THE BINDING OF LOW DENSITY LIPOPROTEIN BY LIVER MEMBRANES IN THE PIG

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SUMMARY

Porcine liver membranes are capable of high affinity binding of homologous low density lipoproteins (LDL). Binding is time and temperature dependant and substrate saturable. High affinity binding sites are half saturated at 11 µg/ml lipoprotein-protein. The binding of ^{125}I-LDL is inhibited by unlabelled homologous LDL, very low density lipoproteins (VLDL) and high density lipoproteins (HDL) and also be human LDL and HDL, but not by unrelated proteins tested. The binding and displacement patterns with membranes from several other porcine tissues are similar to those of liver membranes. These results suggest the presence of "lipoprotein binding sites" in liver membranes which recognize structural features common to the lipoproteins and further indicate that liver membranes are not unique in their ability to bind LDL.

INTRODUCTION

It is not presently known which tissue(s) is normally responsible for the catabolism of low density lipoproteins (LDL). Recent studies in human fibroblasts indicate that the first step in LDL breakdown is binding of the lipoprotein to specific cell surface receptors prior to uptake and lysosomal degradation (1,2). This degradative process is involved in the regulation of cellular cholesterol metabolism. Binding studies in fibroblasts suggest that the LDL receptor recognizes LDL and very low density lipoproteins (VLDL) but not high density lipoproteins (HDL) (2). The common binding of LDL and VLDL is presumably due, in part, to the presence in each of β protein (2). Later studies in human fibroblasts with porcine lipoproteins suggest, however, that the unrelated "arginine-rich" polypeptide is also recognized by the "LDL binding site" (2,3).

Most of the LDL binding studies to date have been performed in cultured cells. The present communication describes the binding of porcine LDL to homologous membranes isolated from the liver and other tissues. The pig is a useful model for binding studies since its plasma lipoproteins are similar to those of the human (4,5). The results indicate that all the tissues studied are capable of LDL-

displacable binding of ^{125}I-LDL. The "LDL binding site" recognizes lipoproteins in general rather than LDL exclusively. This suggests that the lipoprotein structural features recognized by the cell membrane binding site are more general than those observed with fibroblasts.

MATERIALS AND METHODS

Serum and tissues were obtained from freshly killed adult normolipidemic swine. Rabbit antibody to pig serum was prepared by intramuscular injection of 5 mg pig serum in complete Freund's adjavant into rabbits at 10 day intervals over 30 days. Antiserum was obtained 10 days after the last injection and was reactive against VLDL, LDL and HDL. Lipoproteins were isolated from serum in the ultracentrifuge according to established procedures (4) using KBr for density adjustment. They were separated at the following densities (g/ml): VLDL, d<1.006; LDL, d 1.020-1.063; HDL, d 1.12-1.21. They were then washed twice at their respective densities and dialyzed against $1x10^{-3}$ M EDTA, pH 7.4 in 0.15 M NaCl. VLDL, LDL and HDL were each free of contamination by the other lipoproteins as assessed by agarose gel electrophoresis and immunoelectrophoresis using anti-pig serum. Cholesterol, triglyceride and protein compositions of the lipoproteins agreed well with those reported for porcine lipoproteins (4). Except as indicated, plasma membrane fractions were prepared by homogenizing 4g liver in 10 volumes of Krebs-Ringer phosphate buffer, pH 7.4, with a Polytron homogenizer. The fraction which sedimented in 10 minutes at 600-4000 x g was washed and resedimented four times in the same buffer, twice at 4000 x g for 10 minutes and twice at 10,000 x g for 5 minutes. Membranes from other tissues were similarly prepared. ^{125}I-LDL (150-250 cpm/ng LDL protein) was prepared by iodination at pH 10.5 according to the procedure of McFarlane (6). The product was more than 99% precipitable in 10% trichloroacetic acid (TCA) and 82% precipitable with rabbit antibody to pig serum. Approximately 3% of the TCA precipitable radioactivity was associated with lipids. Monoiodinated ^{125}I insulin (1 Ci/umole) was prepared according to Cuatrecasas (7). NAD pyrophosphatase was assayed as previously described (8) and 5' AMPase was determined according to Emmelot, et al (9). Membranes were incubated with ^{125}I-LDL, with or without unlabelled LDL for 4 h at 24°C in Krebs-Ringer phosphate buffer, pH 7.4 which contained 4% bovine serum albumin (BSA) to minimize nonspecific interactions. After incubation membranes were isolated by centrifugation at 10,000 x g through a layer of 0.25 M sucrose in incubation buffer, washed twice with 0.9% NaCl, and membrane bound radioactivity was determined.

