THE EFFECT OF DIFFUSION ON THE BINDING OF MEMBRANE-BOUND RECEPTORS TO COATED PITS

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ABSTRACT We have formulated a kinetic model for the primary steps that occur at the cell membrane during receptor-mediated endocytosis. This model includes the diffusion of receptor molecules, the binding of receptors to coated pits, the loss of coated pits by invagination, and random reinsertion of receptors and coated pits. Using the mechanistic statistical theory of nonequilibrium thermodynamics, we employ this mechanism to calculate the two-dimensional radial distribution of receptors around coated pits at steady state. From this we obtain an equation that describes the effect of receptor diffusion on the rate of binding to coated pits. Our equation does not assume that ligand binding is instantaneous and can be used to assess the effect of diffusion on the binding rate. Using experimental data for low density lipoprotein receptors on fibroblast cells, we conclude that the effect of diffusion on the binding of these receptors to coated pits is no more than 84% diffusion controlled. This corresponds to a dissociation rate constant for receptors on coated pits (k^-) that is much less than the rate constant for invagination of the pits ($\lambda = 3.3 \times 10^{-3}$/s) and a correlation length for the radial distribution function of six times the radius of a coated pit. Although the existing experimental data are compatible with any value of k^-, we obtain a lower bound for the value of the binding constant (k^+) of $2.3 \times 10^{-2}(\mu m)^2$/s. Comparison of the predicted radial distributions with experiment should provide a clear indication of the effect of diffusion on k^+.

INTRODUCTION

Protein molecules, such as insulin, or large molecular clusters, such as low density lipoproteins (LDL), enter cells through different mechanisms from small molecules or hydrated ions. Large molecules are too big for pores, channels, or simple carriers and enter by more complicated routes (1). During the last two decades it has become clear that many large molecules enter cells through a process mediated by mobile receptors situated in the cell membrane (2, 3). After binding to the receptor, the ligand-receptor complex appears to move by diffusion in the plane of the plasma membrane until it encounters specialized patches called coated pits. These patches are visible as fuzzy areas in electron micrographs and are composed predominately of a protein called clathrin (4). Coated pits provide locations where the plasma membrane can invaginate. Although the fate of these invaginations inside the cell is controversial (5, 6), the invaginations function to carry ligands bound to receptors into the cell. This process is called receptor-mediated endocytosis.

Receptor-mediated endocytosis is a remarkable mechanism for gaining entrance to a cell. It has many points for potential control, e.g., negative feedback of internalized ligands on the rate of receptor synthesis, and is known to be the mechanism of entry of a great number of substances into a variety of cells (1, 6, 7). Although few details of the molecular mechanism for individual steps in the process are known, it has been possible to measure membrane diffusion constants for several different receptors (8, 9, 10). The receptors, which are present in the order of 10^4–10^5 copies per cell (11), have been hypothesized to be inserted randomly on the cell surface (1). If this is so, they must diffuse until they are bound in the clathrin-coated pits. The measured diffusion constants are smaller than expected on the basis of viscosity measurements of cell membranes, possibly due to interference by submembrane structures (8, 9, 10). Because diffusion is slow, it has been suggested that the rate of association of receptors and coated pits is a diffusion-controlled rate process (12). Such a process would be the two-dimensional analogue of diffusion-controlled reactions in solution (13), such as fluorescence quenching. The intrinsic rate of these reactions is so rapid that the rate at which reactants diffuse together ends up determining the overall rate of the reaction (14).

Several calculations have been made to assess the effect of diffusion on the binding of receptors to coated pits (12, 15, 16, 17). These calculations are based on Fick's diffusion equation and use ideas borrowed from the Smoluchowski theory for solution phase reactions. Unfortunately, the Smoluchowski theory has a logarithmic divergence in two dimensions. Consequently, it has been necessary to introduce modifications into the Smoluchowski theory or to use the diffusion equation to estimate

rate constants by calculating mean first-capture times for receptors.

Here we present a calculation of the effect of diffusion on the binding rate of receptors by coated pits that is free of the problems of the Smoluchowski theory (18, 19). We rely on the mechanistic statistical theory of nonequilibrium thermodynamics (20, 21) to calculate the steady-state radial-distribution function of receptors around coated pits. This type of calculation has been successfully applied to rapid reactions in solution (19, 22), and accounts for a variety of effects, including the lifetime for reactants, that are not included in the Smoluchowski theory. The theory can also be applied to reactions that are confined to membranes without the divergences that plague the Smoluchowski theory (19).

The calculations here are restricted to a particular model. This model, described in the following section, specifically assumes that receptors are inserted randomly into the membrane, as originally envisioned by Goldstein et al. (1). Recently there has been evidence presented for certain receptors that insertion is not random, but rather is preferential in the neighborhood of coated pits (23). Our calculation, although based on random insertion, gives rise to a structured radial distribution function of unbound receptors around coated pits. This distribution of unbound receptors increases with distance from the pit with a characteristic length, ξ^{-1}. This length depends on the rates of the kinetic processes involved in the process of endocytosis, and we obtain an explicit expression for ξ^{-1} using our model.

Another basic assumption in our model is that receptor molecules are capable of binding to coated pits independently of ligand binding. While this is true for some receptors, such as LDL receptors on fibroblasts (1), other receptors appear to bind to coated pits only when their ligands are already attached (24). Although our calculations can be extended to include this complication, we have not yet done so. The measurements of the movement of receptors by photobleaching experiments have demonstrated that their motion is diffusive in character (8, 9, 10). Nonetheless, it is conceivable that recycling of the plasma membrane induces a mechanical streaming that may be capable of conveying receptors to coated pits (25). Although we do not consider such a mechanism here, our results have some implications for this proposal. According to our findings, even the very small diffusion constant that characterizes LDL receptors on fibroblasts is large enough to account for the observed rate of turnover and distribution of the LDL receptors. While we do not rule out the existence of membrane streaming theoretically, we do conclude that diffusive transport of receptors coupled with the kinetics of binding and unbinding from coated pits provides a quantitative explanation of existing data for LDL receptors on fibroblasts.

