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Mathematical Modeling of Intracellular Membrane Transport: Receptor-Mediated Endocytosis and Degradation of Low-Density Lipoproteins

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Abstract—Receptor-mediated endocytosis of low-density lipoproteins, their transport within endosomes, and subsequent degradation in lysosomes are essential components of the molecular system for cholesterol homeostasis in vertebrate cells. The system under study is also an example of clathrin-mediated endocytosis, a possible way of cell communication with the environment. Construction of a detailed mathematical model of this system would allow comprehensive study of mechanisms and kinetics of molecular processes and evaluation of the effect of various mutations, disorders, and environmental changes on the system operation. Receptor-mediated endocytosis of low-density lipoprotein particles and their subsequent degradation in the cell have been modeled. A network of mono- and bimolecular reactions best describing the system has been proposed. The results of calculation of kinetic parameters of the molecular system obtained with the model are in agreement with experimental evidence.

Key words: mathematical modeling, low-density lipoproteins, receptor, endocytosis, degradation

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INTRODUCTION

Plasma membrane, endoplasmic reticulum, Golgi apparatus, and other organelles form a unified membrane system of a eukaryotic cell. It is the place of synthesis of lipids and membrane proteins and their exchange mediated by directional controlled intracellular membrane transport [1]. Vesicular transport and the endosomal compartment are essential parts of this system. They support interaction between cells and environment and transport of proteins and lipids between organelles and compartments [2]. The endosomal compartment is a complex dynamic system adaptable to environmental factors. Several biosynthesis and recycling pathways can be recognized: (1) macropinocytosis, (2) phagocytosis, (3) clathrin-mediated endocytosis, (4) caveolin-mediated endocytosis, and (5) clathrin- and caveolin-independent endocytosis [3]. In this paper, we propose a mathematical model of receptor-mediated endocytosis of low-density lipoproteins (LDL), their transport in the endosome system, and subsequent degradation in lysosomes as an example of clathrin-mediated endocytosis [4–8]. For this model, a succession of mono- and bimolecular reactions is proposed that satisfactorily describes the system in terms of chemical kinetics. It has been shown that the predicted kinetic characteristics of elements of the molecular system are in agreement with experimental data.

THE MOLECULAR SYSTEM

Receptor-mediated endocytosis of LDL particles and their subsequent degradation in lysosomes involve several steps: (1) exposure of receptor molecules on the cell surface, (2) receptor binding to a ligand (LDL particle), (3) formation of a clathrin-coated pit around the receptors, (4) separation of the clathrin-coated vesicle, (5) formation of early endosomes and release of receptors from ligands therein, (6) return of receptors to the cell surface, (7) formation of late endosomes and their transport to lysosomes, (8) fusion of late endosomes with lysosomes, and (9) LDL degradation in lysosomes [3–10]. Each of these stages is a complex subsystem, involving numerous molecular events. For example, formation of a clathrin-coated pit and separation of the vesicle from the cell membrane requires many molecular adapter complexes: AP2 (adapter protein 2), epsin, dynamin, actin, etc. These complexes are bound to clathrin and receptors to form a so-called molecular scaffold, inducing vesicle formation [3, 6–11]. The involvement of clathrin-coated vesicles in different salvage pathways is determined by the fact that individual receptors possess additional signals for specific adapter proteins. The adapter for the LDL receptor is the autosomal recessive hypercholesterolemia protein, which mediates the interaction between the receptor and clathrin. This interaction plays the key role in the ability of the receptors to undergo endocytosis [3, 12, 13].

THE MATHEMATICAL MODEL

We propose an outline of receptor-mediated endocytosis of LDL particles followed by LDL degradation in lysosomes (Fig. 1a). A chemokinetic model of this system has been developed in terms of mono- and bimolecular reactions. Here is this model in differential form:

$$\begin{cases} \frac{dx_1}{dt} = -k_1x_1x_2 + k_2x_3 - k_1x_1x_4 + k_2x_5 - k_1x_1x_6 + k_2x_7 \\ \frac{dx_2}{dt} = -k_1x_1x_2 + k_2x_3 - k_3x_2 + k_4x_4 + k_8x_8 \\ \frac{dx_3}{dt} = k_1x_1x_2 - k_2x_3 - k_3x_3 + k_4x_5 \\ \frac{dx_4}{dt} = -k_1x_1x_4 + k_2x_5 + k_3x_2 + k_6x_6 - (k_4 + k_5)x_4 \\ \frac{dx_5}{dt} = k_1x_1x_4 - k_2x_5 + k_3x_3 + k_6x_7 - (k_4 + k_5)x_5 \\ \frac{dx_6}{dt} = -k_1x_1x_6 + k_2x_7 + k_5x_4 - (k_6 + k_7)x_6 \\ \frac{dx_7}{dt} = k_1x_1x_6 - k_2x_7 + k_5x_5 - (k_6 + k_7)x_7 \\ \frac{dx_8}{dt} = k_7x_6 - k_8x_8 \\ \frac{dx_9}{dt} = k_7x_7 - k_8x_9 \\ \frac{dx_{10}}{dt} = k_8(x_8 + x_9) - k_{10}x_{10} \\ \frac{dx_{11}}{dt} = k_8x_9 - k_9x_{11} \\ \frac{dx_{12}}{dt} = k_9(x_{11} - x_{12}) \\ \frac{dx_{13}}{dt} = k_9(x_{12} - x_{13}) - k_{11}x_{13} + k_{12}x_{14} \\ \frac{dx_{14}}{dt} = k_{11}x_{13} - k_{12}x_{14} \\ \frac{dx_{15}}{dt} = k_9x_{13} - k_{13}x_{15} \\ \frac{dx_{16}}{dt} = k_{13}x_{15} - k_{14}x_{16}, \end{cases} \quad (1)$$

where x_i are concentrations of LDL particles and their receptors in the corresponding states (Fig. 1a, Table 1) and k_i are the rate constants for the processes (Fig. 1a, Table 2).

The most appropriate set of model parameters was obtained by numerical analysis (Table 2). It provides a proper description of experimental data on LDL internalization and degradation kinetics (Fig. 1b).

ANALYSIS OF THE MODEL

Internalization of LDL Receptors

It is known that formation of a clathrin-coated vesicle able to be internalized into the cell takes some time, which is determined by the formation of the molecular scaffold that induces separation of the vesicle from the plasma membrane [9–10]. This process involves many molecular events, and its modeling is difficult. We analyzed three simplified versions of the pathway of LDL receptor internalization in a clathrin-coated vesicle to choose the best one. In the first version, receptors on the cell surface belong to a single pool, and each of them is internalized at a certain rate. In the second version, the receptors belong to two pools, exchanging with each other, and only one of the pools is involved in endocytosis. In the third version, the receptors on the cell surface are divided into three exchanging pools: (1) receptors not linked to proteins forming the coated pit (x_2 and x_3 in Fig. 1a), (2) receptors in nascent coated pits (x_4 and x_5 in Fig. 1a), and (3) receptors in coated pits ready to separate (x_6 and x_7 in Fig. 1a). Receptors of each of the pools can absorb LDL particles (x_1 in Fig. 1a) from the milieu.

Model (1) with the specified parameter values $k_{3\cdot} \longrightarrow \infty$, $k_{4\cdot} \longrightarrow 0$, $k_{5\cdot} \longrightarrow \infty$, and $k_{6\cdot} \longrightarrow 0$ (Fig. 1a) corresponds to the version with a single receptor pool on the cell surface. Its analysis shows that the first simplest version does not describe the experimental evidence reported in [5]. In this model, the amount of receptors bound to the labeled ligand on the cell surface exponentially decreases virtually to zero, whereas in the experiment 10% remain on the surface (Fig. 2a).

Model (1) with fixed parameter values $k_{3\cdot} \longrightarrow \infty$, $k_{4\cdot} \longrightarrow \infty$ (Fig. 1a) corresponds to the version with two exchanging pools. It provides a more accurate description of the experimental data. Nevertheless, the predicted absorption of receptors on the cell surfaces within the first 2 h slightly exceeds that in the experiment (Fig. 2b).

The third version of LDL receptor internalization provides the best basis for model (1), yielding an adequate description of the kinetics of the process (Fig. 1b).

The mean time of LDL receptor internalization in a coated pit is taken in the model to be 2 min ($k_7 = 10^{-2}$ s^{-1}, Fig. 1a), which allows adequate approximation of the kinetic curve of internalization of LDL-bound receptors (Fig. 1b, ▲) at the initial stage (the first 5–10 min) of rapid uptake of about 70–80% of the receptors in the coated vesicles (x_8 and x_9 in Fig. 1a). After 20 min, the kinetic curve flattens out because of system “buffering” by receptors present in nascent coated pits but not ready for internalization. Estimated rate constants for exchange between the three receptors pools (k_i, where

