Chicken yolk contains bona fide high density lipoprotein particles

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Abstract Lipoproteins, the major nutrient source for developing embryos in egg-laying species, are thought to be transported from the circulation of the hen to the yolk of growing oocytes. In order to fully understand the contribution of the different lipoprotein species to oocyte growth, yolk formation, and embryo development, we have started to elucidate the relationships between the high density lipoproteins (HDL) in serum with the hitherto uncharacterized yolk HDL fraction. Immunoblotting with antibodies against apolipoprotein (apo) A-I, the major protein moiety of circulating HDL, revealed, for the first time, significant amounts of this protein in yolk. Importantly, yolk apoA-I was an integral component of bona fide lipoprotein particles: *i)* the apoA-I-containing particles could be purified by ultracentrifugal flotation and immunoaffinity chromatography on immobilized anti-apoA-I IgG; *ii)* the particles resembled serum HDL in ultrastructural, chemical, and biochemical aspects; and *iii)* in particular, these particles contained another major apolipoprotein, apo II. To date, apo II has been assumed to be unique to the very low density lipoprotein (VLDL) and HDL fractions of laying hen serum. Its residence on yolk HDL particles, together with the other results, strongly implies that yolk HDL, at least to a large part, is derived from serum. This implication is supported by the presence of apoA-I in oocytic coated vesicles. However, an oocyte plasma membrane receptor for the transport of HDL could not be identified; furthermore, immunoelectron microscopy demonstrated that yolk HDL particles do not colocalize with VLDL, known to be endocytosed via a specific receptor. Thus, these studies have revealed that HDL particles are taken up into the oocyte from the serum of the laying hen, and are deposited into the yolk by a mechanism distinct from that involved in the uptake of other yolk lipoproteins.—**Vieira, P. M., A. V. Vieira, E. J. Sanders, E. Steyrer, J. Nimpf, and W. J. Schneider.** Chicken yolk contains bona fide high density lipoprotein particles. *J. Lipid Res.* 1995. **36:** 601–610.

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In oviparous (egg-laying) species, the yolk of the oocyte contains nutrients necessary for development of the embryo. Most of the yolk components are derived from plasma precursors that are synthesized in the liver under the control of estrogen. In the chicken, the major yolk precursors are the lipoproteins, very low density lipoprotein (VLDL), and vitellogenin (VTG). The plasma levels of VLDL and VTG increase dramatically at the onset of egg laying in hens, and upon estrogen treatment of roosters (1, 2). Apolipoprotein (apo) B, the major protein component of VLDL, is strongly induced by estrogen at both the transcriptional and translational levels (3). Estrogen also changes the apolipoprotein composition of VLDL by inducing the synthesis of apo VLDL-II (apo II) (4), a small homodimer of disulfide-linked subunits with 82 amino acid residues each (5). Apo II has been demonstrated only in mature female birds where it associates with all lipoprotein fractions. It has no known structural homologue in mammals; however, in analogy to mammalian apoC-III, apo II inhibits endothelial lipoprotein lipase (6). This inhibition has been proposed as the mechanism responsible for the transport of intact triglyceride-rich VLDL particles, rather than lipolyzed derivatives thereof, into the oocytes (6).

The specific and efficient uptake by growing oocytes of the yolk precursors, VLDL and VTG, from the plasma occurs via receptor-mediated endocytosis. The key molecule is a 95-kDa receptor that is specifically expressed in oocytes and is responsible for the uptake of both lipoproteins (7–12). The 95-kDa VLDL/VTG receptor has been shown to recognize apoB of VLDL (13) and the lipovitellin domain of VTG (14), and to belong to the

Abbreviations: apo, apolipoprotein; BNHS, N-hydroxysuccinimidobiotin; ECL, enhanced chemiluminescence; HDL, high density lipoprotein; HRP, horseradish peroxidase; LDL, low density lipoprotein; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBS, Tris-buffered saline; TCA, trichloroacetic acid; VLDL, very low density lipoprotein; VTG, vitellogenin.

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low density lipoprotein (LDL) receptor family (15, 16).

In contrast to the well-studied contribution of VLDL and VTG to yolk formation, that of high density lipoprotein (HDL) has not yet been investigated. This is surprising in the light of the fact that laying hens, while producing large amounts of VLDL, also have considerable levels of plasma HDL (17, 18). In roosters it is the main lipoprotein fraction (19), and high levels of HDL are found after hatching (20). These substantial HDL levels in the chick presumably arise from both massive de novo synthesis (20, 21) and yolk utilization by the embryo. Chicken apoA-I (28 kDa), the main protein component of HDL, is highly homologous to its mammalian counterpart (22–25). ApoA-II, another known component of mammalian HDL fractions, or a structural homologue thereof, has not been demonstrated in the chicken (26).

Plasma levels of apoA-I in roosters, in contrast to those of apo II, are apparently refractory to estrogen treatment (27). In mature hens, plasma levels of HDL have been reported to be lower than in immature birds, but such analysis may be skewed by the dramatically elevated levels of VLDL in such animals (18). Nevertheless, estrogen has an effect on the lipid and apolipoprotein composition of chicken HDL: the HDL particles of laying hens or estrogenized roosters harbor apo II and are enriched in triglycerides (2, 18).

Early studies on egg yolk composition have suggested the presence of small amounts of a high density lipoprotein component in the oocyte (28). The exact nature and composition of this fraction, however, were not determined. Antibodies against the unfractionated egg yolk high density material showed crossreactivity with plasma HDL and VLDL (29), but in the absence of apolipoprotein analysis these data remained obscure.

