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Effect of linear reinsertion of receptor on the distribution of receptors around coated pits

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Abstract

We consider a linear receptor reinsertion step in our kinetic model of the primary steps occurring in receptor-mediated endocytosis. In contrast with our previous zeroth order receptor reinsertion assumption, here we consider a first order process, and we study the effect of receptor diffusion on the trapping rate constant (k^+) and the radial distribution of receptors around coated pits (g_{rp}). Using experimental data for low density lipoproteins (LDL) receptors on fibroblast cells, we find that the trapping of receptors by coated pits is diffusion-controlled for any value of the escaping rate constant (k^-). This result is significantly different from our previous findings. In fact, for a zeroth order process, we find that either diffusion has no effect on k^+ or, at the most, receptor trapping is 84% diffusion-controlled. Moreover, we find values for the receptor reinsertion rate constant (κ), which range between 15% of the pit's invagination rate constant, λ, and three halves λ. In addition, the ratio κ/λ is equal to the ratio of the concentration of receptors in pits with respect to the internalized receptors. Comparison between the experimental radial distribution of receptors around pits and the theoretical should provide an indication of the diffusion effect on k^+.

Keywords: Endocytosis; Receptors; Coated pits; Diffusion; Keizer's theory

1. Introduction

Receptor-mediated endocytosis is a process that enables cells to transport macromolecules across the plasma membrane. Macromolecules that utilize this pathway include polypeptides, hormones, growth factors, low density lipoproteins [LDL), transferrin, proteins modified for degradation, and some antibodies. In addition, viruses and bacterial toxins gain entry to the cell by using this process [1]. The receptors that participate in endocytosis are integral membrane proteins synthesized by the cell and inserted into the plasma membrane [2]. The receptors are specific for each macromolecule that gains entry through receptor-mediated endocytosis. Once in the membrane, the receptors distribute in different ways depending on the receptor and cell type. Epidermal growth factor (EGF) receptor is found randomly distributed on the cell surface [3]. Once bound to its ligand, the EGF ligand–receptor complex moves to regions of the cell membrane

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known as coated pits. In contrast, LDL receptors tend to cluster in these coated regions [4]. Once in these coated regions, receptors are immobilized and internalized.

The coated pits derive their name from a lattice formed by a protein called clathrin and the cytoplasmic surface of the cell [5]. On the average, each cell may have between 500 to 1500 coated pits on its surface [6]. These coated regions invaginate and form coated vesicles which continually lose their coats, forming endocytic vesicles called endosomes [7]. This process occurs on average approximately every 20 seconds, and it is continual and independent of any other process. In some cells, the coated pits are situated above actin filament bundles. Therefore, the coated pits are able to interact with the cytoskeleton [8], but how the clathrin recycles to the cytoplasmic surface is not known.

An important aspect of the internalization of receptors is their recycling. Experiments with fibroblasts in culture have shown that the internalization of α_2 macroglobulin molecules is of the order of 2×10^6 molecules per hour [9]. At this rate, the cell is unable to synthesize receptors fast enough to replace the internalized receptors. Using this example, one could assume that receptors are immediately recycled to the cell surface and reutilized. A fraction of receptors probably end up in the lysosomes and are degraded. One could also assume that the synthesis of receptors occurs in response to this degradation, and this is a zeroth order process.

Receptor synthesis is partially responsible for keeping a steady surface receptor concentration. But in some cases, synthesis is a slow process compared with receptor–ligand internalization. For example, when cells were incubated with α macroglobulin ^{125}I–trypsin complexes ($\alpha M^{125}I$–T) at 37°C, accumulation of $\alpha M^{125}I$–T occurred until the saturation point (8.8 fmol $\alpha M^{125}I$–T per μg cell protein) was reached [10]. The $\alpha M^{125}I$–T is internalized via receptor-mediated endocytosis, and it is degraded in the lysosomes at a minimum rate of 35% per hour [10]. This means that when the system reaches the saturation point, $\alpha M^{125}I$–T internalization occurs at a rate of 3.1 fmol $\alpha M^{125}I$–T per μg protein per hour. It has been observed that surface receptors bind 0.7 fmol $\alpha M^{125}I$–T per μg protein, and that the synthesis of receptors occurs at a rate of 15% per hour [10].

This experiment suggests that receptors must be reutilized. If this is the case, experimental data can be interpreted consistently with a rate of ligand uptake proportional to the number of surface receptors. The following experiment is consistent with the previous assumption. When cells were treated with cyclohexamide for four hours at 37°C, their receptor synthesis and internalization were altered. If later the cells were incubated with $\alpha M^{125}I$–T at 0°C, a 50% reduction in ligand binding, compared with control cells, was observed. The same percentage was observed for cells incubated at 37°C. From these experiments, the amount of $\alpha M^{125}I$–T taken up per hour divided by the bound $\alpha M^{125}I$–T would give us the number of times each receptor was used. Thus, surface receptors in controlled cells and cyclohexamide treated cells were utilized 5–10 times per hour [10].

