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LOW DENSITY LIPOPROTEIN RECEPTOR REGULATION

KINETIC MODELS

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The macromolecular species distribution in a receptor-mediated endocytotic pathway was computer simulated based on kinetic data reported in the literature. In the proposed model, the rapidity with which the recycled receptor is shuttled to the cell surface is indicated by the magnitude of k_{-3}, the shuttling constant. The magnitude of k_{-3} will vary with the experimental conditions, but when this value is large, the internalized receptor is shuttled back to the cell surface with a traverse time of 14 min. Under steady-state conditions, after the cells have been incubated in the presence of LDL for 5 h (M.S. Brown and J.L. Goldstein, Cell 9 (1976) 663), the time required for a receptor to traverse the entire endocytotic pathway is 52 min. Our simulation suggests that normal LDL binding in such a short-term experiment may be independent of receptor synthesis. Thus, the degradation of LDL and resultant build-up of cholesterol would have no apparent inhibitory effect on the down-regulation of receptor synthesis.

1. Introduction

In the broad sense, several properties of the cell surface receptor are responsible for its efficient mediation and modulation of adsorptive endocytosis [1–5]: (i) The receptor is regulated by the extent of exposure of cells to ligand in vitro, i.e., continuous exposure causes down-regulation of receptors in the protein hormones [6–15]. (ii) In other systems which transport plasma lipoproteins, the receptor is recycled back to the cell surface [1–5]. After binding and internalization of the low density lipoprotein (LDL) particle, an unoccupied receptor appears on the cell surface, allowing continuous binding and internalization [1–5]. (iii) Receptor-ligand complexes are internalized within a few minutes and the cell surface receptors migrate continuously to mediate the endocytosis [4].

The fate and action of the cholesterol thus introduced into the cell are, apart from its utilization for membrane synthesis, of critical importance to this pathway [16–20]: (i) Cholesterol suppresses the activity of hydroxymethylglutaryl-CoA reductase, the rate-limiting enzyme in cholesterol synthesis; (ii) it activates acyl-CoA : cholesteryl acyltransferase (ACAT), which reesterifies some of the incoming cholesterol for storage as cholesteryl ester in the cytoplasm; and (iii) oxycholesterol (such as 7-ketocholesterol, 6-ketocholesterol), as well as 25-hydroxycholesterol, suppresses the synthesis of the LDL receptor in vivo [21–26].

Current structural information strongly indi-

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cates that LDL receptors are transmembrane acidic glycoproteins with a molecular weight of 164 000 which mediate endocytosis through coated pits [19,20,27]. In normal fibroblast cells incubated with LDL at a concentration of 20 μg/ml, a maximum of approx. 250 000 LDL molecules could be bound to each cell [28]. This translates into approximately six receptors which are segregated from resident membrane glycoproteins in each of 5000 coated pits per cell. In most patients with homozygous familial hypercholesterolemia (FH), Tolleshaug et al. [20] have stated that no such segregation occurs. Brown and Goldstein [37] report results for a subject with normal binding, but internalization-negative cells. If receptors are segregated in the coated pits without LDL bound, it appears that they may still be internalized. The internalized LDL-receptor complex releases receptors into the intracellular compartments to be recycled by an endosome [3,20,27,29–33] while the LDL is degraded in the lysosome.

In this communication, a kinetic model is described for the recycling of LDL receptors and the macromolecular species distribution of the receptor in human skin fibroblasts in the steady state. This kinetic simulation confirms earlier reported findings that oxycholesterol or hydroxycholesterol down-regulates the synthesis of cell surface receptor, suggesting that under such conditions, the binding of normal LDL is independent of receptor regulation.

2. Kinetics of LDL receptor function

The kinetics of LDL receptor function have been studied by measuring the binding of ^{125}I-LDL to the cell surface receptor in intact cultured fibroblast monolayers in situ. When ^{125}I-LDL is incubated with monolayers of normal fibroblasts at 4°C, binding to the receptor occurs, but no internalization takes place [28,34–36].

The amount of ^{125}I-LDL bound to the surface receptor (R^sL) can be distinguished from the amount internalized (R^mL) by treatment of the cells with heparin, which selectively releases the ^{125}I-LDL bound to the cell surface [37].

Brown and Goldstein [35,37] were the first to show that exposure of fibroblasts to LDL or free cholesterol in vitro caused a decrease, or down-regulation, in the level of cell surface LDL receptor activity.

3. Proposed kinetic pathway for regulation of LDL-receptor metabolism in fibroblast cells: theoretical model

3.1. Regulation of the LDL receptor in the fibroblasts

Our theoretical model for the kinetic pathway for the regulation of LDL receptor metabolism is based on the experiments of Brown and Goldstein [35,37], which indicated that the activity of the receptor was under feedback regulation. Either added LDL or added sterol reduced ^{125}I-LDL binding, apparently by suppressing receptor synthesis. By controlling the activity of this receptor, the cells regulated the entry of LDL and therefore the cellular cholesterol content.

In these experiments, fibroblasts were grown in medium containing 10% fetal calf serum; on the sixth day, monolayers were washed with phosphate-buffered saline (PBS) and medium containing 10% human lipoprotein-deficient serum was added. On day 7 of cell growth, each monolayer received 2 ml of 5% human lipoprotein-deficient serum containing 10 μg/ml of ^{125}I-LDL. After 5 h of incubation at 37°C, the medium was removed and its content of ^{125}I-labeled trichloroacetic acid-soluble material was measured. The cell monolayers were then washed and the amounts of heparin-releasable and heparin-resistant ^{125}I-LDL were determined as described by Brown and Goldstein [37].

