1977) for the high-affinity class of sites and from 1×10^{-6} (Wolff et al., 1977) to 8.6×10^{-4} M (Watterson et al., 1976) for the low-affinity class of sites.

The dashed line in Figure 4 represents a best-fit computer-calculated curve for the oxidized modulator, calculated assuming the oxidized protein possesses two classes of Ca^{2+}-binding sites with apparent dissociation constants for Ca^{2+} of $4.25 \pm 1.54 \times 10^{-10}$ and $3.83 \pm 0.46 \times 10^{-4}$ M. In view of the negligible conformational change associated with it, the high-affinity ($K_d = 4.25 \times 10^{-10}$) site(s) are probably an artifact of the mathematical treatment of the data and do not truly exist. Thus, the affinity for Ca^{2+} of sites whose occupancy results in the major conformational change in the molecule has been reduced by two to three orders of magnitude in the oxidized modulator protein. In view of the circular dichroism spectra in Figure 3, this change in affinity probably reflects the conformational differences between the Ca^{2+} free forms of native and oxidized modulator rather than any conformational difference between the Ca^{2+}-bound states.

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Structural Organization of the Lipoprotein HDL_c from Atherosclerotic Swine. Structural Features Relating the Particle Surface and Core†

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ABSTRACT: The plasma lipoprotein HDL_c from miniature swine fed a high-cholesterol, saturated-fat diet exhibits a thermal transition (temperature range 25-45 °C) of its core-located cholesterol esters. This transition from an ordered, smectic-like structure to a more disordered structure is similar to that described for human plasma low-density lipoprotein (LDL). Small-angle X-ray scattering measurements demonstrate that HDL_c is a spherical particle (~180-Å diameter) intermediate in size between human LDL (~220 Å) and normal high-density lipoprotein (~100 Å). The electron-density profile of HDL_c below the transition (10 °C) exhibits a single core-located electron-density peak associated with a region of overlapping steroid moieties of the cholesterol esters arranged in a layered structure in the particle core. This electron-density profile may be compared to that for human LDL (below the transition temperature) which exhibits two core-located electron-density peaks due to layered cholesterol esters. Thus, the smaller size of HDL_c results in one fewer molecular units in the repeating, layered cholesterol ester organization. Comparison of the electron-density profiles for HDL_c and LDL demonstrates a common structural feature. The region of overlapping steroid moieties juxtaposed to the surface-located phospholipids and apoproteins is positioned at a constant distance from the particle surface in HDL_c and LDL. This constant structural feature relating the core-located cholesterol esters and the particle surface suggests a common interaction between the phospholipid and proteins at the surface and the initial layer of cholesterol esters in the particle core.

Human plasma low-density lipoprotein (LDL[1]) undergoes a reversible thermal transition between 20 and 45 °C (Deckelbaum et al., 1975, 1977). This transition is associated with a change from an ordered to a more disordered organization of the cholesterol esters localized in the core of the quasi-spherical LDL particle. Recently, we have used models of the molecular organization of the cholesterol esters in LDL to interpret the X-ray small-angle scattering profiles obtained from these assemblies and, hence, derive information on the molecular packing of the cholesterol esters below and above the transition (Atkinson et al., 1977). At 10 °C, the cholesterol esters are arranged in the core of the LDL particle in a radially repeating organization with a molecular packing similar to that of the smectic phase exhibited by the isolated esters. At 45 °C, this regular radial repeating organization is absent and the organization of the cholesterol esters is less ordered.

The lipoprotein designated HDL_c, which appears in the plasma of cholesterol-fed miniature swine, has α_2 mobility and an apoprotein composition including the arginine-rich and A-1 apoproteins (LDL apo-B is absent) (Mahley et al., 1975).

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[1] Abbreviations used: LDL, low-density lipoprotein; HDL, high-density lipoprotein.

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TABLE I: Percent Chemical Composition of HDL_c Fractions.

	fraction		
	1[a]	2[b]	3[c]
protein	15.8	20.3	25.7
phospholipid	17.5	29.3	32.5
cholesterol ester	56.3	42.5	35.0
cholesterol	9.9	7.5	6.2
triglyceride	0.5	0.5	0.6

[a] $1.02 < d < 1.04$ g/mL. [b] $1.04 < d < 1.06$ g/mL. [c] $1.06 < d < 1.09$ g/mL.