Here we have identified, isolated, characterized, and ultrastructurally localized bona fide HDL particles present in chicken oocytes. The data are consistent with the notion that a mechanism different from that for VLDL is involved in the targeting of HDL from serum to yolk.

EXPERIMENTAL PROCEDURES

Experimental animals

White Leghorn laying hens and roosters were obtained from Heindl Co. (Vienna, Austria) and maintained as previously described (7). New Zealand White rabbits used for production of antibodies were obtained from the "Forschungsinstitut für Versuchstierzucht und Tierhaltung," Himberg, Austria.

Preparation of antibodies

Polyclonal antibodies were raised in rabbits by repeated subcutaneous injections of the purified antigens. For the first injection, the antigens were mixed with Freund's complete adjuvant (Sigma); for subsequent injections, Freund's incomplete adjuvant was used. For anti-apoB antibodies, apolipoproteins from purified laying hen VLDL were separated by 4.5–18% gradient SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The gel was stained with 4 M sodium acetate and apoB was isolated from the stained gel slice by electroelution. For anti-apoA-I antibodies, HDL was isolated from rooster plasma as described previously (13), and the intact lipoprotein particles were used for immunization. Anti-apo VLDL II was prepared against the purified apolipoprotein as described earlier (13). IgG fractions were purified from rabbit sera on protein-A Sepharose CL-4B (Pharmacia) (30).

Preparation of lipoproteins

VLDL from laying hen plasma (31) and yolk (32) and HDL from rooster and laying hen plasma (13) were isolated by sequential ultracentrifugation as described. HDL from laying hen plasma was additionally purified by immunoaffinity chromatography using an anti-apoA-I column: the polyclonal antibody was coupled to CNBr-activated Sepharose 4B (Pharmacia) according to manufacturer's instructions using a ratio of 20 mg of IgG per gram of dry gel. The ultracentrifuged lipoprotein sample was dialyzed against phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4) prior to the application to the column. After extensive washing with equilibration buffer (PBS) the bound proteins were eluted with 100 mM glycine/1 M acetic acid, pH 3, into tubes containing 250 μl 1 M K_2HPO_4. The protein peak was detected by measuring the absorbance at 280 nm and the peak fractions were dialyzed against PBS prior to analysis.

For the purification of apoA-I-containing particles from yolk, yolk from laid eggs was diluted with 4 vol of PBS and centrifuged for 15 min at 9,000 rpm (SS-34 rotor, Sorvall) to remove insoluble material. The supernatant was ultracentrifuged overnight at 100,000 *g* to remove yolk VLDL. The content of the bottom half of the tube was collected, adjusted to d 1.20 g/ml with KBr and ultracentrifuged at 100,000 *g* for 48 h. The top 3 ml was collected, adjusted to a density of 1.20 g/ml with KBr and applied to the bottom of a 14 × 95 mm tube (Beckmann). A step gradient was formed by overlaying the sample solution with 3 ml each of KBr solutions with the densities of 1.12, 1.08, and 1.00 g/ml (top). The centrifugation was performed in a SW-40.1 rotor (Beckmann) at 39,000 rpm overnight at 4°C. One-ml fractions were collected and analyzed by SDS-PAGE. The apoA-I-containing fractions were dialyzed overnight against PBS and subjected to immunoaffinity chromatography on an anti-apoA-I column as described for the purification of HDL from laying hen serum.

Biotinylation of lipoproteins

For the biotinylation of HDL and VLDL, 0.15 μmol N-hydroxysuccinimidobiotin (BNHS; Sigma) in 2.5 μl dimethylformamide per mg of lipoprotein was added to the lipoproteins in PBS (33). The mixture was incubated at room temperature for 1 h. Free BNHS was removed by dialysis against Tris-buffered saline (TBS; 150 mM NaCl, 50 mM Tris/HCl, pH 7.5). The success of the biotinylation reaction was tested by probing the immobilized ligands on nitrocellulose with streptavidin-HRP (Amersham; diluted 1:1000), followed by the chemiluminescence detection using the ECL-kit (Amersham) according to the supplied protocol.

Gel electrophoresis and Western blotting

One-dimensional gradient (4.5–18%) SDS-PAGE was performed according to Laemmli (34) using a minigel system (Bio-Rad, Mini-Protean™ II Slab Cell). Electrophoresis was performed at 180 V for 60 min. Samples containing 50 mM dithiothreitol were heated at 95°C for 5 min. Broad range M_r standards (Bio-Rad) were used. Protein bands were stained with Coomassie Brilliant blue and gels were destained with 10% acetic acid. Electrophoretic transfer of the proteins to nitrocellulose (Bio-Rad, pore size 0.45 μm) was performed in transfer buffer (26 mM Tris, 192 mM glycine) for 2 h at 200 mA, on ice, using the Bio-Rad Mini Transblot system. The transferred proteins were stained with 0.2% Ponceau S in 3% (w/v) TCA and destained with water. Western blotting was performed using Protein-A-HRP (Sigma) followed by a chemiluminescence detection method (ECL-system; Amersham) according to the manufacturer's instructions. Nitrocellulose membranes were exposed for 0.1–5 min on Hyperfilm™-ECL (Amersham). Protein concentrations were determined according to Lowry et al. (35).

