The influence of transport on the kinetics of binding to surface receptors: application to cells and BIAcore

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Accurate estimation of biomolecular reaction rates from binding data, when ligands in solution bind to receptors on the surfaces of cells or biosensors, requires an understanding of the contributions of both molecular transport and reaction. Efficient estimation of parameters requires relatively simple models. In this review, we give conditions under which various transport effects are negligible and identify simple binding models that incorporate the effects of transport, when transport cannot be neglected. We consider effects of diffusion of ligands to cell or biosensor surfaces, flow in a BIAcore biosensor, and distribution of receptors in a dextran layer above the sensor surface. We also give conditions under which soluble receptors can be expected to compete effectively with surface-bound receptors. Copyright © 1999 John Wiley & Sons, Ltd.

Introduction

In biological systems, chemical reactions often occur between reactants that are not well mixed. Frequently one of the reactants is confined to a surface while the other is in solution. Because the reactant in solution must be transported to the surface before it can react with the surface molecule, the kinetics of binding can differ significantly from the kinetics expected when both reactants are well mixed. The purpose of this review is to understand (1) under what conditions transport influences the kinetics of binding and dissociation and (2) how one can quantitatively describe the kinetics when transport cannot be neglected. First we will briefly review binding kinetics when the reactants are well mixed, then we will consider binding when one of the reactants is confined to a sphere and the other reactant diffuses to the surface, and finally we will consider a BIAcore flow cell, where one of the reactants is confined to a flat surface and the other reactant is transported by flow and diffusion.

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Abbreviations used: PDE, partial differential equation; RU, resonance units; SPR, surface plasmon resonance.

Binding in solution

When reactants are well mixed we have from the law of mass action that, for a monovalent ligand at concentration C and a monovalent soluble receptor at concentration R, the rate of change of the concentration B of bound complex is

$$\mathrm{d}B/\mathrm{d}t = k_\mathrm{a}CR - k_\mathrm{d}B \qquad (1)$$

If the total receptor concentration is R_T and the total ligand concentration is C_T then, using mass conservation

$$R_\mathrm{T} = R + B \qquad C_\mathrm{T} = C + B \qquad (2)$$

Substituting eq. (2) into eq. (1) we obtain a single ordinary differential equation that describes the rate at which bound complex is formed.

If ligands and receptors are uniformly dispersed in solution, then, with the possible exception of very small diffusional transients, the rate coefficients k_a and k_d are constant. For this reason they are referred to as rate constants, k_a as the association or forward rate constant and k_d as the dissociation or reverse rate constant. k_a and k_d are constants in the sense that they are independent of time and of the concentrations of the reactants, although they may depend on other parameters such as temperature and viscosity.
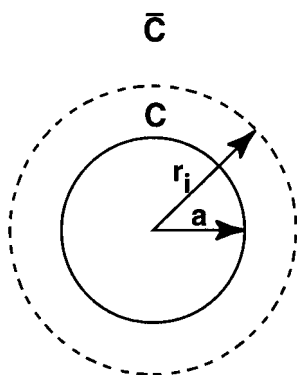
Note that in eq. (1) the initial rate of binding is proportional to the concentration R of free receptors. When the system is well mixed, receptors act independently so that, for example, doubling the concentration of free receptors in solution will double the rate at which bound complexes initially form. We will see that when transport

influences the binding kinetics, the surface reactants no longer act independently.

Binding of ligands to receptors on spherical surfaces

The first step for both biological cells and biosensors in detecting a specific biomolecule in solution is the binding of that molecule to a surface receptor. Because detection starts with the formation of a bound complex on a surface, the possibility arises that the kinetics of binding may be influenced by the transport of the biomolecule to the surface. If the chemical reaction is slow compared to transport, the binding kinetics are unaffected by transport and the system acts as if it were well mixed. If the chemical reaction at the surface is fast, for example if the forward rate constant for the reaction or the receptor surface density is high, the binding kinetics will be affected or even dominated by transport. (What we mean by 'fast' and 'slow' should become clear shortly.)

When ligands diffuse in solution and bind to cell surface receptors, the ligand concentration will vary both in space and time. A diffusion–reaction process such as this is described by a partial differential equation (PDE), which is reviewed in the Appendix. However, let us consider a simpler model that approximates the continuous spatial change in the ligand concentration by dividing the volume outside the surface into discrete compartments, in each of which the ligand concentration is uniform. For binding of diffusing ligands to receptors on a spherical cell, the standard approximation divides the space outside the sphere into two compartments (Fig. 1), an outer compartment where the ligand concentration is that of the bulk solution, $\bar{C}$, and an inner compartment where the ligand concentration, C, changes because ligands bind to, and dissociate from, receptors and because ligands are transported to and from the outer compartment. Let us look at a single cell, with receptors distributed uniformly over its surface. At time $t = 0$, the ligand concentration is uniform, except in a

small region about the cell (the inner compartment), where the concentration is 0, i.e. at $t = 0$, $\bar{C} = C_T$ for $r \geq r_i$ and $C = 0$ for $r_i > r \geq a$. Initially, ligands diffuse rapidly into the inner compartment and then begin to bind to cell surface receptors. Because the problem has radial symmetry, the net flux is only in the radial direction. We let V_i denote the volume of the inner compartment, A the surface area of the cell, and R the concentration of free receptors on the cell surface. For example, if A is measured in cm^2, then R is measured in receptors/cm^2. Then the quantities V_iC, AR and AB are the total number of ligands in the inner compartment and the total number of free receptors and bound receptors on the cell surface. The free ligand concentration in the inner compartment and the bound ligand concentration on the cell surface obey the following equations:

$$V_i dC/dt = -k_aARC + k_dAB + k_+(\bar{C} - C) \quad (3)$$
$$dB/dt = k_aRC - k_dB \quad (4)$$

where k_+ is the rate constant that characterizes diffusion between the inner and outer compartments.

