Physical Properties of the Dimyristoylphosphatidylcholine Vesicle and of Complexes Formed by Its Interaction with Apolipoprotein C-III†

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ABSTRACT: The structure of a single bilayer vesicle of dimyristoylphosphatidylcholine has been characterized by sedimentation, densimetry, and light-scattering measurements. The molecular weight, partial specific volume, Stokes radius, and degree of hydration were found to be 2.68×10^6, 0.972 cm^3/g, 125 Å, and 0.86 g/g, respectively. From these quantities, a spherically symmetrical model has been derived that features a phospholipid bilayer 35.5 Å thick and a hydration shell 9.3 Å thick. This particle was shown to bind apolipoprotein C-III (apoC-III) up to 0.08 g/g without loss of its original vesicular structure. At protein–lipid ratios in excess of 0.08 g/g, sedimentation, gel chromatography, and light-scattering measurements indicated a dramatic decrease in Stokes radius and molecular weight. The sedimentation data showed these parameters to become constant at protein–lipid ratios in excess of 0.25 g/g. In this region, the Stokes radius and molecular weight were found to be ~80 Å and 442 000, respectively. Within the constraints of these values and other data, several models for this complex are discussed.

In recent years, considerable attention has been focused on multicomponent biological systems in which lipid–protein interactions were operative. Some of the representative systems of major interest have been cytochrome oxidase (Jost et al., 1973), red cell glycoproteins (Marchesi et al., 1976), adenosinetriphosphatase (Kimelberg, 1975), myelin basic protein (Liebes et al., 1976), and apolipoproteins (Morrisett et al., 1975). Much of the effort of this laboratory has been directed toward the goal of elucidating the micro- and macromolecular structure of lipid–protein complexes resulting from the interaction of phospholipid vesicles with apolipoproteins, with the hope that an understanding of these systems will permit the development and refinement of accurate models for intact native lipoproteins. Previous work has indicated that one of the apolipoproteins of human very low density lipoproteins, apoC-III,[1] forms a complex with bilamellar vesicles of egg yolk phosphatidylcholine (0.23 g of protein/g of lipid) by intercalating into the bilayer without destroying the initial structural integrity of the vesicles (Morrisett et al., 1974). ApoC-III has also been shown to interact with vesicles of dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) when the lipid is at or above its gel to liquid-crystalline phase transition temperature (Träuble et al., 1974; Pownall et al., 1974, 1977). In the case of DMPC vesicles, the binding of apoC-III (0.26 g/g) causes an elevation of the transition temperature (normally observed at 24 °C); an apparent decrease in the size of the cooperative unit undergoing this transition is suggested by paramagnetic resonance data (Novosad et al., 1976). To date, no detailed study of the hydrodynamic properties of the DMPC bilamellar vesicle or its complex with apoC-III has been reported. In this report, these physical properties are presented and structural models are proposed for both the DMPC vesicle and its complex. These results are contrasted with the previous study of the egg yolk phosphatidylcholine–apoC-III system (Morrisett et al., 1974) with respect to overall structure and complex formation. In this study, it is shown that DMPC vesicles not only bind apoC-III but also undergo a structural alteration at protein–lipid ratios above 0.08 g/g to a species of smaller molecular weight.

Experimental Section

Materials. ApoC-III containing one residue of sialic acid was isolated from very low density lipoproteins as described previously (Morrisett et al., 1973). Precaution was taken to minimize apoprotein self-association by treating with 6 M guanidine hydrochloride followed by exhaustive dialysis immediately before use. A single buffer (standard buffer) consisting of 100 mM NaCl, 10 mM Tris, 1 mM NaN_3, 1 mM EDTA, pH 7.40, was used throughout this study. Crystalline DMPC was obtained from Sigma Chemical Co.; it migrated as a single species upon thin layers of silica gel G eluted with either $CHCl_3$–CH_3OH–H_2O (65:25:4) or $CHCl_3$–CH_3OH–HOAc–H_2O (80:13:8:0.8) and had a fatty acid composition of 99.5% myristic acid, as judged from gas chromatography (10% Silar 10 C/Supelcoport[2]). Deuterium oxide (99.75 atom % ^2H) was purchased from J. T. Baker Chemical Co.

Methods. Instrumentation. Partial specific volumes were calculated from density measurements (Kratky et al., 1973) performed on a DMA-02D Mettler-Paar densimeter (Anton-Paar, Graz, Austria). Its temperature was monitored to an accuracy of ±0.001 °C with a Model TM 401 high-sensitivity thermometer (Technical Hardware, Inc., Fullerton,

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[1] Abbreviations used are: DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; apoC-III, an apolipoprotein (molecular weight 9300) containing one sialic acid residue and isolated from human very low density lipoproteins; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; EDTA, (ethylenedinitrilo)tetraacetic acid.

