Approximating the Effects of Diffusion on Reversible Reactions at the Cell Surface: Ligand-Receptor Kinetics

Byron Goldstein and Micah Dembo
Theoretical Biology and Biophysics Group, Theoretical Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545 USA

ABSTRACT We consider the problem of determining the time dependence of the bound ligand concentration for the reversible binding of a diffusing monovalent ligand to receptors uniformly distributed over the surface of a spherical cell. We start by formulating a boundary value problem that captures the essential physics of this situation. We then introduce a systematic approximation scheme based on the method of weighted residuals. By this means we convert the initial boundary value problem into a simpler problem that requires solving only a small number of ordinary differential equations. We show how, at the lowest order of approximation, the method can be used to obtain modified chemical rate equations where, in place of fundamental rate constants, effective rate coefficients appear. These rate coefficients are functions of the ligand diffusion coefficient, the cell radius, the receptor density and other variables. We compare exact and approximate solutions and discuss under what conditions the approximate equations can be used. We also apply the method of weighted residuals to obtain approximate descriptions of the binding kinetics when (1) there are two different cell surface receptor populations that bind the ligand and (2) the cell secretes a ligand that can bind back to receptors on the cell (autocrine binding).

INTRODUCTION

In biological systems chemical reactions often occur between reactants that are not well mixed. In many cases one of the reactants is confined to a surface while the other is distributed over a volume. Here we consider one important example, the reversible binding of ligands to cell surface receptors.

For a ligand to bind to a cell surface receptor it must first diffuse to the surface of the cell and then react with a receptor. Therefore, one expects that to predict the kinetics of ligand-cell surface receptor binding one must solve a diffusion-reaction problem with appropriate boundary and initial conditions. Surprisingly, this is rarely done. Instead, when ligand-cell surface receptor binding studies are analyzed, the full diffusion-reaction problem is approximated by using a modified chemical rate equation (an ordinary differential equation (ODE)). This modified rate equation has the same form as for a well mixed system, except that the rate constants have an unusual interpretation. They are in fact effective rate coefficients that are nonlinear functions of the density of cell surface receptor (DeLisi, 1983; Goldstein et al., 1989).

The purpose of introducing effective rate coefficients is to try to approximate two related effects that arise when receptors are confined to cell surfaces. The first such effect (rebinding) occurs because a ligand that dissociates from one receptor has a high probability of reacting with another receptor on the same surface instead of escaping into solution. Such rebinding can significantly slow the rate of dissociation (Goldstein et al., 1989). At high receptor densities the forward kinetics of a reaction is also affected because of competition or screening. The rate of binding to the cell is no longer proportional to the number of free receptors on the cell surface but saturates with increasing receptor number as receptors compete for ligand and the reaction between ligand and cell becomes diffusion limited (Schwartz, 1976; Berg and Purcell, 1977; Erickson et al., 1987.)

Accounting for rebinding and competition effects by introducing some sort of effective rate constants has intuitive appeal. However, because of the ad hoc nature of this procedure, there are many uncertainties associated with the resulting modified chemical rate equation. The first concerns the accuracy of its solutions compared with those of the full reaction-diffusion problem. If the accuracy is not acceptable, how can one improve it? How can one generalize the equation to more complicated binding problems, e.g., competing receptor populations, other geometries, and autocrine secretion of ligand?

This paper is in two parts, the spirits of which are quite different. In the first part (the following section) we review the modified chemical rate equation, which we refer to as the ad hoc approximation. Using heuristic arguments we generalize it to two cases: (1) that of autocrine binding where a cell secretes a ligand that can bind back to the cell surface and (2) that of a cell with two receptor populations on its surface that bind the same ligand with different fundamental rate constants.

In the second part of the paper we start from the diffusion-reaction problem. In a systematic manner, we introduce an approximation scheme that converts the problem of solving a diffusion equation with nonlinear boundary conditions into that of solving a set of coupled nonlinear ODE. We accomplish this by using the method of weighted residuals (MWR). In the Appendix we show how the results in the first part obtained in a purely ad hoc way, can be obtained systematically by using the MWR. We also show how the MWR can

Received for publication 19 April 1994 and in final form 25 January 1995.
Address reprint requests to Dr. Byron Goldstein, Theoretical Biology and Biophysics Group, Theoretical Division, T-10, MS K710, Los Alamos National Laboratory, Los Alamos, NM 87545. Tel.: 505-667-6538; Fax: 505-665-3493; E-mail:bxg@lanl.gov.

0006-3495/95/04/1222/09 $2.00

be used to obtain an equation that reduces to the ad hoc result at long times but is more accurate than the ad hoc equation at short times. Finally, for two examples for which the diffusion-reaction problem is exactly solvable, we compare the exact solution to the solutions of the ad hoc equation and the equation obtained by using the MWR.

THE AD HOC APPROXIMATION

Before considering ligands binding to receptors confined to surfaces, let us review the binding of ligands to receptors in solution. From the law of mass action we have that, for a ligand at concentration L and a receptor at concentration R, and their bimolecular complex (bound receptor) at concentration B,

$$\dot{B} = k_{on}LR - k_{off}B. \tag{1}$$

If there are a total of R_T receptors and L_T ligands, then, using mass conservation,

$$R_T = R + B, \tag{2a}$$

and

$$L_T = L + B. \tag{2b}$$

Substituting Eq. 2, a and b, into Eq. 1 we obtain a single nonlinear ODE that describes the rate at which bound complex is formed.

If L, R, and B are monodispersed in solution, then, with the possible exception of very small diffusional transients, the rate coefficients k_{on} and k_{off} are constant. To be more precise, these rate coefficients are independent of the time and of the concentrations of the reactants, although they may depend on hidden parameters such as the temperature and viscosity. Because k_{on} and k_{off} possess these qualities they are sometimes referred to as fundamental rate constants.

