Physical Properties of Lipid–Protein Complexes Formed by the Interaction of Dimyristoylphosphatidylcholine and Human High-Density Apolipoprotein A-II†

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ABSTRACT: Apolipoprotein A-II (apoA-II) from human plasma high-density lipoproteins associates with dimyristoylphosphatidylcholine (DMPC) to give complexes whose structure is determined by the temperature at which the reaction is conducted. The temperature dependence is related to the gel → liquid crystalline transition temperature, T_c, of DMPC which occurs at 23.9 °C. At $T < T_c$ (20 °C), $T = T_c$, and $T > T_c$ (30 °C), three different complexes can be isolated. At 20 °C, a 75:1 (molar ratio of lipid to protein) complex is formed. This complex has a molecular weight (M_r) of 343 000, a Stokes radius, R_s, of 65 Å, and a partial specific volume ($\bar{v}$) of 0.914 mL/g. At 24 °C, two different complexes may be formed. One is similar to the one formed at 20 °C and the other is a complex with a DMPC:apoA-II ratio of 240:1; the corresponding physical constants for the latter complex are M_r = 1 580 000, R_s = 120 Å, and $\bar{v}$ = 0.948 mL/g. This complex is asymmetric, having a frictional coefficient f/f_0 = 1.20. At 30 °C, a 45:1 complex was formed; for this complex, M_r = 229 000, R_s = 57 Å, and $\bar{v}$ = 0.892 mL/g. Electron microscopy reveals that the negatively stained complexes are arranged in rouleaux having subunits with average dimensions of 175 × 60, 250 × 62, and 500 × 55 Å for the 45:1, 75:1, and 240:1 complexes, respectively. The multiple lipid–protein species formed by apoA-II and DMPC suggest the possible existence of more than one macromolecular species of lipid and apoA-II in the plasma.

Apolipoprotein A-II (apoA-II),[1] the second most abundant protein of the human high density lipoproteins (HDL) (Jackson et al., 1976), has been studied in several laboratories. Its amino acid sequence has been reported by Brewer et al. (1972); apoA-II contains two identical polypeptide chains of 77 amino acid residues which are linked by a disulfide bond at residue 6. It self-associates at low concentrations, but no common model of this process has been reported by the investigators studying this phenomenon (Gwynne et al., 1975; Teng et al., 1978; Stone & Reynolds, 1975; Vitello & Scanu, 1976). A recent communication (Ritter & Scanu, 1977) suggests that oligomeric apoA-II preferentially associates with isolated HDL lipids.

The affinity of apoA-I and apoA-II for HDL appears to differ greatly. Using differential scanning calorimetry, Tall et al. (1975, 1977) observed the thermally induced release of apoA-I at lower temperatures than that of apoA-II. Nichols et al. (1976) have shown that lipid-free apoA-I is released from HDL at low concentrations of guanidine–HCl, but at higher concentrations there is also release of apoA-II. Incubation of HDL with phospholipid multilamellar liposomes effects the selective removal of apoA-I with fusion of the residual apoA-II-rich lipoprotein (Tall & Small, 1977). ApoA-I readily associates with human HDL to form apoA-I-enriched HDL particles (Shepherd et al., 1977). Pownall et al. (1978a) have shown that lipid-free apoC proteins displace apoA-I from HDL to form a lipoprotein particle enriched in apoC. Similarly, Lagocki & Scanu (1978) have reported that human apoA-II dissociates apoA-I from canine HDL to form an apoA-II-enriched lipoprotein. These results suggest that apoA-I easily transfers to and from HDL whereas apoA-II is more strongly associated with HDL and is less easily displaced.

None of the above studies establishes whether the association of apoA-I or apoA-II with HDL is kinetically or thermodynamically controlled. Pownall et al. (1978b) have studied the kinetics of apoA-I association with DMPC and DPPC and found that the rate of association depends on the physical state of the lipid. Below the transition temperature (T_c) of DMPC its rate of association with apoA-I is slow; the rate is fastest at T_c and decreases to nil at 30 °C. Incorporation of cholesterol into the DMPC liposomes accelerated the reaction rate at all temperatures studied (Pownall et al., 1979). It was suggested that the relatively high rate of association of apoA-I with DMPC at T_c was due to selective binding at lattice defects between the gel and liquid crystalline phases and that the addition of cholesterol increased the number of defects, thereby enhancing the rate of association.

