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High-affinity binding of lower-density lipoproteins to chicken oocyte membranes

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Oocyte membrane fragments bind specifically radioiodinated VLD lipoprotein (very-low density lipoprotein) and LD lipoprotein (low-density lipoprotein). Competitive binding assays showed 2–3 times more VLD lipoprotein than LD lipoprotein bound at 4°C. Equilibrium-binding data revealed the presence of one class of non-interacting sites for VLD lipoprotein (k_D 12 μg/ml) and co-operative binding for LD lipoprotein. The binding of VLD lipoprotein showed a distinct pH maximum at 5.3, whereas an indistinct maximum at about pH 7.3 was observed for LD lipoprotein. Unlabelled VLD lipoprotein did compete with ^{125}I-labelled LD lipoprotein binding, but unlabelled LD lipoprotein did not compete with ^{125}I-labelled VLD lipoprotein binding. VLD lipoprotein binding was inhibited by HD lipoprotein (high-density lipoprotein), but not by lysozyme, collagen, poly-L-lysine or poly-L-arginine; LD lipoprotein binding was inhibited by lysozyme and collagen, but not by HD lipoprotein. On the basis of these studies, we suggest that: (1) VLD lipoprotein and LD lipoprotein enter the oocytes by a receptor-mediated transport mechanism; (2) the receptors for VLD lipoprotein and LD lipoprotein are distinct; and (3) the binding of LD lipoprotein to chicken oocyte membranes differs from that to other cell types.

The importance of binding of LD lipoprotein to specific cell-surface receptors has been demonstrated with cultured human skin fibroblasts (Brown & Goldstein, 1979). After binding, the LD lipoprotein receptor complex is internalized via coated vesicles (Roth & Porter, 1964; Roth *et al.*, 1976; Anderson *et al.*, 1977). The internalized LD lipoprotein is catabolized in the lysosomes and exerts a regulatory role on cholesterol synthesis and storage.

Electron-microscopic observations have shown that lipoprotein particles, probably VLD lipoprotein and LD lipoprotein, are transferred from blood to the surface of chicken oocytes and incorporated into the cells by a mechanism similar to that which is responsible for the incorporation of LD lipoprotein into fibroblasts, i.e. by adsorptive endocytosis (Perry & Gilbert, 1979). This suggests that a selective transport mechanism is the basis for the transfer. Since the first step in selective protein uptake involves interaction with specific surface receptors, the present study was undertaken to determine whether specific receptors exist for VLD lipoprotein and/or LD lipoprotein on oocyte membranes. Furthermore, since selective protein uptake is particularly manifested in oogenesis, e.g. the uptake of vitellogenin by chicken oocytes (Yusko & Roth, 1976), we decided to investigate whether a selective process included the uptake of VLD lipoprotein and/or LD lipoprotein.

Several lines of evidence implicate VLD lipoprotein as the main carrier of cholesterol and other lipids to chicken oocytes: (1) concomitant with the onset of egg production, plasma VLD lipoprotein increases from a trace amount up to about 2000 mg/100 ml (Schjeide, 1954; Yu *et al.*, 1976); (2) direct transfer of VLD lipoprotein from hen blood to the egg yolk was shown to occur *in vivo* (Holdsworth *et al.*, 1974); (3) a comparison of the lipid content of the various lipoproteins of plasma and yolk indicated that VLD lipoprotein was selectively transferred to oocytes (Gornall & Kuksis, 1973); (4) and VLD lipoprotein isolated from egg yolk was identical immunochemically, electrophoretically and analytically with respect to amino acid content and end-groups with serum VLD lipoprotein (Hillyard *et*

Abbreviations used: VLD lipoprotein, very-low-density lipoprotein ($d < 1.006$); LD lipoprotein, low-density lipoprotein (d 1.006–1.063); HD lipoprotein, high-density lipoprotein (d 1.063–1.210).

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al., 1972). However, LD lipoprotein has also been implicated in lipid transport into the oocytes (Bacon *et al.*, 1973; Jordanov & Boyadjieva-Michailova, 1974; Perry & Gilbert, 1979). It was therefore necessary to include both VLD lipoprotein and LD lipoprotein in the present study in order to determine by which mechanism lipids enter the oocytes.

