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# Low-density-lipoprotein binding by mast-cell granules

## Demonstration of binding of apolipoprotein B to heparin proteoglycan of exocytosed granules

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1. To study the interaction between low-density lipoprotein (LDL) and granules from rat serosal mast cells *in vitro*, mast cells were stimulated with the degranulating agent 48/80 to induce exocytosis of the secretory granules. Subsequent incubation of the exocytosed granules with $^{125}$I-LDL resulted in binding of the labelled LDL to the granules. When increasing amounts of agent 48/80 were added to mast-cell suspensions, a dose-dependent release of granules was observed and a parallel increase in the amount of $^{125}$I-LDL bound to granules resulted. 2. $^{125}$I-LDL bound to a single class of high-affinity binding sites on the granules. At saturation, 105 ng of LDL were bound per $\mu$g of granule protein. 3. The lipoprotein binding to mast-cell granules was apolipoprotein(apo)-B+E-specific. Thus $^{125}$I-LDL binding to the granules was effectively competed for by LDL (apo-B) or by dimyristoyl phosphatidylcholine vesicles containing apo-E, but not by high-density lipoprotein ($HDL_3$) containing apo-AI as their major protein component. 4. Neutralization by acetylation of the positively charged amino groups of apo-B of LDL or presence of a high ionic strength in the incubation medium prevented LDL from binding to the granules, indicating the presence of ionic interactions between the positively charged amino acids of LDL and negatively charged groups of the granules. 5. It could be demonstrated that LDL bound to the negatively charged heparin proteoglycan of the granules. Thus treatment of granules with heparinase resulted in loss of their ability to bind LDL, and substances known to bind to heparin, such as Toluidine Blue, avidin, lipoprotein lipase, fibronectin and protamine, all effectively competed with LDL for binding to the granules. The results show that LDL is efficiently bound to the heparin proteoglycan component of mast-cell granules once the mast cells are stimulated to release their granules into the extracellular space.

## INTRODUCTION

The role of mast cells in the immediate-type hypersensitivity reactions is well established. On antigen challenge, the mast cells are activated and release specific cytoplasmic organelles, the secretory granules, into the extracellular space (Ishizaka & Ishizaka, 1984). The granules contain preformed mediators of the immediate-type hypersensitivity reactions such as histamine and, in addition, proteolytic enzymes embedded in a heparin proteoglycan matrix. Most of the mediators stored in the secretory granules are freely soluble in physiological buffers (Schwartz & Austen, 1984). Hence, after degranulation of mast cells or after suspending purified granules in physiological buffers, the soluble mediators are released from the granules. In contrast, the major protein component of granules, composed of neutral proteinases, is not released but instead remains tightly bound to the heparin proteoglycan matrix of the granules (Schwartz & Austen, 1984). The function of the formed insoluble heparin–proteinase complexes, i.e. of extracellular granules, has remained obscure.

We have recently found that the extracellularly located granules of mast cells are able to interact with LDL by degrading the apo-B component of LDL (Kokkonen & Kovanen, 1985). The present studies were designed to test whether LDL binds to the granules, and if so, whether LDL, known to bind to various glycosaminoglycans, including heparin (Iverius, 1972), would bind to the heparin proteoglycan of the mast-cell granules. The results indicate that LDL is able to bind to the mast-cell granules once the granules are released from mast cells into the extracellular space, and that it is the heparin proteoglycan of the granules to which LDL binds on formation of an LDL–granule complex.

## MATERIALS AND METHODS

### Materials and animals

$Na^{125}I$ (13–17 mCi/$\mu$g) and $Na_2{}^{35}SO_4$ (> 5 mCi/$\mu$g) were purchased from Amersham International. Heparinase (500 units/mg of protein), avidin, 2-mercaptoethylamine (cysteamine), bovine serum albumin and soybean trypsin inhibitor were obtained from Sigma. Toluidine Blue was purchased from Fluka. DMPC was obtained from KSV-Chemicals Ltd. Dulbecco's PBS was purchased from Gibco. Fibronectin from human plasma was kindly given by Dr. Antti Vaheri, Department of Virology, University of Helsinki, Helsinki, Finland. Lipoprotein lipase from bovine milk, and purified human apo-E2 and apo-AI were kindly given by Dr. Timo Kuusi, Third Department of Medicine, University of Helsinki, Helsinki, Finland. Compound 48/80 was kindly given by AB Leo. Male Wistar rats weighing between 300 and 500 g were obtained from Orion Ltd.

Abbreviations used: LDL, low-density lipoprotein; HDL, high-density lipoprotein; apo-B etc., apolipoprotein B etc.; DMPC, dimyristoyl phosphatidylcholine; PBS, phosphate-buffered saline (137 mM-NaCl/2.7 mM-KCl/8.1 mM-$Na_2HPO_4$/0.9 mM-$CaCl_2$/1.1 mM-$KH_2PO_4$/0.5 mM-$MgCl_2$, pH 7.3).

