THERMODYNAMICS OF CELL ADHESION II

Freely Mobile Repellers

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ABSTRACT The equilibrium adhesion of a cell or vesicle to a substrate is analyzed in a theoretical model in which two types of mobile molecules in the cell membrane are of interest: receptors that can form bonds with fixed ligands in the substrate and repellers that repel the substrate. If the repulsion between the repeller molecule and substrate is greater than kT, there is substantial redistribution of the repellers from the contact area. Coexisting equilibrium states are observed having comparable free energies (a) with unstretched bonds and repeller redistribution and (b) with stretched bonds and partial redistribution.

INTRODUCTION

This work extends our previous treatment of equilibrium adhesion of a biological cell to a substrate mediated by specific chemical bonds. In earlier models of cell adhesion (1–3) the "receptor" molecules mediating the adhesion were treated as mobile in the cell membrane while the components of the glycocalyx that are responsible for nonspecific repulsion were assumed to be immobile. The novel aspect of this investigation is that those cell membrane molecules that mediate repulsion through coulomb and steric effects, the repellers, are treated as laterally mobile in the membrane. Their redistribution from the contact area between cell and substrate, although accompanied by a decrease in entropy, may result in a net decrease in free energy if the repulsive potential energy is great enough; in fact, one finds that equilibrium adhesion occurs in spite of a large repulsive potential energy and that the repeller concentration in the contact area is reduced.

GLOSSARY

Definitions		Dimensions
A	Contact area between cell and substrate	(ℓ^2)
A_T	Total surface area of a cell	(ℓ^2)
$\mathcal{A}$	Total surface area of substrate ($\mathcal{A} > A_T/2$)	(ℓ^2)
B	Number of receptor-ligand bonds	
C_0	Concentration in the standard state; let $C_0 = 1$ molecule/cm^2	(ℓ^{-2})
kT	Boltzmann constant × temperature, °Kelvin	$(m\ell^2t^{-2})$
R_T	Total (bonded and unbonded) receptor number on cell	
S	Distance between substrate and cell within A	(ℓ)
Z	Number of repellers in contact area	
Z_T	Total number of repellers on cell	
γ	Repulsion coefficient/repeller molecule	$(m\ell^3t^{-2})$
κ	Spring constant for receptor-ligand bond	(mt^{-2})
$\mu_B^o(\sigma)$	Free energy per bonded receptor-ligand molecule with the length σ, (standard state)	$(m\ell^2t^{-2})$
μ_L^o	Free energy per ligand molecule (standard state)	$(m\ell^2t^{-2})$
μ_R^o	Free energy per receptor molecule (standard state)	$(m\ell^2t^{-2})$
$\mu_z^o(\infty)$	Free energy per repeller molecule infinitely far from the substrate (standard state)	$(m\ell^2t^{-2})$
ρ_L	Ligand density on substrate	(ℓ^{-2})
σ	Unstressed receptor-ligand bond length	(ℓ)
τ	Screening length for the repulsion	(ℓ)

"Mean" Parameters

$A_T = 10^{-6}$ cm^2
$kT = 4 \times 10^{-14}$ erg
$K(\sigma) = 10^{-8}$ cm^2
$R_T = 10^5$
$Z_T = 10^6$
$\gamma = 8 \times 10^{-20}$ dynes · cm^2
$\kappa = 10^{-1}$ dynes/cm
$\rho_L = 10^{11}$ cm^{-2}
$\sigma = 2 \times 10^{-6}$ cm
$\tau = 10^{-5}$ cm

$RA = 1$
$RZ = 10^{-1}$
$EC = 10^3$
$XK = 10$
$GM = 1$
$TU = 5$

FREE ENERGY OF ADHESION

The adhesion is characterized by four variables: the area of contact, A; the number of bonds, B; the separation distance

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between the substrate and cell surface, S; and the number of repeller molecules in the contact area, Z. We determine these variables by minimizing the free energy. The receptor-ligand bonds are assumed to occur within the contact area A and to have an unstressed length (σ) and a potential energy with a length dependence of a Hooke's Law spring. Each repeller molecule is assumed to have a repulsive potential energy of interaction with the substrate of the form (2, 3) $S^{-1} \exp - \{S/\tau\}$; furthermore, repellers outside of the contact area are assumed to be so far from the substrate that their repulsive potential energy is negligible. The ligand molecules are assumed to be at fixed sites on the substrate.

