Ligand-Receptor Interaction Rates in the Presence of Convective Mass Transport

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ABSTRACT The rate of binding of a ligand to receptors on the cell surface can be diffusion limited. We analyze the kinetics of binding, diffusion-limited in a stationary liquid, in the presence of convective mass transport. We derive a formula that expresses the reaction kinetics in terms of the mass transfer coefficient. A moderately transport-limited kinetics is not readily recognizable from the shape of the binding curve and may lead to erroneous estimates of the rate coefficients. We apply our results to practically important cases: a cell suspension in a stirred volume of liquid and a confluent cell colony under a laminar stream. Using typical numbers characterizing the ligand-receptor interactions, we show that stirring and perfusion can be important factors determining the reaction rates. With the confluent colony, the early reaction kinetics requires a different treatment, and we provide it for the case of low receptor occupancy. We show that, even with a fast perfusion, a cell monolayer can transiently generate a zone of depletion of the ligand, and that would affect the early stages of the reaction. Our results are expressed in a simple analytical form and can be used for the design and interpretation of experimental data.

INTRODUCTION

The problem of measurement of the association and dissociation rate constants of ligand binding to cell surface receptors has received much attention in the biological literature (Maguire et al., 1977; Sklar, 1987; Bylund and Toews, 1993). The simplest reaction between a ligand and receptor is represented as

$$\text{Ligand} + \text{Receptor} \leftrightarrow \text{Complex}, \tag{1}$$

which obeys the rate law

$$\frac{dC}{dt} = k_{on}LR_o - (k_{on}L + k_{off})C, \tag{2}$$

where C is the surface concentration of ligand-receptor complexes (mol/cm^2), R_o is the total surface concentration of the receptors (mol/cm^2), and L is the volumetric concentration of the free ligand (mol/cm^3). If the receptors on the cell surface are sufficiently numerous and of high affinity, it may happen that the rate of removal of the ligand by the cell would be comparable to the rate at which new ligand is supplied by diffusion. Alternatively, if we are considering dissociation initiated by an abrupt dilution of the sample, the ligand released from the receptors may bind back to the cell before it is dispersed by diffusion. In both cases, the ligand concentration near the cell surface L_s, which actually determines the binding rate, will be different from the bulk concentration L_o: it would be less than L_o if the ligand flows toward the cell and greater than L_o if the ligand flows away from the cell. This situation will be referred here to as diffusion-limited. Thus, two sets of rate coefficients can be introduced: apparent rates $k'_{on(off)}$, which assume $L = L_o$ in Eq. 2, and molecular rates $k_{on(off)}$, which assume $L = L_s$.

Diffusion-limited binding to a cell should be distinguished from diffusion-limited binding to an isolated receptor. Binding to a single receptor is regarded as diffusion-limited if the association rate constant is on the order of 10^8–10^9 M^{-1}s^{-1} (Lauffenberger and Linderman, 1993). Such receptors create depletion zones whose size is approximately the size of the receptor. When receptors are positioned on a cell surface, they may also create a depletion zone that would extend over the characteristic size of the cell. This effect depends on both the association rate of individual receptors and their surface density (Lauffenberger and Linderman, 1993). We will be interested in this larger-scale effect, and the term "diffusion-limited" will be used to refer to a situation when binding to receptors on a surface proceeds at a rate different than binding to isolated receptors. The molecular rate k_{on} in Eq. 2 characterizes binding to isolated receptors that may or may not be diffusion-limited by itself. If it is diffusion-limited, the ligand concentration L_s, if measured very close to the receptor, will be different from L_s at points on the surface distant from the receptor. Therefore we have to more clearly define L_s. Concentration L_s should be interpreted as the surface concentration distant from the receptor (DeLisi and Wiegel, 1981). If the receptors occupy only a small fraction of the cell surface, this concentration will be very close to the average concentration. If k_{on} is not diffusion limited, L_s will be the same everywhere at the surface, and there will be no ambiguity.

The molecular rates are often of primary interest, because they reflect the inherent properties of receptors, and in this respect the diffusion-limited conditions in kinetic measurements are undesirable. Then the question is, what must be

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done to bring the experimentally accessible apparent association/dissociation rates close to the molecular rates? Stirring and perfusion are the common ways to facilitate ligand exchange between the cells and the environment. Here we show how to decide whether stirring of a cell suspension or perfusion of a confluent cell monolayer under given conditions would permit direct experimental measurement of the molecular rates. Our approach is quite general and can be applied to a variety of conditions. It has certain limitations, though; thus, with the confluent cell colony, the early reaction kinetics requires a special treatment and we provide it for the case of a low receptor occupancy.

In our treatment we use results from the heat and mass transport theory. The mass transport theory has been developed and successfully applied to industrial processes for decades. However, the need for a similar approach in quantitative cell biology is frequently overlooked. This work is an attempt to bring the importance of the problem to the attention of experimental biologists and to present readily applied formulas for analyzing the effect of convection on the ligand-receptor reaction rates.