When cells were incubated continuously with ^{125}I-LDL at 37°C, they established a steady state in which the rate of uptake of ^{125}I-LDL was equal to the rate of degradation.

3.2. Formulation of the kinetic models for LDL-receptor regulation

A kinetic model is proposed for the regulation of LDL-receptor metabolism. It is assumed that

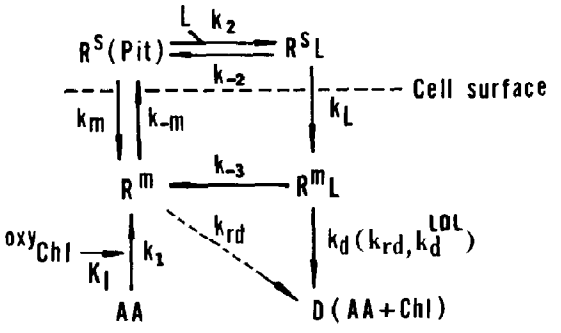

Fig. 1. Proposed kinetic pathway for regulation of LDL receptor metabolism in fibroblast cells. During receptor-mediated endocytosis, LDL binds to a specific cell surface receptor, and the receptor-ligand complex is internalized in clathrin-coated pits. Most ligands dissociate from their receptors in endosomes due to the low pH [41–43]. Ligands (LDL) are then transported to lysosomes, where they are degraded. The receptors could cluster together at one pole of the endosome and bud off in a new vesicle that shuttles back to the cell surface ($R^mL \rightarrow R^s$). [R^s] (pit) represents unoccupied surface receptor in cell surface pits; [R^sL], surface receptor-LDL complex (heparin-sensitive fraction). [R^mL] is the intracellular receptor-LDL complex (heparin-insensitive fraction) which releases the receptor [R^m] into the intracellular compartments to be recycled. AA, amino acids; Chl, cholesterol; oxy_{Chl}, oxycholesterol.

the degree of recycling depends on the reverse rate process which we denote as the shuttling constant (k_{-3}), by which the LDL-receptor complex [R^mL] reverts to the intracellular recycled receptor [R^m]. We propose a multi-step, irreversible ordered sequence model incorporating cholesterol concentration into the kinetic model to explain cell surface receptor regulation.

The up- and down-regulation kinetics of receptor synthesis and recycling based on the numerical solution for five differential equations [38,39] are shown here.

$$\frac{d[R^s]}{dt} = k_{-m}[R^m] - k_m[R^s] + k_{-2}[R^sL] - k_2[R^s][L]$$

$$\frac{d[R^m]}{dt} = k_1[A] + k_m[R^s] - k_{-m}[R^m] + k_{-3}[R^mL] - k_{rd}[R^m]$$

$$\frac{d[R^sL]}{dt} = k_2[R^s][L] - (k_{-2} + k_L)[R^sL] \qquad (1)$$

$$\frac{d[R^mL]}{dt} = k_L[R^sL] - (k_{-3} + k_d)[R^mL]$$

$$\frac{d[D]}{dt} = k_d^{LDL}[R^mL] + k_{rd}[R^m]$$

where [A] is the amino acid concentration, [R^m] the concentration of intracellular receptors, and k_1[A] the receptor synthesis rate. k_i represents the rate constant of each pathway indicated in fig. 1. [D] is the concentration of degradation product and k_d a first-order rate constant for degradation of [R^mL], which includes the constants for the degradation of receptor and LDL (k_{rd} and k_d^{LDL}). The rate constant for internalization is k_L, while k_{-3} represents the shuttling constant for movement and dissociation of receptor from R^mL. k_{-m} is the rate constant for the movement and appearance of internalized or newly synthesized receptor at the cell surface. k_m is the rate constant for self internalization of the cell surface receptor which is not bound to LDL.

Experiments under steady-state conditions, described elsewhere, indicate that k_{-m} will be much larger than k_m in the presence of LDL, while k_{rd} and k_{-2} approach zero. Assuming that the rate of synthesis remains constant over the 5 h of the experiment [37], the above equations become

$$\frac{d[R^s]}{dt} = k_1[A] - k_2[R^s][L] + k_3[R^mL]$$

$$\frac{d[R^sL]}{dt} = k_2[R^s][L] - k_L[R^sL] \qquad (2)$$

$$\frac{d[R^mL]}{dt} = k_L[R^sL] - (k_3 + k_d)[R^mL]$$

From eq. 2, the cell surface receptor concentration, [R^s], may be expressed as

$$[R^s] = \frac{k_1[A]}{a}(1 - e^{-at}) - \frac{k_2[L]k_{-3}}{(k_{-3} + k_d)(a - k_L)} \times [R^s]_0(e^{-k_L t} - e^{-at}) + [R^s]_0 e^{-at} \qquad (3)$$

where $a = (k_2[L]k_d)/(k_{-3} + k_d)$ and $[R^sL] = 0$ on day 7 at initial time, $t = 0$, for convenience in solving the differential equations.