Given the experimental evidence [11], one could conclude that for certain cells, receptors and ligands, receptor recycling is responsible for the steady surface concentration of receptors in receptor-mediated endocytosis. Furthermore, we could assume that the synthesis contribution to the receptor concentration is negligible. Based on these considerations, we extend our model of receptor-mediated endocytosis [12,13] by including a first order receptor internalization. For this model, we study the effect of receptor surface diffusion on the distribution of receptors around coated pits. In the next section, we discuss our model and the modifications to include reinsertion of receptors as a first order process. Also, we calculate the trapping rate constant within the Fluctuation–Dissipation (F–D) theory [14,15]. In Section 3, we consider the effect of receptor diffusion on the trapping of LDL receptors by coated pits, and we analyze the radial distribution function of receptors around coated pits. Finally, in section 4 we discuss our results and compare them with our previous model calculation of the random pit reinsertion with random zeroth order receptor reinsertion.

2. Linear receptor reinsertion

In receptor mediated-endocytosis, the internalization of a macromolecule involves the binding of the ligand to the receptor. In recent studies, Linderman and Lauffenburger [11] and Kaplan [16] analyzed the kinetics of ligand uptake, and their results suggest that the reinsertion of receptors is a first order process. This result is in contrast with the assumption that receptor reinsertion is entirely due to receptor synthesis, which is a zeroth order process. Moreover, for certain systems the biosynthesis cannot account for the steady state surface concentration of receptors that is observed. Our present discussion of receptor-mediated endocytosis is based on the observed steady state surface concentration of receptors, and the slow, compared with ligand uptake, biosynthesis of receptors. In order to include the previous observations, we modify our minimal model of receptor-mediated endocytosis [12,13]. Namely, we extend the number of extensive variables and include the reinsertion of internalized receptors. Therefore, we consider five elementary processes. The first process is the trapping of receptors by a pit

$$\mathrm{R} + \mathrm{P}(j\mathrm{R}) \underset{k^-}{\overset{k^+}{\rightleftharpoons}} \mathrm{P}((j+1)\mathrm{R}), \tag{1}$$

where R stands for receptor, P(jR) is a pit with j receptors, and $k^+(k^-)$ is the rate constant for the trapping (escaping) process. The invagination of a pit with j receptors is the second process and is represented as

$$\mathrm{P}(j\mathrm{R}) \overset{\lambda}{\rightarrow} j\mathrm{IR}, \tag{2}$$

where λ is the rate of invagination, and IR represents the internalized receptor, which is the new extensive variable. The third process is the reinsertion of a pit. In our model, we consider a zeroth order random reinsertion of pits with K_p being the rate of pit reinsertion. The next process is the reinsertion of the internalized receptor, and it is represented as

$$\mathrm{IR} \rightarrow \mathrm{R}, \tag{3}$$

with κ being the rate of constant for this process. Finally, we consider mobile receptors that can diffuse on the cell surface. This last process is characterized by a diffusion coefficient, D.

Following the F–D theory [12–15], we get the following equations for the average number densities of the different species

$$\frac{\mathrm{d}\bar{n}_\mathrm{P}}{\mathrm{d}t} = -\lambda\bar{n}_\mathrm{P} + K_\mathrm{P} \tag{4}$$

$$\frac{\mathrm{d}\bar{n}_\mathrm{R}}{\mathrm{d}t} = -k^+\bar{n}_\mathrm{P}\bar{n}_\mathrm{R} + k^-\bar{n}_\mathrm{RP} + \kappa\bar{n}_\mathrm{IR} \tag{5}$$

$$\frac{\mathrm{d}\bar{n}_\mathrm{RP}}{\mathrm{d}t} = k^+\bar{n}_\mathrm{R}\bar{n}_\mathrm{P} - k^-\bar{n}_\mathrm{RP} - \lambda\bar{n}_\mathrm{RP} \tag{6}$$

$$\frac{\mathrm{d}\bar{n}_\mathrm{IR}}{\mathrm{d}t} = \lambda\bar{n}_\mathrm{RP} - \kappa\bar{n}_\mathrm{IR}. \tag{7a}$$

In eqs. (5–7a) we have defined the average number of receptors in pits, n_RP, as:

$$\bar{n}_\mathrm{RP} = \sum_{j=0} \bar{n}_{j\mathrm{P}}, \tag{7b}$$

where $\bar{n}_{j\mathrm{P}}$ is the average number of pits with j receptors. In a compact notation, we define the vector $\bar{n}^\mathrm{T} = (\bar{n}_\mathrm{P}, \bar{n}_\mathrm{R}, \bar{n}_\mathrm{RP}, \bar{n}_\mathrm{IR})$ and rewrite eqs. (4)–(7) as

$$\frac{\mathrm{d}\bar{n}}{\mathrm{d}t} = \boldsymbol{R}(\bar{n}), \tag{8}$$

where $\boldsymbol{R}$ represents a vector related nonlinearly with $\bar{n}$. In this model, we consider a constraint in the number receptors, namely

$$\bar{n}_\mathrm{RP} + \bar{n}_\mathrm{R} + \bar{n}_\mathrm{IR} = \mathrm{Const.} \equiv \bar{n}_\mathrm{T}. \tag{9}$$

