THE EFFECT OF RECEPTOR CLUSTERING ON DIFFUSION-LIMITED FORWARD RATE CONSTANTS

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ABSTRACT The effect of receptor clustering on the diffusion-limited forward rate constant (k_+) is studied theoretically by modeling cell surface receptors by hemispheres distributed on a plane. We give both exact results and bounds. The exact results are obtained using an electrostatic analogue and applying the method of the images. Accurate upper bounds on k_+ are found from a variational principle.

INTRODUCTION

Distributed on the surface of cells are a variety of receptors that mediate the uptake of specific biological molecules from the cell's environment. Diffusion-limited forward rate constants have been calculated for the case where the receptors are uniformly distributed over the cell surface (1–3). However, in many situations binding sites are preclustered on the plasma membrane.

Clustering of binding sites may occur because the receptor molecule, although uniformly distributed over the membrane is itself multivalent, or because receptors are clustered in the plane of the membrane. For example, although anitbodies appear not to be clustered in the absence of ligand on the surface of lymphocytes, mast cells, and basophils, each antibody has two identical binding sites and, hence, the binding sites are clustered in pairs (4). The insulin receptor, with its symmetric structure of two heavy and two light chains, is most likely also multivalent (5). An important example of preclustered receptors are the low-density lipoprotein (LDL) receptors on fibroblasts (6, 7). These receptors are clustered in structures known as coated pits, with an average coated pit containing ~20 LDL receptors (8). These receptors also appear to be clustered in smaller groups (5 or less) outside of coated pits (R. G. W. Anderson, personal communication). To complicate matters, multivalent receptors can also be preclustered. For example, the insulin receptor on some cell types is aggregated (9).

In this paper we first consider the general relation between diffusion-limited forward rate constants and the problem of calculating the capacitance in electrostatics. We then look at the simplest example of clustering on the cell surface: two binding sites in proximity. We model the binding sites as hemispheres and then calculate exactly the diffusion-limited forward rate constant. This result is used to discuss binding to the cell surface when such receptor pairs are uniformly distributed over the cell surface.

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GENERAL CONSIDERATIONS

We model the cell membrane as an infinite plane and consider binding sites of arbitrary shape anchored to this plane (see Fig. 1). To calculate the diffusion-limited forward rate constant, one has to solve the steady-state diffusion equation

$$\nabla^2 c = 0 \tag{1}$$

for c, the ligand concentration, under the following boundary conditions:

$$c = c(\infty) \tag{2a}$$

at large distances from the binding sites;

$$c = 0 \tag{2b}$$

at the surfaces of the binding sites; and

$$\frac{\partial c}{\partial n} = 0 \tag{2c}$$

everywhere on the plane outside the binding sites. Here $\partial c/\partial n$ denotes the normal derivative of the concentration.

To demonstrate the connection with electrostatics we follow Berg and Purcell (1) and set

$$c = c(\infty)(1 - \phi/\phi_0). \tag{3}$$

FIGURE 1 Basic geometry of cell membrane and binding site. The shaded area shows a receptor of arbitrary shape; its mirror image in the plane of the membrane is indicated by a dashed line.

Substituion of Eq. 3 into Eqs. 1 and 2 shows that ϕ has the following properties;

$$\nabla^2\phi = 0 \tag{4}$$

subject to the bondary conditions,

$$\phi = 0$$

far from the boundaries;

$$\phi = \phi_0 \tag{5b}$$

on the binding sites, and

$$\frac{\partial\phi}{\partial n} = 0 \tag{5c}$$

everywhere on the plane outside the binding sites. This last boundary condition is automatically satisfied provided ϕ is defined on both sides of the membrane and the binding site below the membrane is defined as the mirror image of the binding site above the membrane (see Fig. 1). Eqs. 4 and 5 show that ϕ is the electrostatic potential outside a system of conductors with the same shape as the binding sites, with $\phi = \phi_0$ on their surface.