THEORY

We first describe the effect of mass transport rate on the kinetics of ligand binding in general terms, without specifying the mechanism of mass transport. The ligand exchange between the cell surface and the environment can be characterized using the mass transfer coefficient, which is a convenient approach when the exact solution cannot be obtained (Cussler, 1984). Let the mass flow rate (the amount of material transferred through a unit area in a unit time) be Φ (mol $\cdot$ cm^{-2} $\cdot$ s^{-1}). The mass transfer coefficient k_t is defined as

$$k_t = \frac{\Phi}{L_1 - L_2}, \tag{3}$$

where L_1 and L_2 refer to two concentrations characteristic of the system (Cussler, 1984). It follows from this definition that the units of k_t are cm/s. The flow of ligand to the surface is equal to the reaction rate:

$$\Phi = \frac{dC}{dt}, \tag{4}$$

and L_1 and L_2 correspond to L_o and L_s. The relationship between the apparent rates, derived in the assumption of $L = L_o$, and molecular rates can be found from the system of two equations:

$$\frac{dC}{dt} = k_t(L_o - L_s) = k_{on}L_sR_o - (k_{on}L_s + k_{off})C \tag{5a}$$

$$\frac{dC}{dt} = k_t(L_o - L_s) = k'_{on}L_oR_o - (k'_{on}L_o + k'_{off})C. \tag{5b}$$

Substituting L_s from (5a) into (5b) we find

$$\frac{k'_{on(off)}}{k_{on(off)}} = \frac{k_t}{k_t + k_{on}(R_o - C)} \tag{6a}$$

or

$$\frac{k'_{on(off)}}{k_{on(off)}} = 1 - \frac{k'_{on}(R_o - C)}{k_t}. \tag{6b}$$

Note that, in general, the ratio of the apparent rate coefficients to molecular constants is a function of time due to the dependence of C on time. Equation 6 remains valid when R_o or the molecular rate constants change with time, as they do during endocytosis, up-regulation, phosphorylation, etc.

Equation 6 can be used to determine whether the rates measured in the experiment are close to the molecular rates. Theoretically, they can even be used to calculate the molecular constants given the apparent rates and receptor concentration; however, in experiments in cell biology, k_t is seldom known with high precision and therefore a better approach is to ensure that $k_t \gg k_{on}(R_o - C)$ or $k_t \gg k'_{on}(R_o - C)$. It follows from Eq. 6 that these two conditions are equivalent. Because $R_o \geq R_o - C$, a simpler condition $k_{dif} \gg k'_{on}R_o$ can be used for practical purposes.

Formulae equivalent to (6a), expressed usually in terms of the receptor number and the area of the absorbing surface of the receptor, have been well known for purely diffusion transport (Lauffenberger and Linderman, 1993). A more general formulation we are using here makes it possible to analyze a broader variety of cases. When mass transport has two independent components, diffusion and convection, the overall mass transfer coefficient can be represented by the sum of two terms:

$$k_t = k_{dif} + k_{con}, \tag{7}$$

where k_{dif} and k_{con} are the contributions due to diffusion and convection, respectively (Cussler, 1984). Thus the analysis of any particular experimental system should include the following steps:

1) Determine if $k_{dif} \gg k'_{on}R_o$. It is convenient to introduce a parameter p_{dif} describing the effectiveness of diffusion:

$$p_{dif} = \frac{k_{dif}}{k_{on}R_o} \tag{8}$$

Corresponding parameters for convection p_{con} or mass transfer in general p_t can be defined similarly. The condition $k_{dif} \gg k_{on}R_o$ is then the same as $p_{dif} \gg 1$; if this is true, then even in a still liquid the experimentally accessible apparent rates would be close to the molecular rates.

2) If $p_{dif} \sim 1$ (or $k_{dif} \sim k'_{on}R_o$), then, at least while $C \ll R_o$, the apparent and molecular rate constants might differ significantly because of local depletion or accumulation of the ligand, and ensuring measurement of the molecular rate constants would require additional convective transport. Then we need to be able to predict whether convection can enhance k_t sufficiently to meet the condition $p_t = p_{dif} +$

$p_{con} \gg 1$. The convective mass transfer coefficient k_{con} has been determined for a number of specific experimental conditions. The aim of the following sections is to evaluate the magnitudes of p_{dif} and p_{con} for systems important in cell biology.

Equation 6 relate the molecular rate coefficients to the apparent rates and the mass transfer coefficient. However, the value that is directly obtained in experiments is $C(t)$, and it is important to know how $C(t)$ is affected by a limited rate of mass transfer. To integrate Eq. 5b, we need to assume that k_t does not depend on time; that is, we restrict ourselves to processes that are quasi-stationary with respect to mass transfer (Example 4 below provides the case when this is not true). Equation (5b) can be rearranged into an integratable form by substituting the apparent rates for molecular rates using Eq. 6a and separating the variables:

$$\frac{k_t + k_{on}R_o - k_{on}C}{k_t k_{on}L_oR_o - k_t(k_{on}L_o + k_{off})C}\,dC = dt. \tag{9}$$

So far we have been dealing with the rate coefficients regardless of the particular experimental conditions; now we need to specify them. Suppose that we are interested in the kinetics of binding of the ligand added at $t = 0$ to cells that have no prebound ligand; the initial condition is thus $C = 0$ at $t = 0$. Then integration yields

$$\frac{C_\infty}{p_tR_o}\left(\frac{C}{C_\infty}\right) - \left(1 + \frac{1}{p_t} - \frac{C_\infty}{p_tR_o}\right)\ln\left(1 - \frac{C}{C_\infty}\right) = k_+t, \tag{10}$$

where

$$k_+ = k_{on}L_o + k_{off} \tag{11}$$

is the relaxation rate at an infinitely fast transport of the ligand, and

$$C_\infty = \frac{k_{on}}{k_+}L_oR_o \tag{12}$$

is the equilibrium concentration of ligand-receptor complexes.

