Kinetics of ligand equilibration between tubular and vesicular parts of the endosome

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The kinetics of ligand equilibration between the tubular and vesicular parts of the endosome are studied for ligands diffusing in the vesicle and in a narrow cylindrical tubule attached to it. The key quantity in our analysis is the fraction of ligands in the vesicle at time t, $P_{ves}(t)$. We derive an expression for the Laplace transform of $P_{ves}(t)$ as a function of the vesicle volume and the length and radius of the tubule as well as the ligand diffusion coefficients in the vesicle and in the tubule. This transform is used to find the average equilibration time as a function of the system parameters.

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Receptor-mediated endocytosis is a mechanism that allows cells to incorporate a wide variety of extracellular materials bound to receptors on the cell surface. Such materials include peptide hormones, growth factors, cytokines, plasma glycoproteins, lysosomal enzymes, toxins, and viruses [1]. Different aspects and characteristic features of this process have been understood at the molecular level as discussed in the literature [1–10]. A schematic diagram of the endocytic cycle is shown in Fig. 1. The cycle is initiated by the binding of ligand molecules to specific cell surface receptors. Receptor-ligand complexes accumulate in localized regions on the cell surface. These regions invaginate and pinch off to form intracellular vesicles which carry the complexes. It is thought that several vesicles then form larger intracellular bodies, the endosomes. Electron micrographs of endosomes indicate that the endosome consists of a central chamber whose diameter is approximately 0.2–0.8 μm and one or more attached tubules whose diameters ranges from 0.01 to 0.06 μm [7,8,11]. While 60%–70% of the endosome volume is found in the vesicle, 60%–70% of the surface area belongs to the tubule [12].

A sorting process occurs in which the cell determines the destination of endocytosed molecules. Some of the molecules, mainly receptors, return to the cell surface to bind new ligands. This is termed receptor recycling. The ligand molecules are delivered to lysosomes where they degrade. Before a physical separation the receptor and ligand dissociate. Then they are distributed to different regions of the endosome: receptors go to the tubules and ligands mainly remain in the lumen of the vesicle. The tubules are believed to be intermediates in the recycling of receptor molecules back to the cell surface. Receptors and ligands in the tubules will be recycled when the tubules break their connection with the vesicles. The time during which receptors and ligands are allowed to redistribute between tubular and vesicular parts of the endosome is termed the sorting time.

In a pioneering paper Linderman and Lauffenburger (LL) proposed a diffusion model to account for the kinetics of separation [13]. They postulate that receptors diffuse on the spherical surface of the vesicle until they are irreversibly trapped on the surface of the tubule. Once a receptor reaches a tubule, it remains there permanently and is unable to diffuse from the tubule back to the vesicle. With a physiologically realistic choice of parameter values, LL predict that during a sorting time of 5 min 90% of the receptors are in the tubule. This prediction is in good agreement with experimental findings.

After the receptor-ligand complexes dissociate the ligands diffuse in the bulk of the endosome. Being initially in the vesicle they begin to equilibrate between the vesicle and tubule. The LL model assumes that the kinetics of equilibration is controlled by a search for the entrance to the tubule. In fact, this search is not the rate-limiting step, since when a ligand reaches the tubule entrance it is much more likely to return to the vesicle than to diffuse into the tubule [14]. Therefore the equilibration of ligands takes a much longer

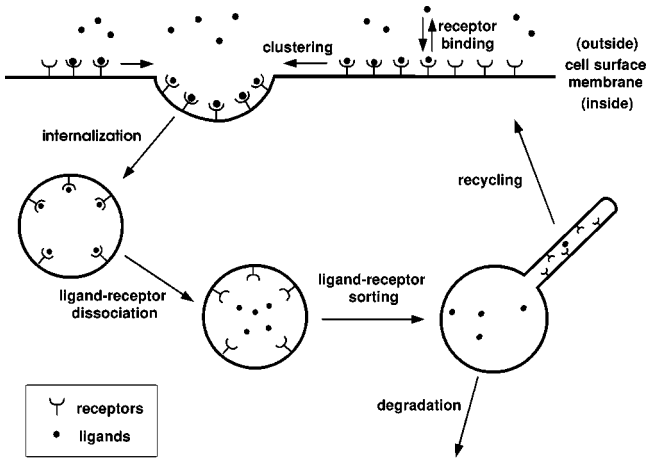

FIG. 1. Schematic diagram of receptor-mediated endocytosis. The receptors bind extracellular ligand molecules. Receptor-ligand complexes accumulate in localized regions on the cell surface. Complexes are internalized when the cell membrane regions invaginate and pinch off to form small intracellular vesicles containing complexes. Receptors and ligands are sorted in the endosome, allowing receptor recycling to the cell surface where they bind new ligands and ligand delivery to lysosomes where they degrade.