Our calculation does not assume an instantaneous intrinsic rate of receptor binding to coated pits. Thus it can be used to assess the effect of diffusion on the binding process. With the exception of the dissociation rate constant of receptors from coated pits, k^-, the parameters needed for calculating the binding rate constant, k^+, of LDL receptors on fibroblasts have been measured. In the section entitled Diffusion-controlled Binding of LDL Receptors, we carry out the calculation of k^+ assuming that binding is diffusion controlled. We find that the experimental data for LDL receptors is incompatible with the assumption of diffusion control. In the section entitled Combined Diffusion-Reaction Control: LDL Receptors, we drop this assumption. Our calculations there show that no matter what the size of the dissociation rate constant of receptors from coated pits, the binding rate is no more than 84% diffusion controlled. Hence we conclude that while diffusion may have a significant effect in the receptor mediated endocytosis of LDL, the existing data are compatible with no diffusion effects. In the section entitled The Radial Distribution Function: LDL Receptors, we evaluate the radial distribution function for LDL receptors around coated pits. We find that receptors are depleted within a range of about six times the radius of a coated pit. If the dissociation rate constant, k^-, is small enough, this phenomenon should be observable in images of fibroblast membranes quenched from 37°C. This provides a new prediction for testing the effect of diffusion in the random insertion model. Finally, in the section entitled Relationship to Smoluchowski Theory, we examine the relationship of the present theory to earlier calculations based on the modified Smoluchowski theory.

MODEL OF CELL MEMBRANE PROCESSES

Three elementary processes are included in our model of the membrane-based events that occur in receptor-mediated endocytosis (12, 16, 17). We first consider the process of invagination in which coated pits are removed from the active membrane surface. We treat this as a first-order rate process dependent only on the total number of coated pits, N_p, present at a given time. Thus invagination is assumed to be independent of the number of receptors bound to the coated pit. If the area of the membrane is A, then the time rate of change of the number density, $\rho_p = N_p/A$, of coated pits due to invaginations is

$$(d\rho_p/dt)_{invag} = -\lambda\rho_p, \tag{1}$$

where λ^{-1} is the average lifetime of a coated pit before invagination. To account for the fact that coated pits are recycled from the invagination process, we must add to this a term K_p to account for the reinsertion of pits. For simplicity we treat this term as a constant, so that the rate of change of ρ_p is given by

$$d\rho_p/dt = -\lambda\rho_p + K_p. \tag{2}$$

Two molecular processes are important for the membrane bound receptors. First, we assume that the receptors are mobile so that they diffuse through the plasma membrane. Thus at any position $\mathbf{r} = (x, y)$ in the membrane surface, the local number density of free receptors, $\rho_r(\mathbf{r}, t)$, changes according to Fick's law. This imples that

$$[\partial\rho_r(\mathbf{r}, t)/\partial t]_{diff} = D\nabla^2\rho_r(\mathbf{r}, t), \tag{3}$$

where D is the diffusion constant for the receptors. Again, for simplicity, we assume that the diffusion constant for a receptor is independent of

whether or not it carries a bound ligand. This is probably a good assumption since the magnitude of receptor diffusion constants is probably dominated by the cytoplasmic constraints.

The local number density of free receptors also changes because of the binding of receptors to coated pits. This is a nonlinear process that involves encounters between a free receptor and a coated pit. To make this plain, we distinguish among coated pits that have $j = 1,2,3,\ldots$ receptors bound to them. If the number of pits with j receptors bound is N_{pj}, then their surface density is $\rho_{pj} = N_{pj}/A$. The elementary binding process can be represented by

$$p_j + r \underset{(j+1)k^-}{\overset{k^+}{\rightleftharpoons}} p_{j+1}, \tag{4}$$

where p_j represents a coated pit with j receptors and r is an unbound receptor. The rate constant for the unbinding step is proportional to $(j + 1)$ since any of the $(j + 1)$ bound receptors could be escaping. Thus $1/k^-$ is the average lifetime of a receptor in a pit. This process leads to the following kinetic equations for the change in ρ_r

$$(\partial\rho_r/\partial t)_j = -k^+\rho_r\rho_{pj} + (j + 1)k^-\rho_{p,j+1}, \tag{5}$$

where $j \geq 0$. If both sides are summed over j, we get the net effect on ρ_r of the binding process, which is

$$(\partial\rho_r/\partial t)_{bind} = k^+\rho_r\rho_p + k^-(N_{rp}/A), \tag{6}$$

where $N_{rp} = \Sigma_j\, j\rho_{pj}A$ is the total number of receptors in pits. Since the receptors in pits are localized in the pits, their physical number density is N_{rp}/A_p, where A_p is the total area occupied by the pits. This is in practice ~1% of the total membrane area under steady state conditions at 37°C. For simplicity in the resulting equations we use instead the density N_{rp}/A, which is the overall number density of receptors in pits. Thus

$$\rho_{rp} = N_{rp}/A = \sum_j j\rho_{pj}. \tag{7}$$

In this notation

$$(\partial\rho_r/\partial t)_{bind} = -k^+\rho_r\rho_p + k^-\rho_{rp}. \tag{8}$$

Finally, we must consider a contribution to the local density of receptors that comes from reinsertion of receptors into the membrane. Assuming that this is uncorrelated with the density of the coated pits, we treat this term as a positive constant, K_r. Adding to this term to Eqs. 3 and 8, it results that

$$\partial\rho_r/\partial t = -k^+\rho_p\rho_r + k^-\rho_{rp} + D\nabla^2\rho_r + K_r. \tag{9}$$

Using Eq. 6 and the definition of ρ_{rp} in Eq. 7, we find that binding changes the overall density of receptors in pits according to

$$(\partial\rho_{rp}/\partial t)_{bind} = k^+\rho_r\rho_p - k^-\rho_{rp}. \tag{10}$$

This term must be supplemented with the rate of loss of receptors from the membrane when the pits invaginate. As we have assumed that this process is independent of the number of receptors in a pit, it follows

$$(\partial\rho_{pj}/\partial t)_{invag} = -\lambda\rho_{pj}, \tag{11}$$

so that multiplying Eq. 11 by j and summing as indicated in Eq. 7 gives

$$(\partial\rho_{rp}/\partial t)_{invag} = -\lambda\rho_{rp}. \tag{12}$$

Adding Eqs. 10 and 12 together gives the total effect of invagination and binding on ρ_{rp} as

$$\partial\rho_{rp}/\partial t = k^+\rho_p\rho_r - k^-\rho_{rp} - \lambda\rho_{rp}. \tag{13}$$

Eqs. 2, 9, and 13 are the basic kinetic equations of our model.