With this constraint, the steady state solutions to eqs. (4)–(7) are given by

$$\bar{n}^{\mathrm{ss}}_\mathrm{IR}/\bar{n}^{\mathrm{ss}}_\mathrm{RP} = \lambda/\kappa \tag{10}$$

$$\bar{n}^{\mathrm{ss}}_\mathrm{P} = K_\mathrm{P}/\lambda \tag{11}$$

$$\frac{\bar{n}^{\mathrm{ss}}_\mathrm{R}}{\bar{n}^{\mathrm{ss}}_\mathrm{RP}} = \left[\frac{k^- + \lambda}{k^+\bar{n}^{\mathrm{ss}}_\mathrm{P}}\right] \tag{12}$$

$$\bar{n}^{\mathrm{ss}}_\mathrm{RP} = \frac{\bar{n}_\mathrm{T}}{1 + \dfrac{\lambda(\lambda + k^-)}{k^+K_\mathrm{P}} + \dfrac{\lambda}{\kappa}}. \tag{13}$$

Fluctuations about these averages will eventually yield information on the distribution of re-

ceptors around pits. Therefore, we have to analyze the statistical properties of fluctuations in the number density. According to the F–D theory [12–15], the fluctuations in the number densities satisfy the following stochastic differential equation

$$\frac{\partial \delta \boldsymbol{n}}{\partial t} = \mathbf{H}\delta \boldsymbol{n} + \tilde{\mathbf{f}}, \tag{14}$$

where $\delta \boldsymbol{n}^{\mathrm{T}} = (\delta n_{\mathrm{P}}, \delta n_{\mathrm{R}}, \delta n_{\mathrm{RP}})$. Notice that the constraint in the number of receptors reduce the number of independent fluctuations i.e., $\delta n_{\mathrm{IR}} = -\delta n_{\mathrm{R}} - \delta n_{\mathrm{RP}}$. At steady state $\tilde{\mathbf{f}}$ is a multivariant Gaussian white noise; the noise vanishes on the average and has the covariance matrix

$$\langle \tilde{\mathbf{f}}(\boldsymbol{r}, t)\tilde{\mathbf{f}}^{\mathrm{T}}(\boldsymbol{r}', t')\rangle = \boldsymbol{\gamma}(\boldsymbol{r}, \boldsymbol{r}')\delta(t - t'), \tag{15}$$

where $\delta(x)$ is the Dirac function. The relaxation matrix, $\mathbf{H}$, is given by

$$H_{ij} \equiv \left[\frac{\partial R_i}{\partial n_j}\right]_{\bar{n}_{\mathrm{ss}}}. \tag{16}$$

Thus, eq. (14) is a linearization of eqs. (4)–(7), and $\mathbf{H}$ is given by

$$\mathbf{H} = \begin{bmatrix} -\lambda & 0 & 0 \\ -k^{+}\bar{n}_{\mathrm{R}}^{\mathrm{ss}} & D\,\nabla^2 - k^{+}\bar{n}_{\mathrm{P}}^{\mathrm{ss}} - \kappa & k^{-} - \kappa \\ k^{+}\bar{n}_{\mathrm{R}}^{\mathrm{ss}} & k^{+}\bar{n}_{\mathrm{P}}^{\mathrm{ss}} & -(k^{-} + \lambda) \end{bmatrix}. \tag{17}$$

The covariance matrix, $\boldsymbol{\gamma}$, is determined following the postulates of the F–D theory [12–15] and the molecular mechanism described by eqs. (1)–(3). If one follows the procedure described elsewhere [12,13], one obtains the following expression

$$\boldsymbol{\gamma} = \begin{bmatrix} \lambda\bar{n}_{\mathrm{P}}^{\mathrm{ss}} & 0 & \lambda\bar{n}_{\mathrm{RP}}^{\mathrm{ss}} \\ 0 & -2\bar{n}_{\mathrm{R}}^{\mathrm{ss}} D\,\nabla_r^2 + k^{+}\bar{n}_{\mathrm{R}}^{\mathrm{ss}}\bar{n}_{\mathrm{P}}^{\mathrm{ss}} + (k^{-} + \lambda)\bar{n}_{\mathrm{RP}}^{\mathrm{ss}} & -k^{+}\bar{n}_{\mathrm{R}}^{\mathrm{ss}}\bar{n}_{\mathrm{P}}^{\mathrm{ss}} - k^{-}\bar{n}_{\mathrm{RP}}^{\mathrm{ss}} \\ \lambda\bar{n}_{\mathrm{RP}}^{\mathrm{ss}} & -k^{+}\bar{n}_{\mathrm{R}}^{\mathrm{ss}}\bar{n}_{\mathrm{P}}^{\mathrm{ss}} - k^{-}\bar{n}_{\mathrm{RP}}^{\mathrm{ss}} & k^{+}\bar{n}_{\mathrm{R}}^{\mathrm{ss}}\bar{n}_{\mathrm{P}}^{\mathrm{ss}} + k^{-}\bar{n}_{\mathrm{RP}}^{\mathrm{ss}} + \lambda\langle n\rangle\bar{n}_{\mathrm{RP}}^{\mathrm{ss}} \end{bmatrix}\delta(\boldsymbol{r} - \boldsymbol{r}'), \tag{18}$$

where $\langle n\rangle$ is given by [12]

$$\langle n\rangle = \sum_j j^2 \frac{\bar{n}_{\mathrm{P}j}^{\mathrm{ss}}}{\bar{n}_{\mathrm{RP}}^{\mathrm{ss}}}. \tag{19}$$