Now consider the ad hoc treatment of binding of ligands to cell surface receptors (reviewed in DeLisi, 1983; Goldstein, 1989). In this approximation one assumes that the chemical rate equation for surface binding has the form

$$\dot{B} = k_f\bar{L}R - k_rB. \tag{3}$$

This is the same as Eq. 1 except that the fundamental rate constants k_{on} and k_{off}, are replaced by effective rate coefficients, k_f and k_r, and the uniform concentration L is replaced by $\bar{L}$, the ligand concentration far from the cell. $\bar{L}$ is sometimes called the bulk ligand concentration. The effective rate coefficients, which are not constant but functions of the free receptor site concentration, are introduced to account for competition and rebinding effects that arise when the ligand concentration is nonuniform.

If binding is modeled as follows: (1) diffusion of the ligand to the cell surface; (2) formation of an encounter complex (free ligand in close proximity to the cell surface); and (3) binding to receptors on the surface and if the encounter complex is in a quasi-steady state, then (Eigen, 1974, Shoup and Szabo, 1982)

$$k_f = \frac{k_{on}}{1 + Rk_{on}/k_+}, \tag{4a}$$

$$k_r = \frac{k_{off}}{1 + Rk_{on}/k_+}, \tag{4b}$$

where k_+ is the diffusion limited forward rate constant, obtained by solving the diffusion equation in the steady state with perfectly absorbing boundary condition ($L = 0$ at $r = a$). For a ligand diffusing to a sphere that is very much larger than itself,

$$k_+ = 4\pi Da, \tag{4c}$$

where a is the radius of the sphere and D the diffusion coefficient of the ligand.

When the number of receptors on a cell is small, competition and rebinding are negligible and the effective rate coefficients and fundamental rate constants are essentially equal. As the number of receptors becomes large the effective forward rate coefficient for binding to a cell saturates because of competition effects and the so-called diffusion limit is approached:

$$\lim_{R\to\infty} Rk_f \to k_+. \tag{5a}$$

As Rk_f, the effective forward rate coefficient for the entire cell approaches a finite limit, $k_f \to 0$. In this limit, competition dominates and the effective forward rate coefficient for a single receptor goes to zero. Large receptor densities also cause rebinding effects to slow dissociation. In the limit of infinite receptor density, ligands never escape the cell surface:

$$\lim_{R\to\infty} k_r \to 0. \tag{5b}$$

The difference between k_{off} and k_r is solely a result of rebinding (Berg, 1978). We can therefore define the following useful quantities:

$$\frac{k_r}{k_{off}} = \frac{1}{1 + Rk_{on}/k_+} = \text{fraction of dissociations that escape} \tag{6a}$$

$$\left(1 - \frac{k_r}{k_{off}}\right) = \frac{Rk_{on}/k_+}{1 + Rk_{on}/k_+} = \text{fraction of dissociations that rebind} \tag{6b}$$

and

$$\gamma = \frac{k_+}{Rk_{on}} = \frac{4\pi Da}{Rk_{on}} = \text{ratio of escape to rebinding probabilities} \tag{6c}$$

Autocrine binding

Consider a cell that secretes a ligand at a rate S and has receptors on its surface that can bind the ligand. Let the

secretion occur through a mechanism that releases ligands uniformly over the cell surface. A ligand released from the cell surface through secretion or through dissociation from a receptor will have the same probability of rebinding, which is given by Eq. 6b. Therefore, in the presence of secretion, the modified chemical rate equation, Eq. 3, becomes

$$\dot{B} = k_f \bar{L} R - k_r B + (1 - k_r/k_{off})S. \quad (7)$$

If the only source of ligand is the cell's secretion, then, far from the cell, the ligand concentration will be negligible and $\bar{L} = 0$. Note that, in the steady state ($\dot{B} = 0$ and S = constant), Eq. 7 predicts that, for an isolated cell in the absence of an external source of ligand, $B = KRS/4\pi Da$, where $K = k_{on}/k_{off}$. This is the result obtained by solving the diffusion equation. Autocrine binding has previously been modeled by using Brownian dynamics simulation techniques, compartmental analysis, and a model that is a hybrid of these (Forsten and Lauffenburger, 1992, 1994a, b). The simplicity of Eq. 7 is attractive, but a comparative study of the published autocrine binding models is needed before definitive comments about their relative merits can be made.

Two receptor populations binding the same ligand

Consider a cell that has on its surface two receptor populations, type 1 and type 2, that bind the same ligand with different rate constants. We can think of all dissociations from type 2 receptors as acting as sources for type 1 receptors, i.e., $S_1 = -\dot{B}_2$. Therefore, from Eq. 7,

$$\dot{B}_1 = k_{f1} \bar{L} R_1 - k_{r1} B_1 - (1 - k_{r1}/k_{off1})\dot{B}_2 \quad (8a)$$

and, similarly,

$$\dot{B}_2 = k_{f2} \bar{L} R_2 - k_{r2} B_2 - (1 - k_{r2}/k_{off2})\dot{B}_1. \quad (8b)$$

We see from these equations that the presence of a second receptor population influences the binding to, and dissociation of, ligands from the first receptor population. For example, in a dissociation experiment, ligands dissociating from population 2 ($\dot{B}_2 \leq 0$) will contribute a positive term to Eq. 8a and slow dissociation of ligands from population 1.

We show in the Appendix that when type 1 and type 2 receptors have identical fundamental rate constants Eq. 8, a and b, reduces to Eq. 3.

THE DIFFUSION-REACTION PROBLEM

Let a cell of radius a be centered at the origin of spherical coordinates. The evolution of the ligand concentration at radial distance $r > a$ is governed by the diffusion equation,

$$\partial_t L = \frac{D}{r^2} \partial_r r^2 \partial_r L. \quad (9)$$

At the surface of the sphere, $r = a$, this equation is subject to the boundary condition that the net flux into the sphere is equal to the rate of change of the bound ligand concentration:

$$4\pi a^2 D \partial_r L = \dot{B}. \quad (10a)$$

The reaction rate can be expressed in terms of the fundamental rate constants and the ligand concentration at the surface $L_a = L(a, t)$:

$$\dot{B} = k_{on} L_a R - k_{off} B. \quad (10b)$$

The boundary condition, Eq. 10a, is a continuum representation that distributes the effects of the receptors uniformly over the cell surface. In this we depart from others who have regarded receptors as discrete absorbing patches distributed randomly on a reflecting surface (Zwanzig and Szabo, 1991). In summary, our fundamental model is based on three assumptions. (1) Outside the cell, the ligand obeys the diffusion equation, Eq. 9. (2) For all time there is radial symmetry; one small area on the cell surface is the same as any other. (3) At the cell surface, reaction between ligands and receptors is governed by a local statement of the law of mass action. In practical terms this means that reacting species can be regarded as well mixed over distance scales that are small compared with the cell radius.

