Journal of Supramolecular Structure 8:223–234 (1978)
Transmembrane Signaling 1–12

Inhibition of the Binding of Low-Density Lipoprotein to Its Cell Surface Receptor in Human Fibroblasts by Positively Charged Proteins

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A group of proteins and polyamino acids with positively charged domains were shown to inhibit the binding of ^{125}I-LDL to its receptor on the surface of human fibroblasts. The list of inhibitory proteins included platelet factor 4 (which has a cluster of lysine residues at its carboxyl terminus), two lysine-rich histones, poly-L-lysines of chain length greater than 4, and protamine. These proteins were effective in the concentration range of 5–50 μg/ml. Two other positively charged proteins, lysozyme and avidin, did not inhibit ^{125}I-LDL binding. Kinetic studies suggested that protamine was not acting simply as a competitive inhibitor with regard to the LDL receptor. In light of previous data showing that polyanions such as heparin and polyphosphates also inhibit ^{125}I-LDL binding to its cell surface receptor, the current findings suggest that charge interactions are important in this binding reaction. In a related series of studies, a number of glycoproteins and their asialo derivatives as well as a number of sugar phosphates failed to inhibit ^{125}I-LDL binding to its receptor in fibroblasts.

Key words: low-density lipoprotein, cell surface receptor, fibroblasts, platelet factor 4, histones, protamine, poly-L-lysine, glycoproteins, cholesterol

Cultured human fibroblasts obtain the cholesterol required for structural and regulatory purposes by means of a cell surface receptor. This receptor binds the major cholesterol-carrying lipoprotein in human plasma, low-density lipoprotein [1–3]. The receptor-bound LDL is internalized by adsorptive endocytosis and carried to cellular lysosomes where its protein and cholesteryl ester components are hydrolyzed. The

Abbreviations: FH–familial hypercholesterolemia; HMG CoA reductase–3-hydroxy-3-methyl-glutaryl Coenzyme A reductase; LDL–low-density lipoprotein.

Received for publication March 29, 1978; accepted April 28, 1978.

0091-7419/78/0803-0223$02.30

liberated cholesterol is used for cell membrane synthesis. This cholesterol also regulates three metabolic events. First, it suppresses the activity of 3-hydroxy-3-methylglutaryl Coenzyme A reductase (HMG CoA reductase), thereby suppressing cholesterol synthesis within the cell. Second, it activates an acyl Coenzyme A: cholesterol acyltransferase, producing a reesterification of the LDL-derived cholesterol. Third, it suppresses the synthesis of the LDL receptor itself, thereby preventing an overaccumulation of cholesterol by the cell [1–3].

Previous studies have suggested that the binding of LDL to the receptor involves an ionic interaction between positively charged amino acids of the apoprotein B component of the lipoprotein and negatively charged groups on the receptor [4]. Thus, binding of ^{125}I-LDL to the cell surface of fibroblasts can be inhibited by compounds with regions of multiple negative charges, such as heparin, dextran sulfate, and polyphosphates [2, 4]. Such polyanions are known to bind to positively charged groups on the protein component of LDL [5]. Moreover, acetylation of the positively charged free amino groups of LDL has been shown to prevent the binding of the lipoprotein to the fibroblast receptor [6]. Similarly, selective modification of either the arginine residues of LDL-protein with cyclohexanedione [7] or the lysine residues of LDL-protein with diketene (Mahley, RW, personal communication) also destroys the ability of the lipoprotein to bind to the receptor. Finally, animal and human lipoproteins that contain an apoprotein that is rich in arginine residues, termed the arginine-rich protein or apoprotein E, can also bind to the LDL receptor in human fibroblasts and can be taken up and hydrolyzed through the LDL receptor pathway [8, 9].

The current studies were undertaken to determine whether other proteins or polyamino acids that have regions of strong positive charge could inhibit the binding of ^{125}I-LDL to the LDL receptor. The most detailed studies were carried out with human platelet factor 4. Like LDL, this 7,800-dalton protein binds avidly to heparin [10–13]. Recently, the carboxyl terminal region of platelet factor 4 was shown to contain a sequence in which pairs of lysine residues are separated by pairs of hydrophobic residues (either isoleucine or leucine residues) [12, 13]. Clusters of positively charged amino acids also occur at two other sites along the peptide chain of platelet factor 4. In designing the current studies, we reasoned that the cluster of positively charged residues in platelet factor 4 which are believed to be the site of its interaction with heparin [12, 13] might allow platelet factor 4 to inhibit the binding of ^{125}I-LDL to the LDL receptor. In addition, we have studied a variety of other molecules that contain domains of strong positive charge, such as histones, synthetic polylysines of various chain lengths, and protamine. The results indicate that each of these compounds is able to inhibit the binding of ^{125}I-LDL to the LDL receptor.