Ligand blotting

For ligand blotting experiments, oocyte membrane extracts were prepared exactly as described previously (7). After SDS-PAGE (16 μg of membrane protein/lane) under non-reducing conditions, the proteins were transferred to nitrocellulose as described above. For blocking, 5% (w/v) bovine serum albumin in ligand blot buffer (20 mM Tris, 90 mM NaCl, 2 mM $CaCl_2$, pH 8.0) was used. The biotinylated ligand (10 μg/ml) was added in the blocking buffer and the incubation was performed for 90 min. After washing with ligand blot buffer, streptavidin-HRP (Amersham; 1:1000) was used in an ECL reaction to detect the bound ligand.

Preparation of coated vesicles

Ovaries were dissected from laying hens and placed in ice-cold buffer (0.1 M 2(*N*-morpholino)ethane sulfonic acid, 1 mM EGTA, 0.5 mM $MgCl_2$, 3 mM NaN_3, 1 mM phenylmethyl sulfonylfluoride, 5 μM leupeptin, pH 6.5). Follicles of 1–8 mm diameter were removed from the ovary, cut free of thecal cell layers, and coated vesicles were prepared using a low-sucrose method (36). Vesicles prepared in this fashion have previously been characterized both structurally and biochemically, and have been shown to be true clathrin-coated vesicles with fewer than 2% of non-coated vesicular structures (12, 37).

Electron microscopy

For the analysis of the size and appearance of the HDL particles studied here, the HDL solutions (100 μg/ml) were transferred to activated carbon-coated copper grids, washed twice by placing the grids on top of a drop of deionized water, and stained with sodium phosphotungstate (1%, w/v). Samples were examined in a Philips EM 300 electron microscope with an instrumental magnification of 35,500 ×. For the localization of apoA-I in the yolk, immunogold cytochemistry was performed as described previously (12) using the rabbit anti-apoA-I IgG as primary antibody.

Lipid analyses

Total and free cholesterol levels were determined using the enzymatic CHOD-iodide-method test kit (Merck, Germany). Enzymatic determination of phospholipids and triglycerides was performed with test kits (Bio-Merieux, France) as recommended by the manufacturer.

RESULTS

When rooster serum, laying hen serum, and yolk were analyzed by immunoblotting with anti-apoA-I IgG, an antigen of identical electrophoretic mobility was detected in all three samples (**Fig. 1**). Its apparent molecular mass corresponded to that of apoA-I (28 kDa). No additional bands were visualized, and non-immune IgG did not reveal any reactive band (not shown). The concentration of apoA-I in the yolk was in the same order of magnitude as that in the serum of the laying hen (cf. legend to Fig. 1). The novel finding that apoA-I is present in the yolk prompted us to further investigate the nature of the immunoreactive material.

In order to determine whether, like its counterpart in the circulation, yolk apoA-I was associated with lipoprotein particles, we isolated the apoA-I-containing fraction from yolk. After initial ultracentrifugation of yolk at its own density, VLDL was removed from the top of the tube, and the bottom half, which contained all of the detectable apoA-I, was adjusted to d 1.20 g/ml. In the subsequent ultracentrifugation, apoA-I floated to the top of the tube. This suggested that yolk apoA-I was present in lipoprotein particles with relatively high density. The apoA-I