Also, from eq. 2, the concentration of cell surface-bound LDL may be expressed as

$$[R^sL] = \frac{k_2[L]}{k_L}\left\{\frac{k_1[A]}{a}(1-e^{-at}) - \frac{(k_2[L]-k_L)}{(a-k_L)} \times [R^s]_0(e^{-k_L t} - e^{-at})\right\} \quad (4)$$

Thus, the concentration of internalized LDL-receptor complex is

$$[R^mL] = \frac{k_2[L]}{(k_{-3}+k_d)}\left\{\frac{k_1[A]}{a}(1-e^{-at}) - \frac{(k_2[L]-k_L)}{(a-k_L)}[R^s]_0(e^{-k_L t} - e^{-at})\right\} \quad (5)$$

These expressions are derived in detail in eqs. A9–A11 of appendix A.

Experimentally, the $[R^sL]$ can be distinguished from $[R^mL]$ as follows [37]: cells are incubated with ^{125}I-LDL at 37°C until the steady state is established. The fibroblast monolayers are then rapidly chilled to stop internalization, washed extensively to remove unbound ^{125}I-LDL, and then incubated at 4°C with heparin, which releases the ^{125}I-LDL that is bound to the receptor on the cell surface. The amount of ^{125}I-LDL that remains associated with the cell after such heparin treatment represents $[R^mL]$ that has been internalized by the fibroblast cell (see appendix A).

It is now possible to evaluate all the rate constants using eqs. 4 and 5 and the data reported by Brown and Goldstein [37]. During the period of dynamic equilibrium, at $t = 5$ h incubation at 37°C, the amount of ^{125}I-LDL bound to the receptor and the amount of ^{125}I-LDL contained within the cell remain constant, while the amount of ^{125}I-LDL degradation product appearing in the culture medium increases at a linear rate.

The treatment of washing monolayers to remove unbound ^{125}I-LDL offers an advantage since $[R^s]$, the free receptor in the coated pits, can be evaluated in the steady state.

The total cell receptor concentration is expressed as $[R]_T = [R^s] + [R^sL] + [R^mL] + [R^m]$. The receptors present in the coated pits may represent receptors which have been newly synthesized, recycled, or both.

It is possible to evaluate the total concentration of cell surface receptor and distribution of intracellular receptor-LDL complex as a function of time based on eq. 1. In the steady state, the cellular synthesis rate of receptor remains constant at 1.98 pmol/h [35].

The effect of the degradation of LDL and the resultant build-up of cholesterol on the macromolecular species distribution in the steady state may be simulated based on the numerical solution of five differential equations (eq. 1), where $d[D]/dt = k_d^{LDL}[R^mL]$. The concentration of cholesterol in the system may be determined from the rate expression for degradation,

$$[D] = \frac{k_2[L]}{(k_{-3}+k_d)}\left\{\frac{k_1[A]}{a}(1-e^{-at}) - \frac{k_2[L]-k_L}{(a-k_L)}[R^s]_0(e^{-k_L t} - e^{-at})\right\} k_d^{LDL} t \quad (6)$$

The influence of the rate of degradation on the other kinetic processes involved in endocytosis and receptor regulation may then be evaluated, based on the rate expressions of eq. 1 and the determination of the endocytotic species distribution.

3.3. Computational procedure

Using a North Star computer system with a floating point BASIC, the three rate equations for free, bound, and internalized receptor ($d[R^s]/dt$, $d[R^sL]/dt$, and $d[R^mL]/dt$) were solved and the concentration of each species plotted as a function of LDL concentration or time (an Olis Graphics subroutine, FPZBASIC, was used in plotting the functions: Olis Model No. 3600, 140 Featherwood Drive, Athens, GA).

Two methods were used to solve the differential equations [38–40]. In the first, the equations were simplified by the assumptions $k_{-2} \ll k_2$ and $k_{-2} \ll k_d$ and then solved algebraically using eqs. 3–5. Letting $[R^s]_0$, the concentration of unbound cell surface receptor at time zero, be equivalent to 1550 ng/mg protein and using the following values, $k_1[A] = 6.0$ ng mg^{-1} protein h^{-1}, $k_2 = 0.09$ ng mg^{-1} h^{-1}, $k_{-3} = 1.56$ h^{-1}, $k_L = 12$ h^{-1}, as

Table 1

Kinetic parameters for receptor-mediated endocytosis: low density lipoprotein

These parameters represent the best available data currently reported in the literature, and were compiled from several sources.