At equilibrium there is a uniform distribution of each type of molecule both inside and outside the contact area[1]. For this model, the free energy[2] is

$$\begin{aligned} G(A,B,S,Z) &= (R_T - B)\left(\mu_R^\circ + kT \ln\left\{\frac{R_T - B}{A_T C_0}\right\}\right) \\ &+ (\rho_L A - B)\left(\mu_L^\circ + kT \ln\left\{\frac{\rho_L A - B}{A C_0}\right\}\right) \\ &+ \rho_L(\mathcal{A} - A)\left(\mu_L^\circ + kT \ln\left\{\frac{\rho_L}{C_0}\right\}\right) \\ &+ B\left(\mu_B^\circ(\sigma) + \frac{\kappa}{2}(S - \sigma)^2 + kT \ln\left\{\frac{B}{AC_0}\right\}\right) \\ &+ Z\left(\mu_Z^\circ(\infty) + \frac{\gamma}{S}\exp - \{S/\tau\} + kT \ln\left\{\frac{Z}{AC_0}\right\}\right) \\ &+ (Z_T - Z)\left(\mu_Z^\circ(\infty) + kT \ln\left\{\frac{Z_T - Z}{(A_T - A)C_0}\right\}\right). \quad (1) \end{aligned}$$

(The letters in Eq. 1 are defined in the Glossary.)

In order of appearance, the terms of Eq. 1 (inside square brackets) are the free energy due to unbound receptors, unbound ligands in the contact area, unbound ligands outside the contact area, receptor-ligand bonds, repellers in the contact area, and the repellers outside the contact area. The arguments of the logarithms are the activities of the respective substances; we assume an ideal dilute solution and take the activities to be the concentrations.

A reduction of the complexity of Eq. 1 results after the substitution of dimensionless variables for the four variables and the selection of six dimensionless parameters constructed from the fourteen parameters given in the Glossary.

The dimensionless variables chosen are

$$a = A/A_T,\ b = B/R_T,\quad s = (S - \sigma)/\sigma,\ z = Z/Z_T. \quad (2)$$

The dimensionless parameters are indicated by paired capital letters as follows:

$$\begin{aligned} &RA = R_T/\rho_L A_T,\quad RZ = R_T/Z_T,\quad EC = K(\sigma)R_T/A_T, \\ &XK = \kappa\sigma^2/kT,\quad GM = \gamma/\sigma kT,\quad TU = \tau/\sigma. \end{aligned} \quad (3)$$

It is also convenient to choose a dimensionless free energy,

$$g(a, b, s, z) = G(A, B, S, Z)/Z_T kT. \quad (4)$$

$K(\sigma)$, which appears in Eq. 3, is the equilibrium constant for the surface equilibrium between receptors, ligands and receptors bonded with ligands. The following relation holds between $K(\sigma)$ and the standard state chemical potentials[3]

$$K(\sigma) = \frac{[B]}{[L][R]} = C_0^{-1} \exp\{(\mu_L^\circ + \mu_R^\circ - \mu_B^\circ(\sigma) + kT)/kT\}. \quad (5)$$

It is found that when Eqs. 2–4 are substituted into Eq. 1, that $g(a_1, b_1, s_1, z_1) - g(a_2, b_2, s_2, z_2)$ is dependent only on the combined parameters given in Eq. 3. Thus all possible equilibrium states may be discovered as the six parameters in Eq. 3 are varied. If we define the "zero" of the free energy to be the value of $g(0, 0, \infty, 0)$ (infinite separation), then equilibrium states can be found from examination of $\Delta g(a, b, s, z)$

$$\begin{aligned} \Delta g(a, b, s, z) &= g(a, b, s, z) - g(0, 0, \infty, 0) \\ &= \ln\left\{\frac{1 - z}{1 - a}\right\} + z\left[GM \exp - \{(s + 1)/TU\}/(s + 1)\right. \\ &+ \ln\left\{\frac{z}{a}\right\} - \ln\left\{\frac{1 - z}{1 - a}\right\}\Big] \\ &+ \frac{RZ}{RA} \cdot a \ln\left\{1 - \frac{RAb}{a}\right\} + RZ \ln\{1 - b\} \\ &+ RZ \cdot b\Big[1 - \ln\{EC\} - \ln\{1 - b\} \\ &- \ln\left\{\frac{a}{RA \cdot b} - 1\right\} + \frac{XK}{2}s^2\Big]. \end{aligned} \quad (6)$$

The local minima of $g(a, b, s, z)$ correspond to equilibrium states. However, we impose another constraint on a: that it be less than or equal to a maximum that we take to be ½ (the cell cannot have more than half its surface area in contact with the substrate). Due to the form of $g(a, b, s, z)$, the remaining three variables cannot pass outside of their acceptable ranges, $0 \leqq b, z \leqq 1$ and $0 \leqq s \leqq \infty$.