Using this information, we want to calculate the density–density correlation function, which is linked to the radial distribution function, g_{ij}, namely

$$\langle \delta n_i(\boldsymbol{r})\delta n_j(\boldsymbol{r}')\rangle^{\mathrm{SS}} = \sigma_{ij}^{\mathrm{ss}}(\boldsymbol{r}, \boldsymbol{r}') = \bar{n}_i^{\mathrm{ss}}\bar{n}_j^{\mathrm{ss}}\left[g_{ij}(\boldsymbol{r}, \boldsymbol{r}') - 1\right], \tag{20}$$

where the angular bracket represents the static average over the steady-state ensemble. In order to accomplish this task, we have to transform eq. (17)–(18) to the Fourier space. In Fourier space, we have the following expression for the fluctuation–dissipation theorem [14]

$$\hat{\mathbf{H}}(\boldsymbol{k})\hat{\boldsymbol{\sigma}}(\boldsymbol{k}) + \hat{\boldsymbol{\sigma}}(\boldsymbol{k})\hat{\mathbf{H}}(\boldsymbol{k}) = -\hat{\boldsymbol{\gamma}}(\boldsymbol{k}), \tag{21}$$

where

$$\hat{\sigma}_{ij}(\boldsymbol{k}, \boldsymbol{k}') = \frac{1}{(2\pi)^4}\int d\boldsymbol{r}\int d\boldsymbol{r}'\, e^{i\boldsymbol{k}\cdot\boldsymbol{r} - i\boldsymbol{k}'\cdot\boldsymbol{r}'}\sigma_{ij}(\boldsymbol{r}, \boldsymbol{r}') \equiv \hat{\sigma}_{ij}(\boldsymbol{k})\frac{\delta(\boldsymbol{k} - \boldsymbol{k}')}{(2\pi)^2}. \tag{22a}$$

After a lengthy but otherwise straightforward calculation, one finds that the pit–receptor correlation function, $\hat{\sigma}_{12}$, is

$$\hat{\sigma}_{ij}(\boldsymbol{k}) = -\frac{k^{+}\bar{n}_{\mathrm{P}}^{\mathrm{ss}}\bar{n}_{\mathrm{R}}^{\mathrm{ss}}}{2D}\frac{\bar{b}(\kappa)}{k^2 + \xi^2(\kappa)}, \tag{22b}$$

where we have defined the following quantities

$$\bar{b}(\kappa) = \frac{2 + 3\kappa/\lambda + k^-\kappa/\lambda^2}{[1 + k^-/\lambda][2 + k^-/\lambda]} \tag{23}$$

$$\xi^2(\kappa) = \frac{bk^+\bar{n}_P^{ss} + \lambda + \kappa}{D} \tag{24a}$$

$$b = \frac{2 + \kappa/\lambda}{2 + k^-/\lambda}\,. \tag{24b}$$

Finally, the inverse Fourier transform gives the pit–receptor density–density correlation function in r-space

$$\sigma_{PR}(|r - r'|) = -\frac{k^+\bar{n}_P^{ss}\bar{n}_R^{ss}}{4\pi D}\bar{b}(\kappa)K_0(\xi(\kappa)|r - r'|), \tag{25a}$$

where K_0 is the McDonald function (also called a modified Bessel function) of order zero [17]. From this density–density correlation function, the radial distribution of receptors around pits in r-space is readily obtained

$$g_{PR}(|r - r'|) = 1 - \frac{k^+\bar{b}(\kappa)}{4\pi D}K_0(\xi(\kappa)|r - r'|). \tag{25b}$$

Information about the average spatial distribution of receptors around coated pits is given by the radial distribution function. This information can also be used to calculate the binding rate constant k^+. For this purpose we recall the application of the F–D theory to bimolecular processes [14,15,18]. In this approach, we need to know the so-called intrinsic reactivity, k°, and use the following expression for the bimolecular rate constant for circular symmetry in two dimensions

$$k^+ = 2\pi\int_0^\infty k^\circ(r)g_{PR}(r)r\,dr. \tag{26}$$

The simplest type of reactivity function assumes a unique reactive distance, in this case, the radius of the pit, R. Therefore, we can express the intrinsic reactivity as

$$k^\circ(r) = \frac{k^\circ}{2\pi r}\delta(r - R). \tag{27}$$

Thus, eq. (26) reduces to

$$k^+ = k^\circ g_{PR}(R). \tag{28}$$

Equation (28) is a general result in the sense that it includes the diffusion controlled case, which is obtained when $k^\circ/k^+ \gg 1$, as well as the possibility that diffusion is only partially rate limiting i.e., $k^\circ/k^+ \approx 1$. These conclusions are evident if we consider eqs. (26) and (28) and solve for k^+. This calculation yields the following result

$$\frac{k^+}{4\pi D} = \frac{\dfrac{k^\circ}{4\pi D}}{1 + \dfrac{k^\circ}{4\pi D}\bar{b}(\kappa)K_0(\xi(\kappa)R)}. \tag{29}$$