Parameters	Low density lipoprotein (Class II), skin fibroblasts (monolayer experiments)	
(A) Receptor synthesis	$[R^s]$ or $[R^m]$ [35]	$[R^s]$ [35]
$k_1[A]$ (ng mg protein^{-1} h^{-1})	6.0	6.0
(B) Ligand binding	$k_2 = 0.46$ h^{-1} μg^{-1} ml [L] = 42.86 μg/ml	$[k_2L] = 18$ h^{-1} $k_2 = 0.09$ h^{-1} μg^{-1} ml
k_2X (min^{-1} or h^{-1})	20.0 h^{-1} [4,37]	
$t_{1/2}$ (min)	2.1 min	2.3 min
t_{mean}	3.0	3.2
(C) Dissociation of ligand from bound receptor, $[L] = \dot{X}$	$[R^sL] \rightarrow [R^s]$	$[R^sL] \rightarrow [R^s]$
k_{-2} (min^{-1} or h^{-1})	–	–
$t_{1/2}$	–	–
t_{mean}	–	–
(D) Internalization of surface receptor	$[R^sL] \rightarrow [R^mL]$ [3,4]	$[R^sL] \rightarrow [R^mL]$ [3,4]
k_L (min^{-1} or h^{-1})	12 h^{-1}	12 h^{-1}
$t_{1/2}$	3.46	3.46
t_{mean}	5.0	5.0
(E) Movement of internalized receptors	$[R^mL] \rightarrow [R^m]$	$[R^mL] \rightarrow [R^m]$
k_{-3} (min^{-1} or h^{-1})	1.57 h^{-1}	1.57 h^{-1}
$t_{1/2}$	–	26.5 min
t_{mean}	–	38.2
(F) Appearance of internalized receptor at the cell surface	$[R^m] \rightleftarrows [R^s]$	$[R^m] \rightleftarrows [R^s]$
k_m or k_X (min^{-1} or h^{-1}) (k_m)	10.0 h^{-1}	10.0 h^{-1}
$t_{1/2}$ ($t^m_{1/2}$)	4.2 min	4.2 min
t_{mean} (t^m_{mean})	6.0	6.0
(G) Dissociation of ligand from recycled receptor-bound ligand complex	–	–
k_0 (min^{-1} or h^{-1})	–	–
$t_{1/2}$	–	–
t_{mean}	–	–
(H) Degradation of receptor-ligand complex	$[R^mL] \rightarrow D$	$[R^mL] \rightarrow D$
k_d (k_{rd}, k_d^{LDL}) (h^{-1})	0.05	0.05
$t_{1/2}$	13.9 h	13.9 h
t_{mean}	20.0	20.0
(I) Degradation of LDL from $[R^mL]$	$[R^mL] \rightarrow D$	$[R^mL] \rightarrow D$
k_d^{LDL} (h^{-1})	0.91 h^{-1}	0.91 h^{-1}
$t_{1/2}$ (min)	45.7 min	45.7 min
t_{mean}	65.9 min	65.9 min
t_{mean}, time required for a receptor to traverse the entire endocytotic cycle (t_R)	$t_R = [k_2L]^{-1} + [k_L]^{-1} + [k_{-3}]^{-1} + [k_m]^{-1}$ = 52 min $t_R = 12.0$ min [35] $t_R = [k_2L]^{-1} + [k_L]^{-1} + [k_{-m}]^{-1} = 14$ min	

shown in table 1, plots of $[R^s]$, $[R^mL]$, and $[R^sL]$ were generated to see the effect of varying the rate constants.

In the second method, the four differential equations (eq. 1) were solved simultaneously using the fifth-order Runge-Kutta formula [40]. Here Δt, the step-size increment, was set at 0.01 h and the error obtained using $\Delta t/2$ was found to be 1.1×10^{-4}. Values and initial conditions were as follows: $[R^m]_0 = 600$ ng/mg protein, $[R^s]_0 = 1550$ ng/mg protein, at $t = 0$, as taken from the data of Brown and Goldstein [37]. $k_{rd} = 0.1\ h^{-1}$, $k_m = 0.1\ h^{-1}$, $k_{-m} = 10\ h^{-1}$ and LDL = 10 μg/ml, as shown in table 1.

Using eq. 1, and $d[D]/dt = k_d^{LDL}[R^mL]$, the five differential equations were solved simultaneously. The results were then plotted and compared to those from eq. 9.

4. Results

Fig. 2 shows the simulation of cell surface receptor binding and the distribution of endocytotic species in a steady state in human fibroblasts as a function of ^{125}I-LDL concentration. Experimental data for heparin-releasable $[R^sL]$ and heparin-resistant $[R^mL]$ fractions were taken from figs. 1A and B of ref. 37, and the kinetic simulation was based on eqs. 3–5. The global goodness of fit of the R^2 value was 0.9983.

In Brown and Goldstein's experiment, the monolayers were incubated for 5 h at 37°C in the presence of varying concentrations of ^{125}I-LDL. In the kinetic simulation shown in fig. 2, at $t = 0$, $[R^s]_0$ was evaluated to be 1550 ng/mg protein and LDL concentration varied from 0 to 70 nmol, based on Brown and Goldstein's figs. 1A and B. The rate constant for ligand binding, $k_2[L]$, was determined to be 20 h^{-1} and the shuttling constant, k_{-3}, 1.57 h^{-1}; the internalization constant, k_L, was 12 h^{-1} and the degradation constant, k_d, was 0.05 h^{-1}. The rate of synthesis remains constant at 1.98 pmol mg^{-1} protein h^{-1}, equivalent to 6.0 ng mg^{-1} protein h^{-1} [35].

Cell surface receptor binding and macromolecular species distribution of $[R^s]$, $[R^mL]$, and $[R^sL]$ in the same system were simulated as a function of