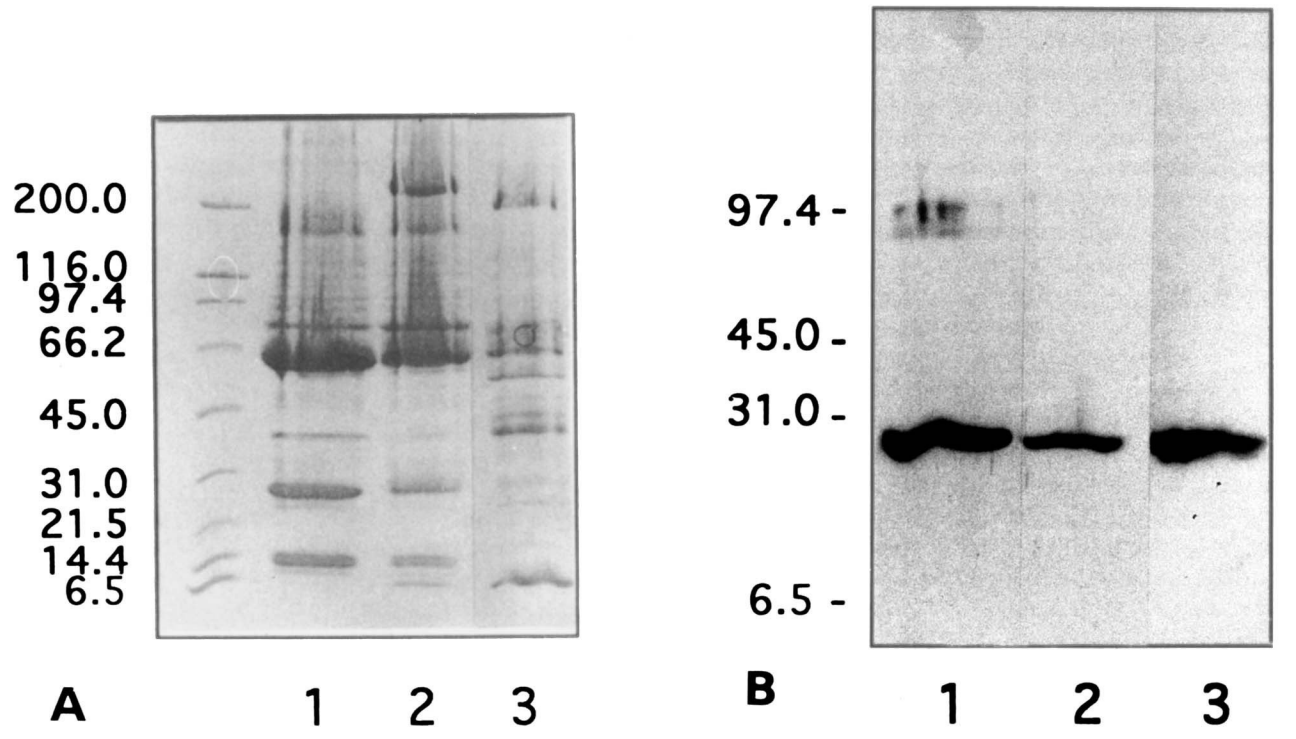

Fig. 1. Analysis of chicken serum and yolk for the presence of apolipoprotein A-I. SDS-PAGE and Coomassie blue staining (A) and immunoblotting of serum and yolk samples with anti-apoA-I IgG (8 μg/ml) (B) were performed as described in Experimental Procedures. Lanes 1: 1 μl rooster serum; lanes 2: 1 μl laying hen serum; lanes 3: 1 μl of yolk previously diluted with 4 vol of PBS. In (B), lanes 1 and 2 were exposed for 10 sec and lane 3 for 2 min. The positions of migration of M_r standards (kDa) are shown on the left.

fraction from this step was further subjected to density gradient ultracentrifugation, which separated the bulk of remaining apoB-containing particles (such as VLDL and possibly LDL) from those particles harboring apoA-I, as determined by immunoblotting (**Fig. 2**). As previously reported (32, 38), apoB of oocytic VLDL (termed yolk VLDL) becomes postendocytotically proteolyzed into distinct fragments (range, approx. 220 kDa to 40 kDa); the immunoreactive fragments can be seen in Fig. 2, panel A. As expected, apoB accumulates in the top fractions of the density gradient, and apoA-I at substantially higher density (Fig. 2, panel B); the major apoA-I-containing peak was present at a density of 1.15 g/ml. A few density gradient fractions appeared to contain both apolipoproteins. The purification of HDL from laying hen serum using the same ultracentrifugation protocol also resulted in overlapping of apoB and apoA-I in some fractions (data not shown). To determine whether such preparations contain a mixture of apoB-containing particles with unusually high density as well as true HDL particles, these fractions from yolk and laying hen serum were subjected to anti-apoA-I immunoaffinity chromatography as described under Experimental Procedures. The bound lipoproteins were eluted and their apolipoprotein composition was analyzed by SDS-PAGE (**Fig. 3**) and immunoblotting (**Fig. 4**). The major protein band of 28 kDa in the HDL preparations from both laying hen serum (Fig. 3, lane 2) and yolk (Fig. 3, lane 3) corresponded to apoA-I in the rooster lipoprotein (Fig. 3, lane 4). Furthermore, the size of the smaller protein in the two samples from the laying hen corresponded to the known molecular weight of apo II. No other protein bands corresponding to known apolipoproteins were detected in any of the samples.

To further characterize the HDL fractions, we performed immunoblot analysis with antibodies against apoA-I, apoB, and apo II. The major band of the yolk particles was confirmed to be apoA-I (Fig. 4A); a higher M_r band recognized by the anti-apoA-I antibody in all three samples is most likely a dimeric form of apoA-I (39). The lower M_r bands seen in laying hen serum and yolk HDL (cf. Fig. 3) were confirmed to be apo II, which migrates as two bands on SDS-PAGE under reducing conditions ((13), Fig. 4C). In agreement with previously published results (13), rooster HDL was devoid of apo II. ApoB could not be detected in any of the laying hen HDL preparations (Fig. 4B, lanes 2 and 3), demonstrating the successful removal of apoB-containing material on the anti-apoA-I affinity column. Rooster HDL prepared by ultracentrifugation was also free of apoB (Fig. 4B, lane 1).

The apoA-I-containing particles from yolk, rooster and laying hen serum were further studied by electron microscopy to compare the relative size and shape of the