We now assume that, after a short transient during which the ligand concentration rises rapidly in the inner compartment but there is negligible binding, the ligand concentration changes slowly with time. If this is so we can set $dC/dt = 0$. This is called a quasi-steady-state approximation (Segel and Slemrod, 1989). It does not mean that C is constant in time, only that it changes slowly. (For a justification of this approximation see Mason *et al.*, 1999.) Basically, for the binding phase, the approximation is valid when the characteristic time for transport, the time to diffuse across the distance of a cell radius, a^2/D, is short compared to the time for the chemical reaction, $1/k_aR_T$. When the left side of eq. (3) is set equal to zero, one can solve for C and use the equation obtained to eliminate this variable from eq. (4). This yields the following equation for B:

$$dB/dt = k_a^e R\bar{C} - k_d^e B \quad (5)$$

where we have introduced effective association and dissociation rate coefficients:

$$k_f^e = \frac{k_a}{1 + k_aRA/k_+} \quad k_d^e = \frac{k_d}{1 + k_aRA/k_+} \quad (6)$$

Equation (5) describes the kinetics of binding when receptors are confined to a spherical surface. It has the same form as eq. (1), the well-mixed case, except that the true rate constants are replaced by effective rate coefficients that depend on the concentration of free receptors, a quantity that is changing in time as binding or dissociation proceeds. (It is fortuitous that the volume of the inner compartment, whose size we don't know, has dropped out of the problem. Its value is only important during the initial transient when binding is negligible.) k_+ is the diffusion-limited forward rate constant (Smoluchowski, 1917). [This identification is made by considering the limiting flux of ligand to the cell surface, as the receptor density $R \to \infty$ in eqs (5) and (6).] For two spherical particles whose radii sum to a and whose diffusion coefficients sum to D

$$k_+ = 4\pi Da \quad (7)$$

For a molecule interacting with a cell, to an excellent approximation, a is the cell radius and D the ligand diffusion

Figure 1. A compartment model for ligands binding to and dissociating from receptors on a spherical surface of radius a. The space outside the sphere is divided into an inner region, $a < r \leq r_i$, where the ligand concentration is C, and an outer region, $r > r_i$, where the ligand concentration equals the bulk concentration $\bar{C}$.

coefficient. (The expression for k_+ agrees with our intuition. The faster the diffusion and the bigger the cell, the faster a molecule in solution will find the cell.) We now can quantify what we mean when we say transport influences the kinetics of binding when the chemical reaction is fast compared to transport. From eq. (6), the effective rate coefficients will differ from the true rate constants when $k_a RA/(4\pi Da) \geq 1$.

The effective rate coefficients correct, in an approximate way, for two related effects that arise when receptors are confined to surfaces. One effect, rebinding, occurs because a ligand that dissociates from one receptor has a non-zero probability of reacting with another receptor on the same surface instead of escaping into solution. The quantity $1/(1 + k_a RA/k_+)$, which is the ratio of the effective dissociations rate coefficient to the true dissociation rate constant [eq. (6)], is the fraction of dissociations that lead to a true separation of the ligand from the cell (Berg, 1978). When $k_a RA/(4\pi Da) \gg 1$ this fraction will be small and dissociation from the cell surface will be much slower than dissociation from an isolated receptor. At receptor densities where rebinding occurs, the other effect, competition among receptors for ligand, alters the forward kinetics. 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.) From eq. (6) we see that, as the number of receptors is increased, the rate at which ligands bind to the cell goes from $k_a CRA$ to an upper limit, $4\pi DaC$, which is independent of receptor density. It is not surprising that, when the receptor density is high enough to influence dissociation, this same density will influence the forward kinetics as well. The ratio of these rate coefficients equals a constant, the true equilibrium binding constant, i.e. $K = k_a/k_d = k_a^e/k_d^e$. This is required if eq. (5) is to go to the correct limit at equilibrium.

Let us now consider dissociation, where at the start of the dissociation phase, $t = 0$, the external concentration is set equal to zero. Equation (5) then becomes:

$$dB/dt = -\frac{k_d}{1 + k_a RA/k_+} B \qquad (8)$$

At $t = 0$ we denote the bound ligand concentration as B_0 and the fraction of sites bound as $f_0 = B_0/R_T$. In eq. (8), k_+ is again given by eq. (7). (k_+ is now the diffusion-limited rate constant for transport away from the cell. Although in this case it equals the diffusion-limited rate constant for transport to the cell, for some geometries and transport processes this will not be so.)