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They were kept in constant light and were provided with water and laboratory chow (Orion Ltd.) *ad libitum*.

### Isolation of mast cells and mast-cell granules

The isolation of the rat serosal mast cells (i.e. mast cells from peritoneal and pleural cavities) was performed by density-gradient centrifugation in Ficoll as described previously (Kokkonen & Kovanen, 1985). Mast-cell granules were isolated from purified mast cells as described previously (Kokkonen & Kovanen, 1985). The amount of granules is expressed in terms of granule protein.

### Preparation of $^{35}$S-labelled granules

The heparin component of mast-cell granules was radiolabelled *in vitro* by incubating purified rat mast cells [$(5–10) \times 10^6$] with 1 mCi of $Na_2{}^{35}SO_4$ for 4 h as previously described by Yurt *et al.* (1977). After incubation the granules were isolated as described above. $^{35}$S-labelled material in mast-cell granules was identified as heparin by treating the granules with heparinase (Linker & Hovingh, 1972). For this purpose, the $^{35}$S-labelled granules were incubated with heparinase in buffer C [100 mM-sodium acetate (pH 7.0)/1.0 mM-calcium acetate/bovine serum albumin (1 mg/ml)/ soybean trypsin inhibitor (2.5 mg/ml)] at 37 °C for 15 min. To separate the $^{35}$S-labelled degradation products from granules, the granules were sedimented by centrifuging at 12000 *g* for 5 min and the amounts of $^{35}$S radioactivity in the supernatants and in the granule pellets were measured (LKB–Wallac RackBeta 1215 liquid-scintillation counter). The extent of heparin degradation by heparinase is expressed as the percentage of $^{35}$S-labelled material released from the granules into the supernatants. Similar values for the extent of heparin degradation could also be obtained if the $^{35}$S-labelled degradation products were separated from the granules by gel filtration on a Sepharose 4B column (Pharmacia) (Atkins *et al.*, 1985). Therefore the $^{35}$S radioactivity present in the supernatants could be used as a quantitative measure of degradation of granule heparin.

### Preparation and iodination of lipoproteins

Human lipoprotein fractions were prepared from the plasma of fasted healthy donors as previously described (Kokkonen & Kovanen, 1985). LDL [*d* (relative density) = 1.019–1.050) and $HDL_3$ (*d* = 1.125–1.215) were fractionated by sequential ultracentrifugation (Havel *et al.*, 1955) using solid KBr for density adjustment (Radding & Steinberg, 1960). The concentration of each lipoprotein is expressed in terms of its protein concentration. Lipoprotein purity was judged by cellulose acetate electrophoresis (Lipoprotein Electrophoresis; Helena Laboratories) where LDL and $HDL_3$ migrated as single homogeneous bands. Gradient-gel electrophoresis in 0.2% (w/v) SDS (gradient gel PAA 4/30; Pharmacia) revealed that LDL contained only apo-B, and $HDL_3$ contained apo-AI as its major apolipoprotein and no apo-B or apo-E. LDL was iodinated by the ICl method (McFarlane, 1958; Bilheimer *et al.*, 1972) as previously described (Kokkonen & Kovanen, 1985). The ranges of the specific radioactivities of $^{125}$I-LDL were 100–220 c.p.m./ng of protein. For each experiment $^{125}$I-LDL was diluted with unlabelled LDL to give the specific radioactivities indicated in the Figure legends.

### Preparation of apolipoprotein–DMPC complexes

Apo-E2 was first treated with cysteamine to convert the cysteine residues of the apo-E2 into positively charged lysine analogues (Weisgraber *et al.*, 1982). DMPC vesicles were prepared by the procedure of Roth *et al.* (1977). The gradient-gel electrophoresis of the apo-E–DMPC and apo-AI–DMPC complexes showed single bands of apo-E and apo-AI respectively.

### Binding of $^{125}$I-labelled lipoproteins to mast-cell granules

The standard binding assay was conducted in 90 $\mu$l of buffer A [150 mM-NaCl/bovine serum albumin (10 mg/ml)/soybean trypsin inhibitor (1.2 mg/ml)/ 5 mM-Tris/HCl, pH 7.4], containing the granules (2.5–20 $\mu$g of granule protein), and the indicated concentrations of $^{125}$I-labelled or unlabelled lipoproteins. Neither bovine serum albumin nor trypsin inhibitor influenced the binding of $^{125}$I-LDL to the granules. The binding reactions were initiated by adding the granules, and the incubations were carried out at 0 °C in stoppered plastic tubes in an ice bath for the indicated times. To determine the amount of granule-bound labelled lipoproteins, a portion (80 $\mu$l) of the reaction mixture was layered on to 170 $\mu$l of medium consisting of 0.25 M-sucrose, bovine serum albumin (10 mg/ml) and 5 mM-Tris/HCl, pH 7.4. The tubes were then centrifuged at 12000 *g* for 15 min at 4 °C. The supernatant of each tube was removed by aspiration and the area of the tube containing the granule pellet was sliced with a scalpel and counted for its $^{125}$I radioactivity (LKB–Wallac CliniGamma 1272 $\gamma$-radiation counter). The results are expressed as ng of LDL protein bound per $\mu$g of granule protein.