HDL_c is a cholesterol ester rich particle and is intermediate in size between LDL and normal high-density lipoproteins (HDL). Unlike normal HDL, HDL_c is bound by the cell-surface LDL receptor, internalized, and catabolized by cells (Mahley and Innerarity, 1977). In this communication, we are concerned with the description of the molecular packing of the cholesterol esters in this lipoprotein.

Differential scanning calorimetric and X-ray small-angle scattering evidence indicate that the cholesterol esters in this lipoprotein can, like LDL, also form an organized microdomain in the core of the lipoprotein (Tall et al., 1977). This organized domain similarly undergoes a cooperative transition to a more disordered state. However, since LDL and HDL_c exhibit this order–disorder transition whereas normal HDL does not (Tall et al., 1977), HDL_c may represent a lower size limit for lipoprotein particles in which the cholesterol esters can adopt an organized structure. Thus, a structural description of the cholesterol ester organization in HDL_c and comparison with the organization in normal LDL may lead to information on the details of the interaction between the cholesterol esters and the other lipid and protein components. This may provide a description of the structural principles governing lipoprotein assembly.

Materials and Methods

Swine LDL and HDL_c were isolated from plasma by ultracentrifugation at a density in the range 1.02–1.063 g/mL and purified by Geon-Pevikon block electrophoresis as previously described (Mahley and Weisgraber, 1974). Lipoproteins were identified by paper electrophoresis and chemical composition (Mahley and Weisgraber, 1974).

X-ray scattering measurements were made on samples, (ca. 200 mg/mL concentration) sealed in 1-mm capillary tubes, using Cu Kα radiation from an Elliott GX-6 rotating anode generator. X rays were collimated using double-mirror focusing optics as previously described (Atkinson et al., 1977). Photographically recorded scattering patterns were quantitated using a Joyce-Loebl Model IIICS microdensitometer. Measurements were made on HDL_c fractions of density 1.02–1.04 (fraction 1), 1.04–1.06 (fraction 2), and 1.06–1.07 g/mL (fraction 3). Table I summarizes the compositional data for these HDL_c fractions (Mahley et al., 1975; Tall et al., 1977).

Results and Interpretation

Scattering patterns (Figures 1a and 3a) for HDL_c (Tall et al., 1977) show a series of well-resolved scattering maxima similar to the scattering observed from normal human LDL (Atkinson et al., 1977; Laggner et al., 1976; Tardieu et al., 1976; Muller et al., 1978). These maxima are indicative of the quasispherical morphology of the HDL_c and LDL particles. The scattering profiles obtained for HDL_c from each fraction were qualitatively similar. However, fraction 1 was more monodisperse than fractions 2 and 3, as evidenced by the sharpness of the scattering maxima. Therefore, analysis of the data obtained from fraction 1 is presented.

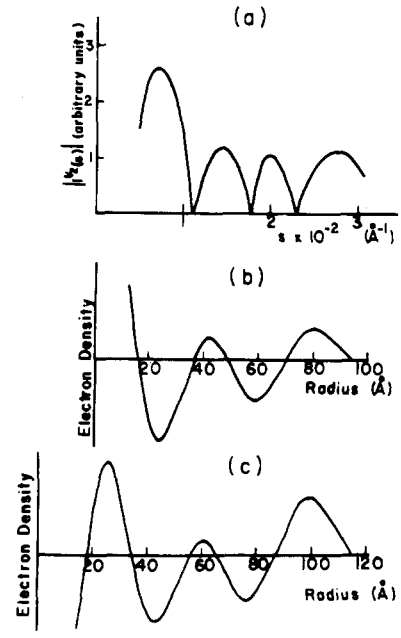

FIGURE 1: (a) Structure factor $|I^{1/2}(s)|$ for HDL_c at 10 °C. In the experimental data for HDL_c, the minima in the scattering do not go to zero between maxima as expected for perfectly spherical particles. Therefore, following subtraction of the background scattering from the sample tube and water, the positions of the maxima were used to interpolate the zero's. This treatment assumes that the nonzero minima are due mainly to polydispersity and only to a small degree of deviation from perfect spherical symmetry. The signs of the maxima were chosen alternately positive and negative according to the minimum-wavelength principle and agreement with the model calculations. (b) Radial electron-density profile for HDL_c at 10 °C obtained by spherical Fourier transformation of the data in a. (c) Radial electron-density profile for normal human LDL at 10 °C taken from Atkinson et al. (1977).