for the diffusion-controlled case, the system has to satisfy the condition

$$k^\circ \gg \frac{4\pi D}{\bar{b}(\kappa)K_0(\xi(\kappa)R)}, \tag{30}$$

which yields the following expression for the diffusion-controlled rate constant

$$k_{DC}^+ = \frac{4\pi D}{\bar{b}(\kappa)K_0(\xi(\kappa)R)}. \tag{31}$$

Under the assumption of a diffusion-controlled process, and using the F–D theory, we obtained an expression for the rate constant based on our extended model of receptor-mediated endocytosis. This theoretical result has to be consistent with the solution of the macroscopic rate equations (4)–(7). In particular, eq. (31) and eq. (12) have to be consistent, if diffusion of the receptors on the cell membrane is the rate determining step. In this case, the self consistency of the model yields the following equations:

$$K_0\left[R\sqrt{\frac{\lambda}{D}}x\right] = \frac{2\pi D}{\lambda}\frac{2 + k^-/\lambda}{1 + 3\kappa/2\lambda + k^-\kappa/2\lambda^2}\frac{\bar{n}_p}{\bar{\rho}}, \tag{32}$$

where we have defined

$$\bar{\rho} = \bar{n}_{RP}/\bar{n}_R \tag{33}$$

$$x = 1 + 2\bar{\rho}\frac{1 + k^-/\lambda}{2 + k^-/\lambda}\left[1 + \frac{\kappa}{2\lambda}\right] + \frac{\kappa}{\lambda}. \tag{34}$$

At steady state, the value of the ratio of receptors in coated pits to the number of receptors outside coated pits is known for LDL receptors on fibroblasts. Other parameters are known for this system except for κ and k^-. Using these data, we can explore the values of κ and k^- which are consistent with eq. (32).

In the case when the receptors escape from the coated pits, we have two extreme cases. The first and most physically plausible case considers trapped receptors, or $k^-/\lambda \ll 1$. In this case, one gets in general

$$2\pi D\frac{\bar{n}_P^{ss}}{\bar{\rho}}\frac{2}{1 + 3\kappa/2\lambda} = K_o\left[R\sqrt{\frac{\lambda}{D}x}\right], \tag{35}$$

with

$$x = 1 + \bar{\rho}[1 + \kappa/2\lambda] + \kappa/\lambda. \tag{36}$$

For the second case, where $(k^-/\lambda) \gg 1$, one obtains

$$2\pi D\frac{\bar{n}_P^{ss}}{\bar{\rho}}\frac{2}{\kappa/\lambda} = K_o\left[R\sqrt{\frac{\lambda}{D}x}\right], \tag{37}$$

with

$$x = 1 + 2\bar{\rho}[1 + \kappa/2\lambda] + \kappa/\lambda. \tag{38}$$

Equations (35) and (37) are the main result of this section. In the present model, the new parameter, κ/λ, allows a solution to the consistency equations, eqs. (35)–(37). Namely, we fixed the value of k^-/λ and solve for κ/λ. The solutions to these transcendental equations will support the assumption of diffusion as the rate determining step. These results contrast with the findings based on our original model where a solution to equivalent transcendental equations do not exist [12,13]. Therefore, the effect of diffusion is partial in the original model, and the assumption of total diffusion control is not consistent. In the present model and using data of LDL receptors on fibroblasts, we will explore, in the next section, the values of the parameter κ/λ, which are consistent with the assumption of diffusion control.

3. The effect of diffusion on the trapping of LDL receptors

In this section, we use LDL receptors data to test quantitatively our results. First, we will use the experimental parameters in eqs. (35) and (37) and find the value of κ/λ, which yields the ratio of the concentration of receptors in pits with respect to the concentration of internalized receptors. For example from Table 1, we get the following general transcendental equation:

$$K_o[0.086\sqrt{x}] = 1.2\left[\frac{2 + k^-/\lambda}{1 + 3\kappa/2\lambda} + k^-\kappa/2\lambda^2\right] \tag{39a}$$

with

$$x = 1 + 4.44\left[1 + \frac{\kappa}{2\lambda}\right]\frac{1 + k/\lambda}{2 + k^-/\lambda} + \kappa/\lambda. \tag{39b}$$

Notice that we have in this model two unknown parameters κ/λ and k^-/λ. From this result, we can analyze two limiting cases. The first considers no escaping from the pit i.e. $k^-/\lambda \ll 1$. This limit yields, from eq. (39), a transcendental equation for κ/λ

$$K_o\left[0.086\sqrt{1 + 2.22\left[1 + \frac{\kappa}{2\lambda}\right] + \frac{\kappa}{\lambda}}\right] = 2.4\left[\frac{1}{1 + 3\kappa/2\lambda}\right]. \tag{40}$$

For the second case, which considers fast escaping or $k^-/\lambda \gg 1$, we have

$$K_o\left[0.086\sqrt{1 + 4.44\left[1 + \frac{\kappa}{2\lambda}\right] + \frac{\kappa}{\lambda}}\right] = 2.4\left[\frac{1}{k/\lambda}\right]. \tag{41}$$

These two limits bound the values of κ/λ which are consistent with a diffusion controlled process.

Table 1

Characteristic parameters for LDL receptors

Parameter	Symbol	Value	Source
Radius of coated pits	R	0.10 μm	[19,20]
Receptor diffusion constant	D_A	4.5×10^{-3} (μm^2/s)	[3,20]
Steady-state density of coated pits (37°C)	n_P^{ss}	0.31 (μm)$^{-2}$	[19,20]
Number ratio of receptors in pits to receptors out of pits	$\bar{\rho}$	2.2	[19,20]
Invagination rate constant	λ	3.3×10^{-3} s^{-1}	[19,20]

Therefore, we limit our analysis to these cases. However, a partial diffusion effect cannot be neglected since the values of the experimental parameters have fairly large uncertainties [13].

