Ligand-receptor binding in the presence of a diffusion gradient

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Received on November 20, 1978.

Abstract

When, by means of surface receptors, a cell senses external levels of a chemical, the amount actually sensed can be strongly influenced by how fast the chemical diffuses away from (or towards) the cell. For a given binding affinity, the effect of diffusion becomes significant for small values of the parameter $\left((l^2 k_{-1})/D\right)$ where l is of the order of the cell size, k_{-1} the dissociation rate of binding and D the diffusion coefficient of the chemical.

Key words: Receptor, binding, Dictyostelium, cyclic AMP.

1. Introduction

In many situations involving the binding of a ligand to receptors, the quantity of interest is the number of bound receptors rather than their concentration; for instance in the case of a hormone binding to receptors on its target cell. In estimating this number, it is customary to make a few simplifying assumptions. The purpose of this paper is to point out that these assumptions can lead to quite incorrect estimates of the real binding in some situations. We restrict our attention to "normal" binding. This will mean that all receptor sites are identical and non-interacting, and that dissociation of the bound ligand is a first-order reaction.

The existence of one possible source of error is well known, though this is not usually stressed[1]. The usual relation for estimating binding:

$$\frac{N}{N_0} = \frac{B}{B_0} = \frac{C}{K_{0.5} + C} \tag{1}$$

refers to *free* substrate concentrations C, whereas in its place one often finds C_0 $(= C + B)$, the total concentration, since only it is known beforehand. Here N, B are respectively the number and concentration of bound receptors, and the suffix 0 refers to maximum values. Substituting C_0 in terms of C in (1) leads us to the second problem, which is that there is no simple result for N in terms of N_0, C_0 and $K_{0\cdot5}$ which does not involve B_0. This gives rise to difficulties in situations where the ligand level varies spatially, for instance, due to diffusion on account of concentration gradients.

2. An accurate expression for binding

After putting $C_0 - B$ in place of C in (1), simple algebra yields

$$B = 1/2\ [(B_0 + K_{0\cdot5} + C_0) - \{(B_0 + K_{0\cdot5} + C_0)^2 - 4B_0\, C_0\}^{1/2}] \tag{2}$$

The argument leading to an approximate value of B is made as follows:

(*a*) Suppose that relative to the substrate concentration, the affinity is low, that is, $C_0 \ll K_{0\cdot5}$;

(*b*) now, if most of the substrate stays free, that is $B \ll C_0$, one can

(*c*) substitute C_0 in place of C in (1), giving

$$\frac{N'}{N_0} = \frac{B'}{B_0} = \frac{C_0}{C_0 + K_{0\cdot5}} \simeq \frac{C_0}{K_{0\cdot5}} \tag{3}$$

where primes refer to approximate quantities. However, conclusion (*b*) is correct only if the additional assumption—usually not explicitly stated—is made that $B_0 \ll K_{0\cdot5}$. In particular, if $B_0/K_{0\cdot5}$ is large, B' is a very poor approximation to B at low values of $C_0/K_{0\cdot5}$. Table I gives an instance of this.

As already mentioned, expression (2) is not new, in the sense that (1) is always understood to refer to the free substrate concentration; it is only that the precise nature of the approximation involved in using (3) is not made explicit. Granted that (2) is the correct expression to use, a basic difficulty arises when one tries to extract from it an expression for N in situations where C_0 is not uniformly distributed in space.

Table I

C_0	B^1	B
0·1	0·91	0·09
1·0	5·00	0·90
10·0	9·09	7·30
100·0	9·90	9·89

Legend: Approximate (B^1) and exact (B) values of bound substrate receptor complex. $B_0 = 10$, $K_{0\cdot5} = 1$. The notation is as in equations (1) and (2) of the text. Using the approximate expression can sometimes lead to absurd values for the binding (first two rows).

3. Binding in the presence of a diffusiou gradient

It is impossible to go from (2) to an expression for N without a knowledge of the volume within which the binding reaction takes place; this is obvious, since one needs to convert a number density into a number. In the usual equilibrium binding assay, this is no problem. Consider however the case of a hormone diffusing towards, or away from, a cell. What is the appropriate volume within which the receptor molecules are effectively in equilibrium with the substrate? Our intention here is to consider one way of approaching this question, and more importantly, to point out that the "standard" approximation—expression (3)—can lead to serious errors *even* within the range of validity expected of it under conditions where the ligand is uniformly distributed in space.

