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DENSITY AND COMPOSITION MODELS FOR LIPOPROTEINS *

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Two theoretical models for lipoprotein particle structure are proposed, based on density and composition and utilizing the Stokes' coefficient as evaluated from the Maude-Whitemore expression, using analytical ultracentrifugation. These density and composition models are compared with the Corey-Pauling-Koltun space-filling model proposed by Verdery and Nichols, and that proposed by Shen et al., which has a sharply defined boundary between the hydrophobic core and the amphipathic layer surrounding it. Results of our calculations of the percentage of total apolar lipid accommodated in the outer hydrophobic core —28, 64 and 94% for LDL, HDL_2 and HDL_3, respectively—suggest that there may be differences among these three lipoprotein classes in both the packing of cholesteryl ester and triglyceride as well as the conformation of apolipoproteins at the particle's surface.

1. Introduction

Although the exact topological arrangement of the phospholipid (PL) and protein in lipoproteins is unknown, Lindgren et al. [1] proposed a model for high-density lipoprotein (HDL) structure which featured a core of triglyceride, cholesterol and cholesteryl ester surrounded by an outer shell of apolipoproteins. The most widely accepted models for the two major human HDL subclasses have been based on small-angle X-ray scattering data [2]. Scanu [2] proposed that the phospholipid polar head groups and the protein are in the outer hydrophilic layer, while the cholesteryl esters, triglycerides and the acyl chains of the phospholipids are in a hydrophobic core.

Most recently, Huang [3] has proposed a spatial orientation model for a phospholipid-cholesterol complex at the interface of a bilayer in which the 3β-hydroxyl group on C3 cholesterol is hydrogen bonded with the C1 carbonyl oxygen of phospholipid, in a low dielectric environment. This model suggests that in lipoproteins the surface layer is primarily composed of phospholipid, cholesterol and proteins.

Hydrodynamic [4] and electron microscopic studies [5] have shown HDL_3 and LDL to be fairly compact, nearly spherical particles. Several researchers, examining HDL_3 [6–8] and HDL_2 [7–10], have independently concluded that the two types of particles are spherical [11,12] with the protein moieties located in the outer shell. Verdery and Nichols [13] used simple calculations of the size, area, volume and composition of HDLs [13–15] to reach the conclusion that lipoproteins exist as globular particles. In X-ray scattering studies on serum LDL, Luzzati et al. [16] argued that the LDL particle is more likely to have a tetrahedral symmetry, with a convoluted surface. These findings are supported by freeze-etching electron microscopic studies by Gulik-Krzywicki et al. [17] and Aggerbeck et al. [18]. The particle and core radii of both HDLs and LDLs have been determined by small-angle X-ray scattering measurements [19,20].

We propose an alternative, hydrodynamic method for the evaluation of the core radius of HDL or LDL from the particle-to-core weight ratio, based on the composition of the particle and the densities and weight fractions of the constituent components. These data can also be used to evaluate the particle radii from the following

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expression based on Stokes' Law:

$$R^2 = \left[9\eta s^0/2(\rho_p - \rho_s)\right].$$

The second method of calculating particle radius from experimentally obtained values of the sedimentation coefficient $s^0_{20,w}$ was to use a semi-empirical expression which has proved useful in describing concentrated lipoprotein particles with diameters in the range of 200–1000 Å. Originally developed by Maude and Whitemore [21],

$$R^2 = \left[9\eta s_{app}/2(\rho_p - \rho_s)\right] F(\phi),$$

where $F(\phi)$ is a function describing the volume fraction of the particles in solution [21–27]. Noting that no extrapolation of sedimentation coefficient at infinite dilution is required, this expression simplifies to $F(\phi) = (1-\phi)^{-\alpha}$, for a hard sphere as $\phi \to 0$. α is the proportionality constant for the sedimentation property of the particle, $s^0 = s_{app}(1 + \alpha\phi)$, and varies with size, shape and sedimentation rate—leading to a change in sedimentation velocity by a factor of $(1 + \alpha\phi)$. Note that $F(\phi) = s^0/s_{app}$ *.

This third method employs the following expression based on a theoretical analysis by Batchelor [25,27] and Barnea and Mizrahi [26]: *

$$R^2 = \left\{\left[9\eta s_{app}/2(\rho_p - \rho_s)\right]\left[1/(1-\alpha\phi)\right]\right\},$$

and applies only in dilute solution where the volume fraction ϕ of lipoprotein particles is below 2%.

In the present communication, we describe a convenient method for determination of the core radius, R_1 (provided that accurate values of R_2, the particle radius, and ρ_2, the particle density, have been determined), and for the evaluation of the amount of water bound by the three lipoprotein classes—LDL, HDL_2 and HDL_3. Based on these hydrodynamic parameters, we propose two theoretical models for lipoprotein structure.

* $R = [9\eta s^0/2(\rho_p - \rho_s)]^{1/2}$, $R = [9\eta s_{app}/2(\rho_p - \rho_s)]^{1/2} F(\phi)^{1/2}$, and with a proper ratio yields $(s^0)^{1/2}/(s_{app})^{1/2} = F(\phi)^{1/2}$ and the sedimentation coefficient at infinite dilution for a sphere, $s^0 = \bar{v}_p(\rho_p - \rho_s)D/RT$ as the frictional coefficient, $f = \bar{v}_p(\rho_p - \rho_s)/s^0$. Here η is the viscosity of the solvent and ρ_p and ρ_s are the densities of particle and solvent, respectively.

2. Density and composition models for lipoproteins [28]

Two theoretical models for lipoprotein particle structure are proposed, (see fig. 1), based on density, ρ, and composition, utilizing the Stokes' coefficient as evaluated from the Maude-Whitemore expression [21], in the Model E analytical ultracentrifuge.