In the first case, the value $\kappa/\lambda = 0.151$ solves eq. (40). This result is related to a ratio of the concentration through the steady state solutions of eq. (7), namely eq. (10). Combining this result with the experimental value of $\bar{\rho}$, one can relate the concentrations of receptors and receptors in pits with internalized receptors. Finally, since the total number of receptors is constant, we obtained the fraction of internalized receptors, receptors and receptors in pits. According to our model and the values for LDL receptors on fibroblasts, we predict that 82.0% of the receptors are internalized, 12.4% of receptors are in pits, and 5.6% of receptors are on the cell surface outside of pits. Even though we do not have experimental ratios, our results are plausible. For example in the case of EGF receptors, we find that the binding of EGF is reduced by an 80% of the initial binding capacity. For example in the presence of excess EGF steady state binding of EGF is just 20% of the initial value. Recalling that EGF receptors are only trapped when bound to a ligand, one could say that 80% of the EGF receptors are internalized.

For the second limiting case, $k^- \gg \lambda$, we find the solution of the transcendental equation (41) to be $\kappa/\lambda = 1.685$. This value predicts the following concentration ratios: 29.0% for internalized receptors, 48.8% for receptors in pits, and 22.2% for free receptors on the cell surface. Therefore, the values of κ/λ, which are consistent with a diffusion controlled process, lie in the interval [0.151, 1.675]. The receptor fractions predicted by our model range between 82.0% to 29.0% for internalized receptors, 12.4% to 48.8% for receptors in pits, and 5.6% to 22.2% for free receptors.

As we mentioned previously, a combined diffusion-reaction controlled process cannot be neglected since the experimental parameters observe large uncertainties. In this case, we consider the ratio

$$y = k^+/k^o \tag{42}$$

Using this ratio, we can rewrite eq. (29) as

$$y = \frac{1}{1 + k^+/k_D y} \tag{43}$$

or

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

If we use the steady state relation given by eq. (12) and the definition of the diffusion-controlled rate constant, we get

$$y = 1 - \frac{\bar{\rho}}{\bar{n}_P^{ss}} \frac{k^- + \lambda}{4\pi D} \bar{b}(\kappa) K_0(\xi(\kappa) R). \tag{45}$$

Using the definition of $\bar{b}(\kappa)$, eq. (45) reduces to

$$y = 1 - \frac{\lambda}{2\pi D} \frac{1 + \dfrac{3\kappa}{2\lambda} + \dfrac{k^-\kappa}{2\lambda^2}}{2 + \dfrac{k^-}{\lambda}} \frac{\bar{\rho}}{\bar{n}_P} K_0\left[R\sqrt{\frac{\lambda}{D}x}\right], \tag{46}$$

where x is defined by eq. (34). If we use the value of the parameters for LDL receptors on fibroblasts, we get an equation for y with κ/λ and k^-/λ as independent variables

$$y = 1 - \frac{1}{1.2}\left[\frac{1 + \dfrac{3\kappa}{2\lambda} + \dfrac{k^-\kappa}{2\lambda^2}}{2 + \dfrac{k^-}{\lambda}}\right] K_0(0.086\sqrt{x}), \tag{47}$$

where x is given by eq. (39b). In principle, the

value of κ/λ is given by the ratio in concentrations of receptors in pits with respect to internalized receptors, as described by eq. (10). Thus, a connection with a measurable quantity exists. At this point, different approaches can be taken depending on the experimental data. For example, if the parameters κ/λ and k^-/λ are known, the partial importance of diffusion can be estimated; that is, if two parameters are known, the third can be estimated. Unfortunately, k^-/λ and y have not been measured and, in general, are difficult to obtain experimentally. Therefore, we will only the case where escaping is not allowed. In this case, $k^-/\lambda \ll 1$, and eq. (47) reduces to

$$y = 1 - \frac{1}{1.2}\left[\frac{1+3\kappa/2\lambda}{2}\right]K_0(0.086\sqrt{x}), \qquad (48)$$

with x given by eq. (38). Whether or not the process is diffusion-controlled will be determined by κ/λ, which is related to the fraction of receptors in pits and internalized pits. As we have shown in the previous section, if the value is 0.151, the process is diffusion-controlled. If this is not the case, and once the value of κ/λ has been determined, eq. (48) will predict the percentage of diffusion dependence. Finally, since the value of the ratio of the population of receptors in pits with respect to internalized receptors has not been reported, and the value of 0.151 is consistent with diffusion control, we will not pursue any further the analysis of a partial diffusion effect. Instead, we consider the radial distribution of receptors around coated pits.