[2] Commercial DMPC as obtained from Sigma when chromatographed by high-pressure liquid chromatography was found to contain 3–4% free myristic acid.

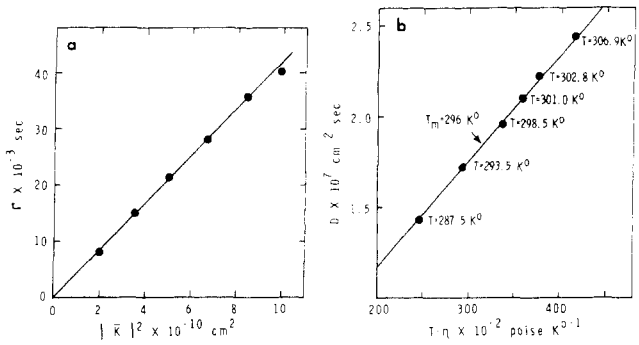

FIGURE 1: (a) Decay rate Γ as a function of the square of the scattering wave vector $|\bar{K}|^2$ for a solution of dimyristoylphosphatidylcholine vesicles. $T = 28$ °C, $\lambda_0 = 4579$ Å, concentration = $\simeq 1$ mg/mL. (b) Comparison of calculated (—) (Stokes–Einstein equation) and measured (●) translational diffusion coefficients as a function of T/η for a suspension of dimyristoylphosphatidylcholine vesicles in a buffer containing 100 mM NaCl, 10 mM Tris, 1 mM NaN_3, 1 mM EDTA (pH 7.4). η is the solvent viscosity; $|\bar{K}|^2 = 6.69 \times 10^{10}$ cm^{-2}.

Calif.) and controlled with a water bath to a precision of ±0.01 °C. The translational diffusion coefficient and corresponding Stokes radii were determined by quasielastic light scattering (Pecora, 1964). For a monodisperse solution of particles undergoing Brownian motion, the autocorrelation function decays exponentially, with the correlation time, τ_c, given by the equation

$$\tau_c = (2D|\bar{K}|^2)^{-1} \quad (1)$$

where $|\bar{K}|$ is the scattering wave vector

$$|\bar{K}| = \left(\frac{4\pi n}{\lambda_0}\right) \sin(\theta/2) \quad (2)$$

where n is the refractive index, θ the scattering angle, and λ_0 the wavelength of incident light. Light-scattering measurements were performed using an argon ion laser (Spectra Physics Model 165) as a source of incident light (λ_0 4579 Å). The laser beam was operated at a power level of 60–100 mW and was focused at the center of a scattering cell (10 × 4 × 45 mm) containing the sample which had been filtered through a 0.22-μm Millipore membrane.

Analytical ultracentrifugal measurements were performed on a Beckman Model E instrument equipped with an RTIC temperature control. Sedimentation equilibrium and velocity experiments were carried out as described previously (Morrisett et al., 1974). The isodensity point of the vesicles in deuterium oxide was determined by sedimentation analysis in a manner similar to that of Huang and Charlton (1971). Sedimentation coefficients were obtained at vesicle concentrations of 0.5 to 2.0 mg/mL. In order to obtain the desired protein–lipid ratios in the sedimentation studies, precisely measured volumes of DMPC vesicles and apoC-III at known concentration were transferred with Glenco microsyringes to the centrifuge cells. The solutions were mixed by manually rotating the closed cells.

For gel filtration chromatography, a water-jacketed column (28 °C) of Sepharose 6B (0.9 × 180 cm) was operated in the downward flow mode at a rate of 3–6 mL/h. Homogeneous samples of DMPC bilayer vesicles were prepared by chromatography of 40 mg of phospholipid which had been initially dispersed by sonication in 1 mL of standard buffer (Morrisett et al., 1974). The DMPC–apoC-III complexes were prepared by dropwise addition of the apoprotein to a vesicle solution until a protein–lipid weight ratio of about 0.33 was attained. The resulting complex was then chromatographed on the Sepharose 6B column and was found to elute at a volume between that of pure DMPC vesicles and the apoprotein.

Phospholipid concentration was determined by the method of Bartlett (1959). Protein concentration was determined by amino acid analysis or by absorbance at 280 nm ($E_{280} = 2.67$ mL mg^{-1} cm^{-1}).

