Competition between solution and cell surface receptors for ligand

Dissociation of hapten bound to surface antibody in the presence of solution antibody

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ABSTRACT We present a joint theoretical and experimental study on the effects of competition for ligand between receptors in solution and receptors on cell surfaces. We focus on the following experiment: After ligand and cell surface receptors equilibrate, solution receptors are introduced, and the dissociation of surface bound ligand is monitored. We derive theoretical expressions for the dissociation rate and compare with experiment. In a standard dissociation experiment (no solution receptors present) dissociation may be slowed by rebinding, i.e., at high receptor densities a ligand that dissociates from one receptor may rebind to other receptors before separating from the cell. Our theory predicts that rebinding will be prevented when $S \gg N^2\kappa_{on}/(16\pi^2 D a^4)$, where S is the free receptor site concentration in solution, N the number of free surface receptor sites per cell, κ_{on} the forward rate constant for ligand-receptor binding in solution, D the diffusion coefficient of the ligand, and a the cell radius. The predicted concentration of solution receptors needed to prevent rebinding is proportional to the square of the cell surface receptor density. The experimental system used in these studies consists of a monovalent ligand, 2,4-dinitrophenyl (DNP)-aminocaproyl-L-tyrosine (DCT), that reversibly binds to a monoclonal anti-DNP immunoglobulin E (IgE). This IgE is both a solution receptor and, when anchored to its high affinity Fc_ϵ receptor on rat basophilic leukemia (RBL) cells, a surface receptor. For RBL cells with 6×10^5 binding sites per cell, our theory predicts that to prevent DCT rebinding to cell surface IgE during dissociation requires $S \gg 2{,}400$ nM. We show that for $S = 200$–$1{,}700$ nM, the dissociation rate of DCT from surface IgE is substantially slower than from solution IgE where no rebinding occurs. Other predictions are also tested and shown to be consistent with experiment.

INTRODUCTION

The binding of a ligand to a cell surface receptor is the first step in a cascade of events that leads to the generation of a transmembrane signal. In many cases such binding occurs in the presence of receptors in solution that compete with cell surface receptors for the ligand. For example, *B* cells are stimulated by antigen binding to their surface immunoglobulin, and this occurs *in vivo* in the presence of secreted antibodies of the same specificity. In many allergic reactions of the immediate hypersensitive type, solution antibody (usually IgG) competes for the same antigenic sites with IgE that is bound via high affinity Fc_ϵ receptors to the surface of mast cells and basophils (Lichtenstein et al., 1968; Ottesen et al., 1981; Golden et al., 1982). Retroviruses, including human immunodeficiency virus (HIV), shed envelope proteins (Gelderbom et al., 1987) which may compete with their surface counterparts for antibody that bind these proteins. The envelope glycoprotein gp120 of HIV-1 binds to its cellular receptor, CD4, on T cells, macrophage, and other cell types. Soluble forms of CD4 have been produced and, *in vitro*, have been used to compete with cell surface CD4 and prevent the attachment of HIV-1 to human T cells (Smith et al., 1987; Fisher et al., 1988; Hussey et al., 1988; Deen et al., 1988, Traunecker et al., 1988). Patients with HTLV-I-positive adult T cell leukemia have greatly elevated levels of a low affinity receptor (the Tac antigen) that binds interleukin 2 (IL 2). The HTLV-I-positive T cell line HUT 102B2 releases a soluble form of this receptor which binds IL 2 normally and therefore can compete with cell surface IL 2 receptors for this ligand (Rubin et al., 1986).

We seek to study the effects on a ligand-cell surface receptor system of adding solution receptors that bind the same ligand. We start with an equilibrium solution of ligand and cell surface receptors. We then add solution receptors and follow the kinetics of dissociation of surface bound ligand. At equilibrium, bound ligands are constantly dissociating from cell surface receptors and moving into solution while, at the same rate, free ligands are moving from solution and binding to cell surface receptors. When solution receptors are added, some free ligand that in the absence of these receptors would have returned to bind to cell surface receptors, binds instead to receptors

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in solution. Still, other ligands may rebind to surface receptors many times before binding to a receptor in solution. With time the system will move to a new equilibrium. When the surface receptor density is sufficiently high rebinding can greatly slow the dissociation of bound ligand from the cell surface (Erickson et al., 1987). One obvious question which we attempt to answer here is: What concentration of solution receptors is required to block rebinding? A more general question that we address is: How does the kinetics of dissociation depend on the concentrations of solution and cell surface receptors?

First we present a theoretical description of the dissociation, which is based on previous theoretical studies that have been used successfully to describe the effects of cell surface receptor density on the binding and dissociation of ligands from cell surfaces when no solution receptors are present (Berg and Purcell, 1977; Berg 1978; DeLisi 1980, 1981; DeLisi and Wiegel, 1981; Shoup and Szabo, 1982). We then study experimentally the dissociation of a monovalent ligand; 2,4-dinitrophenyl (DNP)-aminocaproyl-L-tyrosine (DCT), that reversibly binds to a bivalent receptor, a monoclonal anti-DNP immunoglobulin E (anti-DNP IgE) (Liu et al., 1980). This anti-DNP IgE is both a solution receptor and, when anchored to its high affinity Fc_ϵ receptor on rat basophilic leukemia (RBL) cells, a cell surface receptor. Previously we used this system to study the effects of cell surface receptor density on the rate of ligand binding to cell surface receptors, in the absence of solution receptors (Erickson et al., 1987). This experimental system is well suited for the present studies because we can control both the solution and cell surface receptor concentrations, varying the latter concentration between 0 and ~6×10^5 sites/cell.

MATERIALS AND METHODS

Sensitization of cells with labeled IgE and fluorescence measurement of bound ligand

Procedures for maintaining the RBL (subline 2H3) cells, for preparation of labeled IgE, and for binding IgE to its receptors on the RBL cells have been described previously (Erickson et al., 1986; Erickson et al., 1987). Cell receptors for IgE were saturated with varying ratios of ^{125}I- and fluorescein-5-isothiocyanate (FITC) labeled mouse IgE (specific for DNP) and unlabeled rat IgE (which does not bind DNP) such that the cells had varying surface densities of the labeled anti-DNP IgE. Fluorescence microscopy of the labeled cells showed a characteristic smooth green ring stain around the equator of the spherical cells and essentially no intracellular fluorescence. The cell concentration was determined by counting with a microscope and hemocytometer. The specific activity of ^{125}I-IgE, determined as previously described (Erickson et al., 1986), allowed us to calculate the number of receptors for DNP ligands per cell.

The proportionate decrease in FITC-IgE fluorescence that accompanies Fab site occupation by DNP-ligands has been described in detail previously (Erickson et al., 1986). All fluorescence recordings were made as previously described on a spectrofluorimeter (model 8000; SLM Instruments, Inc., Urbana, IL) in ratio mode with FITC excitation and emission wavelength 490 and 526 nm, respectively. A CS-71 longpass filter (No. 3384: Corning Glass Works, Corning Science Products, Corning NY) was used in the emission port to reduce scattered light contributions. The spectrofluorimeter was interfaced with an AST Premium 286 computer for direct data acquisition.

For each dissociation experiment, 2 ml of a labeled cell suspension or labeled IgE in solution were placed in a 10 × 10 × 48 mm acrylic cuvette and stirred continuously. Sufficient DCT was added via microcapillary tubes to saturate the Fab binding sites, thereby quenching the FITC fluorescence (maximally by ~20%). After the fluorescence decrease was complete, varying amounts of unlabeled anti-DNP IgE were added to the suspension with a calibrated Finnpipette (Labsystems OY, Pulttitie 9, 00810, Helsinki 81, Finland). Mixing times were <2 s. The rate of dissociation of the DCT from the Fab sites was monitored as the consequent increase of fluorescence with time. Data points were recorded at 2.4 s intervals. All experiments were done at 15°C.