One can solve eq. (8), with $R_T - B$ substituted for R, to obtain the following transcendental equation for the fraction of ligands that remain bound at time t, $b(t) = B(t)/B_0$:

$$k_d t = -(1 + \delta)\ln b - \delta f_0(1 - b) \qquad (9)$$

where

$$\delta = k_a R_T A/k_+ \qquad (10)$$

The half-life for dissociation can be obtained by setting $b = 1/2$ in eq. (9), i.e.

$$t_{1/2} = (\ln 2/k_d)[1 + \delta - \delta f_0/(2\ln 2)] \qquad (11)$$

Equation (9) predicts that as dissociation proceeds it slows as more receptors on the cell become free and the probability of rebinding increases. This slowing of the rate of dissociation has been observed, for example, for small haptens dissociating from cell surface antibody (Goldstein et al., 1989).

Let us consider a simple case where the initial number of bound receptors is small so that we can assume that during the entire course of dissociation $R = R_T$. For this case eq. (8) predicts

$$b = e^{-\tilde{k}_d^e t} \qquad (12)$$

where $\tilde{k}_d^e$ denotes the effective dissociation rate coefficient, eq. (6), when $R = R_T$. Even if initially a large fraction of receptors are occupied, at long times, when most of the sites are free, the two-compartment model predicts exponential decay with the true dissociation constant replaced by the effective dissociation coefficient. This prediction is wrong. As dissociation proceeds, the concentration gradient of free ligand decreases and diffusion of ligand away from the cell slows. Eventually, a quasi-steady state between bound and free ligand is established and diffusion away from the sphere rather than dissociation from receptors on the sphere becomes the rate limiting step. At long times the concentration of bound ligand does not decay exponentially, but rather as $t^{-3/2}$ (Carslaw and Jaeger, 1959; Berg, 1978; Goldstein and Dembo, 1995). In particular,

$$b = \frac{1}{2\kappa\pi^{1/2}(Dt/a^2)^{3/2}} + \frac{3(2/\bar{\delta} + 2 - \kappa)}{4\kappa^2\pi^{1/2}(Dt/a^2)^{5/2}} + \cdots \qquad (13)$$

where

$$\kappa = 4\pi a^3/(KRA) \qquad (14)$$

Note that the leading term in eq. (13) depends on the equilibrium constant K rather than the dissociation rate constant k_d.

The time, t^*, that characterizes the transition from exponential decay to $t^{-3/2}$ decay occurs when the two terms on the right of eq. (13) are of equal magnitude. Equating these terms in eq. (13), one can show that

$$t^* = \frac{3}{\tilde{k}_d^e}\left(1 - \frac{a^2 \tilde{k}_d^e}{2D}\right) \qquad (15)$$

Under most conditions the characteristic time for a ligand to diffuse across a cell, a^2/D, is short compared to the mean time, $1/\tilde{k}_d^e$, for a ligand to escape from a cell that has R_TA free receptors. When $1 \gg a^2\tilde{k}_d^e/(2D)$, $t^* \approx 3/\tilde{k}_d^e$. From eq. (12) we estimate that decay is exponential until about 5% of the initially bound ligands remain. Except at very long times, eq. (11) gives a good description of the dissociation process.

Binding kinetics in a BIAcore flow cell

In a BIAcore flow cell (Biacore AB, Uppsala, Sweden), one of the reactants is immobilized on a sensor chip. We call it the receptor, in analogy to a receptor on a cell surface, although it is commonly referred to as the immobilized ligand. The other reactant, called the analyte, enters at one

a

b
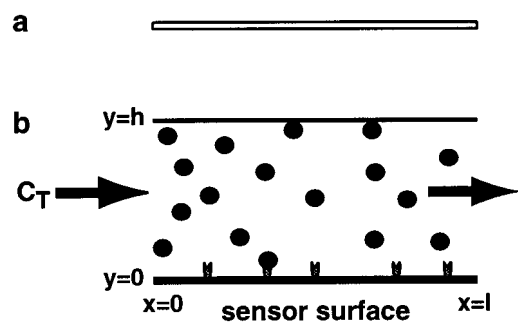
Figure 2. Cross section of a BIAcore flow cell, of length *l* and height *h*. The top panel (a) reflects the true aspect ratio, $h/l = 0.02$. In the lower panel (b), receptors are shown immobilized on a sensor chip on the bottom of the flow cell. (This convention is common in schematic diagrams of the BIAcore flow cell, although in the apparatus, the sensor chip is actually on the top surface.) The analyte enters the flow cell from the left, at a concentration C_T, flows through the chamber, and flows out at the right end.

end at a concentration C_T, flows past the sensor surface and leaves at the other end (Fig. 2). Along the flow cell the flow is laminar with a parabolic velocity profile. The flow velocity is zero at the top ($y = h$) and bottom ($y = 0$) boundaries and maximal, equal to v_c, in the center ($y = h/2$), i.e. the velocity $v(y)$ at a height y above the sensor surface is

$$v(y) = 4v_c(y/h)[1 - (y/h)] \quad (16)$$

Note that the average velocity $\langle v \rangle = 2v_c/3$ and the flow rate $Q = \langle v \rangle hw$, where h and w are the height and width of the flow cell. Because the velocity is zero at the sensor surface, diffusion as well as flow is important in transporting the ligand to the sensor surface.