Results and Discussion

Physical Properties and Structural Model of DMPC Vesicles. In initial light-scattering studies, DMPC vesicles were noted to aggregate with time. Over the temperature range studied (14–34 °C), it was found that these vesicles had aggregated significantly after 12 h, as evidenced by both an increase in the correlation time, τ_c, and nonexponential behavior of the autocorrelation function. In addition, the size of the vesicles was also found to vary with the volume required for their elution from the gel filtration column. Therefore, to ensure a vesicle population of standard size and to minimize the effect of aggregation, experiments were performed with samples taken from the peak fractions of the elution profile and all samples were measured only after Millipore filtration (0.22-μm pore size) and temperature equilibration (30 min).

The plot of the decay rate, Γ ($\Gamma = (\tau_c)^{-1}$), as a function of $|\bar{K}|^2$ for quasielastic light scattering was linear at 28 °C. This finding indicates that the dispersions of these vesicles were uniform with respect to particle size (Figure 1a). In addition, the translational diffusion coefficient as determined from measurements of the correlation time, τ_c, was found not to be concentration dependent between 0.5–2.5 mg/mL. From these data, the translational diffusion coefficient, reduced to standard conditions, was $D_{20,w} = 1.72 \pm 0.06 \times 10^{-7}$ cm^2/s. The mean particle diameter determined from the Stokes–Einstein equation was 250 ± 8 Å.

$$R_0 = \frac{kT}{6\pi\eta D} \quad (3)$$

R_0 is the hydrodynamic radius, k is the Boltzmann constant, T is the absolute temperature, D is the diffusion coefficient, and η is the solution viscosity.

The physical parameters of the vesicle were also obtained independently at this temperature by sedimentation analysis. The values of molecular weight, $M = 2.73 \times 10^6$, sedimentation coefficient, $s_{20,w} = 5.50 \pm 0.15$ S, and partial specific volume, $\bar{v} = 0.972$ cm^3/g, determined from sedimentation velocity and densimetry experiments were combined in the Svedberg equation to yield the diffusion coefficient. The parameter, $D_{20,w}$ and corresponding diameter were computed to be 1.66×10^{-7} cm^2/s and 258 Å, respectively, in excellent agreement with the light-scattering data.

The value of the translational diffusion coefficient of the DMPC vesicle obtained by quasielastic light scattering and sedimentation analysis in this study is almost an order of magnitude lower than that obtained previously by a pulsed-gradient Fourier transform NMR technique (McDonald and Vanderkooi, 1975). In that study, the diffusion coefficient obtained in a D_2O–phosphate buffer at 37 °C was 1.9×10^{-6} cm^2/s. If the viscosity of the buffer is assumed to be equal to the viscosity of D_2O at 37 °C, a mean particle radius of 15 Å can be computed for the DMPC vesicle. This value is approximately a factor of 2 less than the bilayer thickness of the vesicle as proposed in this study (see discussion below), and of the order of the molecular size of an isolated DMPC molecule. It is clear from the above calculation that the translational diffusion coefficient determined by the NMR technique is

TABLE I: Physical Properties of Dimyristoylphosphatidylcholine Single Bilayer Vesicles Measured at 28 °C in Buffer b Consisting of 100 mM NaCl, 10 mM Tris, 1 mM NaN_3, 1 mM EDTA, pH 7.4.

$D_{28,b}$[a]	2.10×10^{-7} cm^2 s^{-1}
$D_{20,w}$	1.72×10^{-7} cm^2 s^{-1}
$s_{28,b}$[b]	5.99 S
$s_{20,w}$	5.50 S
R_0[c]	125 Å
$\bar{v}$[d]	0.972 cm^3 g^{-1}
M	(2.73[e], 2.63[f])10^6
δ[g]	0.86 g of H_2O/g of lipid

[a] Translational diffusion coefficient determined by quasielastic light scattering. [b] Sedimentation coefficient. [c] Stokes radius calculated from the diffusion coefficient using the Stokes–Einstein equation. [d] Partial specific volume determined by sedimentation velocity in D_2O and by mechanical oscillator densimetry. [e] Molecular weight determined by sedimentation equilibrium. [f] Molecular weight calculated from experimentally determined values of $s_{20,w}$ and $D_{20,w}$ using the Svedberg equation. [g] Degree of hydration calculated from eq 4.

inconsistent with that expected for the Brownian motion of an individual DMPC single bilayer vesicle.

The translational diffusion coefficient of a molecule in solution must be linearly dependent on the quantity T/η, if the molecular dimensions remain unchanged as a function of temperature. The translational diffusion coefficient of DMPC vesicles measured as a function of temperature exhibited such a linear dependence (Figure 1b) over a temperature range of 14–34 °C. It is clear that no significant alteration in the hydrodynamic diameter of the vesicle is associated with the well-documented phase transition at $T_m \sim 23$ °C (Hinz and Sturtevant, 1972; Shimshick and McConnell, 1973; Novosad et al., 1976).