Materials and methods

Carrier-free $Na^{125}I$ was obtained from Amersham/Searle, Arlington Heights, IL, U.S.A. Tricine {*N*-[2-hydroxy-1,1-bis(hydroxymethyl)-ethyl]glycine}, bovine serum albumin (Cohn fraction V), collagen (Type III) from calf skin, poly-L-arginine hydrochloride (Type III-B), poly-L-lysine hydrobromide (Type I-B) and dextran sulphate (sodium salt) (mol.wts. 40000 and 500000) were purchased from Sigma Chemical Co. Dextran sulphate (potassium salt), heparin (lithium salt) and Aquacide II-A were purchased from Calbiochem–Behring Corp. Lysozyme was purchased from Worthington Biochemical Corp. Bio-Rad Protein Assay Kit was purchased from Bio-Rad Laboratories. Siliclad (Prosil-28) was from PCR Research Chemicals (Gainesville, FL, U.S.A.), Eagle's minimum essential medium, non-essential amino acids solution and frozen chicken serum were from Grand Island Biological Co. Fresh (not frozen) chicken plasma was purchased from Krutulis Laboratories, Bridgeport, NY, U.S.A.

Oocyte membrane fragments

Oocytes (about 2cm in diameter) were obtained from the ovaries of freshly killed White Leghorn hens, either from a local slaughterhouse or from an in-house colony of laying hens. Oocytes were immediately placed in ice-cold Dulbecco's phosphate-buffered saline (Dulbecco & Vogt, 1954), slit, drained of yolk and gently shaken in phosphate-buffered saline to remove adherent yolk. The fragments used for these studies consisted of the oocyte plasma membrane, a perivitelline layer, a monolayer of follicle cells, and the basement membrane. After dissection, the fragments were placed in phosphate-buffered saline at 4°C for 2h. Immediately before incubation, the diced fragments, about 2.0 ± 0.5mm in diameter, were rapidly washed once in phosphate-buffered saline and placed in the incubation medium, which was minimum essential medium supplemented with 20mM-Tricine, pH 7.3, 1% (v/v) non-essential amino acids and lipoprotein-deficient chicken serum (2.5mg of protein/ml). Similar medium has been used in the study of LD lipoprotein binding to various cell types (Brown & Goldstein, 1974; Goldstein *et al.*, 1976, 1979).

When binding was studied as a function of pH, the pH of the incubation medium was adjusted with 1M-NaCl or -NaOH. pH was measured at 4°C and rechecked over a period of several hours, during which period the pH remained constant. The washes after incubation were performed with buffer B (see under 'Binding studies') of the appropriate pH.

Lipoproteins and lipoprotein-deficient serum

The lipoproteins were prepared by standard techniques from fresh chicken plasma containing Na_2EDTA (1mg/ml) by sequential ultracentrifugation (Havel *et al.*, 1955; Chapman *et al.*, 1977). HD lipoprotein was isolated from rooster plasma. Lipoprotein-deficient serum was prepared from frozen chicken serum with solid KBr at 1.210g/ml (Radding & Steinberg, 1960). After centrifugation for $3 \times 10^6 g$-h and re-centrifugation, lipoprotein-deficient serum was stored at −20°C, whereas the lipoproteins were kept at 4°C.

The lipoproteins and lipoprotein-deficient serum were dialysed for at least 36h at 4°C against three changes, each of at least 50vol., of 0.15M-NaCl/0.3mM-Na_2EDTA, pH 7.3.

The concentrations of lipoproteins and lipoprotein-deficient serum were determined by the Bio-Rad protein assay with albumin as a standard, after determining that the assay gave results comparable with those obtained by the method of Lowry *et al.* (1951).

The purity of the lipoproteins was evaluated by electrophoresis in agarose gel (Noble, 1968) and in 3%-polyacrylamide gel (Masket *et al.*, 1973).