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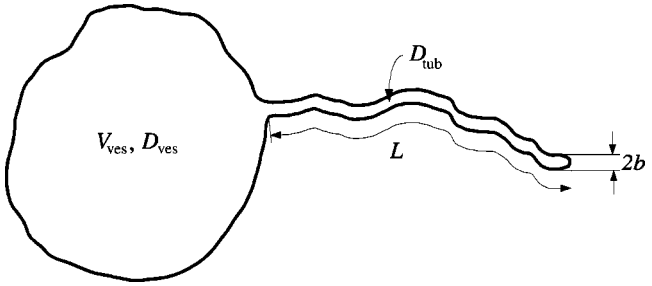

FIG. 2. Endosome is modeled as a chamber of volume V_{ves} to which a thin cylindrical tubule of radius b and length L is attached.

time than that predicted by the LL model.

In the present paper we study the kinetics of ligand equilibration in the geometry shown in Fig. 2. We assume that the ligands do not interact and analyze the transitions of a single ligand between the vesicle and tubule. The ligand equilibration is identical to equilibration in a two-state non-Markovian system represented by the kinetic scheme

$$\text{vesicle} \underset{\varphi_{tub}(t)}{\overset{\varphi_{ves}(t)}{\rightleftarrows}} \text{tubule}, \tag{1}$$

where $\varphi_{ves}(t)$ and $\varphi_{tub}(t)$ are probability densities for the ligand lifetime in the vesicle and tubule, respectively.

The density $\varphi_{ves}(t)$ depends on the volume of the vesicle, V_{ves}, the radius b of the cylindrical tubule, and on the ligand diffusion coefficient in the vesicle, D_{ves}. This density is a single exponential when the radius b is small compared to the radius of curvature of the vesicle wall as recently shown in [15]:

$$\varphi_{ves}(t)=ke^{-kt}, \quad k=\frac{4bD_{ves}}{V_{ves}}. \tag{2}$$

In contrast to $\varphi_{ves}(t)$, the density $\varphi_{tub}(t)$ is multiexponential. It depends on the tubule length L and radius b, as well as the ligand diffusion coefficients in the tubule, D_{tub}, and in the vesicle, D_{ves}, which may differ (in a narrow tubule D_{tub} may be smaller than D_{ves}). The expression for the Laplace transform of $\varphi_{tub}(t)$ is derived in the Appendix.

In this Brief Report we derive an exact solution for the kinetics of ligand equilibration described by the two-state model in Eq. (1). In particular, we find an expression for the average equilibration time $\bar{t}_{eq}$, which has the form

$$\bar{t}_{eq}=\frac{V_{ves}}{V_{ves}+V_{tub}}\left(\frac{\pi bL}{4D_{ves}}+\frac{L^2}{3D_{tub}}\right), \tag{3}$$

where $V_{tub}=\pi b^2 L$ is the volume of the tubule. For physiologically reasonable values of the parameters our estimate for the average equilibration time is about 15 times or more greater than that predicted by the LL model. Nevertheless, this time is well below the sorting time, which means that the ligands have enough time to equilibrate between the vesicle and tubule.

Consider a ligand which is in the vesicle at $t=0$. A key quantity in our analysis is the probability that the ligand will be in the vesicle at time t, $P_{ves}(t)$. For noninteracting ligands $P_{ves}(t)$ is the fraction of the total number of the ligands that are in the vesicle at time t. The probability $P_{ves}(t)$ satisfies

$$\frac{dP_{ves}(t)}{dt}=-kP_{ves}(t)+k\int_0^t \varphi_{tub}(\tau)P_{ves}(t-\tau)d\tau. \tag{4}$$

The first term on the right-hand side represents those realizations of the ligand trajectory which escape from the vesicle and enter the tubule at time t. The second term accounts for realizations which enter the tubule at time $t-\tau$, spend time τ in the tubule, and return to the vesicle at time t. The initial condition for the last equation is $P_{ves}(0)=1$.