The sedimentation coefficient, which is also sensitive to changes in hydrodynamic diameter, *was* found to vary with temperature. The quantity, $s_{20,w}$, was found to be 5.50 and 6.59 S when measured at 28 and 23 °C, respectively. However, it was found that the increase in sedimentation coefficient can be accounted for by an observed decrease in the partial specific volume from 0.972 cm^3/g at 28 °C to 0.963 cm^3/g at 23 °C as measured by the flotation technique in D_2O. The sedimentation coefficient is seen to be a very sensitive probe of changes within the bilayer because of its dependence on the partial specific volume through the quantity, $1 - \bar{v}\rho$. It is a necessary consequence that, if the volume of the bilayer decreases while maintaining a constant hydrodynamic volume, either the bilayer thickness must decrease or the hydration must increase.

In Table I are summarized values for various physical properties of DMPC vesicles at 28 °C: the diffusion and sedimentation coefficients, Stokes radius, partial specific volume, molecular weight, and degree of hydration, δ. This last parameter is determined by relating the hydrodynamic volume to the volume occupied by the lipid mass in the vesicle (Tanford, 1961).

$$\delta = \left(\left(\frac{4\pi}{3}\right) NR_0^3 - M\bar{v}\right) \Big/ M\bar{v}_1 \qquad (4)$$

Thus, combining the data obtained from the sedimentation and diffusion experiments with $M = 2.68 \times 10^6$, $R_0 = 125$ Å, $\bar{v}_1$, the partial specific volume of bulk water, equal to 1.0038 cm^3/g, and N, Avogadro's number.

From the physical properties listed in Table I, a model can be proposed for the structure of the DMPC vesicle which is characterized by the following features: (a) a spherical bilayer of thickness BL; (b) an internal aqueous compartment of radius R_I; (c) an external aqueous shell of thickness ΔR; (d) water in the hydrated regions whose density does not differ significantly from that of bulk water. All of these features are incorporated into the model shown in Figure 2. The dimensions of the structure are obtained from the expression for the volume of a bilayer previously derived from egg yolk phosphatidylcholine vesicles (eq 12, Morrisett et al., 1974). In the present study, a value of 35.5 Å (Laggner, P., Kratky, O., Gotto, A. M., and Morrisett, J. D., in preparation) is used for the thickness of the DMPC bilayer.[3] The dimensions of ΔR and R_I are calculated to be 9.3 and 80.2 Å, respectively, which are consistent with the degree of hydration found for the vesicle. It should be noted here that the independently derived hydration shell thickness for the DMPC vesicle is in good agreement with that found previously for the egg yolk phosphatidylcholine vesicle.

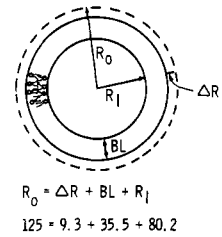

FIGURE 2: Structural model of the DMPC vesicle. R_0 is the hydrodynamic radius, BL is the bilayer thickness, ΔR is the hydration-shell thickness, and R_I is the radius of the inner aqueous compartment.

Structure of the Complex Resulting from Interaction of the DMPC Vesicle with ApoC-III. The DMPC vesicle as characterized and discussed in the above section interacts with apoC-III to form two structurally different complexes.

The formation of these two types of complexes is demonstrated in Figure 3 where the sedimentation coefficient, $s_{28,b}$, observed for a sedimenting vesicle is plotted against the ratio of apoC-III to phospholipid in the system. The DMPC vesicle alone sediments with an observed sedimentation coefficient of 6.0 S, increasing to approximately 10 S when the protein–lipid ratio is increased from 0 to 0.08 g/g. This observed increase in sedimentation coefficient indicates that the protein binds to the vesicle and that binding causes not only an increase in its molecular weight, but also a decrease in its partial specific volume because of the greater density of the protein. Assuming that the protein is simply intercalated into the bilayer without disrupting the vesicle structure nor increasing its dimensions, one may calculate the molecular weight, M, from the equation

$$M = M_0(1 + x) \qquad (5)$$

where x is equal to the protein–lipid mass ratio and M_0 is the initial molecular weight of the vesicle.