To analyze Eq. 10, consider three limiting cases:

1) $p_t \gg 1$. In this limit the reaction is not limited by transport rate, and we obtain the familiar exponential form:

$$C = C_\infty(1 - e^{-k_+t}). \tag{13}$$

2) $C_\infty/p_tR_o \ll 1$. This condition can be interpreted as an arbitrary p_t and a low ligand concentration (much less than the dissociation constant k_{off}/k_{on}); it is also equivalent to $k_t/k_{on}C_\infty \gg 1$, which imposes less strict limitations on k_t than the condition $p_t \gg 1$. In this case we also have the exponential dependence, with $k_+ \approx k_{off}$ and reduced rate constants, according to (6a):

$$C = C_\infty(1 - e^{-(p_t/1+p_t)\cdot k_{off}t}). \tag{14}$$

3) Finally, for an arbitrary p_t and $C_\infty/R_o \to 1$ (L_o is much greater than the dissociation constant so that the receptors would be saturated when the system reached equilibrium), Eq. 10 becomes

$$\frac{1}{p_t}\left(\frac{C}{C_\infty}\right) - \ln\left(1 - \frac{C}{C_\infty}\right) = k_+t \tag{15}$$

Limitations due to a finite transport rate are important only at the early stages of binding, while the flow to the cell surface is fast. As binding approaches saturation, the flow rate decreases, and the transport can easily provide this amount of ligand without impeding the reaction. Although the early kinetics of ligand binding at high ligand concentrations is slower than exponential, an attempt to approximate this nonexponential binding curve with a single exponent may lead to a deceptively good fit. For instance, for $p_t = 1$, the rate constants based on the binding data during the time required to saturate 99.5% of the receptors will be underestimated by 43% (and by 50% at a low ligand concentration, according to Eq. 14) with a slight (5%) overestimation of the number of binding sites (Fig. 1 *A*). For a smaller value of $p = 0.2$, the curve can be more easily distinguished from the best-fit exponent (Fig. 1 *B*). Whether the exponent is generated by adjusting both the receptor number and binding rate (curve *a*) or the binding rate only while holding the receptor number constant (curve *b*), the characteristic linear stretch of the transport-limited binding curve in the beginning with an abrupt saturation may sometimes enable one to diagnose the kinetics severely limited by transport.

We now consider applications of the result (6) to four biologically important situations: diffusion-dominated transport to a spherical cell in suspension, mixed diffusional/convective transport to a spherical cell in a stirred suspension, and diffusion- and convection-dominated transport to a confluent monolayer of cells in a laminar flow. We shall notice, however, that in the latter case the binding at sufficiently early times is diffusion dominated. Because no

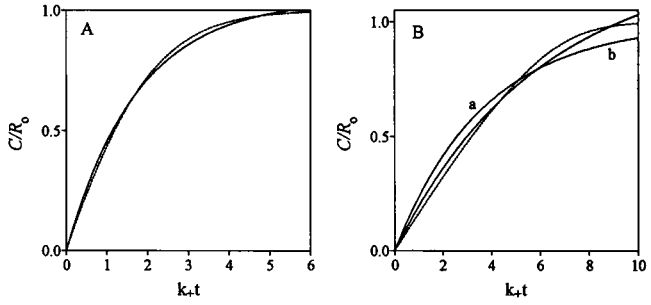

FIGURE 1 Kinetics of the transport-limited formation of ligand-receptor complexes, expressed as the ratio of the concentration of occupied receptors at time t to that at $t = \infty$ at a high ligand concentration. Theoretical binding curves (*dotted lines*) for $p_t = 1$ (*A*) and $p_t = 0.2$ (*B*) and the best-fitted one-site exponential association curves of the type $Y[1 - \exp(-xt)]$ (*solid lines*) generated by the GraphPad Prizm computer program are shown. To generate the curve *b* in (*B*), the parameter Y was set to the correct value of 1; for the curve *a* both parameters Y and X were optimized.

constant value of k_t describes this process, we must directly solve the diffusion problem. Because of the mathematical complexity of the general case, we give the rigorous solution for low ligand concentrations only.

Example 1. Isolated spherical cell: diffusional transport

The apparent rates for the purely diffusional transport between an isolated spherical cell and the environment can be easily obtained by using the mass transfer approach. Let the cell diameter be d. If the time t_e required for diffusion processes to reach equilibrium on the scale of a cell size is much shorter than the ligand-receptor reaction times ($t_e \approx d^2/D$, where D is the diffusion coefficient, and for $d = 10$ μm and $D = 10^{-6}$ cm^2/s it is close to 1 s), the ligand concentration can be determined from the spherically symmetric steady-state diffusion equation. Its solution for the space outside the sphere is

$$L = \frac{A}{r} + L_o, \tag{16}$$

where r is the radial coordinate ($r \geq d/2$) and A is a parameter independent of spatial coordinates, determined by the boundary conditions. The flow at the cell surface is given by

$$\Phi = -D\frac{4A}{d^2}. \tag{17}$$

Dividing it by

$$L_o - L_s = L_o - L\left(\frac{d}{2}\right) = \frac{2A}{d}, \tag{18}$$

we have

$$k_{dif} = \frac{2D}{d}. \tag{19}$$

Substituting it for k_t in Eq. 6a we obtain

$$\frac{k'_{on(off)}}{k_{on(off)}} = \frac{\frac{2D}{d}}{\frac{2D}{d} + k_{on}(R_o - C)}, \tag{20}$$

which is similar to equations that can be found in Shoup and Szabo (1982) and Lauffenberger and Linderman (1993). Even though this is not a new result, we provide it for completeness. It also follows from our derivation that formula (20) holds for a variety of boundary conditions at the cell surface and not only for linear proportionality between the concentration at the cell surface and the flow as assumed in the cited works. For instance, it can be a reversible binding with slowly (on the scale of d^2/D) varying parameters.