It is convenient to solve Eq. (4) by using Laplace transforms. According to Eq. (4) the Laplace transform of $P_{ves}(t)$ $[\hat{P}_{ves}(s)=\int_0^\infty e^{-st}P_{ves}(t)dt]$ satisfies

$$s\hat{P}_{ves}(s)-1=-k\hat{P}_{ves}(s)+k\hat{\varphi}_{tub}(s)\hat{P}_{ves}(s), \tag{5}$$

where $\hat{\varphi}_{tub}(s)$ is the Laplace transform of $\varphi_{tub}(t)$ derived in the Appendix:

$$\hat{\varphi}_{tub}(s)=\int_0^\infty \varphi_{tub}(t)e^{-st}dt$$

$$=\frac{\kappa}{\kappa+\sqrt{sD_{tub}}\tanh\left(L\sqrt{\frac{s}{D_{tub}}}\right)}, \quad \kappa=\frac{4D_{ves}}{\pi b}. \tag{6}$$

Solving Eq. (5) one finds

$$\hat{P}_{ves}(s)=\frac{1}{s+k[1-\hat{\varphi}_{tub}(s)]}. \tag{7}$$

As $s\to 0$, $\hat{P}_{ves}(s)$ approaches $[s(1+k\bar{t}_{tub})]^{-1}=P_{ves}^{eq}/s$, where $\bar{t}_{tub}=-\varphi'_{tub}(0)$ is the average lifetime of the ligand in the tubule and P_{ves}^{eq} is the probability of finding the ligand in the vesicle at equilibrium. Using the relation $\bar{t}_{tub}=L/\kappa$ $=\pi bL/(4D_{ves})$, which follows from Eq. (6), together with the expression for k in Eq. (2) one finds

$$P_{ves}^{eq}=\frac{1}{1+k\bar{t}_{tub}}=\frac{V_{ves}}{V_{ves}+V_{tub}}. \tag{8}$$

It is convenient to describe equilibration in terms of a relaxation function $R(t)$, which decays monotonically from unity at $t=0$ to zero as $t\to\infty$. The probability $P_{ves}(t)$ can be expressed in terms of $R(t)$ as

$$P_{ves}(t)=P_{ves}^{eq}+(1-P_{ves}^{eq})R(t). \tag{9}$$

The Laplace transform of $R(t)$ is

$$\hat{R}(s)=\frac{\hat{P}_{ves}(s)-\frac{1}{s}P_{ves}^{eq}}{1-P_{ves}^{eq}}=\frac{s\bar{t}_{tub}-1+\hat{\varphi}_{tub}(s)}{s\bar{t}_{tub}\{s+k[1-\hat{\varphi}_{tub}(s)]\}}. \tag{10}$$

When writing the second expression for $\hat{R}(s)$ we have used the relations in Eqs. (7) and (8). Brownian dynamics simulations were run to check the assumptions used in developing the theory. These simulations showed excellent agreement between $R(t)$ calculated numerically and the theoretical prediction.

The function $R(t)$ can only be found by inverting the Laplace transform numerically. A convenient parameter that characterizes the ligand equilibration is the average equilibration time $\bar{t}_{\mathrm{eq}}$, defined by

$$\bar{t}_{\mathrm{eq}} = \int_0^\infty R(t)dt = \hat{R}(0) = \frac{\hat{\varphi}''_{\mathrm{tub}}(0)}{2\bar{t}_{\mathrm{tub}}(1+k\bar{t}_{\mathrm{tub}})}. \tag{11}$$

It follows from Eq. (6) that $\hat{\varphi}''_{\mathrm{tub}}(0) = 2\bar{t}_{\mathrm{tub}}[\bar{t}_{\mathrm{tub}} + L^2/(3D_{\mathrm{tub}})]$. When this is substituted into Eq. (11) one finds

$$\bar{t}_{\mathrm{eq}} = P_{\mathrm{eq}}\left(\bar{t}_{\mathrm{tub}} + \frac{L^2}{3D_{\mathrm{tub}}}\right) = \frac{V_{\mathrm{ves}}}{V_{\mathrm{ves}}+V_{\mathrm{tub}}}\left(\frac{\pi bL}{4D_{\mathrm{ves}}} + \frac{L^2}{3D_{\mathrm{tub}}}\right). \tag{12}$$

This is the result given in Eq. (3).