The immobilization of the receptor is usually accomplished by coupling it to a dextran layer that extends approximately 100 nm out from the sensor surface (sensor chip CM5), 0.2% of the height of the flow cell. Flow cells are also used with shorter dextran chains (sensor chip F1), with receptors directly coupled to the sensor surface (sensor chip C1), or with vesicles having receptors on their surface coupled to the sensor surface (sensor chip L1). Detection of binding is based on the optical phenomenon of surface plasmon resonance (SPR, Garland, 1996). The SPR response detects changes in the index of refraction caused by mass changes at the sensor surface. These changes are brought about by the binding of analyte to receptor. By continuously monitoring the SPR signal, the time course of binding is followed in real time. After binding, buffer alone may be introduced to monitor the dissociation kinetics.

In the ideal situation, neither the transport of ligand to the sensor surface nor its transport within the dextran layer influences the binding kinetics. This happens when transport is fast compared to binding. In this case the analyte concentration rapidly becomes uniform in space and constant in time, equal to the injection concentration C_T. Equation (1) with $C = C_T$ describes the kinetics and can be used to determine k_a and k_d.

When is the dextran layer thin?

When can we ignore the dextran layer and treat the flow cell

as if the receptors were directly coupled to a flat surface? For the dextran layer to 'appear' thin to the analyte, the height of the dextran layer must be small compared to the average distance the analyte travels before it binds. This condition insures that the binding of the analyte to the dextran layer will be uniform. If the density of binding sites is too high, binding will first occur near the top of the dextran layer. As these sites fill up, analyte will have to diffuse farther in the layer to find free sites. Under these conditions the dextran layer will influence the kinetics of binding (Schuck, 1996). Further, because the SPR signal depends to some extent on the distance the bound analyte is from the sensor surface, analyte bound early (near the top of the dextran layer) will contribute less to the SPR signal than analyte bound late (near the bottom of the layer).

Consider an analyte that diffuses in the dextran layer with a diffusion coefficient $D_i \leq D$. Since the flow velocity is parabolic [eq. (16)], and equal to zero at the sensor surface, we assume there is negligible flow in the dextran layer. [If the dextran layer has no effect on the flow field outside the layer then at the top of the layer, $y = 100$ nm, and from eq. (16) $v = 0.002v_c$.] Let d be the height of the dextran layer, R_T the surface receptor density (receptors/cm^2), and R_T/d the receptor concentration in the layer (receptors/cm^3). For an analyte diffusing in a medium with uniformly distributed binding sites, the probability that the analyte is not bound after traveling a distance y into the medium is:

$$P(y) = \exp[-(y/\lambda)] \quad (17)$$

where the mean free path $\lambda = [dD_i/(k_aR_T)]^{1/2}$. Thus, we can ignore the dextran layer when $\lambda \gg d$ or equivalently when

$$k_a \ll D_i/(dR_T) \quad (18)$$

In obtaining eq. (18) we ignored flow in the dextran layer, even though models for the dextran layer indicate that flow penetrates into the layer (Witz, 1999). When there is flow in the layer, transport is faster, the mean free path longer and transport effects less pronounced in the layer than predicted when only diffusion is assumed to occur.
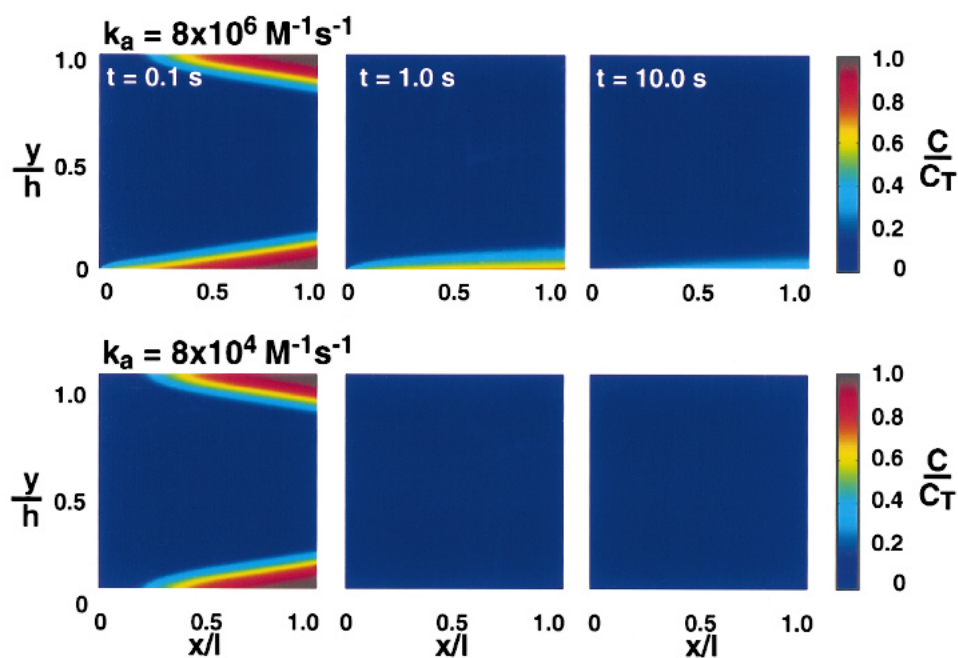
To get a feeling for when the dextran layer can influence transport let us evaluate eq. (18) for the following hypothetical case: a 30 kDa analyte in solution has a diffusion coefficient $D = 3 \times 10^{-6}$ cm^2/s which is reduced in the layer to $D_i = 1 \times 10^{-6}$ cm^2/s. At maximal binding the SPR response is 60 resonance units (RU). Since 1 RU $= 10^{-10}$ g/cm^2, $R_T = 2 \times 10^{-10}$ M cm. For a CM5 chip, $d = 5 \times 10^{-5}$ cm. From eq. (18), when $k_a \geq 10^8$/(M·s), the mean free path is equal to or shorter than the height of the dextran layer and transport within the layer may influence the kinetics of binding.