The typical values of the receptor numbers per cell are 5×10^2 to 5×10^5 (Lauffenberger and Linderman, 1993), which for an average-sized cell with a surface area of 300 μm^2 converts into $R_o \approx 0.3$–300 fmol/cm^2. The ligand-receptor association rates are typically within 10^4-10^7 M^{-1} s^{-1} (Lauffenberger and Linderman, 1993; Wank et al., 1983; Sklar et al., 1985; Maguire et al., 1977), providing the range for the reaction term $k_{on}R_o$ between 3×10^{-9} and 3×10^{-3} cm/s. Diffusion coefficients for biological molecules vary from 10^{-7} to 10^{-5} cm^2/s (Cantor and Schimmel, 1980); if $d = 10$ μm, we can see that the diffusional term $2D/d$ can vary between 10^{-4} and 10^{-2} cm/s and thus can be comparable to the reaction term $k_{on}R_o$. For example, binding of the nerve growth factor to PC12 cells with 160,000 receptors/cell and association rate constants on the order of 10^7 cm^2/s (Woodruff and Neet, 1986) appears to be partially diffusion limited.

Example 2. Isolated spherical cell: mixing

We consider a suspension of cells with a rotating bar that creates turbulent mixing. The problem of mass transfer in a turbulent flow to spherical particles has been treated by many authors (Harriott, 1962; Calderbank, 1967; Levins and Glastonbury, 1972; Batchelor, 1980; Armenante and Kirwan, 1989). The majority of investigators used the Froessling equation

$$\frac{k_t d}{D} = 2 + B\mathbf{Re}^{n_1}\mathbf{Sc}^{n_2}, \tag{21}$$

derived originally for laminar flow around a particle and modified it to include turbulent conditions (Harriott, 1962). In this equation, $\mathbf{Re} = \rho v l/\mu$ (Reynolds number; ρ and μ are the density and viscosity of the liquid, respectively, and v and l are the velocity and length characteristic of the motion), $\mathbf{Sc} = \mu/D\rho$ (Schmidt number), and B, n_1 and n_2 are coefficients. As we have seen earlier (Eq. 19), the constant 2 on the right side describes the diffusional transport; the term $B\mathbf{Re}^{n_1}\mathbf{Sc}^{n_2}$ represents the contribution of convection. Definition of the Reynolds number allows a certain freedom in choosing the parameters. The characteristic length is, naturally, the cell diameter. The characteristic velocity has been chosen in different ways by different authors; a good agreement with experimental data has been achieved by setting it equal to the root mean square velocity in the eddies (Levins and Glastonbury, 1972; Armenante and Kirwan, 1989). The latter can be expressed through the power input per unit mass of fluid ϵ. If a particle is smaller than the minimal eddy size, a condition that can be expressed as

$$d \ll [\mu^3/\rho^3\epsilon]^{1/4} \tag{22}$$

then the Reynolds number is

$$\mathbf{Re} = d^{4/3}\epsilon^{1/3}\rho/\mu \tag{23}$$

(Armenante and Kirwan, 1989). The best fit of experimental data, obtained with 10 μm neutral density particles, to (21)

was achieved by setting $B = n_1 = 0.52$, $n_2 = 1/3$ (Armenante and Kirwan, 1989); the final correlation is thus

$$\frac{k_t d}{D} = 2 + 0.52\left(\frac{\rho}{\mu}\right)^{0.29} \epsilon^{0.17} d^{0.69} D^{-0.33}. \quad (24)$$

The quantity ϵ is related to the total power P dissipated by a rotating impeller as $p = V\rho\epsilon$, where V is the stirred volume. To find the total power input, the following relationship is used:

$$P = \rho L^5 n^3 \cdot f(\mathbf{Re}_i), \quad (25)$$

where L is the diameter of the impeller, n is its revolution speed (rev/s), and $f(\mathbf{Re}_i)$ is an experimentally determined function of the Reynolds number of the impeller ($\mathbf{Re}_i = \rho n L^2/\mu$) (Bates et al., 1966).

For conditions normally used in experiments with biological cells, Eq. 24 can be written in a more usable form. With the substitution of (25) and the values typical of water-based solutions, $\rho = 1$ g/cm^2 and $\mu = 0.01$ g/cm · s, into Eq. 24, and taking into account that for impeller Reynolds numbers between 10 and 1000, $f(\mathbf{Re}_i)^{0.17} \approx 1$ (Bates et al., 1966), we obtain an equation

$$k_t \approx \frac{2D}{d}(1 + V^{-0.17} L^{0.85} n^{0.51} d^{0.69} D^{-0.33}) \quad (26)$$

$$= \frac{2D}{d} + 2V^{-0.17} L^{0.85} n^{0.51} d^{-0.31} D^{0.67}.$$

All of the quantities in Eq. 26 are expressed in the CGS unit system.