### Other assays

Histamine was determined by fluorimetry as described previously (Kokkonen & Kovanen, 1985). Protein was determined by the procedure of Lowry *et al.* (1951), with bovine serum albumin as a standard.

The results given in the Figures are representative of experiments repeated at least three times.

## RESULTS

### Binding of $^{125}$I-LDL to exocytosed mast-cell granules

To induce specific and non-cytotoxic degranulation of mast cells, i.e. exocytosis of secretory granules, we treated mast cells with the compound 48/80 (5 $\mu$g/ml) (Röhlich *et al.*, 1971). To demonstrate the extrusion of the granules, the cells were removed by centrifugation (150 *g* for 5 min), and the supernatants were centrifuged at a centrifugal force (12000 *g* for 15 min) sufficient to sediment granules. The presence of granules in the 12000 *g* sediment could be verified by measuring the activity of the proteolytic enzyme chymase in the sediment. Chymase, the major proteolytic enzyme of the granules, is not released from the granules after degranulation and can thus be used as a granule marker (Schwartz & Austen, 1984). All of the chymase activity present originally in the 150 *g* supernatant could be recovered in the 12000 *g* sediment, demonstrating quantitative sedimentation of the released granules at the given centrifugal force (results not shown). In addition, electron-microscopic examination showed that the 12000 *g* sediment consisted solely of mast-cell granules. When the resuspended 12000 *g* sediment (granules) was

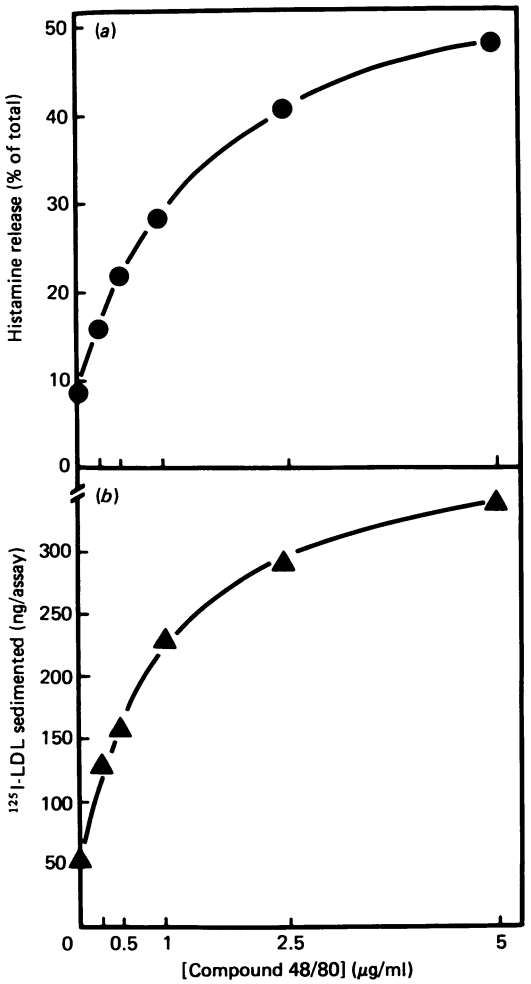


**Fig. 1. Degranulation of mast cells (*a*) and sedimentation of $^{125}$I-LDL (*b*) as functions of compound 48/80 concentration**

Each assay contained $2 \times 10^5$ purified mast cells in 100 μl of PBS containing 10 mg of human serum albumin/ml and 5.6 mM-glucose. The mast cells were first incubated at 37 °C for 15 min, after which the indicated amounts of compound 48/80 (dissolved in the above buffer) were added, and the incubations were continued for 5 min to allow mast-cell degranulation to be completed. After incubation the mast cells were sedimented by centrifugation at 150 *g* for 5 min, and 50 μl of each supernatant was removed and centrifuged at 12000 *g* for 15 min. The formed sediments were washed once by centrifuging at 12000 *g* for 5 min and resuspended in 100 μl of the above buffer containing 25 μg of $^{125}$I-LDL (19 c.p.m./ng). After incubation at 37 °C for 5 min, a portion (90 μl) of the reaction mixture was layered on to separation medium and the amount of $^{125}$I-LDL sedimented was determined as described in the Materials and methods section. For calculation of the amount of histamine released (percentage of total), the contents of the histamine in the supernatants and in the sedimented mast cells were determined as described in the Materials and methods section.

incubated with $^{125}$I-LDL at 37 °C for 5 min, and subsequently centrifuged to sediment the granules, a pellet containing $^{125}$I radioactivity was formed. No sedimentation of $^{125}$I-LDL was observed without granules. The co-sedimentation of $^{125}$I-LDL with the granules suggested that the labelled LDL bound to the granules, i.e. that complexes between LDL and the granules were formed.