2.1. Model A

For lipoproteins having a cholesteryl ester-triglyceride core, the model is expressed:

$$\rho_2 = \rho_1(W_2/W_1)(R_1/R_2)^3,$$

where ρ_2 and ρ_1 are the particle and core densities; R_2 and R_1 are particle and core Stokes' radii, and W_2 and W_1 are the particle and core weights, respectively.

2.2. Model B

The partitioning model, representing the formation of an interface layer between the core and outer layer due to interdigitation of either cholesteryl ester or triglyceride with outer layer components, is expressed:

$$\rho_2 = \rho_0(W_2/W_0)(R_0/R_2)^3,$$

where $(W_1/W_0) = (R_1/R_0)^3(\rho_1/\rho_0)$ and $(W_2/W_1) = (R_2/R_1)^3(\rho_2/\rho_1)$; ρ_0 is the core density, R_0 and R_1 are the core and core + interface layer radii and W_0 and W_1 are the core and core + interface layer weights. The interdigitation characteristic of the partitioning model would be expected to influence the conformation of the apolipoproteins at the surface of the particle.

3. Materials and methods

3.1. Preparation of lipoproteins

LDL (ρ 1.006–1.063), HDL_2 (ρ 1.063–1.125) and HDL_3 (ρ 1.125–1.210) were isolated from hu-

man plasma by differential density centrifugation followed by gel filtration chromatography as previously described [29–31]. LDL was dialyzed against 0.05 M phosphate ($NaH_2PO_4/NaHPO_4$) buffer, pH 7.4, ionic strength 0.1 at 4°C. HDL_2 and HDL_3 were dialyzed against 0.1 M sodium phosphate buffer, pH 7.4, ionic strength 0.2 at 4°C.

3.2. Density measurements

Density measurements were made with a bulb and capillary pycnometer of the Lipkin type, at 20.0 and 25°C. Triplicate measurements were made on each solvent and sample. With a 5 ml pycnometer, the agreement between measured densities was ± 0.0001 g/ml or better in all cases.

3.3. Viscosity measurements

Viscosities were measured with an Ostwald-Cannon (Fisher No. 25) viscometer of 5 ml capacity. The viscometer was immersed in a constant temperature bath at $25.00 + 0.02$°C. This particular viscometer had an efflux time of ≈ 500 s using distilled water at 25°C. Values of viscosity (in poise) were corrected to 20°C by multiplying by 1.02 for 0.05 M and 1.04 for 0.1 M sodium phosphate buffer, pH 7.4.

3.4. Sedimentation velocity and equilibrium measurements

Sedimentation equilibrium experiments were performed in a Spinco/Beckman Model E analytical ultracentrifuge equipped with RTIC unit. Runs were made at 7447 rpm for LDL, 10589 rpm for HDL_3 and 8225 rpm for HDL_2. Runs to determine the concentration dependence of molecular weight were made at 20°C, using an Yphantis six-channel centerpiece made from carbon-filled epoxy resin, at an initial concentration of ≈ 0.1 g/dl, running three samples simultaneously in H_2O and D_2O. The total sample volume in the cell was 0.11 ml, giving a column height of 3 mm.

The calculation of the molecular weight distribution and $[\mathrm{d} \ln C/\mathrm{d}(x^2)]$ were based on the Yphantis [32] and Richards and Schachman [33] manipulation, using a computer program which can be modified for use with an Amdahl 470/0-2 unit (modified IBM 360/1800, University of Flordia's Circa computing facilities), plotting $\ln J$ or $\ln C$ as a function of x^2. The weight average molecular weights of lipoproteins were calculated from the slope of a plot of $\ln C$ versus x^2 in H_2O and D_2O. The $\ln C$ versus x^2 data were fitted to a least-square polynomial and the values of $[\mathrm{d} \ln C/\mathrm{d}(x^2)]$ were calculated by a modification of the sliding, five-point, least-square quadratic treatment of Yphantis [32].

3.5. The partial specific volumes of LDL, HDL_2 and HDL_3

The partial specific volume of LDL was determined by Edelstein and Schachman's method [34] based on the expression:

$$(1 - \bar{v}_p \rho_{D_2O}) A_{H_2O} / (1 - \bar{v}_p \rho_{H_2O}) A_{D_2O} = 1.$$

Rearrangement of this expression yields:

$$\bar{v}_p = (A_{H_2O} - A_{D_2O}) / (A_{H_2O} \rho_{D_2O} - A_{D_2O} \rho_{H_2O}).$$

where $A = [\mathrm{d} \ln C/\mathrm{d}(x^2)]$ and ρ is the density of the solvent. The effective specific volume, ϕ', was also determined by the sedimentation-viscosity method for each density class based on the following expression. ϕ' is used here, since the partial specific volume includes preferential hydration and salts.

$$(1 - \phi' \rho_{H_2O})(s^0\eta)_{H_2O} / (1 - \phi' \rho_{D_2O})(s^0\eta)_{D_2O} = 1$$

Rearrangement of this expression yields:

$$\phi' = (s^0\eta)_{H_2O} - (s^0\eta)_{D_2O} / (s^0\eta)_{H_2O} \rho_{D_2O} - (s^0\eta)_{D_2O} \rho_{H_2O},$$

all runs being made at 52640 rpm. The calculated ϕ' was compared with the graphical analysis of a plot of $s^0\eta$ as a function of solvent densities in H_2O and D_2O, where $s^0\eta = M(1 - \phi' \rho_s)/6\pi r$. s^0 is the sedimentation coefficient at infinite dilution. ρ_s and η are the solvent density and viscosity, respectively, and r is the radius of the spherical particle.