Therefore, we simultaneously seek local minima of the unconstrained function of four variables $g(a, b, s, z)$ and of the constrained function of three variables $g(\frac{1}{2}, b, s, z)$. To

[1] A more detailed description of a similar but less complex model can be found in reference 1.

[2] We chose the Gibbs free energy, G, because we assume the process of cell adhesion occurs at constant temperature and pressure. For details of the thermodynamics of solutions, see Lewis and Randall (1961) (4).

[3] $K(\sigma)$ does not vary with C_0, the concentration of the standard state. $K(\sigma)$ is a function of temperature. A reasonable value of $K(\sigma)$ is 10^{-8} cm.^{2} Eq. 5 is derived from Eq. A2. (This arises from a bulk equilibrium constant of 10^7/Molar and a membrane thickness of 10^{-6} cm).

be an equilibrium state, the latter must also satisfy

$$\left.\frac{\partial g}{\partial a}\right|_{a=1/2} < 0.$$

The methods used to find the minima are in the Appendix.

RESULTS

The dependence of the equilibrium adhesion upon the dimensionless parameters is intuitive. In spite of the "algebraic complexity" of the problem, only a few types of functional dependence on the parameters are observed.

To begin, we vary only one parameter at a time, while the others remain at their "mean" values given in the Glossary. One axis on each of the plots is *RA*. Since *RA* is the average receptor density divided by the average ligand density, decreasing *RA* can be thought of as increasing the ligand density (see Eq. 3).

Figs. 1 *a–c* show the four variables and also the free energy $g(a, b, s, z)$ as functions of *RA*, with *EC* increasing from Fig. 1 *a* to *c*. The four variables reach the same