RESULTS

The plasma membrane preparations used for these experiments were enriched 4-5 fold over liver homogenates based on membrane marker enzyme activities and insulin-displacable ^{125}I-insulin binding (Table IA). The binding of ^{125}I-LDL by liver membranes was saturable. Half saturation was achieved at 11 $\pm$ 4 µg/ml (mean $\pm$ S.E.M.). This was determined from three experiments by extrapolation of the high affinity portion of Scatchard plots of saturation data (Figure 1). All subsequent experiments were conducted at ^{125}I-LDL concentrations of about 5 µg/ml since at this concentration binding was primarily to high affinity membrane sites. LDL

Table I. LDL Binding by Various Pig Liver Fractions

Fraction	Nucleotide Pyrophosphatase		5' AMPase		Insulin Displacable ^{125}I-insulin Binding		LDL Displacable ^{125}I-LDL Binding	
	Activity*	Purification	Activity	Purification	ng/mg membrane protein	Purification	ng/mg membrane protein	Purification
A. Fractions Prepared in Krebs-Ringer Phosphate Buffer								
Homogenate	0.19	1	0.24	1	(0.079)‡	1	(16.3)	1
600-4000 x g pellet	0.86	4.5	1.05	4.4	0.330	4.2	76.3	4.7
B. Fractions Prepared in 0.25 M Sucrose								
Homogenate	(0.25)	1	(0.33)	1	(0.02)	1	(78.4)	1
600 x g pellet	0.60	2.4	1.22	3.1	0.070	3.3	197.5	2.5
12,000 x g pellet (Mitochondria)	0.45	1.8	0.86	2.6	0.038	1.8	242.0	3.1
Heavy membranes (12000 x g pellet)	1.19	4.76	1.63	4.9	0.105	5.0	383.5	4.9
Light membranes 45000 x g pellet	1.41	5.6	2.57	7.8	0.068	3.2	469.8	6.0

A. Membrane fractions were prepared as described in Methods. B. Liver fractions were prepared according to Chang, et al. (19) in 10 volumes 0.005 M Tris pH 7.4 containing 0.25 M Sucrose. Insulin binding: incubation system contained 5.03 ng/ml ^{125}I-insulin with and without 50 µg/ml unlabelled insulin. LDL-binding: incubation system contained 5 ug/ml ^{125}I LDL with and without 500 ug/ml ^{125}I-LDL. After incubation, mixtures were layered on 4 ml 0.25 M Sucrose in Krebs-Ringer phosphate buffer containing 4% BSA and centrifuged for 30 min at 50,000 x g. Pellets were rinsed with 0.9% NaCl and radioactivity was determined.

* Enzyme activities expressed in umole/hr/mg membrane protein. Enzyme assays performed at 37°C.

‡ ( ) indicates calculated value. Actual enzyme and binding assays were performed on pellet obtained by centrifugation of homogenate at 105,000 x g for 1 hr. Binding assays were performed as above except that membranes were isolated from the incubation mixtures by centrifugation at 105,000 x g for 1 hr. Calculated values assume 28% of total liver protein is particulate based on protein content of 105,000 x g pellet and supernatant fractions of liver homogenate determined separately.

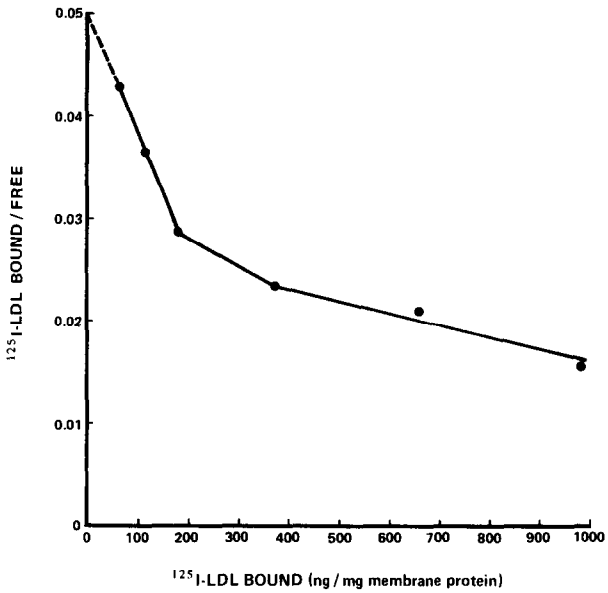

Figure 1. Representative Scatchard plot of the concentration dependant binding of ^{125}I-LDL to porcine liver membrane. Incubations were performed as described in Methods. Incubation systems contained 1.62-130 µg/ml ^{125}I-LDL protein.

binding was time and temperature dependent. It reached a maximum at 4 h and increased 3-3.5 fold as the temperature was increased from 24°C to 35°C. Binding was linear up to membrane concentrations of about 1 mg/ml.