When does transport in the flow cell influence binding?

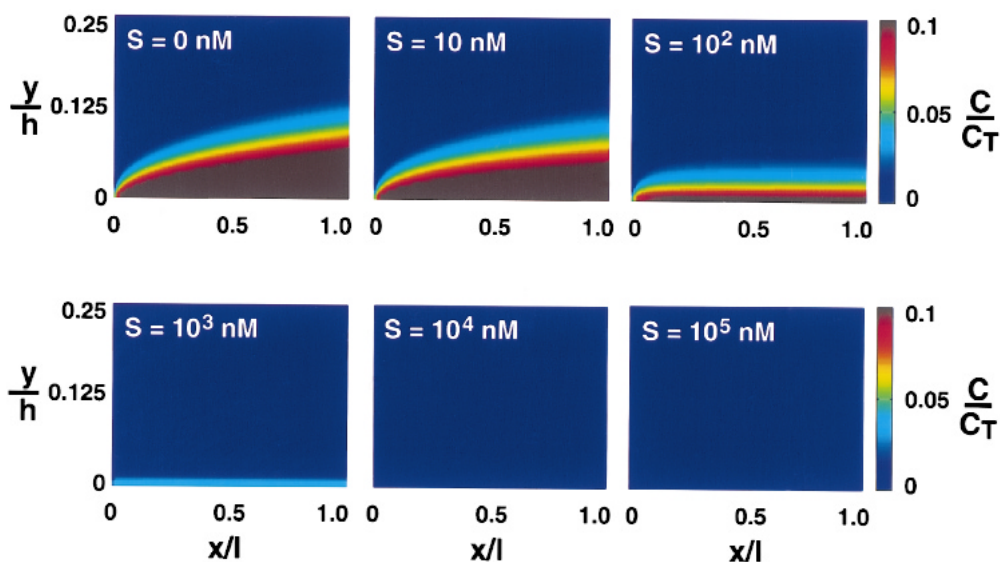In the remainder of the review we assume that eq. (18) is satisfied and that we can ignore the dextran layer and treat the flow cell as if receptors are uniformly distributed on a flat surface. We now want to know under what conditions transport (flow and diffusion) of the analyte to the sensor surface influences the binding kinetics. When transport is fast compared to binding, the chemical reaction determines
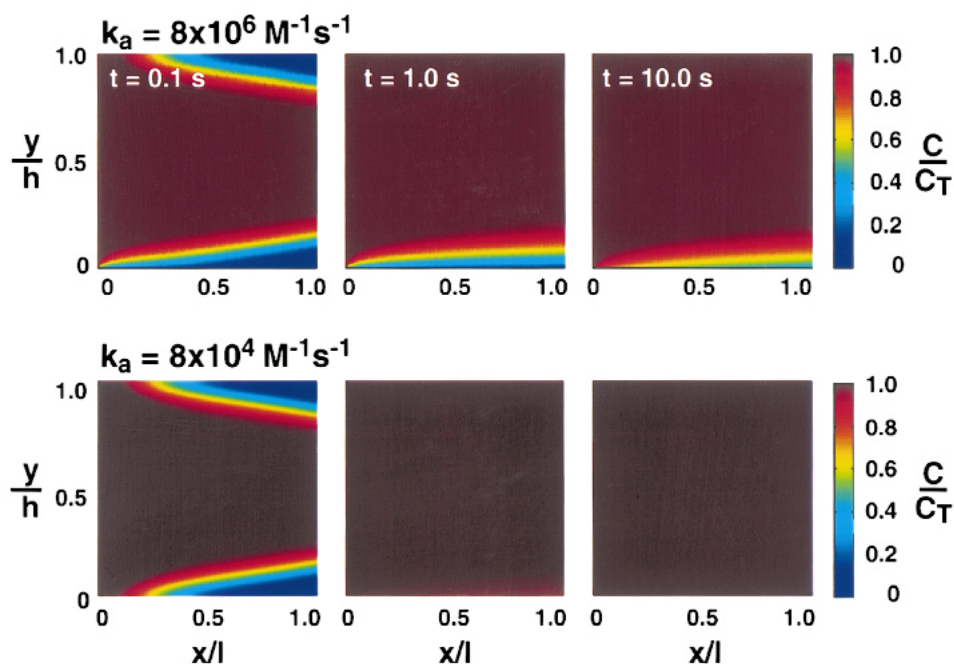
Plate 1. Predicted spatial variation in the concentration of free analyte, during the association phase, at times 0.1, 1.0 and 10 s. Simulations are based on the full PDE model. The top three panels illustrate the case where association is transport-limited ($k_a = 8 \times 10^6$/(M s) and $k_a R_T / \langle k_M \rangle = 4.8$). The lower three panels illustrate the reaction limit, i.e. the case where the reaction is slow relative to transport ($k_a = 8 \times 10^4$/(M s) and $k_a R_T / \langle k_M \rangle = 0.048$). Other parameter values used in the simulations are $h = 5 \times 10^{-3}$ cm, $l = 0.24$ cm, $k_d = 0.2$ s^{-1}, $v_c = 10$ cm/s, $D = 10^{-6}$ cm^2/s, $C_T = 25$ nM, and $R_T = 1.25$ nM cm. For a 14 kDa analyte, R_T corresponds to 175 RU at maximal binding.