MATERIALS AND METHODS

Materials

L-Lysine, poly-L-lysine (Type IV, mol wt 4,000–15,000), poly-L-lysine (Type I-B, mol wt > 70,000), succinylated poly-L-lysine (Type I-B, mol wt > 50,000), poly-L-glutamic acid (Type II-B, mol wt 15,000–50,000), histone f_1 (Type V-S, calf thymus), histone f_{2b} (Type VII-S, calf thymus), thyroglobulin (Type I, bovine), ceruloplasmin (Type III, human), transferrin (Grade II, human), invertase (Grade VI, Baker's yeast), mannan (Baker's yeast), sugar phosphates, avidin, lysozyme, and protamine chloride (Grade V, salmon

sperm) were purchased from Sigma Chemical Co, $(L\text{-lysine})_4$ and $(L\text{-lysine})_{8\text{-}12}$ were obtained from Miles Laboratories, Inc; α-1-antitrypsin (human) was purchased from Worthington Biochemical Corp; ovomucoid and fetuin were obtained from Grand Island Biological Co; and ovalbumin was obtained from Pharmacia. Asialo-ovomucoid, asialo-α-1-antitrypsin, and asialo-fetuin were prepared by acid hydrolysis at 80° as described by Stockert, Morell, and Steinberg [14]. Human platelet factor 4 was isolated and purified to homogeneity as previously described [12]. The purified platelet factor 4 preparation was kept at 4° in a 1 M NaCl solution at a protein concentration of 1 mg/ml. ^{125}I-labeled platelet factor 4 was prepared by a modification of the method of Handin and Cohen [11]. Other chemicals and tissue culture materials were obtained from sources as previously reported [4].

Lipoproteins

Human LDL (density 1.019–1.063 g/ml) and lipoprotein-deficient serum (density > 1.215 g/ml) were obtained from the plasma of individual healthy subjects and prepared by differential ultracentrifugation [15]. The concentration of LDL is expressed in terms of its protein content. ^{125}I-labeled LDL was prepared as previously described [16].

Cells

Human fibroblasts from normal subjects and from a patient with the receptor-negative form of familial hypercholesterolemia were grown in monolayers as previously described [4]. All experiments were performed using a standard format. Confluent monolayers of cells from stock flasks were dissociated with 0.05% trypsin–0.02% ethylenediaminetetraacetic acid solution. On day 0, a total of 8×10^4 fibroblasts were seeded into each 60-mm Petri dish containing 3 ml of growth medium [4] with 10% (v/v) fetal calf serum. On day 3, the medium was replaced with 3 ml of fresh growth medium containing 10% fetal calf serum. On day 5, when the cells were in late logarithmic growth, each monolayer was washed with 3 ml of Dulbecco's phosphate-buffered saline, after which 2 ml of medium A (growth medium containing 10% lipoprotein-deficient serum) was added. All experiments were initiated on day 7 after the cells had been incubated with lipoprotein-deficient serum for 48 h.

Assays

The amounts of surface-bound ^{125}I-LDL (dextran sulfate-releasable ^{125}I-LDL), intracellular ^{125}I-LDL (dextran sulfate-resistant ^{125}I-LDL), and degraded ^{125}I-LDL were measured in intact fibroblast monolayers by previously described methods [4, 17]. Similar methods were used for measurement of the total binding (surface-bound plus intracellular) and degradation of ^{125}I-platelet factor 4 by fibroblast monolayers. The incorporation of [^{14}C]oleate into cholesteryl [^{14}C]esters by intact fibroblast monolayers [18] and the activity of HMG CoA reductase in detergent-solubilized extracts of fibroblasts [15] were determined by the referenced methods. The protein content of extracts and whole cells was determined by the method of Lowry, et al [19] with bovine serum albumin as a standard.

RESULTS

Figure 1 shows that increasing concentrations of highly purified platelet factor 4 were able to inhibit the binding of ^{125}I-LDL to the cell surface LDL receptor in intact