Our model is essentially that proposed by Wofsy and Goldstein (12, 16, 17). The differences are that we explicitly retain the dissociation rate, $k^-\rho_{rp}$, in our calculations; we treat the rate of recycling of pits and rate of insertion of receptors as constants; and, finally, we explicitly include the effect of diffusion of the receptors. Like earlier models, we have made several simplifying assumptions. First, we have assumed that insertion of receptors is uncorrelated to the location of the pits. Second, we have assumed that receptors are free to bind to pits whether or not they are carrying ligands. Third, we assume that the binding of a ligand does not modify the diffusion constant of a receptor. All these restrictions, in principle, can be relaxed in our model.

CALCULATION OF THE DENSITY FLUCTUATIONS

Eqs. 2, 9, and 13, which define the dynamics of our model, describe only what happens in the cell membrane on the average. In other words, if we examine a particular section of the membrane that contains a large number of receptors and pits, these equations give us the expected behavior. However, in a large section of membrane one actually has a distribution of densities of receptors and pits. Thus, there are density fluctuations (20, 21). These fluctuations are not included in the average description, although it is known that the same events that describe the average dynamics can be used to describe these fluctuations (18, 19, 20, 21).

Since the coated pits may, in principle, occupy any spatial position in the membrane, they are distributed, on the average, uniformly in a membrane. The same holds true for the receptors. Thus, on the average, our model implies that

$$d\bar{\rho}_p/dt = -\lambda\bar{\rho}_p + K_p, \tag{14}$$

and

$$\begin{aligned} d\bar{\rho}_r/dt &= -k^+\bar{\rho}_p\bar{\rho}_r + k^-\bar{\rho}_{rp} + K_r, \\ d\bar{\rho}_{rp}/dt &= k^+\bar{\rho}_p\bar{\rho}_r - k^-\bar{\rho}_{rp} - \lambda\bar{\rho}_{rp}, \end{aligned} \tag{15}$$

since diffusion effects vanish in a system that is uniform on the average. Eqs. 14 and 15 can be used to obtain the steady state values of $\bar{\rho}_p$, $\bar{\rho}_r$, and $\bar{\rho}_{rp}$, that is, the constant values when transient time dependence has vanished. This means that the left-hand sides of Eqs. 14 and 15 are zero. The solutions to the steady state equations are

$$\rho_r^{ss} = \frac{\rho_{rp}^{ss}(\lambda + k^-)}{\rho_p^{ss}k^+} \tag{16}$$

$$\rho_{rp}^{ss} = K_r/\lambda \tag{17}$$

$$\rho_p^{ss} = K_p/\lambda. \tag{18}$$

We are interested in calculating fluctuations about these averages. In particular we are interested in obtaining the average number density of unbound receptors around a coated pit, $\rho_{p,r}$. This function can be obtained from the statistical fluctuations in density at steady state using the relationship (18, 19, 22)

$$\langle\delta\rho_p(\mathbf{r})\delta\rho_r(\mathbf{r}')\rangle^{ss} = \rho_p^{ss}[\rho_{p,r}(|\mathbf{r} - \mathbf{r}'|) - \rho_r^{ss}] \tag{19}$$

where the angular brackets represent the statistical average over the steady state distribution function and $\delta\rho_i(\mathbf{r}) = \rho_i(\mathbf{r}) - \rho_i^{ss}$, the fluctuation of the density around its steady state value. It is conventional to write the function $\rho_{p,r}(|\mathbf{r} - \mathbf{r}'|)$ in terms of the radial distribution function (26), $g_{p,r}$, which is defined by $g_{p,r} = \rho_{p,r}/\rho_r$. Thus the radial distribution function can be calculated from the formula

$$g_{p,r}(|\mathbf{r} - \mathbf{r}'|) = 1 + \langle\delta\rho_p(\mathbf{r})\delta\rho_r(\mathbf{r}')\rangle^{ss}/\rho_p^{ss}\rho_r^{ss}. \tag{20}$$

The radial distribution function gives information about the average spatial distribution of receptors around coated pits.

The radial distribution function can also be used to calculate the

binding rate constant, k^+. The formula (18, 19, 22) requires a knowledge of $k^0(r)$, which is called the intrinsic reaction rate constant, and for circular symmetry in two dimensions is

$$k^+ = 2\pi \int_0^\infty k^0(r) g_{p,r}(r) r dr. \tag{21}$$

The simplest type of reactivity function dictates that binding occurs only at the radius, R, of the pit with an intrinsic rate constant, k^0. Thus $k^0(r) = k^0\delta(r - R)/2\pi r$, and so Eq. 21 implies that (19)

$$k^+ = k^0 g_{p,r}(R). \tag{22}$$

The reaction will be diffusion controlled when $k^0/k^+ \gg 1$ (an intrinsically rapid reaction) for which one recovers the Smoluchowski or sink boundary condition (13) $g_{p,r}(R) = 0$. Eq. 22, however, is more general and includes the possibility that diffusion is only partially rate limiting, i.e., $k^0/k^+ \approx 1$.