Parameter estimation

Our parameter estimates were obtained using the International Mathematics and Statistics Library (IMSL) routine ZXSSQ, which is based on a finite difference, Levenberg-Marquardt algorithm for solving nonlinear least squares problems.

For a monovalent ligand binding to a receptor site in solution we determined the equilibrium binding constant K as follows: The concentration of bound ligand $L^* = KR_TL/(1 + KL)$, where $L = L_T - L^*$ is the free ligand concentration, L_T the total ligand concentration and R_T the total receptor site (Fab sites) concentration. Solving for L^* we have that

$$L^* = \frac{1 + KL_T + KR_T - [(1 + KL_T + KR_T)^2 - 4K^2R_TL_T]^{1/2}}{2K}.$$

L^* is related to the relative fluorescence F, since binding leads to quenching of the fluorescence. In particular, $L^*/L_T = 1 - (F - F_1)/(F_2 - F_1)$, where F_1 is the relative fluorescence when all receptor sites are free and F_2 is the relative fluorescence when all receptor sites are filled. To determine K we fit the above equation to a fluorescence titration curve taking as free parameters K, F_1, and F_2.

RESULTS

Theoretical

When receptors are clustered in space, as when they are confined to cell surfaces, their rates of binding can be quite different than when they are uniformly distributed in solution. When the density of free cell surface receptors is sufficiently high, a ligand will rapidly bind to a receptor once it is near the cell surface, and the rate at which the ligand binds to a receptor will be limited by the rate at which the ligand diffuses to the cell surface. For such high cell surface receptor densities, theory predicts (Berg and Purcell, 1977; Berg, 1978; DeLisi, 1980, 1981; DeLisi and Wiegel, 1981; Brunn, 1981; Shoup and Szabo, 1982) that both the forward and reverse rate constants for ligand-receptor binding will be reduced. The forward rate constant will be reduced because nearby receptors compete for the same ligand. The reverse rate constant will be reduced because a ligand that dissociates from one receptor is likely to bind to another receptor, rather than move away from the cell. If k_f and k_r are the forward and reverse rate constants for the binding of a ligand to a monovalent cell surface receptor, then theory predicts that for a cell with N free binding sites on its surface (Shoup and Szabo, 1982)

$$k_f = \frac{\kappa_{on}}{1 + N\kappa_{on}/k_+} \qquad (1)$$

$$k_r = \frac{\kappa_{off}}{1 + N\kappa_{on}/k_+}, \qquad (2)$$

where κ_{on} and κ_{off} are the reaction limited forward and reverse rate constants for the binding of a ligand to an isolated cell surface receptor (i.e., to a cell surface receptor when the average separation distance between receptors is very large and the time to diffuse to the cell is negligible) and k_+ is the diffusion limited forward rate constant for binding of a ligand to the cell. The rate constant k_+ characterizes the transport of ligands from solution to the vicinity of the cell surface, while the rate constant κ_{on} characterizes the binding of ligands to single receptors.

If the cell is modeled as a sphere of radius a then k_+ is just the Smoluchowski diffusion-controlled rate constant, i.e.,

$$k_+ = 4\pi D\mathrm{a}, \qquad (3)$$

where D is the diffusion coefficient of the ligand. With this value of k_+ we see from Eq. 2 that the quantity $1/(1 + N\kappa_{on}/4\pi D\mathrm{a})$ is the fraction of molecular dissociations that lead to true separations of the ligand from the cell (Berg, 1978). When $N\kappa_{on}/(4\pi D\mathrm{a}) \gg 1$ this fraction will be small and dissociation from the cell surface will be much slower than dissociation from an isolated receptor.

Eqs. 1–3 were obtained assuming there were no receptors in solution. When receptors are present in solution Eqs. 1 and 2 are still valid, but k_+ is no longer given by Eq. 3. As we show in the Appendix, if we let S be the concentration of free receptor binding sites in solution, and assume that S is sufficiently large that we can treat the binding of a ligand to a solution receptor as irreversible, then

$$k_+ = 4\pi D\mathrm{a}[1 + (\kappa_{on}S\mathrm{a}^2/D)^{1/2}]. \qquad (4)$$

Note that k_+ increases with increasing free receptor concentration. This must be so if increasing S is to prevent rebinding. In particular, Eqs. 1 and 2 show that as k_+ increases, the effects of diffusion decrease until, when $N\kappa_{on}/k_+ \ll 1$, they become negligible. The dependence of k_+ on S is discussed in more detail in the Appendix.

In Eq. 4 κ_{on} is the forward rate constant for the binding of a ligand to a receptor in solution. We have assumed that κ_{on} is the same for a receptor in solution and on a cell surface. (If this is not so the κ_{on} in Eqs. 1 and 2 will differ from the κ_{on} in Eq. 4. For the experimental system we use here, we have directly determined κ_{on} for IgE both in solution and on RBL cells, and shown them to be the same (Erickson et al., 1987). Thus, the ligand binding properties of our receptor, a monoclonal anti-DNP IgE (Liu et al., 1980), are unchanged when the IgE is bound to its Fc_ϵ receptor on RBL cells.

From Eqs. 1, 2, and 4 we predict that the rate constants for a ligand interacting with a cell surface receptor in the presence of receptors in solution with identical binding properties are:

$$k_f = \frac{\kappa_{on}}{1 + \dfrac{N\kappa_{on}}{4\pi D\mathrm{a}[1 + (\kappa_{on}S\mathrm{a}^2/D)^{1/2}]}} \qquad (5)$$

$$k_r = \frac{\kappa_{off}}{1 + \dfrac{N\kappa_{on}}{4\pi D\mathrm{a}[1 + (\kappa_{on}S\mathrm{a}^2/D)^{1/2}]}} \qquad (6)$$

We can now determine when the presence of receptors in solution will block the rebinding of dissociated ligand to the cell surface. From Eq. 2 we see rebinding will be negligible and $k_r \approx \kappa_{off}$ when $N\kappa_{on}/k_+ \ll 1$. It follows from Eqs. 4 and 6 that this inequality will always be satisfied if $N\kappa_{on}/(4\pi D\mathrm{a}(\kappa_{on}S\mathrm{a}^2/D)^{1/2}) \ll 1$. This inequality can be rewritten in the following form:

$$S \gg \frac{\kappa_{on}}{D}\left[\frac{N}{4\pi\mathrm{a}^2}\right]^2. \qquad (7a)$$

When this inequality is satisfied the concentration of free receptor sites in solution, S, is sufficiently high to prevent rebinding. The most interesting feature of this result is that the solution concentration required to prevent rebinding increases with the square of the free surface receptor concentration.

It is instructive to rewrite Eq. 7a in the following form

$$S \gg \frac{N}{4\pi\mathrm{a}^2\lambda}. \qquad (7b)$$

The quantity $\lambda = \sqrt{D/(\kappa_{on}S)}$ is the screening length and, as discussed in the Appendix, is the distance from the cell surface over which most of the variation in the ligand concentration occurs. This inequality indicates that when $a \gg \lambda$, the cell surface receptors have an effective three dimensional concentration that can be obtained by uniformly distributing them in a shell about the cell whose height equals the screening length. (This effective three dimensional concentration is only appropriate for the competition experiment we are considering and is not a general prescription for obtaining effective three dimensional concentrations for two dimensional surface concentrations.)