$k_a = 8 \times 10^6 \ M^{-1}s^{-1}$

t = 0.1 s   t = 1.0 s   t = 10.0 s

$\frac{y}{h}$

$\frac{C}{C_T}$

$k_a = 8 \times 10^4 \ M^{-1}s^{-1}$

$\frac{y}{h}$

$\frac{C}{C_T}$

x/l   x/l   x/l

Plate 2. Predicred spatial variation in the concentration of free analyte, during the dissociation phase. The cases and parameters are the same as for Plate 1.

S = 0 nM   S = 10 nM $S = 10^2$ nM

$\frac{y}{h}$

$\frac{C}{C_T}$

$S = 10^3$ nM $S = 10^4$ nM $S = 10^5$ nM

$\frac{y}{h}$

$\frac{C}{C_T}$

x/l   x/l   x/l

Plate 3. Predicred distribution of free analyte, 1 s after dissociation is initiated, for six concentrations of soluble receptor in the dissociation phase. Other parameters are given in the text.

the binding and eq. (1) describes the kinetics. Recall that this requires that the rate of binding to the surface, $k_a R_T A$, be slow compared to the rate of transport to the surface, k_+, i.e. that $1 \gg k_a R_T A/k_+$ where, in the present case, A is the area of the sensor surface, k_+ is the transport-limited rate constant of the analyte in the BIAcore flow cell and, as before, R_T is the total surface receptor density.

In a BIAcore flow cell, transport will depend on the position x along the sensor surface. (This was not true in the previous section where transport was by diffusion alone.) Analyte will be transported fastest to positions closest to the inlet. During dissociation, rebinding will be more likely to occur at positions further along the flow cell. To a good approximation (Lok *et al.*, 1983)

$$k_+(x) = Ak_M(x) = \frac{A}{\Gamma(4/3)}\left(\frac{4v_cD^2}{9hx}\right)^{1/3} \approx 0.855A\left(\frac{v_cD^2}{hx}\right)^{1/3} \tag{19}$$

where x is the position along the sensor surface (Fig. 2), h is the height of the flow cell, v_c is the flow velocity in the center of the flow cell, $\Gamma(4/3) = 0.9064\ldots$ (Γ denotes the gamma function), and k_M is the mass transport coefficient. As expected, eq. (19) predicts that transport is fastest near the inlet (small x) and increases with increasing velocity and increasing diffusion coefficient. The derivation of eq. (19) ignores reflection of analyte from the upper boundary in Fig. 2. This is an excellent approximation when the time to traverse the length of the cell, $l/\langle v \rangle$, is short compared with the time to diffuse from the sensor surface to the reflecting boundary, $h^2/(4D)$. This is the usual situation in a BIAcore experiment.

Averaging eq. (19) over l, the length of the sensor surface, we obtain

$$\langle k_M \rangle = \frac{1}{\Gamma(4/3)}\left(\frac{3v_cD^2}{2hl}\right)^{1/3} \approx 1.282\left(\frac{v_cD^2}{hl}\right)^{1/3} \tag{20}$$

One can estimate when transport influences binding by calculating when $k_a R_T A/\langle k_+ \rangle \geq 1$ or equivalently $k_a R_T/\langle k_M \rangle \geq 1$. The influence of transport on the binding kinetics can be reduced by increasing the flow rate, but because $\langle k_M \rangle$ depends on v_c to the $\frac{1}{3}$ power, changing the flow rate by a factor of 100 will only change the transport coefficient by a factor of 4.64.

To get a feeling for the size of $\langle k_M \rangle$ we note that for a standard flow cell, with $h = 5 \times 10^{-3}$ cm, $w = 5 \times 10^{-2}$ cm and $l = 0.24$ cm, a flow rate of 100 μl/min corresponds to $v_c = 10$ cm/s. For an analyte with $D = 1 \times 10^{-6}$ cm^2/s and this flow rate, $\langle k_M \rangle = 2.6 \times 10^{-3}$ cm/s.

