EFFECT OF MEMBRANE FLOW ON THE CAPTURE OF RECEPTORS BY COATED PITS

Theoretical Results

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ABSTRACT Coated pits trap cell surface receptors and mediate their internalization. Once internalized, many receptors recycle back to the cell surface. When recycled receptors are inserted into the plasma membrane, they move until they are again trapped in coated pits. The mechanisms for moving receptors from their insertion sites to coated pits are unknown. Unaided diffusion as the transport mechanism is consistent with the observed kinetics of receptor recycling. Another candidate for the transport mechanism is convection. For receptors that recycle to random positions on the cell surface, or to restricted regions about coated pits, we assess the importance of convective flow in the transport of receptors to coated pits. First we consider local flows set up by the formation of coated pits and their transformation into coated visicles. As coated pits form and round into coated vesicles, surrounding membrane is drawn inward, creating flows directed toward the coated pit centers. We show that unless the lifetime of a coated pit is very short, 10 s or less, such local flows have a negligible effect on the time it takes receptors to reach coated pits. We also show that they are unlikely to be the mechanism that keeps receptors that have reached coated pits trapped within coated pits until they are internalized. Finally we calculate the mean time τ for a diffusing receptor to reach a coated pit in the presence of membrane flow that is constant in magnitude and direction, as may occur on moving cells. We show that for typical membrane flow velocities, τ can be reduced significantly from its value in the absence of flow. For example, a velocity $v = 2.8$ μm/min cuts the mean transport time in half.

INTRODUCTION

Coated pits (Roth and Porter, 1964) are transient structures, attached to the cytoplasmic side of the plasma membrane, that trap cell surface receptors. They internalize the trapped receptors, as well as plasma membrane, by rounding up and forming coated vesicles (reviewed in Anderson and Kaplan, 1983). Once internalized many receptors including those for low-density lipoprotein (LDL) (Basu et al., 1981; Brown et al., 1982), transferrin (Bleil and Bretscher, 1982; Harding et al., 1983; Klausner et al., 1983), asialoglycoproteins (Tanabe et al., 1969; Steer and Ashwell, 1980; Schwartz et al., 1982), α-2-macroglobulin (Kaplan, 1980; Van Leuven et al., 1981), and insulin (Marshall et al., 1981) recycle back to the cell surface. When recycled receptors are inserted into the plasma membrane they move in the plane of the membrane until they are again trapped by coated pits. The mechanisms reponsible for moving recycled receptors from their insertion sites to coated pits are unknown.

On human fibroblasts an LDL receptor in one cycle spends ~2–3 min outside a coated pit (reviewed in Klausner et al., 1985). Calculations show that this is approximately the time it would take an LDL receptor to move from its insertion site to a coated pit if LDL receptors were randomly inserted into the plasma membrane, moved by pure diffusion, and bound rapidly and irreversibly once they reached a coated pit (Goldstein et al., 1984; Keizer et al., 1985). Some experiments with human fibroblasts suggest that LDL receptors are not randomly inserted into the plasma membrane, but rather return to regions of the cell surface where coated pits form (Robenek and Hesz, 1983; Robenek et al., 1983). We have estimated that such preferential insertion could reduce by ~50% the average time it takes an LDL receptor to diffuse to a coated pit (Wofsy et al., 1985).

Although unaided diffusion as the transport mechanism of LDL receptors to coated pits is consistent with the observed kinetics of LDL receptor recycling, its role in such transport is unknown. Another candidate for the transport mechanism is convection. In this study, for receptors that recycle to random positions on the cell surface, we assess the importance of convective flow in the transport of receptors to coated pits. First we consider local

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flows set up by the formation of coated pits and their transformation to coated vesicles. As coated pits form and round up, surrounding membrane must be pulled in to form coated vesicles. This will cause membrane to flow toward the center of coated pits. We investigate the effect of such radial flow on the rate of transport of receptors to coated pits. We also investigate whether this type of flow can, in addition to transporting receptors to coated pits, play a role in trapping them in coated pits.

Next we consider bulk membrane flow as may occur, for example, on moving cells. Evidence for such flow comes from the observation that when the leading edge of a moving cell comes in contact with a small particle, the particle often adheres to the cell surface and is transported backward (Ingram, 1969; Abercrombie et al., 1970; Harris and Dunn, 1972). The motion of such adherent particles is consistent with particles undergoing Brownian motion in the presence of a constant convective flow (Dembo and Harris, 1981). For receptors experiencing this same type of motion we calculate their rate of transport to coated pits and their mean time to find a coated pit.

THEORY

We consider a dilute system of traps (the coated pits) that are randomly distributed on a two-dimensional surface. The traps are taken to be dilute because on human fibroblasts coated pits cover 1% of the cell surface at 37°C. (Coated pits cover 2% of the cell surface at 4°C [Anderson et al., 1976; Orci et al., 1978], but when the temperature is raised to 37°C the number of coated pits on the surface is reduced by half [Anderson et al., 1977].) The assumption that the traps are randomly distributed is an idealization. Coated pits appear to be partially ordered on human fibroblasts. They tend to be linearly aligned over intracellular fibers (Anderson et al., 1978). Whether traps are ordered or disordered makes only a small difference in the rate at which a trap captures receptors provided the traps are dilutely distributed over the entire surface of the cell. For calculations similar to those presented in this paper for a dilute system of perfectly ordered traps see Echavarría-Heras (1986).

In most of the work that follows we consider receptors that are inserted into the plasma membrane at random locations. We also briefly consider receptors that are inserted into the plasma membrane in restricted regions about coated pits (Robenek and Hesz, 1983; Robenek et al., 1983). Elsewhere (Goldstein and Wiegel, 1988) we consider receptors on spreading or fully spread cells that return to the cell surface at the cell's periphery (Bretscher, 1983; Hopkins, 1985).

Our aim is to estimate the effect of membrane flow on the mean capture time, τ, i.e., the average time it takes for a newly inserted receptor to be trapped by a coated pit. The mean capture time has a simple relation to the forward rate constant, k_f, for the trapping of a receptor by a coated pit, and the cell surface density of coated pits, P:

$$\tau = 1/(k_f P). \tag{1}$$

We estimate τ by first estimating k_f.