3.6. Density and composition model for lipoproteins

3.6.1. Model A (fig. 1A)

This model assumes the lipoprotein particle to be a sphere of radius R_2 and density ρ_2 with a core radius R_1 and density ρ_1 consisting of triglyceride (TG) and cholesteryl ester (CE), and a surface thickness of L_A consisting of protein, phospholipid, unesterified cholesterol, carbohydrate, and other lipids (e.g., sphingolipid). The core radius, R_1, is calculated based on the constituent density, ρ_i, and weight fraction, W_i, of each component given in tables 1 and 2.

$$W_2/W_1 = f_2/f_1 = (V_2\rho_2)/(V_1\rho_1) = (R_2/R_1)^3(\rho_2/\rho_1) \quad (1)$$

where f_1 is the weight fraction of the core and $f_2 = 1$.

$$R_1 = R_2[f_1\rho_2/\rho_1]^{1/3} \quad (2)$$

The density of the core, ρ_1, is calculated from:

$$\rho_1 = (f_{TG}\rho_{TG}/f_1) + (f_{CE}\rho_{CE}/f_1). \quad (3)$$

The anhydrous R_2 was calculated from Stokes' law,

$$R_2 = [9s^0\eta/2(\rho_2 - \rho_s)]^{1/2}. \quad (4)$$

where η is the viscosity of solvent (0.01022 P for 0.05 M and 0.01042 P for 0.1 M phosphate at 20°C) and ρ_2 is the particle density. The density of solvent ρ_s, measured by pycnometry at 20°C, was found to be 1.0036 for 0.05 M and 1.0126 for 0.1 M phosphate. The Stokes' radius of the hydrated particle, $\bar{R}_H$, for a spherical particle is expressed as:

$$\bar{R}_H = [3\bar{M}_w\phi'/4\pi N]^{1/3} \quad (5)$$

where $\bar{M}_w$ is the weight average molecular weight, and N is Avogadro's number. The amount of water bound, $\bar{X}_H$, in g/g particle is:

$$\bar{X}_H = \rho_s[\phi' - (4\pi NR_2^3/3\bar{M}_w)]. \quad (6)$$

3.6.2. Model B (fig. 1B)

This model also assumes each lipoprotein particle to be a sphere of radius R_2 and density ρ_2, as in the case of Model A. However, this model also induces an interface region of thickness L_B, resulting from partitioning or interdigitation of triglyceride or cholesteryl ester, or both, from the core. The thickness of L_B depends upon the weight fraction of the interface region, f_B, which may vary from zero to the weight fraction of either cholesteryl ester or triglyceride in the particle. Thus, partitioning by cholesteryl ester and by triglyceride were considered separately in two different models, namely B_1 and B_2. Interdigitation by cholesterol from the surface into the interface region, which was considered previously [28], may not be feasible since the β-conformation proposed by Huang [3] would strongly interact with surface

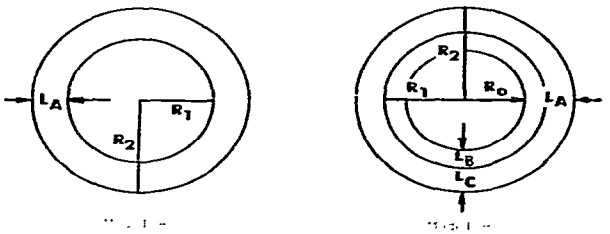

Fig. 1. Two models for lipoprotein structure. Model A is a particle of radius R_2 with a core consisting of triglyceride and cholesteryl ester of radius R_1, and a surface consisting of phospholipid, cholesterol, protein, and carbohydrate of thickness L_A. Model B includes an interface region of thickness L_B consisting of either cholesteryl ester, triglyceride, or both and a surface of thickness L_C. The core radius varies from R_0 to R_1.

Table 1

Densities and partial specific volumes of some constituent molecules

Molecule	$\bar{v}$ (cc/g)	ρ (g/cc)
Triglyceride	1.093	0.915
Cholesteryl ester	1.044 (1.042) [a]	0.958 (0.9578)
Unesterified cholesterol	0.968	1.033
Phospholipid	0.970	1.031
Protein (HDL)	0.740	1.351
Protein (LDL)	0.725	1.379
Hexose	0.613	1.631
Hexosamine	0.666	1.502
Sialic Acid	0.584	1.712

[a] Tardieu et al. [19] and Janiak et al. [42].

Table 2

Percent composition of lipoprotein [a]
TG, triglyceride; CE, cholesteryl ester; UC, unesterified cholesterol; PL, phospholipid; CHO, carbohydrate.

Lipoprotein	Protein	TG	CE	UC	PL	CHO + other lipid	Core	Surface
LDL	23.0	6.4	41.3	6.7	21.6	1.0	47.7	52.3
HDL_2	40.0	3.4	13.1	4.4	35.2	3.9	16.5	83.5
HDL_3	56.0	3.2	9.4	2.2	27.0	2.2	12.6	87.4

[a] The chain compositions of the lipids are reported by Scanu and Kruski [43]. Adapted from Scanu et al. [44], Skipski [46], Eisenberg [45] and Osborne and Brewer [49].

components. Also, even if partitioning of cholesterol occurred, our calculations indicate that the amount would be too small to detect experimentally in any of the three density classes.

3.6.3. Model B_1

Interdigitation by cholesteryl ester (CE) does not affect the surface, since the interface region is considered to be arising from the core. That is, in model B_1, R_1 and ρ_1 are equal to R_1 and ρ_1 in model A and are constant. R_1 represents the maximum value that R_0 can achieve when $f_B = 0$ (i.e., no partitioning, the same as model A). Otherwise, R_0 and ρ_0 of model B_1 vary as a function of f_B where $0 \leq f_B \leq f_{CE}$. Therefore,

$$f_0 = f_1 - f_B \text{ and } \rho_0 = \rho_1 + (d\rho_0/df_0)f_B \qquad (10)$$

A simplified expression for the core radius of model B_1 analogous to eq. (2) is:

$$R_0 = R_2[f_0\rho_2/\rho_0]^{1/3} \qquad (11)$$

A proper substitution of f_0 and ρ_0 into eq. (11) gives:

$$R_0 = R_2\{(f_1 - f_B)\rho_2/[\rho_1 + (d\rho_0/df_0)f_B]\}^{1/3} \qquad (12)$$

3.6.4. Model B_2

Interdigitation by triglyceride (TG) from the core into the interface region likewise does not affect the surface, but only the core. Therefore, eqs. (10)–(12) also apply to this model, except $0 \leq f_B \leq f_{TG}$. Since some portions of the protein may extend into the inner hydrophobic core, it is reasonable to assume that this has some effect on the conformation of the apolipoprotein at the particle surface.