Accounting for transport with a two-compartment model

The SPR signal from a BIAcore does not report the amount bound as a function of x, but instead reports an average amount bound, the average being over a central section of the sensor surface. Surprisingly, a two-compartment model like the one discussed for the sphere gives an excellent description of the binding kinetics (the time dependence of the bound concentration averaged over the sensor surface)

when transport is significant (Myszka *et al.*, 1997, 1998). To obtain the appropriate equations for the BIAcore one replaces k_+ with $A\langle k_M \rangle$ and $\overline{C}$ with C_T in eq. (3), or equivalently in eqs (5) and (6). Calling the height of the inner compartment $h_i = V_i/A$ and defining the quantities $\tilde{B} = B/h_i$, $\tilde{R} = R/h_i$, $\tilde{R}_T = R_T/h_i$, and $\tilde{k}_M = k_M/h_i$, eqs (3) and (4) become

$$dC/dt = -k_aC(\tilde{R}_T - \tilde{B}) + k_d\tilde{B} + \tilde{k}_M(C_T - C) \tag{21}$$
$$d\tilde{B}/dt = k_aC(\tilde{R}_T - \tilde{B}) - k_d\tilde{B} \tag{22}$$

These are the equations that are used in the popular CLAMP and BIAevaluation 3.0 software packages when accounting for transport effects. In these programs the concentrations R, R_T and B are measured in RU, the concentrations C and C_T in M, and the parameters k_a in 1/(M·s), k_d in 1/s and k_M in cm/s. This is equivalent to setting $h_i = 1$ RU/M. As we pointed out for the sphere, and which holds for the BIAcore flow cell as well, after a brief initial transient the binding kinetics are independent of the size of the inner compartment. Because the data are insensitive to the value of h_i it is usual to set $h_i = 1$ RU/M. This avoids having to divide the data values by h_i before fitting the data. This choice of the value for h_i has nothing to do with the physical height of the inner compartment. To obtain k_M in cm/s from the parameter determined by the least squares fit of the data, $\tilde{k}_M$ in 1/s, one must multiply by 10^{-7} and divide by the molecular weight of the analyte, i.e.

$$\begin{aligned} k_M &= (1\text{RU/M})\tilde{k}_M = (10^{-7}\text{cm g/mol})\tilde{k}_M \\ &= (10^{-7}\text{cm/MW})\tilde{k}_M \end{aligned} \tag{23}$$

where we have used the conversion factor 1 RU $= 10^{-10}$ g/cm^2.

Simulating the effects of transport

To better understand the effects of transport we have used the full PDE model to simulate the processes that take place in a BIAcore flow cell when transport in the dextran layer can be ignored (discussed in the Appendix). It is instructive to look at the predicted distribution of free analyte in the flow cell when the kinetics are transport-limited and when they are reaction-limited. In Plate 1, the binding phase, and plate 2, the dissociation phase, these cases are illustrated. In the transport-limited case, $k_a = 8\times10^6$/(M·s) and $k_aR_T/\langle k_M \rangle = 4.8$, while in the reaction-limited case $k_a = 8\times10^4$/(M·s) and $k_a R_T/\langle k_M \rangle = 0.048$. All the other parameters in the simulations are the same and given in plate 1. (Recall that when $k_aR_T/\langle k_M \rangle \geq 1$, transport influences binding, while when $k_aR_T/\langle k_M \rangle \leq 1$, binding is reaction-limited and transport has no influence.) Plate 1 shows snapshots of the free analyte concentration at three times, 0.1, 1.0 and 10 s. In the simulation $v_c = 10$ cm/s and $l = 0.24$ cm, so in the center, $y = h/2$, the initial front traverses the flow cell in 0.023 s. For the reaction-limited case one sees a symmetric parabolic flow pattern spread out somewhat by diffusion, but by 1 s the concentration of analyte is uniform throughout the flow cell. For the transport-limited case, at 0.1 s one sees that the predicted flow pattern is not symmetric. The binding surface has distorted the pattern because binding is fast compared to

transport. At 1 and 10 s, there are still strong gradients in the analyte concentration. For the parameters used in this simulation, treating the system as if it were well mixed will yield poor estimates of the rate constants (Myszka *et al.*, 1998). In Plate 2, simulations for the dissociation phase are shown and similar arguments apply.

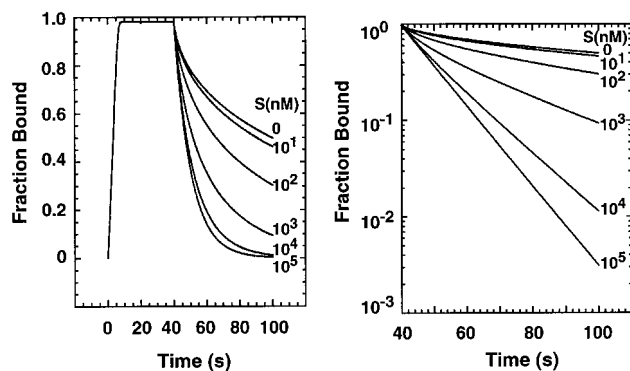## Blocking rebinding with soluble receptor

When rebinding occurs, an accurate determination of k_d can sometimes be obtained by using soluble receptor in the dissociation phase rather than buffer alone. At a high enough soluble receptor concentration, rebinding will be blocked and the fraction of sites bound during dissociation will be given by $b = \exp(-k_d t)$.