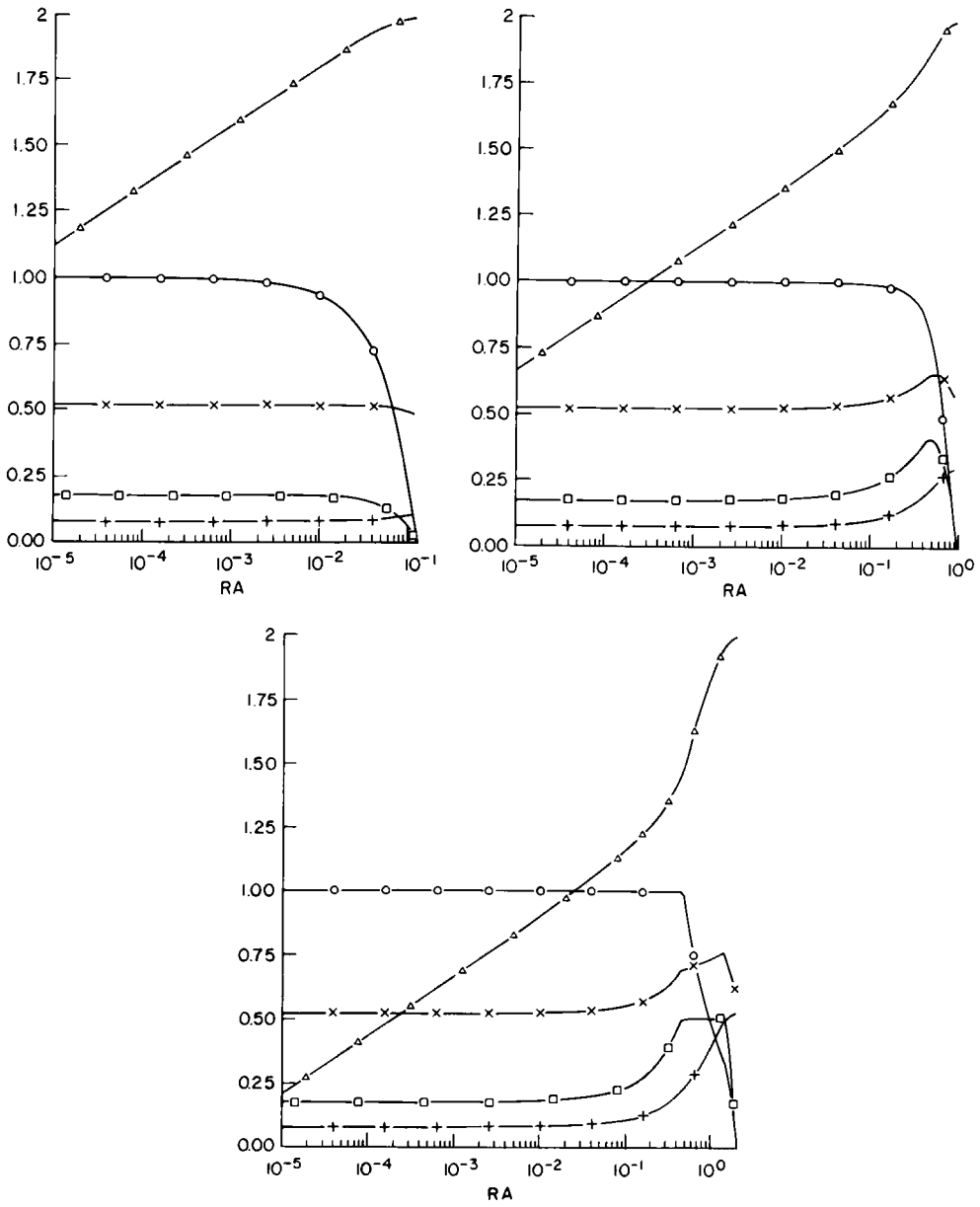

FIGURE 1 Fixed parameters are as follows: $RZ = 0.1$, $XK = 10.0$, $GM = 1.0$, $TU = 5.0$. In *a*, $EC = 10^0$; in *b*, $EC = 10^2$; in *c*, $EC = 10^4$. *x* axis: $\log_{10} RA$; $-5.0 \leq \log_{10} RA \leq 1.0$. *y* axis: $0.0 \leq y \leq 2.0$. The five curves in each figure are as follows: the curve labeled with squares is *a*, the fraction of the surface in the contact area, $(0 \leq a \leq \frac{1}{2})$; the curve labeled with triangles is the free energy, $\Delta g(a, b, x, z) + 2.0$; the curve labeled with circles is *b*, the fraction of receptors that are bonded; the curve labeled with plus signs is *s*, the separation from the substrate in units of the unstressed bond length minus one bond length; and the curve labeled by *x*s is z/a, the fraction of the initial repeller concentration remaining in the contact area.

asymptotes for small $RA(\simeq 10^{-6})$ independent of the value of EC. The behavior of this model is qualitatively similar to that described for the simpler model (1). For the "mean" parameter values (see Glossary) the threshold for adhesion, defined as the minimum ligand density at which the contact area first becomes nonzero, is 6×10^{11} mol/cm^2 for both models.

Instead of plotting the repeller number z, we plot z/a which is the fraction of the initial concentration in the contact area at equilibrium. Beyond the threshold, when a is monotonic, so is z/a; and when a goes through a maximum, so does z/a. This somewhat surprising behavior occurs as a result of a trade-off between an increasing "entropic penalty" for removing repellers from the contact area as a increases, and the increasing "repulsive penalty" for keeping repellers in the contact area as b grows and s diminishes.

The free energy, g, decreases monotonically. The factor

$$-RZ \cdot b \ln\left\{\frac{a}{RA \cdot b} - 1\right\},$$

in g (Eq. 6) causes the plot of g vs. log RA to be linear as RA goes to zero; this term reflects the number of ways in which bonds can be distributed over the ligands. From Figs. 1 *a–c* and the parameter values in the Glossary, one finds that $RA = 10^{-2}$, $G(A, B, S, Z)$ is on the order of 10^{-2} ergs/cm^2. This value can be substantially increased by increasing R_T, the receptor number, or by increasing ρ_L, the ligand density.

If one plots the contact area vs. RA and EC, one gets the three-dimensional plot, Fig. 2. (The area, a, plotted in Figs. 1 *a–c* can be found by taking vertical sections of Fig. 2). One can see the decreasing dependence of the threshold on EC in the curved baseline. Maximal contact area appears as a relatively narrow plateau.