When the above experiment was repeated using increasing amounts of compound 48/80, increasing amounts of histamine were released into the incubation medium, reflecting a dose-dependent exocytosis of mast-cell granules (Fig. 1*a*). The abilities of the various amounts of released granules to form complexes with LDL was then quantified by measuring the co-sedimentation of labelled LDL and the granules. It appeared that the formation of LDL–granule complexes closely paralleled the increase in histamine release, i.e. the release of granules into the extracellular space (Fig. 1*b*). At maximal stimulation of degranulation, the amount of histamine in the extracellular space was 5.7-fold higher and the amount of LDL–granule complexes formed was 6.2-fold higher than without stimulation. Hence, a quantitative relationship between the amount of granules released and the amount of formation of LDL–granule complexes could be established.

### Characteristics of $^{125}$I-LDL binding to isolated mast-cell granules

The above experiments demonstrated that, after stimulation of mast cells, the exocytosed mast-cell granules are able to bind $^{125}$I-LDL. To examine the binding phenomenon in more detail, we conducted a series of experiments with isolated mast-cell granules obtained from lysed mast cells, a method which yields large amounts of granules (Thon & Uvnäs, 1966). Since mast-cell granules are also able to interact with LDL by degrading it (Kokkonen & Kovanen, 1985), we carried out the experiments at 0 °C to be able to study binding alone without concomitant degradation of $^{125}$I-LDL.

When $^{125}$I-LDL was incubated with isolated mast-cell granules at 0 °C, the labelled lipoprotein was rapidly bound to the granules, with equilibrium achieved in less than 1 min. On incubation of granules with increasing concentrations of $^{125}$I-LDL a two-component saturation curve for the binding was obtained (Fig. 2*a*). The high-affinity component of this binding curve showed evidence of saturation at about 25 μg of LDL protein/ml. At higher concentrations of $^{125}$I-LDL a small non-saturable component of the binding curve became apparent. When the binding reaction was conducted in the presence of excess unlabelled LDL, the high-affinity component of the $^{125}$I-LDL binding curve was abolished, indicating that the unlabelled LDL was competing with the $^{125}$I-LDL for a limited number of binding sites.

The difference between the $^{125}$I-LDL binding in the absence and the presence of excess unlabelled LDL gives a measure of the high-affinity binding. A Scatchard plot of the high-affinity binding curve produced a straight line with intercepts giving a value of 6 μg of LDL protein/ml (equivalent to 10 nM-LDL) for the concentration of $^{125}$I-LDL producing half-maximal binding (Fig. 2*b*). The maximal binding was 105 ng/μg of granule protein. When the above experiment was repeated at 37 °C in the presence of soybean trypsin inhibitor to prevent LDL degradation (Kokkonen & Kovanen, 1985), a two-component saturation curve similar to that observed at 0 °C was obtained (results not shown). On the basis of the assumptions that each granule contains approx. 50 fg of protein (Schwartz & Austen, 1984; Helander & Bloom, 1973), and that the protein content of LDL