4. Results

4.1. Sedimentation velocity and viscosity measurements

All schlieren patterns for the three lipoprotein classes (LDL, HDL_2 and HDL_3) were fairly symmetrical in both H_2O and D_2O. However, a marked linearity in $g_{(s)}/g_{(s)max} = (s_{app} - \bar{s})^2$[35, 36] for LDL samples was observed at a concentration of 3.82 mg/ml [27] at both the trailing and leading edges for fixed values of the apparent unimodal distribution function of a single solute [37,38]. In contrast, the values of $g_{(s)}/g_{(s)max}$ were nonlinear for HDL_2 and HDL_3. Analysis of the sedimentation behavior of both HDL_2 and HDL_3 samples ranging in concentration from 0.4 to 3.2 mg/ml shows the concentration dependence of the sedimentation coefficient. After a proper correction for viscosity and density, the concentration dependence proportionality constant, α, for LDL samples was found to be 0.0086 ± 0.001 ml/g, i.e., $s_{app} = s^0/(1 + \alpha C)$.

A plot of $1/s$ versus C for HDL_2 and HDL_3 in 40% D_2O, after extrapolation of a least-squares line through each data point, gave infinite dilution sedimentation coefficients of $s^0_{20,w} = 3.25$ and $s^0_{20,w} = 3.29$, respectively. In water, these values were $s^0_{20,w} = 4.97$ for HDL_2 and $s^0_{20,w} = 4.56$ for HDL_3. As summarized in table 3, LDL in 10% D_2O gave $s^0_{20,w} = 5.74$ and in water, $s^0_{20,w} = 7.94$. The concentration dependence parameters, α, for HDL_2 and HDL_3 were determined to be 15.30 ± 0.01 and

Table 3

Sedimentation coefficient, effective partial specific volume and viscosities of solvent of LDL, HDL_2 and HDL_3.

Lipoprotein	Solvent [a]	$s^0_{20,w}$	ϕ' [b]	η(cP)
LDL	H_2O	7.94	0.9517	1.0220
	10% D_2O	5.74		1.0482
HDL_2	H_2O	4.97	0.8891	1.0421
	40% D_2O	3.25		1.1443
HDL_3	H_2O	4.56	0.8586	1.0421
	40% D_2O	3.29		1.1443

[a] Solvent system is composed of sodium phosphate buffer, $\mu=0.1$, for LDL. $\mu=0.2$ for HDL_2 and HDL_3, pH 7.4. Viscosity and densities of solvent as a function of temperature were adapted from 'Density and Partial Specific Volume of D_2O', Vol. a-i, 1943, Texas Eng. Expt. Stat., Texas A&M University, and Robinson and Stokes [52]. The expression, $1/s$ versus c proportionality constant, α, for LDL is 0.009 ml/g, 15.3 ml/g for HDL_2, 14.2 ml/g for HDL_3.

[b] Calculated by the sedimentation-viscosity method.

14.2 ± 0.01 ml/g, respectively. The viscosities in H_2O and D_2O were converted to poise and multiplied by a viscosity correction factor (η/η^0) for phosphate at 20°C (1.02 for LDL, 1.04 for HDL_2 and HDL_3).

The hydrated densities of HDL_2 and HDL_3 were found to be 1.1247 ($\phi'=0.8891$) and 1.1647 ($\phi'=0.8586$), respectively, by the sedimentation-viscosity method. The hydrated density of LDL is 1.0457 ± 0.012 ($\phi=0.9563$) by the Edelstein-Schachman method and 1.0508 ($\phi=0.9517$) by the sedimentation-viscosity method. It should be noted that these values for hydrated density may not represent the true reciprocal of the partial specific volume, $\bar{v}_p$, but rather are a measure of the hydration and/or salts bound with the lipoproteins.

Several average values of the effective partial specific volume, ϕ', and effective particle density, ρ_2, were used to evaluate the core radius, R_1, the radius of the particle, R_p, and the hydration thickness, $\bar{L}_H$, shown in tables 4 and 5.

4.2. Sedimentation equilibrium in H_2O and D_2O

The slopes of a plot of $\ln J$ or $\ln C$ versus x^2 for LDL in water and in 10% D_2O were used to evaluate the effective specific volume, ϕ', as described in the previous section. The effective specific volume derived from the slope of $(A)_{H_2O} = [d \ln C/d(x^2)] = 1.0754 \pm 0.001$ and $(A)_{D_2O} = 0.7634 \pm 0.02$ was determined to be 0.9563 ± 0.005 ml/g and the particle density $\rho_2 = 1.0457$. This represents $\approx 0.5\%$ difference in the effective specific volume from one previously calculated from sedimentation-viscosity data. Both differ from a previously published $\bar{v}_p$ for LDL of 0.9630 ± 0.004 [29,39] by about 1%.

Our results show that the apparent weight average molecular weights of several preparations of

Table 4

Weight average molecular weights, and the Stokes' radii of LDL, HDL_2 and HDL_3

R_2 was calculated from the sedimentation-viscosity expression.

Lipoprotein	ϕ'	ρ_2	$M_w(\times 10^{-6})$	R_2(A)
LDL	0.9630	1.0384	2.74×10^6 [a]	101.51
	0.9563	1.0457	2.76×10^6	101.48
	0.9517	1.0508	1.80×10^6	87.96
			± 0.019	
HDL_2	0.9050	1.1050	0.400 [b]	50.22
	0.8891	1.1247	0.474	45.60
			± 0.004	
HDL_3	0.8670	1.1534	0.175 [c]	38.97
	0.8586	1.1647	0.240	37.50
			± 0.005	

[a] Hammond and Fisher [39]; Fisher et al. [29]; Jeffrey et al. [38].

