Interaction of cholesterol ester and triglyceride in human plasma very low density lipoprotein[1]

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Abstract The properties of human plasma very low density lipoproteins (VLDL), low density lipoproteins (LDL), and their extracted lipids were compared using calorimetric, X-ray scattering, and polarizing microscopy techniques. Intact LDL, and cholesterol esters isolated from LDL and VLDL each undergo reversible changes in their physical state around body temperature. These transitions are associated with ordered liquid crystalline to liquid phase changes of the cholesterol esters. In contrast to LDL, VLDL has no reversible transitions and shows no evidence of ordered liquid crystalline structures between 10 and 45°C. Therefore, unlike LDL, VLDL does not contain a separate cholesterol ester region capable of undergoing cooperative melting. Solubility studies at 37°C of cholesterol esters and triglyceride isolated from VLDL show that even at a weight ratio of 1:1, which greatly exceeds the relative amount of cholesterol esters in VLDL, cholesterol ester is completely soluble in triglyceride. Thus, the cholesterol ester in VLDL is not sequestered in a separate domain within VLDL, but is dissolved in the liquid core of the particle.

Supplementary key words low density lipoprotein · lipoprotein structure · lipid phase behavior · scanning calorimetry

Chylomicrons and very low density lipoproteins (VLDL) are triglyceride (TG)-rich particles that contain B-apoprotein and cholesterol ester (CE). TG and CE in these particles are catabolized, at least in some animals, by dissimilar pathways (1–4). TG is removed mainly in extrahepatic tissues, whereas CE is taken up by the liver (1–4). Coincident with TG removal from chylomicrons and VLDL in peripheral tissue, a smaller cholesterol-rich remnant is formed (5) that is rapidly removed by the liver (3). While the details of metabolism of the individual chemical constituents of the remnant are not completely understood, in rats only about 10% of B-apoprotein and less than 5% of CE of low density lipoprotein (LDL) are derived from VLDL (6,7). In contrast, in man nearly all VLDL B-apoprotein becomes associated with LDL (8). This fact suggests that human VLDL may give rise to LDL via the intermediate formation of remnants.

If LDL is a catabolic product of VLDL, is it formed by selective removal of TG and phospholipid to leave a particle rich in CE and B protein? Or is it possible that intact LDL exists within, or fused to, VLDL and is released during catabolism of the VLDL particle?

We have shown that intact LDL has a CE-rich core surrounded by the polar lipid and protein constituents (9). This core undergoes a reversible transition near body temperature due to an ordered liquid crystalline–liquid[4] phase change of CE within the intact LDL. This transition results from the melting of adjacent CE molecules and probably accounts for the induced circular dichroism of carotenoids in LDL observed between 37 and 2°C (10). Using calorimetry, X-ray scattering, and polarizing microscopy, we have searched for a CE-rich region in VLDL. We have not found such a region and conclude that CE is dissolved in a liquid TG core of VLDL. Thus, during formation of LDL from VLDL, there is probably a selective concentration of CE and B protein as other components are removed during catabolism of VLDL.

METHODS

Preparation and analysis of lipoproteins

Plasma was separated from blood (in 1 mg EDTA/ml) of normolipemic male donors after a 15–16 hr

Abbreviations: VLDL, very low density lipoprotein(s), LDL, low density lipoprotein(s); TG, triglyceride(s); CE, cholesterol ester(s); TLC, thin-layer chromatography; DSC, differential scanning calorimetry.

[1] A part of this work has been published in abstract form (21).

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[4] Liquid crystals are the intermediate phases existing between the crystalline solid and the liquid state of some organic compounds. Liquid crystals have some properties of a crystal (e.g., long range order of molecules) and some of a liquid (e.g., fluidity).

fast. VLDL at plasma density d 1.006 and LDL between salt densities d 1.025–1.050 g/ml were isolated by repetitive ultracentrifugation in a Beckman 40.3 rotor (Spinco Div., Palo Alto, CA) for 18 hr at 40,000 rpm at 13°C as previously described (9). Lack of contamination with serum proteins and other lipoproteins was confirmed by immunodiffusion and immunoelectrophoresis (11). VLDL was concentrated by centrifugation at d 1.050. The concentrated VLDL (4–10 g/dl) was then dialyzed to d 1.006 in 0.19 M NaCl and 0.1% EDTA pH 8.5. Samples of VLDL or LDL requiring further concentration (15–20 g/dl) were dialyzed and concentrated simultaneously in a collodion vacuum dialysis apparatus (Schleicher and Schuell, Inc., Keene, NH). Lipoprotein concentrations were determined by protein content (12) and duplicate dry weight determinations. Experiments on lipoproteins were performed immediately after preparation.