To obtain the radial distribution function we calculate the pit-density, receptor-density correlation function indicated on the right-hand side of Eq. 20 using the mechanistic statistical theory of nonequilibrium thermodynamics (20, 21). According to that theory fluctuations in the densities of coated pits, receptors in pits and unbound receptors can be obtained by linearizing Eqs. 2, 9, and 13 around steady state and then adding appropriate random terms. The resulting equations are

$$\partial\delta\rho_p/\partial t = -\lambda\delta\rho_p + \tilde{f}_1 \tag{23}$$

$$\partial\delta\rho_r/\partial t = -k^+\rho_r^{ss}\delta\rho_p - k^+\rho_p^{ss}\delta\rho_r + D\nabla^2\delta\rho_r + k^-\delta\rho_{rp} + \tilde{f}_2 \tag{24}$$

$$\partial\delta\rho_{rp}/\partial t = k^+\rho_r^{ss}\delta\rho_p + k^+\rho_p^{ss}\delta\rho_r - (k^- + \lambda)\delta\rho_{rp} + \tilde{f}_3. \tag{25}$$

The random terms, f_i, are given by the mechanism. They are multivariant white noise that vanish on the average and have the covariant matrix (20, 21)

$$\langle \tilde{\mathbf{f}}(\mathbf{r},t)\tilde{\mathbf{f}}^T(\mathbf{r}',t)\rangle = \gamma(\mathbf{r},\mathbf{r}')\delta(t - t'). \tag{26}$$

The form of γ is obtained in the Appendix

$$\gamma(\mathbf{r},\mathbf{r}') = \begin{bmatrix} \lambda\rho_p^{ss} & 0 & \lambda\rho_{rp}^{ss} \\ 0 & k^+\rho_r^{ss}\rho_p^{ss} + k^-\rho_{rp}^{ss} & -(k^+\rho_r^{ss}\rho_p^{ss} \\ & -2D\nabla_r^2 & +k^-\rho_{rp}^{ss}) \\ \lambda\rho_{rp}^{ss} & -(k^+\rho_r^{ss}\rho_p^{ss} & k^+\rho_r^{ss}\rho_p^{ss} \\ & +k^-\rho_{rp}^{ss}) & +(k^- + \lambda\langle j\rangle)\rho_{rp}^{ss} \end{bmatrix} \delta(\mathbf{r} - \mathbf{r}'), \tag{27}$$

where we have indexed $p = 1$, $r = 2$, and $pr = 3$. Consider, now, the covariance matrix $\langle\delta\rho_i(\mathbf{r})\delta\rho_j(\mathbf{r}')\rangle^{ss} \equiv \sigma_{ij}(\mathbf{r},\mathbf{r}')$. This matrix is known to solve the matrix differential equation

$$H\sigma + \sigma H^T = -\gamma, \tag{28}$$

which is called the steady state fluctuation-dissipation theorem. The matrix H is the linear matrix differential operator in Eqs. 23–25, namely,

$$H(\mathbf{r},\mathbf{r}') = \begin{bmatrix} -\lambda & 0 & 0 \\ -k^+\rho_r^{ss} & -k^+\rho_p^{ss} + D\nabla_r^2 & k^- \\ k^+\rho_r^{ss} & k^+\rho_p^{ss} & -(k^- + \lambda) \end{bmatrix} \delta(\mathbf{r} - \mathbf{r}'). \tag{29}$$

In the notation of Eq. 28 the pit-density receptor-density correlation function is given by $\sigma_{21} = \sigma_{12}$. The solution of Eq. 28 is most easily obtained (19) by two-dimensional Fourier transformation of Eq. 28, i.e.,

$$\sigma_{ij}(\mathbf{k},\mathbf{k}') \equiv \frac{1}{(2\pi)^4}\int d\mathbf{r}\int d\mathbf{r}'\, e^{i\mathbf{k}\cdot\mathbf{r}}e^{i\mathbf{k}'\cdot\mathbf{r}'}\sigma_{ij}(\mathbf{r},\mathbf{r}'). \tag{30}$$

Performing the indicated integrals and solving the resulting algebraic equations we find that

$$\sigma_{12}(\mathbf{k},\mathbf{k}') = \left[\frac{-k^+\rho_r^{ss}\rho_p^{ss}\bar{b}}{2D(\xi^2 + k^2)}\right]\frac{\delta(\mathbf{k} + \mathbf{k}')}{(2\pi)^2}, \tag{31}$$

where

$$\bar{b} = b\lambda/(\lambda + k^-)$$

$$b = 2\lambda/(2\lambda + k^-)$$

$$\xi = \left(\frac{\rho_p^{ss}bk^+ + \lambda}{D}\right)^{1/2}. \tag{32}$$

The inverse Fourier transform is standard (19) and yields

$$\langle\delta\rho_p(\mathbf{r})\delta\rho_r(\mathbf{r}')\rangle^{ss} = \sigma_{12}(|\mathbf{r} - \mathbf{r}'|) = -k^+\frac{\rho_r^{ss}\rho_p^{ss}\bar{b}}{4\pi D}K_0(\xi|\mathbf{r} - \mathbf{r}'|), \tag{33}$$

where K_0 is the McDonald function (also called a modified Bessel function) of order zero (27). Thus using Eq. 20 the radial distribution function is

$$g_{r,p}(r) = 1 - \frac{k^+\bar{b}}{4\pi D}K_0(\xi r). \tag{34}$$

EFFECT OF DIFFUSION ON THE BINDING RATE CONSTANT

The effect of diffusion, if any, on steady state endocytosis, comes through effects on the binding rate constant, k^+. According to the previous section, k^+ can be calculated using Eq. 22. For our model of endocytosis, $g_{r,p}$ is given by Eq. 34, so that Eq. 22 becomes explicitly

$$k^+ = k^0\left[1 - \frac{k^+\bar{b}}{4\pi D}K_0(\xi R)\right]. \tag{35}$$

Since Eq. 32 shows that ξ depends on k^+, Eq. 35 is a transcendental equation for k^+ in terms of λ, k^-, D, k^0, R and ρ_p^{ss}. As a practical matter it has not been possible to measure k^- or k^0. However, Eq. 16, which determines the steady state values of ρ_r^{ss}, provides additional information. It can be written

$$k^+ = \frac{\rho_{rp}^{ss}}{\rho_r^{ss}\rho_p^{ss}}(\lambda + k^-). \tag{36}$$

Eqs. 35 and 36 provide two independent equations for k^+. The steady state values of $\rho = (\rho_{rp}^{ss}/\rho_r^{ss})$ and ρ_p^{ss} needed in Eq. 36 can be estimated from electron micrographs using various labels. The value of λ also has been measured for certain membranes (28). We are not aware of any measurements that have established values for k^- in any system.