The trapping of a receptor by a coated pit can be thought of as a two-step process. First, after a receptor is inserted into the plasma membrane, it moves by diffusion and convection until it is in the right position to react with a coated pit. Then it reacts to form a stable complex. If the forward and reverse rate constants for the transport step are k_+ and k_-, and if the forward rate constant for the reaction step is k_1, then in the steady state (Eigen, 1974; DeLisi, 1980; Shoup and Szabo, 1982)

$$k_f = k_+ k_1/(k_1 + k_-). \tag{2}$$

When the reaction is rapid so that $k_1 \gg k_-$, then the overall forward rate constant is equal to the forward rate constant for transport, i.e.,

$$k_f \approx k_+. \tag{3}$$

We assume that Eq. 3 holds for the interaction of a receptor with a coated pit, i.e., once a receptor interacts with a coated pit it remains trapped for a time that is long compared to the time for it to move away from the coated pit. In this approximation we can treat the trap as if it were a perfect absorber.

Method of Calculating k_+

To calculate k_+ we concentrate on a single perfectly absorbing trap of radius a centered at the origin. We take $c(r)$ to be the surface concentration of receptors a distance r from the trap center. At the boundary of the trap

$$c(a) = 0. \tag{4}$$

In three dimensions, Smoluchowski (1917) calculated k_+ for an infinitely dilute system of traps in the following way. He solved the steady-state diffusion equation for $c(r)$ subject to the boundary conditions that $c(r)$ vanished at the surface of the trap, and was equal to a positive constant, c_∞, at infinity. He then calculated k_+ by calculating the flux into the trap, divided by c_∞. However, in two dimensions there is no solution to the steady-state diffusion equation with these boundary conditions. Unlike in three dimensions, one cannot go to the infinitely dilute limit in two dimensions. This means we must consider the effects of more than a single trap.

Berg and Purcell (1977) got around this problem in the following way. They focused on a single circular trap of radius a with particles (in our case receptors) diffusing about the trap in an annulus of outer radius b. They chose b so that the trap density $P = 1/(\pi b^2)$ and the volume fraction was taken up by traps $\Phi = (a/b)^2$. At b they imposed a reflecting boundary condition.

The rationale for this approach can best be seen by first considering a one-dimensional problem. Suppose that on an infinite line we place perfectly ordered traps of width $2a$ with centers separated by a distance $2b$, so that $\Phi = a/b$. Particles are free to diffuse along the line outside the traps. When a particle is halfway between two traps, because of the periodicity of the traps, it "sees" the identical picture to its left and right. This implies that the exact solution to the infinite trap problem can be obtained by solving a single trap problem where a trap of width $2a$ is centered on a line of length $2b$. At the ends of the line $c(r)$ obeys reflecting boundary conditions. As in the infinite trap problem, $\Phi = a/b$ for the single trap problem. Similarly, in two dimensions, for an infinite system of perfectly ordered traps, there is an equivalent single trap problem over a finite surface. The shape of the outer boundary, where reflecting boundary conditions hold, is determined by the type of periodic array the traps form. For example, if the traps are in a square lattice of side b, then the boundary for the single trap problem is a square of side b.

The Berg–Purcell (1977) approach can be thought of as an approximate way to treat ordered systems of traps. For all ordered systems it approximates the outer boundary by a circle. It is an excellent approximation for ordered traps (Torney and Goldstein, 1987); for disordered traps it is less accurate.

Unlike an ordered system of traps, in a disordered system if one started at the center of a trap and moved radially outward, no matter how far out one went the pattern one saw would never repeat. For a disordered system we cannot reduce the infinite trap problem to a single trap problem. Our approach is to again focus on a single perfectly absorbing circular trap of radius a. We treat the effect of all the other traps by smearing them out over all space (a mean field approximation). For example, if the traps are infinitely long lived, and if receptors are inserted into the membrane uniformly at a rate S (where S has units of receptors per second per cm^2) and then move by pure diffusion with diffusion coefficient D, then in the steady state we assume the receptor concentration about the trap obeys the equation

$$D\nabla^2 c - k_+ P c + S = 0, \qquad a \le r \le \infty, \tag{5}$$

with the boundary conditions that at $r = \infty$ the receptor concentration is finite and at $r = a$ it is zero, i.e., Eq. 4 holds. The effect of the other traps in the mean field approximation is taken into account through the loss term, $k_+ P c$.

In two dimensions

$$k_+ = \frac{2\pi a D}{\langle c \rangle} \frac{\partial c}{\partial r}, \tag{6}$$

where the derivative is evaluated at $r = a$ and $\langle c \rangle$ is the receptor concentration averaged over all space.

To solve for k_+ we first solve Eq. 5 for $c(r)$ and then substitute into Eq. 6. This gives us a transcendental equation for k_+.

In this paper we must modify Eq. 5 to take into account the convective motion of the receptors and the finite lifetime of the traps. In the steady state a recycling process keeps the number of coated pits on the cell surface constant. We assume that when new coated pits form they do so at random locations on the cell surface. When there is convective flow the flux density $\mathbf{j}$ becomes

$$\mathbf{j} = -D\nabla c + \mathbf{v} c, \tag{7}$$

where $\mathbf{v}$ is the flow velocity of the membrane. If λ is the rate at which traps are internalized ($1/\lambda$ is the trap lifetime), then Eq. 5 becomes

$$-\nabla(\nabla \cdot \mathbf{j}) - k_+ P c - \lambda c + S = 0. \tag{8}$$

The term λc in Eq. 8 reflects the effect of the finite lifetime of the traps (Goldstein et al., 1984).