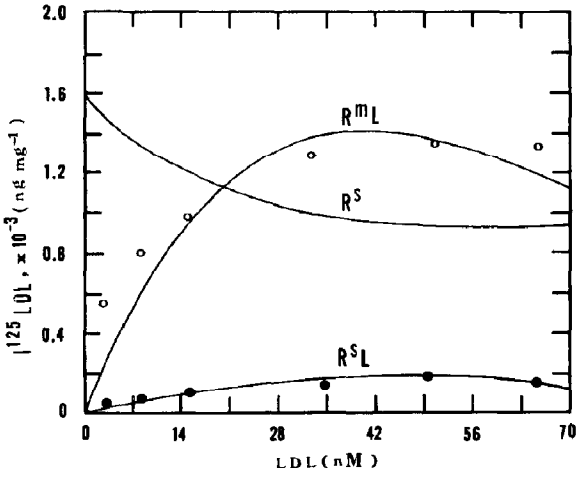

Fig. 2. Cell surface receptor binding and distribution of endocytotic species in a steady state in human fibroblasts as a function of ^{125}IL-LDL concentration. Experimental data were taken from fig. 1A and B of Brown and Goldstein [37]. On day 7 of cell growth, each monolayer was incubated with 0–200 μg protein/ml of ^{125}I-LDL for 5 h at 37°C. Cell surface receptor binding to LDL (R^sL), internalized receptor complex (R^mL), and cell surface free receptor (R^s) in normal human fibroblasts were simulated in a kinetic model based on differential equations (eqs. 3–5). Assuming the initial conditions, $t = 5$ h incubation, $[R^s]_0 = 1550$ ng/mg protein, R = unit activity of LDL binding, LDL concentrations varied from 0 to 70 nmol. $k_2 = 0.08\ h^{-1}\ \mu g^{-1}$ ml; under saturating conditions, [LDL] = 220 μg/ml where $k_2[L] = 20\ h^{-1}$. $k_{-3} = 1.57\ h^{-1}$, $k_d = 0.05\ h^{-1}$, $k_L = 12\ h^{-1}$, $k_1[A] = 6$ ng mg^{-1} protein h^{-1}, and [LDL] = 10 μg/ml. The system reached a steady state within 130 min, at which point the amount of ^{125}I-LDL contained within the cell and the amount of ^{125}I-LDL bound to the receptor remained relatively constant, as did the level of unbound surface receptor. This plot was generated based on eqs. 3–5 and then used to evaluate the rate constants, k_i, where $a = (k_2[L]k_d)/(k_{-3} + k_d)$. (○) Heparin-resistant fraction, (●) surface receptor-bound LDL.

time. The total number of receptors $[R]_T = [R^s] + [R^mL] + [R^sL] + [R^m]$ in which the species $[R^s]$ consists of both newly synthesized and recycled receptors at the cell surface. Within 20 min, the species distribution reached a steady state. Under such steady-state conditions, the time required for a receptor to traverse the entire endocytotic cycle was found to be 52 min, in contrast to the mean time of 12 min reported by Brown et al. [3]. However, if the magnitude of the shuttling constant, k_{-3}, is very large, the traverse time is 14