Were the value of k^0 known, Eqs. 35 and 36 could be used to establish the values of both k^+ and k^-. We carry out a calculation of this sort in the following section. Alternatively, if one knows k^-, then Eqs. 35 and 36 can be used to establish the values of k^+ and k^0. This calculation is

carried out in the section entitled Combined Diffusion-Reaction Control: LDL Receptors.

DIFFUSION-CONTROLLED BINDING OF LDL RECEPTORS

As mentioned in the Introduction, our model is appropriate for receptor mediated endocytosis of LDL receptors on fibroblasts. Evaluation of the effect of diffusion on the binding rate using modifications of the Smoluchowski theory has led to the conclusion that the binding reaction is diffusion controlled. In the context of the present theory this implies that the intrinsic rate constant, k^0, is very large. More specifically, solving Eq. 35 we see that

$$k^+ = \frac{4\pi D[\bar{b}K_0(\xi R)]^{-1}k^0}{k^0 + 4\pi D[\bar{b}K_0(\xi R)]^{-1}}. \tag{37}$$

The reaction will be diffusion-controlled when

$$k^0 \gg 4\pi D[\bar{b}K_0(\xi R)]^{-1}, \tag{38}$$

in which case

$$k^+ = 4\pi D/\bar{b}K_0(\xi R), \tag{39}$$

with $\bar{b}$ and ξ given in Eq. 32. Notice that k^0 no longer appears in Eq. 39. Thus under the assumption that the reaction is diffusion controlled, we can attempt to find simultaneous solutions of Eqs. 39 and 36 for k^+ and k^-. To do this we eliminate k^+ from Eq. 39 using Eq. 36. We find that

$$\frac{2\pi D\rho_p^{ss}(2+f)}{\lambda\rho} = K_0\left(R\left(\frac{\lambda}{D}\right)^{1/2}\right)\left[1 + 2\rho\left(\frac{1+f}{2+f}\right)\right]^{1/2}), \tag{40}$$

where $f = k^-/\lambda$. Because of the way we defined ρ_{rp}^{ss}, $\rho = \rho_{rp}^{ss}/\rho_r^{ss}$ is the ratio of the number of receptors in coated pits to the number of receptors out of the pits at steady state. Its value is known for LDL receptors on fibroblasts. Since the value of λ, as well as all other parameters in Eq. 40, are known, we can solve Eq. 40 for k^-. Using the values in Table I and defining $x = (1 + f)/(2 + f)$, Eq. 40 becomes

$$1.2 = (1 - x)K_0(0.086[1 + 4.4x]^{1/2}). \tag{41}$$

Notice that x is bounded below by $\frac{1}{2}$ and above by 1. Thus we need to find an x in this range that solves Eq. 41. For $\frac{1}{2} \le x \le 1$, the argument of K_0 in Eq. 41 varies between 0.15 and 0.20, and the value (27) of K_0 varies between 2.01 and 1.75. Since $(1 - x)$ varies between 0.5 and 0 over this range, Eq. 41 has no solution for LDL receptors based on the values in Table I. Because the values in Table I are taken from experiments that have a fairly large uncertainty, it is not possible to state categorically that the binding of LDL receptors is not diffusion controlled. Indeed, by using a somewhat different set of values, which are still plausible experimentally, it is possible to obtain a solution to Eq. 41. We explore the importance of diffusion on the binding of LDL receptors further in the next section by dropping the assumption of diffusion control.

TABLE I
CHARACTERISTIC PARAMETERS FOR LDL RECEPTORS ON FIBROBLASTS

Parameter	Symbol	Value	Source
Radius of coated pits	R	0.10 μm	Reference 17
Receptor diffusion constant	D	4.5×10^{-3} $(\mu m)^2/s$	Reference 8
Steady state density of coated pits (37°C)	ρ_p^{ss}	0.31 $(\mu m)^{-2}$	Reference 17
Invagination rate constant	λ	$3.3 \times 10^{-3}/s$	Reference 17
Number ratio of receptors in pits to receptors out of pits	$\rho = \rho_{rp}^{ss}/\rho_r^{ss}$	2.2	Reference 17

COMBINED DIFFUSION-REACTION CONTROL: LDL RECEPTORS

In the previous section we assumed the intrinsic binding rate was so rapid that the binding process was completely under diffusion control. Here we relax that assumption and allow for the possibility that the intrinsic reaction rate, k^0, is not indefinitely large. Thus the rate constant would be affected by both diffusion and reaction rates. Our analysis is based on the more general expression for k^+ in Eq. 37. For ease in writing we define the diffusion-controlled value of k^+ to be (cf. Eq. 39)

$$k_D = 4\pi D/\bar{b}K_0(\xi R). \tag{42}$$

Thus we can rewrite Eq. 37 in the form

$$k^+ = k_D k^0/(k_D + k^0). \tag{43}$$

To analyze diffusion effects on k^+, we examine the ratio $k^+/k^0 = y$. Dividing both sides by k^0, Eq. 43 can be written

$$y = \frac{1}{1 + k^+/k_D y} \tag{44}$$

or

$$y = 1 - (k^+/k_D). \tag{45}$$

We obtain an explicit expression for y in terms of x by substituting Eq. 36 for k^+ on the right-hand side of 45 and for k^+ in the argument of k_D using the definition of ξ in Eq. 32. This leads to

$$y = 1 - \frac{\rho\lambda(1-x)}{2\pi D\rho_p^{ss}} K_0\left(R\left(\frac{\lambda}{D}\right)^{1/2}[1 + 2\rho x]^{1/2}\right). \tag{46}$$

For LDL receptors we again use Table I to evaluate the

parameters in Eq. 46, finding that

$$y = 1 - \frac{(1 - x)}{1.2} K_0(0.086[1 + 4.44\,x]^{1/2}). \quad (47)$$

Eq. 47 can be used to evaluate the effect of diffusion on k^+ as a function of the value of k^-, the unbinding rate. Recall that

$$x \equiv (1 + k^-/\lambda)/(2 + k^-/\lambda), \quad (48)$$

and that x lies in the range $\frac{1}{2} \leq x \leq 1$. As we have seen in the previous section, there is no value of x for which $(1 - x)K_0$ is as large as 1.2. Thus y must be greater than zero. In other words, k^0 is not indefinitely large and so the reaction cannot be diffusion controlled, as was noted in the previous section.

