 3 could not be accounted for by a one-parameter theory involving a monophasic bonding reaction, with an adjustable off-rate. Indeed, in this case, the experimental curves should be straight lines at high antibody dilution. A three-parameter model involving an intermediate binding state was then considered ("Appendix"). It was found possible to obtain experimental curves fairly similar to experimental data. A typical fit is shown on Fig. 4. The numerical values of fitted parameters are shown on Table III as well as minimal \( \chi^2 \) values. Interestingly, no substantial difference was found between parameters obtained with beads coated with 1/100 and 1/1,000 specific antibodies, in accordance with the hypothesis that we were dealing with single molecular bonds. Further, when the hydrodynamic force was varied on a sevenfold range, no substantial change of these kinetic parameters was found, in accordance with the hypothesis that these forces were well below the threshold required to break ligand–receptor bonds.

However, this agreement between theoretical and experimental data does not formally prove that we were dealing with single molecular bonds. Indeed, similar results could be obtained if a fixed minimal number of bonds were required to mediate cell arrest. Therefore, limiting dilution analysis was performed to address this point.

When Beads Are Coated with Limiting Dilutions of Specific Antibodies, the Binding Rate Is Roughly Proportional to the First Power of the Surface Density of Binding Sites—The binding efficiency parameter was determined on beads coated with specific anti-rabbit immunoglobulin diluted at 1/1000, 1/2500, 1/5000, 1/7500, and 1/10000. This parameter was plotted versus
The distribution of arrest durations was determined on spheres coated with 1/1,000 specific anti-rabbit immunoglobulin antibodies and subjected to the lowest flow rate (11 s⁻¹). A total number of 154 arrests were recorded, and the fraction of arrests lasting at least 3 h following preparation (not shown).

First, glass surfaces were coated with rabbit immunoglobulins as described, and the surface density of these molecules was determined with indirect immunofluorescence and confocal microscopy. No substantial release was detected during the first 3 h following preparation (not shown).

Secondly, particles were labeled on the stage of a confocal microscope. In a representative experiment, the mean fluorescence of unlabeled particles was 140 (S.D. 57 particles). When labeling solution was added, the fluorescence rose to 1446 ± 138 (n = 15) after a 30-min incubation on the microscope stage. Finally, when beads were washed with fresh medium, the fluorescence was not significantly changed 5 min later (1531 ± 78, n = 24). It is concluded that no significant loss of fluorescence occurred during the first 5 min following the removal of labeling molecules.

Thus, bead detachment was at least one 100-fold more rapid than that of isolated molecules. Further, the aforementioned results did not support the hypothesis that shear forces might be responsible for this rapid separation.

DISCUSSION

The main purpose of this work was to achieve a direct determination of the lifetime of ligand-receptor bonds involving particle-bound molecules.

There is a Basic Difference between the Adhesion of Receptor-bearing Beads and Cells to Ligand-coated Surfaces—Recently, several authors reported quantitative data on the efficiency of adhesion between receptor-bearing cells and ligand-coated surfaces in flow chamber. When human granulocytes (17), rat basophilic leukemia cells (9) or murine lymphoid clones (4) were studied, an inverse relationship was found between cell velocity and binding probability per μm displacement. However, in the present study adhesion efficiency was fairly independent of the particle velocity (Table I). We suggest that the explanation for this difference is that in cellular systems receptor-ligand interaction is mainly diffusion-driven. If the limiting parameter is the time required for cell adhesion molecules to pass through the cell-surface contact area (which may be the tip of a microvillus), the adhesion probability will be proportional to the contact time, thus making the adhesion probability per unit length of cell displacement along the surface inversely proportional to the cell velocity. However, lateral diffusion of antibody molecules is not expected on the surface of the beads used in the present study. Therefore, ligand-receptor interaction may be dependent on particle displacement, making the adhesion probability proportional to the bead displacement rather than the contact time, as found in the present study. For this reason, the bead model cannot represent actual cell behavior. However, this absence of diffusion may provide an unique opportunity to study single molecular bonds without a need for excessive receptor dilution, which would make binding events too rare to be subjected to a quantitative study.