The contact area can be plotted as a function of RA and RZ. With $RZ \lesssim 0.5$, the cell will not "round up" even if RA goes to zero, because the repulsion is small. One observes the threshold always occurs with $RA/RZ \cong 10$ and for smaller values there is a relatively rapid transition to maximal contact area.

Similar behavior is seen in a plot of the contact area a, as a function of RA and TU. No "rounding up" is seen for $TU \lesssim 0.85$; the repulsion is effectively screened. The asymptotic (large TU) solution is observed practically if $TU \geq 10$.

If Fig. 3 the contact area is shown as a function of RA and GM, the later parameter giving the magnitude of the repulsion. There is a dramatic change occurring at $GM = 2$. For $GM < 2$, the threshold varies linearly with GM. For $GM > 2$, the threshold is independent of GM. The explanation is that redistribution of the repeller moelcules from the contact area begins when the repulsion per repeller is on the order of kT, which corresponds to $GM = 2$ (see Eq. 3).

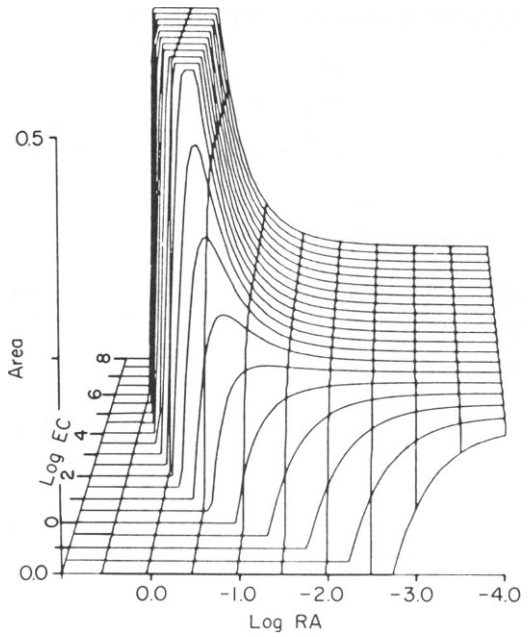

FIGURE 2 Fixed parameters are as follows: $RZ = 0.1$; $XK = 10.0$; $GM = 1.0$; $TU = 5.0$. x axis = $\log_{10} RA$; $1.0 \geq \log_{10} RA \geq -4.0$. y axis = $\log_{10} EC$; $-2.0 \leq \log_{10} EC \leq 8.0$. z axis = a; $0 \leq a \leq 0.5$. The contact area, a, is plotted as a function of EC and RA.

With $GM \geq 10$, only a negligible number of repeller molecules will remain in the contact area.

The contact area can be plotted as a function of GM and RZ. For $GM < 2.0$ and $RZ < 0.1$ the threshold is reached when $\log_{10}(RZ) - \log_{10}(GM) = -1$. However, the threshold abruptly becomes independent of GM for $GM > 2$.

Changes in the "bond stiffness" parameter XK result nearly exclusively in a linear change of the separation distance s.

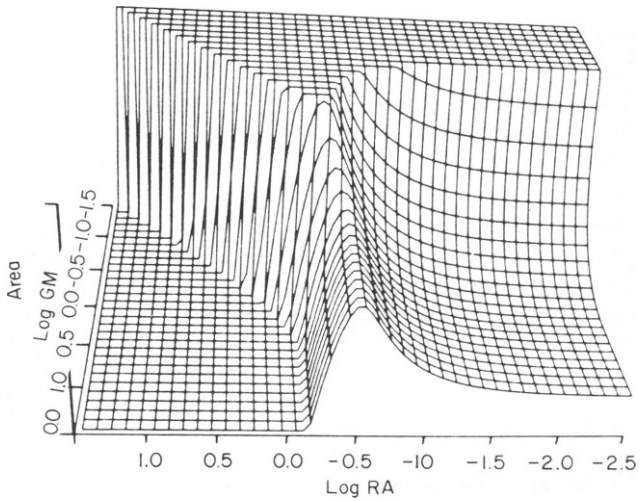

FIGURE 3 Fixed parameters are as follows: $RZ = 0.1$; $EC = 10^3$; $XK = 10.0$; $TU = 10^3$. x axis = $\log_{10} RA$; $-2.5 \leq \log_{10} RA \leq 1.5$. y axis = $\log_{10} GM$; $1.5 \geq \log_{10} GM \geq -1.5$. z axis = a; $0 \leq a \leq 0.5$. The contact area, a, is plotted as a function of RA and GM.