When binding takes place in the presence of non-zero diffusion gradients, one has to solve the combined reaction-diffusion equation for the system. We shall now perform this exercise after making simplifying assumptions about the geometry of the problem and the kinetics. Consider an one-dimensional situation with immobile receptor molecules uniformly distributed at a density B_0 in the region $-l < x < +l$. To begin with ($t = 0$), let the ligand too be uniformly distributed in the same region, at a density C_0. At later times, the ligand will be partly bound to the receptor and partly free; in the free state it can exist anywhere along the x-axis. Let $C(x, t)$ be the concentration of free ligand, $B(x, t)$ that of the bound ligand, that is, of the bound receptor-ligand complex; and $K_{0\cdot5}$ the equilibrium binding constant. As the quantity of interest to be estimated consider $\langle B\rangle_m$, the maximum value of the spatial average of B taken in the range $-l < x < +l$. Notice that this is proportional to N_m, the maximum value for the total *number* of bound receptors. Before proceeding to the calculation, we note the naive result, which follows on assuming that diffusion is sufficiently slow to be taken to be rate-limiting. Suppose further that the objections raised regarding (3) are not relevant and one can take it for granted that (*a*) most of the ligand is free and (*b*) binding is of low affinity. This means assuming

$$B_0 \lesssim K_{0\cdot5}, \quad C_0 \ll K_{0\cdot5} \tag{4}$$

Then one has

$$\langle B\rangle'_m \simeq B_0 \frac{C_0}{K_{0\cdot5}} \tag{5}$$

where the prime once again refers to the standard approximation. Note that $\langle B\rangle'_m \ll B_0$.
The correct formulation of the problem is as follows:

for $t > 0$,

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - \frac{\partial B}{\partial t}, \quad |x| < l$$

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}, \quad |x| \geqslant l$$

$$\frac{\partial B}{\partial t} = k_1 B_0 C - k_{-1} B, \quad |x| < l$$

and at

$$t = 0,\ \ B = 0,\ C = C_0,\ \ |x| > l, \qquad \text{and } C = 0 \text{ elsewhere.} \tag{6}$$

D is the diffusion coefficient of the ligand and $K_{0\cdot5} = k_{-1}/k_1$. Implicit in the equation for $\partial B/\partial t$ is the assumption that only a small fraction of the total number of receptors is occupied: otherwise the collision term should have been $k_1\ (B_0 - B)\,C$ instead of $k_1,\ B_0,\ C$. The method of solving (6) is outlined in the appendix[2]. What one finds is that for large values of the dimensionless parameter l^2k_{-1}/D,

$$\langle B(x,\ t)\rangle \simeq B_0 \frac{\langle C(x,\ t)\rangle}{\langle C(x,t)\rangle + K_{0\cdot5}} \simeq B_0\ \frac{C_0}{K_{0\cdot5}} = \langle B\rangle'_m \text{ from (4)}$$

However, for small values of l^2k_{-1}/D,

$$\langle B(x,t)\rangle \simeq \frac{B_0\,C_0}{K_{0\cdot5}}\ \frac{l}{\sqrt{D}}\left(\frac{1}{\sqrt{\pi t}} - \frac{\alpha_1}{\sqrt{t^3}}\right) \text{ when } t \text{ is large,} \tag{7}$$

and

$$\langle B(x,t)\rangle \simeq k,\ B_0\,C_0\,(t - \alpha_2\sqrt{t^3}) \text{ when } t \text{ is small,} \tag{8}$$

with α_1 and α_2 both positive. By ignoring the terms containing α_1, α_2 and putting

$$\langle B\rangle_m = k_1,\ B_0\,C_0\,t = \frac{B_0\,C_0}{K_{0\cdot5}}\ \frac{l}{\sqrt{D}}\ \frac{1}{\sqrt{\pi t}} \tag{9}$$

one gets an upper limit for $\langle B\rangle_m$. This results in

$$\langle B\rangle_m = \frac{B_0\,C_0}{K_{0\cdot5}}\left(\frac{l^2k_{-1}}{\pi D}\right)^{1/3} \tag{10}$$

Clearly this is different from (5), and for a given $k_{0\cdot5}$ one has

$$\frac{\langle B\rangle_m}{\langle B\rangle'_m} = \left(\frac{l^2k_{-1}}{\pi D}\right)^{1/3} \tag{11}$$

Now, $l^2/D \equiv \tau_D$ is of the order of time for diffusion to be effective over a distance l, and $1/k_{-1} = \tau_E$ is of the order of time for ligand-receptor binding to equilibrate in the absence of diffusion. Therefore, one expects $\langle B\rangle_m$ to be significantly smaller than $\langle B\rangle'_m$ when $\tau_D \ll \tau_E$ Crudely speaking, when this holds the ligand can "diffuse away before it can bind," so that any estimate of binding which ignores diffusion will be an overestimate. As seen from (7) through (9), the expression for $\langle B\rangle_m$ desired here is *already* an overestimate, so that the actual ratio of $\langle B\rangle_m$ to $\langle B\rangle'_m$ will be smaller than that indicated by (11).