Modeling Local Membrane Flow Induced by Coated Pit Formation

As a coated pit forms and rounds up to become a coated vesicle, it pulls nearby membrane radially inward. By the time the coated vesicle has pinched off from the cell surface, an amount of membrane is pulled in that is equal to the surface area of a coated vesicle, $4\pi a_{cv}^2$, where a_{cv} is the coated vesicle radius. The nature of the radial flow depends on the kinetics of coated pit and coated vesicle formation. The detailed kinetics for particular cell types and the possibility of differences among cell types are still open questions. The potassium depletion experiments of Larkin et al. (1986) suggest that new coated pits form initially as planar clathrin lattices and then round up. On the other hand, Pearse and Crowther (1987) argue that curvature is constantly being built into the coated pit. In the latter case, membrane would be incorporated into the coated pit continuously until the coated pit budded off as a coated vesicle, whereas in the former case, an initial period of pit formation would be followed by a period of rounding without further growth.

If we knew how the pit radius and surface area changed with time, so that we could obtain the time dependence of membrane flow, in theory we could write a kinetic equation for the concentration of receptors a distance r from the pit center at time t after the pit begins to form. Because the radius of the pit, as seen on the cell surface, increases as the pit forms and goes to zero when the vesicle pinches off, the equation would have to be solved with a moving boundary. In the absence of definitive kinetic information, we approximate the changing coated pit radius by a fixed average value. This greatly simplifies the problem and, because in two dimensions k_+ depends only weakly on the radius of the trap, seems reasonable. When we average this equation over time with a fixed boundary we obtain Eq. 8 where $\mathbf{v}$ is the time average lipid membrane flow velocity.

To obtain a form for $\mathbf{v}$ at a distance r from the coated pit center, we note that at a point outside the coated pit the equation of continuity holds, i.e.,

$$\nabla \cdot (\rho \mathbf{v}) = 0, \tag{9}$$

where ρ is the membrane lipid density. We assume that we can approximate the surface outside the membrane as a plane so that Eq. 9 becomes

$$\frac{1}{r}\frac{\partial}{\partial r}(r\mathbf{v}) = 0, \tag{10}$$

which implies that the magnitude of the velocity $v \sim 1/r$. As noted above, during the lifetime of a coated pit, $1/\lambda$, an amount of membrane of area $4\pi a_{cv}^2$ is pulled in. If $\langle v \rangle$ is the time average of the velocity of the membrane at the boundary of the pit, then $2\pi a \langle v \rangle = 4\pi a_{cv}^2 \lambda$. It follows that

$$\mathbf{v} = -\frac{\beta}{r}\hat{\mathbf{r}}. \tag{11}$$

where $\beta = 2a_{cv}^2\lambda$, and $\hat{\mathbf{r}}$ is the unit vector in the radial direction. With this form for $\mathbf{v}$, Eq. 8 becomes

$$\frac{\partial^2 c}{\partial r^2} + (1 + 2\nu)\frac{1}{r}\frac{\partial c}{\partial r} - \gamma^2 c + \frac{S}{D} = 0, \tag{12a}$$

where

$$\nu = \beta/(2D) = a_{cv}^2\lambda/D, \tag{12b}$$

and

$$\gamma = [(k_+P + \lambda)/D]^{1/2}. \tag{12c}$$

The solution to Eq. 12a with $c(a) = 0$ and $c(\infty)$ finite is (see Appendix for details)

$$c(r) = \frac{S}{\gamma^2 D}[1 - (a/r)^\nu K_\nu(\gamma r)/K_\nu(\gamma a)], \tag{13}$$

where the K_ν are modified Bessel functions of order ν.

When we substitute Eq. 13 into Eq. 6 we obtain the following equation for k_+:

$$k_+ = 2\pi a D\gamma K_{\nu+1}(\gamma a)/K_\nu(\gamma a). \tag{14}$$

In the limit as D goes to zero we can show from Eq. 14 that $k_+ = 4\pi a_{cv}^2\lambda$, i.e., without diffusion the only receptors that are captured are those imbedded in the internalized membrane. Previously we presented a model for the trapping of diffusing receptors by a coated pit where, when the coated pit appears, it instantly traps all receptors within its boundary (Goldstein et al., 1984). For this model $k_+ = \pi a^2 \lambda$ in the limit as D goes to zero. If we take $a = 2a_{cv}$, so that in both models the same amount of area is internalized by a coated pit, the models are identical when $D = 0$. When $D \neq 0$, the model presented in this paper includes the effect of local membrane flow whereas the previous model does not. Because the membrane flow is radial inward, this will increase the calculated value of k_+ compared with the previous model. A second difference between the models is that previously we assumed the coated pits were ordered rather than randomly distributed. The differences that arise because of this are considered in the Discussion section.

In the Results section we present numerical solutions of Eq. 14. Even though the derivation was based on a mean field approximation, Eq. 14 agrees with the result of Keizer (1981) for the special case of infinitely long-lived traps ($\lambda = 0$), in the absence of flow ($\nu = 0$), and in the dilute limit ($\gamma a \ll 1$).

Modifications When Receptors Are Inserted Near Coated Pits

If receptors do not recycle to random locations on the cell surface but instead to restricted areas about coated pits, we must modify Eq. 12a. To model such recycling we follow Wofsy et al. (1985) and assume receptors are only inserted into the plasma membrane in annuli about coated pits. We take the inner radius of the insertion region to equal the coated pit radius a and the outer radius to equal s. (Wofsy et al. [1985], who considered restricted insertion of receptors for ordered traps in the absence of flow, inserted receptors within m coated pit radii of the center of a coated pit so that $s = ma$.) We take S_1 to be the rate per unit area at which receptors are inserted into the annuli. We again focus on a single coated pit, taking into account the effects of the other coated pits by using a mean field approximation. We treat absorption as before, through a term $-k_+Pc$ in the diffusion equation, e.g., see Eq. 5 or Eq. 12a. Just as we uniformly smear out the absorption by the other traps, so we also smear out the insertion rate about the other traps. The equivalent of Eq. 12a now becomes

$$\frac{\partial^2 c}{\partial r^2} + (1 + 2\nu)\frac{1}{r}\frac{\partial c}{\partial r} - \gamma^2 c + \frac{S_1 + S_2}{D} = 0 \quad a \le r \le s \tag{15a}$$

$$\frac{\partial^2 c}{\partial r^2} + (1 + 2\nu)\frac{1}{r}\frac{\partial c}{\partial r} - \gamma^2 c + \frac{S_2}{D} = 0 \qquad s \le r \tag{15b}$$

If the traps were infinitely long lived then S_2 would simply be the effective insertion rate due to the insertion of receptors in the annuli about all the other traps. However, when a trap disappears, receptors that were a distance r from the trap center are randomly distributed with respect to the other trap centers. In effect, these receptors are redistributed. S_2 is therefore the sum of the effective insertion rate and the redistribution rate.