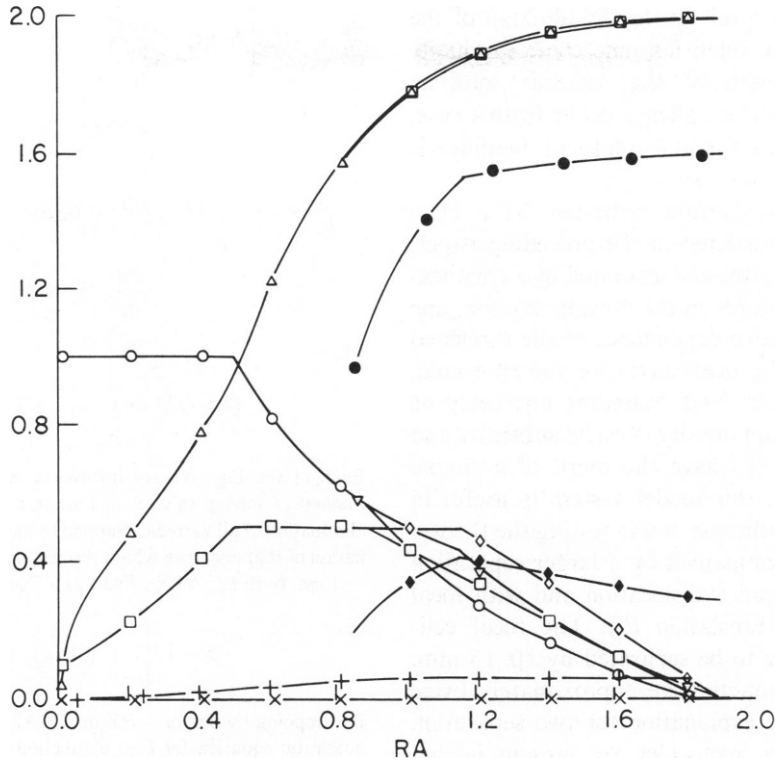

FIGURE 4 Fixed parameters are as follows: $EC = 10^8$; $GM = 12.0$; $XK = 3.75$; $RZ = 0.1$; $TU = 2.0$. x axis = RA; $0 < RA \leq 2.0$. y axis = RA; $0 \leq y \leq 2.0$. There are 10 curves plotted, five for each of the equilibrium states; coexistence is from $\sim 0.84 \leq RA \leq 1.85$. The close state has the same labels for the curves as in Figs. 1 *a–c*: (*a*) squares; (*b*) circles; ($\Delta g + 2$) triangles pointing up; (*s*), pulses; (z/a), *xs*. For the distant state the curves are labeled as follows: (*a*) open diamonds; (*b*) triangles pointing down, ($\Delta g + 2$), squares; (*s*), solid circles; (z/a), solid diamonds. The thresholds and free energies for the two states are slightly different; the free energy for the distant state is greater with $0.84 \leq RA \leq 0.95$ and smaller with $0.95 \leq R \leq 1.85$.

Dual Equilibrium States

A novel situation occurs because of the ability of the repellers to redistribute. For a particular combination of the parameters: large GM, $O(10)$; medium TU, $O(2)$; and small XK, $O(1)$, one observes two equilibrium states with the same parameters over an interval on the RA axis. Their free energies on contact areas and bond numbers can be comparable, but they differ substantially in their values of s and z. In addition to the commonly observed closely bonded state with small s and z, there is a distantly bonded equilibrium state with large s and moderately large z. With the separation, s, on the order of TU, the repulsion is decreased; and at the same time, because XK is small, the penalty for stretching the bonds is not large. The parameter GM must be large to ensure that a local minimum of the free energy occurs for large s. Thus, dual minima occur in g even though the repulsive energy decreases monotonically with s,