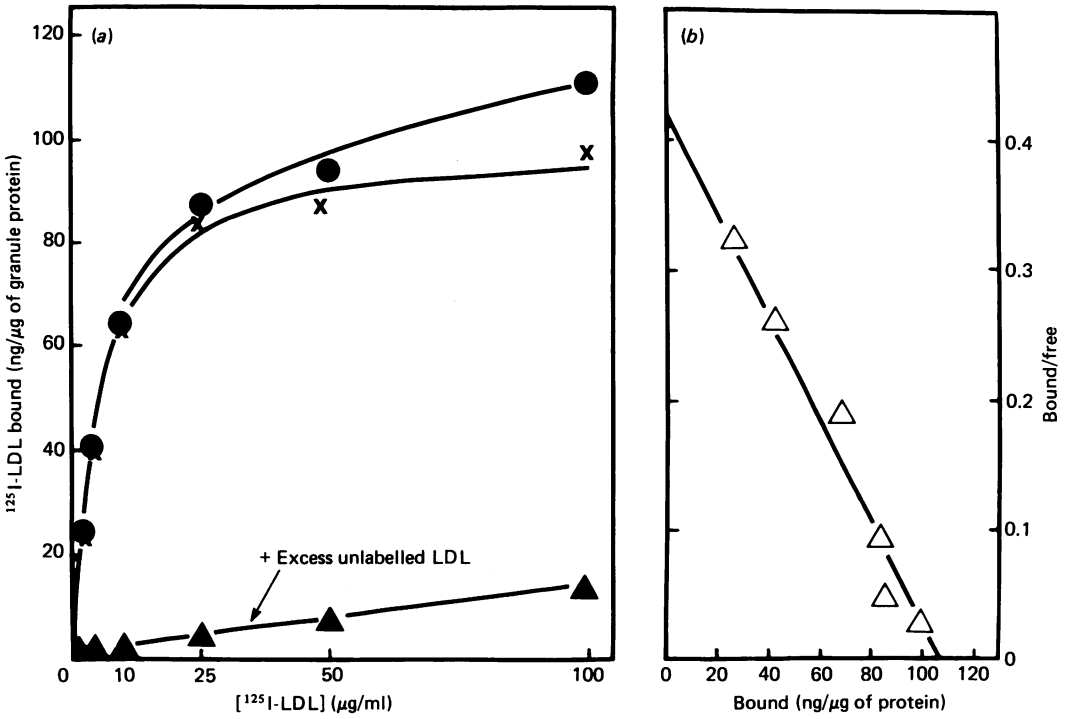


**Fig. 2. Saturation curve for the binding of $^{125}$I-LDL to mast-cell granules**

(*a*) Each binding-reaction mixture contained in 90 μl of buffer A, 2.5 μg of granules and the indicated concentrations of $^{125}$I-LDL (28 c.p.m./ng) in the absence (●) or presence (▲) of 2 mg of unlabelled LDL/ml. After incubation at 0 °C for 60 min, the amounts of $^{125}$I-LDL bound to granules were determined. The high-affinity binding (×) was calculated by subtracting the amount of $^{125}$I-LDL bound in the presence of excess unlabelled LDL (non-specific binding) from that bound in the absence of excess unlabelled LDL (total binding). (*b*) Scatchard plot of the high-affinity binding data. 'Bound/free' is the amount of bound $^{125}$I-LDL (ng of protein/ml) divided by the amount of unbound lipoprotein in the reaction mixture (ng of protein/ml).

($M_r$ $2.6 \times 10^6$) is 24%, it could be calculated that, at saturation, each granule bound approx. 5000 particles of LDL.

To test the effect of apo-B modifications on LDL binding to mast-cell granules, LDL was treated with either acetic anhydride (Basu *et al.*, 1976) or with cyclohexanedione (Mahley *et al.*, 1977) to yield LDL in which the positively charged groups of lysine or arginine respectively are neutralized. The binding of acetyl-$^{125}$I-LDL and cyclohexanedione–$^{125}$I-LDL to mast-cell granules was totally abolished (results not shown), demonstrating the critical role of positively charged amino acids of apo-B in the observed binding phenomenon.

The above results suggested the presence of ionic interactions between the positively charged groups of LDL and negatively charged groups of the granules. To study the presence of ionic interactions, the binding of $^{125}$I-LDL to mast-cell granules was tested at NaCl ionic strengths of up to 0.5 (500 mM-NaCl). Under these experimental conditions the sedimentation of granules was not influenced (measured using $^{35}$S-labelled granules). As shown in Fig. 3, the binding of the labelled LDL to the granules was optimal at an ionic strength of 0.10. Above the physiological ionic strength, 0.15, binding of the labelled LDL to the granules was strongly inhibited. The strong influence of ionic strength on the formation of an LDL–granule complex and the shown requirement for positively charged groups on LDL indicated the presence of ionic interactions between apo-B and granules in the binding of LDL to the granules.

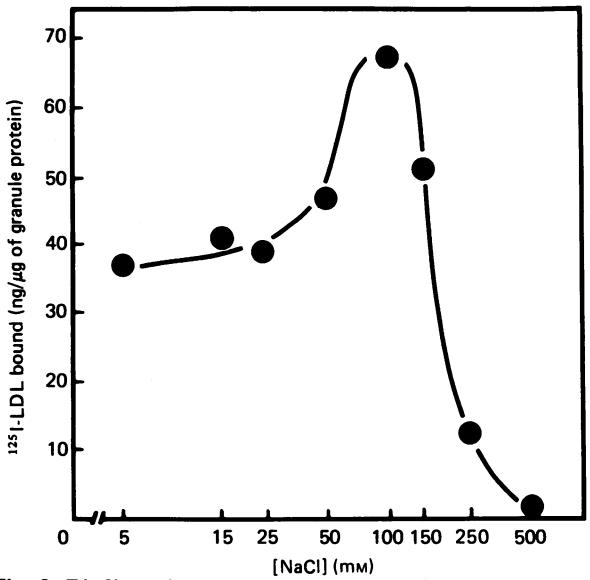


**Fig. 3. Binding of $^{125}$I-LDL to mast cell granules as a function of ionic strength**

A 5 μg portion of granules was preincubated at 0 °C for 30 min in 85 μl of buffers containing 10 mg of bovine serum albumin/ml, 1.2 mg of trypsin inhibitor/ml, 5 mM-Tris/HCl, pH 7.4, and various amounts of NaCl to give the indicated concentrations. After the preincubation period, each tube received 2 μg of $^{125}$I-LDL (33 c.p.m./ng), and the incubation was continued at 0 °C for 30 min, after which the amounts of $^{125}$I-LDL bound to granules were determined.