The Arrests Observed with Particles Coated with Low Concentration of Specific Antibodies and anti-rabbit immunoglobulin diluted at 1/1,000, 1/2,500, 1/5,000, 1/7,500, and 1/10,000. They were then driven along rabbit immunoglobulin-coated glass surfaces with a wall shear rate of 11 s⁻¹. The fraction of beads displaying at least one arrest was calculated and used to determine the binding parameter b using Equation 2. Each point was determined after studying between 69 and 321 individual beads. The uncertainty on the determination of the fraction of beads with at least one arrest was calculated following Ref. 23 and is shown as an error bar (± S.D.). The slope of the regression line is 1.08.

| Shear rate (s⁻¹) | Dilution of anti-rabbit Ig | k_d | k_s | k_x | χ² |
|------------------|---------------------------|-----|-----|-----|----|
| 11               | 100                       | 0.84| 0.5 | 0.005| 28.0|
| 11               | 1000                      | 0.88| 0.46| 0.008| 13.9|
| 22               | 1000                      | 0.70| 0.15| 0.12 | 5.9 |
| 44               | 1000                      | 0.82| 0.28| 0.03 | 13.1|
| 44               | 1000                      | 0.46| 0.48| 0.006| 17.6|
| 44               | 1000                      | 0.40| 0.30| 0.02 | 8.65|
| 72               | 1000                      | 0.51| 0.28| 0.009| 21.6|
| 72               | 1000                      | 0.55| 0.36| 0.046| 5.58|

The Arrests Observed with Particles Coated with Low Concentration of Specific Antibodies and anti-rabbit immunoglobulin diluted at 1/1,000, 1/2,500, 1/5,000, 1/7,500, and 1/10,000. They were then driven along rabbit immunoglobulin-coated glass surfaces with a wall shear rate of 11 s⁻¹. The fraction of beads displaying at least one arrest was calculated and used to determine the binding parameter b using Equation 2. Each point was determined after studying between 69 and 321 individual beads. The uncertainty on the determination of the fraction of beads with at least one arrest was calculated following Ref. 23 and is shown as an error bar (± S.D.). The slope of the regression line is 1.08.
centrations of Specific Antibodies Are Mainly Initiated by Single Ligand-Receptor Interactions—As shown on Table I, even when specific antibodies were diluted 1/1000 with irrelevant antibodies, beads displayed much more frequent arrests than when they were coated with 100% irrelevant antibodies. Therefore, even with 1/1000 antibody concentration, most arrests were mediated by specific bonds. Two arguments support the view that these arrests were mainly due to the formation of a single bond.

First, when specific antibodies were diluted 1/1000, the site density was about 3.5 sites/μm². The contact area between the bead and the surface may be defined as the area where the distance between surfaces is less than the sum L of the lengths of a rabbit immunoglobulin (on the chamber floor) and a mouse immunoglobulin (on the bead). From elementary geometrical formula, this area is 2πaL, where a is the sphere radius. Because L is about 0.02 μm (corresponding to four times the length of a Fc or Fab fragment of an immunoglobulin molecule (26)) and a is 1.4 μm, the contact area is about 0.17 μm². If there is on average less than one mouse anti-rabbit Ig molecule in this region, it is quite unlikely that there would be two molecules simultaneously interacting with an antigen site on the surface.

Second, if n bonds were required to mediate a detectable arrest, the arrest probability would vary as the nth power of specific antibody concentration under conditions of limiting dilution (see Appendix 2 of Ref. 27). As shown on Fig. 5, limiting dilution experiments support the hypothesis that arrests are mediated by a single bond, because the logarithm of arrest probability varied as the 0.8th power of the logarithm of antibody concentration.

Our Experimental System May Yield Fairly Precise Information on the Influence of a Force on the Lifetime of Ligand-Receptor Association—Our approach may yield at the same time precise kinetic and mechanical data. Indeed, according to Goldman, Cox, and Brenner (18), a sphere deposited on a plane under a laminar shear flow of shear rate \( \Gamma \) is subjected to a drag force \( F \) and torque \( \Gamma' \) given by:

\[
F = 1.7005 \times 6 \pi a \mu G \quad \text{(Eq. 4)}
\]

\[
\Gamma' = 0.9440 \times 4 \pi a^2 \mu G \quad \text{(Eq. 5)}
\]

where \( a \) is the sphere radius and \( \mu \) is the medium viscosity. As shown on Fig. 6, the force \( F \) experienced by a single bond of length \( L \) much smaller than \( a \) is 

\[
T = (F + \Gamma')/a \sqrt{a/2L} \quad \text{(Eq. 6)}
\]

Using 20 nm for \( L \) (see Ref. 26 and above) and considering spheres of 1.4-μm radius embedded in a medium of 0.001 Pas viscosity, such as water at 20 °C, we obtain:

\[
T = 0.51 G \text{ piconewton} \quad \text{(Eq. 7)}
\]

where \( G \) is in s⁻¹. Thus, under our experimental conditions, the applied force \( T \) ranged between 5.6 and 36.7 piconewtons. It must be emphasized that this estimate is only weakly dependent on the numerical value of parameter \( L \). The results shown on Tables II and III suggest that the lifetime of antigen-antibody bonds we studied was not substantially reduced by this treatment. This conclusion is consistent with previous estimates of binding strength (6, 10, 28, 29). Further, our experimental system may provide additional information by allowing simultaneous determination of applied force and bond dissociation rate.