The inward diffusive flux, J, into the binding sites is given by the surface integral over the binding site

$$J = D\int \nabla c \cdot \mathbf{n}\mathrm{d}S \tag{6}$$

where D is the diffusion coefficient of the ligand, $\mathbf{n}$ the outward pointing unit vector and $\mathrm{d}S$ the surface element. Because of the symmetry of the problem with respect to the plane of the membrane, we can as well write

$$J = (D/2)\int' \nabla c \cdot \mathbf{n}\mathrm{d}S \tag{7}$$

where the prime indicates that the surface integral extends over the binding site and its mirror image. Using Eq. 3 we can cast this in a form familiar from electrostatics

$$J = -\frac{Dc(\infty)}{2\phi_0}\int' \nabla\phi \cdot \mathbf{n}\mathrm{d}S \tag{8}$$

$$J = -\frac{Dc(\infty)}{2\phi_0}\int' \nabla^2\phi \cdot \mathrm{d}V \tag{9}$$

where we have used Gauss' theorem, and the volume integral is extended over the interiors of the binding sites and their mirror images. Using Poisson's equation in the meter-kilogram-second (mks) systems[1]

$$\nabla^2\phi = -\rho/\epsilon_0, \tag{10}$$

where ρ is the charge density and ϵ_0 is the dielectric constant of the vacuum, Eq. 9 becomes

$$J = \frac{Dc(\infty)Q}{2\epsilon_0\phi_0} \tag{11}$$

where Q equals the total charge enclosed by the binding sites and their mirror images when they are treated as conducting surfaces held at a constant potential ϕ_0. In terms of the capacitance C,

$$J = \frac{Dc(\infty)}{2\epsilon_0}C. \tag{12}$$

Thus, for receptors of any geometry where the capacitance is known, Eq. 12 can be used to calculate the diffusive flux. The diffusion-limited forward rate constant k_+ is then obtained from the expression (see, for example, reference 11)

$$k_+ = J/c(\infty). \tag{13}$$

If the explicit calculation of the capacitance is not feasible, a variational principle could be of practical use. To derive such a principle, we note that the energy U of the system of conductors under consideration can be written in the forms (see, for example reference 12)

$$U = \frac{1}{2}C\phi_0^2, \tag{14a}$$

and

$$U = \frac{\epsilon_0}{2}\int' (\nabla\phi)^2\,\mathrm{d}V. \tag{14b}$$

Hence, by combination, one has the expression

$$C = \frac{\epsilon_0}{\phi_0^2}\int' (\nabla\phi)^2\,\mathrm{d}V. \tag{15}$$

Now consider the problem to calculate the minimum of the integral

$$L[\psi] = \frac{\epsilon_0}{\phi_0^2}\int' (\nabla\psi)^2\,\mathrm{d}V \tag{16}$$

over those functions ψ that tend to ϕ_0 at the surface of the conductors and to 0 at infinity. As the Euler-Lagrange equation for this variation problem is

$$\nabla^2\psi = 0, \tag{17}$$

We use the mks system of units since that is the system of units used in reference 10, the most comprehensive collection of results in electrostatics.

one sees that the solution is exactly the electrostatic potential ϕ, and that $L[\phi] = C$, because of Eq. 16. In summary, we have found

$$C = \min_{\Psi} \frac{\epsilon_0}{\phi_0^2} \int' (\nabla\Psi)^2 dV \tag{18}$$

which is the well-known variational principle for the capacitance (see, Chapter 19 of reference 12).

A useful corollary of this variational principle for the capacitance is found if one compares the capacitances $C(B')$ and $C(B'')$ of two conductors B' and B'', which have the property that B' can be located entirely inside B''. In this case, the class of functions ψ that tend to ϕ_0 on B' includes the class of functions ψ that tend to ϕ_0 on B''. Hence, one finds

$$C(B') \leqq C(B''). \tag{19}$$

A second useful inequality that follows from the variational principal states that if A' and A'' are two nonoverlapping conductors, and if $A' + A''$ denotes the conductor that consists of the two pieces A' and A'', then

$$C(A' + A'') \leqq C(A') + C(A''). \tag{20}$$

A nonvariational proof is given in reference 13. These inequalities can be used to derive upper and lower bounds for the flux into a receptor of arbitrary shape.