Far from the cell we require that the ligand concentration become independent of position. This leads to the Dirichlet condition:

$$\lim_{r \to \infty} L(r, t) = \text{constant} = \bar{L}. \quad (11)$$

The initial condition compatible with Eq. 11 is

$$L(r, 0) = \text{constant} = \bar{L}. \quad (12)$$

The total number of cell surface receptors is conserved. This means that Eq. 2a still holds, except now R, R_T, and B are surface concentrations.

In the present problem, ligand is neither internalized nor degraded. Thus, the total ligand (free in solution plus bound to the cell surface) is a constant in time, and

$$4\pi \int_a^\infty L(r, t) r^2 \, dr + B(t) = 4\pi \int_a^\infty \bar{L} r^2 \, dr + B_0, \quad (13)$$

where B_0 is the initial level of bound ligand.

Eq. 13 can be obtained directly by integrating the diffusion equation, Eq. 9, and applying the boundary conditions, Eq. 10, a and b. Rearranging the conservation law for ligand, Eq. 13 becomes

$$4\pi \int_a^\infty (L(r, t) - \bar{L}) r^2 \, dr = (B_0 - B(t)). \quad (14)$$

THE METHOD OF WEIGHTED RESIDUALS

We now approximate the partial differential equation for the reaction of diffusing ligands with receptors on a surface by

a simpler problem involving only a finite number of ODE. A general framework for accomplishing this is the MWR (Fletcher, 1984). To apply this method to the present problem it is convenient to introduce the following nondimensional variables corresponding to distance, time, and ligand concentration:

$$x \equiv r/a, \quad \tau \equiv Dt/a^2, \quad \ell = 4\pi a^3(L - \bar{L}). \tag{15}$$

In terms of these variables the diffusion equation for ligand, Eq. 9a, becomes

$$\partial_\tau \ell = \frac{1}{x^2}\,\partial_x x^2 \partial_x \ell. \tag{16a}$$

The boundary conditions at $x = 1$ and $x = \infty$ are

$$\partial_x \ell = \dot{B} = \kappa_{on}(\ell(1, \tau) + \bar{\ell})R - \kappa_{off}B, \tag{16b}$$

and

$$\lim_{x\to\infty} \ell(x, \tau) = 0. \tag{16c}$$

In these equations we have introduced the following notation:

$$\dot{B} \equiv \frac{dB}{d\tau}, \qquad \kappa_{on} \equiv \frac{k_{on}}{(4\pi Da)}, \qquad \kappa_{off} \equiv \frac{k_{off}a^2}{D}, \qquad \bar{\ell} \equiv 4\pi a^3 \bar{L}. \tag{16d}$$

The initial conditions for nondimensional free and bound ligand are

$$\ell(x, 0) = 0, \quad \text{and} \quad B(0) = B_0. \tag{16e}$$

Integrating Eq. 16a over the volume outside the unit sphere and applying the boundary conditions we obtain

$$\partial_\tau \int_1^\infty \ell(x, \tau)x^2\,dx = \int_1^\infty \partial_x x^2 \partial_x \ell = x^2 \partial_x \ell(x)\Big|_1^\infty = -\dot{B}. \tag{17}$$

Integrating Eq. 17 with respect to τ and using the initial conditions we derive a nondimensional form of the conservation law for mass:

$$\int_1^\infty \ell(x, \tau)x^2\,dx = -(B(\tau) - B_0). \tag{18}$$

The next step in the MWR is to separate the problem of satisfying boundary conditions and constraints from the problem of satisfying the partial differential equation itself. This is done by expanding the dependent variable in terms of a set of generalized basis functions, $\psi_k(x)$. Thus, we write the ligand concentration as a sum of the form

$$\ell(x, \tau) = \ell_p(x, \tau) + \sum_{k=1}^{\infty} \alpha_k(\tau)\psi_k(x). \tag{19}$$

The leading term in this expansion, the particular function ℓ_p, is chosen so as to identically satisfy the boundary conditions, Eqs. (16b) and (16c), and the conservation law, Eq. (18). Thus, the ψ_k, which are independent of τ, need to satisfy only the homogeneous form of the boundary conditions and constraints. For the current problem the homogeneous boundary conditions imply that the partial derivatives of the ψ_k with respect to x must vanish at $x = 1$ and that the functions themselves must vanish at $x = \infty$. The homogeneous form of the integral constraints of mass conservation implies that, for all k,

$$\int_1^\infty \psi_k(x)x^2\,dx = 0. \tag{20}$$

Note that because of this equation all the ψ_k must approach zero as x approaches infinity faster than $1/x^3$. We conclude that a reasonable way to construct the basis functions is by taking linear combinations of three consecutive powers of $1/x$ in such a way as to satisfy these requirements. Without loss of generality we can also take $\psi_k(1) = 1$. It can be easily verified that the basis functions obtained by this procedure are

$$\psi_k = -k(k + 3)x^{-(k+3)} + (k + 1)(2k + 5)x^{-(k+4)} - (k + 2)^2 x^{-(k+5)}, \tag{21}$$

where $k = 1, 2, \cdots$.

Because of the required properties of ψ_k and ℓ_p, Eq. 19 will always satisfy the boundary conditions and the conservation of mass for any choice of the amplitude coefficients, $\alpha_k(\tau)$.