In contrast to the scattering profile for LDL, which shows five subsidary maxima, the scattering pattern for HDL_c at 10 °C (Figure 1a) exhibits four maxima at larger angular spacings, consistent with a particle size smaller than LDL. The scattering profile for LDL is characterized by the high relative intensity of the fifth subsidiary maximum at $1/36$ Å^{-1}. In the case of HDL_c, it is the fourth subsidiary maximum centered at $1/36$ Å^{-1} which exhibits a similar high relative intensity. At 45 °C, above the temperature of the thermal transition for HDL_c (Tall et al., 1977), this maximum at $1/36$ Å^{-1} is absent from the scattering pattern. However, the three maxima at smaller angles remain unchanged, demonstrating that the spherical morphology is not disrupted at 45 °C. These changes in the scattering pattern correlate with the thermal transition observed calorimetrically, are reversible, and correspond to similar changes observed for human LDL (Tall et al., 1977).

Figure 1a,b illustrates the structure factor $|I^{1}/_{2}(s)|$ for HDL_c at 10 °C and the spherically averaged electron-density distribution of the HDL_c particle calculated by Fourier transformation of these data. The electron-density profile clearly demonstrates that the outer radius of the particle is ~90 Å, intermediate between HDL (~50 Å) and LDL (~110 Å). The electron-density distribution shows that the overall structural arrangement of a hydrocarbon core region surrounded by a shell of high electron density which is characteristic of normal HDL and LDL also applies in the case of HDL_c. The surface location of the protein and polar phos-

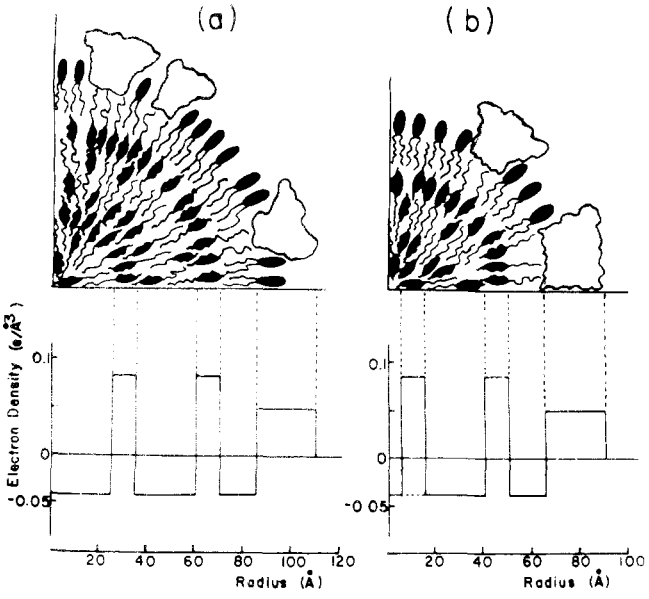

FIGURE 2: Schematic representation of the packing of cholesterol esters and the model electron-density distribution for: (a) normal human LDL at 10 °C (Atkinson et al., 1977) and (b) HDL_c at 10 °C. The packing of the cholesterol esters is based on perturbations of the packing of crystalline cholesteryl myristate (Craven and DeTitta, 1976). For L the first electron-density peak in the core region due to overlapping steroid moieties is centered at a radius of 35 Å, equivalent to one molecular length in the perturbed packing. For HDL_c the first electron-density peak is located at 10 Å, corresponding to orienting the first molecular layer of cholesterol esters with the steroid nucleus toward the center of the particle. The dotted line through the first electron-density peak in the model distribution for HDL_c illustrates the removal of the inner region of overlapping steroid moieties from the model as described in the text.