Using the result obtained in this section, we can analyzed the radial distribution function of receptors around coated pits. Using eq. (25b), this function is given by the following expression:

$$g_{RP}(|r-r'|) = 1 - \frac{k^+\bar{b}(\kappa)}{4\pi D}K_0\left[R\xi(\kappa)\frac{|r-r'|}{R}\right], \qquad (49)$$

where we have defined the following relation:

$$\xi(\kappa) = \sqrt{\frac{\lambda}{D}}\sqrt{\frac{bk^+\bar{n}_P^{ss}}{\lambda} + 1 + \frac{\kappa}{\lambda}}, \qquad (50)$$

and b is given yb eq. (24b). Furthermore, if we use eq. (12), we can eliminate k^+, and eq. (50) reduces to

$$g_{RP}(|r-r'|) = 1 - \frac{\lambda}{\bar{n}_P^{ss}}\left[\frac{\bar{n}_{RP}^{ss}}{\bar{n}_R^{ss}}\right]\left[1+\frac{k^-}{\lambda}\right]\frac{\bar{b}(\kappa)}{4\pi D} \times K_0\left[R\sqrt{\frac{\lambda}{D}}\sqrt{b\frac{\bar{n}_{RP}^{ss}}{\bar{n}_R^{ss}}\left[1+\frac{k^-}{\lambda}\right]+1+\frac{\kappa}{\lambda}} \times \frac{|r-r'|}{R}\right]. \qquad (51)$$

Using experiments values from Table 1, we find that eq. (51) can be reduced to

$$g_{RP}(|r-r'|) = 1 - f_1\left[\frac{\kappa}{\lambda}, \frac{k^-}{\lambda}\right]K_0 \times \left[0.086\sqrt{f_2\left[\frac{\kappa}{\lambda}, \frac{k^-}{\lambda}\right]}\frac{|r-r'|}{R}\right], \qquad (52)$$

where we have defined the following relations:

$$f_1\left[\frac{\kappa}{\lambda}, \frac{k^-}{\lambda}\right] = \frac{2+3\frac{\kappa}{\lambda}+\frac{\kappa}{\lambda}\frac{k^-}{\lambda}}{2.4\left[2+\frac{k^-}{\lambda}\right]} \qquad (53)$$

$$f_2\left[\frac{\kappa}{\lambda}, \frac{k^-}{\lambda}\right] = 1 + \frac{\kappa}{\lambda} + 4.44\left[1+\frac{\kappa}{2\lambda}\right]\frac{1+k^-/\lambda}{2+k^-/\lambda}. \qquad (54)$$

From eq. (52), the two limiting cases, $k^-/\lambda \gg 1$ and $k^-/\lambda \ll 1$, can be considered. For these limits, we have the values of κ/λ which are consistent with a diffusion-controlled receptor trapping. Therefore in the diffusion-controlled case and for $k^-/\lambda \ll 1$, we find the following expression:

$$g_{RP}(|r-r'|) = 1 - 0.511K_0\left[0.162\frac{|r-r'|}{R}\right], \qquad (55)$$

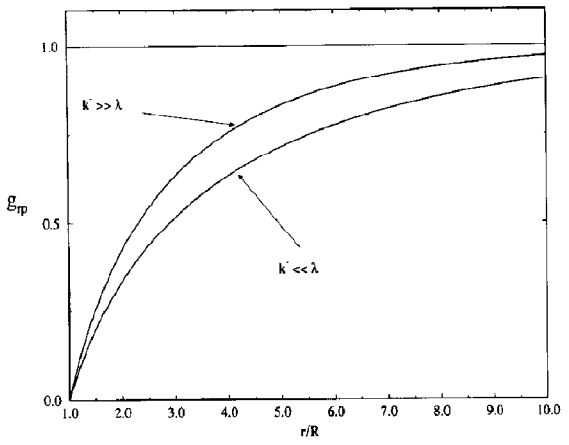

Fig. 1. Radial distribution of LDL receptors around coated pits for linear receptor reinsertion. In this case, we have used parameters from Table 1 and eqs. (55) and (56).

and for $k^-/\lambda \gg 1$, we have

$$g_{RP}(|\boldsymbol{r}-\boldsymbol{r}'|) = 1 - 0.702 K_0\left[0.283\frac{|\boldsymbol{r}-\boldsymbol{r}'|}{R}\right]. \quad (56)$$

Figure 1 depicts the radial distribution function of LDL-receptors around coated pits on fibroblasts for the limiting cases $k^-/\lambda \gg 1$ and $k^-/\lambda \ll 1$. In contrast with our previous models [12,13], this system shows minor changes in the distribution of LDL-receptors when the ratio k^-/λ is varied between its two limiting values. Also, notice that the value of g_{RP} is zero when $|\boldsymbol{r}-\boldsymbol{r}'| = R$. This condition reflects a consistency between the diffusion-controlled receptor trapping assumption in the present model and LDL data on fibroblasts.

4. Discussion

Although only few details of the molecular mechanism for individual steps involved in receptor-mediated endocytosis are known, we can safely assume that reinsertion of receptors can either be first or zeroth order processes. This assumption with the well accepted random pit reinsertion form the fundamental features of the model of receptor-mediated endocytosis considered here. Also an important and open question that the model calculations try to explain is whether the actual receptor trapping by coated pits is a diffusion-controlled process or not. As we have argued before, the slow receptor diffusion on the cell membrane is not enough to assume a diffusion-controlled receptor trapping. Rather than consider receptor diffusion as the only indicator, we have to compare it with the actual interaction occurring when the receptor reaches the pit boundary. Thus actual knowledge of the molecular process at contact between the pit and the receptor is needed. Once the trapping mechanism is understood, the characteristic diffusion time for receptor trapping has to be compared with the characteristic time. In order to say that receptor trapping is diffusion-controlled, the characteristic diffusion time has to be much greater than the characteristic trapping time.