With these expressions for ligand-cell surface receptor rate constants in the presence of solution receptors, Eqs. 5 and 6, we can write down the chemical rate equations that describe the kinetics of binding and dissociation for this system. Calling N and N_T the free and total cell surface receptor site concentration, S and S_T the free and total solution receptor site concentration, and L and L_T the free and total ligand concentration, we have that

$$\frac{dN}{dt} = -k_f L N + k_r (N_T - N) \quad (8)$$

$$\frac{dS}{dt} = -\kappa_{on} L S + k_{off}(S_T - S) \quad (9)$$

$$L_T = L + (S_T - S) + (N_T - N)\rho/6.02 \times 10^{11}, \quad (10)$$

where ρ is the cell concentration in cells/ml. The units of N_T and N are sites/cell while all other concentrations are in nanomolars. Below we will use the following notation: a bar over N indicates a bulk cell surface receptor site concentration in nanomolars, e.g., $\overline{N} = N\rho/6.02 \times 10^{11}$. Note that k_f and k_r are not constant in Eq. 8, but functions of S and N, given by Eqs. 5 and 6.

We derived Eqs. 5 and 6 in the steady state. We now use these steady-state rate constants in Eqs. 8 and 9, which describe the time evolution of the system. We expect that as long as there are not rapid changes in the concentrations these steady state expressions will give a good description of the dissociation of ligand from the cell surface. As yet we do not have a rigorous condition that specifies when such a formulation is valid. However, for the parameter values used here we have found good agreement between the predictions obtained by numerically solving Eqs. 8–10 and the numerical solutions of the partial differential reaction diffusion problem with spherical symmetry (Torney and Goldstein, unpublished results).

For a dissociation experiment ligand and cell surface receptor are first in equilibrium and no solution receptor is present, and then the experiment is initiated at $t = 0$ when solution receptors at concentration S_T are added. The initial ($t = 0$) concentrations are

$$S_0 = S_T$$

$$\overline{N}_0 = \frac{-(KL_T - K\overline{N}_T + 1) + [(KL_T - K\overline{N}_T + 1)^2 + 4K\overline{N}_T]^{1/2}}{2K} \quad (11)$$

$$L_0 = L_T - \overline{N}_T + \overline{N}_0,$$

where $K = \kappa_{on}/\kappa_{off} = k_f/k_r$. The expressions for $\overline{N}_0$ and L_0 were obtained by solving Eqs. 8 and 10 at equilibrium ($dN/dt = 0$) in the absence of solution receptors ($S_T = S = 0$).

In general, for a given set of initial concentrations, we must solve Eqs. 8–10 by numerical integration. However, when a high concentration of solution receptors is added so that the free ligand concentration in solution rapidly falls to its final equilibrium value, we obtain considerable simplification. If S_{eq} and L_{eq} are the equilibrium values of the free solution receptor and free ligand concentrations at the end of the experiment, then for large solution receptor concentrations we expect S and L to rapidly take on these values and then remain essentially constant as N slowly decays to its final value. In the Appendix we use the methods of Segel and Slemrod (1989) to derive conditions under which this quasi-equilibrium approximation is valid.

When we set $L = L_{eq}$ in Eq. 8 and $S = S_{eq}$ in Eqs. 5 and 6, and substitute these expressions into Eq. 8, we can integrate Eq. 8 to obtain the following transcendental equation for $n = N/N_T$, the fraction of free surface receptor sites, as a function of t:

$$\Phi N_T n_{eq}(n - n_0) + n_{eq}(1 + \Phi N_T n_{eq}) \cdot \ln\left[\frac{n_{eq} - n}{n_{eq} - n_0}\right] = -\kappa_{off} t, \quad (12a)$$

where,

$$\Phi = \frac{\kappa_{on}}{4\pi Da[1 + (\kappa_{on} S_{eq} a^2/D)^{1/2}]}. \quad (12b)$$

$n_0 = N_0/N_t$ is the fraction of cell surface binding sites that are free at the start of the experiment ($t = 0$), $n_{eq} = N_{eq}/N_T$ is the fraction free at the end of the experiment ($t = \infty$), and n is the fraction free at time t.

For comparison with experimental data, it is useful to write Eq. 12a in terms of the following variable:

$$x = \frac{n - n_0}{n_{eq} - n_0}. \quad (13)$$

We see that $x = 0$ at $t = 0$ and $x = 1$ at $t = \infty$.

In terms of x, Eq. 12a becomes

$$(1 - \delta)x + \ln(1 - x) = -k_m(\infty)t, \quad (14)$$

where

$$\delta = \frac{1 + \Phi N_0}{1 + \Phi N_{eq}} = \frac{k_m(\infty)}{k_m(0)} \leq 1 \quad (15)$$

$$k_m = k_r + k_f L_{eq} = \frac{\kappa_{off} + \kappa_{on} L_{eq}}{1 + \Phi N}. \quad (16)$$

The parameter $k_m(\infty)$ is obtained from Eq. 16 by setting $N = N_{eq}$ and is the value k_m approaches as $t \longrightarrow \infty$. The initial rate constant for dissociation, $k_m(0)$, is obtained from Eq. 16 by setting $N = N_0$. That $\delta = k_m(\infty)/k_m(0)$ comes from the definitions of $k_m(\infty)$ and $k_m(0)$.

Note the short and long time limits of Eq. 14. As $t \longrightarrow 0$, $x \longrightarrow 0$, and therefore $\ln(1 - x) \approx -x$. When we substitute this approximation into Eq. 14 we find that

$$\lim_{t \to 0} x = k_m(0)t\,. \tag{17a}$$

In the limit that $t \longrightarrow \infty$, $x \longrightarrow 1$ and therefore the logarithmic term in Eq. 14 becomes infinite while the linear term remains finite. We can therefore neglect the linear term so that

$$\lim_{t \to \infty} x = 1 - e^{-k_m(\infty)t}\,. \tag{17b}$$

Eqs. 17a and b show that the initial and final rate constants for dissociation from a cell surface receptor are given by Eq. 16 with $N = N_0$ and $N = N_T$, respectively. The maximum rate of dissociation occurs at the start of the experiment when the number of free cell surface receptor sites is a minimum. As dissociation continues more sites become free, which increases the likelihood of rebinding and therefore slows dissociation.

In summary, we have derived an approximate equation, Eq. 14, which describes the dissociation of bound ligand from cell surface receptors in the presence of a high concentration of solution receptors. In the Appendix we discuss in detail the conditions under which Eq. 14 is valid.

In Table 1 we list the symbols, and their definitions, that we will frequently use in the text.

TABLE 1 **List of frequently used symbols**

Symbol	Definition
a	Cell radius
D	Diffusion coefficient of the ligand in solution
δ	Ratio of $k_m(\infty)$ to $k_m(0)$
F	Relative fluorescence
F_{max}	Relative fluorescence after dissociation has gone to completion
F_{min}	Relative fluorescence immediately after addition of solution IgE
k_f	Forward rate constant for the binding of a ligand to a cell surface receptor binding site
k_r	Reverse rate constant for the dissociation of a ligand from a cell surface receptor binding site
κ_{on}	Forward rate constant for the binding of a ligand to a receptor binding site in solution
κ_{off}	Reverse rate constant for the dissociation of a ligand from a receptor binding site in solution
k_+	Diffusion limited forward rate constant for the binding of a ligand to a cell
$k_m(0)$	Measured rate constant for dissociation at start of the experiment
$k_m(\infty)$	Measured rate constant for dissociation at completion of the experiment
K	Equilibrium constant for binding of a ligand to a receptor binding site ($K = k_f/k_r = \kappa_{on}/\kappa_{off}$)
L	Concentration of free ligand in solution (nanomolars)
L_T	Total concentration of ligand (nanomolar)
L_0	Concentration of free ligand at the start of the experiment (nanomolars)
L_{eq}	Equilibrium concentration of free ligand at the end of the experiment (nanomolars)
N	Number of free receptor binding sites per cell
N_T	Total number of receptor binding sites per cell
N_0	Number of free receptor binding sites per cell at the start of the experiment
N_{eq}	Equilibrium number of receptor binding sites per cell at the end of the experiment (nanomolars)
ρ	Cell concentration (cells/milliliters)
S	Concentration of free receptor binding sites in solution (nanomolars)
S_T	Total concentration of receptor binding sites in solution (nanomolars)
S_0	Concentration of free receptor binding sites in solution at the start of the experiment (nanomolars)
S_{eq}	Equilibrium concentration of free receptor binding sites in solution at the end of the experiment (nanomolars)
x	Fraction of ligand dissociation that has occurred by time t