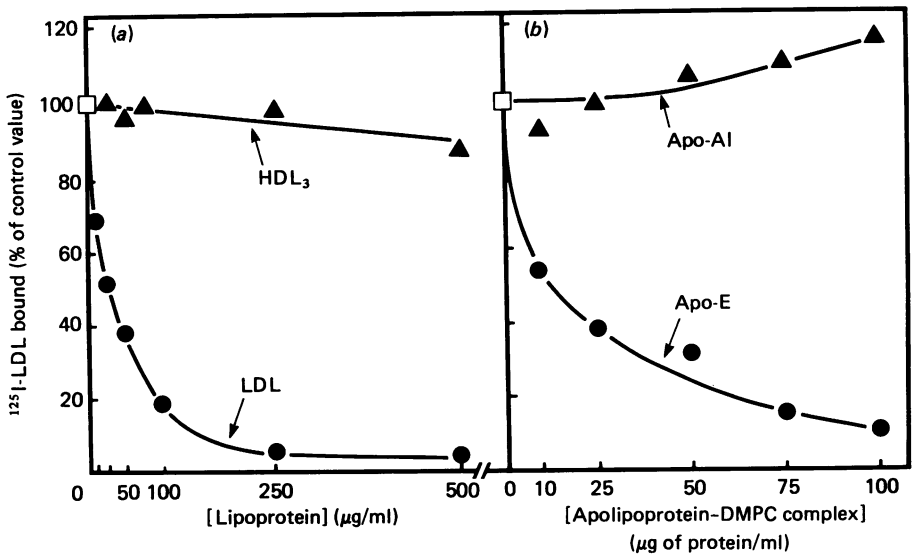


**Fig. 4. Comparison of the abilities of unlabelled LDL and $HDL_3$ (*a*), and apo-E–DMPC complexes and apo-AI–DMPC complexes (*b*) to compete with $^{125}$I-LDL for binding to mast-cell granules**

(*a*) Each binding-reaction mixture contained in 90 μl of buffer A, 5 μg of granules, 20 μg of $^{125}$I-LDL (29 c.p.m./ng)/ml and the indicated concentrations of either unlabelled LDL (●) or $HDL_3$ (▲). After incubation at 0 °C for 60 min, the amounts of $^{125}$I-LDL bound to granules were determined. The 100% control value for $^{125}$I-LDL bound in the absence of unlabelled lipoproteins was 163 ng/μg of granule protein. (*b*) Apolipoprotein–DMPC complexes were prepared as described in the Materials and methods section. Each binding-reaction mixture contained, in 90 μl of buffer A, 2.5 μg of granules, 1 μg of $^{125}$I-LDL (104 c.p.m./ng)/ml and the indicated concentrations of either apo-E–DMPC complexes (●) or apo-AI–DMPC complexes (▲). After incubation at 0 °C for 60 min, the amounts of $^{125}$I-LDL bound to granules were determined. The 100% control value for $^{125}$I-LDL bound in the absence of apolipoprotein–DMPC complexes was 11 ng/μg of granule protein.

### Apolipoprotein specificity of lipoprotein binding to mast-cell granules

After having defined the role of apo-B in the binding of LDL to mast-cell granules, we studied whether lipoproteins devoid of apo-B would also bind to the granules. For this purpose we compared the abilities of LDL (apo-B) and of $HDL_3$ (devoid of apo-B and apo-E) containing apo-AI as its major protein component to compete with $^{125}$I-LDL for binding to the granules. As shown in Fig. 4(*a*), the binding of $^{125}$I-LDL was effectively competed for by unlabelled LDL but not by unlabelled $HDL_3$. In addition we could show that $^{125}$I-$HDL_3$ completely lacked the ability to bind to mast-cell granules (results not shown).

To study further the apolipoprotein specificity of the binding phenomenon, we prepared apolipoprotein–DMPC complexes containing either purified human apo-E or apo-AI, and compared their abilities to compete with $^{125}$I-LDL for binding to mast-cell granules (Fig. 4*b*). It appeared that the binding of the labelled LDL to the granules could be competed for by the apo-E–DMPC complexes, but not by the apo-AI–DMPC complexes. The above results with lipoproteins and apolipoprotein–DMPC complexes revealed specificity of the binding phenomenon in that the apolipoproteins B and E, but not AI, are able to bind to mast-cell granules.

### Characterization of the LDL-binding site on mast-cell granules

In the final series of experiments we studied the nature of the binding site responsible for the observed binding of the apolipoproteins B and E to mast-cell granules. The mast-cell granules are known to contain a negatively charged heparin proteoglycan (Schwartz & Austen, 1984) which, by virtue of its negative charge, might have interacted with the positively charged amino acids of apo-B and apo-E (Mahley *et al.*, 1979). To test this hypothesis we treated mast-cell granules with heparinase, an enzyme known to degrade heparin specifically (Linker & Hovingh, 1972). To be able to quantify the degradation of granule heparin, we labelled the sulphate groups of heparin with $^{35}$S (Yurt *et al.*, 1977). When the $^{35}$S-labelled granules were incubated with heparinase to yield maximal degradation of granule heparin (77% loss of $^{35}$S label), and then allowed to bind $^{125}$I-LDL, it was found that the amount of LDL binding was on average only 21% of that obtained with untreated $^{35}$S-granules (Fig. 5). This result indicated that granule heparin was essential for binding of LDL to the granules. In a control experiment we could demonstrate that chondroitinase ABC had no effect on the binding of $^{125}$I-LDL to mast-cell granules (results not shown).