Lipid analyses and preparation

Lipids were extracted from lipoproteins using at least 20 volumes of chloroform–methanol 2:1 (v/v) (13). Analysis of lipid composition was performed by quantitative thin-layer chromatography (TLC) (14) as modified by Katz, Shipley, and Small (15). In five VLDL preparations, TG constituted 60.4 ± 4.3 (mean ± SEM) weight percent of the total lipids; CE 14.2 ± 2.9%; free cholesterol 6.4 ± 1.0%; and phospholipids 19.0 ± 1.4%. Protein was assumed to constitute 8% of the weight of the particle (16). LDL lipids (from six preparations) contained 2.7 ± 0.2% TG; 55.8 ± 1.4% CE; 11.9 ± 0.2% free cholesterol; and 29.5 ± 3.0% phospholipid, with apoprotein contributing 20.8 ± 0.8% of the particle weight. Individual lipid classes were isolated by preparative TLC using 20 × 20 cm plates coated with silica gel G (Merck, Darmstadt, Germany) in the solvent system hexane–ether 96:4, eluted with chloroform–methanol 2:1 and stored at −20°C under nitrogen. Before use, solvents were evaporated under a nitrogen stream and further dried in a vacuum desicator at room temperature for 24 hr.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) measures the difference in power input necessary to maintain equal heating or cooling rates for both sample and inert reference. On cooling, for example, if the sample crystallizes, heat is given off and less heat must be applied to the sample to maintain equal temperature with the reference (equal to the heat of fusion). This difference in power input causes a deviation from baseline and the area under the resulting peak is proportional to the enthalpy (ΔH) of the heat of fusion. If no transition occurs in the sample, a straight baseline is recorded. Further, transitions will only be observed if there are domains of like molecules large enough to permit cooperative melting or crystallization.

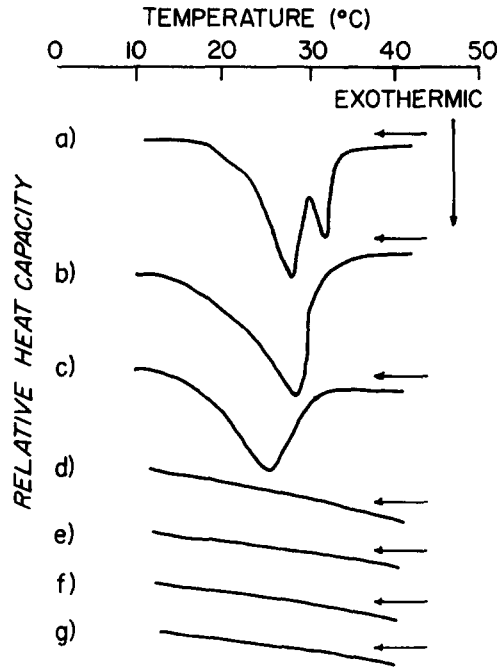

Fig. 1. Differential scanning calorimetry cooling curves of LDL, VLDL, and lipids extracted from VLDL. The cooling rate was 5°C/min at a full scale sensitivity of 0.1 mcal/sec, except b and c which were at 0.2 mcal/sec. Number in parentheses refers to mg of cholesterol ester in sample. (a) VLDL cholesterol ester 100% (0.42); (b) VLDL cholesterol esters with 6.4% VLDL triglyceride (1.07); (c) LDL (0.72); (d) VLDL (0.74); (e) VLDL triglyceride 100%; (f) VLDL triglyceride with 21% VLDL cholesterol ester (0.32); (g) VLDL triglyceride with 48% VLDL cholesterol ester (0.71).

Five preparations of VLDL and six of LDL were studied with a Perkin-Elmer DSC-2 differential scanning calorimeter (Perkin-Elmer Corp., Norwalk, CT). On three occasions, both VLDL and LDL were obtained from the same donors. Ten to 70 μl of lipoprotein solutions varying in concentration from 30 to 210 mg/ml were sealed in sample pans and an equivalent volume of buffer solution sealed in reference pans. Isolated VLDL and LDL lipids were placed in sealed pans and studied over a similar temperature range. Mixtures of CE and TG were prepared by weighing the individual components into sample pans and equilibrating at 55°C, a temperature at which both lipids are melted. All samples were run at least in duplicate and temperature runs were repeated a minimum of twice with consistent results.

X-Ray scattering/diffraction

Three VLDL preparations were studied at concentrations of 75–170 mg/ml. Results were compared with that previously obtained for LDL (9). Specimens were sealed in 1 mm diameter Lindemann glass tubes and placed in a variable temperature sample holder. X-Ray scattering/diffraction patterns were recorded with a focusing Elliott toroidal mirror optics, utilizing nickel filtered CuKα radiation from an Elliott GX-6 rotating anode generator, with exposure times of 3–12 hr.