Equation (26) determines whether or not stirring is sufficient to enhance ligand exchange. Stirring is effective when the convection term on the right side, which can be controlled by choosing the sample volume, length of the bar, and rotation speed, is made comparable or greater than the diffusion term $2D/d$. Eventually we not only want the convection term to be comparable to the diffusion term, but also to make k_t large enough to ensure that $p_t \gg 1$. Substituting (26) into the expression (8) for p_t, we obtain a condition from which the required parameters of mixing V, l, and n can be found:

$$V^{-0.17} l^{0.85} n^{0.51} + d^{-0.69} D^{0.33} \gg 0.5 d^{0.31} D^{-0.67} k_{on} R. \quad (27)$$

As an example, consider a typical small-scale laboratory experiment: cells 10 μm in diameter are interacting with a ligand while being suspended in a volume of 1 ml and stirred with a small bar. First we have to check the applicability of Eq. 26. To do that, we need to estimate the power input. Let a 0.9-cm bar rotate at 2 rev/s. The impeller Reynolds number $\mathbf{Re}_i = \rho n L^2/\mu$ in our setup is 160. For $\mathbf{Re}$ in this range, f is close to unity (Bates et al., 1966); then, from (25), the power input in our system is 5 erg/s. The precise knowledge of the stirring power is not critical, because it enters formula (22) with the exponent 1/4. The right part of (22) is equal to 240 μm, which is much greater than the typical cell size. Therefore we can use (26). Substitution of the above numerical values into (26) shows that the ratio of the flow-dependent to diffusional transport is expected to be 0.5–2.2 for the range of diffusion coefficients from 10^{-5} to 10^{-7} cm^2/s and thus the overall transport rate can exceed the transport rate due to diffusion alone by the factor of 1.5–3. These estimates demonstrate that, if diffusion is rate limiting ($p_{dif} \geq 1$), stirring can effectively facilitate the exchange of molecules between cells and solution. It would be less effective for small cells: for a 1-μm bacteria the effectiveness of stirring relative to diffusion would be 5 times lower. On the other hand, it is less likely that binding to a small cell will be diffusion limited because of a larger diffusion term $2D/d$.

Example 3. A confluent colony on the plane: no flow

Consider a confluent colony at $z = 0$, sufficiently large so as to neglect the effect of the boundaries (we shall define this condition later). Let the ligand be introduced in the volume at time $t = 0$, so that the initial conditions are $L = L_o$ and $C = 0$. We need to solve the diffusion equation with respect to L:

$$\frac{\partial L}{\partial t} = D\frac{\partial^2 L}{\partial z^2}. \quad (28)$$

We first restrict ourselves to the case of low receptor occupancy. Then the boundary conditions are

$$\frac{dC}{dt} = D\nabla L_s = k_{on} R_o L_s - k_{off} C. \quad (29)$$

The symbol ∇L_s denotes the gradient at the boundary. The solution is obtained by applying a Laplace transform (similar problems can be found in Thompson et al., 1981, and Stenberg and Stilbert, 1986). For C and L_s we have

$$\frac{C}{C_\infty} = 1 - e^{-\tau} - \int_0^\tau e^{(\tau'-\tau)} h(\tau') d\tau', \quad (30)$$

$$\frac{L_s}{L_o} = 1 - h(\tau), \quad (31)$$

where

$$h(\tau) = \frac{1}{\sqrt{1-p'^2}}\{e^{\tau(1-\sqrt{1-p'^2})^2/p'^2} \operatorname{erfc}[\sqrt{\tau}(1-\sqrt{1-p'^2})/p'^2]$$

$$- e^{\tau(1+\sqrt{1-p'^2})^2/p'^2} \operatorname{erfc}[\sqrt{\tau}(1+\sqrt{1-p'^2})/p'^2]\}, \quad (32)$$

where τ is the normalized time

$$\tau = t \cdot k_{off} \quad (33)$$

and

$$p' = \frac{2\sqrt{Dk_{off}}}{k_{on}R_o}, \quad (34)$$

is a parameter analogous to p_{dif}. Indeed, the numerator in (34) can be viewed as $2D/d'$, where d' is not the cell diameter as before but the average distance (with the coefficient $1/\sqrt{2}$) traveled by a diffusing molecule over the characteristic time of this process, which is $1/k_{off}$:

$$d' = \sqrt{D/k_{off}}. \quad (35)$$

The ratio C/C_∞ is plotted versus τ in Fig. 2 for different values of p'. The upper curve ($p' = \infty$) corresponds to an infinitely fast diffusion and is the standard binding curve. For $p' \leq 3$ the reaction is diffusion limited and is considerably slower than at $p' = \infty$. Because for many ligand-receptor systems k_{off} assumes the value from less than 0.0001 to 0.1 s^{-1} yielding for the numerator in (34) the range of 10^{-5}–10^{-3} cm/s, the diffusion-limited binding to a confluent monolayer of cells must be a common phenomenon. The parameter d' also defines whether the approximation of the infinite colony is valid. If the true colony size is a, the effect of the boundaries can be regarded as insignificant only when $a \gg d'$. For the above range of D and k_{off}, $d' = 0.01$–0.4 mm.

The onset of depletion may be rapid (Fig. 3). For $4D/(k_{on}R_o)^2 = 10$ it takes only 0.1 s to reduce L_s by 20% and 1 s to reduce it by more then 40% if $k_{off} \leq 0.1$ s^{-1}. These numbers give an idea of how intensive the liquid renewal at the surface must be to counterbalance the developing depletion zone. Later the concentration recovers as the ligand binding approaches equilibrium and the absorption rate decreases.

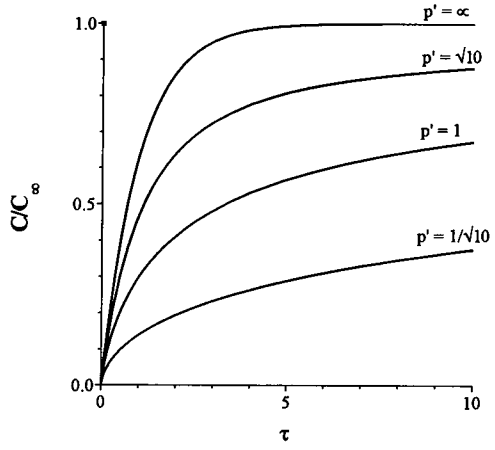

FIGURE 2 Kinetics of formation of ligand-receptor complexes, expressed as the ratio of the concentration of occupied receptors at time t to that at $t = \infty$ at a low ligand concentration ($C_\infty/R \ll 1$) and different values of p' (see Eq. 34). If there were a continuous perfusion with a laminar flow, these curves would correctly describe ligand binding only as long as $\tau \ll k_{off}t_e$ (Eq. 37).