[b,c] Hazelwood [7]; Schumaker and Adams [47]; Shore and Shore [48].

Table 5

Radius and hydration radius of lipoproteins: Model A.

Lipoprotein	R_1(Å)	L_A(Å)	R_2(Å)	$\bar{L}_H$(Å)	$\bar{x}$(g/g)	$L_H/L_A(\phi')$
LDL($\bar{v}_p$)	71.02	16.94	87.96	13.52	0.338	0.692
LDL(ϕ')	81.94	19.54	101.48			
HDL_2($\bar{v}_p$)	26.47	19.13	45.60	7.88		
HDL_2(ϕ')	31.04	22.44	53.48		0.389	0.351
HDL_3($\bar{v}_p$)	20.14	17.36	37.50	5.89		
HDL_3(ϕ')	23.31	20.08	43.39		0.308	0.293

LDL in 0.05 M sodium phosphate buffer, pH 7.4, varied from 2.77×10^6 to 3.4×10^6. These values are in fairly good agreement with results obtained by other methods. In sedimentation equilibrium experiments employing Laplace transforms [40], Jeffrey et al. [38] reported an apparent weight average molecular weight of 2.74×10^6. The use of the Svedberg expression with a value of $D^0_{20,w} = 1.9 \times 10^{-7}$ cm^2/s and $s^0_{20,w} = 8.28$ at $\rho_2 = 1.03212$ g/ml gave a molecular weight of 2.74×10^6. Using a plot of $s^0\eta$ versus ρ_s, solvent density, Fisher et al. [41] obtained a value of 2.8×10^6.

Our sedimentation velocity and viscosity measurements at a molecular weight of 2.8×10^6 give an effective partial specific volume of 0.9517, as shown in tables 3 and 4, in fair agreement with the previously reported value of 0.963 ± 0.004 ml/g [41]. Based on eq. (4), the particle radii R_2 of LDL, HDL_2 and HDL_3 were determined to be 87.96, 45.60 and 37.50 Å, respectively.

The weight average molecular weights of HDL_2 and HDL_3 shown in table 4 are slightly higher than those reported by Hazelwood [7], perhaps due to the associating effect of the Hofmeister series of phosphate anions in the 0.1 M sodium phosphate buffer [28].

The effective partial specific volumes of HDL_2 and HDL_3 determined at $(s^0\eta)_{H_2O}$ and $(s^0\eta)_{D_2O}$ at 20°C, for several different preparations in H_2O and 40% D_2O, were determined to be 0.8891 and 0.8586, respectively. These values determined by sedimentation-viscosity measurements are ≈ 1–1.8% lower than those of $\bar{v}_p = 0.9050$ for HDL_2 and $\bar{v}_p = 0.8670$ for HDL_3 reported by Hazelwood [7].

4.3. Determination of radius and hydration radius: Model A

Values of the radius and hydration radius of various lipoproteins, determined based on Model A and utilizing eqs. (1)–(6), are given in table 5. Our values for R_2 and R_1 for LDL are in fairly good agreement with those previously reported from small-angle X-ray scattering data [19], i.e., 90.2–93.0 Å for the radius of gyration of the high-density region (R_2 of our Model A) and 75.4–78.6 Å for the low-density core structure (our R_1). The thickness of the surface layer, L_A, is 16.94 Å for anhydrous LDL, 19.54 Å for hydrated LDL. The hydration layer, L_H, evaluated from eqs. (4) and (5) measured 13.52 Å. The difference in L_A for hydrated and anhydrous LDL suggests that $\approx$ 2.6 Å of the water layer, L_H, is outside the surface layer. The amount of water bound was found to be 0.338 g/g LDL, in agreement with the value of 0.34 g water/g LDL particles previously reported by Fisher et al. [29].

Our values for R_1 and R_2 for HDL_3 seen in table 5 are also in fair agreement with those of Tardieu et al. [19], i.e., 47.4 Å for the radius of gyration of the high-density region and 29.8 Å for the low-density region (core). We note that the thickness of the surface water layer increases to 3.3 Å in HDL_2 and 2.7 Å in HDL_3 with the amount of water bound being 0.389 g/g HDL_2 and 0.308 g/g HDL_3. Indeed, the amount of water bound in all three lipoprotein classes appears to be about the same. Our results also suggest that as the ratio of L_H to L_A increases, the binding effect of a Hofmeister series of anions on the lipoprotein particles also increases.

The core density, ρ_1 in R_1 for LDL was found to be 0.9522 g/ml. These values for HDL_2 and HDL_3 were 0.9491 and 0.9471 g/ml, respectively. The slight variance may be attributed to a variation in the ratio of cholesteryl ester to triglyceride in the core, that is 8.4:1 for LDL, 5.1:1 for HDL_2 and 3.9:1 for HDL_3. We note that as the core density and core volume decrease, the degree of hydration remains relatively constant, as seen from table 5.