In practice, the infinite sum in Eq. 19 must be truncated. Let us keep matters simple by seeing what happens when we include only one term in the expansion, i.e.,

$$\ell(x, \tau) \approx \ell_1(x, \tau) \equiv \ell_p(x, \tau) + \alpha_1(\tau)\psi_1(x). \tag{22}$$

Substituting Eq. 22 into both sides of Eq. 16a and subtracting, we obtain an equation for the so-called first residual:

$$R_1 \equiv \dot{\ell}_p + \dot{\alpha}_1(\tau)\psi_1(x) - \phi_p - \alpha_1(\tau)\phi_1(x), \tag{23a}$$

where,

$$\phi_p \equiv \frac{1}{x^2}\,\partial_x x^2\,\partial_x \ell_p. \tag{23b}$$

and

$$\phi_1 \equiv \frac{1}{x^2}\,\partial_x x^2 \partial_x \psi_1. \tag{23c}$$

We define two functions, $f(x)$ and $g(x)$, as being orthogonal if the inner product,

$$\langle f, g\rangle = \langle g, f\rangle \equiv \int_1^\infty f(x)g(x)x^2\,dx, \tag{24}$$

is identically zero. The only function that is orthogonal to a complete set of functions is the zero function. Consequently,

we obtain the best approximation to the solution of our boundary value problem by choosing the time-dependent coefficient α_1 such that R_1 is orthogonal, or nearly orthogonal, to as many members of a complete set of weighting functions w_j as possible.

A simple choice for the weighting functions in our problem is

$$w_j \equiv (j + 1)x^{-j} \qquad j = 0, 1, 2, \cdots. \tag{25}$$

The fact that this set is complete follows from the existence of the Laurent expansion for any function, analytic on the exterior of the sphere and vanishing at infinity.

Notice that the w_j have a natural ordering and that they are bracketed at one extreme by the constant function $w_o = 1$ and at the other extreme by the delta function $w_\infty = \delta(x - 1)$. Thus, to make R_1 nearly orthogonal to a large number of w_j, it is a good strategy to choose the adjustable parameters so that R_1 is orthogonal to w_0 and w_∞.

Much of the art of efficiently using the MWR lies in the construction of the particular function, ℓ_p. In general, we would like a way to pick particular functions that work for all geometries and does not rely on deep insight or great cleverness. Our approach is to require that ℓ_p be an eigenfunction of the diffusion operator, i.e.,

$$\frac{1}{x^2}\partial_x x^2 \partial_x \ell_p = \beta^2 \ell_p. \tag{26}$$

In the current example this implies that ℓ_p is of the form

$$(a_1/x)\exp(-\beta x) + (a_2/x)\exp(+\beta x), \tag{27}$$

where a_1, a_2, and β are arbitrary functions of τ. These functions are then determined by requiring that the boundary conditions and the conservation of mass be exactly satisfied. This leads to the result

$$\ell_p = \frac{-\dot{B}}{(1 + \beta)x}\exp(-\beta(x - 1)), \tag{28a}$$

where

$$\beta \equiv \sqrt{\frac{\dot{B}}{B(\tau) - B(0)}}. \tag{28b}$$

Kinetic equations for cell surface binding

The N = 0 approximation

The simplest approximation for ℓ is obtained by setting $\alpha_1 = 0$ in Eq. 22, i.e., taking $\ell \approx \ell_p$.

Substituting the expression for ℓ_p, Eq. 28a, into Eq. 16b we obtain

$$\dot{B} = \kappa_{on}(-\dot{B}/(1 + \beta) + \bar{\ell})R - \kappa_{off}B \tag{29a}$$

or, after rearranging terms,

$$\dot{B} = \frac{\kappa_{on}}{1 + \kappa_{on}R/(1 + \beta)}\bar{\ell}R - \frac{\kappa_{off}}{1 + \kappa_{on}R/(1 + \beta)}B. \tag{29b}$$

This result is similar to the ad hoc result, Eqs. 3 and 4, a and b, except for the factor of β. The two results become identical only if we set $\beta = 0$. The presence of β in Eq. 29b produces an improvement over the ad hoc result at small times (cf. comparison between approximate and exact results, below). At large times, β approaches zero and Eq. 29b reduces to the ad hoc result. In the Appendix we present a class of particular functions that lead directly to the ad hoc approximation.

The N = 1 approximation

To obtain the first order correction to Eq. 29b we begin by taking the inner product of R_1, Eq. 23a with w_0 and w_∞.

$$\dot{\alpha}_1\langle 1, \psi_1\rangle = -\langle 1, \bar{\ell}_p\rangle + \langle 1, \phi_p\rangle + \alpha_1\langle 1, \phi_1\rangle. \tag{30a}$$

$$\dot{\alpha}_1\langle \delta(x - 1), \psi_1\rangle = -\langle \delta(x - 1), \dot{\ell}_p\rangle + \langle \delta(x - 1), \phi_p\rangle + \alpha_1\langle \delta(x - 1), \phi_1\rangle. \tag{30b}$$

After evaluating the inner products it is easy to see that Eq. 30a is identically zero. This comes about because the particular function and the basis functions already satisfy conservation of mass exactly (Eqs. 18 and 20). Evaluating the inner products in Eq. 30b and noting that $\phi_1(1) = -38$, we obtain

$$\frac{d}{d\tau}(\alpha_1 + \ell_p(1)) = \beta^2 \ell_p(1) - 38\alpha_1. \tag{31}$$

Letting $\ell_a = \alpha_1 + \ell_p(1)$ and substituting $\ell_p(1) = -\dot{B}/(1 + \beta)$, Eq. 31 becomes

$$\dot{\ell}_a = -(\beta^2 + 38)\dot{B}/(1 + \beta) - 38\ell_a, \tag{32a}$$

where β is given by Eq. 28b. This equation is coupled to the equation for $\dot{B}$ that is obtained by substituting $\ell(1, \tau) \approx \ell_1(1) = \ell_a$ into Eq. 16b.

$$\dot{B} = \kappa_{on}(\ell_a + \bar{\ell})R - \kappa_{off}B, \tag{32b}$$

where, from Eq. 2a, $R = R_T - B$.

From Eq. 16e, it follows that Eq. 32, a and b, are subject to the initial conditions $\ell_a = 0$ and $B = B_0$.