Experimental

To study the dissociation of ligand from cell surface receptors in the presence of solution receptors we use a monoclonal anti-DNP IgE (Liu et al., 1980) as both the solution and the cell surface receptor. The IgE, when acting as a cell surface receptor, is bound to high affinity Fc_ϵ receptors on RBL cells. Estimates of the half-life for dissociation of IgE from its Fc_ϵ receptor on RBL cells range from 7 to 45 h (Isersky et al., 1979; Wank et al., 1983). Because these times are much longer than the times for our kinetic experiments, which typically run for <30 min, the IgE concentration on the RBL cell surface remains constant during our experiments. To measure the rate at which surface IgE binding sites that were initially occupied by ligand become free, we use a fluorescein-modified IgE as the cell surface receptor. Previously we showed that the fraction of IgE sites bound to ligand can be determined by measuring the fluorescence quenching that accompanies DNP binding to fluorescein isothiocyanate (FITC) labeled IgE (Erickson et al., 1986). Here we use this technique to measure the kinetics of dissociation.

Fig. 1 shows the results of a typical kinetic dissociation experiment. Plotted is the relative fluorescence as a function of time. Initially only RBL cells with FITC-IgE

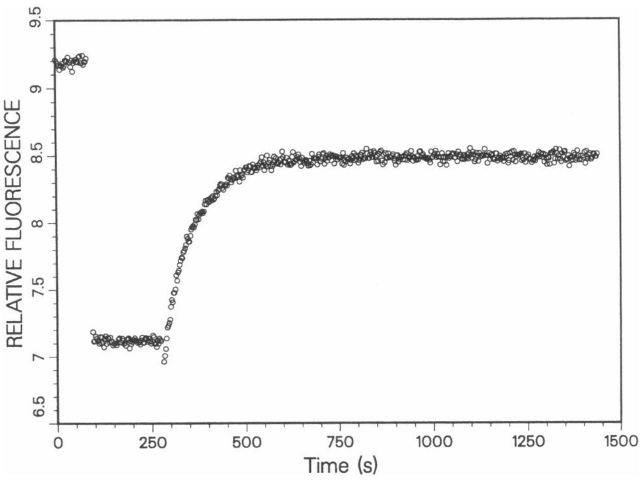

FIGURE 1 A typical kinetic dissociation experiment at 15°C. At $t = 0$ RBL cells with 5.7×10^5 Fab sites/cell (2.85×10^5 FITC IgE/cell) are present in solution. At $t = 82$ s DCT is added to a final concentration of 13.2 nM. The DCT binds to the FITC-IgE, fills a large fraction of the binding sites, and quenches the fluorescence (~20% of the total signal). At $t = 274$ s solution receptor (unlabeled IgE) is added at a final concentration of 942 nM, the DCT begins to dissociate and the fluorescence starts to recover.

on their surface are present (the first plateau at ~ 9.2). Next, a high concentration of the monovalent ligand DCT is added. The DCT binds to the FITC-IgE quenching the fluorescence and a new equilibrium is rapidly established (the second plateau at ~ 7.2). Lastly, the solution receptors (unlabeled monoclonal IgE) are added, and the fluorescence recovers as the DCT dissociates from cell surface IgE binding sites.

After the solution receptors are added, the fraction of ligand dissociation that has occurred by time t, Eq. 13, is related to the relative fluorescence by the expression

$$x = (F - F_{\min})/(F_{\max} - F_{\min}), \tag{18}$$

where $F_{\max}$ is the value of the relative fluorescence after dissociation has gone to completion (the third plateau at ~8.5), $F_{\min}$ is the value of the relative fluorescence immediately after the addition of unlabeled IgE, and F is the relative fluorescence at time t. The addition of the solution IgE alters the fluorescence. (If we add the solution IgE before the DCT, the relative fluorescence drops to ~8.5.) This alteration cannot be accounted for by dilution corrections alone. For this reason $F_{\min}$ is lower than the value of the second plateau, but its exact value is unknown. This can be seen in Fig. 1 where the first three data points after the start of dissociation are lower than the original minimum fluorescence. From Eq. 18 it follows that

$$F = (F_{\max} - F_{\min})x + F_{\min}. \tag{19}$$

To see whether dissociation of ligand from IgE is slower when these receptors are clustered on cells compared with when they are in solution we first do a series of dissociation experiments with different concentrations of solution (S_T) and cell surface receptor (N_T). As described below, for each of these experiments we determine $k_m(\infty)$. (Recall that $k_m(\infty)$ is the off rate constant for dissociation of a ligand from a cell surface receptor as dissociation goes to completion.) To see if rebinding is occurring we also determine $k_m(\infty)$ for dissociation of DCT from FITC-IgE in solution, in the presence of large concentrations of unlabeled IgE, when there are no cells present.

To determine $k_m(\infty)$ from a fluorescence recovery experiment we fit Eq. 19 to the data, calculating x numerically from Eq. 14. In our nonlinear least squares data fitting routine we take as free parameters: $k_m(\infty)$, δ, $F_{\min}$, and $F_{\max}$. The parameters δ and $F_{\min}$ are sensitive to the exact time at which the experiment starts, i.e., the time at which the solution receptors are added. Small errors in the starting time can lead to large errors in these two parameters. In our experiment there is an uncertainty in the starting time of ~2 s and thus, there may be large errors in the values we obtain for $k_m(0)$ and δ. However, the parameters $k_m(\infty)$ and $F_{\max}$ are unaffected by errors in the starting time (see the Appendix for an explanation of why this is so).

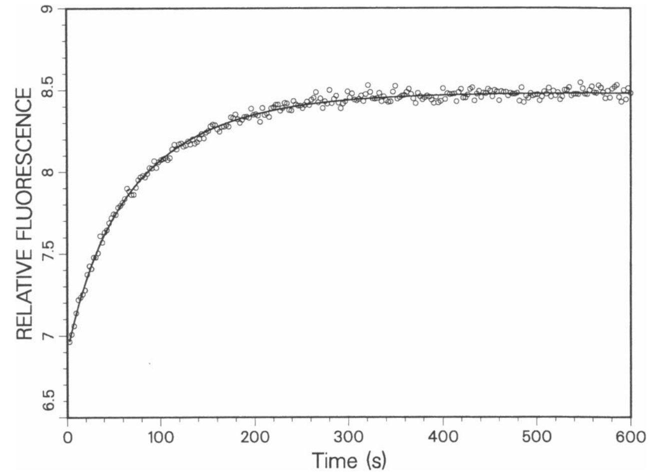

FIGURE 2 Determination of the reverse rate constant $k_m(\infty)$ for dissociation of DCT from cell surface FITC-IgE at 15°C. The data are from Fig. 1. Shown is the first 10 min of the fluorescence recovery, i.e., $t = 0$ corresponds to the time when unlabeled IgE is added. The solid curve was obtained from a nonlinear least squares fit of Eq. 19 to the complete recovery curve (483 data points going out to $t = 1,159$ s). The variable x in Eq. 19 was calculated from Eq. 14 numerically. In the fitting procedure the parameters $k_m(\infty)$, δ, $F_{\min}$, and $F_{\max}$ were allowed to vary. The best values for these parameters (in the least squares sense) were found to be: $k_m(\infty) = 1.07 \times 10^{-2}\ \mathrm{s}^{-1}$, $\delta = 0.63$, $F_{\min} = 6.90$, and $F_{\max} = 8.48$. The results of fitting thirteen such experiments are listed in Table 3.