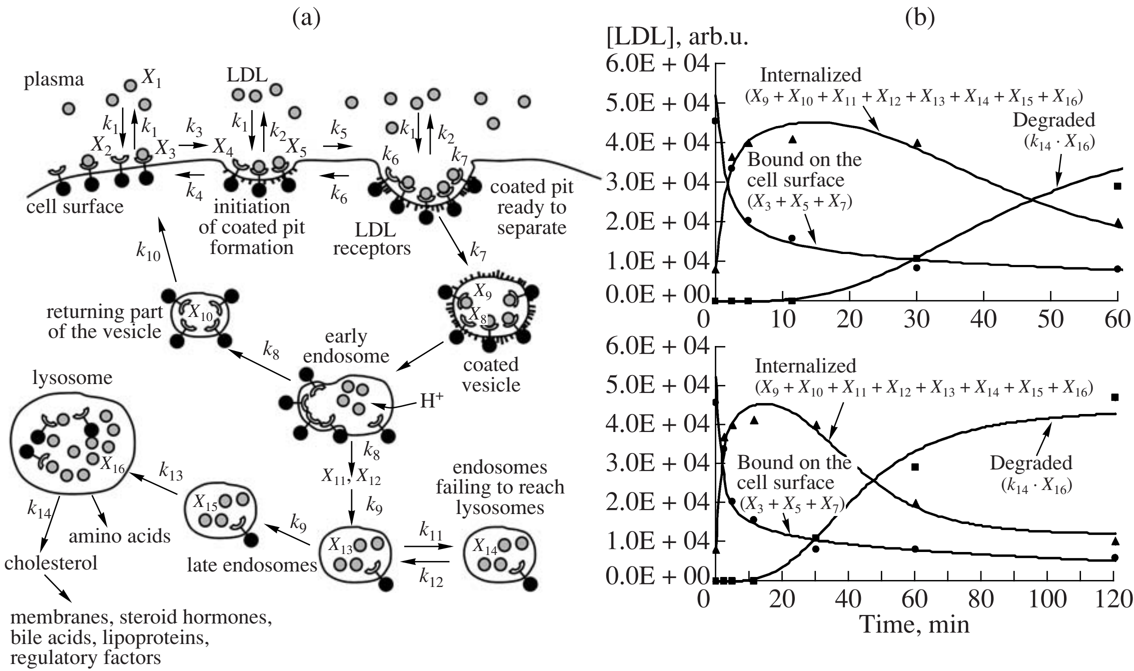

Fig. 1. (a) Diagram of the receptor-mediated endocytosis of LDL particles followed by LDL degradation in lysosomes. In this model, receptors on the cell surface are divided into three exchanging pools: receptors not bound to proteins coating pits; receptors in nascent coated pits, and receptors in coated pits ready to separate. After internalization, the receptors and LDL particles enter coated vesicles, which are then fused with smooth vesicles to form early endosomes, where the receptors and ligands are separated. The receptors return to the cell surface in returning vesicles, and the LDL particles are transported to lysosomes in endosomes. To model the delay of the transport of late endosomes and their fusion to lysosomes, these endosomes are divided into five exchanging pools (see text). (b) Comparison of the prediction from mathematical model (1) (solid curves) with the experimental data on the kinetics of receptor internalization and LDL degradation [5]. Designations for experimental data: ●, the amount of receptor-bound LDL particles on the cell surface; ▲, concentration of internalized LDL particles; ■, concentration of degraded LDL particles. The parameter values at which the predicted kinetic curves were calculated are shown in Table 2. The initial distribution of labeled LDL–receptor complexes was taken as follows: not bound to proteins coating the pit, 16.7%; in nascent coated pits, 16.7%; in coated pits ready to separate, 53%; and in coated vesicles, 13.6%.

$i = 3, 4, 5, 6$; Fig. 1a) are shown in Table 2. The characteristic exchange times (τ_i) are calculated as $\tau_i = 1/k_i$.

Recycling of LDL Receptors

After absorption, coated vesicles contact with small cell organelles, smooth vesicles, to form early endosomes [14]. In these endosomes, the low pH (5.9) generated by vacuolar proton ATPases causes separation of receptors and ligands [3]. The receptors (x_{10}; Fig. 1a) are exposed again to the plasma membrane within the returning vesicles, whereas the LDL particles within late endosomes (x_i, where i = 11, 12, 13, 14, and 15; Fig. 1a) are transported to lysosomes. The total time of receptor recycling in the model is 20 min, which is in agreement with experimental data [14]. This time is composed of characteristic times for the formation of the coated vesicle (2 min; $k_7 = 10^{-2}$ s^{-1}; Fig. 1a), formation of the early endosome and separation of LDL particles and receptors (8 min, $k_8 = 2 \times 10^{-3}$ s^{-1}; Fig. 1a), and return of LDL receptors to the cell surface (10 min, $k_{10} = 1.67 \times 10^{-3}$ s^{-1}; Fig. 1a).