In the Appendix, starting with Eq. 15 we derive the following expression for k_+:

$$k_+ = \frac{2\pi D a\gamma K_{\nu+1}(\gamma a)/K_\nu(\gamma a)}{1 - \dfrac{2\gamma a}{(\gamma s)^2 - (\gamma a)^2}\dfrac{K_{\nu+1}(\gamma a)}{K_\nu(\gamma a)}[1 - (s/a)^{\nu+1}K_{\nu+1}(\gamma s)/K_{\nu+1}(\gamma a)]}. \tag{16}$$

Eq. 16 has the property that as s goes to infinity, so that there is no longer any preferential insertion, it reduces to Eq. 14. Also, as s goes to a, so that receptors are instantly inserted into coated pits, k_+ goes to infinity, i.e., the mean capture time goes to zero.

Local Membrane Flow and the Escape from Coated Pits

We wish to know whether the radial flow induced by the formation and invagination of a coated pit can play a significant role in keeping receptors in coated pits. As a crude model we assume that the flow velocity inside a coated pit is given by Eq. 11. This neglects the curvature of the coated pit. More importantly, we know Eq. 11 cannot hold near $r = 0$. By assuming it does and allowing the velocity to become infinite at $r = 0$, we will overestimate the importance of the flow in opposing the escape of receptors from coated pits.

We calculate the function $w(r)$, the mean time for a particle starting from position r $(0 \leq r \leq a)$ to reach the outer radius of the pit, $r = a$. From the definition of $w(r)$ it follows that $w(a) = 0$. In the Appendix we show that $w(r)$ satisfies the equation

$$D_0 \frac{\partial^2 w}{\partial r^2} + \frac{D_0 - \beta}{r} \frac{\partial w}{\partial r} + 1 = 0. \tag{17}$$

The solution of Eq. 17 with $w(a) = 0$ and $w(r)$ finite at $r = 0$ is

$$w(r) = \frac{a^2 - r^2}{2(2D_0 - \beta)}. \tag{18}$$

If we assume a particle is equally likely to be anywhere within the coated pit, then on averaging Eq. 18 over the area of the coated pit we find that the mean escape time

$$\tau_e \equiv \langle w(r) \rangle = \frac{a^2}{4(2D_0 - \beta)}. \tag{19}$$

When $\beta = 2D_0$ the time for a particle to escape becomes infinite. Although this result arises because we allowed the velocity to become infinite at $r = 0$, it suggests that the velocity can trap particles in coated pits when $\beta \geq 2D_0$, or equivalently,

$$a_{cv}^2 \lambda / D_0 \geq 1. \tag{20}$$

Modeling Capture by Coated Pits in the Presence of Bulk Flow

We now consider a coated pit in a region of the plasma membrane where bulk membrane flow is occurring, as might be the case on a moving cell. Suppose that far from the pit the membrane flow velocity is constant so that

$$\mathbf{v} = v\hat{\mathbf{i}}, \tag{21}$$

where $\hat{\mathbf{i}}$ is the unit vector in the x direction. It is not known what the flow lines will look like near the coated pit. However, the coated pit is permeable to membrane so the deviation from straight line flow in the vicinity of the coated pit may be small. We shall neglect such deviations and assume that everywhere outside the coated pit Eq. 21 holds. Making this approximation will lead to an overestimate of the capture rate because particles that would be swept around the coated pit will, in this approximation, be swept into it. We also neglect the radial flow caused by the formation of the pit, as described by Eq. 11. The justification for this is given in the Results section.

With the velocity given by Eq. 21, Eq. 8 becomes

$$D\nabla^2 c + v\frac{\partial c}{\partial x} - (k_+ P + \lambda)c + S = 0. \tag{22}$$

It is useful to define the following parameters:

$$\alpha = v/(2D), \tag{23}$$

$$\delta = (\alpha^2 + \gamma^2)^{1/2}, \tag{24}$$

where γ is given by Eq. 12c. In the Appendix we show that for the appropriate boundary conditions Eq. 22 has the following solution:

$$c(r, \theta) = S/(k_+ P) + \exp(-\alpha x) g(r, \theta), \tag{25}$$

where:

$$g(r, \theta) = -\frac{S}{k_+ P}\left[\frac{I_0(\alpha a)}{K_0(\delta a)} K_0(\delta r) + 2\sum_{m=1}^{\infty} \frac{I_m(\alpha a)}{K_m(\delta a)} K_m(\delta r) \cos(m\theta)\right]. \tag{26}$$

Then we use Eqs. 25 and 26 and a modified form of Eq. 6 to derive the following equation for k_+:

$$k_+ = 2\pi D a \delta \left[I_0{}^2(\alpha a) \frac{K_1(\delta a)}{K_0(\delta a)} + \sum_{m=1}^{\infty} (-1)^m I_m{}^2(\alpha a) \frac{K_{m-1}(\delta a) + K_{m+1}(\delta a)}{K_m(\delta a)} \right]. \tag{27}$$

RESULTS

When a coated pit is formed and rounds into a coated vesicle, membrane is pulled in creating a radial flow inwards that may help trap receptors. The average flow velocity a distance r away from the center of a coated pit is given by Eq. 11. The maximum average velocity outside the coated pit occurs at $r = a$ and is given by the expression

$$v_{max} = 2a_{cv}^2 \lambda / a, \tag{28}$$

where a_{cv} is the radius of a coated vesicle, a the radius of a coated pit, and λ the rate at which coated pits are internalized. The average radius of a coated pit is 0.1 μm (Heuser, 1980). On human fibroblasts the lifetime of a coated pit is ~5 min so $\lambda \approx 0.2\ \text{min}^{-1} = 3.3 \times 10^{-3}\ \text{s}^{-1}$. (For a discussion of the parameter values used in this paper see Goldstein et al., 1984). If we take $a_{cv} = a/2$, then v_{max} = 0.01 μm/min. This is 100 times smaller than typical bulk flow velocities generated on moving cells.