TABLE 2 Determination of the off rate constant κ_{off} for dissociation of DCT from FITC-IgE in solution from six experiments

$k_m(\infty)(\times 10^{-2}\,s^{-1})$	$S_T(\times 10^2\,nM)$	$L_T(nM)$	$\kappa_{off}(\times 10^{-2}\,s^{-1})$
2.70	7.90	15.6	2.65
2.64	4.00	15.8	2.54
2.75	2.40	16.1	2.57
2.75	1.61	16.1	2.50
2.63	3.20	16.0	2.50
2.73	4.00	16.0	2.62
			2.56 ± 0.06

For DCT dissociating from FITC-IgE in solution in the presence of a high concentration of unlabeled IgE, $k_m(\infty) = \kappa_{off} + \kappa_{on}L_{eq} \approx \kappa_{off}(1 - L_T/S_T)$. κ_{on} and κ_{off} are the forward and reverse rate constants for DCT binding to a single IgE binding site, L_{eq} is the final DCT concentration, and L_T and S_T are the total concentrations of DCT and unlabeled IgE, respectively. We determined $k_m(\infty)$ from a nonlinear least squares fit of Eq. 19, with $x = 1 - \exp(-k_m(\infty)t)$, to dissociation experiments such as the one shown in Fig.4. In addition, we also obtained values for F_{min} and F_{max} (results not shown).

In Fig. 2 we show the theoretical fit (Eq. (19) with x calculated from Eq. 14 to the dissociation experiment shown in Fig. 1. In this experiment we used 8.8×10^6 cells/ml with an average receptor site concentration of 5.7×10^5 sites/cell, which corresponds to a solution concentration of 8.35 nM. Dissociation was initiated by the addition of solution receptors (unlabeled IgE) at a final site concentration of 942 nM. From our fit we found the following value for the dissociation rate constant: $k_m(\infty) = 1.07 \times 10^{-2}\,s^{-1}$. This is ~2.4 times slower than the dissociation constant we determined for DCT dissociating from FITC-IgE in solution, where in six experiments we found that $\kappa_{off} = 2.56 \pm 0.06 \times 10^{-2}\,s^{-1}$ (see Table 2). In all we carried out thirteen experiments including the one shown in Fig. 1, where the dissociation of DCT from cell surface FITC-IgE was initiated by the addition of unlabeled solution IgE. The parameters determined from these experiments are given in Table 3.

Our theory predicts that as DCT dissociates from cell surface binding sites its rate of dissociation constantly decreases from an initial rate $k_m(0)$ to a final rate $k_m(\infty)$. As time proceeds more surface sites become free, more rebinding occurs, and dissociation slows. This slowing can be seen in Fig. 3 where the data in Fig. 2 is replotted as a log-linear plot of $(1 - F_{max}/F_{min})$ vs. t. The initial slope of such a plot equals $k_m(0)$ and the final slope approaches $k_m(\infty)$. The solid line in Fig. 3 is the straight line fit to only the first ten data points, corresponding to the first 25 s of the experiment. The slope of this curve equals $1.60 \times 10^{-2}\,s^{-1}$. This estimate of $k_m(0)$ compares well with the estimate obtained by fitting the complete curve in Fig. 2 where, from the determinations of δ and $k_m(\infty)$, we have that $k_m(0) = \delta k(\infty) = 1.69 \times 10^{-2}\,s^{-1}$.

In Figs. 4 and 5 we carry out a similar analysis for the dissociation of DCT from FITC-IgE in solution. The solid line in Fig. 5 is the straight line fit of the first 10 data points ($t < 25$ s) with slope $k_m(0) = 2.67 \times 10^{-2}\,s^{-1}$. Fitting the data for the entire curve in Fig. 4 yields $k_m(\infty) = 2.70 \times 10^{-2}\,s^{-1}$. Unlike dissociation from cell surface receptors (Figs. 2 and 3), we observe no systematic slowing when DCT dissociates from receptors in solution.

Finally, we test how the variation in $k_m(\infty)$ with S_T and N_T compares with that predicted by the theory. From Eqs.

TABLE 3 Least square estimates of the parameters $k_m(\infty)$ and δ for thirteen ligand (DCT)-cell surface receptor (FITC-IgE) dissociation experiments

$N_T(\times 10^5$ sites/cell)	$S_T(nM)$	$L_T(nM)$	$S_{eq}(nM)$	$k_m(\infty)(\times 10^{-2}\,s^{-1})$	δ
6.00	1540	13.0	1527	1.04	0.60
6.00	942	13.2	929	0.90	0.43
6.00	712	13.3	699	1.03	0.55
6.00	478	13.4	465	0.95	0.50
5.70	1540	13.0	1527	1.05	0.58
5.70	942	13.2	929	1.07	0.63
5.70	478	13.4	465	1.01	0.60
3.00	712	3.96	708	1.63	0.61
3.00	400	4.00	396	1.74	0.63
3.00	242	4.02	238	1.82	0.74
1.75	712	3.96	708	1.92	0.87
1.75	400	4.00	396	1.74	0.66
1.75	242	4.02	238	1.46	0.54

$k_m(\infty)$ is the reverse rate constant for dissociation of DCT from cell surface FITC-IgE at the end of the experiment when all binding sites are free. $\delta = k_m(\infty)/k_m(0)$, where $k_m(0)$ is the reverse rate constant for dissociation at the start of the experiment when a large fraction of the cell surface FITC-IgE are bound. N_T, S_T, and L_T are the concentrations of cell surface FITC-IgE binding sites, the unlabeled solution IgE binding sites, and the DCT, respectively. S_{eq} is the final concentration of free unlabeled solution IgE binding sites and was estimated using Eq. 22. We use the data in the first four columns to test Eq. 20 (see Fig. 6). In addition to determining $k_m(\infty)$ and δ we also determined F_{min} and F_{max}, the maximum and minimum relative fluorescence (results not shown).

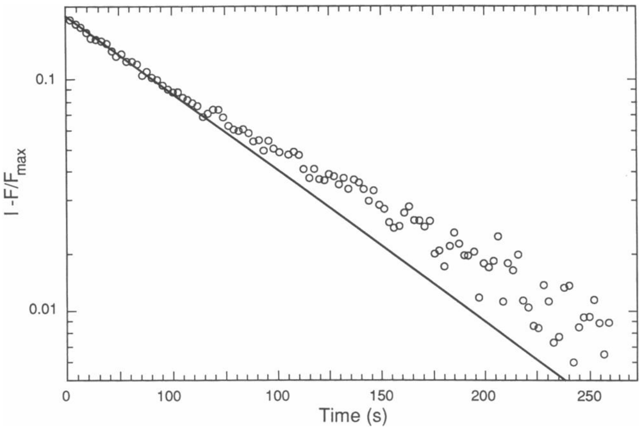

FIGURE 3 The slowing of the rate of dissociation of DCT from cell surface IgE. Our theory predicts that at short times a plot of $\ln(1-F/F_{max})$ vs. t, where F is the relative fluorescence at time t and $F_{max} = 8.48$ is the relative fluorescence after dissociation has gone to completion, will be a straight line with slope equal to $k_m(0)$, the initial dissociation rate. (This follows from Eq. 17a. At short times, i.e., $1 \gg k_m(0)t$, $x \approx k_m(0)t$ and therefore, since $k_m(0)t$ is small, $x \approx 1 - \exp(-k_m(0)t)$. Fitting a straight line to the first ten data points (*solid line*) gives a slope of $1.60 \times 10^{-2}\text{s}^{-1}$. As t increases dissociation slows as can be seen by the concave upward deviation of the data from the straight line. Because the $\ln(1-F/F_{max})$ is infinite when $F=F_{max}$, we cannot plot the long time data for when F fluctuates above F_{max} the function becomes undefined.