The model analyzed in the present paper takes into account the effect of diffusion in a tubule on ligand equilibration between the tubule and vesicle. This extends the LL model which identifies the kinetics of equilibration with the kinetics of the ligand's first entrance into the tubule. According to the LL model the relaxation function satisfies Eq. (4), omitting the second term on the right-hand side. In consequence, the LL relaxation function is $R_{\mathrm{LL}}(t) = \exp(-kt)$, which implies that the average equilibration time is k^{-1}. The ratio of the average equilibration time in Eq. (12) to the time k^{-1} is

$$k\bar{t}_{\mathrm{eq}} = \frac{V_{\mathrm{tub}} + \dfrac{4D_{\mathrm{ves}}}{3D_{\mathrm{tub}}}bL^2}{V_{\mathrm{ves}}+V_{\mathrm{tub}}}. \tag{13}$$

If we assume that the vesicle is a sphere of radius R and if we choose the values of the geometric parameters from the range reported in [12], $R = 0.2\ \mu$m, $b = 0.04\ \mu$m, and $L = 4\ \mu$m, we find that $V_{\mathrm{tub}} = 0.6V_{\mathrm{ves}}$ and $bL^2 \approx 30V_{\mathrm{tub}}$. If we additionally assume that $D_{\mathrm{ves}} = D_{\mathrm{tub}}$, we find that $k\bar{t}_{\mathrm{eq}} \approx 15$. With this choice of parameters our model predicts an average equilibration time 15 times larger than that predicted by the LL model. In fact, in a narrow tubule D_{tub} may be smaller than D_{ves}. If so, $k\bar{t}_{\mathrm{eq}}$ will be even greater than 15.

To calculate the value of $\bar{t}_{\mathrm{eq}}$ we assume that $D_{\mathrm{ves}} = D_{\mathrm{tub}} = 10^{-7}$ cm^2/sec [13]. Making use of the geometric parameters in Eq. (12) we find that $\bar{t}_{\mathrm{eq}} \approx 1/3$ sec. This time is well below the sorting time, which means that the ligands have more than enough time to equilibrate between the tubule and the vesicle.

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APPENDIX: THE PROBABILITY DENSITY FOR THE LIGAND LIFETIME IN THE TUBULE

Our derivation is based on the replacement of three-dimensional diffusion in a cylindrical tubule by one-dimensional diffusion in an interval, an approximation suggested in [16]. The tubule of length L is replaced by an interval $0 < x < L$ in which $x = 0$ corresponds to the end of the tubule in contact with the vesicle. Consider a ligand that enters the tubule at time $t = 0$. The propagator—or probability density for finding that ligand at x at time t—will be denoted by $g(x,t|0)$. This satisfies the diffusion equation

$$\frac{\partial g(x,t|0)}{\partial t} = D_{\mathrm{tub}}\frac{\partial^2 g(x,t|0)}{\partial x^2}. \tag{A1}$$

The propagator satisfies a reflecting boundary condition at $x = L$:

$$\left.\frac{\partial g(x,t|0)}{\partial L}\right|_{x=L} = 0. \tag{A2}$$

A ligand that reaches the point $x = 0$ can either enter the vesicle or return to the tubule, which is equivalent to regarding the origin as being a partially absorbing endpoint of the interval. The boundary condition at this point is

$$D_{\mathrm{tub}}\left.\frac{\partial g(x,t|0)}{\partial L}\right|_{x=0} = \kappa g(0,t|0), \tag{A3}$$

where an expression for κ, derived in [16,17], is given by $\kappa = 4D_{\mathrm{ves}}/(\pi b)$.

The left-hand side of Eq. (A3) is the probability flux escaping from the tubule at time t. This flux is just the probability density for the ligand lifetime in the tubule, $\varphi(t)$. This means that $\varphi(t) = \kappa g(0,t|0)$. The Laplace transform of Eq. (A1) is

$$D_{\mathrm{tub}}\frac{d^2\hat{g}(x,s|0)}{dx^2} = s\hat{g}(x,s|0) - \delta(x), \tag{A4}$$

where the δ function is a consequence of the initial condition placing the ligand initially at $x = 0$. This equation is to be solved subject to the Laplace transforms of the boundary conditions in Eqs. (A2) and (A3). Since Eq. (A4) is a linear equation, it can be solved by elementary methods. Making use of the relation $\hat{\varphi}(s) = \kappa\hat{g}(0,s|0)$ one can derive the expression for $\hat{\varphi}(s)$ given in Eq. (6).

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