In Fig. 1 we show the effects of radial flow on the diffusion limited forward rate constant, k_+, when receptors are randomly inserted into the plasma membrane and then diffuse with a diffusion coefficient D in the plane of the membrane. The solid (dashed) curves correspond to the model where radial flow is present (absent). They were obtained by solving Eq. 14 numerically for k_+. The middle curves are for $D = 4.5 \times 10^{-11}$ cm^2/s, the measured diffusion coefficient of mobile LDL receptors on a mutant human fibroblast cell line (Barak and Webb, 1982). As the lifetime of a coated pit decreases (λ increases) the radial flow velocity increases because membrane is pulled in at a faster rate to form the same size coated vesicle. This is why in Fig. 1, as λ increases, k_+ increases more rapidly in the presence than in the absence of radial flow. However, for the parameter values of interest (Fig. 1 with $\lambda = 3.3 \times 10^{-3}$ s^{-1}) the effect of the flow induced by the formation of a coated pit is negligible. The smaller the diffusion coefficient of the receptor, the greater the effect of radial flow, but even for $D = 1.0 \times 10^{-12}$ cm^2/s, radial flow has little effect for $\lambda < 0.1$ s^{-1}. Thus, as far as trapping of receptors is concerned, such flow can be ignored for coated pits with lifetimes >10s.

Because the radial flow velocity decreases with the distance from the center of a coated pit (see Eq. 11) we expect it to be most effective in enhancing the capture of receptors near coated pits. There is some evidence that LDL receptors may be inserted into the plasma membrane in restricted regions, called plaques, about coated pits (Robenek and Hesz, 1983; Robenek et al., 1983). In Fig. 2 we show the effect of restricted insertion on k_+ when receptors are recycled to plaques of inner radius a and outer radius s. The curve in Fig. 2 was obtained by solving Eq. 16 numerically for k_+, for parameter values that characterize LDL receptors on human fibroblasts. When the same calculation is done with the same parameter values, but the radial flow set equal to zero, we obtain essentially the same results as shown in Fig. 2. Again, the effect of radial flow is negligible until the lifetime of the coated pit is $\leq$10 s.

When receptors are uniformly inserted over the entire surface the calculated mean time to get to a coated pit, τ = 4.1 min, and $k_+ = 1.3 \times 10^{-10}$ cm^2/s. To double k_+ and halve τ requires a plaque radius $s = 5.2a$ (see Fig. 2). In obtaining this result we assumed that the coated pits were randomly distributed on the cell surface. Previously we carried out the same calculation, but assumed the coated pits were ordered and found that to halve τ required a plaque radius $s = 3.2a$ (Wofsy et al., 1985). Restricted insertion of receptors near coated pits is much more effective in reducing τ when the coated pits are randomly distributed.

Although radial flow appears not to be important in enhancing the capture of cell surface receptors by coated pits, it may be that once receptors are in coated pits radial flow helps keep them there. The idea is attractive because it offers the possibility of explaining why different receptors, whose cytoplasmic tails have little or no homology, can aggregate in the same coated pit. Suppose that receptors diffuse more slowly when in coated pits than when out

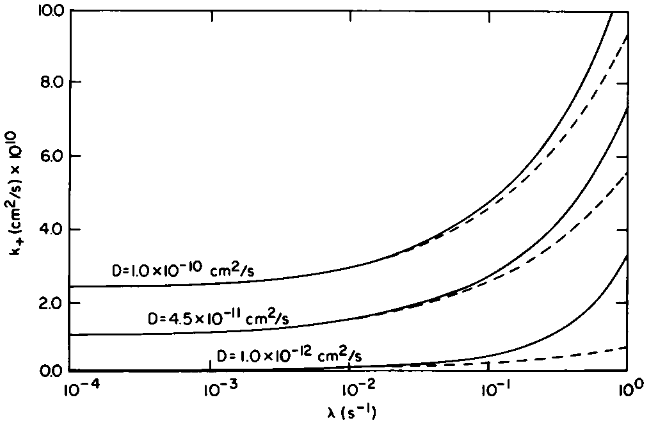

FIGURE 1 The effect of radial flow induced by coated pit formation on the diffusion limited forward rate constant, k_+, for a cell surface receptor interacting with a coated pit, as a function of λ, the rate at which coated pits internalize. The faster coated pits internalize, the greater the flow velocity they induce. The solid curves include the flow; the dashed curves do not. D is the receptor diffusion coefficient. The curves were obtained by solving Eq. 14 numerically for k_+. For the solid curves v was given by Eq. 12b, and for the dashed curves $v = 0$. The following parameter values were used: coated pit radius, a = 0.1 μm; coated vesicle radius, a_{cv} = 0.05 μm; fraction of cell surface area covered by coated pits, $A = \pi a^2 P$ = 0.01.

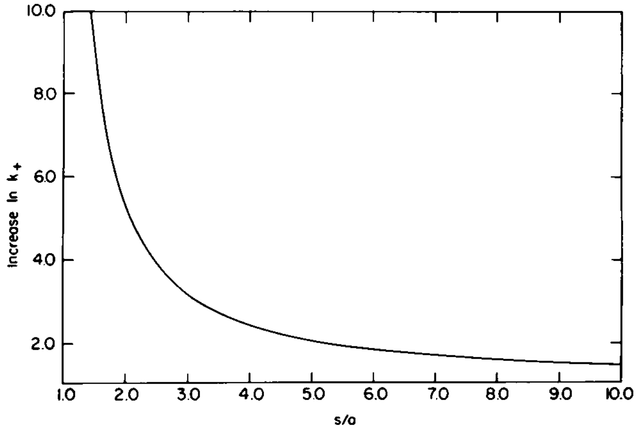

FIGURE 2 The effect of restricted insertion on the diffusion limited forward rate constant, k_+, when receptors are inserted into the membrane about coated pits in annuli of inner radius a, the coated pit radius, and outer radius s, the plaque radius. The curves were obtained by solving Eq. 16 numerically for k_+. We used the following parameter values, which are characteristic of human fibroblasts. Average lifetime of a coated pit $1/\lambda$ = 5 min, so that λ = 0.2 min^{-1} = 3.3×10^{-3} s^{-1}; coated pit radius, a = 0.1 μm; coated vesicle radius, a_{cv} = 0.05 μm; fraction of the cell surface area covered by coated pits, $A = \pi a^2 P$ = 0.01; LDL diffusion coefficient $D = 4.5 \times 10^{-11}$ cm^2/s.