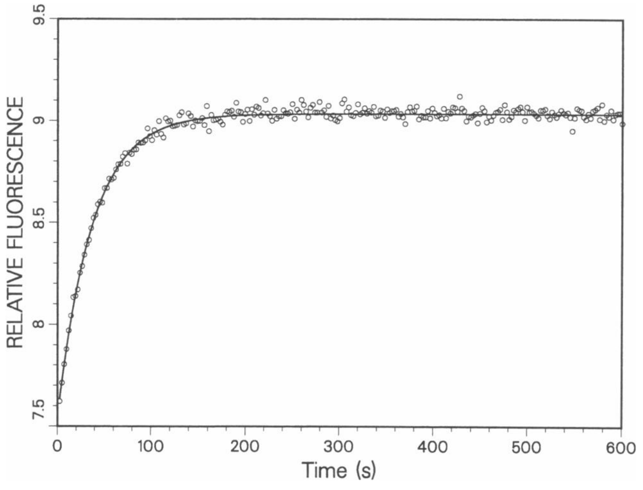

FIGURE 4 Determination of the reverse rate constant $k_m(\infty)$ for the dissociation of DCT from solution FITC-IgE at 15°C. 8.8 nM of FITC-IgE was allowed to equilibrate with 15.6 nM DCT. At $t = 0$ unlabeled IgE was added at a final concentration of 790 nM. The solid curve was obtained from a nonlinear least squares fit of Eq. 19 to the complete recovery curve (478 data points going out to $t = 1147$ s). In Eq. 19 we took $x = 1 - \exp(-k_m(\infty)t)$. The parameter $k_m(\infty)$, F_{min} and F_{max} were allowed to vary. The best fit values for these parameters (in the least squares sense) were found to be: $k_m(\infty) = 2.70 \times 10^{-2}\text{s}^{-1}$, $F_{min} = 7.54$ and $F_{max} = 9.03$. The results of six experiments are listed in Table 2.

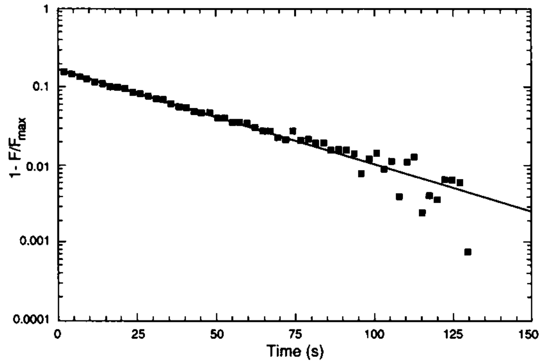

FIGURE 5 The rate of dissociation of DCT from solution FITC-IgE is constant with time. Shown is a plot of $\ln(1-F/F_{max})$ vs. t for the first 130 s of the data in Fig. 4 F is the relative fluorescence at time t and $F_{max} = 9.03$ is the relative fluorescence after dissociation has gone to completion. Fitting a straight line to the first data points (*solid line*) gives a slope of 2.67×10^{-2} s^{-1}. Fitting the data for the entire experiment (see Fig. 4) gives $k_m(\infty) = 2.70 \times 10^{-2}$ s^{-1} time.

12b and 16 we see that the theory predicts that

$$k_m(\infty) = \frac{\kappa_{off}(1 + KL_{eq})}{1 + \dfrac{\alpha N_{eq}}{1 + (4\pi a^3 \alpha S_{eq})^{1/2}}}, \tag{20}$$

where

$$\alpha = \kappa_{on}/(4\pi Da). \tag{21}$$

We show in the Appendix that for the large solution receptor concentrations we use, the following approximations hold:

$$\begin{aligned} KL_{eq} &\approx L_T/S_T \\ N_{eq} &\approx N_T(1 - L_T/S_T) \\ S_{eq} &\approx S_T(1 - L_T/S_T). \end{aligned} \tag{22}$$

To test Eq. 20 we fit it to the data given in Table 3, taking as free parameters the off rate constant for dissociation of DCT from FITC-IgE in solution, κ_{off}, and the lumped parameter $\alpha = \kappa_{on}/(4\pi Da)$. We hold the RBL cell radius fixed at 4 μm. The least squares fit to the data yields the following values for the parameters: $\kappa_{off} = 2.73 \times 10^{-2}$ s^{-1} and $\alpha = 6.91 \times 10^{-6}$ sites^{-1}. Because $k_m(\infty)$ is a function of two variables, the data are predicted to fall on a family of curves rather than a single curve. In Fig. 6, two particular curves are shown (*solid lines*). Both curves were calculated from Eq. 20 using the best fit values of the parameters. The upper curve was obtained from Eq. 20 by setting S_{eq} equal to the smallest value it takes on in Table 3, while the lower curve was obtained by setting S_{eq} equal to the largest value it takes on in Table 3. If there were no noise in the data, the theory predicts that all the

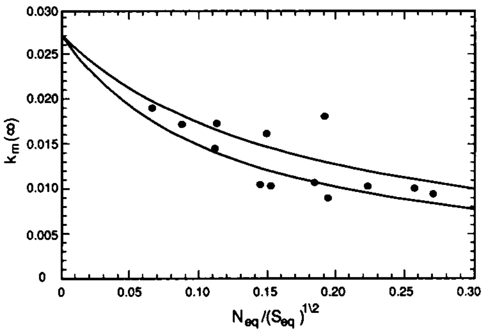

FIGURE 6 Variation of the reverse rate constant $k_m(\infty)$ with cell surface FITC-IgE and solution unlabeled IgE. Plotted is $k_m(\infty)$ vs. $N_{eq}/(S_{eq})^{1/2}$ for the data in Table 3. N_{eq} is the concentration of free cell surface FITC-IgE binding sites and S_{eq} is the concentration of free unlabeled IgE binding sites in solution, after dissociation has gone to completion. In this plot S_{eq} is in nanomolars and the units of N_{eq} are 10^5 sites/cell. A nonlinear least squares fit of the theoretical expression for $k_m(\infty)$, Eq. 20, to the data in Table 3 with two free parameters and the cell radius $a = 4\ \mu m$, yielded: $\kappa_{off} = 2.73 \times 10^{-2}\ s^{-1}$ and $\alpha = 6.91 \times 10^{-6}\ sites^{-1}$. The solid curves were calculated from Eq. 20 using the best fit values for the parameters, with $a = 4\ \mu m$. For the upper and lower curves respectively, S_{eq} was held fixed at its smallest and highest value in Table 3. The theory predicts that the data should fall on or between these curves.

data points should fall either on, or between these two curves.