Iodination

A sample (1.5mg) of either VLD or LD lipoprotein was iodinated with 5mCi of $Na^{125}I$ by the ICl method of McFarlane (1956). Unbound iodine was separated by dialysis. The final specific radioactivity was about 6×10^8c.p.m./mg for LD lipoprotein and 2.5×10^8c.p.m./mg for VLD lipoprotein. In these preparations, 98% of the radioactivity was precipitated with 15% (w/v) trichloroacetic acid.

Binding studies

Membrane fragments (about 30mg wet wt.) were incubated in Siliclad-treated glass tubes with 1ml of incubation medium containing either ^{125}I-labelled VLD lipoprotein or ^{125}I-labelled LD lipoprotein with and without the respective unlabelled lipoprotein as indicated. The fragments were incubated for the indicated time at 4°C with shaking at 80 oscillations/min. Incubation was terminated by centrifugation for 15000*g*-min at 4°C, and they were washed with 4×2ml of buffer B (0.15M-NaCl/50mM-Tris/HCl, pH 7.3) containing 2mg of albumin/ml, followed by two more washes of 2ml each with buffer B containing no albumin (Goldstein *et al.*, 1976, 1979). Radioactivity of the tubes and the fragments was determined by γ-radiation spectrometry.

Specific binding was determined as the difference between ^{125}I-labelled lipoprotein bound in the presence of labelled lipoprotein alone and that bound in the presence of labelled lipoprotein and unlabelled lipoprotein at a concentration more than 50 times the k_D. Each assay was performed in triplicate.

Release of bound ^{125}I-labelled VLD lipoprotein and ^{125}I-labelled LD lipoprotein

After radioactivity counting, the fragments were incubated for 1h at 4°C on a shaker with 1ml of buffer C (50mM-NaCl/20mM-Tricine, pH 7.3) containing dextran sulphate (sodium salt), dextran sulphate (potassium salt) or heparin (lithium salt). Incubation was terminated by centrifugation for 15000*g*-min at 4°C. The supernatants were removed by aspiration, counted for radioactivity and compared with ^{125}I-labelled lipoprotein bound in the presence of labelled lipoprotein alone initially, which represented total binding.

Protein content of the fragments

After the binding was determined, 0.1ml of 10M-NaOH was added to each tube overnight at room temperature. The protein content was determined as described by Lowry *et al.* (1951) after the addition of 0.9ml of distilled water to each tube. Albumin, dissolved similarly, was used as a standard. Typically the mean value of the protein content of the fragments in any experiment was $278 \pm 16\mu g$ (±S.E.M.).

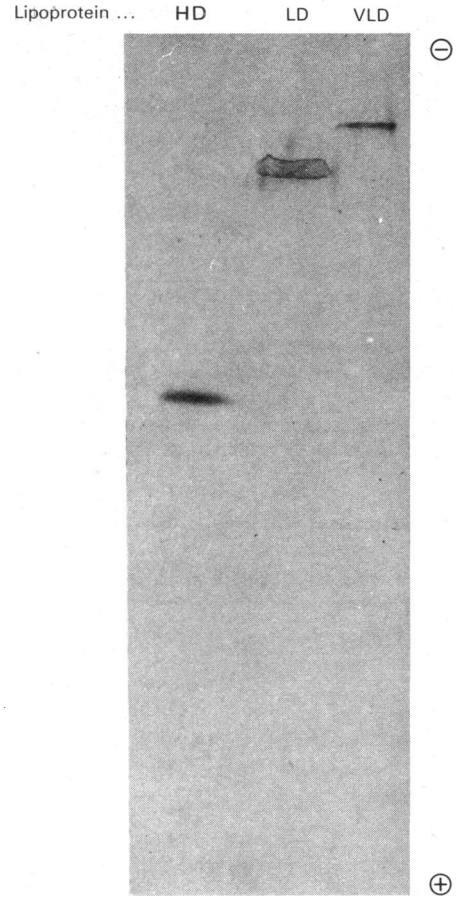

Fig. 1. *Polyacrylamide-gel electrophoresis of plasma HD, LD and VLD lipoproteins*

Each sample contained 25μg of lipoprotein prestained with Sudan Black. The separating gel was 3% (w/v) acrylamide; the upper loading gel was 2.5% acrylamide.