AN EXACT RESULT FOR A CLUSTER OF TWO BINDING SITES

In this section, we will consider the geometry displayed in Fig. 2 consisting of two identical hemispheres of radius a with centers separated by a distance b, where $b \geqq 2a$. This problem was solved by Smythe (10) using the method of images. In the context of diffusion, Samson and Deutch (14) used this solution to obtain the diffusion-limited forward rate constant for two identical spheres in solution. In our notation, the total flux into both hemispheres is

$$J = 4\pi Dc(\infty)a \sinh\beta \sum_{n=1}^{\infty} (-1)^{n+1} \operatorname{csch} n\beta \tag{21a}$$

$$\operatorname{csch}\beta = \frac{b}{2a}. \tag{21b}$$

In the limit $b \to \infty$, in which the two hemispheres become independent, the flux approaches $4\pi Dc(\infty)a$, which is twice the flux into a single hemisphere. In the opposite limit, in which the spheres are made to touch, $b = 2a$ and the flux approaches the value

$$J = 4\pi Dc(\infty)a \sum_{n=1}^{\infty} \frac{(-1)^{n+1}}{n} = 4\pi Dc(\infty)a \ln 2. \tag{22}$$

Because J is an increasing function of the distance b between hemispheres, the result shows that clustering of two receptors in the cell membrane will decrease their diffusion limited forward rate constant. In terms of the diffusion-limited forward rate constants for the cluster, k_{2+}, and the isolated single receptor, k_{1+}, Eq. 22 shows that

$$2k_{1+} \geqq k_{2+} \geqq 2k_{1+}\ln 2. \tag{23}$$

In the next section we shall show that the clustering of receptors of any shape and number will always decrease the diffusion-limited forward rate constant.

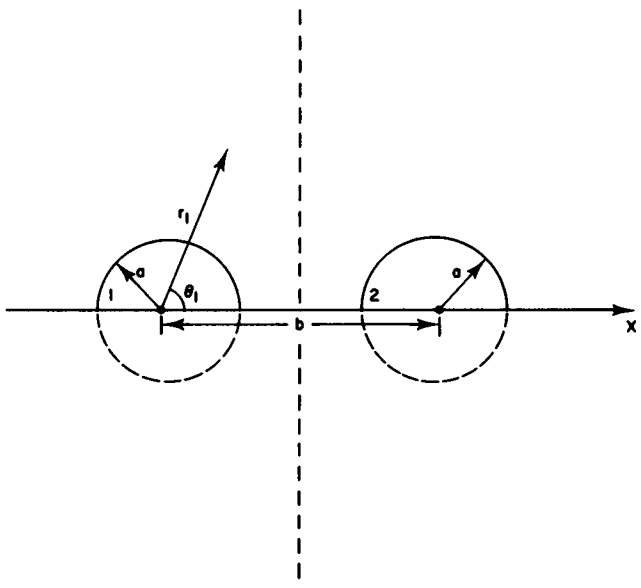

FIGURE 2 Geometry consisting of two identical hemispheres, and their mirror images; discussed in text.