The value of $2.73 \times 10^{-2}\ s^{-1}$ for κ_{off} that we determined from fitting Eq. (20) to the data in Table 3 agrees with the value $2.56 \times 10^{-2}\ s^{-1}$ that we directly determined for DCT dissociating from FITC-IgE in solution (see Table 2). Also, the value we determined for α is consistent with what we know about the values of the individual parameters that make up α. To estimate α we need to know κ_{on}, D and a. Because we know κ_{off}, we chose to determine κ_{on} by determining the equilibrium constant $K = \kappa_{on}/\kappa_{off}$ for DCT binding to FITC-IgE in solution (see Fig. 7). From three experiments $K = 1.86 \pm 0.42\ nM^{-1}$ and therefore, for the value of κ_{off} from Table 2, we have that $\kappa_{on} = 4.76 \pm 1.19 \times 10^{-2}\ nM^{-1}\ s^{-1} = 7.91 \pm 1.98 \times 10^{-14}\ cm^3/s$. We have not determined D directly, but for a small ligand such as DCT at 15° C, we expect that $D \approx 5 - 10 \times 10^{-6}\ cm^2/s$. The radius of an RBL cell $a \approx 4\ \mu m$. Putting these values into the expression for α, Eq. 21, we find that $\alpha \approx 1.2 - 3.9 \times 10^{-6}\ sites^{-1}$. From our fit of the data we found $\alpha = 6.9 \times 10^{-6}\ sites^{-1}$, which is within experimental error of the estimated range.

DISCUSSION

We have presented a theory to describe the kinetics of dissociation of ligands from cell surface receptors in the presence of solution receptors that also bind the ligand. A major result of the theory, Eqs. 5 and 6, is the prediction of the dependence of the ligand-cell surface receptor association and dissociation rate constants on the free cell surface receptor concentration, N, and the free solution receptor concentrations, S. In a dissociation experiment when no solution receptor is present, dissociation of the ligand from cell surface receptors may be much slower than dissociation from a receptor in solution because the ligand may rebind many times to free cell surface receptors before achieving a true separation from the cell surface. From Eq. 6 it follows that such rebinding will be prevented from occurring when $S \gg N^2\kappa_{on}/(16\pi^2 D a^4)$. Note that this inequality is independent of the size of the receptor. The effective concentration of the surface receptors that the solution receptors need to overcome, is not, as one might have guessed, simply the surface site density divided by the height of the receptor. The effective concentration does not depend linearly on N, but rather is proportional to N^2. For our experimental system the single-site forward rate constant $\kappa_{on} = 7.9 \times 10^{-14}\ cm^3/s$ at 15°C and the RBL cell radius $a \approx 4\ \mu m$. We have not determined the diffusion coefficient of the ligand (DCT) but a reasonable estimate for D at 15°C is $5 \times 10^{-6}\ cm^2/s$. For these parameter values we predict that if $N = 6 \times 10^5$ sites/cell, then to prevent rebinding $S \gg 2{,}400$ nM. When we carried out dissociation experiments in the presence of solution receptor concentrations ranging from 1,540 to 478 nM, the rate constant for dissociation of DCT from cell surface IgE was ~ 2.5 times slower than for dissociation from solution IgE. As we lowered the cell surface receptor density the rate of dissociation from cell surface IgE increased, but even for $N = 1.75 \times 10^5$ sites/cell and

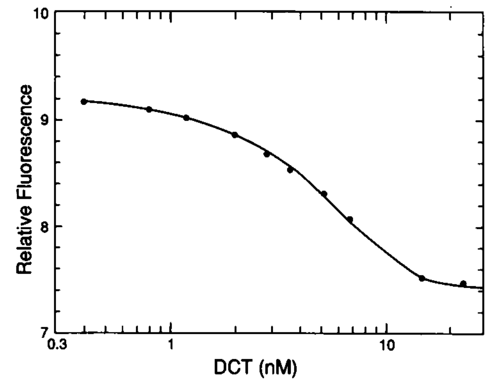

FIGURE 7 Equilibrium binding of DCT to FITC-IgE in solution at 15°C. The FITC-IgE concentration was 8.8 nM. Plotted is the relative fluorescence vs the total DCT concentration. The solid curve was obtained from a nonlinear least squares fit of the data (see Materials and Methods) and yielded an equilibrium constant $K = 1.61$ (nanomolars)$^{-1}$. Two additional experiments were performed with an FITC-IgE concentration of 9.7 nM. For the three experiments $K = 1.86 \pm 0.42$ (nM)$^{-1}$.

S = 712–242 nM, the reverse rate constant was substantially slower than for dissociation from solution IgE. These results are summarized in Table 3.

Another prediction of the theory is that as the ligand dissociates from cell surface binding sites the rate constant for dissociation decreases from an initial rate $k_m(0)$ to a final rate $k_m(\infty)$, because with time more surface binding sites become free and more rebinding occurs. We were able to demonstrate directly that this slowing down occurs (see Fig. 3). Typically, in our experiments where a large fraction of surface receptor sites were initially bound, the rate of dissociation slowed by a factor of two during the course of dissociation. Finally, we were able to test the predicted form for the rate constant, Eq. 6, and show it was consistent with experiment. However, the experimental errors were such that this was not a rigorous test of the theory.

The system we studied exhibited rebinding effects, i.e., a reduction in the reverse rate constant for ligand-cell surface IgE dissociation, at high IgE concentrations ($\geq 1.0 \times 10^5$ sites/cell). Previously we showed that the forward rate constant decreased with increasing cell surface IgE concentration (Erickson et al., 1987). A similar effect had been seen for the binding of phage lambda to receptors on *Escherichia coli* (Schwartz, 1976). Recently Wiley (1988) observed that dissociation of epidermal growth factor (EGF) from its receptor on A431 cells ($2 - 4 \times 10^6$ EGF sites/cell) was much slower when the initial number of EGF receptor sites bound was smaller than when most of the sites were filled. Abbot and Nelsestuen (1987) observed binding in the diffusion limit of blood coagulation factor Va light chain to its receptor on membrane vesicles. We expect that for many systems the kinetics of binding and dissociation are altered at high cell surface receptor densities. The theory presented here shows how the presence of solution receptors that compete with the cell surface receptors for the ligand, further changes the kinetics.

APPENDIX

Diffusive rate constant in the presence of solution receptors

To obtain the diffusive rate constant k_+ for the binding of a ligand with diffusion coefficient D, to a cell of radius a, in the presence of solution receptors whose free binding site concentration is S, we calculate the steady state flux into a perfectly absorbing sphere of radius a. Because the sphere is perfectly absorbing, at the surface of the sphere the free ligand concentration is zero, i.e., $C(a) = 0$. Far from the sphere C is finite. We modify the standard formulation of Smoluchowski in two ways. (a) Outside the sphere we allow ligands to both diffuse and be absorbed by solution receptors. (b) To maintain a steady state we insert ligands at a constant rate Q uniformly throughout space to replace the ligands that are absorbed by either the sphere or the solution receptors. In the Smoluchowski approach a steady state is maintained by holding the ligand concentration constant at infinity. Once solution receptors are introduced that absorb ligands uniformly outside the sphere, it is no longer possible to maintain a nonzero steady state in this way. If κ_{on} is the forward rate constant for the binding of a ligand to a receptor in solution then in the steady state

$$0 = D\nabla^2 C - \kappa_{on} S C + Q\ , \tag{A1}$$

where we have assumed that S is sufficiently large so that dissociation from solution receptors can be neglected. In particular, this requires that the distance a ligand travels when it is free, $\sqrt{D/(\kappa_{on}S)}$, be small compared with the distance it travels when it is bound to a receptor, $\sqrt{D_S/\kappa_{off}}$, where D_S is the diffusion coefficient of the ligand-receptor complex. Thus, a necessary condition for Eq. A1 to be valid is that $KS \gg D/D_S$ (Dembo and Goldstein, unpublished result). For our ligand (DCT) and receptor (IgE), $D/D_S \approx 10^2$, and $K \approx 2\ \text{nM}^{-1}$. Thus, we require that $S \gg 50$ nM.