Although the molecular details are still not fully known, our model calculations look for consistency between experimental data and the diffusion-controlled trapping assumption. The model of receptor-mediated endocytosis considered here has been introduced in previous work [2,13], where we have assumed a random and zeroth order pit and receptor reinsertion. For these as-

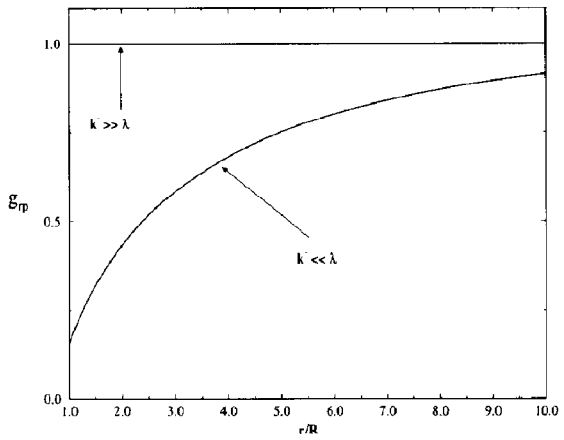

Fig. 2. Radial distribution of LDL receptors around coated pits for random receptor reinsertion. In this case, we have used parameters from Table 1 and eq. (55) from Ref. [12].

sumptions, the model is *not* consistent with a diffusion-controlled receptor trapping for LDL internalization by fibroblasts. This conclusion is depicted in Fig. 2, where the line $g_{rp} = 1$ represents no diffusion effect. And, the value of the radial distribution different from zero at $r = R$ implies a partial diffusion effect.

In contrast, in the present work we have considered a different receptor reinsertion mechanism. Instead of the zeroth order, we have assumed a first order reinsertion. This assumption is consistent with the idea that receptors are reused several times before they are degraded. For this reinsertion mechanism, we have found that the the model is consistent with a diffusion-controlled receptor trapping. This conclusion is depicted by Fig. 1. Notice that g_{rp} is equal to zero at $r = R$ in both limit cases. Moreover, using Table 1, we get quantitative values for the reinsertion coefficient κ and values for the ratio of the concentration of receptors in pits with respect to internalized receptors.

One of the most interesting differences is depicted in Fig. 3, where we notice that in the case of random receptor reinsertion diffusion has no effect on the distribution of receptors around coated pits, i.e., $g_{rp} = 1$. In contrast, for linear reinsertion, receptor trapping is diffusion-controlled, i.e., $g_{rp}(R) = 0$, and affects the distribution of receptors around the coated pits. Also, we

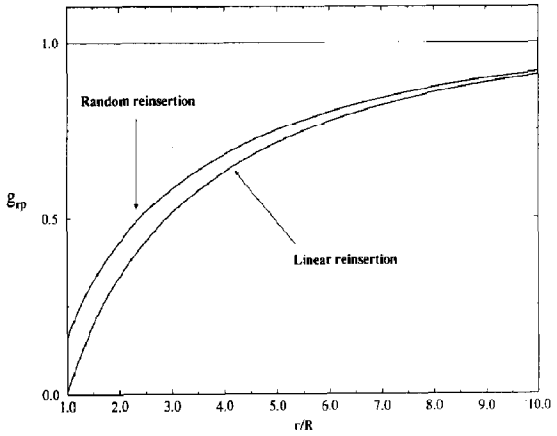

Fig. 3. Direct comparison of the radial distribution of LDL receptors around coated pits between the random and linear receptor reinsertion mechanisms, when $k^- \ll \lambda$.

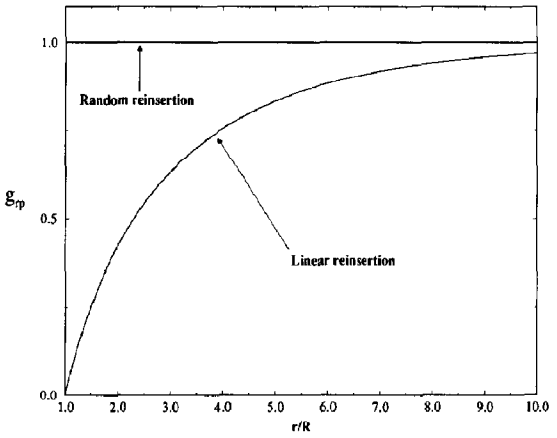

Fig. 4. Direct comparison of the radial distribution of LDL receptors around coated pits between the random and linear receptor reinsertion mechanisms, when $k^- \gg \lambda$.

notice that in the case $k^- \gg \lambda$, Fig. 4, the models yield different prediction. Namely, linear reinsertion is consistent with a diffusion-controlled receptor trapping while random reinsertion is not and only a partial diffusion effect is present, i.e., $g_{rp}(R) \neq 0$.

Although our method is able to give exact relations, it is sensitive to experimental parameters. Due to large uncertainties in the measured quantities, we cannot draw a definite conclusion is to whether receptor trapping is diffusion-controlled or not. However, our model calculations can help to elucidate and understand the processes involved in receptor-mediated endocytosis if new refined measurements, details of the pit–receptor boundary mechanism and determination of the radial distribution of receptors around pits are obtained.

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