To find k^+ and k^0 for different values of k^-, we need only assume a value of k^- and then use Eqs. 47 and 48. The smallest value that x can have is $\frac{1}{2}$, corresponding to $k^- = 0$. According to Eq. 47 this gives

$$y = 1 - 0.42\,K_0(0.15) = 0.16. \quad (49)$$

Thus

$$k^+ = 0.16\,k^0, \quad (50)$$

which means that the reaction rate is less than that predicted by diffusion control, but that diffusion still has a predominant effect. Indeed, combining Eqs. 45 and 50 gives

$$k^+ = 0.84\,k_D, \quad (51)$$

so that the reaction is ~84% diffusion controlled. For values of k^- greater than zero, the effect of diffusion decreases. For example, for $x = 0.67$, $k^- = \lambda$ and the reaction is only 53% diffusion controlled. Finally, if k^- is greater than λ by a factor of 10 or so, then $y \approx 1$ and diffusion will have no effect on the binding reaction.

The effect of an increased value of the dissociation rate constant, k^-, on the observed binding constant, k^+, is easy to understand. When k^- is zero, the reaction is 84% diffusion controlled. In terms of the radial distribution function in Eq. 34 this means that the density of receptors at the radius, R, of a coated pit is depleted by 84% of its average value in the membrane. This is the cause of the resultant decrease in the observed value of k^+. A nonzero value of k^- means that there is a reinjection of receptors into the membrane near R. The effect of this reinjection is small if the average residence time of a receptor in a pit, i.e., $1/k^-$, is much longer than the lifetime of a coated pit, i.e., $1/\lambda$. In other words, as long as $\lambda \gg k^-$, which corresponds to $x \approx \frac{1}{2}$, dissociation will be ineffective in countering the slowing effect of diffusion. However, for $x \approx 1$, $k^- \gg \lambda$, and so the residence time of a receptor in a coated pit is much shorter than the lifetime of a pit. This implies that reinjection of receptors from the coated pit into the membrane is an important process and will raise the receptor density near the pit. This will counteract the effect of diffusion.

To evaluate the magnitude of k^+ for the range of (unknown) values of k^- for LDL receptors, it is simplest to use Eq. 36. Employing the values in Table I, this equation can be written

$$k^+ = [2.3x/(1 - x)] \times [10^{-2}(\mu m)^2 s^{-1}]. \quad (52)$$

The smallest possible value of x is $\frac{1}{2}$, corresponding to $k^- = 0$. This provides the same lower bound for k^+ obtained by Wofsy and Goldstein (16, 17), namely,

$$2.3 \times 10^{-2}(\mu m)^2 s^{-1} \leq k^+. \quad (53)$$

If k^- is comparable to λ, the value of k^+ increases. Thus for $k^- = \lambda$, $x = 0.67$ and the calculated value of k^+ is $4.6 \times 10^{-2}\,(m)^2 s^{-1}$. We can set an upperbound for k^+ by using an expression for k^0 equal to the two-dimensional collisional rate of an LDL-receptor complex with a coated pit, namely

$$k^0 \leq 2R(2\pi k_B T/m)^{1/2}. \quad (54)$$

Here k_B is Boltzmann's constant, T is the absolute temperature, and m is the mass of a receptor-LDL complex. Estimating (16, 17) the mass of an LDL-receptor complex to be 3×10^6 daltons, gives an upper-limit value for k^0 of $\sim 5 \times 10^5 (\mu m)^2/s$. The only way that k^+ could achieve such a high value would be in the complete absence of diffusion effects. The experimental data for LDL receptors could support such a value only if k^- were much larger than λ and binding occurred with every encounter of an LDL receptor and a coated pit.

RADIAL DISTRIBUTION FUNCTION: LDL RECEPTORS

The existing data on LDL receptors do not provide a conclusive test of the effect of receptor diffusion on endocytosis. As we showed in the previous section, diffusion will have a large effect (84%) if the dissociation reaction is slow and no effect if the dissociation reaction is rapid. Our calculations, however, suggest an experimental method that may help to differentiate between these two extreme possibilities. Because our calculation is carried out for the stationary state, we have been able to determine the radial distribution function of receptors around coated pits. That result is given by the formal expression in Eq. 34. Using the expression for $\bar{b}$ in Eq. 32, the expression for k^+ in Eq. 47 and the values in Table I, the radial distribution function for LDL receptors can be written

$$g_{r,p}(r) = 1 - 0.84(1 - x)K_0(0.086[1 + 4.4x]^{1/2} r/R). \quad (55)$$

The quantity

$$\xi^{-1} = R/(0.086[1 + 4.4\,x]^{1/2}) \quad (56)$$

in Eq. 55 is the correlation length. Its value is rather insensitive to the value of x. It varies from 6.5 R when $x =$

½ to 5.0 R when $x = 1$, and provides a measure of the length scale on which the radial distribution of density changes.

Perhaps the most striking feature of this radial distribution function is its dependence on the value of $x = (1 + k^-/\lambda)/(2 + k^-/\lambda)$. When k^- is much greater than λ, $x \approx 1$ and $g_{r,p}(r) \approx 1$. This means that the density of receptors adjacent to a coated pit is identical to its average value in the membrane and indicates, as discussed in the section entitled Diffusion-controlled Binding of LDL Receptors, that diffusion has no effect on the binding process. The maximum effect of diffusion occurs when $k^- = 0$ and $x = ½$, which gives

$$g_{r,p}(r) = 1 - 0.42\, K_0(r/6.5\, R). \tag{57}$$

This expression, which is graphed in Fig. 1, shows a pronounced dip at the radius of the pit, corresponding to the removal of receptors by the pit and the inability of diffusion to replace them. As Eq. 57 shows, the correlation length of the radial distribution function is 6.5 R when $k^- = 0$. This length is appreciably longer than the radius of a pit and yet much shorter than the average distance between coated pits, which is the order (16, 17) of 18 R. Thus it seems reasonable to expect that membrane imaging techniques should be able to detect such a decrease in density of receptors near coated pits, if it exists.