Because there is spherical symmetry, Eq. A1 can be written as follows:

$$0 = \frac{1}{r^2}\frac{d}{dr}\left[r^2\frac{dC}{dr}\right] - \frac{C}{\lambda^2} + \frac{Q}{D}, \tag{A2}$$

where the screening length $\lambda = \sqrt{D/(\kappa_{on}S)}$, and r is the radial distance from the center of the sphere. We shall assume that S can be treated as being constant in space, i.e., that S is sufficiently large that the binding of the ligand takes up a negligible fraction of free solution receptor sites. With the given boundary conditions, and taking S constant, the solution to Eq. A2 is

$$C(r) = C_\infty\left[1 - \frac{a}{r}e^{-(r-a)/\lambda}\right] \tag{A3}$$

where $C_\infty = Q/(\kappa_{on}S)$.

The rate constant k_+ equals the flux into the sphere divided by the average concentration outside the sphere, i.e.,

$$k_+ = \frac{4\pi D a^2 \mathrm{d}C/\mathrm{d}r}{C_\infty}, \tag{A4}$$

where $\mathrm{d}C/\mathrm{d}r$ is evaluated at $r = a$. Thus, from Eqs. A3 and A4 we find that

$$k_+ = 4\pi Da[1 + a/\lambda) = 4\pi Da[1 + (\kappa_{on}Sa^2/D)^{1/2}]. \tag{A5}$$

Note that in the limit that $S \rightarrow 0$, we recover the standard result that $k_+ = 4\pi Da$.

Although at first it may seem surprising that the presence of solution receptors that bind the ligand increases k_+, we can see from Eq. A4 why this is so. The presence of solution receptors increases the gradient of the ligand concentration in the vicinity of the absorbing sphere. In the presence of solution receptors most of the variation in $c(r)$ occurs within a few screening lengths of the cell surface. In terms of the screening length Eq. 4 becomes $k_+ = 4\pi Da(1 + a/\lambda)$. When λ is comparable with or smaller than the cell radius, k_+ in the presence of solution receptors differs appreciably from k_+ when they are absent.

Effect on parameter estimation of errors in the initial starting time

After a short initial transient x is given by Eq. 17b and therefore at long times Eq. 19 becomes

$$F = F_{\max} - (F_{\max} - F_{\min})e^{-k_m(\infty)t}. \tag{B1}$$

Suppose that there is an error in the starting time so that the experiments starts at $t = t_0$ rather than at $t = 0$. Then the correct expression for F is

$$F = F_{\max} - (F_{\max} - F_{\min})e^{-k_m(\infty)(t-t_0)}. \tag{B2}$$

Note however that both Eqs. B1 and B2 are of the same form, i.e.,

$$F = F_{\max} - Ae^{-k_m(\infty)}, \tag{B3}$$

where A is a lumped parameter. Thus if we fit the same data with Eq. B1, allowing $F_{\max}$, $F_{\min}$ and $k_m(\infty)$ to be free parameters, or Eq. B2, allowing $F_{\max}$, $F_{\min}$, $k_m(\infty)$ and t_0 to be free parameters, we will obtain the same values for $F_{\max}$, $k_m(\infty)$ and A.

Because Eq. B1 is not correct at very short times, the argument we have presented is not rigorous, but it strongly suggests that when we use Eq. 19 with the correct expression for x, Eq. 14, errors in t_0 will have little effect on the values obtained for $F_{\max}$ and $k_m(\infty)$. To see if this is so we have fit Eq. 19 to all the cell dissociation experiments using two different expressions for x. First we fit the data using Eq. 14 to calculate x, the results of which are given in Table 2. Then we introduced an additional free parameter t_0 by changing t to t-t_0 in Eq. 14 and refit all the data (results not shown). The values we obtained for $F_{\max}$ and $k(\infty)$ did not change while the values of δ and $F_{\min}$ did.

Approximate values for L_{eq}, N_{eq} and S_{eq}

At equilibrium $dN/dt = dS/dt = 0$ in Eqs. 8 and 9 and $N = N_{eq}$, $S = S_{eq}$ and $L = L_{eq}$ in Eqs. 8–10. We are interested in the case when we add a large concentration of solution receptors ($KS_{eq} \gg 1$) that bind most of the ligand so that the free ligand concentration is close to zero, i.e., $1 \gg KL_{eq}$. For this to hold we must have $S_T \gg L_T$. From Eqs. 8 and 9 it follows that

$$N_{eq} = \frac{N_T}{1 + KL_{eq}} \approx N_T(1 - KL_{eq}) \tag{C1}$$

$$S_{eq} = \frac{S_T}{1 + KL_{eq}} \approx S_T(1 - KL_{eq}). \tag{C2}$$

Substituting these expressions into Eq. 10 and solving for L_{eq} we obtain the following result

$$KL_{eq} = \frac{KL_T}{1 + K\overline{N}_T + KS_T} = \frac{L_T}{S_T}\left[1 - \frac{1 + K\overline{N}_T}{KS_T} \dots\right]. \tag{C3}$$

It follows from Eq. C3 that $KL_{eq} \approx L_T/S_T$ when $S_T \gg N_T$. In all our cell dissociation experiments (see Table 2) the concentrations where chosen so that $KS_T \gg 1$, $S_T \gg \overline{N}_T$, and $S_T \gg L_T$.

Conditions for validity of the quasi-equilibrium approximation (QEA)

To obtain conditions for when the use of the QEA is valid in deriving Eq. 14 we follow Segel and Slemrod (1989). In what follows we assume that the solution receptor concentration is in large excess, i.e., $S_T \gg L_T$ and $S_T \gg \overline{N}_T$.

We expect that after the solution receptor is added there will be an initial transient as ligands in solution bind to these added receptors. At very short times the binding will appear irreversible, i.e.,

$$\frac{dL}{dT} \approx -\kappa_{on} S_T L, \tag{D1}$$

where at $t = 0$, $S = S_T$. Thus, the characteristic time of this initial transient is $t_c = 1/(\kappa_{on} S_T)$.

We want the duration of this transient to be short compared with the characteristic time it takes for the ligand to dissociate from the cell surface. When we solved Eq. 8 in the QEA we found that at long times (Eq. 17b) the rate of decay of ligand bound on the surface equals $k_m(\infty) = (\kappa_{off} + \kappa_{on} L_{eq})/(1 + \Phi N_{eq})$. Therefore the characteristic time for this slow dissociation $t_S = 1/k_m(\infty)$. The condition that $t_S \gg t_c$ is therefore that

$$|\Delta N/N_0| \approx \frac{1}{N_0}(dN/dt)_{\max} \times t_c\,. \tag{D3}$$

If at $t = 0$ we neglect any binding to the surface then from Eq. 8

$$(dN/dt)_{\max} \approx k_r N_T(1 - N_0/N_T) \tag{D3}$$

$$\frac{1}{N_0}(dN/dt)_{\max} \times t_c \approx \frac{1}{1 + \Phi N_0}\frac{1}{KS_T}\frac{1 - n_0}{n_0}, \tag{D4}$$

where $n_0 = N_0/N_T$. Thus for the initial condition we used to be valid in the QEA we must have

$$(1 + \Phi N_0)KS_T \gg (1 - n_0)/n_0\,. \tag{D5}$$

The largest ligand concentration we used was 13.4 nM. For this value of L_0 we estimate that $n_0 \approx 0.04$. Because $K \approx 2\ \text{nM}^{-1}$ this condition requires that $(1 + \Phi N_0)KS_T \approx 24$ nM. Because ΦN_0 is small we therefore need $S_T \approx 12$ nM. This condition is more restrictive than the first condition, Eq. D2. When we compare numerical solutions of Eqs. 8–10 with results obtained from the QEA, Eq. 14, we find that we get good agreement for $S_T \geq 200$ nM.

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