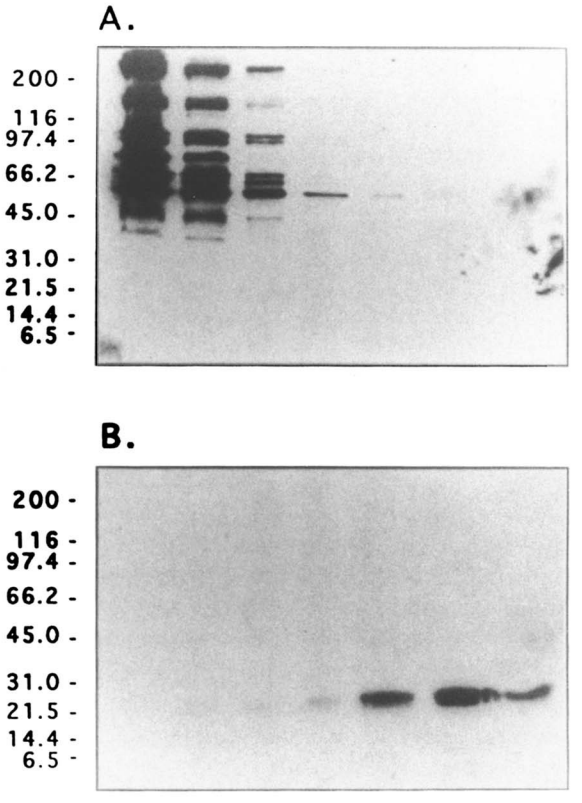

Fig. 2. Immunoblotting analysis of the distribution of apolipoproteins B and A-I after density gradient centrifugation of yolk. Each lane contains 5 μl of successive gradient fractions obtained as described in Experimental Procedures. Lanes from left to right represent seven fractions from the top to the bottom of the ultracentrifuge tube, with densities of 1.006, 1.022, 1.044, 1.082, 1.123, 1.172, and 1.220 g/ml, respectively. Immunoblotting was performed with 8 μg/ml of anti-apoB IgG (A) and 8 μg/ml of anti-apoA-I IgG (B) and developed by the ECL method. Exposure was for 10 sec. The positions of migration of *M*, standards (kDa) are shown on the left.

different HDL (**Fig. 5**). All three preparations appeared as spherical particles with an average size of approximately 10 nm, similar to that of the typical mammalian HDL particles (40, 41). Discs or discoidal particles were not observed. Next, the chemical composition of the three apoA-I-containing particles was determined. The affinity-purified yolk particle was shown to have a composition compatible with being a bona fide HDL fraction, as it did not differ significantly from that of rooster and laying hen serum HDL (**Table 1**). The main components of the yolk HDL were protein (33%), cholesteryl ester (26%), and phospholipid (23%). The significant amount of cholesteryl ester in the HDL yolk fraction prompted us to examine the composition of total yolk, as an earlier study (28) reported the absence of cholesteryl esters from yolk. Our analysis showed that cholesteryl esters represented 1.3% of the yolk mass, presumably contributed to a large extent by the HDL fraction.

We also used immunoelectron microscopy with a gold-labeled anti-apoA-I antibody to localize the HDL particles in yolk. The storage compartment within the yolk consists of membrane-delimited organelles, the so-called yolk spheres (42). These pseudo-endosomes reach a diameter of 140 μm, and contain within them an electron-lucent phase with interspersed electron-dense granular substructures (42). Previously, VLDL particles that are endocytosed by a specific receptor in the plasma membrane of the oocyte (9) have been demonstrated, together with the receptor, in the electron-lucent phase of yolk spheres (12, 15). In contrast, yolk HDL particles were found to be concentrated in the electron-dense granules (**Fig. 6**), which are thought (43) to consist mainly of storage forms of VTG, itself a very high density lipoprotein.

The data to this point strongly suggested that yolk HDL is mainly derived from the serum HDL pool via uptake by the oocyte, a cell with very high endocytic activity (12, 42). To obtain further support for this notion, we prepared clathrin-coated vesicles from follicles and analyzed

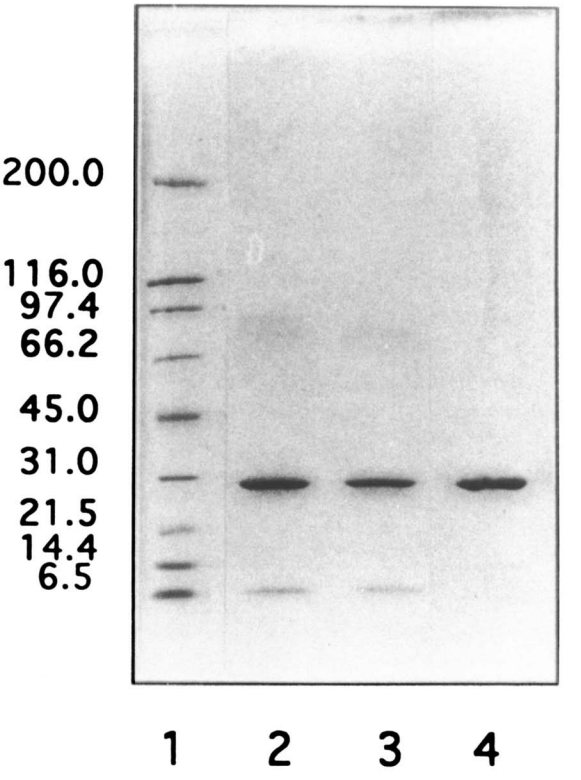

Fig. 3. SDS-PAGE analysis of purified chicken apoA-I-containing lipoproteins. Laying hen serum and yolk HDLs were purified by ultracentrifugation followed by immunoaffinity chromatography, and rooster HDL was purified by ultracentrifugation as described in Experimental Procedures. Aliquots of the isolated fractions (5 μg protein) were subjected to SDS-PAGE followed by Coomassie blue staining. Lane 1, *M*, standards (kDa); lane 2, laying hen serum; lane 3, yolk, and lane 4, rooster serum.