[3] This dimension represents the distance between maximum electron density peaks (presumably the phosphorus–phosphorus interatomic distance across the bilayer). The bilayer thickness used in the previous study (Morrisett et al., 1974) cannot be directly compared here because of the difference in chemical nature of the vesicles and temperature (10°C) employed. The relationship between hydration shell thickness and bilayer thickness is more tightly constrained in the case of the DMPC vesicles because of the larger radius; e.g. DMPC vesicles with a bilayer thickness taken to be 45 Å would have an unreasonable hydration shell thickness of 16 Å. However, egg yolk vesicles with a bilayer thickness taken to be 38 Å rather than 45 Å would have a reasonable hydration shell thickness of 5 Å.

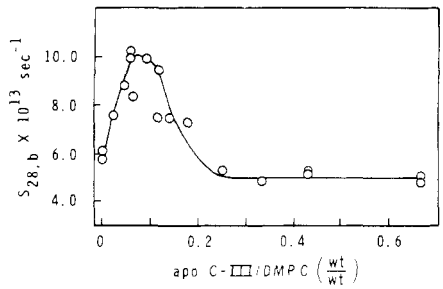

FIGURE 3: Sedimentation velocity experiment in which the observed sedimentation coefficient of the apoC-III–DMPC complex was measured as a function of protein–lipid mass ratio. Conditions: temperature, 28 °C; solvent, 100 mM NaCl, 10 mM Tris, 1 mM EDTA, 1 mM NaN_3 (pH 7.4); speed, 52 640 rpm.

Consider the complex resulting from interaction of apoC-III and DMPC *at* a mass ratio of 0.08 g/g. If the molecular weight of the vesicle alone is taken to be 2.68×10^6 (Table I), the molecular weight for the complex is then computed to be 2.89×10^6. The partial specific volume of the complex, $\bar{v}_c$, can be computed by simple additivity of masses (assuming no volume changes occur) according to the equation

$$\bar{v}_c = (x\bar{v}_p + \bar{v}_v)/(1 + x) \quad (6)$$

Hence, if x is taken as 0.08 g/g, $\bar{v}_p$ as 0.723 cm^3/g (Morrisett et al., 1974), and $\bar{v}_v$ as 0.972 cm^3/g (Table I), the partial specific volume of the complex, $\bar{v}_c$, is computed to be 0.954 cm^3/g. The calculated value of s, assuming no shape or size change, will then be

$$s = s_0(M/M_0)^{2/3}(1 - 0.954\,\rho)/(1 - 0.972\,\rho)$$
$$= 1.75 s_0 = 10.5\ \text{S} \quad (7)$$

This value of s is in excellent agreement with the experimentally observed value of 10 S, and suggests that *at 0.08* g/g, apoC-III binds to DMPC vesicles by a mechanism similar or identical to that observed for egg yolk phosphatidylcholine vesicles *at all ratios* studied (up to 0.6 g/g) (Morrisett et al., 1974).

As the protein–lipid ratio is increased above 0.08 g/g, the sedimentation coefficient of the resulting complex decreases drastically, until a minimal value of 5 S is reached at 0.25 g/g. Since this protein-rich complex exhibits a smaller sedimentation coefficient than the protein-poor one characterized above, the immediate conclusion is that the former is asymmetric and/or of a lower molecular weight. Significantly, the breakdown of the protein–DMPC complex is complete at 0.25 g/g, whereas the egg yolk phosphatidylcholine–apoC-III complex is saturated without disintegration at 0.23 g/g. Apparently, the loosely packed, unsaturated acyl chains of the egg yolk phosphatidylcholine bilayer can accommodate considerably more protein without allowing the vesicle to lose its structural integrity.

The binding of apoC-III to DMPC vesicles was also observed by gel filtration chromatography. At a protein–lipid ratio of less than 0.08 g/g, the complex elutes at essentially the same volume as the pure single bilayer DMPC vesicle. When this ratio is increased to 0.25 g/g, the elution volume also increases (Table II), suggesting that the complex has assumed a new structure of lower molecular weight and/or smaller volume. The Stokes radius of this complex was calculated by the method of Ackers (1967) from its elution volume (62.0 mL) and the column parameters determined from elution volumes of several proteins of known size (Table II). The computed Stokes radius for the DMPC–apoC-III complex

TABLE II: Stokes Radius Determination of DMPC–ApoC-III Complex by Gel Filtration at 28 °C on Sepharose 6B (0.9 × 180 cm).