Polarizing light microscopy

TG and CE extracted from lipoproteins were examined by direct light and between crossed polarizers on a Zeiss NL standard microscope equipped with a controlled heating and cooling stage (17). To ensure homogeneous sampling, mixtures of the two lipids previously run on the calorimeter were taken directly from the DSC pans at 55°C and placed on microscope slides.

RESULTS

DSC cooling curves[5] of VLDL, LDL, and their extracted lipids are shown in **Fig. 1.** CE isolated from LDL (Fig. 1a) demonstrated the two typical liquid–liquid crystal transitions of cholesterol esters (17): the liquid to cholesteric transition and the cholesteric to smectic phase transition.[6] As in pure CE model systems,[7] the addition of small amounts of VLDL-TG abolished the cholesteric transition in VLDL-CE and broadened the smectic transition (Fig. 1b) so that the shape is similar to that seen in intact LDL (Fig. 1c). Although addition of small amounts (4–12%) of TG changed the shape of the transition, the total enthalpy (1.0 ± 0.1 cal/g CE, mean ± 1 SD, n = 4) is similar to pure CE (1.0 ± 0.1 cal/g CE, n = 5). The lower enthalpy (0.7 ± 0.1 cal/g CE, n = 6) observed in LDL reflects limitations imposed on the CE by the LDL particle structure (9). Liquid–liquid crystalline transitions were not seen (Fig. 1d) in any of the five VLDLs studied, although the VLDL samples contained amounts of CE equivalent to LDL samples. TG extracted from VLDL showed no transitions on cooling from 45 to 10°C (Fig. 1e). Also, mixtures of CE and TG at the same weight ratio as in VLDL (Fig. 1f) or higher (Fig. 1g) showed no liquid–liquid crystal transitions.

X-Ray scattering studies were performed on VLDL at 10°C, a temperature at which LDL always shows a strong maximum corresponding to a Bragg spacing of 36 Å **(Fig. 2a).** This characteristic spacing at 36 Å is associated with an ordered liquid crystalline state of the CE in intact LDL (9). In contrast, although VLDL has been studied with CE concentrations up to 22 mg/ml, it showed no evidence of liquid crystal structure in that a 36 Å maximum is not seen (Fig. 2b). LDL, studied with CE concentrations as low as 5 mg/ml clearly showed the 36 Å spacing.

To determine if the amount of CE present in VLDL could be solubilized in the TG of VLDL at body temperature, mixtures of VLDL-CE and VLDL-TG containing up to 48% of the total weight as CE were examined by hot stage polarizing microscopy. Mixtures were heated to 45°C and cooled to 37°C. All were observed to be isotropic liquid solutions at 37°C. No change in state occurred at this temperature during 21 days of observation. Similar mixtures were cooled to 10°C at a rate of 1°C/min and, within 1 hr after cooling, all mixtures grew birefringent CE crystals directly from the liquid. On reheating to 37°C the crystals again melted. Since crystallization does not occur over long periods at 37°C and crystals previously precipitated by cooling melt by 37°C, the equilibrium physical state of VLDL-CE up to a 1:1 weight ratio to TG at 37°C is a liquid solution in which CE is dissolved in liquid TG.

DISCUSSION

In this study we provide evidence that CE in human VLDL are not sequestered in a separate domain within the particle. If such a domain existed, we would expect a similar liquid–liquid crystal transition in VLDL as in LDL, as VLDL particles contain

[5] Cooling rather than heating runs were used in comparing liquid crystalline phase behavior of isolated cholesterol esters with intact lipoproteins. The liquid crystalline phases of most cholesterol esters are monotropic with respect to the crystalline phase, that is, they have a lower transition temperature than the melting point of the crystal and only appear when the melt is cooled below the melting point of the crystal (17). The liquid crystalline phases in isolated lipoprotein cholesterol esters are also monotropic. Further, they are unstable, and true crystals often form from the liquid crystals shortly after the latter phase appears. Thus heating runs may show transitions representing combined melts of both true crystals and liquid crystals and obscure true liquid crystalline behavior. Furthermore, the cooling runs are adequate to characterize liquid–liquid crystal transitions of cholesterol ester since these transitions show little, if any supercooling (17).

[6] In cholesteric liquid crystals, the cholesterol ester molecules are ordered in helices about an axis at right angles to the long axis of the molecules, whereas in the smectic liquid crystalline state, planar arrays of cholesterol esters are regularly stacked with a periodicity of 36 Å.

[7] Small, D. M. Unpublished work.

at least as many CE molecules as LDL particles.[8] Also, VLDL shows no 36 Å maximum characteristic of the CE liquid crystal domain of LDL. Thus, LDL does not exist as a separate entity within or fused to VLDL. The amount of CE per VLDL sample was more than sufficient to allow for observation of CE liquid crystal transitions with the calorimeter used. In fact, a cooperative transition involving only 20% of the CE molecules would have been easily detectable.