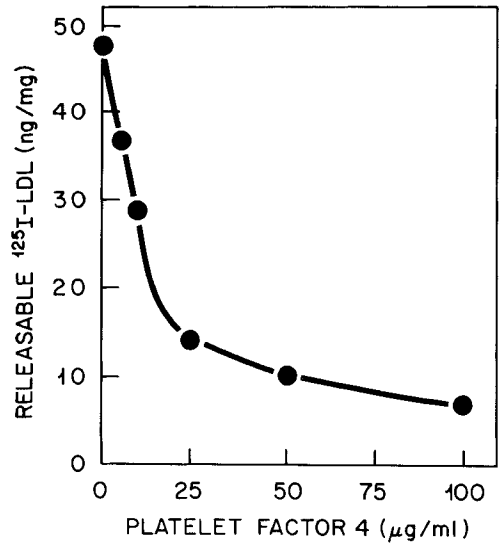

Fig 1. Inhibition of the binding of ^{125}I-LDL to monolayers of fibroblasts at 4° by platelet factor 4. On day 7 of cell growth, each monolayer received 2 ml of ice-cold medium A, 0.2 ml of 1 M NaCl containing the indicated concentration of platelet factor 4, and 5 μg protein per milliliter of ^{125}I-LDL (207 cpm/ng). After incubation at 4° for 2 h, the cells were washed extensively [4] and the amount of dextran sulfate-releasable ^{125}I-LDL was determined.

fibroblasts at 4°. When tested against ^{125}I-LDL at 5 μg/ml, platelet factor 4 at a concentration of 10 μg/ml was able to inhibit the receptor binding by 50%.

Platelet factor 4 also inhibited the binding of ^{125}I-LDL to the receptor at 37° (Fig 2A). The inhibition of surface binding by platelet factor 4 led to a reduction in the rate of internalization and degradation of ^{125}I-LDL by fibroblasts (Figs 2B and 2C). When compared on a weight basis, platelet factor 4 and unlabeled LDL were approximately equally effective in inhibiting ^{125}I-LDL for receptor binding. However, considering that the molecular weight of platelet factor 4 is 7,800 [12] and the molecular weight of LDL-protein is approximately 500,000 [20], it is clear that inhibition by platelet factor 4 required a higher molar concentration of protein than did unlabeled LDL.

As expected from its ability to inhibit the binding, uptake, and degradation of ^{125}I-LDL, platelet factor 4 also prevented the stimulation of cholesterol esterification that ordinarily occurs when LDL is added to fibroblasts. Thus, as shown in Figure 3, platelet factor 4 at a concentration of 10 μg/ml produced nearly complete inhibition of the incorporation of [^{14}C]oleate into cholesteryl [^{14}C]oleate by fibroblasts that had been incubated with 10 μg/ml of LDL. Platelet factor 4 also prevented the LDL-mediated suppression of HMG CoA reductase activity (Table I).

In an attempt to determine whether platelet factor 4 binds to the LDL receptor, we labeled the protein with ^{125}I. Table II shows that when this ^{125}I-platelet factor 4 was incubated with fibroblasts for 5 h at 37° a relatively large percentage of the material (10–15%) adhered either to the cells or to other material in the culture dish despite vigorous washing (designated as "bound" radioactivity in Table II). A smaller amount of the protein (1–2% of total amount added to medium) was degraded to trichloroacetic acid-soluble material and excreted into the culture medium in a 5-h interval. Neither the amount bound nor the amount degraded was significantly reduced in the presence of a large excess of native LDL,

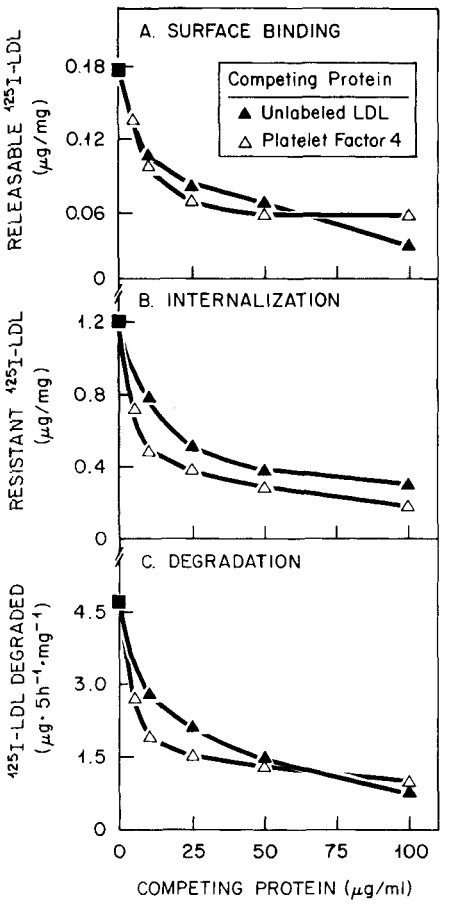

Fig 2. Inhibition of ^{125}I-LDL metabolism in monolayers of fibroblasts by platelet factor 4. On day 7 of cell growth, each monolayer received 2 ml of medium A, 0.2 ml of 1 M NaCl containing the indicated concentration of either unlabeled LDL (▲) or platelet factor 4 (△), and 25 μg protein/ml of ^{125}I-LDL (98 cpm/ng). After incubation for 5 h at 37°, the medium was removed and its content of ^{125}I-labeled trichloroacetic acid-soluble (noniodide) material (C) was measured. The cell monolayers were then washed extensively [4], after which the amounts of dextran sulfate-releasable ^{125}I-LDL (A) and dextran sulfate-resistant ^{125}I-LDL (B) were determined.