To better understand the physical meaning of Eq. 32, a and b, it is useful to translate the equations into dimensional form:

$$Vd(L_a - \bar{L})/dt = -dB/dt - (38DV/a^2)(L_a - \bar{L}) \tag{33a}$$

$$dB/dt = k_{on}L_aR - k_{off}B \tag{33b}$$

where

$$\frac{V}{4\pi a^3} = \frac{(1 + \beta)}{(38 + \beta^2)} = \frac{D(B - B_0) + \frac{1}{2}\sqrt{D(B - B_0)a^2 dB/dt}}{38D(B - B_0) + a^2 dB/dt} \tag{33c}$$

In this form one can see the direct analogy between the result of applying the MWR and a standard three-compartment analysis of ligand binding. In the latter analysis there is ligand bound on the surface, ligand in an intermediate layer

immediately adjacent to the cell, and ligand in the bulk solution (see Fig. 1). The quantities L_a and V correspond to the concentration and volume of the intermediate compartment and the quantity $38DV/a^2$ corresponds to the transport coefficient between the intermediate layer and the bulk.

The benefit of the MWR lies in that the transport coefficients of the compartment model are directly expressed in terms of the known physical variables. In addition one can see that the volume of the intermediate compartment is not a constant but depends on the instantaneous rate of binding through dB/dt and on the past history of binding through $B - B_0$. Notice that at small times $B - B_0 \approx tdB/dt$ and $V/(4\pi a^3) \to \sqrt{Dt/(4a^2)}$. This means that there is an integrable singularity at $t = 0$ in the equation for dL_a/dt, Eq. 33a. At large times a^2dB/dt is small compared with $D(B - B_0)$ and $V/(4\pi a^3) \to 1/38$. The characteristic time for the transition from one regime to the other is $t_c = a^2/(4D)$. In many applications this time will be short and in these cases it will be a good approximation to take V as a constant equal to $4\pi a^3/38$, i.e., the width of the intermediate compartment equal to $a/38$. In this limit, the term in Eq. 33a describing diffusive transport between the intermediate and outer compartments equals $-4\pi a^2D(L_a - \bar{L})/a$, indicating that the characteristic diffusional length equals the cell radius.

To recover the ad hoc approximation we need to assume not only that $V \approx$ constant but that the intermediate compartment is also in quasi-equilibrium; $dL_a/dt \approx 0$ in Eq. 33a. Under these conditions, $L_a \approx \bar{L} - \dot{B}/(4\pi Da)$. When this result is substituted into Eq. 33b, we directly obtain the ad hoc approximation.

Comparison between approximate and exact results

Irreversible binding

We now compare solutions to the approximate equations we have obtained with exact solutions of our fundamental equation, Eq. 16a, for two special cases of the boundary conditions. The first case, irreversible binding, corresponds to the boundary condition of constant positive concentration, $\bar{L}$ = constant > 0 at $r = \infty$, and to the boundary condition of irreversible binding at $r = a$:

$$4\pi Da^2\partial_r L = \dot{B} = \frac{4\pi Da}{\gamma} L(a, t) \tag{34}$$

where $\gamma = 4\pi Da/(k_{on}R)$ is the ratio of the escape probability to the rebinding probability (see Eq. 6c). Although this case is highly unphysical, we discuss it because it allows for a simple test of our approximate equations.

In the case of irreversible binding, where initially $B = 0$, the exact solution is (Collins and Kimball, 1949)

$$B(\tau) = \ell \int_0^\tau \Phi(\tau)\, d\tau \tag{35}$$

where Φ, the nondimensional flux, is:

$$\Phi = \frac{1}{1 + \gamma}\left(1 + \frac{1}{\gamma} e^{y^2} \operatorname{erfc}(y)\right) \tag{36a}$$

$$y = \tau(1 + 1/\gamma)^2 \tag{36b}$$

We shall use the subscripts *exact*, MWR_0, and *AH* to refer to the solution of the diffusion equation, Eq. 16a; the solution of Eq. 29b, which is the $N = 0$ approximation in the MWR; and the solution of Eq. 3, which is the ad hoc approximation.

At short times, $\tau \ll 1$:

$$\Phi_{exact} \approx \frac{1}{\gamma} - \frac{2}{\sqrt{\pi}\gamma^2}\sqrt{\tau} \tag{37a}$$

$$\Phi_{MWR_0} \approx \frac{1}{\gamma} - \frac{1}{\gamma^2}\sqrt{\tau} \tag{37b}$$

$$\Phi_{AH} = \frac{1}{1 + \gamma}. \tag{37c}$$

At long times, $\tau \gg 1$:

$$\Phi_{exact} \approx \frac{1}{1 + \gamma} - \frac{1}{(1 + \gamma)^2}\frac{1}{\sqrt{\tau}} \tag{38a}$$

$$\Phi_{MWR_0} \approx \frac{1}{1 + \gamma} - \frac{1 - \gamma}{1 + \gamma}\frac{1}{\sqrt{\tau}} \tag{38b}$$

$$\Phi_{AH} = \frac{1}{1 + \gamma}. \tag{38c}$$

The flux in the ad hoc approximation is a constant that equals the limit that the exact result approaches at long times. However, at short times the ad hoc result, Eq. 37c, has the wrong limit. This is not surprising inasmuch as, at $\tau = 0$, the ad hoc equation is incorrect. Initially the ligand concentration is uniform just as in a well mixed system and therefore the binding is characterized by the fundamental, not the effective, rate constants. The MWR_0 solution has the correct short and long time limits. At long times the second order term approaches the exact result for $\gamma \ll 1$, but for $\gamma \geq 1$ is of the wrong sign.

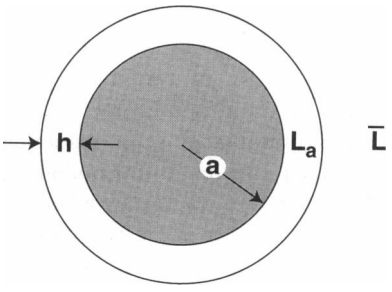

FIGURE 1 The geometry of a three-compartment model. The volume of the intermediate compartment $V = 4\pi(a + h)^3 - 4\pi a^3$. After an initial transient $V = 4\pi a^3/38$. On the surface of the cell of radius a the concentration of bound ligand equals B. In the intermediate compartment, $a < r < a + h$, the ligand concentration is L_a and outside this compartment, $r > a + h$, the ligand concentration equals $\bar{L}$, the bulk value.