Model systems of CE and TG have proved useful in predicting the phase behavior of lipids found in lipoproteins. At 37°C cholesteryl oleate is soluble in triolein up to a weight ratio of 1:3 (17). VLDL-CE is even more soluble in VLDL-TG at 37°C, at least up to a weight ratio of 1:1 which far exceeds the CE/TG ratio in intact VLDL (1:4.3). Thus, because CE is not sequestered in a separate domain in VLDL, as it is in LDL, it is probably dissolved in VLDL core TG.

While most of the TG is removed when rat VLDL is catabolized to remnant or intermediate density lipoproteins, there is a relatively small change in the number of cholesterol ester molecules per particle (5, 20). How much of this CE is found in LDL in man is not known. Faergeman and Havel suggest that, although only 3.4% of labeled CE of VLDL transferred to LDL in the rat 1 hr after injection, the fraction of VLDL that completes this conversion may be larger in man (7). The half-life of labeled rat VLDL apoprotein B is less than 10 min, and less than 10% of the B apoprotein of VLDL is transferred to rat LDL (6). In contrast, the half time for disappearance of labeled VLDL apoprotein B in humans is about 200 min, and all apoprotein B of VLDL is transferred to LDL (8). Further, all apoprotein B in LDL is derived from VLDL (8). Thus, in man, it is quite possible that VLDL-CE becomes LDL-CE, although the action of plasma lecithin: cholesterol acyltransferase and the transfer of cholesterol molecules between lipoprotein particles may be an important part of this process. Since CE and TG are mutually soluble in VLDL, the metabolism of VLDL probably includes selective removal of TG and the resulting concentration of CE in the lipoprotein core.

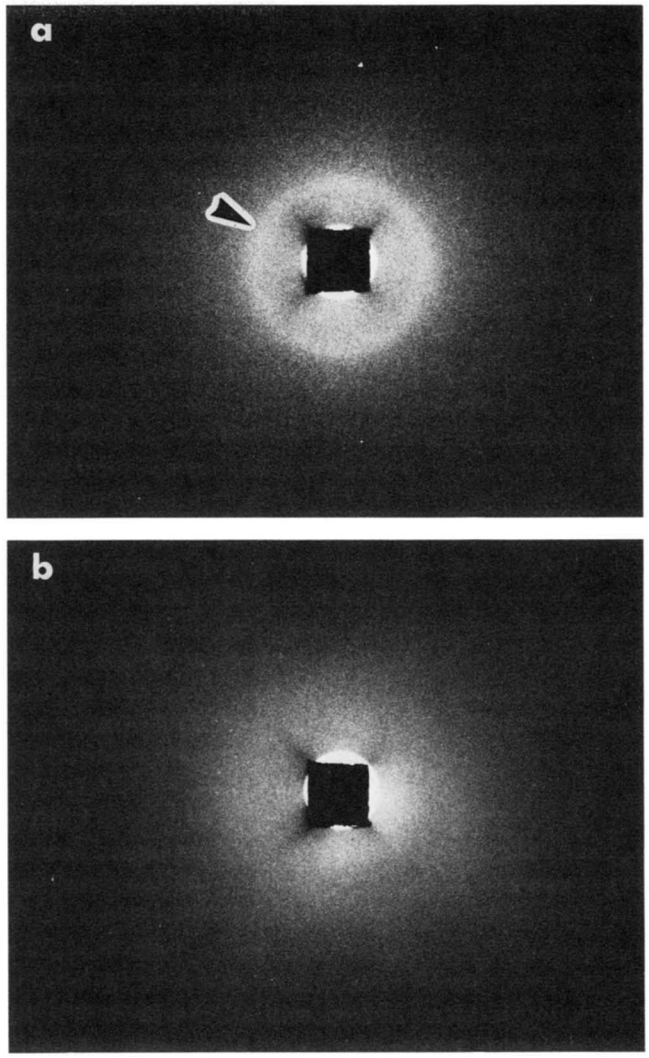

Fig. 2. X-Ray scattering pattern of LDL and VLDL at 10°C. (a) LDL solution containing 75 mg cholesterol ester per ml showing the fringe (arrow) corresponding to the 36 Å spacing, which represents domains of liquid crystalline cholesterol ester in LDL. The 36 Å spacing is clearly seen at lower concentrations of LDL. (b) In VLDL (cholesterol ester concentration 22 mg/ml) this fringe is never present.

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[8] The number of cholesterol ester molecules is calculated using the molecular weight of the lipoprotein particle and the weight percent of cholesterol esters, assuming a molecular weight for the latter of 644 daltons. For example, from the data of A. Gustafson et al. (18) even the smallest VLDL particles (S_f 20–50, mean molecular weight 6×10^6 daltons) contain 1500 cholesterol ester molecules. This compares with about 1300 cholesterol ester molecules per LDL particle (19).

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