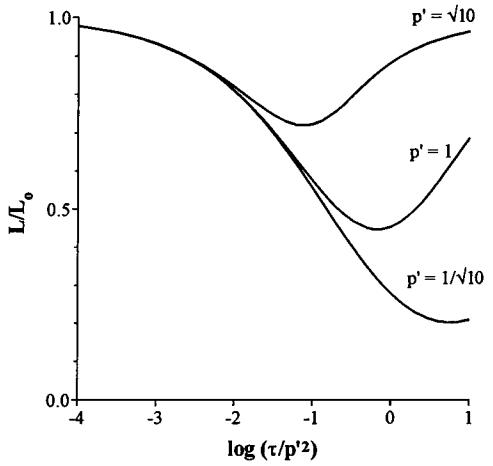

FIGURE 3 Development of the depletion above the cell colony. The ratio of the ligand concentration at $z = 0$ to that at $z = \infty$ is plotted against a dimensionless function of time. The conditions are specified in the legend to Fig. 2.

The solution to Eq. 28 for an arbitrary ligand concentration cannot be as readily obtained. A reasonable estimate can, however, be made. The general case is different from the low ligand concentration limit in that k_{off} in boundary condition (29) must be replaced with $k_{off} + k_{on}L_s$. If there were no depletion, it would be a constant parameter $k_{off} + k_{on}L_o$, which would also replace k_{off} in the definitions of τ (Eq. 33) and p' (Eq. 34). An increased p' relative to a low concentration case also means less depletion, so the actual L_s will be always greater than the minimum value of L_s at a low ligand concentration. The rate coefficients enter p' under the square root; if L_s and L_o are within the same order of magnitude, the square root will further reduce the difference, and the magnitude of

$$p' = \frac{2\sqrt{D(k_{off} + k_{on}L_o)}}{k_{on}R_o} \quad (36)$$

can be used to roughly evaluate the importance of diffusion effects in binding kinetics.

Example 4. Laminar flow over a colony: short times

If binding to a confluent colony in a stationary liquid is diffusion limited (p' is on the order of unity or less), one may try to enhance the ligand exchange by continuously replacing the liquid. Let the same confluent cell colony occupy a strip-shaped area with a width a on the inside wall of a flat channel with a width h ($h \gg d'$). A laminar stream with the average velocity v (which in practice can be calculated by dividing the flow rate by the cross section of the channel) flows through the channel. To use the data for the steady-state mass transfer between the flow and the wall (see below), we must estimate the time t_e at which the steady state with respect to mass transfer is established. This

is approximately equal to the time required for diffusion to spread over the distance δ into the stream where the flow velocity becomes comparable to the diffision velocity. As long as $\delta \ll h$ (which can be verified by solving the following equation for δ), the stream velocity is equal to the distance from the wall multiplied by the velocity gradient at the wall (for the flat channel, the latter equals $6v/h$), and so

$$t_e \approx \frac{\delta^2}{2D} = \frac{ah}{6v\delta} = 0.24\left(\frac{ah}{v\sqrt{D}}\right)^{2/3}. \quad (37)$$

For $a = h = 0.3$ cm, $v = 1$ cm/s, and $D = 10^5$–10^7 cm^2/s, the equilibration time is 2–11 s. During this time, the reaction is occurring within a narrow space adjacent to the colony, which is little disturbed by the flow; therefore the results for the stationary case from the previous section are applicable. Thus, even in the presence of a flow, the early stages of the reaction (at $t < t_e$) can be diffusion limited. The parameters in the kinetic experiment using laminar flow should be chosen so as to make t_e much less than the ligand-receptor reaction time.

Example 5. Laminar flow over a colony: long times

At $t > t_e$ we can use the expression for the steady-state mass transfer coefficient:

$$k_t \approx \left(\frac{D^2 v}{ah}\right)^{1/3} \quad (38)$$

(Kays and Perkins, 1985). For the same parameter values as in the previous section, k_t lies between 5×10^{-5} and 10^{-3} cm/s, which overlaps with the likely range of $k_{on}R_o$ (see above). Therefore, a continuous flow may permit the experimental measurement of the molecular rate constants. The required values of the parameters a, h, and v must be chosen according to

$$\left(\frac{v}{ah}\right)^{1/3} \gg D^{-2/3}k_{on}R \quad (39)$$

The formulas and typical values for the examples considered above are summarized in Table 1.

DISCUSSION

Equations (6) and (10) describe the ligand-receptor binding under a variety of conditions. They utilize the mass transfer coefficient, which has been determined, theoretically and experimentally, for many stationary systems (Cussler, 1984). Equations equivalent to (6) have been well known in biology, however, for the particular case of diffusional transport only. The integral Eq. 10, as far as we know, has not been previously reported. The major limitation of Eq. 10 (but not of Eq. 6) is the assumption that the mass transfer coefficient does not change with time. Because we are interested in reaction kinetics, this is equivalent to having a characteristic reaction time much greater then the relaxation time for the transport process. The validity of this assumption must be verified for each system.