Results

Purity of lipoproteins

A typical run in polyacrylamide gel is shown in Fig. 1. VLD lipoprotein did not enter the 3% separating gel, but remained at the interphase between the 2.5% loading gel and the separating gel. HD lipoprotein moved further away from the origin than did LD lipoprotein. On agarose electrophoresis, the lipoproteins moved as described by Hearn & Bensadoun (1975).

Time course of binding

The time course of total and specific binding for ^{125}I-labelled VLD lipoprotein and ^{125}I-labelled LD lipoprotein is shown in Fig. 2. Since specific binding was maximal after 30min, this period was used routinely. Further studies are necessary to explain the decrease in binding with time of incubation. It should be pointed out, however, that it is not likely to be due to degradation of tissue, in that 15 and 30min incubation of fragments with ^{125}I-labelled LD lipoprotein at the end of the time-course study gave the same high amounts bound as at the beginning.

Consistently, more VLD lipoprotein bound specifically than did LD lipoprotein (85 and about 43% of total binding respectively).

pH optima

VLD lipoprotein showed a clear maximum in specific binding at pH5.3 (Fig. 3). The specific binding of LD lipoprotein varied only slightly from pH 4.3 to 8.3, with a minor peak at pH 7.3.

Temperature-dependence

The amounts of VLD lipoprotein specifically bound were not significantly different at 4 and 20°C, whereas specifically bound LD lipoprotein increased from 0.62 ± 0.31 (S.E.M.) at 4°C to $1.00 \pm 0.30\mu g$/mg of protein at 20°C. All experiments were

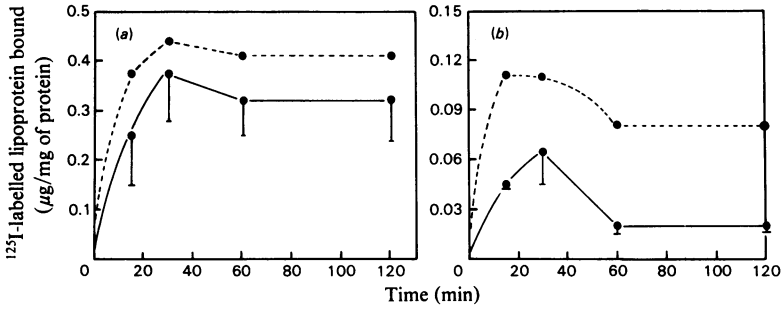

Fig. 2. *Time course of total (----) and specific (——) binding of (a) ^{125}I-labelled VLD lipoprotein at 10μg/ml (363 c.p.m./ng) and (b) ^{125}I-labelled LD lipoprotein at 2μg/ml (72 c.p.m./ng) with and without 500μg of unlabelled VLD lipoprotein/ml or 100μg of unlabelled LD lipoprotein/ml at pH 7.3 and 4°C*

After incubation, the fragments were washed as described in the Materials and methods section. Each value is the mean for triplicate determinations; the S.E.M. is shown for specific binding.

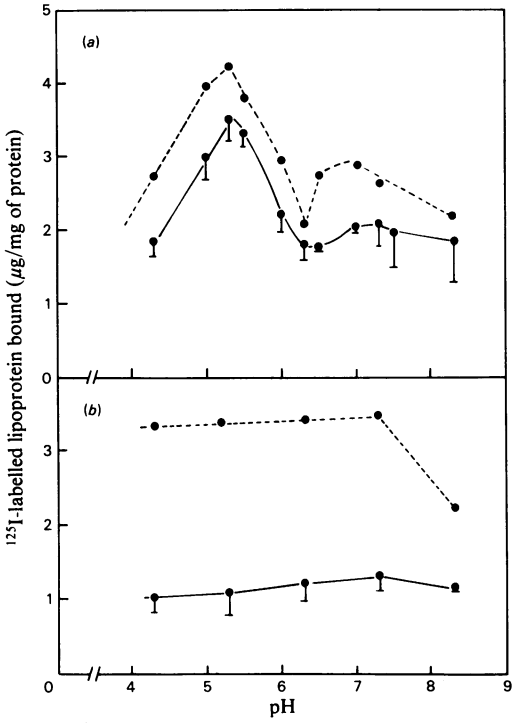

Fig. 3. *Total (----) and specific (——) binding of (a) ^{125}I-labelled VLD lipoprotein at 20μg/ml (64 c.p.m./ng) and (b) ^{125}I-labelled LD lipoprotein at 10μg/ml (302 c.p.m./ng) to membrane fragments as a function of pH*