$$\frac{GM}{s+1}\exp-\left\{\frac{s+1}{TU}\right\}.$$

Fig. 4 shows an example of two coexisting equilibrium states. The coexistence is over a range of RA from ~0.84 to 1.85. (The bond stiffness parameter, XK, at 3.75, is approximately one-third of the "mean" value [Glossary]; GM, the repulsion parameter, at 25 is large. TU is 2.0, close to its "mean" value.) The distant state has a lower threshold and a lower free energy[4] over most of the range of coexistence. Both the distant state and the close state reach $a = \frac{1}{2}$, and both are at $a = \frac{1}{2}$ for $RA \doteq 0.86$. The bond numbers approach a similar value as RA goes to 0.841 from higher values. Both separation distances decrease as RA decreases, but it is more apparent for the distant state. The ratio z/a is ~0 for the close state, but it is on the order of 0.25 and goes through a maximum for the distant state. No other types of dual equilibrium states are observed.

DISCUSSION

The molecular details of cell-substrate adhesion remain to be elucidated. It is thought that many distinct classes of molecules are involved in cell-cell adhesion and recognition (5, 6); our model demonstrates the complicated adhesive behavior one expects with only two types of cellular molecules: receptors and repellers.

One expects redistribution of repeller molecules if the repulsion per molecule is on the order of kT. With observed redistribution, further increases in the repulsion per mole-

[4]The actual free energy difference between the two states would be of the order of $10^4\,kT$ if the dimensionless free energies differed by 1% and if Z_T were 10^6 (see Eq. 4).

cule are not expected to result in the de-adhesion of the cell. To increase coulomb repulsion, one could gradually increase the Debye length of the aqueous solution. Although de-adhesion will not always occur in this case, the free energy, and hence the strength of binding, is diminished.

Thresholds for cell-substrate adhesion have been observed (7) and were predicted in the preceding paper; they are characteristic of adhesion mediated by a chemical equilibrium. From the figures in the Results section, one can construct the qualitative dependence of the threshold upon the parameters. The predictions for the threshold, spreading, maximal contact, and "rounding up" could be tested by varying the ligand density. Vesicle-substrate and vesicle-vesicle adhesions (8) have the merit of a simple geometry and chemistry; this model system is useful in modeling cell-substrate adhesion and in testing the theory.

Cell locomotion is accompanied by a larger separation from the substrate in rapid translocation and with focal close contacts in slow translation (9). The focal cell-substrate contacts appear to be separated by 10–15 mm, and the more distant contacts have approximately twice that value. The probable explanation for two separation distances is that different molecules are present in and around the two distinct types of adhesion. However, this model proposes that one molecular species can simultaneously mediate two types of adhesion. Although the distant and close adhesions have comparable free energies, the distant state in general exists only for a narrow domain of the parameters. This lability may be important for cell translocation where any bonds formed must soon be broken. In an experiment, one might observe a transition from a distant to close adhesive separation as the ligand density is increased and that the transition may either be abrupt or may involve coexistent close and distant adhesions. It may also be true that a kinetic barrier will prevent the cell from achieving a close adhesion from a distant adhesion, and thus the cells will be observed to adhere only over a limited range of ligand density.

Metabolic inhibitors and a temperature of 4°C can reduce the strength of cell-substrate adhesion by a factor of 10 (6, 10). One could argue that cytoskeletal rearrangements, receptor recruitment, and chemical modification of the substrate each may result in the strengthening of adhesion. Each could be incorporated in the model, at least approximately, by changing the parameters and the constraint on the contact area. Moreover, lipid vesicles containing only pure Neural Cell Adhesion Molecules aggregate (11). Therefore, this model should be useful in the clarification of important features of cell-substrate adhesion.

APPENDIX

To have an unconstrained minimum, the four first partial derivatives of $g(a, b, s, z)$ must vanish; this yields the following four equations:

$$\frac{\partial g}{\partial a} = \frac{RZ}{RA} \cdot \ln\left\{1 - \frac{RA \cdot b}{a}\right\} + \frac{1 - z}{1 - a} - \frac{z}{a} = 0 \quad \text{(A1)}$$

$$\frac{\partial g}{\partial b} = -RZ\left[\ln EC - \frac{XKs^2}{2} + \ln(1 - b) + \ln\left\{\frac{a}{RA \cdot b} - 1\right\}\right] = 0 \quad \text{(A2)}$$

$$\frac{\partial g}{\partial s} = b \cdot s \cdot XK \cdot RZ - zQ\left[(s + 1)^{-1} + TU^{-1}\right] = 0 \quad \text{(A3)}$$

$$\frac{\partial g}{\partial z} = Q + \ln\frac{z}{a} - \ln\left\{\frac{1 - z}{1 - a}\right\} = 0, \quad \text{(A4)}$$

where

$$Q = GM \exp -\{(s + 1)/TU\}/(s + 1). \quad \text{(A5)}$$

Each of the Eqs. A1–A4 involves a different triple of variables. The method of solving this set of four transcendental equations consists of elimination of all variables but one by substitution, and then the determination of that one upon which the remaining three depend.