TABLE I. Prevention of LDL-Mediated Suppression of HMG CoA Reductase Activity by Platelet Factor 4

Addition to medium	HMG CoA reductase activity (pmoles·min^{-1}·mg protein^{-1})
None	138
LDL (5 μg/ml)	27
Platelet factor 4 (40 μg/ml)	169
LDL + platelet factor 4	121

On day 7 of cell growth, each monolayer of fibroblasts received 2 ml of medium A and 0.1 ml of 1 M NaCl containing the indicated addition. After incubation for 6 h at 37°, the cells were harvested for measurement of HMG CoA reductase activity. Each value represents the average of duplicate incubations.

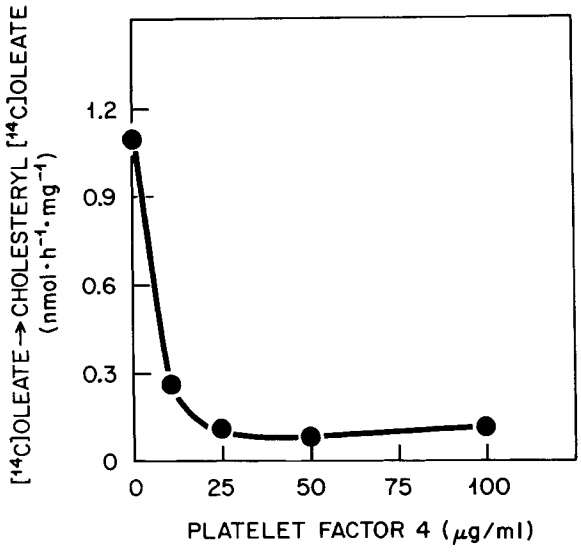

Fig 3. Inhibition of the LDL-mediated stimulation of cholesteryl ester formation in monolayers of fibroblasts by platelet factor 4. On day 7 of cell growth, each monolayer received 2 ml of medium A, 10 μg protein per milliliter of unlabeled LDL, and 0.2 ml of 1 M NaCl containing the indicated concentration of platelet factor 4. After incubation for 5 h at 37°, the cells were pulsed-labeled for 2 h at 37° with 0.1 mM [^{14}C]oleate-albumin (13,200 cpm/nmole), after which the cellular content of cholesteryl [^{14}C]oleate was determined. The amount of cholesteryl [^{14}C]oleate formed in parallel monolayers to which no LDL and no platelet factor 4 were added was 0.03 nmol·h^{1}·mg protein^{1}.

TABLE II. Binding and Degradation of ^{125}I-Platelet Factor 4 and ^{125}I-LDL in Normal and Familial Hypercholesterolemia (FH) Homozygote Fibroblasts at 37°

	^{125}I-platelet factor 4 (ng·5h^{-1}·mg^{-1})					^{125}I-LDL (ng·5h^{-1}·mg^{-1})				
					Degraded					Degraded
Cell strain	Bound		Degraded		Bound	Bound		Degraded		Bound
	− LDL (a)	+ LDL	− LDL (b)	+ LDL	(b/a)	− LDL (a)	+ LDL	− LDL (b)	+ LDL	(b/a)
Normal	29	24	4.5	2.8	0.16	1120	71	4000	300	3.6
FH homozygote	41	37	3.2	2.7	0.07	34	11	170	174	–

On day 7 of cell growth, each monolayer received 2 ml of medium A and either 32 ng/ml of ^{125}I-platelet factor 4 (30,000 cpm/ng) or 10 μg protein/ml of ^{125}I-LDL (261 cpm/ng) in the absence or presence of 480 μg protein/ml of unlabeled LDL as indicated. After incubation for 5 h at 37°, the medium was removed and its content of ^{125}I-labeled trichloroacetic acid-soluble (noniodide) material was measured. The cell monolayers were then washed extensively [4], after which the total amount of ^{125}I-platelet factor 4 or ^{125}I-LDL bound to cells (surface-bound + intracellular) was determined as previously described [16]. Each value represents the average of duplicate incubations. The values for degradation of ^{125}I-platelet factor 4 and ^{125}I-LDL represent the cell-dependent rate of proteolysis, ie, the difference between values obtained in the presence and absence of cells [16]. The content of total cellular protein averaged 250 μg/dish.

indicating that the LDL receptors did not constitute an appreciable fraction of the binding sites for platelet factor 4. This conclusion was further supported by the observation that the binding and degradation of ^{125}I-platelet factor 4 were similar in cells from a normal subject and cells from a patient with homozygous familial hypercholesterolemia that have a nearly total deficiency of LDL receptors (Table II).