of coated pits. This could come about, for example, because the cytoplasmic tail of the receptor interacts with clathrin or one of the other proteins that make up the latticelike coat. Just as cell surface proteins diffuse more slowly in the presence of cytoskeleton, so we might expect the clathrin coat to further hinder diffusion. We showed in the previous section that local radial flow would be sufficient to prevent receptors from diffusing out of coated pits when

$$D_0 \leq a_{cv}^2 \lambda, \tag{29}$$

where D_0 is the receptor diffusion coefficient inside coated pits. For $a_{cv} = 0.05$ μm and $\lambda = 3.3 \times 10^{-3}$ s^{-1}, inequality (29) predicts that D_0 must be $\leq 8.3 \times 10^{-14}$ cm^2/s before the radial flow can effectively prevent receptors from diffusing out of coated pits. This seems to be too small a diffusion coefficient to be produced by nonspecific interactions.

Bulk membrane flow may occur when a cell moves. We have modeled the capture of receptors by coated pits in the presence of a membrane flow which is constant in magnitude and direction (see Eq. 21). In the model, we have neglected the local radial flow induced by the formation of the coated pit because we have shown that for the parameter values of interest such flow has a negligible effect on the capture of receptors. In Fig. 3 we show the effect of such a flow on k_+ for parameter values that characterize the LDL receptor on human fibroblasts. Dembo and Harris (1981) analyzed the motion of particles that adhered to the leading lamella of crawling chick heart fibroblasts. For each of the particles analyzed there was a constant nonrandom component to the velocity which ranged from 0.8 to 5.7×10^{-6} cm/s, with an average value of 2.9×10^{-6} cm/s. We see from Fig. 3 that for such bulk flow velocities there is a measurable increase in k_+. For the range of velocities reported by Dembo and Harris (1981), $k_+ = 1.4\text{–}3.0 \times 10^{-10}$ cm^2/s, which corresponds to a mean capture time $\tau = 230 - 108$ s. In the absence of flow, $k_+ = 1.3 \times 10^{-10}$ cm^2/s and $\tau = 248$ s. Thus, typical bulk flow velocities can reduce the time it takes a receptor to reach a coated pit by 50% or more. However, there are provisos to this claim. We have neglected any deviation from straight line flow in the vicinity of the coated pit (see discussion after Eq. 21). If such deviations are important, then the values we obtained for k_+ are too high. We have also assumed that coated pits are not swept along in the flow, so that the bulk flow velocity is the relative velocity of the membrane to the coated pit.

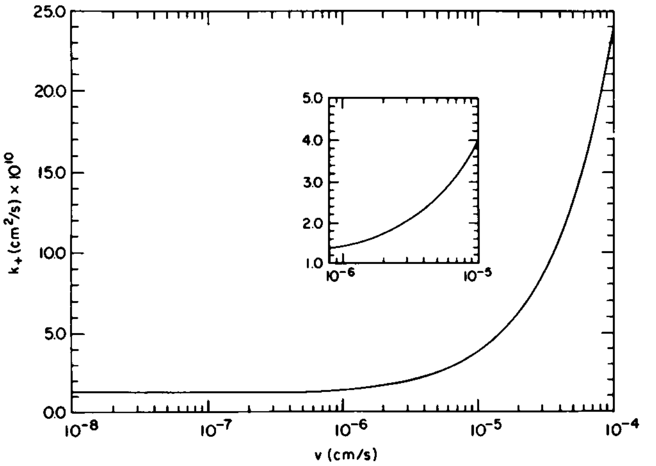

FIGURE 3 The effect of bulk flow on the diffusion limited forward rate constant, k_+. We have assumed that there is a membrane flow velocity that is constant in magnitude, v, and direction, relative to the coated pits. The curve was obtained by solving Eq. 27 for k_+. The parameter values used characterize LDL receptors on human fibroblasts: $a = 0.1$ μm; $A = \pi a^2 P = 0.01$; $\lambda = 0.2$ min^{-1} = 3.3×10^{-3} s^{-1}; $D = 4.5 \times 10^{-11}$ cm^2/s. The insert corresponds to the range of velocities reported by Dembo and Harris (1981).

DISCUSSION

Many receptors that mediate the endocytosis of extracellular ligands recycle from the surface to the cell interior and back again (reviewed in Brown et al., 1983). During the cycle, receptors are inserted into the plasma membrane and then move to coated pits, where they are trapped and internalized. The mechanism by which they move from their insertion sites in the plasma membrane to coated pits is unknown. Here we have modeled the movement of receptors to coated pits by assuming that the receptors can both diffuse on the cell surface and be transported by membrane flow.

First we considered local flows set up by the formation of coated vesicles. We assumed that as coated pits assemble and round up to form coated vesicles they pull in membrane, creating a flow directed inward toward the center of the coated pit. The flow velocity is maximal at the boundary of the coated pit and is inversely proportional to the distance from the center of the coated pit. To estimate the magnitude of the velocity we neglected the detailed kinetics of coated pit formation and assumed that the rate at which membrane was pulled in during the lifetime of the coated pit was constant in time. We estimated that for a coated pit with a lifetime of 5 min, the maximum average velocity of the membrane being pulled in is 0.01 μm/min (see Eq. 28). We showed that such local flows have a negligible effect on the time it takes receptors to reach coated pits. Only if the lifetime of a coated pit were ≤ 10 s would the velocity generated by its formation significantly reduce the time for a receptor to reach a coated pit.