First, from Eq. A4, we find z as a function of a and s,

$$z = 1/\left\{1 + [(1/a) - 1] \exp\{Q\}\right\}. \quad \text{(A6)}$$

By dropping the factor $-RZ$ in Eq. A2 and exponentiating, one finds a quadratic equation for b as a function of a and s. We select one root because it alone satisfies

$$1 \geqq b \text{ and } 1 \geqq RAb/a \geqq 0 \quad \text{(A7)}$$

necessary for equilibrium (see Eq. A1). The root is

$$b = (2RA)^{-1}\{R + a - [(R + a)^2 - 4aRA]^{1/2}\}, \quad \text{(A8)}$$

with

$$R = RA(1 + \exp\{XKs^2/2\}/EC). \quad \text{(A9)}$$

Substituting Eq. A6 into Eq. A3 gives another expression for b as a function of a and s;

$$b = P/\left\{1 + [(1/a) - 1] \exp\{Q\}\right\}, \quad \text{(A10)}$$

with

$$P = Q[(s + 1)^{-1} + TU^{-1}]/(s \cdot XK \cdot RZ). \quad \text{(A11)}$$

Noting that the functions P, Q, and R (Eqs. A11, A5, and A9) depend only on the variable s, we now solve for a as a function of s by eliminating b from Eqs. A8 and A10.

This involves a cubic equation in a, with three real roots, one of which is equal to zero. The root corresponding to Eq. A8 is

$$a = \Big\{e^Q[(e^Q - 1)/P + \tfrac{1}{2}] - \tfrac{1}{2}[R(e^Q - 1) + RAP] - \{\tfrac{1}{4}[e^Q(1 - RA) + RA(1 - P)]^2 + \tfrac{1}{2}(R - RA)(e^Q - 1)[e^Q + RA(P + e^Q - 1) + \tfrac{1}{2}(R - RA)(e^Q - 1)]\}^{1/2}\Big\} \div \Big\{(e^Q - 1)[1 + (e^Q - 1)/P]\Big\}. \quad \text{(A12)}$$

Substituting Eq. A12 into Eqs. A6 and A8 gives b and z as functions of s, and substituting the resulting expressions into Eq. A1 gives one transcendental equation for s whose roots are the stationary points of $g(a, b, s, z)$. For a fixed set of parameters, the roots of Eq. A1, considered as a function of s, were found numerically[5] ($0 \leq s \leq 10$).

For a simultaneous solution of Eqs. A1–A4 to be a local minimum, it is necessary and sufficient that the square matrix H of second partial derivatives have all four characteristic values greater than zero (14). The matrix, H, can be constructed easily from the first partial derivatives given in Eqs. A1–A4; it is a symmetric matrix, and therefore all of its characteristic values are real. The characteristic polynomial of the matrix has the form (15)

$$X^4 - \alpha X^3 + \beta X^2 - \gamma X + \delta = 0.$$

The four roots of this equation (the characteristic values of H) will be positive if and only if

$$\alpha > 0, \beta > 0, \gamma > 0, \text{and } \delta > 0.$$

We use this criterion to select from the stationary points satisfying Eqs. A1–A4 those that correspond to minima (and equilibrium states[6]).

Received for publication 11 February 1985.

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[5]The numerical subroutine used to find the roots of Eq. A1 is called Zeroin (12, 13). The Fortran program used in the solution of this problem is available upon request; computation and graphics were done on a CDC 7600.

[6]Local minima of the constrained function $g(\frac{1}{2}, b, s, z)$ were found with an identical method; one simultaneously solves Eqs. A2, A3, and A4 with $a = \frac{1}{2}$; this gives the same equations for z and b (Eqs. A6, A8; $a = \frac{1}{2}$), and one then substitutes into Eq. A3 to get a transcendental equation in s whose roots are found numerically. In this case, H is a 3×3 matrix.