The data for ^{125}I-platelet factor 4 binding and degradation resemble those previously obtained with ^{125}I-LDL that had been rendered cationic by coupling with N,N-dimethyl-1, 3-propanediamine [21]. With both of these cationic proteins a large proportion of the protein binds to the cell, and a small fraction of the bound material is degraded (compare ratios of degradation/binding for ^{125}I-platelet factor 4 and ^{125}I-LDL in Table II). Despite the relative inefficiency of the coupling process for binding and degradation for platelet factor 4, the amount degraded is larger than can be explained by bulk fluid endocytosis. Thus, a small fraction of the bound material appears to enter the cell by adsorptive endocytosis.

The data in Figure 4 demonstrate that two other positively charged proteins, histone f_1 and histone f_{2b}, were able to inhibit ^{125}I-LDL binding to the LDL receptor at 4°. However, not all positively charged proteins were effective inhibitors. As shown in Figure 5 two equally positively charged protiens, egg white lysozyme and avidin, did not inhibit ^{125}I-LDL binding at concentrations as high as 250 μg/ml. On the other hand, protamine, which is known to bind tightly to heparin, was as effective as unlabeled LDL in inhibiting ^{125}I-LDL binding to the LDL receptor site. These data suggested that some configuration

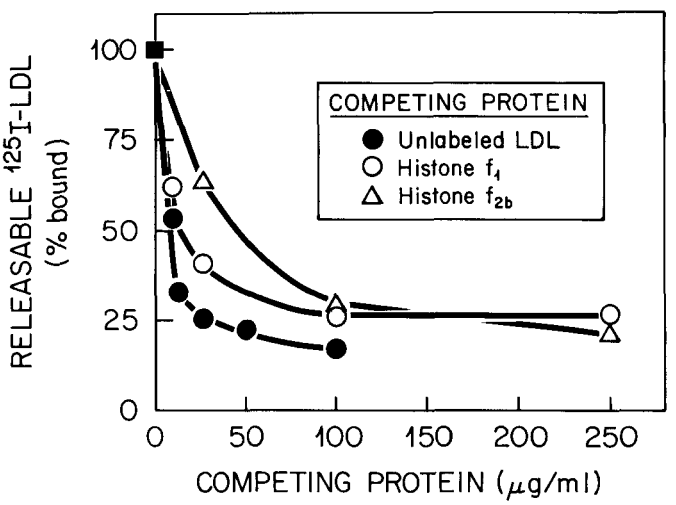

Fig 4. Inhibition of the binding of ^{125}I-LDL to monolayers of fibroblasts at 4° by histones. On day 7 of cell growth, each monolayer received 2 ml of ice-cold medium A, the indicated concentration of the indicated competing protein, and 5 μg protein per milliliter of ^{125}I-LDL (495 cpm/ng). After incubation for 2 h at 4°, the monolayers were washed extensively and the amount of dextran sulfate-releasable ^{125}I-LDL was determined. The 100% value for ^{125}I-LDL bound in the absence of any competing protein (■) was 41 ng/mg.

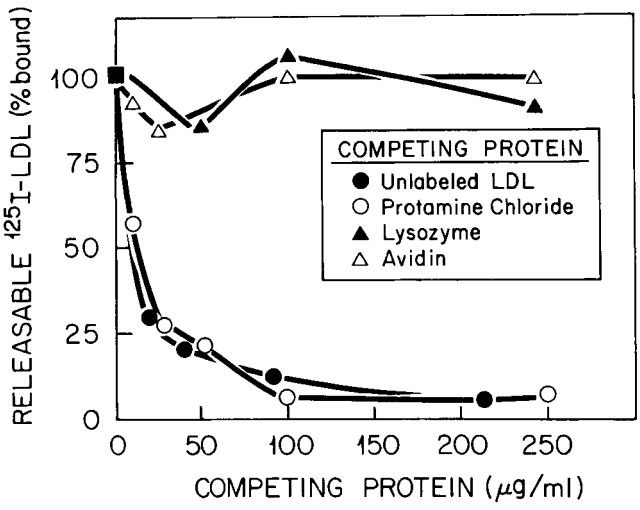

Fig 5. Effects of positively charged proteins on the binding of ^{125}I-LDL to monolayers of fibroblasts at 4°. On day 7 of cell growth, each monolayer received 2 ml of ice-cold medium A, the indicated concentration of the indicated competing protein, and 10 μg protein per milliliter of ^{125}I-LDL (479 cpm/ng). After incubation for 2 h at 4°, the monolayers were extensively washed and the amount of dextran sulfate-releasable ^{125}I-LDL was determined. The 100% value for ^{125}I-LDL bound in the absence of any competing protein (■) was 68 ng/mg.

of positive charges was necessary to achieve inhibition of binding of ^{125}I-LDL to the LDL receptor.