Dissociation from a cell with a constant free receptor concentration

A second exact solution exists for the case of dissociation of bound ligand when the free receptor concentration, R, is constant, which holds approximately when $R_T \gg B_0$. In this case the boundary condition at $r = \infty$ is $L = \bar{L} = 0$. After linearizing the reaction rate, we obtain for the boundary condition at $r = a$:

$$4\pi Da^2 \partial_r L = \dot{B} = \frac{4\pi Da}{\gamma}\left(L(r,t) - \frac{\kappa}{4\pi a^3 B}\right). \quad (39)$$

In the above equations, γ is as defined previously (Eq. 6c) and

$$\kappa = 4\pi a^3 k_{off}/(k_{on}R) \quad (40)$$

is a nondimensional dissociation constant.

When the initial bound ligand is B_0, the exact solution is (see pp. 349–350 in Carslaw and Jaeger, 1959)

$$B(\tau) = \frac{2\kappa B(0)}{\pi}\int_0^\infty \frac{e^{-\pi u^2}u^2\,du}{[u^2(\gamma+1)-\kappa]^2 + [\gamma u^3 - \kappa u]^2}. \quad (41)$$

At short times, $\tau \ll 1$:

$$\frac{B_{exact}}{B_0} \approx 1 - \frac{\kappa}{\gamma}\tau \quad (42a)$$

$$\frac{B_{MWR_0}}{B_0} \approx 1 - \frac{\kappa}{\gamma}\tau \quad (42b)$$

$$\frac{B_{AH}}{B_0} \approx 1 - \frac{\kappa}{1+\gamma}\tau. \quad (42c)$$

At long times, $\tau \gg 1$:

$$\frac{B_{exact}}{B_0} \approx \frac{1}{2\kappa\pi^{1/2}\tau^{3/2}} + \frac{3(2\gamma+2-\kappa)}{4\kappa^2\pi^{1/2}\tau^{5/2}} \quad (43a)$$

$$\frac{B_{MWR_0}}{B_0} \approx \exp\left(-\frac{\kappa}{1+\gamma}\tau\right) \quad (43b)$$

$$\frac{B_{AH}}{B_0} = \exp\left(-\frac{\kappa}{1+\gamma}\tau\right). \quad (43c)$$

Again, at short times the MWR_0 solution goes to the correct limit whereas the ad hoc solution does not. The ad hoc and MWR_0 equations, Eqs. 9 and 29b, differ only at short times, $\tau \leq 1$. If the change in the bound ligand concentration is negligible during this transient period the MWR_0 and ad hoc solutions will be identical. From Eq. 42a, we see that this occurs when $\kappa/\gamma \ll 1$. When this condition is met, we can set $\beta = 0$ in Eq. 29b and Eq. 33c. In Fig. 2, *a* and *b*, where $\gamma/\kappa = 10^{-4}$ and 10^{-2}, the exact, ad hoc, and MWR_0 solutions all agree at short times. In Fig. 2, *c* and *d*, where $\gamma/\kappa = 1$ and 10, there is no longer agreement at short times between the ad hoc and MWR_0.

At long times neither of the approximate solutions predict the correct behavior. Both predict exponential decay, whereas the decay of the exact solution is proportional to $\tau^{-3/2}$. The time that characterizes the transition from exponential decay (and the validity of the approximate solutions) to $\tau^{-3/2}$ decay (and the failure of these approximations) occurs when the two terms on the right of Eq. 43a are of equal magnitude. Calling this nondimensional time τ^* we have

$$\tau^* = \frac{3(\gamma+1)}{\kappa} - \frac{3}{2} = \frac{3D}{a^2 k_r} - \frac{3}{2} \quad (44)$$

where k_r is the effective reverse rate constant given by Eq. 4b.

Eq. 44 shows that if dissociation of ligand from cell surface receptors is slow compared with diffusion away from the cell, i.e., if $2D/a^2 \gg k_r$, then $\tau^* \gg 1$. This means that both the MWR_0 and the ad hoc treatments will be excellent approximations over the wide range of times $0 \leq \tau \leq \tau^*$. However, the approximations will break down after a very short time when ligands rapidly dissociate and slowly diffuse, i.e., when $k_r \gg 2D/a^2$ or, equivalently, $2(\gamma+1)/\kappa \ll 1$. In Fig. 2, *a* and *b*, where $2(\gamma+1)/\kappa = 2 \times 10^4$ and 2×10^2, the exact and approximate solutions agree to within a few percent until 0.01% or less of the bound ligand remains on the cell surface. In Fig. 2, *c* and *d*, where $2(\gamma+1)/\kappa =$ 2.2 and 0.22, the breakdown in the approximate treatments occurs much sooner.

For association, the MWR_0 is a good approximation at both short and long times for all parameter values. The ad hoc approximation fails to give correct behavior at short times if $\gamma < 1$. Comparisons with exact solutions in the case of dissociation provide a more stringent test of the two solutions. Both the MWR_0 and the ad hoc approximation fail at suf-