Eluted particle	Elution vol (mL)	Stokes radius (Å) This study	Stokes radius (Å) Previous study
DMPC *multi*bilayer vesicles	40.0 (V_0)		
Thyroglobulin (bovine)	51.5	92.0[a]	88.5[c]
Fatty acid synthetase (chicken)	59.5	78.0[a]	81.5[d]
DMPC–apoC-III complex	62.0	72.0[b]	
Catalase (bovine)	73.0	49.0[a]	52.3[e]
$^{22}Na^+$	120.0 (V_t)		

[a] Determined from quasielastic light-scattering measurements. [b] Computed by the method of Ackers (1967) from the other elution volume and Stokes radius data given in this Table. [c] Edelhoch (1960). [d] Stoops et al. (1975). [e] Sumner and Gralén (1935).

TABLE III: Physical Properties of a Dimyristoylphosphatidylcholine–ApoC-III Complex (0.08 g of Protein/g of Lipid). Measured at 28 °C in Buffer b Consisting of 100 mM NaCl, 10 mM Tris, 1 mM NaN_3, 1 mM EDTA, pH 7.4.

x[a]	0.08 g of protein/g of lipid
M_{calcd}[b]	2.89×10^6
$\bar{v}_{calcd}$[c]	0.954 cm^3/g
$s_{28,b}$	10.5 S[d], 10.0 S[e]

[a] Protein–lipid weight ratio determined by sedimentation velocity. See Figure 4. [b] Molecular weight calculated on basis of $x = 0.08$ and value of 2.69×10^6 for DMPC vesicle alone. [c] Partial specific volume calculated assuming linear additivity of $\bar{v}$'s for DMPC vesicle (0.972) and apoC-III (0.723) with $x = 0.08$. See eq 6. [d] Sedimentation coefficient in buffer computed on basis of M_{calcd} and $\bar{v}_{calcd}$ above. [e] Sedimentation coefficient determined experimentally.

with a protein–lipid ratio of 0.259 g/g is 72 ± 4 Å (Table III).

Complexes resulting from protein–lipid mixtures were also studied at ratios above 0.25 g/g by quasielastic light scattering. After 30 min of incubation at 28 °C, the correlation time, τ_c, decreased approximately 25%, indicating that the hydrodynamic size of the resultant particle was less than that of the original DMPC vesicle. A complete characterization of the particle by this technique was not possible because the autocorrelation function of such mixtures did not decay exponentially. This suggested that the solutions of the complex did not contain particles of uniform size. Low concentrations (<5%) of aggregated forms of the complex make disproportionally large contributions to the autocorrelation function which is proportional to the square of both the concentration and molecular weight of the scattering particle. The presence of these aggregates would not influence the basic interpretation of the sedimentation and chromatography experiments.

The molecular weight of the DMPC–apoC-III complex may also be determined by sedimentation equilibrium measurements which yield directly the quantity $M(1 - \bar{v}\rho)$. However, the molecular weight and partial specific volume are not directly deducible. One may approximate the partial specific volume by the additivity rule, as given by eq 6. Using a mass ratio of 0.26 g/g, a partial specific volume of 0.921 cm^3/g is calculated for the small DMPC–apoC-III complex. By con-

ducting the sedimentation equilibrium experiment in H_2O and D_2O according to the method of Edelstein and Schachman (1967), the molecular weight and partial specific volume of the 0.26 g/g complex were determined, simultaneously. In standard buffer containing H_2O, at 29.8 °C, the quantity $M(1 - \bar{v}\rho)$ was found to be 42 500. In standard buffer containing 10% D_2O, at 29.8 °C, this same quantity was found to be 37 100. Assuming the molecular weight is constant upon addition of D_2O to the system, the partial specific volume and molecular weight are simultaneously calculated to be 0.923 ± 0.015 cm^3/g and 554 000 ± 101 000, respectively.

An independent measure of the partial specific volume was also obtained by densimetry. The value determined for the DMPC–apoC-III complex with a stoichiometry of 0.26 g/g was found to be 0.905 ± 0.001 cm^3/g. Using this value, the molecular weights computed from the sedimentation equilibrium data in H_2O standard buffer and D_2O standard buffer are 449 000 and 435 000, respectively. The mean of these values, 442 000, is taken to be the molecular weight of the DMPC–apoC-III breakdown complex. Therefore, at a protein–lipid mass ratio of 0.26 g/g, there must be approximately 10 protein molecules and approximately 515 DMPC molecules per particle entity.

Combining the molecular weight, the partial specific volume, and the determined sedimentation coefficient, the Stokes radius may be calculated from the following equation:

$$R_0 = M(1 - \bar{v}\rho)/(6\pi\eta Ns) = 86 \pm 5 \text{ Å} \qquad (8)$$

The reason for the discrepancy between the value obtained for R_0 from sedimentation (86 Å) and from gel chromatography (72 Å) is not clear. It is possible that there is some interaction of the complex with matrix-bound lipids (Huang, 1969) which retards elution of the complex and leads to a smaller observed Stokes radius (Table IV).