Even though local membrane flow caused by coated pit formation does not enhance the rate at which receptors reach coated pits, it is possible that such flow can aid in keeping receptors in coated pits once they get there. If the flow velocity is large enough or equivalently the diffusion coefficient small enough, the receptor will be unable to diffuse away from the pit during its lifetime. The condition for this to occur is given by Eq. 29. If the lifetime of a coated pit is 5 min, Eq. 29 predicts that the diffusion coefficient inside the coated pit, D_0, must be $\leq 8.3 \times 10^{-14}$

cm^2/s before the local flow can effectively keep receptors trapped in coated pits. If the lifetime were somewhat shorter, say 1 min, than D_0 must be $\leq 4.2 \times 10^{-13}$ cm^2/s. Because receptor diffusion coefficients outside of coated pits are 10^2–10^4 times larger than this, it seems unlikely that radial flow set up by coated vesicle formation is the mechanism for keeping receptors trapped in coated pits.

When cells move, bulk membrane flow can occur. We calculated the mean time for a diffusing receptor to reach a coated pit in the presence of membrane flow that is constant in magnitude and direction. For typical membrane flow velocities, such a flow can substantially reduce the mean time τ for a receptor to reach a coated pit. For example, for a velocity $v = 1$ μm/min, $\tau/\tau_0 = 0.7$, whereas for $v = 2.8$ μm/min, $\tau/\tau_0 = 0.5$ (τ_0 is the mean time in the absence of flow).

We assumed coated pits were randomly distributed on the cell surface and recycled to random locations. For parameter values that characterize LDL receptors on stationary human fibroblasts (no bulk membrane flow), we calculated that $\tau_0 = 248$ s. Previously we carried out a similar calculation for coated pits that were ordered rather than randomly distributed on the cell surface (Goldstein et al., 1984) and found that $\tau_0 = 173$ s. On human fibroblasts coated pits tend to align in linear arrays (Anderson et al., 1978, 1980) so that they are neither perfectly ordered nor perfectly disordered. For these cells our calculations suggest that $\tau_0 \approx 3$–4 min. From published experiments we have estimated that $\tau_0 \leq 2.4 \pm 1.4$ min (reviewed in Klausner et al., 1985). Thus the calculated and experimental value of τ in the absence of bulk membrane flow are in agreement. We have shown that local membrane flow induced by the formation and invagination of coated pits has a negligible effect on the trapping of receptors. Thus, a model for stationary cells (no bulk membrane flow) where LDL receptors are randomly inserted into the plasma membrane and then move by pure diffusion until they encounter and are rapidly trapped by coated pits is consistent with experiment. However, because of the large errors in the determination of the parameter values, a mean capture time that is ~50% smaller than that calculated for pure diffusion would also be consistent with experiment. Such a reduction could come about if LDL receptors were preferentially inserted near coated pits, as has been suggested by Robenek and his collaborators (Robenek and Hesz, 1983; Robenek et al., 1983). We have shown here and elsewhere (Wofsy et al., 1985) that a reduction of 50% could come about if the insertion of LDL receptors were confined to annuli about coated pits of outer radii ~3–5 coated pit radii long.

APPENDIX

Here we solve the equations for the receptor concentration c in the three settings considered, i.e., when there is a local membrane flow induced by coated pit formation, when preferential receptor insertion augments the effect of the local radial flow, and when there is bulk flow. We also derive the equation satisfied by the mean escape time for receptors in coated pits in the presence of a local radial flow.

Local (Radial) Membrane Flow

To transform Eq. 12a into a standard equation with a known solution, we write c in the form:

$$c(r) = S/(\gamma^2 D) + r^{-\nu} u. \tag{A1}$$

Substituting Eq. A1 into Eq. 12a we find that u satisfies

$$z^2 \frac{d^2u}{dz^2} + z\frac{du}{dz} - (z^2 + \nu^2)u = 0, \tag{A2}$$

where $z = \gamma r$. Linearly independent solutions are the modified Bessel functions I_ν and K_ν. The corresponding expression for c that is finite at ∞ and 0 on trap boundary is given by Eq. 13.

Local Flow When Receptors Are Inserted Near Coated Pits

Eqs. 15a and 15b, which describe the receptor concentration c inside and outside the plaque of radius s about the coated pit where receptors are inserted preferentially, have the same form as Eq. 12a. Then subject to the condition that c is constant at ∞, the solution has the form

$$c(r) = \begin{cases} (S_1 + S_2)/(\gamma^2 D) + Ar^{-\nu}K_\nu(\gamma r) + Br^{-\nu}I_\nu(\gamma r) & a \leq r \leq s \\ S_2/(\gamma^2 D) + Cr^{-\nu}K_\nu(\gamma r) & s \leq r \end{cases} \tag{A3}$$

Using the other boundary condition, $c(a) = 0$, and the continuity conditions that the two forms of c and its radial derivative must match at the plaque boundary, $r = s$, we obtain the constants defining c in Eq. A3. We use the resulting expression in conjunction with Eq. 6 to derive Eq. 16 for k_+. The denominator in Eq. 6, $\langle c \rangle$ is also the limiting value of c at ∞ and is given by

$$\langle c \rangle = S_2/(\gamma^2 D). \tag{A4}$$

The numerator in Eq. 6 involves the radial derivative of c at $r = a$ and can be evaluated from the expression obtained for c. Substituting this result and Eq. A4 into Eq. 6, we find:

$$k_+ = 2\pi a D\gamma \frac{K_{\nu+1}(\gamma a)}{K_\nu(\gamma a)} \left\{ 1 + \frac{S_1}{S_2}\left[1 - (s/a)^{\nu+1} \frac{K_{\nu+1}(\gamma s)}{K_{\nu+1}(\gamma a)} \right] \right\} \tag{A5}$$

To evaluate k_+, given the fundamental parameters, we need an expression that does not depend explicitly on the ratio S_1/S_2. We find this by noting that the numerator in Eq. 6, the flux to the trap, can be written in an alternative form. The flux is the rate of loss of receptors to the trap and is balanced by the preferential insertion of receptors, at the rate S_1, into the annular plaque about the trap. Combining the resulting expression for the flux with Eq. A4 yields a simple expression for the diffusion limited forward rate constant:

$$k_+ = (S_1/S_2)\pi(s^2 - a^2)\gamma^2 D. \tag{A6}$$

Solving for S_1/S_2 in Eq. A6, substituting the result into Eq. A5, and solving for k_+, we obtain Eq. 16.