To assess whether the positively charged proteins were inhibiting ^{125}I-LDL binding by competing with the lipoprotein for binding to the LDL receptor, we tested the ability of protamine to inhibit ^{125}I-LDL binding in the presence of two different concentrations of ^{125}I-LDL. The data in Figure 6 show that the same concentration of protamine (7 μg/ml) was required to achieve 50% inhibition of binding when ^{125}I-LDL was present at 2 μg/ml (a nonsaturating level) or 10 μg/ml (a saturating level). This observation suggests that protamine was not simply competing directly with ^{125}I-LDL for receptor binding. If protamine were competing directly with ^{125}I-LDL for the same receptor site in a reversible fashion, a higher concentration of protamine would be required to inhibit binding by 50% when the lipoprotein was present at the higher levels as opposed to the lower level. In other experiments not shown, we have demonstrated that the protamine effect is, however, readily reversible. Thus, prior incubation of the cells with protamine, followed by washing to remove unbound protamine, did not cause inhibition of binding when ^{125}I-LDL was added subsequently

To pursue the putative configurational requirement of positively charged molecules further, we tested the ability of poly-L-lysine chains of varying lengths to inhibit ^{125}I-LDL binding. The data in Figure 7A demonstrate that L-lysine itself did not inhibit ^{125}I-LDL binding when added at concentrations as high as 250 μg/ml. Furthermore, a poly-L-lysine preparation consisting of chains of four lysine residues was relatively weak in its ability to inhibit. On the other hand, a poly-L-lysine preparation of chain length 8–12 residues showed 50% inhibition at a concentration of 30 μg/ml, and a poly-L-lysine preparation of 25–96 residues showed 50% inhibition at a concentration of 10 μg/ml (Fig 7A). As expected from the preceeding data, succinylation of the lysine residues of poly-L-lysine

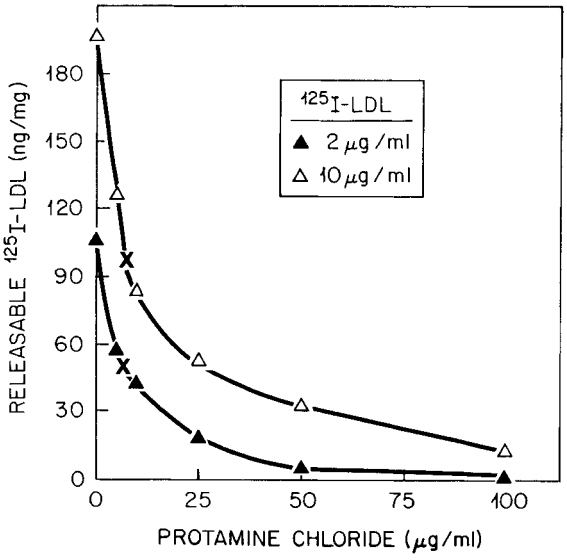

Fig 6. Inhibition of the binding of ^{125}I-LDL to monolayers of fibroblasts at 4° by varying concentrations of protamine. On day 7 of cell growth, each monolayer received 2 ml of ice-cold medium A, the indicated concentration of protamine chloride, and either 2 μg protein per milliliter (▲) or 10 μg protein per milliliter (△) of ^{125}I-LDL (201 cpm/ng). After incubation for 2 h at 4°, the monolayers were washed extensive and the amount of dextran sulfate-releasable ^{125}I-LDL was determined. Each value represents the average of duplicate incubations. The symbol X shows the point of 50% inhibition of binding for each concentration of ^{125}I-LDL.