We have now undertaken a study on the dynamics and thermodynamics of the association of apoA-II and DMPC (Massey et al., 1980, 1981). According to the initial experimental conditions, especially temperature, three stable apoA-II complexes can be formed. In this study we report the physicochemical properties of these complexes. The characterization of the complexes is a necessary prerequisite to a rational understanding of the dynamics and thermodynamic properties of the association of DMPC and apoA-II. Indirect evidence suggests that apoA-II and apoA-I function differently in HDL. A thorough study of the structure, dynamics, and thermodynamics of association of apoA-II and DMPC could be an important component in our predictions of the relative rates of transfer of apoA-II among lipoproteins, the relative stability

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[1] Abbreviations used: PC, phosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; T_c, gel → liquid crystalline transition temperature; HDL, high-density lipoproteins; apoA-II, apolipoprotein A-II, a protein of human plasma HDL; apoA-I, apolipoprotein A-I, the most abundant protein of human plasma HDL; apoC, apolipoproteins C-I, C-II, and C-III, which are minor protein components of HDL; M/M, molar ratio of lipid to protein.

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of apoA-II in HDL, and the role of apoA-II in maintaining the structural integrity of HDL.

Experimental Procedures

Materials

HDL from normal female subjects were precipitated with sodium phosphotungstate by the procedure of Burstein & Scholnick (1973) and purified by the density flotation method of Scanu (1966). After removal of the KBr by dialysis and lyophilization, the HDL were delipidated at 4 °C by multiple extractions with diethyl ether/ethanol (3:1). After being dried, the apoHDL (~250 mg) was solubilized in 6 M guanidine–HCl and applied to a 2.5 × 200 cm column of Sephacryl S-200 superfine from Pharmacia Fine Chemicals, Piscataway, NJ. The column was eluted with a buffer of 3.0 M guanidine–HCl, 0.1 M Tris, pH 8.6, and 0.01% EDTA. The central portions of apoA-II fractions from two purifications were pooled, concentrated, and rechromatographed. The central portion of the apoA-II peak was combined; these fractions were devoid of other protein components as verified by amino acid analysis and the absence of reactivity with antibodies from human serum albumin, low density lipoprotein, and apoA-I. Only one band was resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis even when the gels were overloaded with sample. Immediately prior to each experiment, the required quantity of apoA-II was dissolved in 6 M guanidine–HCl and eluted on a Bio-Rad P-2 desalting column; this procedure provided us with a protein preparation whose physical properties were highly reproducible. ^{125}I-Labeled apoA-II, a gift from Dr. Simon J. T. Mao, coeluted with apoA-II on Sephadex G-150 in 3.0 M guanidine–HCl. This protein contained an average of 0.9 ^{125}I label/apoA-II molecule. ^{3}H- and ^{14}C-labeled DMPC were prepared and purified as previously described (Pownall et al., 1978b). A final purification was achieved on a Waters LC 500 preparative liquid chromatograph. Thin-layer chromatography of all phospholipids on silica gel plates eluted with chloroform/methanol/water (65:25:4) gave only one spot as visualized by a phospholipid specific spray (Dittmer & Lester, 1964) and by charring. Gas chromatographic analysis of the methyl esters obtained by transesterification of DMPC showed the ester composition to be >99% methyl myristate. All buffer salts were obtained from Fisher Scientific. Catalase and ferritin were from Calbiochem, San Diego, CA. Cytochrome *c* was from Sanyo Co. Ltd. Tokyo, Japan. [^{14}C]Glucose and [^{3}H]inulin were from New England Nuclear, Boston, MA. Sepharose Cl-4B was from Pharmacia Fine Chemicals, Piscataway, NJ. Phospholipid concentrations were routinely determined by scintillation counting and verified by the method of Bartlett (1959). Protein concentrations were determined by using ^{125}I-labeled apoA-II and verified by absorption at 275 nm (ϵ_{275} = 0.67 mL mg^{-1} cm^{-1}) or by method of Lowry et al. (1951).