Table 6

Model B_1: Interdigitation by cholesteryl ester

The table shows variations in the inner core radius, R_0, the interface thickness, L_B, and the surface plus interface thickness, L_A, for Model B_1 as a function of the weight fraction of cholesteryl ester (CE) partitioning into the interface region, f_B, according to the following equations: $R_0 = R_2\{(f_1 - f_B)\rho_2/[\rho_1 - (d\rho_0/df_0)f_B]\}^{1/3}$ [a], where $0 \leq f_B \leq f_{CE}$. [b] $L_B = R_1 - R_0$, and $L_A = L_B + L_C = R_2 - R_0$. [c] The other necessary parameters for evaluation of R_0 for Model B_1 are given by: [a] R_2 is calculated from $R^2 = 9s^0n/2(\rho_2 - \rho_s)$ and the true radius, $R_{2(T)}$ from $R_{2(T)} = [3M\bar{v}/4\pi N]^{1/3}$. [b] f_{CE} is the weight fraction of cholesteryl ester in the whole particle from table 2 and f_B was arbitrarily chosen in five equal increments for this table. [c] The core plus interface radius, R_1, and the surface thickness, L_c, are constant in this model.

f_B	R_0(Å)	L_B(Å)	L_A(Å)	$R_{0(T)}$(Å)	$L_{B(T)}$(Å)	$L_{A(T)}$(Å)
LDL at $\rho_2 = 1.0508$						
0	71.02	0	16.94	81.94	0	19.54
0.0826	66.49	4.53	21.47	76.71	5.23	24.77
0.1652	59.97	11.05	27.99	69.18	12.76	32.30
0.2478	54.26	16.76	33.70	62.60	19.34	38.88
0.3304	47.44	23.58	40.52	54.73	27.21	46.75
0.4130	35.90	35.12	52.06	41.41	40.53	60.07
$R_2 = 87.96$ Å; $R_1 = 71.02$ Å; $L_c = 16.94$ Å; $R_{2(T)} = 101.48$ Å; $R_{1(T)} = 81.94$ Å; $L_{c(T)} = 19.54$ Å						
HDL_2 at $\rho_2 = 1.1247$						
0	26.47	0	19.13	31.04	0	22.44
0.0262	24.92	1.55	20.68	29.23	1.81	24.25
0.0524	23.19	3.28	22.41	27.20	3.84	26.28
0.0786	21.18	5.29	24.42	24.84	6.20	28.64
0.1048	18.73	7.74	26.87	21.97	9.07	31.51
0.1310	15.45	11.02	30.15	18.12	12.92	35.36
$R_2 = 45.60$ A; $R_1 = 26.47$ A; $L_c = 19.13$ Å; $R_{2(T)} = 53.48$ Å; $R_{1(T)} = 31.04$ Å; $L_{c(T)} = 22.44$ A						
HDL_3 at $\rho_2 = 1.1647$						
0	20.14	0	17.36	23.31	0	20.08
0.0188	19.04	1.10	18.46	22.03	1.28	21.36
0.0376	17.82	2.32	19.68	20.62	2.69	22.77
0.0564	16.42	3.72	21.08	18.99	4.32	24.40
0.0752	14.75	5.39	22.75	17.06	6.25	26.33
0.0940	12.61	7.53	24.89	14.60	8.71	28.79
$R_2 = 37.50$ Å; $R_1 = 20.14$ Å; $L_c = 17.36$ Å; $R_{2(T)} = 43.39$ Å; $R_{1(T)} = 23.31$ Å; $L_{c(T)} = 20.08$ A						

Lipoprotein	f_1	ρ_1	$(d\rho_0/df_0)_{CE} = (\rho_1 - \rho_{TG})/(f_1 - f_{TG})$	$\bar{x}$
LDL	0.477	0.9522	0.0901	0.338
HDL_2	0.165	0.9491	0.2603	0.389
HDL_3	0.126	0.9471	0.3415	0.308

4.4. Radius and hydration radius of lipoproteins: Model B_1

Table 6 shows the variations in the inner core radius, R_0, the interface thickness, L_B, and the surface plus interface thickness, L_A, for Model B_1 as a function of the weight fraction of cholesteryl ester (CE) partitioning into the interface region, f_B, according to eq. (12), where R_2 is calculated from the Maude-Whitemore expression and $R_{2(T)}$ from $R_{2(T)} = [3M\bar{v}/4\pi N]^{1/3}$ and f_{CE} is the weight fraction of cholesteryl ester in the whole particle from table 2 and f_B is arbitrarily chosen in five equal increments for table 6, noting that the core plus interface radius, R_1, and the surface thickness, L_c, are constant in this model.

Table 7

Model B_2: Interdigitation by triglyceride.

The table shows variations in the inner core radius, R_0, the interface thickness, L_B, and the surface plus interface thickness, L_A, for Model B_2 as a function of the weight fraction of triglyceride (TG) partitioning into the interface region, f_B, according to the following equations: $R_0 = R_2\{[(f_1 - f_B)\rho_2/[\rho_1 + (d\rho_0/df_0)f_B]\}^{1/3}$ [a], where $0 \leq f_B \leq f_{TG}$ [b], $L_B = R_1 - R_0$, and $L_A = L_B + L_C = R_2 - R_0$. [c] The other necessary parameters for evaluation of R_0 for Model B_2 are given by: [a] R_2 is calculated from $R^2 = 9s^0 n / 2(\rho_2 - \rho_s)$ and the true radius, $R_{2(T)}$ from $R_{2(T)} = [3M\bar{v}/4\pi N]^{1/3}$. [b] f_{TG} is the weight fraction of triglyceride in the whole particle from table 2 and f_B was arbitrarily chosen in five equal increments for this table. [c] The core plus interface radius, R_1, and the surface thickness, L_c, are constant in this model.