The Distribution of Arrest Durations Is Quantitatively Consistent with a Model Involving a Two-step Association between Individual Ligand and Receptor Molecules—A first point supporting the validity of our model is that the slopes of experimental detachment curves (Table II) were fairly similar when the specific antibody concentration was 1/100 and 1/1000. This is in accordance with the single bond hypothesis.

More quantitatively, as exemplified on Fig. 4, the overall pattern of binding curves displayed on Fig. 3 could be reproduced with theoretical data based on a two-step model of molecular association involving three adjustable kinetic parameters. As shown on Table III, there was in some cases a significant discrepancy between experimental data and the best theoretical fit. We think that this did not disprove our model, because this discrepancy might be due to the infrequent formation of multiple bonds. Indeed, the agreement between experimental and theoretical curves was on average far better with the highest dilution of specific antibodies.

Further, we wish to emphasize that the existence of an intermediate step seems required to explain the difference between the lifetime of antigen-antibody bonds involving soluble and particle-bound molecules. If we assume that the hydrodynamic drag exerted on bound particles is not sufficient to substantially reduce the bond lifetime, it is difficult to understand why the interaction between flowing beads and substrate lasted only a few seconds. Indeed, the lifetime of adhesions between soluble rabbit anti-dinitrophenol antibodies and substrate may be higher than several hours, and the lifetime of interactions between mouse anti-rabbit immunoglobulin and these immunoglobulins is higher than several minutes (see "Results"). This apparent discrepancy is clearly alleviated if the arrests we detected reflected a transient binding state. Further, other studies made on noncovalent ligand-receptor interactions revealed such intermediate states (31–33). Therefore, our three-parameter model may be considered as the simplest

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1 We are grateful to Prof. Ewan Evans for pointing out the importance of the difference of the forces experienced by the sphere and the bond. The problem of determining the force on a single cell exposed to a laminar shear flow on a surface was first addressed by Schmid-Schoenbein et al. (30).
way of interpreting experimental data.²

In conclusion, we visualized the formation and dissociation of individual ligand-receptor bonds between molecules linked to macroscopic bodies.

**APPENDIX**

Theoretical Distribution of Arrest Durations

Following Equation 3, we considered the association between two complementary binding sites (A and B) as a two-step process with an intermediate bound state \((AB)_1\) and a stable bound state \((AB)_2\). We considered a spherical bead (coated with A-type molecules) bound to a surface coated with B-type molecules through a single bond. At time 0, the bond is in state \((AB)_1\). The system evolution is dependent on three kinetic constants \(k_0\), \(k_d\), and \(k_u\) as recalled below:

\[
\frac{k_u}{k_d} = \frac{A + B \equiv (AB)_1 \equiv (AB)_2}{k_u}\quad \text{(Eq. A1)}
\]

Let \(P_1\) and \(P_2\) be the respective probabilities for the bond to be in state \((AB)_1\) and \((AB)_2\). At time 0, \(P_1\) is equal to 1 and \(P_2\) is zero. At time \(t\), the probability that the bead is bound is \(P_1 + P_2\). Using Equation A1, we may write after simple algebraic manipulation:

\[
dP_2/dt = -(k_u + k_+ + k_-) P_1 + k_-(P_1 + P_2)
\]

\[
dP_1 + P_2 = -k_u P_1
\]

(Eq. A2)

This set of equations is readily solved by looking for a linear combination \(V\) of \(P_1\) and \((P_2 + P_1)\) such that Equation A2 yields:

\[
V = P_1 + a(P_1 + P_2)
\]

\[
dV/dt = \lambda V
\]

(Eq. A3)

where \(a\) and \(\lambda\) are constants (34). We find two solutions:

\[
\lambda_1 = -(k_u + k_+ + k_-) \pm \sqrt{(k_u + k_+ + k_-)^2 - 4k_u k_-} \frac{k}{2}
\]

\[
a_1 = k_u/\lambda_1
\]

(Eq. A4)

Using Equations A3 and A4, we obtain:

\[
(P_1 + P_2) = \frac{V}{a_1 - a_2}
\]

(Eq. A5)

Finally, Equations A4 and A5 yield:

\[
P_1 + P_2 = \frac{k_u}{\lambda_1 - \lambda_2}[(1 + a, \exp(\lambda, t)) - (1 + a, \exp(\lambda, t))]
\]

(Eq. A6)