Transport of LDL Particles in Late Endosomes to Lysosomes and Their Degradation

Model (1) with fixed parameters $k_{9\cdot} \longrightarrow \infty$, $k_{11\cdot} \longrightarrow 0$, $k_{12\cdot} \longrightarrow 0$, and $k_{13\cdot} \longrightarrow \infty$ (Fig. 1a) approximates the simplest pathway of LDL transport, where all internalized particles in endosomes reach lysosomes at a certain velocity. Analysis of this version of the model shows that it fails to reproduce the plateau of the kinetic curve of LDL uptake within the time of measurement from 5 to 30 min (Fig. 2a). The plateau is explained by the fact that LDL particles within endosomes reach lysosomes (containing the enzymes degrading the LDL particles) with a delay. To take into account this delay, the model includes four intermingling late endosome pools (x_i, where i = 11, 12, 13, and 15; Fig. 1a), and a reserve pool (x_{14}; Fig. 1a), which models a pool of undegraded LDL in the cell. As evident from Fig. 1b, LDL degradation slows down after 2 h and nearly flattens out. The mean time of transfer from one endosome pool into another was taken to be 8 min ($k_9 = 2 \times 10^{-3}$ s^{-1}; Fig. 1a). The mean time of LDL degradation in a lysosome (x_{16}; Fig. 1a) was evaluated from the kinetic curve

Table 1. Dynamic variables in the mathematical model of receptor-mediated endocytosis of low-density lipoprotein particles and their subsequent degradation in cell lysosomes

Designation	Physical significance: Concentration of
x_1	LDL particles in blood plasma
x_2	free LDL receptors not bound to proteins coating the pit on the plasma membrane
x_3	receptors bound to LDL particles on the plasma membrane but not bound to proteins coating the pit on the plasma membrane
x_4	free LDL receptors on the plasma membrane in nascent coated pits
x_5	receptors bound to LDL particles on the plasma membrane in nascent coated pits
x_6	free LDL receptors on the plasma membrane in coated pits ready to separate
x_7	receptors bound to LDL particles on the plasma membrane in coated pits ready to separate
x_8	free LDL receptors in coated vesicles
x_9	receptors bound to LDL particles in coated vesicles
x_{10}	LDL receptors in vesicles returning to the cell surface
x_{11}	LDL particles in late endosomes (pool I)
x_{12}	LDL particles in late endosomes (pool II)
x_{13}	LDL particles in late endosomes (pool III)
x_{14}	LDL particles in late endosomes (reserve pool; endosomes failing to reach lysosomes)
x_{15}	LDL particles in late endosomes (pool IV)
x_{16}	LDL particles in lysosomes

Table 2. Parameters of the mathematical model of receptor-mediated endocytosis of low-density lipoprotein particles and their subsequent degradation in the cell

Constant	Process	Value	Unit
k_1	Association of LDL particles with receptors on the plasma membrane of the cell	1.11×10^{-5}	$(\text{molecules/cell})^{-1}$ s^{-1}
k_2	Dissociation of LDL particles from receptors on the plasma membrane	10^{-4}	s^{-1}
k_3	Initiation of coated pits	10^{-4}	s^{-1}
k_4	Process reverse of the formation of coated pits	3×10^{-4}	s^{-1}
k_5	Formation of a coated pit ready to separate	1.5×10^{-3}	s^{-1}
k_6	Process reverse of the formation of a coated pit ready to separate	10^{-4}	s^{-1}
k_7	Formation of a coated vesicle	10^{-2}	s^{-1}
k_8	Formation of an early endosome and separation of LDL particles from receptors caused by the low pH produced by vacuolar proton ATPases in the endosome	2×10^{-3}	s^{-1}
k_9	Formation of late endosomes	2×10^{-3}	s^{-1}
k_{10}	Return of LDL receptors to the cell surface	1.67×10^{-3}	s^{-1}
k_{11}	Entrance to the pool of endosomes failing to reach lysosomes	5×10^{-4}	s^{-1}
k_{12}	Egress from the pool of endosomes failing to reach lysosomes	10^{-5}	s^{-1}
k_{13}	Fusion of late endosomes with lysosomes	2×10^{-3}	s^{-1}
k_{14}	Degradation of LDL particles in lysosomes	2.78×10^{-2}	s^{-1}

of LDL degradation in the cell (Fig. 1b, ■) and taken to be 1 min ($k_{14} = 2.78 \times 10^{-2}$ s^{-1}; Fig. 1a).