^{125}I-LDL binding was concentrated in fractions enriched in plasma membranes as shown by the parallel purification of binding capacity and plasma membrane marker enzymes (Table IB). The plasma membrane fractions clearly bound LDL to a greater extent than nuclear and mitochondrial fractions. It is not possible in this experiment however, to determine whether the binding which was detected in nuclear and mitochonchial fractions represented binding to these organelles, to plasma membrane contaminants of the fractions, or both. Unlabelled LDL competed with ^{125}I-LDL for binding as shown in figure 2A. The other major pig lipoproteins were also effective in competing with ^{125}I-LDL (Figure 2A). The effect appears to be specific for the lipoproteins since ovalbumin and fetuin, two unrelated proteins, were not competitors of ^{125}I-LDL binding in concentrations up to 1000 µg/ml.

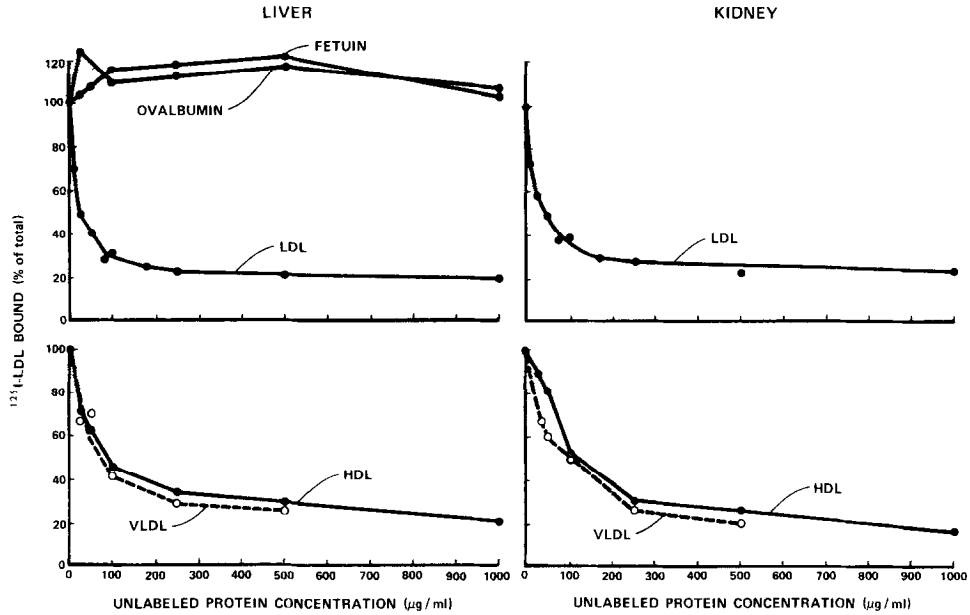

Figure 2. Effect of lipoproteins and unrelated proteins on the binding of ^{125}I-LDL to membranes isolated from A. (left) porcine liver and B. (right) porcine kidney. Incubations were performed as described in Methods. ^{125}I-LDL protein concentration was 5 μg/ml.

The results presented in Table II indicate that both human LDL and human HDL are capable of preventing the binding of pig ^{125}I-LDL to pig liver membranes and are almost as effective as pig LDL itself. Human apoAI and apoAII were both capable of inhibiting the ^{125}I-LDL binding, as were human HDL lipids and phosphatidyl choline. The data suggest that both lipoprotein-protein as well as certain lipids interfere with the LDL-membrane interaction. Dextran and the monosaccharides tested had no effect.

Other Pig Tissues

The binding properties of porcine kidney membranes were similar to those of liver membranes. The binding of ^{125}I-LDL was saturable and displacable by LDL as well as by VLDL and HDL, but not by fetuin (Figure 2B and Table III). A similar pattern of inhibition of ^{125}I-LDL binding was observed in the five other tissues tested (Table III). In each case, both pig LDL and pig HDL inhibited ^{125}I-LDL binding while fetuin was ineffective.

Table II. Inhibition of ^{125}I-LDL Binding to Pig Liver Membranes by Lipoproteins, Lipids and Carbohydrates

	Concentration of Inhibitor (ug/ml)	^{125}I-LDL Displaced* (ng/mg membrane protein)
Pig LDL	500	82.9
Human LDL	500	60.3
Human HDL	500	70.3
Human ApoAI	133	27.6
Human ApoAII	392	41.5
HDL Lipids	500	56.1
Phosphatidyl choline	500	86.1
Cholesteryl linoleate	500	4.7
Dextran (MW 100,000-200,000)	500	9.2
Galactose	417	8.4
Glucose	500	0

Incubations were performed as described in Methods. Lipids were prepared by sonication for 2 min in Krebs-Ringer phosphate pH 7.4 buffer, diluted with an equal volume of the same buffer containing 8% bovine serum albumin and incubated 30 min at 24°C before use. Control incubations contained no membranes.