**REFERENCES**

1. Williams, A. F. (1991) Nature 352, 473–474
2. Van der Mew, A., and Barclay, N. (1994) Trends Biochem. Sci. 19, 354–358
3. Springer, T. A. (1994) Cell 76, 301–314
4. Pierres, A., Tissot, O., Malissen, B., and Bongrand, P. (1994) J. Cell Biol. 125, 945–953
5. Bongrand, P., Claesson, P., and Curtis, A. (eds) (1994) Studying Cell Adhesion, Springer-Verlag, Heidelberg, Germany
6. Tha, S. F., Shuster, J., and Goldsmith, H. L. (1986) Biophys. J. 50, 1117–1126
7. Wattenburger, M., A., Graves, D. J., and Lauffenburger, D. A. (1990) Biophys. J. 57, 765–777
8. Czeis-Roberts, C., Lauffenburger, D. A., and Quinn, J. A. (1990) Biophys. J. 58, 841–856
9. Tempelman, L. A., and Hammer, D. A. (1994) Biophys. J. 66, 1231–1243
10. Evans, E., Berk, D., and Leung, A. (1991) Biophys. J. 59, 838–848
11. Florin, E. L., Moy, V. T., and Gaub, H. E. (1994) Science 264, 415–417
12. Dammer, U., Popescu, O., Wagner, P., Anselmetti, D., Gümmeroth, H.-J., and Misewic, G. (1995) Science 267, 1173–1175
13. Beli, G. I. (1978) Science 200, 618–627
14. Lauffenburger, D. A., and Lindenman, J. J. (1993) Receptors: Models for Binding, Trafficking and Signalling, Oxford University Press, Oxford
15. Hammer, D. A., and Lauffenburger, D. A. (1987) Biophys. J. 52, 475–487
16. Kuo, S. C., and Lauffenburger, D. A. (1993) Biophys. J. 65, 2191–2200
17. Kaplanski, G., Farnerier, C., Tissot, O., Pierres, A., Benoliel, A. M., Alessi, M. C., Kaplanski, S., and Bongrand, P. (1993) Biophys. J. 64, 1922–1933
18. Goldman, A. J., Cox, R. G., and Brenner, H. (1967) Chem. Eng. Sci. 22, 653–660
19. Michi, J., Pieczonka, M., M., Unkëess, J., C., and Silverstein, S. C. (1979) J. Exp. Med. 150, 607–621
20. Pierres, A., Benoliel, A. M., and Bongrand, P. (1994) in Cell Mechanics and Cellular Engineering (Mow, V., C., Guillak, F., Tran-Son-Tay, R., and Hochmuth, R., M., eds) pp. 145–159, Springer-Verlag New York Inc., New York
21. Tissot, O., Pierres, A., Foa, C., Delage, M., and, Bongrand, P. (1992) Biophys. J. 61, 204–215
22. Mege, J. L., Capo, C., Andre, P., Benoliel, A. M., and Bongrand, P. (1990) Biorethology 27, 433–444
23. Snedecor, G. W., and Cochran, W. G. (1980) Statistical Methods, Iowa State University Press, Ames, IA
24. Zaffran, Y., Lepidi, H., Benoliel, A. M., Capo, C., and Bongrand, P. (1993) Blood Cells 19, 115–13
25. Andre, P., Benoliel, A. M., Capo, C., Foa, C., Burfere, M., Boyer, C., Schmitt-Verhulst, A. M., and Bongrand, P. (1990) J. Cell Sci. 20, 618–627
26. Valentine, R. C., and Green, N. M. (1967) J. Mol. Biol. 27, 615–617
27. Capo, C., Guerreau, F., Benoliel, A. M., Bongrand, P., Ryter, A., and Bel, G. (1962) J. Cell Sci. 56, 21–48
28. Teds, D. F. J., Cazen, O., and Goldsmith, H. L. (1993) Biophys. J. 55, 1318–1334
29. Mow, V. T., Florin, E. L., and Gaub, H. E. (1994) Science 266, 257–259
30. Schmid-Schoenbein, G. W., Fung, Y. C., and Zweifach, B. W. (1975) Circ. Res. 36, 173–184
31. Fersht, A. (1985) Enzyme Structure and Mechanism, 2nd Ed., W. H. Freeman & Co., New York
32. Huber, W., Hurst, J., Schlatter, D., Barner, R., Hutseb, J., Kouns, W. C., and Steiner, B. (1995) Eur. J. Biochem. 227, 647–656
33. Beeon, C., and McConnell, H. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 8842–8845
34. Wong, C. W. (1991) Introduction to Mathematical Physics, pp. 102–105, Oxford University Press, New York
35. Alon, R., Hammer, D. A., and Springer, T. (1995) Nature 374, 539–542

² Note that this finding may be dependent on our model. While this paper was being submitted, an analysis of the interaction between blood neutrophils and P-selectin-coated surfaces was reported (35). The lifetime of P-selectin-ligand bonds was estimated at about 1 second in accordance with our estimate of about 2 seconds for neutrophil-E-selectin interaction (17), but no transient state was detected.