The fragments were incubated with and without 750μg of unlabelled VLD lipoprotein/ml or 375μg of unlabelled LD lipoprotein/ml for 30min at 4°C, followed by washings as described in the Materials and methods section. Each value is mean of triplicate determinations; ±S.E.M. is shown for specific binding.

conducted at 4°C to minimize any endogenous enzymic activities that might be enhanced at higher temperatures (Williams, 1979).

Concentration-dependence

Specific binding of VLD lipoprotein (Fig. 4*a*) and of LD lipoprotein (Fig. 4*d*) showed saturation.

To determine reversibility of binding, the fragments were first incubated with either 7.5μg of ^{125}I-labelled VLD lipoprotein/ml or 3.8μg of ^{125}I-labelled LD lipoprotein/ml for 30min. Then half of the tubes received unlabelled lipoprotein at 100 times the k_D, and the other half received lipoprotein-deficient serum at a comparable protein concentration. After incubation for another 30min, 20% less ^{125}I-labelled VLD lipoprotein was bound in the presence of unlabelled VLD lipoprotein than in the presence of lipoprotein-deficient serum. This suggests that 20% of initially bound ^{125}I-labelled VLD lipoprotein was released and possibly exchanged with unlabelled VLD lipoprotein. No decrease in the binding of ^{125}I-labelled LD lipoprotein was observed under similar conditions.

A Scatchard analysis of the binding of VLD lipoprotein suggested the existence of one class of binding sites (Fig. 4*b*) (Scatchard, 1949). The k_D was 12μg/ml or 24nM, based on 10% protein of a total mol.wt. of 5×10^6 (Yu *et al.*, 1976). The intersect of the line with the abscissa gave 1.4μg/mg of protein as the maximum amount of VLD lipoprotein specifically bound.

The concave-downward curvature of the Scatchard plot of the data for LD lipoprotein suggested co-operative binding (Fig. 4*e*), for which k_D varies with amount bound. When the binding data for ^{125}I-labelled LD lipoprotein concentrations greater than 4μg/ml were replotted by the 'Wilkin-