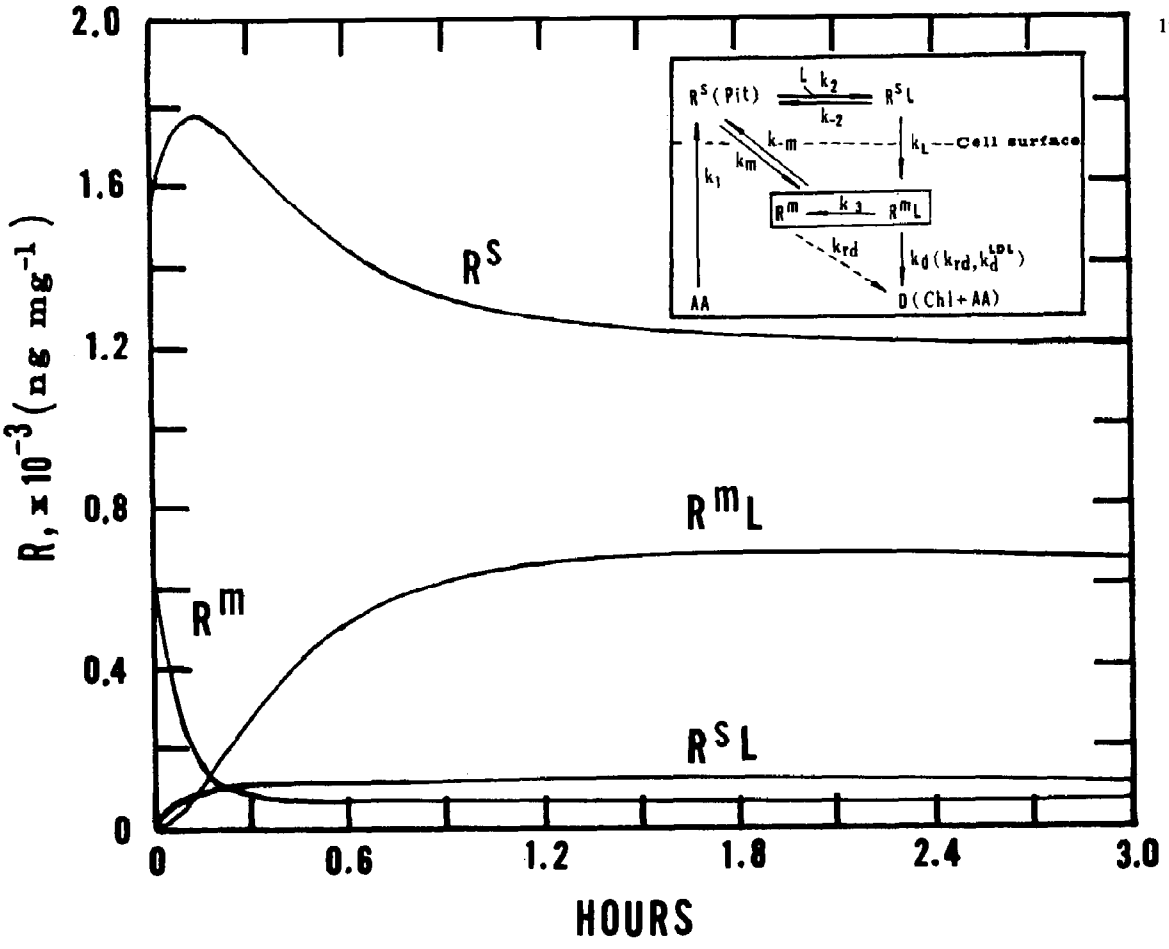

Fig. 3. Macromolecular distribution of the endocytotic species of the LDL-receptor complex in human skin fibroblasts in the steady state at 37°C as a function of time. Kinetic plots shown here were generated by the numerical solution of the four differential equations of eq. 1, where $[R]_T = [R^s]+[R^mL]+[R^sL]+[R^m]$, using the Runge-Kutta approximation method based on the kinetic parameters shown in table 1 and assuming $k_{rd} = 0.1\ h^{-1}$, $k_m = 0.1\ h^{-1}$, $k_{-m} = 10\ h^{-1}$, and [LDL] = 10 μg protein/ml of LDL.

	k_1 [A]	R^m	R^s	R^sL	R^mL
$\frac{d[R^m]}{dt}$	(6.0)	$-(k_{-m}+k_{rd})$	k_m	0	k_{-3}
		$-(10.1)$	(0.1)		(1.57)
$\frac{d[R^s]}{dt}$	0	$+k_m$	$-(k_m+k_2[L])$	0	0
		$+(10.1)$	$-(1.0)$		
$\frac{d[R^sL]}{dt}$	0	0	$k_2 L$	$-k_L$	0
			(0.9)	$-(12.0)$	
$\frac{d[R^mL]}{dt}$ *	0	0	0	k_L	$-(k_{-3}+k_d)$
				(12.0)	$-(1.62)$

* A kinetic plot for R^mL obtained from this simulation is identical within the confidence limits of R^2 to that obtained from Brown and Goldstein's experiment for heparin-resistant ^{125}I-LDL binding to the cell surface receptor (fig. 5c of ref. 37). Within 20 min, the system reaches dynamic equilibrium, such that the amount of ^{125}I-LDL bound to the receptor, R^sL, and the amount of ^{125}I-LDL contained within the cell, R^mL, remain constant, while the level of unbound surface receptor remains high. In the steady state, the time required for a receptor to traverse the entire endocytotic cycle was determined to be 52 min. This is 4-times slower than any previously reported value ($t_R = 12$ min).