If a sphere is the correct model for the complex, the extent of hydration, δ, is computed with eq 4 to be 2.7 g/g. If values for the molecular weight from sedimentation equilibrium and for the Stokes radius from gel chromatography are combined, δ is computed to be 1.22 g/g. Both calculations suggest a rather large degree of hydration. The value obtained for the degree of hydration is dependent on the hydrodynamic model. The following models consider several combinations of asymmetry and hydration to obtain an acceptable physical description for the smaller DMPC–apoC-III complex:

(I) This model assumes the phospholipid molecules are organized in micellar arrangement with all mass (442 000 g/mol) distributed as a sphere. The radius is computed to be 54 Å, the hydration-shell thickness must necessarily be approximately 26 Å if the hydrodynamic radius is taken to be 80 Å.

(II) This model assumes a bilayer vesicle structure. The dimensions of the complex are calculated in a manner similar to that used by Morrisett et al. (1974) for the case of the egg yolk phosphatidylcholine vesicle interacting with apoC-III, or the above case of the DMPC vesicle interacting with apoC-III prior to breakdown. For this model, one computes the hydration shell to have a thickness of about 25 Å (assuming the 35.5-Å bilayer is preserved intact[3]).

(III) A third model assumes the lipids are dispersed as a monolayer 26-Å thick enclosing an aqueous core while interacting with protein on the inside surface. This spherically symmetrical structure would have a hydration shell thickness of 22.5 Å.

(IV) The last model type considered here is asymmetric. One possible asymmetric structure is a prolate ellipsoid of revolution. In order to compute the axes, a reasonable value for the hydration must be assumed. If the hydration of the smaller complex is the same as the DMPC–apoC-III complex at a protein to lipid ratio of 0.08 g/g (0.75 g of H_2O/g of complex), the axial ratio is computed to be 4.4. The minor and major axes are then computed to be 81 and 355 Å, respectively. Alternately, an oblate ellipsoid model would have an axial ratio of 4.75 with minor and major axes of 47 and 222 Å, respectively. It is clear from these calculations that the model proposed by Andrews et al. (1976), where the structure has dimensions of approximately 110 × 55 × 55 Å, does not contain sufficient mass to satisfy the molecular weight and size data obtained in the present study. Their molecular weight data indicated a heterogenous population of particles (M_n, 227 000; M_w, 325 000; M_z, 410 000) which are definitely smaller than the structure investigated here.

The hydration shell of the spherically symmetrical structures in models I, II, and III is much too large and, therefore, essentially unacceptable. Structure II could also be ruled out on the basis of its extremely small radius of curvature on the inner surface (19 Å) which renders the bilayer highly unstable. The structure that results from a protein–lipid mixture >0.08 g/g is more likely to be one involving new protein–lipid structural relationships that are significantly different from those present at ≤0.08 g/g. This view is suggested by the observation that the molecular weight decreased drastically by a factor of 6, and that the partial specific volume was no longer simply related to the composition.

Model III is more tenable than are models I and II. It requires a monolayer thickness of 26 Å which still yields a large hydration value. The monolayer is stabilized by hydration of only one polar head-group-containing surface and, hence, its thickness may not be as well defined as the bilayer thickness which is stabilized by hydration on two such surfaces. It is possible that the mean monolayer thickness is considerably less than the length of a fully extended DMPC molecule; if so, this would allow for a more reasonable hydration-shell thickness (e.g., a monolayer thickness of 13 Å would allow for a hydration-shell thickness of 10 Å).

The asymmetric model IV is probably the most tenable of the four proposed. The protein, apoC-III, does tend to polymerize at least to the trimeric state (Morrisett et al., 1974).

TABLE IV: Physical Properties of a Dimyristoylphosphatidylcholine–ApoC-III Complex (0.25 g of Protein/g of Lipid) Measured at 28 °C in Buffer b Consisting of 100 mM NaCl, 10 mM Tris, 1 mM EDTA, 1 mM NaN_3, pH 7.4.

x	0.26;[a] 0.25[b] g of protein/g of lipid
v	0.921;[c] 0.923;[d] 0.905[e] cm^3/g
M	554 000;[f] 449 000;[g] 435 000[h]
R_s	86 ± 5;[i] 72 ± 4[j] Å
$s_{28,b}$	5.06 S