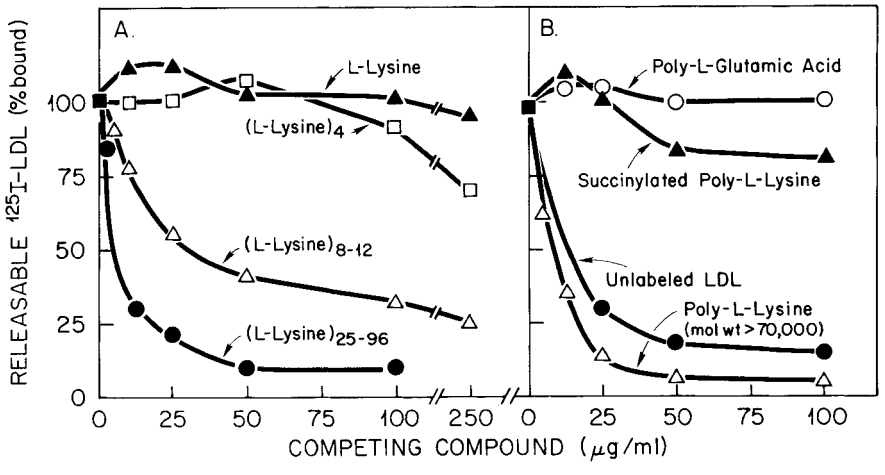

Fig 7. Ability of poly-L-lysines of varying chain length to inhibit the binding of ^{125}I-LDL to monolayers of fibroblasts at 4°. On day 7 of cell growth, each monolayer received 2 ml of ice-cold medium A, the indicated concentration of one of the indicated compounds, and 10 μg protein per milliliter of ^{125}I-LDL (400 cpm/ng). After incubation for 2 h at 4°, the monolayers were washed extensively and the amount o dextran sulfate-releasable ^{125}I-LDL was determined. The 100% values for ^{125}I-LDL bound in the absence of any competing protein (■) were 64 and 44 ng/mg in Experiments A and B, respectively.

blocked the ability of this compound to inhibit ^{125}I-LDL binding (Fig 7B). Moreover, poly-L-glutamic acid, a strontly negatively charged polyamino acid, did not inhibit ^{125}I-LDL binding.

In addition to the above highly charged compounds, we tested the following glycoproteins for their ability to inhibit ^{125}I-LDL binding: thyroglobulin, ceruloplasmin, transferrin, invertase, yeast mannan, ovalbumin, fetuin, α-1-antitrypsin, and ovomucoid. None of these substances caused significant inhibition of ^{125}I-LDL binding, even when added at concentrations in the range of 2–5 mg/ml. The removal of the terminal sialic acid residue from fetuin, α-1-antitrypsin, and ovomucoid did not result in inhibition of ^{125}I-LDL binding, suggesting that the asialoglycoprotein receptor [22] is not involved in the LDL uptake process.

Lysosomal enzymes have been shown to enter fibroblasts through a specific binding reaction in which the cell surface receptors recognize certain sugar phosphates that are attached to the lysosomal enzymes [23, 24]. The following sugar phosphates have been tested for their ability to inhibit ^{125}I-LDL binding: D-mannose-6-phosphate, D-fructose-6-phosphate, D-glucose-6-phosphate, D-galactose-6-phosphate, and D-fructose-1-phosphate. When added at concentrations as high as 10 mM, none of these compounds inhibited the LDL binding reaction. Thus, the LDL receptor appears distinct from the lysosomal enzyme receptor.

Finally, in view of the sensitivity of the LDL binding reaction to positively charged molecules, we tested the ability of three polyamines to inhibit ^{125}I-LDL binding. Spermine, putrescine, and spermidine did not inhibit the binding reaction at concentrations as high as 1 mM.

DISCUSSION

The data in the current paper demonstrate that certain proteins with positively-charged domains inhibit the binding of ^{125}I-LDL to its receptor on the surface of human fibroblasts. The list of inhibitory proteins includes platelet factor 4 (which has a cluster of lysine residues at its carboxyl terminus), two lysine-rich histones (f_1 and f_{2b}), and protamine. Two other proteins that also have strong positive charges – egg white lysozyme and avidin – failed to inhibit ^{125}I-LDL binding. The structural requirements for inhibition were studied with the aid of a series of lysine polymers of varying chain length. The data showed that a minimal chain length of five lysine residues was necessary in order to achieve potent inhibition of ^{125}I-LDL binding to its receptor.

While the phenomenon of inhibition of ^{125}I-LDL binding by certain cationic compounds is clear from these studies, the mechanisms underlying this effect remain unresolved. All of the cationic substances tested are known to bind nonselectively to proteins and other materials with negative charges. They inhibit many reactions in which proteins are involved. In the current circumstance, these polycations might be acting by binding to the LDL receptor, to some adjacent negatively charged structure on the cell surface, to the LDL particle itself, or to some combination of the above. An extensive literature indicates that LDL has a specific ability to bind to polyanionic substances, including polyphosphates, heparin, and other sulfated glycosaminoglycans [5]. Since all of the cationic proteins that inhibited ^{125}I-LDL binding also bind to heparin and other glycosaminoglycans, the simplest interpretation of the current data is that the LDL receptor resembles these glycosaminoglycans in possessing regions of strong negative charge and

that these anionic regions also bind cationic proteins. It is of interest that LDL binds to heparin and other negatively charged substances at neutral pH even though the net charge on the lipoprotein is also negative (the isoelectric point for LDL is 5.28 [25]). Thus, in order to bind to heparin (and perhaps to the LDL receptor) LDL must contain a strongly positively charged domain at some site in its apoprotein B moiety. This situation may be analogous to that of platelet factor 4, which has an isoelectric point of 7.6 [11] but in which the positive charges are segregated at the carboxyl terminus [12, 13]. Whether there is any specific significance to the string of di-lysine residues spaced by pairs of hydrophobic amino acids at the carboxyl terminus of platelet factor 4 is unknown. It would be interesting if apoprotein B of LDL had similar sequences; however, the amino acid sequence of LDL protein is not yet known.