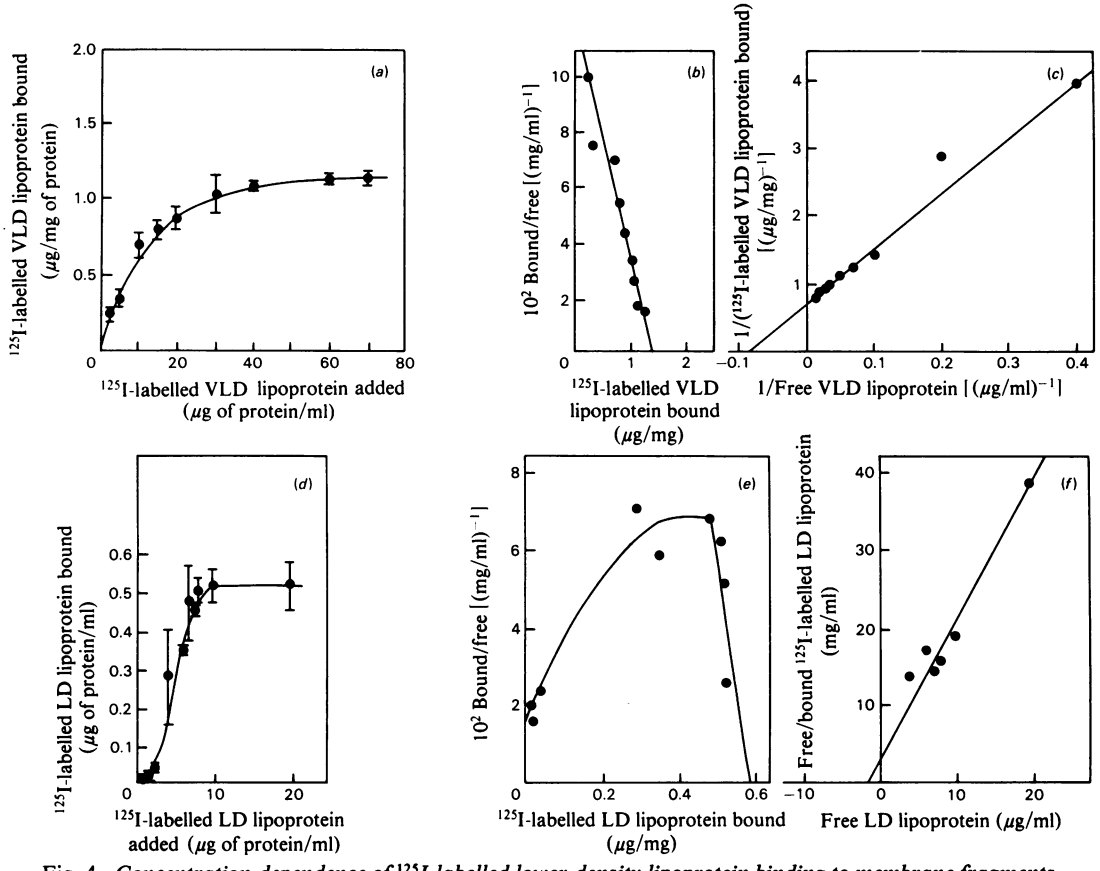

Fig. 4. *Concentration-dependence of ^{125}I-labelled lower-density lipoprotein binding to membrane fragments*

(*a*) Specific binding at the indicated concentrations of ^{125}I-labelled VLD lipoprotein (246c.p.m./ng) incubated with and without 750μg of unlabelled VLD lipoprotein/ml for 30min at 4°C, followed by washings as described in the Materials and methods section. (*b*) Scatchard plot and (*c*) double-reciprocal plot of specific binding of ^{125}I-labelled VLD lipoprotein. (*d*) Specific binding at the indicated concentrations of ^{125}I-labelled LD lipoprotein (417c.p.m./ng) incubated with and without 500μg of unlabelled LD lipoprotein/ml under the same conditions as for VLD lipoprotein. (*e*) Scatchard plot and (*f*) 'Wilkinson inversion' plot of specific binding of ^{125}I-labelled LD lipoprotein. In (*f*), binding data obtained at concentrations greater than 4μg/ml were included in the plot. From (*a*) and (*d*), maximal binding was achieved at about 30μg of ^{125}I-labelled VLD lipoprotein/ml and 10μg of ^{125}I-labelled LD lipoprotein. Each value is mean for triplicate determinations; ± S.E.M. is shown for the values in (*a*) and (*d*).

son inversion' (Gardiner & Ottaway, 1969), as suggested by Rodbard (1973) for co-operative binding, the k_D derived from the intersect of the line with the abscissa was 2μg/ml or 3.4nM, assuming a mol.wt. of 3×10^6, of which 20% is protein (Fig. 4*f*) (Yu *et al.*, 1976). The maximum amount of LD lipoprotein specifically bound, derived from the slope of the Wilkinson plot, was almost 0.6μg/mg of protein.

Iodination

To determine whether iodination alters the binding properties of VLD lipoprotein, we compared the binding of ^{125}I-labelled VLD lipoprotein at one tenth of its k_D value with increasing concentrations of unlabelled VLD lipoprotein (Freychet, 1976), i.e. at varying specific radioactivity (Fig. 4*c*), with that at constant radioactivity (Fig. 4*a*). Analysis by double-reciprocal plots (Fig. 4*c*) gave the same k_D and maximum binding values as those obtained from Scatchard analysis.