*Values given represent amount of ^{125}I-LDL displaced by indicated amounts of inhibitors. Amounts of LDL and HDL are expressed as lipoprotein protein.

DISCUSSION

The existance of specific, high affinity LDL binding sites on the cell surface was first demonstrated using human fibroblasts grown in culture (1,2). Others have recently performed similar studies in different cell types (10-12). Whole cells in these systems not only bind the lipoprotein but also internalize it. Consequently what is measured as binding includes both binding and uptake (13). It has been shown for example that the lipoproteins taken up in some cells may represent a large fraction of the lipoproteins associated with the cell (14,15).

Table III. Inhibition of ^{125}I-LDL Binding by Lipoproteins and Fetuin in Various Pig Tissue Membrane Preparations

Tissue	^{125}I-LDL Displaced (ng/mg membrane protein)		
	LDL	HDL	Fetuin
Liver	62.6	71.8	0
Kidney	55.8	56.0	0.3
Spleen	44.3	43.8	5.9
Heart	124.1	45.1	0
Lung	56.8	49.7	0
Adrenal	112.0	106.0	32.0
Skeletal Muscle	50.9	47.3	0

Incubations were performed as described in Methods. Numbers represent the difference between ^{125}I-LDL bound in the presence and absence of 500 ug/ml of the indicated protein.

Attempts have been made by others to minimize this effect by working at low temperatures to inhibit processes subsequent to binding but this manipulation also reduces specific binding (11). The use of isolated membranes however allows the separation of binding from subsequent uptake and represents a first step in understanding the LDL-binding site interaction.

The in vivo studies of Sniderman, et al (16,17) indicated that porcine liver was capable of rapidly associating with a large fraction of injected ^{125}I-LDL and, in fact, formed the major extravascular pool of LDL. In addition, the ^{125}I-LDL associated with the liver appeared to be present almost entirely in TCA precipitable form. Their studies suggested a specific interaction between the liver and LDL, but its function is presently unknown. Although porcine liver appears not to be the major catabolic site for LDL (16,17)., these observations suggested

that the pig liver plasma membrane might be useful for investigating LDL-membrane interactions.

The binding of ^{125}I-LDL by pig liver cell membrane fractions satisfied those generally accepted criteria for "specific" binding which can be tested in subcellular fractions, such as time and temperature dependance, saturability by excess substrate, and inhibition by unlabelled LDL but not by unrelated proteins. Furthermore, the apparent Kd for the high affinity association was found to be 11 µg/ml, which agrees well with 10-15 µg/ml found for the specific binding of human ^{125}I-LDL to human fibroblasts (1,13). Pig liver LDL binding capacity is concentrated in subcellular fractions enriched in plasma membranes, although the present data do not exclude the possibility that other cellular organelles may also be capable of binding the lipoprotein.

The inhibition of ^{125}I-LDL binding by pig VLDL was not unexpected since human VLDL has been found to effectively inhibit the binding of human ^{125}I-LDL to human fibroblasts. Nor was it surprising that human LDL effectively displaces pig ^{125}I-LDL, since the displacement of human ^{125}I-LDL from human fibroblasts by pig LDL has been observed (18). LDL from both species apparently share common structural features recognized by the cell membrane. The finding that both pig and human HDL also inhibit LDL binding was not predicted however. The HDL from neither species contains significant amounts of β protein or the "arginine rich" polypeptide. The cell membrane must therefore recognize some other structural feature(s) common to all three lipoprotein classes from man and the pig. The inhibition of LDL binding by the major apoproteins of human HDL and by phosphatidyl choline and human HDL lipids suggest that the binding interaction may involve both lipids and apoproteins. It is noteworthy that pig HDL has been found to inhibit the binding of pig ^{125}I-LDL to pig smooth muscle cells in culture (11) even though it does not mediate suppression of HMG CoA reductase in these cells (3).

The present data suggest that the pig liver membrane "LDL binding site" is actually a "lipoprotein binding site" which recognizes structural features common to all the major lipoprotein classes. The similarity of the LDL binding and displacement patterns of membrane preparations from other pig tissues to that of

liver membranes indicates that the liver is not unique in its lipoprotein binding capability. It should be noted, however, that because of differential tissue fragilities, the absolute magnitude of binding displayed by the various membrane preparations in the present work probably do not accurately reflect the relative abilities of the various tissue membranes to bind LDL.

Detailed studies are currently in progress to identify the lipoprotein structural features which are recognized by the "lipoprotein binding site".

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