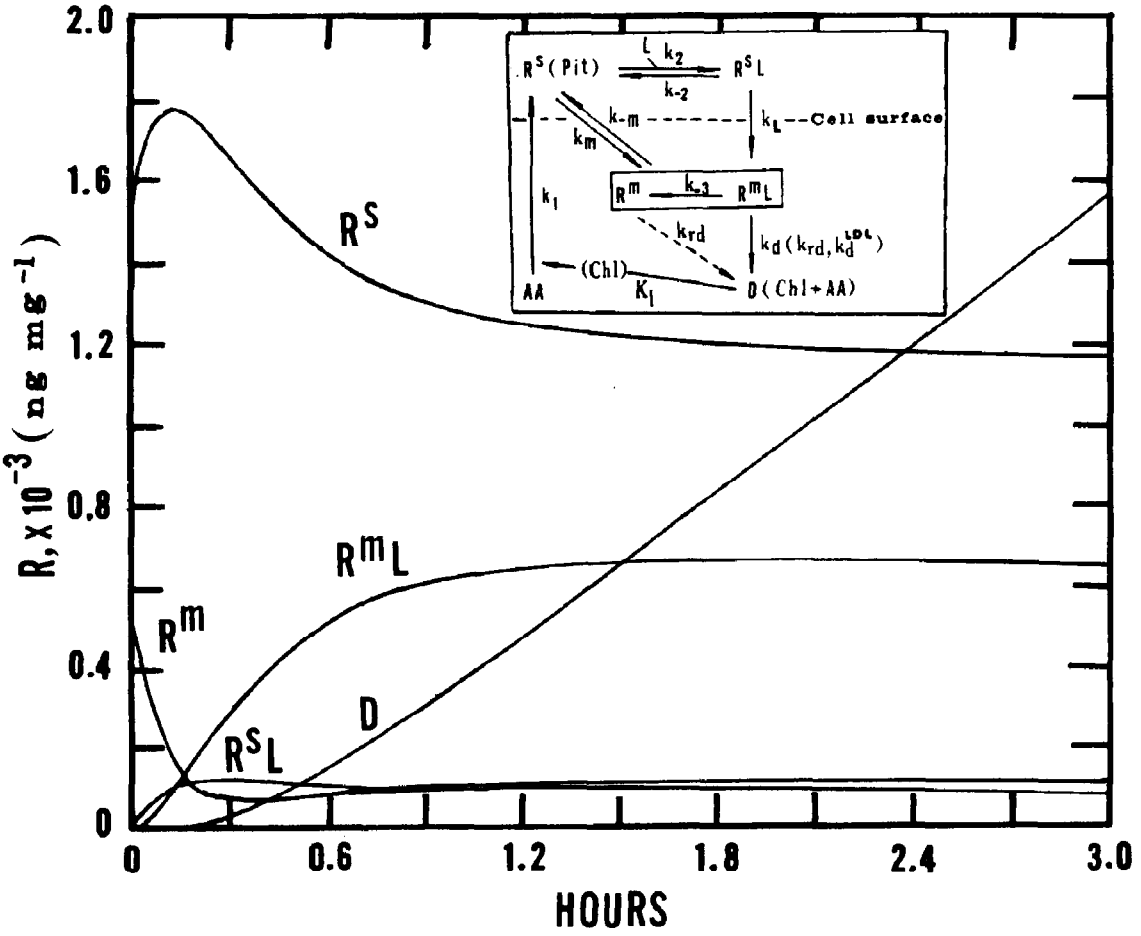

Fig. 4. The effect of cholesterol on the macromolecular species distribution in a steady state in human skin fibroblasts. Kinetic distribution plots were generated by numerical solution of five differential equations (eq. 1) using the Runge-Kutta method. D, concentration of cholesterol. $[R^m]_0 = 600$, $[R^s]_0 = 1550$, $[R^s]_0 = 0$, $[R^mL]_0 = 0$, $[D]_0 = 0$ (ng/mg LDL).

	$k_1[A]$	R^m	R^s	R^sL	R^mL	D
$\frac{d[R^m]}{dt}$	6.0	$-(k_{-m}+k_{rd})$	k_m	0	k_{-3}	0
		$-(10.1)$	0.1		(1.57)	
$\frac{d[R^s]}{dt}$	0	k_m	$-(k_m+k_2[L])$	0	0	0
		(10.0)	$-(1.0)$			
$\frac{d[R^sL]}{dt}$	0	0	$k_2[L]$	$-k_L$	0	0
			(0.9)	$-(12.0)$		
$\frac{d[R^mL]}{dt}$	0	0	0	k_L	$-(k_{-3}+k_d)$	0
				(12.0)	$-(1.62)$	
$\frac{d[D]}{dt}$	0	k_{rd}	0	0	k_d^{LDL}	0
		(0.1)			(0.91)	

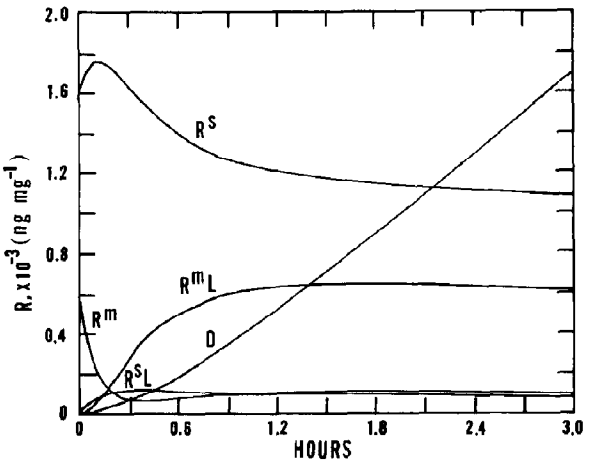

Fig. 5. The effect of cholesterol on the macromolecular species distribution in a steady state in human skin fibroblasts. Kinetic distribution plots were generated by numerical solution of five differential equations using the Runge-Kutta method.

$$\frac{d[R^m]}{dt} = k_1[A] + k_m[R^s] - k_{-m}[R^m] + k_{-3}[R^mL] - k_{rd}[R^m]$$

$$\frac{d[R^s]}{dt} = k_m[R^m] - k_m[R^s] + k_{-2}[R^sL] - k_2[R^s][L]$$

$$\frac{d[R^sL]}{dt} = k_2[R^s][L] - (k_{-2} + k_L)[R^sL]$$

$$\frac{d[R^mL]}{dt} = k_L[R^sL] - (k_{-3} + k_d)[R^mL]$$

$$\frac{d[D]}{dt} = k_d^{LDL}[R^mL] + k_{rd}[R^m]$$

where $k_1[A] = 6.0$ ng mg^{-1} protein h^{-1} and $k_d^{LDL} = 0.91$ h^{-1}, [LDL] = 10 μg/ml. $[R^m]_0 = 600$, $[R^s]_0 = 1550$, $[R^s]_0 = 0$, $[R^mL]_0 = 0$, $[D]_0 = 0$ (ng/mg LDL). Even though the amount of the degradation product, D, increases linearly in the presence of cholesterol, the binding parameters of this system remain the same and the inhibiting effect of cholesterol on the down-regulation of receptor synthesis is too slight to be observed.

	$k_1[A]$	R^m	R^s	R^sL	R^mL	D
$\frac{d[R^m]}{dt}$	6.0	$-(k_{-m} + k_{rd})$ $-(10.5)$	k_m (0.1)	0	k_{-3} (1.57)	0
$\frac{d[R^s]}{dt}$	0	k_{-m} (10.0)	$-(k_m + k_2[L])$ $-(1.0)$	0	0	0
$\frac{d[R^sL]}{dt}$	0	0	$k_2[L]$ (0.9)	$-k_L$ $-(12.0)$	0	0
$\frac{d[R^mL]}{dt}$	0	0	0	k_L (12.0)	$-(k_{-3} + k_d)$ $-(1.62)$	0
$\frac{d[D]}{dt}$	0	k_{rd} (0.05)	0	0	k_d^{LDL} (0.91)	0

min, consistent with reported values [3].