To demonstrate by another method that the binding site on mast-cell granules is heparin, we conducted a series of competition experiments with compounds known to interact with mast-cell-granule heparin or commercial heparin. For this purpose the granules were incubated with Toluidine Blue or avidin, both known to bind to mast-cell-granule heparin (Jaques, 1967; Tharp *et al.*, 1985), or with lipoprotein lipase or plasma fibronectin, both known to bind to commercial heparin (Olivecrona *et al.*, 1971; Stathakis & Mosesson, 1977). All of the compounds tested, i.e. Toluidine Blue and avidin (Fig. 6*a*), as well as lipoprotein lipase and plasma fibronectin (Fig. 6*b*), showed a concentration-dependent inhibition of $^{125}$I-LDL binding to mast-cell granules. In a separate experiment we could demonstrate that

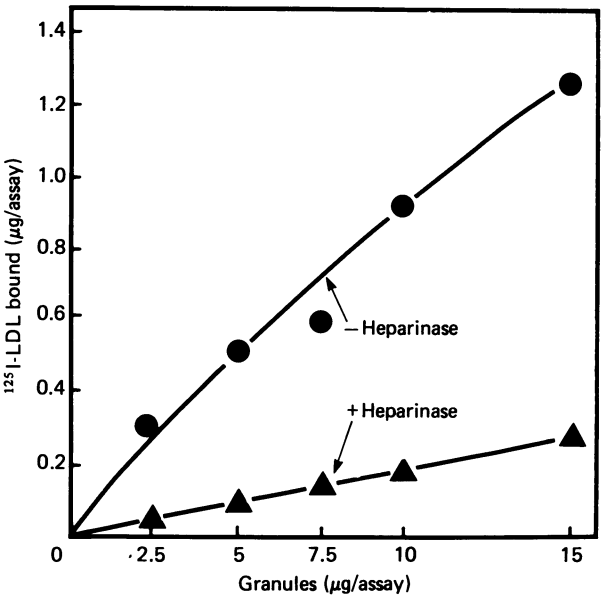


**Fig. 5. Inhibition of $^{125}$I-LDL binding to granules by heparinase**

A 60 μg portion of $^{35}$S-labelled granules (67800 d.p.m.) was incubated with 12 μg of heparinase at 37 °C for 30 min in 500 μl of buffer C as described in the Materials and methods section. In the control experiment no heparinase was added. After incubation with or without heparinase, the granules were sedimented. The sedimented granules were resuspended in 150 mM-NaCl and their protein contents were measured. The binding reactions were conducted in 90 μl of buffer A containing the indicated amounts of either heparinase-treated (▲) or control (●) granules, and 150 μg/ml of $^{125}$I-LDL (13 c.p.m./ng). After incubation at 0 °C for 30 min, the amounts of $^{125}$I-LDL bound to granules were determined. The recoveries of the $^{35}$S-labelled heparinase-treated and control granules in the binding assay were identical (80%).