It also remains possible that the positively charged proteins that inhibit LDL binding may not bind to the receptor site itself, but rather they might bind to adjacent anionic sites, thereby covering up the LDL receptor site through steric hindrance. This latter explanation is given credence by previous observations that positively charged proteins, such as polycationic ferritin, bind at widespread nonspecific sites over the entire surface of fibroblasts at neutral pH [21]. Indeed, in the current studies ^{125}I-platelet factor 4 was shown to bind to fibroblasts in large amounts. The experiment in Table II was performed at a low concentration of ^{125}I-platelet factor 4 (32 ng/ml). In other experiments we have shown that the amount of binding and degradation of ^{125}I-platelet factor 4 increases linearly with platelet factor 4 concentrations up to at least 5 μg/ml, the concentration range necessary to inhibit LDL binding. At this concentration of ^{125}I-platelet factor 4, approximately 500 times as many nanomoles of platelet factor 4 are bound in each dish as compared with the amount of ^{125}I-LDL-protein. It is clear that the vast majority of these binding sites did not represent the LDL receptor, since the binding was similar in normal and familial hypercholesterolemia homozygote cells and since LDL did not compete for the binding. The large amount of binding to other sites prevented us from determining whether platelet factor 4 does bind to the LDL receptor and whether it is taken up and degraded through coated vesicles as is LDL.

Whether the cationic proteins bind to the LDL receptor or to an adjacent site, the binding must be readily reversible, in contrast to the nearly irreversible binding of LDL [2, 3]. This follows from our observation that prior incubation of fibroblasts with protamine or platelet factor 4, followed by washing of the cells before the addition of ^{125}I-LDL, does not lead to any inhibition of ^{125}I-LDL binding (data not shown).

The implications of the current studies for the physiology of the LDL receptor are not yet clear. An extensive series of previous studies has demonstrated that the primary function of this receptor is to bind LDL [1–3]. Thus, the number of receptors in fibroblasts and freshly isolated lymphocytes is strictly regulated by the cholesterol content of the cell, the receptor number increasing sharply when the cells are in need of cholesterol [1–3, 26]. Binding of LDL to the receptor leads to the uptake of LDL, thereby providing cholesterol to the cell. The LDL-derived cholesterol in turn regulates cholesterol metabolism both in cultured cells and in freshly isolated blood cells. Most importantly, when the LDL receptor is genetically nonfunctional as in the human disorder familial hypercholesterolemia, the efficiency of LDL catabolism in the body is reduced so that the lipoprotein accumulates to massive levels in the plasma [26].

It is possible that the ability of positively charged proteins to inhibit the binding of LDL to the receptor might be important in certain pathologic situations. In this regard, the studies with platelet factor 4 are particularly intriguing. This protein is released from

platelets during the clotting reaction. The mean level of platelet factor 4 in fresh anticoagulated human plasma as measured by radioimmunoassay was 4.7 ng/ml [27]. On the other hand, when whole blood was allowed to clot, the mean level of platelet factor 4 rose to 13.2 μg/ml [27]. Although this level is about 60 times less than the concentration of LDL-protein in plasma (800 μg protein per milliliter), it is nevertheless in the range in which inhibition of LDL binding is observed in vitro. Thus, it is conceivable that platelet factor 4 might somehow modulate LDL interaction with the receptor in vivo; or, conversely, LDL might modulate the interaction of platelet factor 4 with heparin and/or with the cell surface glycosaminoglycans of blood vessels that participate in the clotting reaction. Inasmuch as the physiologic role of platelet factor 4 in the clotting mechanism is not yet resolved, these speculations must remain tentative.

ACKNOWLEDGMENTS

This work was supported by NIH grants P01 HL-20948, R01 CA-22409, and R01 HL-22119. T.F.D. holds Faculty Research Award 133 from the American Cancer Society.

Gloria Y. Brunschede and Lynda Letzig provided skillful technical assistance. We thank Carol Mansen and Wendy Womack for their able assistance in the tissue culture laboratory.

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