PROOF THAT RECEPTOR CLUSTERING ALWAYS DECREASES THE DIFFUSION-LIMITED REACTION RATE

To prove that the clustering of receptors of any kind will decrease the flux, we start from the variational principle of the section on General Consideration. Consider an arbitrary complex A consisting of an arbitrary number of receptors of any size. Divide this complex into two nonoverlapping subcomplexes A' and A'', and let $\Phi_{A'}$, and $\Phi_{A''}$ denote the potential of subcomplex A' and A'', respectively. If b denotes the distance between A' and A'', measured in some convenient way, then we know that the potential Φ_A of complex A is a function $\Phi_A(b)$ of this distance. Now, in the limit of infinite separation, the capacities of A, A', A'' are related by

$$C_A(\infty) = C_{A'} + C_{A''}. \tag{24}$$

For a finite value of b we form the trial function

$$\begin{aligned} \Psi_b(\mathbf{r}) &= \phi_{A'}(\mathbf{r}) \quad \text{if} \quad \phi_{A'}(\mathbf{r}) > \phi_{A''}(\mathbf{r}), \\ &= \phi_{A''}(\mathbf{r}) \quad \text{if} \quad \phi_{A''}(\mathbf{r}) > \phi_{A'}(\mathbf{r}). \end{aligned} \tag{25}$$

This trail function goes to zero as r goes to infinity and satisfies the condition $\psi_b(\mathbf{r}) = \phi_0$ on the boundary. Accord-

ing to the variational principle, we now have

$$C_A(b) \leq \frac{\epsilon_0}{\phi_0^2} \int' (\nabla \psi_b)^2 \, dV. \tag{26}$$

Denoting by R' the region of space where $\phi_{A'}(\mathbf{r}) > \phi_{A''}(\mathbf{r})$, and by R'' the region of space where $\phi_{A''}(\mathbf{r}) > \phi_{A'}(\mathbf{r})$ one has

$$\frac{\epsilon_0}{\phi_0^2} \int' (\nabla \Psi_b)^2 dV =$$

$$\frac{\epsilon_0}{\phi_0^2} \int_{R'} (\nabla \phi_{A'})^2 dV + \frac{\epsilon_0}{\phi_0^2} \int_{R''} (\nabla \phi_{A''})^2 dV \tag{27a}$$

$$\leq \frac{\epsilon_0}{\phi_0^2} \int (\nabla \phi_{A'})^2 dV + \frac{\epsilon_0}{\phi_0^2} \int (\nabla \phi_{A''})^2 dV, \tag{27b}$$

where the first (second) integral on the right-hand side of Eq. 27b extends over all space outside subcomplex A' (subcomplex A''). Because of Identity 16 the right-hand side equals $C_{A'} + C_{A''}$. Combination of Eqs. 26 and 27 gives

$$C_A(b) \leq C_{A'} + C_{A''}. \tag{28}$$

From the nature of this proof, it also follows that the equality sign only holds in the limit $b \to \infty$.

UPPER BOUNDS FOR DIFFUSION-LIMITED RATE CONSTANTS

The procedure of the previous section suggests a way to construct an upper bound for the diffusion-limited forward rate constant of an arbitrarily complex of receptors. We demonstrate this method using the model of the section An Exact Result for a Cluster of Two Binding Sites, which consists of two hemispherical binding sites. The advantage of this example is that the exact result is known also.

The basic geometry is indicated in Fig. 2. We shall first consider the case $b \geq 2a$, in which the spheres do not intersect. In this case the trial function equals

$$\psi = \frac{a\phi_0}{r_1}, \qquad (x < 0) \tag{29a}$$

$$\psi = \frac{a\phi_0}{r_2}, \qquad (x > 0) \tag{29b}$$

where r_i denotes the distance to the center of sphere i. Substituting into Eq. 18 and using spherical coordinates θ_1, r_1 as shown in Fig. 2, we find

$$C \leq 4\pi\epsilon_0 a \left(1 + a \int_0^{\pi/2} \sin\theta_1 \, d\theta_1 \int_a^{b/(2\cos\theta_1)} r_1^{-2} \, dr_1\right). \tag{30}$$

Using Eq. 12 and evaluating the integral, we find for the diffusive flux

$$J \leq 4\pi Dac(\infty) \, [1 - (a/2b)]. \tag{31}$$

Comparing Eq. 31 with the exact formula (Eq. 21) we see that this bound is exact for $b \to \infty$. In the limiting case $b = 2a$, the exact flux is $2k_{1+}c(\infty)\ln 2$ and the upper bound, Eq. 31, gives a flux $2k_{1+}c(\infty)3/4$. This bound is too high by only 8%.