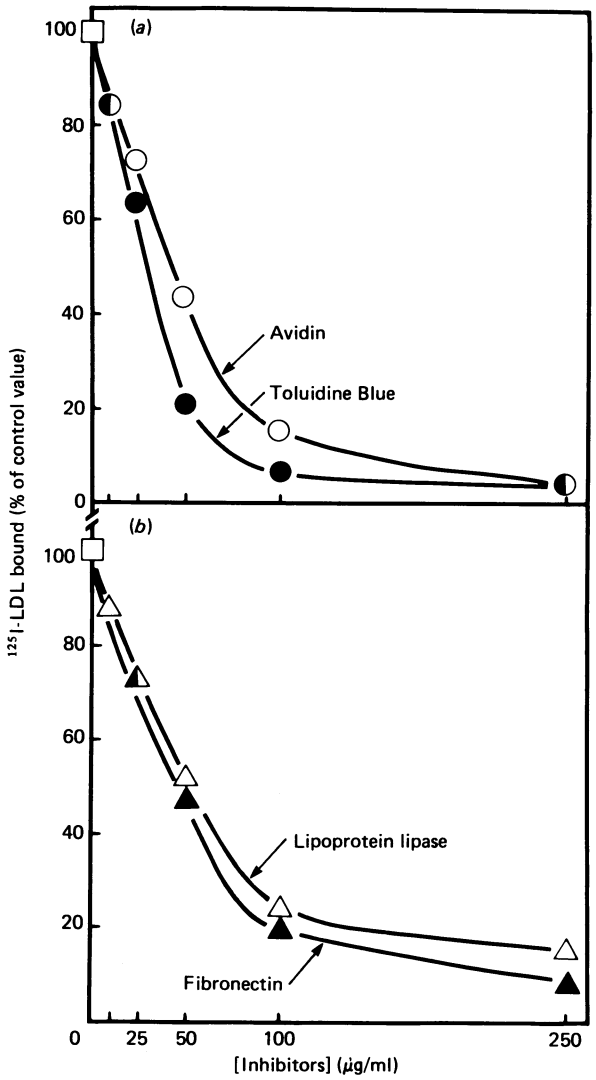


**Fig. 6. Inhibition of $^{125}$I-LDL binding to mast-cell granules by Toluidine Blue and avidin (*a*) and by lipoprotein lipase and fibronectin (*b*)**

The binding reaction with Toluidine Blue (●) was conducted in buffer consisting of 100 mM-NaCl, 10 mg of bovine serum albumin/ml, 1.2 mg of soybean trypsin inhibitor/ml and 50 mM-Tris/HCl, pH 7.4. The experiments with avidin (○), lipoprotein lipase (△) and fibronectin (▲) were conducted in buffer A. Each binding-reaction mixture contained in 90 μl of either buffer, 5 μg of granules, 20 μg of $^{125}$I-LDL (30 c.p.m./ng)/ml and the indicated concentrations of the inhibitors. After incubation at 0 °C for 60 min, the amounts of $^{125}$I-LDL bound to granules were determined. The 100% control values for $^{125}$I-LDL binding in the experiments with Toluidine Blue, avidin, lipoprotein lipase, and fibronectin were 51, 62, 62, and 56 ng/μg of granule protein respectively.

pretreatment of the granules with protamine, a substance known to bind to commercial heparin, effectively abolished the binding of $^{125}$I-LDL to the granules (results not shown). In summary, these results confirmed that it is the heparin component of the granules to which LDL binds on formation of an LDL–granule complex.

## DISCUSSION

The current studies reveal that rat serosal mast cells possess a specific mechanism for complexing extracellular LDL with heparin proteoglycan. The sequence of events leading to formation of the extracellular LDL–heparin proteoglycan complexes involves an initial stimulation of the mast cells, subsequent release of the cytoplasmic secretory granules into the extracellular space and, finally, binding of LDL to the exocytosed granules. We used in our '*in vitro*' model the non-cytotoxic degranulating agent 48/80 to induce a controlled specific degranulation of mast cells. However, a wide variety of stimuli, whether immunological or non-immunological, are known to activate the mast cells and cause their degranulation. Since the process of mast-cell degranulation is identical irrespective of the type of the stimulus (Galli *et al.*, 1984), it is conceivable that any stimulus which leads to mast-cell degranulation will ultimately lead also to interaction between the exocytosed granules and the extracellularly located LDL.

On formation of extracellular LDL–granule complexes, LDL efficiently bound to the heparin proteoglycan of the granules. The efficiency of binding was evident in two ways. First, the binding reaction was very rapid, being completed in less than 1 min. Secondly, the granules had high capacity to bind LDL. Thus, at

saturating concentrations of LDL, the granules did bind, in terms of total weights, about 300 ng of LDL per $\mu$g of granules, i.e. an amount equal to one-third of their weight. Such high binding capacity can be well explained by the high heparin content of the granules. Indeed, in physiological buffers where the soluble mediators are released from the granules, at least 35% of the total weight of the granules is composed of heparin (Schwartz & Austen, 1984).

LDL is known to form complexes with several commercially available (low-$M_r$) glycosaminoglycans, including heparin. Among the glycosaminoglycans tested, heparin demonstrated the highest affinity for LDL (Iverius, 1972). LDL may form complexes also with various naturally occurring proteoglycans. In fact, the interaction of arterial proteoglycans and LDL has been postulated to be an important factor in the entrapment of LDL and the resulting extracellular deposition of lipid in the arterial wall (Berenson *et al.*, 1984). However, unlike other naturally occurring proteoglycans, the heparin proteoglycan is not normally found in the extracellular space (Fransson, 1985). Heparin proteoglycan is an intracellular proteoglycan that is synthesized exclusively in mast cells and stored in their cytoplasmic granules (Fransson, 1985). Consequently, stimulation of mast cells and the ensuing degranulation are required for the extracellular LDL–heparin proteoglycan complexes to form.

The fate of the exocytosed mast-cell granules has remained obscure. Recent studies *in vitro* have demonstrated, however, that mast-cell granules can be phagocytosed and eventually degraded by arterial endothelial cells (Atkins *et al.*, 1985), by cultured fibroblasts (Subba Rao *et al.*, 1983), and by macrophages (Lindahl *et al.*, 1979). If, also, the LDL–granule complexes could be phagocytosed by these cells, then cellular uptake of LDL would follow. It remains to be elucidated whether such uptake mechanism could play a role in the accumulation of LDL cholesterol by macrophages, a phenomenon characteristic of atherosclerosis.

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