If k^- is comparable to λ, the dip in the radial distribution function is less pronounced. For example, for $x = 2/3$, $k^- = \lambda$ and the radial distribution function is

$$g_{r,p}(r) = 1 - 0.28\, K_0(r/5.9\, R). \tag{58}$$

Although the correlation length of $\xi^{-1} = 5.9\ R$ is not changed much by an increased value of k^-, the magnitude of the dip at the origin is greatly decreased. This is illustrated in Fig. 1. This effect is amplified as k^- gets larger, and when k^- exceeds λ by more than a factor of 10,

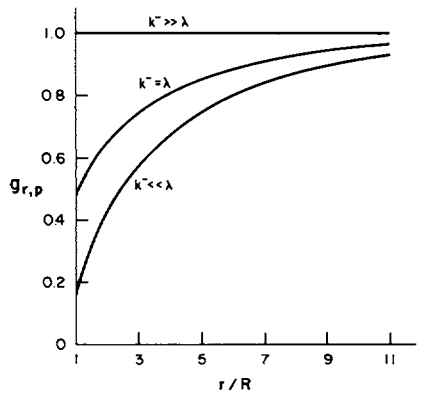

FIGURE 1 The radial distribution function of free LDL receptors around coated pits under three possible conditions. The lowest curve corresponds to $k^- \ll \lambda$ in Eq. 57, the middle curve to $k^- = \lambda$ in Eq. 58, and the upper curve to $k^- \gg \lambda$. The radial distance scale is the radius of a coated pit, R.

the density of receptors near a coated pit is within 90% of its average throughout the membrane.

We believe that membrane-imaging techniques may be able to settle the question of diffusion effects by directly observing the radial distribution function of receptors around coated pits. In these experiments it would be necessary to freeze-in the radial distribution function that develops during active endocytosis at 37°C by rapidly quenching the samples to a lower temperature. The temperature would need to be low enough so that both the recycling of coated pits and receptors as well as their diffusion was stopped. Whether or not this can be achieved in practice is not clear.

Freeze fracture images of LDL receptors and coated pits on fibroblasts have been published (28). In that study the fibroblasts were incubated at 4°C so that recycling of receptors and pits was inhibited. In our model this implies that the lifetime of the pits is effectively infinite, that is $\lambda = 0$. We can analyze this special case using Eqs. 32 and 34. Eq. 32 shows that the coefficient of K_0 in Eq. 34 goes to zero like λ^2, whereas the argument of K_0 vanishes like $\lambda^{1/2}$. Since for small values of its argument (19) $K_0(y) \approx \ln(y/2)$, it follows that $g_{r,p}(r) = 1$ when the lifetime of the coated pits is infinite. This is the expected result for thermodynamic equilibrium (19), which is the prevailing condition when neither pits nor receptors are recycling. Although we have not carried out a detailed analysis of the freeze-fracture images at 4°C, it is our visual impression that the radial distribution function is featureless as predicted by our calculation. We hope that data will become available to carry out an analysis of the radial distribution function under active endocytosis.

RELATIONSHIP TO THE SMOLUCHOWSKI THEORY

The fluctuation theory calculation that we have applied to describe diffusion effects on binding differs in significant ways from the Smoluchowski theory. For the sake of completeness we briefly examine some of the differences and similarities between the two types of calculations. As mentioned in the Introduction, the straightforward application of the Smoluchowski theory in two dimensions leads to a logarithmic divergence from the steady state calculation. Thus several modifications of that theory, which rely on the idea of trapping of diffusing receptors by static sinks, have been proposed (12, 15, 16, 17, 30, 31). The basic idea is to use the numerical value of the surface area occupied by an average sink, i.e., $1/\rho_p^{ss}$, to introduce a characteristic length that removes the divergence. To do this, a disk of radius b having this area, i.e., $\pi b^2 = 1/\rho_p^{ss}$, is associated with each sink. The annular region between $r = R$ and $r = b$ is then used to calculate the average time, t_c, that it takes a receptor in this annulus to reach the boundary of the pit at $r = R$. A reflection boundary condition is used at $r = b$ in order to keep receptors from escaping through the outer boundary.

There are several ways of implementing the calculation of the observed rate constant. One way is to calculate t_c directly from the diffusion equation and use the relationship

$$t_c = 1/k^+\rho_p^{ss}. \tag{59}$$

Another is to relate k^+ to the flux of receptors at the boundary of the sink, in analogy to the usual formulation of the Smoluchowski theory (13). This later approach (16, 17) yields the result originally obtained by Berg and Purcell (31)

$$t_c = \frac{b^2}{2D[1 - (R/b)^2]} \times [\ln(b/R) - \tfrac{3}{4} + (R/b)^2 - (R/b)^4/4]. \tag{60}$$

In the limit of dilute sinks Eq. 60 reduces to

$$t_c = (b^2/2D)[\ln(b/R) - \tfrac{3}{4}]. \tag{61}$$

A similar equation was obtained by Adam and Delbrück (30), however, with the term $\frac{3}{4}$ replaced by $\frac{1}{2}$. This difference between the two results depends on the way in which boundary conditions are chosen (16, 17).