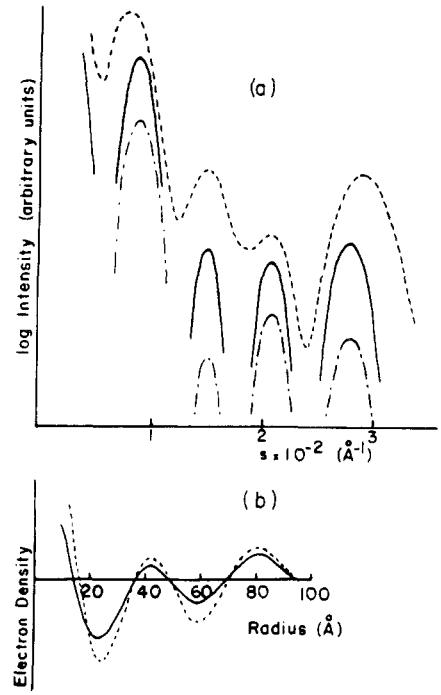

FIGURE 3: (a) X-ray scattering profiles calculated for the model distributions of electron density shown in Figure 2b compared to the scattering observed for HDL_c at 10 °C (- - -). (—) Model in which the first region of overlapping steroid nuclei is located at 10 Å. Note the high relative intensity of the fourth maximum at $1/36$ Å^{-1}, in agreement with experimental observations on HDL_c. (- • -) Model in which the first region of overlapping steroid nuclei has been removed (Figure 2b, dotted line). Note that the third maximum now has a higher relative intensity. (b) Experimentally determined electron-density profile for HDL_c (· · ·) compared to that of the model (—) calculated from the scattering data shown in a (solid line) at the same resolution as obtained experimentally.

pholipid head groups is clearly shown by the surface-located electron-dense peak centered at 80-Å radius. This outer peak is of similar thickness (20–25 Å) to that observed in normal LDL (Atkinson et al., 1977; Laggner et al., 1976, 1977; Muller et al., 1978).

Of particular importance is the single electron-density maximum centered at a radius of 42 Å, located in the core region of the distribution. This electron-density profile showing a single maximum of electron density in the core region may be contrasted with the electron-density distribution of LDL, which shows two electron-density maxima in the core region located at ~30- and ~60-Å radius (Figure 1c). The electron-density distributions for human LDL (Atkinson et al., 1977) and HDL_c, however, show a striking similarity when the two profiles are compared with the surface-located protein/polar group peaks superimposed as shown in Figure 1b,c. The core electron-density maximum located ~40 Å from the surface protein/polar electron density peak (i.e., the peak at 60-Å radius in the profile for LDL and the peak at 42-Å radius in the profile for HDL_c) is a common feature of the electron-density distribution of both LDL and HDL_c at 10 °C.

In the case of LDL below the thermal transition, we have proposed (Atkinson et al., 1977) (see also Laggner et al., 1976, 1977; Muller et al., 1978) that the two electron-density peaks in the core region of the profile arise from regions of superimposed steroid moieties of the cholesterol esters packed in a radial-layered structure. The molecular packing of the cholesterol esters and the resulting electron-density distribution of the model proposed for normal human LDL are shown in Figure 2a. The organization of the cholesterol esters in this model is based on the molecular pair organization observed for the packing in crystalline cholesteryl myristate (Craven and DeTitta, 1976) modified to represent the major HDL_c cholesterol esters, cholesteryl oleate, and linoleate (Tall et al., 1977). The perturbations and modifications of the crystalline packing of cholesteryl myristate involved "melting" of the hydrocarbon chains of the esters, with a concomitant decrease in the length of the hydrocarbon region, together with translations of molecular pairs parallel to the molecular long axis (Atkinson et al., 1977).

The difference in radius of ~20 Å between HDL_c and LDL corresponds to approximately the length of a single cholesterol ester molecule in this perturbed packing, suggesting that the smaller size of HDL_c results from one less molecular unit in the radial-repeating organization. As illustrated in the model shown in Figure 2b, this size difference may be accommodated by the removal of the first molecular layer located at the center of the particle in the model for LDL. Thus, whereas in the model for LDL the regions of overlapping steroids are centered at 30- and 60-Å radius, the removal of the first molecular layer would give regions of overlap at ~10- and at ~45-Å radius.

The X-ray scattering curve calculated for this model distribution of electron density (Figure 2b) with electron-density maxima at ~10 and 45 Å together with a surface-located peak of electron density corresponding to the protein and phospholipid polar groups is shown in Figure 3a compared with the scattering of HDL_c at 10 °C. The calculated scattering profile is in good agreement with that observed experimentally, both reproducing the positions and relative intensities of the subsidiary maxima and accurately reproducing the high relative intensity of the fourth maximum at $1/36$ Å^{-1}. The electron-density profile of the model calculated from the theoretical scattering data at the same resolution obtained experimentally (~20 Å) is shown in Figure 3b. This electron-density distri-

bution is also in good agreement with that derived from the experimental data (Figure 3b), particularly in the region of the outer protein/polar group shell and the electron-density peak at 42-Å radius.