Specificity of binding

Indications of the specificity of the putative receptor(s) are shown in Table 1, in which either ^{125}I-labelled VLD lipoprotein or ^{125}I-labelled LD lipoprotein was incubated with potential competitors.

The dependence of binding on bivalent cations was studied with and without EDTA. When 2mM-EDTA was present, specific binding of VLD lipoprotein decreased by 50% and that of LD lipoprotein by 65%.

Release of ^{125}I-labelled VLD lipoprotein and ^{125}I-labelled LD lipoprotein

The percentages of bound lipoproteins released by various sulphated dextran compounds are shown in Table 2.

Discussion

We wished to investigate the interaction of VLD lipoprotein and LD lipoprotein with the oocytes, with the aim of elucidating the mechanism by which these lipoproteins are taken up by the cells. The first step in receptor-mediated adsorptive endocytosis is the interaction with specific membrane components. We give in the present paper evidence of the existence of such components for VLD lipoprotein and LD lipoprotein. The interaction of the lipoproteins with these components exhibits saturability, high affinity, specificity and, in the case of VLD lipoprotein, reversibility. On the basis of the different reactivity of VLD lipoprotein and LD lipoprotein at different pH values, temperatures, concentrations and in the presence of the potential competitors, we postulate that VLD lipoprotein and LD lipoprotein interact with distinct sites. However, VLD lipoprotein is capable of interacting with the same sites as LD lipoprotein, but not vice versa.

In the hen, LD lipoprotein contains principally apoprotein B, whereas VLD lipoprotein contains two major apoproteins, apoprotein B and apoprotein VLDL-II (Chapman *et al.*, 1977; Williams, 1979). Since (1) LD lipoprotein is not competing with VLD lipoprotein binding and (2) assuming that binding occurs via the apoprotein as in the fibroblast system (Mahley *et al.*, 1977), VLDL-II apparently confers the specificity of VLD lipoprotein binding. Furthermore, since HD lipoprotein inhibits the binding of VLD lipoprotein, apoprotein VLDL-II or an apoprotein with similar binding characteristics is apparently present in HD lipoprotein. When it is considered that apoprotein VLDL-II is of low molecular weight, the corresponding apoprotein in HD lipoprotein appears to be apoprotein C (Raju & Mahadevan, 1976).

Since (1) apoprotein B is common to both VLD lipoprotein and LD lipoprotein and (2) VLD lipoprotein competes with the binding of LD lipoprotein, it appears that apoprotein B confers the specificity of the LD lipoprotein binding, as is the case with human skin fibroblasts (Mahley *et al.*, 1977). However, when the characteristics of LD lipoprotein binding to chicken oocyte membrane fragments and human skin fibroblasts are compared,

Table 1. *Specificity of ^{125}I-labelled lower-density lipoprotein binding*

The ability of a 50-fold molar excess of unlabelled compounds to inhibit the binding of either 15 μg of ^{125}I-labelled VLD lipoprotein (237 c.p.m./ng)/ml or 7.5 μg of ^{125}I-labelled LD lipoprotein (478 c.p.m/ng)/ml was determined. After simultaneous incubation for 30 min at 4°C, the medium was removed by centrifugation and the membrane fragments were washed by a standard procedure. Each value is the mean ± S.E.M. for triplicate determinations. Poly-L-lysine enhanced the binding of VLD lipoprotein about 3-fold and that of LD lipoprotein about 5-fold. Unlabelled VLD- and HD lipoprotein inhibited the binding of ^{125}I-labelled VLD lipoprotein 76 and 35% respectively. Unlabelled VLD lipoprotein and lysozyme inhibited the binding of ^{125}I-labelled LD lipoprotein by 60%; unlabelled LD lipoprotein and collagen inhibited the same binding by 40% and poly-L-arginine by 10%.