The detailed expression for the ratio of $\langle B\rangle_m$ to $\langle B\rangle'_m$ depends on the geometry of the situation, and so one should not expect (11) to be immediately applicable to all cases

in which the ligand concentration in the neighbourhood of a cell varies in time. However, the general observation that given diffusion in free space, $\langle B \rangle_m$ ought to be significantly smaller than $\langle B \rangle'_m$ under these conditions, whenever the ratio $l^2/k_{-1}D$ is small, should be valid. This follows from the remarks on relative time-scales made earlier. As an example of the kinds of numbers involved, consider release of cyclic 3′, 5′ adenosine monophosphate (cAMP) by single cells of *D. discoideum*[3]. The cell surface has receptors to cAMP, and there are reasons for believing that self-stimulation of a cell by its own cAMP release is important. There one has $l \simeq$ the size of a cell $\simeq 10\,\mu$m[4], $k \simeq 1{\cdot}7 \times 10^{-2}\,\text{sec}^{-1}$,[5] $D \simeq 10^{-5}\,\text{cm}^2\,\text{sec}^{-1}$.[6] This gives $(l^2k_1/\pi D)^{1/3} = 0{\cdot}1$. The geometry of diffusion of cAMP released by a cell is 3-dimensional, and depletion of the cAMP level near the cell surface should be even faster than considered here. Thus it is probably safe to say that if cAMP is released in a quick pulse, the number of cell surface receptors stimulated by it is at most 10% of what would normally be expected. A detailed examination of this particular problem will be presented elsewhere.

4. Summary

In considering ligand-receptor binding, one might tend to assume that at low affinity most of the ligand stays unbound. This is not always true, and the assumption that the free and total ligand levels are approximately the same leads to an overestimate of binding. The extent of the overestimate at low ligand concentrations depends on the ratio of the density of receptors to the equilibrium constant of binding; if this ratio is large, the error can be quite high. Another possible source of error in estimating binding is the presence of a diffusion gradient in the spatial distribution of ligand. If the gradient is sharp, the binding expected by ignoring it can be significantly in error. With the particular geometry studied here, the error is once more an overestimate. As a consequence, the true binding in the stimulation of *D. discoideum* receptors by the cell's own cAMP should be less than 10% of the naive estimate if the cell releases the ligand in a sharp pulse.

5. Acknowledgement

This work was supported by grant HCS/DST/407/77 titled " Communication and Pattern Formation in the Cellular Slime Molds ".

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Appendix

The method of Laplace transforms is best for solving equation (6). In outline, it goes as follows:

1. Define the transforms

$$\tilde{B}(x, s) = \int_0^\infty dt\, e^{-st} B(x, t), \quad \tilde{C}(x, s) = \int_0^\infty dt\, e^{-st} C(x, t).$$

2. Use these definitions to convert the equation into a set of algebraic equations for $\tilde{B}$ and $\tilde{C}$ in terms of $B(x, o)$, $C(x, o)$ and other parameters.

3. Re-transform $\tilde{B}(x, s)$, $\tilde{C}(x, s)$ by using the asymptotic relations:

$$(a)\ \tilde{f}(s) = \sum_{n=0}^{\infty} \frac{a_n}{s^{m_n+1}} \Gamma(1 + m_n), \quad s \to \infty$$

$$\Rightarrow f(t) = \sum_{n=0}^{\infty} a_n t^{m_n}, \quad t \to 0 \quad \text{if } m_n > -1$$

$$(b)\ \tilde{f}(s) = \sum_{n=0}^{\infty} a_n s^{\frac{n}{2}-1} \quad s \to 0$$

$$\Rightarrow f(t) = \sum_{n=0}^{\infty} a_n t^{-n/2} \Big/ \Gamma\left(1 - \frac{n}{2}\right), \quad t \to \infty$$

Here $\Gamma(x)$ is the function $\int_0^\infty du\, u^{x-1} e^{-u}$.