What is the soluble receptor concentration needed to block rebinding? Answering this question requires knowing what the effective three-dimensional concentration is of the two-dimensional surface concentration R_T. If the free soluble receptor concentration is S, then the mean free path an analyte travels in solution before binding to a soluble receptor is $\lambda_s = [D/(k_a S)]^{1/2}$. In experiments where solution and surface receptors compete for ligand, it can be shown that the effective three-dimensional surface concentration is R_T/λ_s (Goldstein *et al.*, 1989). Thus, to block rebinding effectively, one needs $S > R_T/\lambda_s$, or equivalently

$$S > k_a R_T^2/D \qquad (24)$$

Although this result was first derived for dissociation from receptors on spherical cells where transport is by diffusion alone, it holds as well for a BIAcore flow cell (unpublished result).

It is often difficult to reach sufficiently high soluble receptor concentrations to effectively compete with surface receptors for analyte, or ligand in the cell surface receptor case. Equation (24) indicates why: the solution concentration required to prevent rebinding increases with the square of the free surface receptor concentration.

In Fig. 3 we simulate the binding and dissociation kinetics when soluble receptor is present in the dissociation phase. The parameters in the simulation were chosen so that rebinding would be significant. In the simulation, $k_a = 5 \times 10^8/(\text{M·s})$, $k_d = 0.1/\text{s}$, $C_T = 10$ nM, $R_T = 1.69 \times 10^{-9}$ M cm, and $D = 10^{-6}$ cm^2/s. For these parameters, eq. (24) predicts that $S > 10^6$ nM is required to block rebinding and allow dissociation to proceed with the true k_d. When rebinding is blocked, the half-life of a bound ligand should equal $\ln 2/k_d = 6.9$ s. In the simulations, when $S = 10$ nM the half-life equals 52.4 s, while when $S = 10^5$ nM it drops to 9.1 s.

Plate 3 shows the predicted distribution of free analyte, 1 s after dissociation is initiated, for different concentrations of soluble receptor in the dissociation phase. As the soluble receptor is increased, the analyte concentration becomes uniform in the x direction and the distance over which it drops to zero in the y direction decreases. This means that the gradients away from the sensor surface become sharper and transport by diffusion away from the surface becomes faster. At high enough receptor concentration, eq. (24), transport is sufficiently fast so that the system is again in the reaction limit. Although for $S \geq 10^4$ nM it appears in Plate 3 that the system is uniformly mixed, if the region near the sensor surface were magnified, a sharp gradient in the analyte concentration would be seen. In Plate 3 all analyte concentrations with $C \geq 0.1 C_r$ are colored the same, the top most color on the scale.

Summary

When receptors are confined to a surface, the transport of ligand to the surface can influence the binding kinetics. We have reviewed conditions which allow one to decide when transport effects are important and when they are negligible. We have also indicated ways to account for transport when it contributes to the binding kinetics. For the BIAcore, if the dextran layer can be taken to be thin, eq. (18), then a two-compartment model gives an excellent description of the binding kinetics and can be used to determine the chemical rate constants and the transport coefficient (Myszka *et al.*, 1997, 1998). However, when transport within the dextran layer influences the binding kinetics (Schuck, 1996), an equivalent simple model for determining the correct chemical rate constants from the binding data has yet to be presented.

Appendix

A full mathematical description of the transport–reaction processes discussed in the text, one that allows for the continuous variation of concentrations in both space and time, takes the form of partial differential equations (PDEs) with appropriate boundary conditions. For the case of ligands diffusing to receptors on cell surfaces, the set of equations is given in Goldstein and Dembo (1995). For the case of a BIAcore flow cell, the equations used to simulate plates 1 and 2 are given in Myszka *et al.* (1998) and Mason *et al.* (1999). The numerical method used to solve the equations is described in Mason *et al.* (1999). The equations

Figure 3. Predicted time courses of binding and dissociation, when soluble receptor is present in the dissociation phase. Results are plotted on a linear scale (left panel) and a log scale (right panel). For the parameters used in the simulation (see the text), rebinding is significant and eq. (24) predicts that a soluble receptor concentration $S > 10^6$ nM is required to block rebinding and allow dissociation to proceed with the true k_d.

used to simulate Fig. 3 and plate 3 are unpublished but are straightforward generalizations of those in Mason *et al.* (1999). Instead of one PDE, in the dissociation phase there are three PDEs, one each for the concentrations of free analyte, free soluble receptor and analyte–soluble receptor complex. In the simulations, the diffusion coefficients of the analyte, soluble receptor and analyte–soluble receptor complex were taken to be the same, but this is not necessary. It was assumed that the soluble receptor could not interact with analyte bound to a surface receptor. This translates into reflecting boundary conditions for the soluble receptor and the analyte–receptor complex at the sensor surface.

Additional references on the binding of ligands to cell surfaces include Berg and Purcell (1977), DeLisi and Wiegel (1981), Schwartz (1976), and Shoup and Szabo (1982). Fundamental references on BIAcore analysis include Christensen (1997), Glaser (1993), Karlsson *et al.* (1994), Schuck (1997), and Yarmush *et al.* (1996).

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