Unlabelled compound	Mol.wt.	VLD lipoprotein bound (μg/mg of protein)	LD lipoprotein bound (μg/mg of protein)
None		3.4 ± 0.9	1.0 ± 0.2
VLD lipoprotein	5.0×10^6	0.8 ± 0.2	0.4 ± 0.0
LD lipoprotein	3.0×10^6	3.4 ± 1.3	0.6 ± 0.2
HD lipoprotein	1.7×10^5	2.2 ± 0.6	1.1 ± 0.2
Collagen	2.9×10^5	3.9 ± 0.6	0.6 ± 0.1
Lysozyme	1.5×10^4	3.4 ± 0.3	0.4 ± 0.1
Poly-L-arginine	1.2×10^5	3.2 ± 0.5	0.9 ± 0.1
Poly-L-lysine	5.0×10^5	11.1 ± 0.5	5.4 ± 0.3

Table 2. *Release of ^{125}I-labelled lower-density lipoproteins by different dextran sulphates*

The membrane fragments were incubated with either 15 μg of ^{125}I-labelled VLD lipoprotein (320 c.p.m./ng)/ml or 7.5 μg of ^{125}I-labelled LD lipoprotein (383 c.p.m./ng)/ml. After the standard washing procedure followed by incubation with dextran sulphate for 1 h at 4°C, the tubes were centrifuged and the supernatants were removed and counted for radioactivity. Each value is the mean for triplicate determinations.

Dextran sulphate	Mol.wt.	Lipoprotein released (% of total binding) ... VLD	LD
Sodium salt (10 mg/ml)	40000	27	6
	500000	9	1
Potassium salt (10 mg/ml)	Not known	20	6

the binding reactions appear to differ in several ways. In LD lipoprotein binding to fibroblasts, basic amino acid residues, e.g. lysine and arginine, inhibit binding (Brown *et al.*, 1978). In contrast, LD lipoprotein binding to oocytes is not inhibited by arginine and is enhanced by lysine. A possible explanation for the enhancement is that lysine may act to 'cross-link' LD lipoprotein with components on the membrane fragments other than their binding sites (Jacobson & Branton, 1977), and the absence of inhibition by arginine shows that this amino acid does not play the same role in binding as it does with fibroblasts (Weisgraber *et al.*, 1978). It is therefore possible that apoprotein B is not the LD lipoprotein component responsible for the binding. An alternative explanation may be that chicken apoprotein B is different from human apoprotein B in the way it binds to the membrane fragments. Differences between the two types have been shown (Chapman *et al.*, 1977).

Irreversibility in LD lipoprotein binding is shared by the human fibroblast system (Goldstein & Brown, 1977) and the chicken oocyte system. In the fibroblast system, the LD lipoprotein–receptor complex is internalized. Although internalization by membrane fragments does not occur, the irreversible binding indicates that the initial interaction is similar in the two systems.

To explain the absence of pH-dependence for LD lipoprotein binding, the high degree of non-specific binding of LD lipoprotein must be considered. Since non-specific binding of LD lipoprotein constitutes a large part of total binding, it is possible that the non-specific pH-independent binding (Basu *et al.*, 1978) obscures the pH-dependent specific binding.

An important property of the LD lipoprotein receptor on fibroblasts is that LD lipoprotein can be dissociated from it by incubation with sulphated polysaccharides and glycosaminoglycans (Goldstein *et al.*, 1976). This property is shared by the binding sites on the oocytes for both VLD- and LD lipoprotein. However, the amounts released are much less (less than 10% of total binding of LD lipoprotein, compared with about 80% for the fibroblasts). Table 2 shows the amounts released by sulphated polysaccharides. Heparin (results not shown) resulted in the same small amounts being released. The relatively low release by dextran sulphate from oocytes may be due to the formation of lipoprotein–dextran sulphate complexes and the subsequent binding of the complexes to sites on the fragments other than the binding sites for the lipoprotein, as observed with mouse macrophages (Basu *et al.*, 1979).

Although the LD lipoprotein binding shares some characteristics with LD lipoprotein binding to cultured human skin fibroblasts (Brown & Goldstein, 1979) and other cell types (Brown *et al.*, 1977), the avian oocyte system has revealed new characteristics of LD lipoprotein binding that were not shared by VLD lipoprotein. These new characteristics may be due to the fact that the interaction of LD lipoprotein with a mixed population of membrane fragments differs from that with intact cells in culture.

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