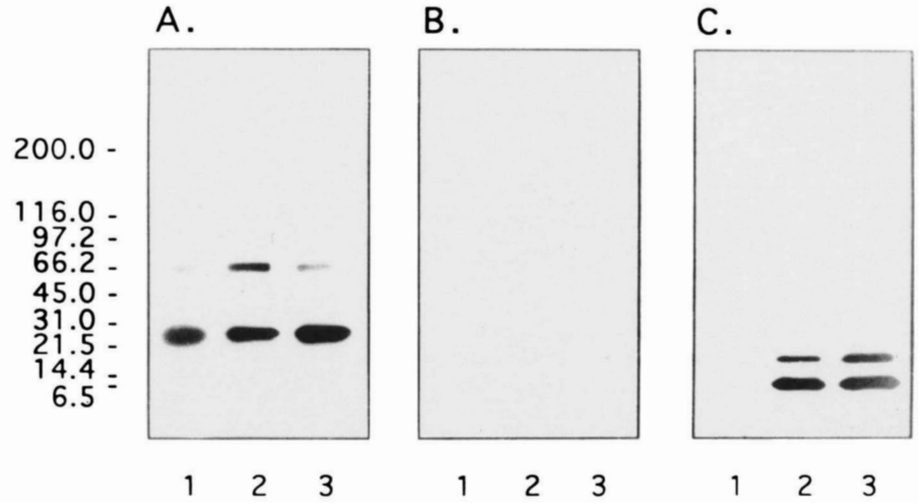

Fig. 4. Immunoblot analysis of chicken HDL for the presence of apolipoproteins A-I, B, and II. The lipoproteins were isolated as described in the legend to Fig. 3. Immunoblotting was performed with 8 μg/ml of anti-apoA-I IgG (A), 8 μg/ml of anti-apoB IgG (B), and 8 μg/ml of anti-apo II (C). The HDL samples (1 μg/lane) were derived from rooster serum (lanes 1); laying hen serum (lanes 2); and yolk (lanes 3). Exposure of the ECL-developed blots was 10 sec for (A) and (C), and 90 sec for (B). The positions of migration of *M*, standards (kDa) are shown on the left.

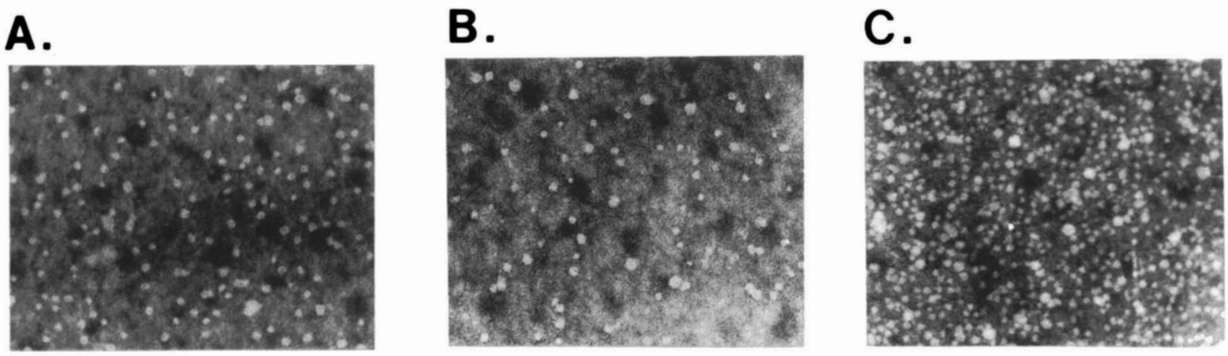

Fig. 5. Ultrastructural analysis of chicken high density lipoproteins. HDL-particles from rooster serum (A), yolk (B), and laying hen serum (C) were prepared as described in the legend to Fig. 3 and analyzed by negative-stain electron microscopy as described in Experimental Procedures. Magnification, × 131,000.

TABLE 1. Chemical composition of chicken apoA-I-containing lipoproteins and total egg yolk

Source	Protein	Triglycerides	Phospholipids	Free Cholesterol	Cholesteryl Esters
Total yolk	33	42	19	4.7	1.3
Laying hen HDL	34	32	15	4	15
Yolk HDL	33	11	23	7	26
Rooster HDL	31	10	24	5	30

Laying hen serum and yolk HDL were prepared by ultracentrifugation and subsequent immunoaffinity chromatography, and rooster HDL was prepared by ultracentrifugation as described in Experimental Procedures. The data are presented as percentage of weight and represent the average of duplicate determinations.

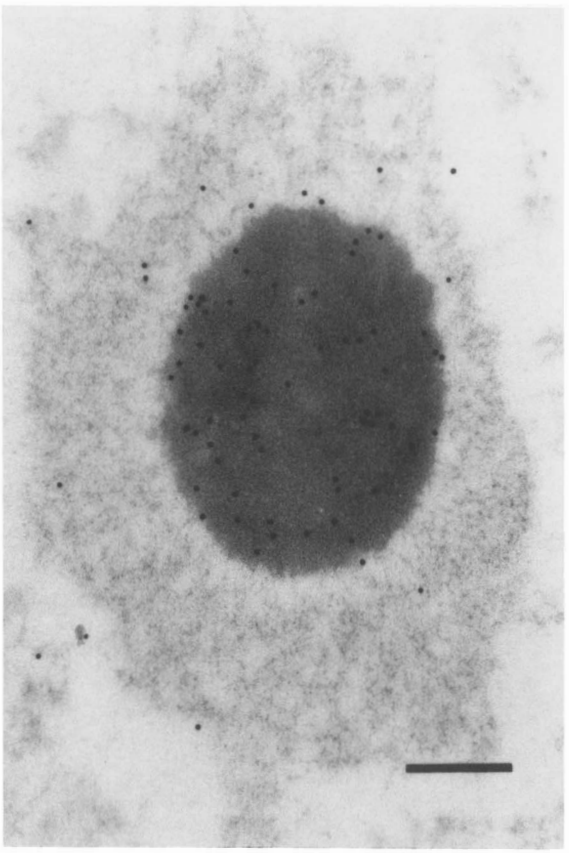

Fig. 6. Immunocytochemical localization of apoA-I in yolk. Yolk was subjected to immunogold (15 nm gold particles) cytochemistry with anti-apoA-I IgG as described in Experimental Procedures. ApoA-I is shown to be concentrated within the electron-dense granules. Magnification, ×46,100. The bar represents 300 nm.

them for the presence of apoA-I by immunoblotting. Indeed, such coated vesicles contained the antigen (**Fig. 7**). VLDL has been shown previously to be endocytosed via a specific plasma membrane receptor into the oocyte (9); colocalization of apoA-I and VLDL in the coated vesicle preparation strongly suggested that yolk HDL is derived from the same compartment as VLDL, i.e., the serum, and is at least transiently present in coated vesicles.