Next we consider the case $0 \leq b \leq 2a$, which corresponds to two identical interpenetrating spheres. It is straightforward to verify that Eq. 31 should be replaced by the inequality

$$J \leq 2\pi Dac(\infty) \, [1 + (b/4a)]. \tag{32}$$

For this case we are aware of the exact result only when $b = a\sqrt{2}$, which is given on p. 135 of reference 10,

$$J = 2\pi Dac(\infty)\left(2 - \frac{1}{\sqrt{2}}\right). \tag{33}$$

The upper bound $2\pi Dac(\infty)[1 + (1/4)\sqrt{2}]$ given by Eq. 32 is too high by <5%.

The method we have used to obtain bounds for the two-hemisphere problem can be extended to obtain bounds on the diffusive flux into any configuration of hemispheres distributed on a plane. For example, it is straightforward to show, using simple trial functions similar to those of Eq. 29, that for a string of n touching hemispheres of equal radius, the centers of which are on a straight line, the diffusion-limited forward rate constant obeys the inequality

$$k_+ \leq \pi Dan \, [1 + (1/n)], \tag{34}$$

where for $n = 1$ the expression is exact.

BINDING TO A CELL

To complete the picture of ligands binding to receptors on cells, we now briefly discuss the calculation of the diffusion-limited forward rate constant for an entire cell. For an arbitrary cluster of receptors, modeled as hemispheres, our method leads to an approximate expression for the diffusion-limited ligand flux

$$J_1 = \alpha Dac(\infty), \tag{35}$$

where the numerical constant α depends on the geometry of the cluster, and a is the radius of a hemisphere. For example, $\alpha = 2\pi$ for a single hemisphere, $\alpha = 4\pi\ln 2$ for two touching hemispheres and $\alpha \leq \pi n \, [1 + (1/n)]$ for n touching hemispheres in a line.

Now consider a spherical cell of radius R, on the surface of which N of these receptor clusters are uniformly distributed. For the simplest case in which there is no nonspecific interaction between the cell and the ligands, the total diffusion-limited ligand flux into the cell (see reference 1) is given by

$$J_N = 4\pi RDc(\infty) \frac{\alpha aN}{4\pi R + \alpha aN}. \tag{36}$$

This expression can be generalized to include nonspecific cell-ligand interactions, as well as nonspherical cell geome-

tries and nonuniform distribution of receptor clusters (2, 3).

Receptor clustering decreases the forward rate constant because more than one binding site competes for the same ligand. Placing receptors on a cell clusters them, even if they are uniformly distributed over the cell surface. This is reflected in Eq. 36 by the dependence of the flux on N (1, 15). If the receptors are not uniformly distributed there is additional clustering and the flux is further reduced.

CONCLUDING REMARKS

The variational principle that formed the basis of the calculations in this paper can be used to analyze any receptor cluster than can be represented by a collection of hemispheres. Additional results for a variety of such clusters will be discussed in a future paper (Weigel and Goldstein, in preparation). The method can also easily be extended to treat diffusion into three-dimensional clusters of spherical receptors.

In this paper we used only the simplest type of trial function for the variational principle, yet the results were remarkably accurate in those cases where comparison with the exact results was possible. The method of images suggests a systematic way to improve the trial function.

In a more biological context the main conclusion of our work is that receptor clustering decreases the diffusion-limited forward rate constant. In the case of two touching hemispheres, this constant is reduced by a factor of ~0.69. As the clusters become larger the reduction becomes more pronounced. Because large clusters of receptors do occur on cell surfaces, for example, LDL receptors in coated pits (6), this may be a significant effect.

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