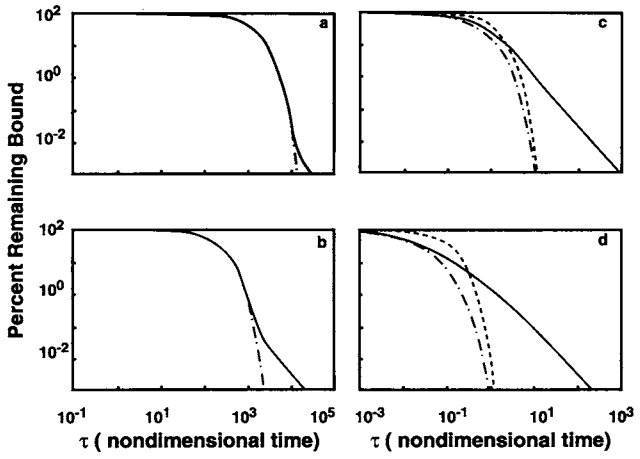

FIGURE 2 Comparison of exact and approximate solutions for dissociation of ligand from cell surface receptors. The solid curves are the exact solutions of the diffusion equation, Eq. 41, when the concentration of free receptors is assumed to be constant and the binding is reversible. The dot-dash curves (· – ·) are solutions of Eq. 29b, the first approximation obtained by using the MWR, and the dashed curves (- - -) are solutions of the ad hoc approximation, Eqs. 3 and 4, a and b. In *a* and *b*, the ad hoc and MWR_0 solutions are indistinguishable. (*a*) $\gamma = 1$ and $\kappa = 0.0001$. (*b*) $\gamma = 1$ and $\kappa = 0.01$. (*c*) $\gamma = 0.1$ and $\kappa = 1$. (*d*) $\gamma = 0.01$ and $\kappa = 10$. $\tau = Dt/a^2$ is the nondimensional time, $\kappa = 4\pi a^3 k_{off}/(k_{on}R)$ is the nondimensional dissociation rate, and $\gamma = 4\pi Da/Rk_{on}$ is the ratio of the escape probability to the rebinding probability.

ficiently long times, but at least the MWR_0 is always correct at short times. One might hope to extend the validity of the MWR_0 to longer times by including additional terms in the expansion. However, we have found that including only a single additional term, i.e., the MWR_1, and simultaneously solving Eq. 32, a and b (results not shown), does not produce the hoped for improvement. This means that inclusion of an intermediate compartment as in Fig. 2 simply improves the solution where it is already quite accurate but does not significantly improve the behavior of the solution at long times in cases where the MWR_0 is bad. This is true even if the intermediate compartment is chosen in an optimal fashion. We conclude that the added complexity of including a few intermediate compartments in the description of ligand binding is rarely justified.

DISCUSSION

To predict how the concentration of ligands bound to cell surface receptors changes in time requires knowing the concentration of free ligands at the cell surface. Because receptors are not uniformly distributed in space but confined to cells, the free ligand concentration will not be uniformly distributed in the volume outside of the cells, except possibly at the start of the experiment and at equilibrium. This means that in general one must solve a diffusion-reaction problem to determine the time development of the bound ligand concentration. As we have formulated the problem, outside of the cell the free ligand concentration obeys the diffusion equation, Eq. 9, far from the cell it is constant, and at the cell surface the net diffusive flux of ligands into the cell equals the rate of change of the bound ligand concentration, Eq. 10b. To solve the diffusion equation with its nonlinear boundary condition at the surface requires numerically solving the partial differential equation.

Starting from the diffuse-reaction problem we have introduced an approximation scheme based on the MWR that allows us to convert the problem to that of solving a set of coupled nonlinear differential equations. In the simplest approximation (MWR_0), we obtain a single differential equation, Eq. 29b, which can be thought of as a chemical rate equation with effective rate constants. The effective rate constants are functions only of the free receptor concentration, except during an initial transient period when they are also functions of the instantaneous rate of binding and the amount bound or dissociated since the start of the experiment. The transient lasts for a time $t \approx a^2/D$, where a is the cell radius and D is the ligand diffusion coefficient. For typical cells, $a \approx 5 \times 10^{-4}$ cm, and the transient period is at most a few seconds long, even for large proteins, $D \approx 10^{-7}$ cm^2/s. If binding is negligible during this time, the effects of the transient can be neglected and the MWR_0 reduces to the ad hoc approximation, which is discussed in the second section of this paper. When ligands dissociate from cell surface receptors the approximate solutions we have discussed all predict that at long times the decay of the concentration of surface bound ligand will be exponential. This is incorrect; solution of the diffusion equation shows that eventually the decay of surface-bound ligand will go as $t^{-3/2}$. However, if diffusion away from the cell is fast compared with dissociation from the cell surface, then the onset of the $t^{-3/2}$ decay will not occur until dissociation is essentially complete. In this parameter range the MWR_0 gives excellent results until less than 0.01% of the initially bound ligand remains on the cell surface. (See Fig. 2, *a* and *b*). If diffusion is slow compared with dissociation, then only at short times is the MWR_0 valid (see Fig. 2, *c* and *d*).

We have also demonstrated how to extend the MWR to obtain higher order approximations. In particular, we obtained one such approximation that required the solution of two coupled ODE. We showed that after an initial transient the equations corresponded to those one would obtain in a standard three-compartment analysis, the three compartments corresponding to surface-bound ligand, free ligand in a layer adjacent to the cell, and ligand in bulk solution (see Fig. 1). What is novel about our result is that it specifies the volume V of the inner compartment to be 1/38 of the volume of the cell. (This value, 1/38, comes from our choice of basis functions, Eq. 21. Other basis functions would give other values, but the basis functions we chose are the natural ones for a spherical geometry.) In the standard compartmental analysis treatment, V is taken as a free parameter. However, we have found that this extension of the MWR does not significantly improve the solution in cases where the MWR_0 is a poor approximation.

Although we have focused on the binding of ligands to receptors on a spherical cell, the MWR can be used in any geometry, even when the diffusion equation has no steady-state solution. It can be generalized to more complicated binding problems as well. We have shown this in the Appendix where we have treated the problems of autocrine binding and binding to cells with two different receptor populations on their surfaces. We expect that the method will find additional applications.

APPENDIX

Obtaining the ad hoc result from the MWR

Consider the following set of particular functions:

$$\ell_p = \frac{a_1}{x}\exp(-\beta(x-1)^n) \qquad n \geq 2 \tag{A1}$$

From the boundary condition at $x = 1$, Eq. 16b, we have that $a_1 = \dot{B}$ and therefore $\ell_p(1, \tau) = -\dot{B}$. Using only the particular function, Eq. A1, to approximate $\ell(1, \tau)$, Eq. 18, we have $\ell(1, \tau) \approx -\dot{B}$. Substituting this expression for $\ell(1, \tau)$ into Eq. 16b and solving for $\dot{B}$ we obtain the ad hoc result.