[a] Protein–lipid weight ratio determined by chemical analysis of complex isolated by gel filtration. [b] Protein–lipid weight ratio determined by sedimentation velocity. See Figure 4. [c] Partial specific volume approximated by weighted additivity of $\bar{v}$'s for DMPC (0.972) and apoC-III (0.723 with x = 0.26). [d] Partial specific volume determined by sedimentation equilibrium in D_2O and H_2O. [e] Partial specific volume determined by mechanical oscillator densimetry. [f] Molecular weight calculated from sedimentation equilibrium alone in D_2O and H_2O. [g] Molecular weight computed using $\bar{v}$ = 0.905 and sedimentation equilibrium data (H_2O). [h] Molecular weight computed using $\bar{v}$ = 0.905 and sedimentation equilibrium data (D_2O). [i] Stokes radius calculated from M, $\bar{v}$, and s according to eq 8. [j] Stokes radius determined by gel filtration. See Table II.

This polymerization could lead to an extended structure that interacts with lipid to satisfy the dimensions presented. A structure containing 10 protein molecules and 515 DMPC molecules per particle entity in an extended structure (e.g., prolate ellipsoid with major axis 355 Å and minor axis 81 Å or an oblate ellipsoid with a major axis 222 Å and a minor axis of 47 Å with hydration, $\delta = 0.75$ g/g) satisfies the hydrodynamic measurements. However, it is not possible to fix the position of the water, lipid, and protein within the structure based on hydrodynamic data alone. That information must await other experimental findings.

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References

Ackers, G. K. (1967), *J. Biol. Chem. 242,* 3026.

Andrews, A. L., Atkinson, D., Barratt, M. D., Finer, E. G., Hauser, H., Henry, R., Leslie, R. B., Owens, N. L., Phillips, M. C., and Robertson, R. N. (1976), *Eur. J. Biochem. 64,* 549.

Atkinson, D., Smith, H. M., Dickson, J., and Austin, J. P. (1976), *Eur. J. Biochem. 64,* 541.

Bartlett, G. R. (1959), *J. Biol. Chem. 234,* 466.

Edelhoch, H. (1960), *J. Biol. Chem. 235,* 1326.

Edelstein, S. J., and Schachman, H. K. (1967), *J. Biol. Chem. 242,* 306.

Hinz, H.-J., and Sturtevant, J. M. (1972), *J. Biol. Chem. 247,* 6071.

Huang, C. (1969), *Biochemistry 8,* 344.

Huang, C., and Charlton, J. P. (1971), *J. Biol. Chem. 246,* 2555.

Jost, P. C., Griffith, O. H., Capaldi, R. A., and Vanderkooi, G. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 480.

Kimelberg, H. K. (1975), *Biochim. Biophys. Acta 413,* 143.

Kratky, O., Leopold, H., and Stabinger, H. (1973), *Methods Enzymol. 27,* 98.

Liebes, L. F., Zand, R., and Phillips, W. D. (1976), *Biochim. Biophys. Acta 427,* 392.

Marchesi, V. T., Furthmayr, H., and Tomita, M. (1976), *Annu. Rev. Biochem. 45,* 667.

McDonald, G. G., and Vanderkooi, J. M. (1975), *Biochemistry 14,* 2125.

Morrisett, J. D., David, J. S. K., Pownall, H. J., and Gotto, A. M. (1973), *Biochemistry 12,* 1290.

Morrisett, J. D., Gallagher, J. G., Aune, K. C., and Gotto, A. M. (1974), *Biochemistry 13,* 4765.

Morrisett, J. D., Jackson, R. L., and Gotto, A. M. (1975), *Annu. Rev. Biochem. 44,* 183.

Novosad, Z., Knapp, R. D., Pownall, H. J., Gotto, A. M., and Morrisett, J. D. (1976), *Biochemistry 15,* 3176.

Pecora, R. (1964), *J. Chem. Phys. 40,* 1604.

Pownall, H. J., Morrisett, J. D., and Gotto, A. M. (1977), *J. Lipid Res. 18,* 14.

Pownall, H. J., Morrisett, J. D., Sparrow, J. T., and Gotto, A. M. (1974), *Biochem. Biophys. Res. Commun. 60,* 779.

Shimshick, E. J., and McConnell, H. M. (1973), *Biochemistry 12,* 2351.

Stoops, J. K., Arslanian, M. J., Oh, Y. H., Aune, K. C., Vanaman, T. C., and Wakil, S. J. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 1940.

Summer, J. B., and Gralén, N. (1935), *J. Biol. Chem. 125,* 33.

Tanford, C. (1961), Physical Chemistry of Macromolecules, New York, N.Y., Wiley, p 340.

Träuble, H., Middelhoff, G., and Brown, V. W. (1974), *FEBS Lett. 49,* 269.