Is the uptake of HDL into the oocyte a receptor-mediated process? We tested whether the yolk apoA-I-containing particles also interact with the oocytic 95-kDa receptor known to mediate the endocytosis of the other yolk lipoprotein precursors, VLDL and VTG (9, 15). For this purpose ligand blot experiments were performed with biotinylated ligands. Oocyte membrane extracts containing the VLDL/VTG receptor were incubated with yolk HDL and, for control purposes, with VLDL from yolk and laying hen serum. The results (**Fig. 8**) demonstrate that HDL does not bind to the VLDL/VTG-receptor or to any other protein present in the oocyte membrane extracts under ligand blotting conditions optimized for lipoprotein ligand binding. In experiments not shown, we have applied different ligand blotting conditions, chemical crosslinking methodology, or affinity chromatography of oocyte membrane extracts on immobilized HDL, but did not identify any molecule with membrane receptor characteristics.

DISCUSSION

Two major components in the yolk of the chicken oocyte, VLDL and VTG, have been well characterized in terms of their receptor-mediated uptake (9, 15), intracellular degradation (32, 38) and localization (12). Earlier studies on the composition of egg yolk have suggested the presence of a small fraction of high density, lipid-containing components in the yolk (28). Immunological similarity of this fraction to both VLDL and HDL from plasma has been reported (29). These somewhat contradictory data could not be explained, because the high density yolk component(s) was not purified or characterized further. Our present studies provide the first identification of the

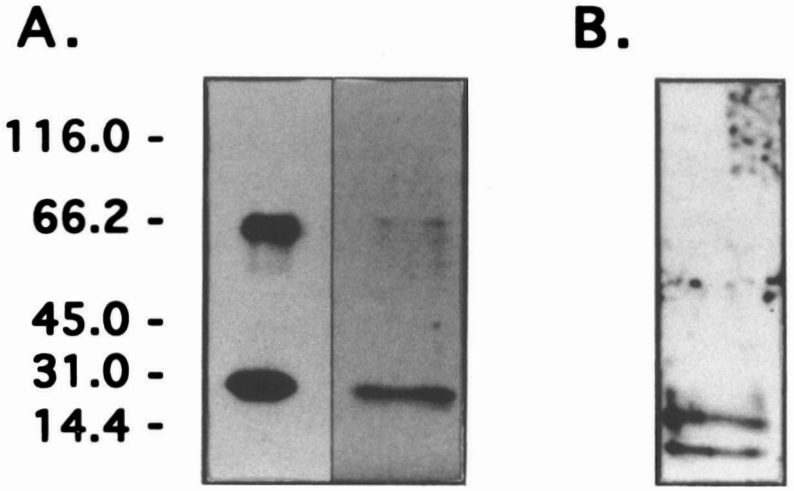

Fig. 7. Immunoblot analysis of oocytic clathrin-coated vesicles for the presence of apoA-I and apo II. In (A), anti-apoA-I IgG (8 μg/ml) was used to probe 1 μg of laying hen serum HDL as control (left lane), and 25 μg of coated vesicle protein (right lane). For the higher M_r band, refer to the text and Fig. 4A. In (B), anti-apo II IgG (8 μg/ml) was used to probe 30 μg of coated vesicle protein. Exposure of the ECL-developed blots was 20 sec for serum HDL and 5 min for the lanes containing coated vesicles. The positions of migration of M_r standards (kDa) are shown on the left.