f_B	R_0(Å)	L_B(Å)	L_A(Å)	$R_{0(T)}$(Å)	$L_{B(T)}$(Å)	$L_{A(T)}$(Å)
LDL at $\rho_2 = 1.0508$						
0	71.02	0	16.94	81.94	0	19.54
0.0128	70.35	0.67	17.61	81.17	0.77	20.31
0.0256	69.67	1.35	18.29	80.38	1.56	21.10
0.0384	68.98	2.04	18.98	79.58	2.36	21.90
0.0512	68.27	2.75	19.69	78.77	3.17	22.71
0.0640	67.55	3.47	20.41	77.94	4.00	23.54
$R_2 = 87.96$ Å; $R_1 = 71.02$ Å; $L_c = 16.94$ Å; $R_{2(T)} = 101.48$ Å; $R_{1(T)} = 81.94$ A; $L_{c(T)} = 19.54$ A						
HDL_2 at $\rho_2 = 1.1247$						
0	26.47	0	19.13	31.04	0	22.44
0.0068	26.08	0.39	19.52	30.59	0.45	22.89
0.0136	25.69	0.78	19.91	30.13	0.91	23.35
0.0204	25.28	1.19	20.32	29.65	1.39	23.83
0.0272	24.86	1.61	20.74	29.16	1.88	24.32
0.0340	24.43	2.04	21.17	28.65	2.39	24.83
$R_2 = 45.60$ Å; $R_1 = 26.47$ Å; $L_c = 19.13$ Å; $R_{2(T)} = 53.48$ Å; $R_{1(T)} = 31.04$ A; $L_{c(T)} = 22.44$ A						
HDL_3 at $\rho_2 = 1.1647$						
0	20.14	0	17.36	23.31	0	20.08
0.0064	19.78	0.36	17.72	22.89	0.42	20.50
0.0128	19.41	0.73	18.09	22.45	0.86	20.94
0.0192	19.02	1.12	18.48	22.01	1.30	21.38
0.0256	18.62	1.52	18.88	21.54	1.77	21.85
0.0320	18.20	1.94	19.30	21.06	2.25	22.33
$R_2 = 37.50$ Å; $R_1 = 20.14$ Å; $L_c = 17.36$ Å; $R_{2(T)} = 43.39$ Å; $R_{1(T)} = 23.31$ Å; $L_{c(T)} = 20.08$ A						

Lipoprotein	f_1	$\rho_1 (d\rho_0/df_0)_{TG} = (\rho_{CE} - \rho_1)/(f_1 - f_{CE})$	$\bar{x}$	
LDL	0.477	0.9522	0.0906	0.338
HDL_2	0.165	0.9491	0.2618	0.389
HDL_3	0.126	0.9471	0.3406	0.308

4.5. Radius and hydration radius of lipoproteins: Model B_2

Table 7 shows the variations in the inner core radius, R_0, the interface thickness, L_B, and the surface plus interface thickness, L_A, for model B_2 as a function of the weight fraction of triglyceride (TG) partitioning into the interface region, f_B, according to eq. (12), where f_{TG} is the weight fraction of triglyceride in the whole particle and $0 \leq f_B \leq f_{TG}$. The degree of hydration $\bar{x}$, in g H_2O/g lipoprotein remains constant in both models.

Table 6 clearly shows that cholesteryl ester (CE) can partition into the acyl chain portion of the phospholipid to a radius of 82 Å, or $R_{I(T)}$ of Model B_1 for LDL, giving a partition region thickness (L_B) of 41 Å. In HDL_2, this distance is $\approx$ 13 Å; in HDL_3, 9 Å. In Model B_2, interdigitation of triglyceride (TG) measured only 2–4 Å in all lipoprotein particles, a distance too slight to be experimentally detected, as seen in table 7. Values for $L_{(A)T}$ in tables 6 and 7 suggest that the surface of these lipoprotein particles is irregular and convoluted.

5. Discussion

5.1. Comparison of density-composition model with CPK space-filling model

5.1.1. High-density lipoproteins (HDL_2 and HDL_3)

A comparison of interest may be made between our Model B, which is based on density and composition data, and the space-filling model for lipoproteins which has been proposed by Verdery and Nichols [13]. As a model for interdigitation or packing of cholesteryl ester and/or triglyceride into the outer hydrophobic core, the Verdery and Nichols model [13] is strictly dependent on the conformation or organization of the protein in the particle. Using Verdery and Nichols' approach, we have reevaluated the volumes of the surface and core regions of lipoprotein particles. Our results show that every molecule of the lipoprotein particle can be accommodated within the volumes of the surface and core regions.

In considering the amount of surface area covered by apolipoprotein, assuming that it is in a spherical conformation, we calculated that the surface area of one apo A-I molecule was 5.18×10^3 Å^2 and that of one apo A-II molecule was 3.67×10^3 Å^2, based on the expression $A = 4\pi[(3/4\pi)(0.74)(M/N)]^{2/3}$. These values were four times larger than those reported by Verdery and Nichols. Assuming that their calculations of surface area covered by lipid are correct, the area available for protein on the surface would be completely covered in both particles, giving a remainder of 18–27% of the protein occupying area beneath the surface in HDL_2 and 13–18% of the protein occupying area beneath the surface in HDL_3.

On the other hand, if the apolipoprotein assumes an extended helical conformation, the area occupied by a helical cylinder (from the Corey-Pauling-Koltun molecular model) with a diameter of 12–18 Å, based on the area πdl, would be $5.68–8.52 \times 10^4$ Å^2 for HDL_2 and $4.00–5.99 \times 10^4$ Å^2 for HDL_3. Hence, the area available for protein on the surface would be completely covered in both particles, with 64–76% of the protein beneath the surface in HDL_3. Such a model would be highly unlikely, as Verdery and Nichols specify that no portions of the protein are inaccessible to enzymatic digestion or succinylation [13].

The protein volume for such a helical cylinder model was calculated to be 4.33×10^5 Å^3 for HDL_2 and 2.90×10^5 Å^3 for HDL_3, where $V = \pi r^2 l$. In HDL_3, this would exceed the total volume of the particle, 2.68×10^5 Å^3. In HDL_2, such a conformation would allow only 0.51×10^5 Å^3 for the outer hydrophobic core volume, ruling out any possibility of phospholipid acyl chains or cholesterol in the outer hydrophobic core. Again, we must conclude that such an extended helical protein conformation is highly unlikely in the HDL particles. Our calculations of the surface and core volumes indicate that the protein is most likely to be in a globular conformation in HDL_2 and HDL_3. The volume available for apolar lipid in the outer hydrophobic core thus becomes particularly significant, since it will directly influence the core packing of cholesteryl ester and triglyceride in the lipoprotein particles.