We considered examples of convection-dependent ligand-receptor binding: an isolated cell with and without mixing and a confluent monolayer with and without perfusion. When cells are studied in suspension, some stirring is always used. From extensive studies of mixing in engineering practice the expression for the mass transfer coefficient is known and can be applied to conditions used in experiments with biological cells. Because the relaxation time for transport on the micrometer scale of the cell size is faster than most of the reactions studied in suspension (however, with the advent of rapid kinetics measurements of ligand-cell interactions in suspension (Neubig and Sklar, 1993) more caution may be required in certain cases), the expression for k_t can be used directly in (6) and (10). From relatively high values of k_{con} for mixing it follows that, when diffusion effects are important, stirring must be taken into account. For example, in a cellular system where the diffusion effects were studied experimentally (Erickson et al., 1987) the data were fit to theoretical formulae by setting $D = 10^{-5}$ cm^2/s. The ligand used in these experiments (2,4-dinitrophenyl aminocaproyl-L-tyrosine) had a molecular mass of about 500 Da, and for molecules of this size one would expect D to be a few times larger. It seems possible that in these experiments stirring contributed to ligand exchange.

Ligand-receptor interaction rates are frequently measured with confluent or nearly confluent adherent cells (Pellanda

TABLE 1 Expressions and typical values of the mass transport coefficients

Process	k_t	Typical range, cm/s
Ligand binding, $k_{on}R$	—	3×10^{-9}–3×10^{-3}
Diffusion, spherical cell	$2D/d$	10^{-4}–10^{-2}
Mixing, spherical cell	$\frac{2D}{d}(1 + V^{-0.17}l^{0.85}n^{0.5}d^{-0.3}D^{0.67})$	$(1.5\text{–}3) \times \frac{2D}{d}$
Monolayer: diffusion or flow at $t < 0.24(ah/vD^{0.5})^{2/3}$	$2\sqrt{D(k_{off} + k_{on}L_o)}$*	10^{-5}–10^{-3}
Monolayer: flow at $t > 0.24(ah/vD^{0.5})^{2/3}$	$(D^2v/ah)^{1/3}$	5×10^{-5}–10^{-3}

*This is only an analog of the mass transport coefficient; however, like with true k_t, its ratio to $k_{on}R$ determines the extent to which diffusion affects the rate of binding.

et al., 1992; Waters et al., 1990; Myers et al., 1987; Pitas et al., 1979; Wiley, 1988). It is not always realized that the conditions for having a diffusion-limited reaction with a confluent colony differ from those with isolated cells. Perfusion with fresh solution is not routinely used in experiments with binding to adherent cells. It appears to be important, however, if $p' \leq 1$ and one wishes to obtain the molecular rate constants directly from the experiment. When $p' \leq 1$, formula (39) allows one to decide whether perfusion under given conditions is going to be sufficient. Some of the perfusion chambers designed for microscopy (e.g., Braga, 1989; Datyner et al., 1985; Scudder et al., 1993; Sevcik et al., 1993; Toyotomi and Momose, 1989) may be useful for such experiments.

A problem analogous to the binding of ligand to cell surface receptors on adherent cells under laminar flow has been reported by Glaser (1993) in modeling the binding of antigen to immobilized antibody under laminar flow. These conditions are utilized for surface plasmon resonance measurements. In that work the problem was solved with numerical computation. Our results are expressed in analytical form, and the analysis of the transient kinetics is added. Even with a fast perfusion, the early stages of the reaction are likely to be affected. Unless t_e is much smaller then the reaction time $1/k_+$ or p' is not large enough to prevent formation of substantial depletion within the time t_e, correct kinetics may not be attainable. Some other method of mixing, more efficient than laminar flow, would be the option.

Table 1 summarizes the examples presented in this work. It can be used to assess whether ligand-receptor binding is transport limited and to design the experimental conditions appropriate for direct measurement of the interaction rates. In choosing the conditions for kinetic experiments, care must be taken to ensure that $p_t = k_t/k_{on}R \gg 1$. The following practical steps can be suggested. 1) Obtain the value of $k'_{on}R$ first without trying to optimize the intensity of convection. 2) Compare it to the value of k_{dif} describing the diffusional mass transfer for the given geometry (isolated cells or a monolayer). 3) If k_t is at least an order of magnitude greater than $k'_{on}R$, the measured apparent rates are close to molecular rates. 4) If k_t is on the same order of magnitude as $k'_{on}R$, then the additional convective mass exchange must be used. The required parameters of stirring or perfusion can be calculated from Table 1 or found from Eq. 27 and Eq. 39.

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REFERENCES

Armenante, P. M., and D. J. Kirwan. 1989. Mass transfer to microparticles in agitated systems. *Chem. Eng. Sci.* 44:2781–2796.

Batchelor, G. K. 1980. Mass transfer from small particles suspended in turbulent fluid. *J. Fluid Mech.* 98:609–623.

Bates, R. L., P. L. Fondy, and J. G. Fenic. 1966. Impeller characteriscits and power. *In* Mixing: Theory and Practice, Vol. 1. V. W. Uhl and J. B. Gray, editors. Academic Press, New York. 111–178.

Braga, P. C. 1989. Versatile perfusion chamber for high-magnification microscopy. *J. Pharmacol. Methods.* 22:1–6.

Bylund, D. B., and M. L. Toews. 1993. Radioligand binding methods: practical guide and tips. *Am. J. Physiol.* 265:L421–L429.

Calderbank, P. H. 1967. Mass transfer. *In* Mixing: Theory and Practice, Vol. 2. V. W. Uhl and J. B. Gray, editors. Academic Press, New York. 1–114.