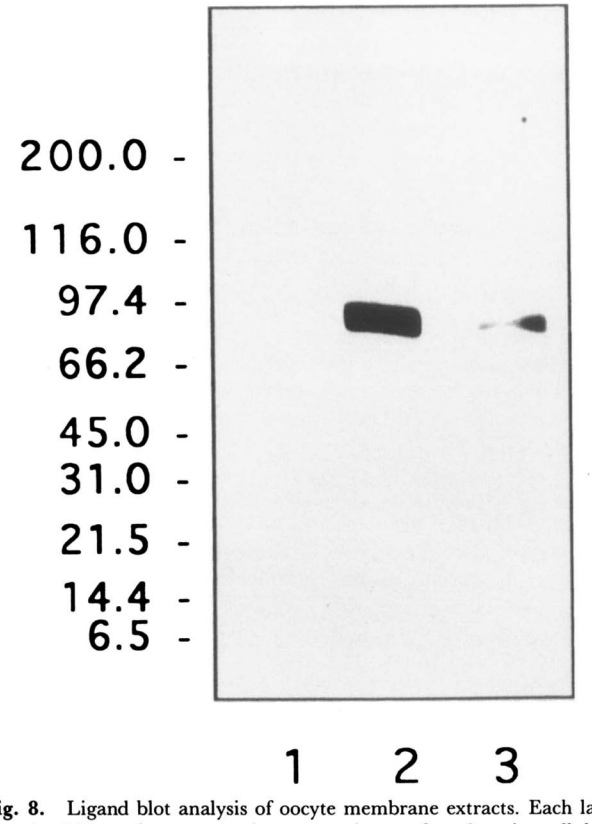

Fig. 8. Ligand blot analysis of oocyte membrane extracts. Each lane contained 16 μg of oocyte membrane protein transferred to nitrocellulose following SDS-PAGE as described in Experimental Procedures. The following biotinylated ligands were used at a concentration of 10 μg/ml: yolk HDL (lane 1), yolk VLDL (lane 2), and laying hen plasma VLDL (lane 3). The ECL-developed blots were exposed for 5 min. The positions of migration of M_r standards (kDa) are shown on the left.

origin, purification, and characterization of the HDL component in the chicken oocyte, and reveal the basis for previous findings.

First, we show that purified yolk HDL, like circulating HDL in the laying hen, contains two apolipoproteins, apoA-I and apo II, and is devoid of apoB. The presence of apo II is consistent with the previous report (29) of crossreactivity between the yolk fraction and plasma VLDL, which carries the bulk of this apolipoprotein, together with apoB. Ultrastructural analysis of yolk HDL demonstrated that the particles were similar in size and shape to the serum HDL particles (Fig. 5). Their chemical analysis (Table 1) also provided data supporting that yolk HDL is a bona fide lipoprotein fraction.

Interestingly, it has been previously reported that neither yolk HDL nor total egg yolk contain any cholesteryl esters (28). Our experiments, however, showed the existence of cholesteryl esters in both the total yolk and yolk HDL (Table 1). In part, this discrepancy may be due to different analytical methods as well as to the fact that we analyzed a highly purified HDL fraction, which represented a small part of the so-called high density fraction examined earlier (28). We conclude that yolk HDL is a significant contributor to the cholesteryl ester pool of yolk. The chemical composition of purified rooster and laying hen serum HDLs resembled those published earlier (28, 44).

These data suggested that yolk HDL is derived from the serum compartment or, possibly, from a pool of HDL secreted from nurse cells present in the follicle, by endocytosis across the oocyte's plasma membrane. Indeed, oocyte clathrin-coated vesicles, which carry both receptor-bound ligands and fluid-phase-borne (i.e., serum-derived) components into cells, contained HDL. Accumulation of apo II, present mainly on VLDL but also on HDL as determined here, in coated vesicles has also been shown previously by gold-label immunocytochemistry (12). Despite colocalization of VLDL and HDL in coated vesicles, within the yolk storage compartments, HDL-apoA-I is found in different substructures than the yolk VLDL. Namely, most of the apoA-I is localized in the electron-dense granules of the storage yolk spheres (Fig. 6), whereas only a small proportion of yolk apo II is present in these granules (12). The bulk of apo II antigen, representing VLDL, is in the electron-lucent phase (12). Hence, HDL and VLDL take different routes to their respective destinies after initially travelling together. In this respect, it is important to note that the oocytic VLDL/VTG receptor is also concentrated in the electron-lucent phase of yolk spheres where VLDL, but not HDL, resides (15).

This previously characterized 95-kDa receptor, a member of the LDL-receptor family (16), is known to be involved in the binding and uptake of the major yolk precursors, VLDL and VTG (9). In concordance with the findings described above, yolk HDL does not bind to this receptor or to any other membrane protein under a variety of conditions (Fig. 8, and data not shown). This is consistent with the earlier finding that neither rooster nor laying hen plasma HDL bind to the oocytic 95-kDa receptor (13). The lack of interaction between yolk HDL and the VLDL/VTG receptor or other receptors also discounts the possibility that the yolk HDL represents an enriched pool of a minor serum HDL species, specifically endocytosed by this receptor(s). It is interesting to note, however, that the chemical composition of the HDL fraction of yolk more closely resembles that of rooster serum HDL than that of hen (Table 1). If a specific receptor(s) does not operate, this may indicate postendocytic modification of the lipid moiety of HDL, as suggested earlier (28).

Thus, the uptake of HDL occurs most likely via mechanism(s) and pathway(s) distinct from that for the major yolk lipoproteins, VLDL and VTG. At present, there is insufficient knowledge about yolk compartmentalization and endocytic vesicle dynamics to make firm conclusions about intracellular ligand itineraries based on the different distributions of VLDL and HDL in yolk spheres. Our present results, however, may indicate a different intra-

oocytic routing of endocytosed, receptor-bound components, such as VLDL, from those that are potentially derived from receptor-independent fluid-phase uptake, such as HDL.

The function(s) of the HDL in the oocyte's yolk is unknown. Considering the inability of the avian ovary to synthesize lipids, yolk HDL might be a source, in particular, for phospholipids in the very demanding biosynthesis of surface membrane in the growing oocyte or in the very early stages of embryo development. Its localization in the compartment of yolk that appears to be phagocytosed by early embryonic cells (38; and cf. ref. 12), might point to a source of lipid for utilization by these cells. The relatively high content of cholesteryl esters suggests that they could serve as building blocks for lipoproteins prior to the expression of cholesterol esterifying enzyme(s) by the embryo. Further experiments are necessary to determine how HDL functions in oocyte growth and/or contributes to the overall nutrient content of the oocyte for utilization by the developing embryo.

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