5.1.2. Low-density lipoprotein (LDL)

The physical parameters of LDL, based on the Corey-Pauling-Koltun space-filling model, are shown in Table 8. In the LDL particle, the sum of the inner and outer hydrophobic core volumes was found to be 3.25×10^6 Å^3, slightly larger than the total apolar lipid volume. Our calculations show that 28% of the triglyceride and cholesteryl ester may be accommodated within the outer hydrophobic core, based on:

$$\underset{\text{(Volume available) for apolar liquid)}}{7.13 \times 10^5 \text{ Å}^3} \div \underset{\text{(Total apolar lipid volume)}}{2.52 \times 10^6 \text{ Å}^3} \times 100 = 28\%$$

Table 8

Physical parameters of LDL based on Corey-Pauling-Koltun space-filling model.

Volumes (in Å^3) of surface and core regions of LDL	
Total volume [a]	4.38×10^6
Polar surface volume [b]	1.03×10^6
Inner ϕ_h core volume [c]	1.62×10^6
Outer ϕ_h core volume [d]	1.73×10^6
Phospholipid acyl chain volume [e]	7.18×10^5
Cholesterol volume [f]	2.99×10^5
Volume available for apolar lipid [g]	7.13×10^5
Triglyceride volume [h]	3.22×10^5
Cholesteryl ester volume [i]	2.20×10^6
Total apolar lipid volume	2.52×10^6

[a] $4/3\ \pi(101.48)^3$. ϕ_h represents the hydrophobic groups.
[b] Protein volume + phospholipid polar head group volume. Protein volume was estimated assuming a sphere with $\bar{v} = 0.725$ ml/g and a molecular weight of 6.34×10^5. The volume of phospholipid polar head groups was estimated assuming 340 Å^3/polar head group and 772 phospholipids/LDL.
[c] $4\pi/3\{[3/4\pi(\text{total volume} - \text{polar surface volume})]^{1/3} - 20\}^3$, assuming acyl chain length of 20 Å/phospholipid.
[d] Total volume − polar surface volume − inner ϕ_h core volume.
[e] Phospholipid (PL) acyl chain volume is 930 Å^3/PL and 772 PL/LDL.
[f] Unesterified cholesterol (UC) volume is 622 Å^3/UC and 480 UC/LDL.
[g] (Outer ϕ_h core volume) − (PL acyl chain volume + cholesterol volume).
[h] Assuming 1542 Å^3/TG and 209 TG/LDL.
[i] Assuming 1248 Å^3/CE and 1760 CE/LDL.

The percentages of total apolar lipid accommodated in the outer hydrophobic core are 28, 64 and 94% for LDL, HDL_2 and HDL_3, respectively, and represent upper limit values of partitioning from the inner hydrophobic core into the outer hydrophobic core. These results suggest potential differences in the packing of cholesteryl esters and triglyceride among the three density classes, with the assumption that all protein is in a globular conformation, occupying only the surface of the particle. However, it is unlikely that this is the case, since protein conformation is dictated by the kinds and amounts of protein structures present as well as the lipid environment. The predicted difference in apolar lipid packing between HDL_2 and HDL_3, for example, may be an artifact generated due to unconsidered differences in protein-protein interactions as a result of different A-I/A-II ratios.

Any deviation from this assumed globular conformation, barring changes in particle radius due to extension of peptide chains into the surrounding medium, means that portions of the protein must extend into the outer hydrophobic core, leaving less room for triglyceride or cholesteryl ester partitioning. This is particularly likely to be the case for LDL, since apo B is insoluble in the absence of amphiphilic compounds such as phospholipid or detergent. That is, a considerable amount of apo B probably extends into the outer hydrophobic core of LDL, interacting with the phospholipid acyl chains and free cholesterol. Therefore, there may indeed be very little room for partitioning of cholesteryl ester and triglyceride in LDL [13]. However, the proteins of the HDLs are not as lipophilic, and although there may be little or no difference in partitioning of cholesteryl ester and triglyceride between HDL_2 and HDL_3, the larger percentages of volume available for apolar lipid in the outer hydrophobic core of the HDLs, which would be accompanied by a significant decrease in the surface curvature [50,51], indicate that there may be a difference in packing of cholesteryl ester and triglyceride between the HDLs and LDL.

Our density and composition Model B proposes a separate and distinct interface region, between the inner and outer hydrophobic cores of the space-filling model, which would consist of a single

component, either cholesteryl ester, triglyceride or both, out of the inner hydrophobic core into a region beneath the outer hydrophobic core, beginning at the ends of the phospholipid acyl chains. This intermediate state of partitioning can be affected by protein conformation only in extreme cases, where protein helices as well as lipophilic side chains may provide interaction sites through which such differential partitioning could take place.

Considering Model B_1, our results indicate that in the HDLs, most of the protein occupies the surface area, since it is completely accessible to enzymatic digestion and succinylation [13]. As seen from table 6, the existence of an interface region may not be significant in these lipoproteins.

In LDL particles containing apo B protein, however, Model B_1 would seem to describe effectively the partitioning properties of cholesteryl ester into the interface region, suggesting that the apo B protein, probably in dimeric form, is interdigitated into the outer hydrophobic core of the LDL. In terms of a space-filling model, the volume occupied by apo B in LDL can be increased from 7.63×10^5 to $0.83-1.15 \times 10^6$ Å^3, assuming 30% extended helices and 70% globular structure. Since there is not sufficient volume in the outer hydrophobic core (7.13×10^5 Å^3) to accommodate such a conformation, some portions of the protein may extend into the inner hydrophobic core, and, through specific interactions with cholesteryl ester or triglyceride, bring about the formation of an interface region as seen in Model B. Such a conformation would still leave unused volume in the outer hydrophobic core ($> 3.68 \times 10^5$ Å^3). It becomes apparent that Model B is strongly dependent on the organization of apolipoprotein in the particle, and that although the model is based upon a sphere, the actual surface of the lipoprotein particle could be highly convoluted and irregularly formed.

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