The plausibility of the modified Smoluchowski theory suggests that there should be a relationship between the Berg-Purcell result and the fluctuation theory calculation. Since the static sinks in the modified Smoluchowski theory have an infinite lifetime, one might imagine that taking the infinite lifetime limit, i.e., $\lambda \to 0$, in the fluctuation theory result in should lead to the Berg-Purcell result. This, however, is not the case. In Eq. 37, for example, we have utilized the average steady state conditions in Eqs. 16–18. Thus, if we take $\lambda \to 0$, it follows that K_p and K_r (the recycling rates of coated pits and receptors) must also approach zero if ρ_{rp}^{ss} and ρ_p^{ss} are to maintain the observed values given in Table I. However, if there is no recycling, the steady state is an equilibrium state and, consequently, the radial-distribution function must be unmodified from its equilibrium form, i.e., $\lim_{\lambda\to 0} g_{r,p}(r) = 1$. Indeed, this is easily verified using Eq. 34 and Eq. 32 since

$$\lim_{\lambda\to 0} \bar{b}K_0(\xi r) = \lim_{\lambda\to 0} \frac{2\lambda^2}{(\lambda + k^-)(2\lambda + k^-)} K_0\left([\lambda/D]^{1/2}\left[\frac{2k^+\rho_p^{ss}}{2\lambda + k^-} + 1\right]\right) = \lim_{\lambda\to 0} \frac{-2\lambda^2}{k^{-2}}\left\{\ln\left([\lambda/D]^{1/2}\left[\frac{2k^+\rho_p^{ss}}{k^-} + 1\right]\right)\right\} = 0. \tag{62}$$

Consequently, Eq. 22 implies that $k^+ = k^o$ for $\lambda = 0$.

The disagreement between fluctuation theory and Eq. 60 arises because of the inconsistency in comparing the Berg-Purcell result (which holds when $\lambda = 0$) and experimental data (which hold for $\lambda \neq 0$). Goldstein and Wofsy (16, 17) have recently modified the Berg-Purcell calculation so that it applies when $\lambda \neq 0$.

The present fluctuation theory-type calculation can be applied to the problem of trapping static sinks in two dimensions, that is, the problem posed by Berg and Purcell. To do so we need to solve the appropriate fluctuation formulae for mobile receptors in a background of static sinks. This has been done already rather generally, and in two-dimensions one finds that (32)

$$g_{r,p}(r) = 1 - \frac{k^+}{2\pi D} K_0([k^+\rho_p^{ss}/D]^{1/2}r). \tag{63}$$

Assuming that the sinks are perfect absorbers means that $g_{r,p}(R) = 0$; or from Eq. 63 that

$$1/k^+ = K_0([k^+\rho_p^{ss}/D]^{1/2}R)/2\pi D. \tag{64}$$

From Eq. 64 it is easy to show that as the density of pits goes to zero

$$k^+\rho_p^{ss}/D = (t_cD)^{-1} \to 0. \tag{65}$$

To compare the solution of Eq. 64 with the Berg-Purcell result in Eq. 61 we use Eq. 59 and the fact that $\pi b^2 = 1/\rho_p^{ss}$ to rewrite Eq. 64 in the form

$$Dt_c = (b^2/2)K_0([t_cD]^{-1/2}R). \tag{66}$$

At low density of pits ρ_p^{ss} we use Eq. 65 and the asymptotic expansion

$$K_0(x) \sim -[\ln(x/2) + \gamma], \tag{67}$$

where $\gamma = 0.5772\ldots$ (Euler's constant) to rewrite Eq. 66 as

$$Dt_c = (b^2/2)\{\ln([t_cD]^{1/2}/R) - \gamma + \ln 2\}. \tag{68}$$

Iterating this expression once gives

$$t_c = (b^2/2D)\{\ln(b/R) - \gamma + \ln\sqrt{2} \; \tfrac{1}{2}\ln(\ln([t_cD]^{1/2}/R) - \gamma + \ln 2)\}. \tag{69}$$

Even though t_c becomes large at low density, the natural logarithm of the final term in Eq. 69 will be small. Thus, approximately, we find at low sink density that

$$t_c = (b^2/2D)[\ln(b/R) - 0.231]. \tag{70}$$

Except for the final term this is identical to the Berg-Purcell and Adam Delbrück results. Since in the fluctuation theory there is no ambiguity associated with boundary conditions, we conclude that Eq. 69 gives the correct low-density limit for trapping time by static sinks in two dimensions.

APPENDIX

The correlation matrix of the random terms, $\tilde{f}_i$, in Eqs. 23–25 is determined by the postulates of the statistical theory of nonequilibrium thermodynamics (20,21). The general form is (19)

$$\gamma_{ij}(\mathbf{r}, \mathbf{r}') = \sum_\kappa \omega_{\kappa i}(V_\kappa^+ + V_\kappa^-)\omega_{\kappa j}\delta(\mathbf{r} - \mathbf{r}'), \tag{A1}$$

where $V_\kappa^\pm$ are the forward and reverse rates of the elementary processes (labeled by κ) causing changes in the variables $\rho_1 = \rho_p$, $\rho_2 = \rho_r$, and $\rho_3 = \rho_{rp}$. The $\omega_{\kappa i}$ are changes in the variable $N_i = A\rho_i$ that occur in the elementary process κ. The elementary processes in the present model are the $j = 1,2, \ldots$ processes of binding indicated in Eq. 4, the $j = 1,2, \ldots$ processes of invagination in Eq. 11, and receptor diffusion. Receptor diffusion involves only the receptors and gives rise to the term in Eq. 27 involving the diffusion constant (19–21). The binding processes effect only the receptors, N_2, and the receptors in pits, N_3. For each value of j, $\omega_{j1} = 0$, $\omega_{j2} = -1$ and $\omega_{j3} = +1$ and $(V_j^+ + V_j^-) = (k^+\rho_{pj}\rho_r + k^-[j+1]\rho_{pj+1})$. Summing over these j processes as indicated in Eq. A1 gives rise to the binding terms in Eq. 27. For the invagination processes $\omega_{j1} = -1$, $\omega_{j2} = 0$, and $\omega_{j3} = -j$ and $(V_j^+ + V_j^-) = \lambda\rho_{pj}$. Summing over j here gives the terms in Eq. 27 involving λ, where in the term γ_{33}

$$\langle j \rangle \equiv \sum_j j^2 \rho_{pj}^{ss}/\rho_{rp}^{ss}. \qquad \text{(A2)}$$

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