Cantor, C. R., and P. R. Schimmel. 1980. Biophysical Chemistry, Part II. W. H. Freeman and Co., San Francisco.

Cussler, E. L. 1984. Diffusion: Mass Transfer in Fluid Systems. Cambridge University Press, Cambridge.

Datyner, N. B., G. A. Gintant, and I. S. Cohen. 1985. Versatile temperature controlled tissue bath for studies of isolated cells using an inverted microscope. *Pflugers Arch.* 403:318–323.

DeLisi, C., and F. W. Wiegel. 1981. Effect of nonspecific forces and finite receptor number on rate constants of ligand-cell bound-receptor interactions. *Proc. Natl. Acad. Sci. USA.* 78:5569–5572.

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.

Glaser, R. W. 1993. Antigen-antibody binding and mass transport by convection and diffusion to a surface: a two-dimensional computer model of binding and dissociation kinetics. *Anal. Biochem.* 213: 152–161.

Harriott, P. 1962. Mass transfer to particles. Part I. *Am. Inst. Chem. Eng. J.* 8:93–101.

Kays, W. M., and H. C. Perkins. 1985. Forced convection, internal flow in ducts. *In* Handbook of Heat Transfer Fundamentals, 2nd ed. W. M. Rohsenow, J. P. Hartnett, and E. N. Ganic, editors. McGraw-Hill, New York. 7.1–7.180.

Lauffenburger, D. A., and J. J. Linderman. 1993. Receptors: Models for Binding, Trafficking, and Signaling. Oxford University Press, New York.

Levins, D. M., and J. R. Glastonbury. 1972. Particle hydrodynamics and mass transfer in a stirred vessel. Part II. Mass transfer. *Trans. Inst. Chem. Eng.* 50:132–146.

Maguire, M. E., E. M. Ross, and A. G. Gilman. 1977. β-Adrenergic receptor: ligand binding properties and the interaction with adenylyl cyclase. *Adv. Cyclic Nucleotide Res.* 8:1–83.

Myers, A. C., J. S. Kovach, and S. Vuk-Pavlovic. 1987. Binding, internalization, and intracellular processing of protein ligands. Derivation of rate constants by computer modeling. *J. Biol. Chem.* 262:6494–6499.

Neubig, R. R., and L. A. Sklar. 1993. Subsecond modulation of formyl peptide-linked guanine nucleotide-binding proteins by guanosine 5′-*O*-(3-thio)triphosphate in permeabilized neutrophils. *Mol. Pharmacol.* 43: 734–740.

Pellanda, A. M., H. P. Gaeggeler, J. D. Horisberger, and B. C. Rossier. 1992. Sodium-independent effect of aldosterone on initial rate of ouabain binding in A6 cells. *Am. J. Physiol.* 262:C899–C906.

Pitas, R. E., T. L. Innerarity, K. S. Arnold, and R. W. Mahley. 1979. Rate and equilibrium constants for binding of apo-E HDLc (a cholesterol-induced lipoprotein) and low density lipoproteins to human fibroblasts: evidence for multiple receptor binding of apo-E HDLc. *Proc. Natl. Acad. Sci. USA.* 76:2311–2315.

Scudder, K. M., G. D. Christian, and J. Ruzicka. 1993. Flow injection fluorescence microscopy: a novel tool for the study of cells through controlled perfusion. *Exp. Cell Res.* 205:197–204.

Sevcik, G., G. D. Guttenberger, and D. Grill. 1993. A perfusion chamber with temperature regulation. *Biotech. Histochem.* 68:229–236.

Shoup, D., and A. Szabo. 1982. Role of diffusion in ligand binding to macromolecules and cell-bound receptors. *Biophys. J.* 40:33–39.

Sklar, L. A. 1987. Real-time spectroscopic analysis of ligand-receptor dynamics. *Annu. Rev. Biophys. Biophys. Chem.* 16:479–506.

Sklar, L. A., J. Sayre, V. M. McNeil, and D. A. Finney. 1985. Competitive binding kinetics in ligand-receptor-competitor systems. *Mol. Pharmacol.* 28:323–330.

Stenberg, M., and L. Stilberg. 1986. External diffusion in solid-phase immunoessays. *J. Theor. Biol.* 120:129–140.

Thompson, N. L., T. P. Burghardt, and D. Axelrod. 1981. Measuring surface dynamics of biomolecules by total internal reflection fluorescence with photobleaching recovery or correlation spectroscopy. *Biophys. J.* 33:435–454.

Toyotomi, S., and Y. Momose. 1989. Temperature-controlled perfusion apparatus for microscope using transparent conducting film heater. *Am. J. Physiol.* 256:C214–C217.

Wank, S. A., C. DeLisi, and H. Metzger. 1983. Analysis of the rate-limiting step in a ligand-cell receptor interaction: the immunoglobulin E system. *Biochemistry.* 22:954–959.

Waters, C. M., K. C. Oberg, G. Carpenter, and K. A. Overholser. 1990. Rate constants for binding, dissociation, and internalization of EGF: effect of receptor occupancy and ligand concentration. *Biochemistry.* 29:3563–3569.

Wiley, H. S. 1988. Anomalous binding of epidermal growth factor to A431 cells is due to the effect of high receptor densities and a saturable endocytic system. *J. Cell Biol.* 107:801–810.

Woodruff, N. R., and K. E. Neet. 1986. β Nerve growth factor binding to PC12 cells. Association kinetics and cooperative interactions. *Biochemistry.* 25:7956–7966.