Mean Escape Time

An instructive way to derive equations satisfied either by concentrations, probability densities or mean hitting times associated with diffusing particles is to take the appropriate limit in equations for random walks approximating the diffusion process. As Berg and Purcell (1977) did in their derivation of a mean hitting time equation for diffusing particles when there is no flow, we will find it most convenient to develop the discrete step equations in a Cartesian coordinate system and then convert the limiting equation to the polar coordinate form of Eq. 17.

To approximate a symmetric diffusion process by a random walk on a square lattice, we say that if a particle is at a point (x, y), then in a time Δt it jumps a distance ξ to any of the four nearest neighbor points $(x \pm \xi, y \pm \xi)$, each with probability 1/4. In our case there is a flow that also affects particle movement. A flow toward the origin, defined by a velocity $v(r)$ for particles a distance r from the origin, carries particles an additional distance $v(r)\Delta t$ radially. The changes in the x and y cordinates due to the flow are then $-v(r)x/r$ and $-v(r)y/r$. Let $w(x, y)$ denote the mean time for a particle at (x, y) to hit a boundary. In the discrete step approximation to the diffusion/flow process, the mean hitting time is the time of the next step, Δt, plus the mean hitting time from the next position, averaged over the four possible positions. That is,

$$\begin{aligned} w(x,y) = \Delta t + \tfrac{1}{4}\,(&w\{x[1 - v(r)/r]\} + \xi, y[1 - v(r)/r]) \\ &+ w\{x[1 - v(r)/r] - \xi, y[1 - v(r)/r]\} \\ &+ w\{x[1 - v(r)/r], y[1 - v(r)/r] + \xi\} \\ &+ w\{x[1 - v(r)/r], y[1 - v(r)/r] - \xi\}), \end{aligned} \tag{A7}$$

The limiting diffusion/flow equation is obtained by letting the time and distance steps Δt and ξ tend to zero in such a way that the mean squared distance traveled due to the diffusion (or random walk) component is proportional to time, i.e., $\xi^2/\Delta t$ is constant. Let $D = 4\xi^2/\Delta t$. Then if we expand the functions in Eq. A7 in power series about (x, y), divide by Δt, and take the limit as Δt tends to 0 with D remaining constant, we obtain

$$D\nabla^2 w - v(r)\left(\frac{x}{r}\frac{\partial w}{\partial x} + \frac{y}{r}\frac{\partial w}{\partial y}\right) + 1 = 0. \tag{A8}$$

The term in parentheses is the scalar product of the gradient of w with the unit vector pointing radially out from the origin. For a radially symmetric function, this is simply $\partial w/\partial r$. Substituting this expression, as well as the polar coordinate form of the Laplacian for radially symmetric functions and the form of the velocity $v(r) = \beta/r$, into Eq. A8, we obtain Eq. 17.

Bulk Flow

In terms of the lumped parameters α and γ defined by Eqs. 23 and 12c, Eq. 22 can be rewritten as

$$\nabla^2 c + 2\alpha\frac{\partial c}{\partial x} - \gamma^2 c + \frac{S}{D} = 1. \tag{A9}$$

As in the case of radial flow, a transformation makes it easier to solve Eq. A9. We define a new function g by

$$c = \frac{S}{\gamma^2 D} + g\exp(-\alpha x). \tag{A10}$$

Then from Eq. 22, g satisfies

$$\nabla^2 g - \delta^2 g = 0, \tag{A11}$$

with δ defined by Eq. 24.

We look for a solution by separating variables, i.e., by finding a complete set of solutions to Eq. A11 of the form $g_m = R_m(r)\Theta_m(\theta)$ and then finding an infinite linear combination g satisfying the boundary conditions

$$g(a, \theta)\exp(-\alpha a\cos\theta) = -S/(\gamma^2 D) \tag{A12a}$$

$$\lim_{r\to\infty}|g(r, \theta)\exp(-\alpha r\cos\theta)| < \infty \qquad \text{for all } \theta. \tag{A12b}$$

These conditions make the concentration 0 at $r = a$ and finite as $r \to \infty$. In addition, solutions must have the symmetry property that $g(r, \theta) = g(r, -\theta)$ for all r and θ.

A solution to Eq. A11 that is finite at ∞ and has the appropriate symmetry is of the form

$$g(r, \theta) = \sum_{n=0}^{\infty} A_m K_m(\delta r)\cos(m\theta), \tag{A13}$$

where K_m is the modified Bessel function of order m.

To determine the constants A_m we substitute Eq. A13 into Eq. A12a. After multiplying by $\cos(m\theta)$ and integrating over θ from 0 to π we find

$$A_0 K_0(\delta a) = -SI_0(\alpha a)/(\gamma^2 D) \tag{A14a}$$

$$A_m K_m(\delta a) = -2SI_m(\alpha a)/(\gamma^2 D) \qquad m \geq 1. \tag{A14b}$$

Eqs. 25 and 26 follow when the A_m defined by Eqs. A14 are substituted into Eq. A13 for g and then c is defined by Eq. A10.

To obtain an expression for k_+, we must modify Eq. 6 to use the average flux at the trap boundary $r = a$ because we no longer have radial symmetry, i.e.,

$$k_+ = \frac{2\pi Da}{\langle c\rangle}\left(\frac{1}{\pi}\int_0^{\pi}\left.\frac{\partial c}{\partial r}\right|_{r=a} d\theta\right). \tag{A15}$$

Using standard formulas and identities to perform the differentiation and integration in Eq. A15, we obtain an expression for k_+ that is the sum of two infinite series: the one given in Eq. 27 and another involving products of the form $I_m I_{m+1}$. The partial sums of the latter series telescope and reduce to a single term: $(-1)^m I_m(\alpha a)I_{m+1}(\alpha a)$. As $m \to \infty$, $I_m(\alpha a) \to 0$; hence the second series converges to 0 and k_+ is given by Eq. 27.

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