Two receptor populations binding the same ligand

When there are two receptor populations on the cell surface that bind the same ligand the boundary condition, Eq. 16b becomes

$$\partial_x \ell = \dot{B} = \dot{B}_1 + \dot{B}_2, \tag{A2a}$$

where

$$\dot{B}_1 = \kappa_{1on}(\ell(1, \tau) + \bar{\ell})R_1 - \kappa_{1off}B_1, \quad \text{(A2b)}$$

and $\dot{B}_2$ satisfies a similar equation. Choosing a particular function as before, Eq. A1, that satisfies the boundary conditions we have $\ell(1, \tau) \approx -\dot{B}$. Substituting into Eq. A2b we obtain

$$\dot{B}_1 = \kappa_{1on}(-\dot{B}_1 - \dot{B}_2 + \bar{\ell})R_1 - \kappa_{1off}B_1. \quad \text{(A3)}$$

Solving for $\dot{B}_1$ we obtain

$$\dot{B}_1 = \frac{\kappa_{1on}}{1 + \kappa_{1on}R_1}\bar{\ell}R_1 - \frac{\kappa_{1off}}{1 + \kappa_{1on}R_1}B_1 - \frac{\kappa_{1on}R_1}{1 + \kappa_{1on}R_1}\dot{B}_2. \quad \text{(A4)}$$

Eq. A4 is the nondimensional form of Eq. 8a.

Autocrine binding

Consider a cell that releases at a constant rate S ligands per second. The nondimensional rate of release is $s = a^2S/D$. If we let $\dot{B}_2 = s$ in Eq. A4, we obtain the nondimensional form of Eq. 7.

The equations for the two receptor population model reduce to the single receptor population model when the rate constants are identical

Using Eq. 8b to eliminate $\dot{B}_2$ from Eq. 8a and rearranging terms, one obtains

$$\dot{B}_1(1 + \delta_1\delta_2) = (1 + \delta_2)(k_{on1}\bar{L}R_1 - k_{off1}B_1) - \delta_1(k_{on2}\bar{L}R_2 - k_{off2}B_2), \quad \text{(A5a)}$$

where $\delta_i = R_ik_{on1}/k_+$. Similarly,

$$\dot{B}_2(1 + \delta_1\delta_2) = (1 + \delta_1)(k_{on2}\bar{L}R_2 - k_{off2}B_2) - \delta_2(k_{on1}\bar{L}R_1 - k_{off1}B_1). \quad \text{(A5b)}$$

Adding Eq. A5, a and b, and setting $k_{on1} = k_{on2} = k_{on}$, $k_{off1} = k_{off2} = k_{off}$, $R = R_1 + R_2$, $B = B_1 + B_2$, and $\dot{B} = \dot{B}_1 + \dot{B}_2$, we obtain

$$(1 + \delta_1\delta_2)\dot{B} = k_{on}\bar{L}R - k_{off}B. \quad \text{(A6)}$$

Dividing through by $(1 + \delta_1\delta_2)$ we obtain Eq. 3.

The authors thank Ronald Beatty for excellent technical assistance.
This work was supported by National Institutes of Health grants GM35556 (B.G.) and AI21002 (M.D.) and performed under the auspices of the U.S. Department of Energy.

REFERENCES

Berg, O. G. 1978. On diffusion-controlled dissociation. *Chem. Phys.* 20: 47–57.

Berg, H. C., and E. M. Purcell. 1977. Physics of chemoreception. *Biophys. J.* 20:193–219.

Carslaw, H. S., and J. C. Jaeger. 1959. Conduction of Heat in Solids, 2nd ed. Oxford University Press, Glasgow. 510 pp.

Collins, F. C., and G. E. Kimball. 1949. Diffusion-controlled reaction rates. *J. Colloid Sci.* 4:425–437.

DeLisi, C. 1983. Role of diffusion regulation in receptor-ligand interactions. *Methods Enzymol.* 93:95–109.

Eigen, M. 1974. Diffusion control in biochemical reactions. *In* Quantum Statistical Mechanics in the Natural Sciences. S. L. Minz and S. M. Wiedermayer, editors. Plenum Press, New York. 37–61.

Erickson, J., B. Goldstein, D. Holowka, and B. Baird. 1987. The effect of receptor density on the forward rate constant for binding of ligands to cell surface receptors. *Biophys. J.* 52:657–662.

Fletcher, C. A. J. 1984. Computational Galerkin Methods. Springer-Verlag, New York. 309 pp.

Forsten, K. E., and D. A. Lauffenburger. 1992. Autocrine ligand binding to cell receptors: mathematical analysis of competition by solution "decoys." *Biophys. J.* 61:518–529.

Forsten, K. E., and D. A. Lauffenburger. 1994a. Probability of autocrine ligand capture by cell surface receptors: implications for ligand secretion measurements. *J. Comput. Biol.* 1:15–23.

Forsten, K. E., and D. A. Lauffenburger. 1994b. The role of low-affinity interleukin-2 receptors in autocrine ligand binding: alternative mechanisms for enhanced binding effect. *Mol. Immunol.* 31:739–751.

Goldstein, B. 1989. Diffusion limited effects of receptor clustering. *Comments Theor. Biol.* 1:109–127.

Goldstein, B., R. G. Posner, D. C. Torney, J. Erickson, D. Holowka, and B. Baird. 1989. Competition between solution and cell surface receptors for ligand: dissociation of hapten bound to surface antibody in the presence of solution antibody. *Biophys. J.* 56:955–966.

Schwartz, M. 1976. The adsorption of coliphage to its host: effect of variations in the surface density of receptor and in phage-receptor affinity. *J. Mol. Biol.* 103:521–536.

Shoup, D., and A. Szabo. 1987. Role of diffusion in ligand binding to macromolecules and cell-bound receptors. *Biophys. J.* 40:33–39.

Zwanzig, R., and A. Szabo. 1991. Time dependent rate of diffusion-influenced ligand binding to receptors on cell surfaces. *Biophys. J.* 60: 671–678.