DISCUSSION

Intracellular membrane transport is one of the key elements of the machinery effecting interaction between the cell and its environment and communication between various cell compartments and organelles. We propose a succession of molecular events best describing the receptor-mediated endocytosis of LDL particles followed by LDL degradation in lysosomes as one of possible pathways of membrane transport in cells. A chemokinetic model of this succession has

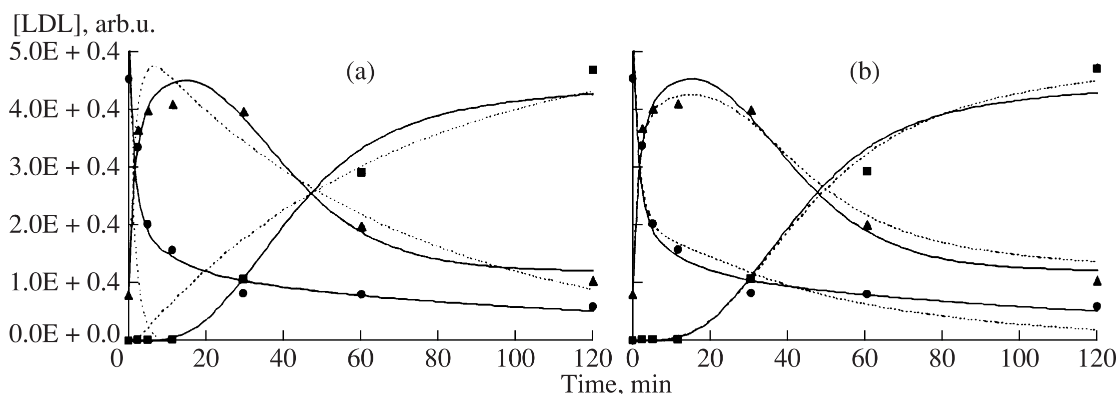

Fig. 2. Comparison of predictions from mathematical model (1) with various parameter values. Solid curves in panels (a) and (b) depict calculation by model (1) with parameter values indicated in Table 2. Dotted curves in panel (a) illustrate calculation from model (1) with parameter values $k_{3'} \longrightarrow \infty$, $k_{4'} \longrightarrow 0$, $k_{5'} \longrightarrow \infty$, $k_{6'} \longrightarrow 0$, $k_{8'} = 2.5 \times 10^{-3}$ s^{-1}, $k_{9'} \longrightarrow \infty$, $k_{11'} \longrightarrow 0$, $k_{12'} \longrightarrow 0$, $k_{13'} \longrightarrow \infty$. Other parameters are as in Table 2. Dotted curves in panel (b) illustrate calculation from model (1) with parameters $k_{3'} \longrightarrow \infty$, $k_{4'} \longrightarrow 0$, $k_{5'} = 3 \times 10^{-4}$ s^{-1}, $k_{6'} = 1 \times 10^{-4}$ s^{-1}. Other parameters are as in Table 2. Symbols in panels (a) and (b) indicate experimental kinetic data on receptor internalization and LDL degradation in the cell [5]. All designations follow Fig. 1.

been constructed and tested in terms of mono- and bimolecular reactions. This model is but a rough approximation to actual complex processes in cells, which are difficult to formalize. The model demonstrates that it is important to account for buffering and delay, which occur everywhere in living systems and reflect the actual kinetics of cell operation (response time of a system etc.).

The molecular system considered here is an example of clathrin-mediated endocytosis. It is known that this kind of endocytosis underlies other cell processes, such as iron ion transport with transferrin and epidermal growth factor [3], with are bound on the cell surface by corresponding receptors and absorbed by the cell by the above-described general mechanisms.

Modern genetical and biochemical analysis and application of up-to-date methods to real-time processing of images of specifically labeled molecular components can reveal fine details of endocytosis and transport in cells [2, 9, 10, 15]. With this knowledge, the mathematical model presented in this work can be applied to describing other pathways of membrane transport in cells. The quantitative and qualitative indices of its molecular components obtained by modern experimental methods can be used for solving inverse problems of determining parameters of new models.

Mathematical modeling of more detailed pathways of intracellular membrane transport would allow combining current knowledge on an integrated conceptual basis. This, in turn, would allow detailed study of mechanisms of molecular processes and prediction of their kinetics by computer simulation, analysis of the effects of various factors on the system (mutations, distortion of system operation mechanisms, environmental changes, etc.), improvement of methods of controlling the system for correction of its abnormal states, and study of pharmacokinetic problems (methods of optimal drug delivery into cells by membrane transport [16]).

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