Resolution artifacts at a radius <20 Å due to the limited angular range of the experimental data preclude a direct confirmation of the layer of superimposed cholesterol moieties in this region. However, calculations for a model (Figure 2b) in which the electron-density peak close to the origin was omitted (i.e., containing only the peaks at ~45 and 80-Å radius) showed that removal of this central region of high electron density results in a scattering profile (Figure 3a) in which the third rather than the fourth subsidiary maximum has a high relative intensity. The experimental data for HDL_c clearly shows a high relative intensity of the fourth subsidiary maximum at $1/36$ Å^{-1}. Thus, the model calculations provide indirect evidence for a region of high electron density at the center of the particle. However, the packing in this region ($R < 20$ Å), which contains less than 5% of the volume of the core region, is undoubtedly highly perturbed due to the small radius of curvature.

Discussion

The similarities in the thermal behavior of the cholesterol esters in swine HDL_c and human and swine LDL suggest a similar structural organization of the esters in both lipoprotein classes (Tall et al., 1976). The qualitative features of the X-ray scattering profiles for HDL_c and LDL, particularly the enhanced relative intensity of the scattering maximum at $1/36$ Å^{-1} (and its disappearance above the calorimetric transition), direct comparison of the electron density profiles of HDL_c and LDL, together with the model calculations further substantiate a common structural arrangement of the cholesterol esters.

The electron-density distribution for HDL_c exhibits a single peak in the electron density of the core region in contrast with the distribution for LDL which exhibits two peaks. These electron-density maxima in the core region of LDL have previously been ascribed to regions of inregister steroid moieties of the cholesterol esters, organized in a radially layered organization (Atkinson et al., 1977). The single electron-density peak in the core region of HDL_c undoubtedly arises from a similar overlap of steroid moieties of the cholesterol esters. The small size of HDL_c results simply in fewer molecular layers in the radial organization.

More important, however, is the observation that the distance between the electron-density maxima due to the overlap of steroid groups juxtaposed to the surface-located protein/polar-group region is similar in HDL_c and LDL (~45 Å peak to peak). This common feature suggests a similar structural arrangement and interactions between the outer layers of cholesterol esters and the layer of protein and phospholipids at the surface of HDL_c and LDL. The model calculations indicate that the distance between the outer edge of the steroid peaks ($R = 50$ Å for HDL_c and $R = 75$ Å for LDL) and the inner edge of the protein/polar surface peak ($R = 65$ Å for HDL_c, $R = 90$ Å for LDL) is 15–20 Å and accommodates the hydrocarbon chains of the phospholipids. This indicates that the first layer of overlapping steroid regions beneath the surface of the lipoprotein abuts the phospholipid acyl chains. With the steroid nucleus located at this position relative to the surface of the particle, either the C_{17} isooctyl side chain or the fatty acid chain of the cholesterol ester may interdigitate with the fatty acid chains of the phospholipid. Which of these alternatives applies cannot be determined at the resolution of this study. However, interdigitation of the cholesterol ester fatty acid chain amongst those of the phospholipid would necessarily expose a large apolar area at the particle surface. This apolar area could be adequately shielded from the aqueous environment by the surface-located apoproteins.

A similar argument may apply in the case of the cholesterol ester organization of normal HDL. The interaction and location of a single layer of cholesterol moieties abutting the phospholipid acyl chains in HDL, however, would account for the 50-Å radius of HDL. Thus, for normal HDL the particle size will not accommodate additional repeating layers of cholesterol esters necessary for an organized domain capable of undergoing thermal rearrangement.

This orienting effect of the surface organization on the core-located cholesterol esters is likely to persist above the thermal transition. Thus, above the thermal transition these surface constraints may result in residual radial alignment of the cholesterol esters, resulting in structural arrangement resembling a nematic or cholesteric phase (i.e., alignment of molecular long axes but not layering of molecules) rather than an isotropic liquid (Tall et al., 1977).

The demonstration of this common structural feature in the molecular organization of HDL_c and LDL points to a "common" interaction between the surface-located phospholipid and protein components and the initial layer of the core-located cholesterol esters. This interaction stabilizing the surface of the lipoprotein particle may apply for all cholesterol ester carrying lipoproteins. The subsequent molecular organization of the cholesterol esters in the bulk of the core of HDL_c, LDL, and perhaps normal HDL may then be described at low temperatures by the number of regularly organized layers.

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