Our simulation suggests that the recycling time is considerably lower under steady-state conditions. Further simulation of the first four differential expressions of eq. 1 permits the analysis of up- and down-regulation of receptor synthesis and recycling in order to determine whether the LDL-mediated decrease in ^{125}I-LDL uptake is indeed due to a decrease in the number of cell surface receptors, as reported by Brown and Goldstein [35,37]. The kinetic plot for [R^mL], the heparin-resistant fraction of ^{125}I-LDL contained within the cell, shown in fig. 3, is similar to Brown and Goldstein's fig. 5C [37].

Furthermore, within 1 h 30 min, the system reaches a dynamic equilibrium, such that the amount of ^{125}I-LDL bound to the receptor, [R^sL], and [R^mL], the amount within the cell, remain constant, while the level of unbound surface receptor, [R^s], remains high and also remains constant. Brown and Goldstein (ref. 37, fig. 5C) reported that the system reached a plateau state in about 3 h.

Considering Brown and Goldstein's findings, one would predict a steady decrease in the level of surface receptor, while in fact this figure shows that the amount of [R^s] quickly levels off at approx. 1300 ng/mg protein. Our simulation suggests that the total amount of surface receptor remains relatively unchanged even though there is an accumulation of cholesterol in the system. The peak in the curve at the onset of this experiment may be due to the washing of the skin fibroblast monolayers, which may cause a rapid translocation of intracellular membrane receptors, [R^m], to the cell surface, as is further evident in the exponential decrease in [R^m] as a function of time. Similar peaks are observed in several of the experimental plots shown by Brown and Goldstein [35].

To what extent, then, does the accumulation of cholesterol affect the regulation of cell surface receptor in this system? The influence of the rate of degradation, and resultant cholesterol accumulation, was simulated based on eq. 6.

The kinetic plot of fig. 3 and the macromolecular distribution of the endocytotic species were compared with the same system, considering the influence of the degradation of LDL and resultant build-up of cholesterol. The effect of cholesterol on the macromolecular species distribution in the steady state, determined from all five differential expressions of eq. 1, is shown in fig. 4.

Simulation shows that even though the amount of the degradation product increases linearly in the presence of cholesterol, the binding parameters of the system remain the same and the inhibitory effect of cholesterol on the down-regulation of receptor synthesis is too slight to be observed in comparing fig. 3 with figs. 4 and 5. In a system in which cholesterol does influence down-regulation, we would expect to see a non-linear increase in the amount of degradation product (see appendix, eq. A13). Indeed, fig. 1C of Brown and Goldstein [37], shows a non-linear increase in LDL degradation with time for normal cells.

5. Discussion

As previously indicated by Brown and Goldstein [37], the receptor-mediated uptake of LDL in cultured cells can be separated into discrete steps. Several mutations which affect some of these steps have been reported; these include receptor-negative, receptor-defective, and internalization-defective mutations. Judicious use of these mutant cells in culture has led to the conclusion that the cholesterol liberated from lysosomal degradation of LDL suppresses the number of cell surface receptors. Presumably, this occurs through inhibition of receptor protein synthesis [35]. Obviously, regulation of surface receptor activity is an important control point in the overall regulation of intracellular cholesterol content.

Kinetic data, derived from the literature, were used in a computer simulation to generate the macromolecular species distribution of the LDL receptor in normal cells under several conditions. In both time-dependent and concentration-dependent studies (for experiments of 5 h duration), the free surface receptor [R^s] quickly reached a steady-state level, but its concentration remained high (figs. 2–5). This would not be expected under conditions of inhibition of receptor synthesis. An increase in the concentration of internalized receptor-LDL complex to a steady-state level was also a

feature (figs. 2–5). It is suggested that a rapid equilibrium occurs between the surface receptor [R^s] and the intracellular receptor [R^m]. In the proposed model for receptor recycling, when the shuttling constant, k_{-3}, is much smaller than the translocation constant, k_{-m}, the traverse time for the receptor is 52 min. Under these conditions, it appears that the release of the receptor from the internalized LDL-receptor complex is slow. This may be one effect of washing the cells and could also be an indicator of the build-up of an intracellular receptor pool. It was not possible to differentiate between cell surface receptor from which bound LDL may have been washed away and receptor newly arrived at the surface after translocation from within the cell.

Under conditions such that k_{-3} is large, i.e., in non-steady-state conditions, the internalized receptor is shuttled back to the surface with a traverse time of only 14 min, suggesting that the receptor is readily released from the internalized LDL-receptor complex and rapidly shuttled to the cell surface (see appendix B).

These kinetic simulations suggest that normal LDL binding in an experiment of short duration may be independent of receptor synthesis and regulation. Our multi-step, irreversible ordered sequence model for the recycling of LDL receptors offers a reasonable explanation for the down-regulation of cell receptors in cultured fibroblasts under steady-state conditions. Simulations based on reported data indicate that the degradation of LDL and resultant release of cholesterol have little or no apparent inhibitory effect on receptor synthesis and regulation in an experiment of only 5 h duration. It has been shown that preincubation with oxycholesterol as well as 25-hydroxycholesterol markedly decreases binding and uptake of ^{125}I-LDL. This effect is greater than that observed with LDL alone or cholesterol alone [35]. Since our results suggest that the accumulation of free cholesterol does not suppress receptor synthesis, it is apparent that further experiments should take into consideration the presence of free cholesterol, oxycholesterol, and 25-hydroxycholesterol. Such studies may help determine whether oxycholesterol may be the factor which suppresses receptor synthesis and regulation. The relative amounts of each of these compounds should be quantified in LDL samples when attempting to assess with any degree of accuracy the effect of cholesterol on receptor synthesis and regulation.

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