Methods

Mixtures of apoA-II and DMPC were analyzed and the complexes isolated by gel filtration over Sepharose Cl-4B in a 90 cm × 1.6 cm water-jacketed column. DMPC and apoA-II in a buffer composed of 8.5% KBr, 0.001 M NaN_3, 0.001 M EDTA, and 0.01 M Tris (pH 7.4)[2] were preincubated separately for 30 min in a Lauda K/2R water bath and were then mixed and incubated in the same bath for 24 h. The mixture was applied to a Sepharose Cl-4B column with the water-jacket temperature the same as the incubation bath. The column had a flow rate of 6–8 mL/h and 1.6-mL fractions were collected. For estimation of the Stokes radius, the column was calibrated with DMPC multibilayer vesicles, DMPC single-bilayer vesicles, ferritin, catalase, cytochrome *c*, and [^{14}C]glucose. The columns were monitored by analysis of ^{125}I-labeled apoA-II and [^{3}H]DMPC by γ counting and liquid scintillation counting, respectively. The recovery of ^{125}I-labeled apoA-II was 98–100%. When all the DMPC reacted with apoA-II, as indicated by complete clearance of liposomal turbidity, there was ~95–100% recovery of [^{3}H]DMPC. Recovery of [^{3}H]DMPC liposomes varied with the temperature at which the columns were run (Massey et al., 1980). This was attributed to the inability of some of the liposomes to completely penetrate the Sepharose Cl-4B gel. Washing the columns with several bed volumes of buffer removed this excess lipid. The peak fractions were pooled and concentrated by using Amicon concentrators and an Amicon UM-10 filter.

Density Gradient Ultracentrifugation. A DMPC–apoA-II complex was adjusted to a final volume of 2.5 mL with 0.5 M CsCl. This solution was placed in the low-density side of a Buchler gradient former. The high-density side contained 2.5 mL of 1.73 M CsCl with 0.01 M Tris, 0.1 M NaCl, and 0.002 M NaN_3, pH 7.4. After each gradient was formed with the aid of a Buchler densiflow, the tubes were centrifuged in a Beckman SW-50.1 rotor at 45 000 rpm for 72 h at 23 °C. Fractions of approximately 250 μL each were collected. Protein and lipid were measured by using ^{125}I-labeled apoA-II and [^{3}H]DMPC. The density was estimated by refractometry on a Bausch and Lomb refractometer.

Analytical Ultracentrifugation and Light Scattering. Partial specific volumes were calculated from density measurements (Kratky et al., 1973) performed on a DMP-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, CA) 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) as previously described (Morrisett et al., 1974; Aune et al., 1977). Analytical ultracentrifugal measurements were performed on a Beckman Model E instrument equipped with an RTIC temperature control. Sedimentation equilibrium experiments were carried out as described previously (Morrisett et al., 1974) by using the meniscus depletion method of Yphantis (1964) and analyzed according to Aune & Timasheff (1971).

Electron Microscopy. Solutions of the DMPC–apoA-II complexes (0.1 mg/mL) in 0.1 M ammonium bicarbonate were used for negative staining. All operations were performed at room temperature, 22 °C. Copper grids coated with Formvar and stabilized by evaporated carbon were used to support a thin film of each sample. All samples were stained with 1% sodium phosphotungstate, pH 7.0, and examined in a Philips 201 electron microscope at an accelerating voltage of 80 kV. The magnifications were checked by a calibration grid (Fullam). Dimensions are the average of more than 25 measurements.

Results and Discussion

Complexes of apoA-II and DMPC were formed by preincubating apoA-II and DMPC separately at either 20, 24, or 30 °C for 30 min, then mixing, and incubating the mixture at the same temperature for 24 h; preincubation is essential

[2] A buffer containing 8.5% KBr is of sufficient density to prevent the settling of DMPC liposomes in experiments that took 24 h whereas without KBr the liposomes settled significantly within 1 h. Having a homogeneous reaction mixture is important in formation of the complex having a 240:1 molar ratio of lipid to protein which appeared to form from the interaction of a 75:1 complex and DMPC liposomes.

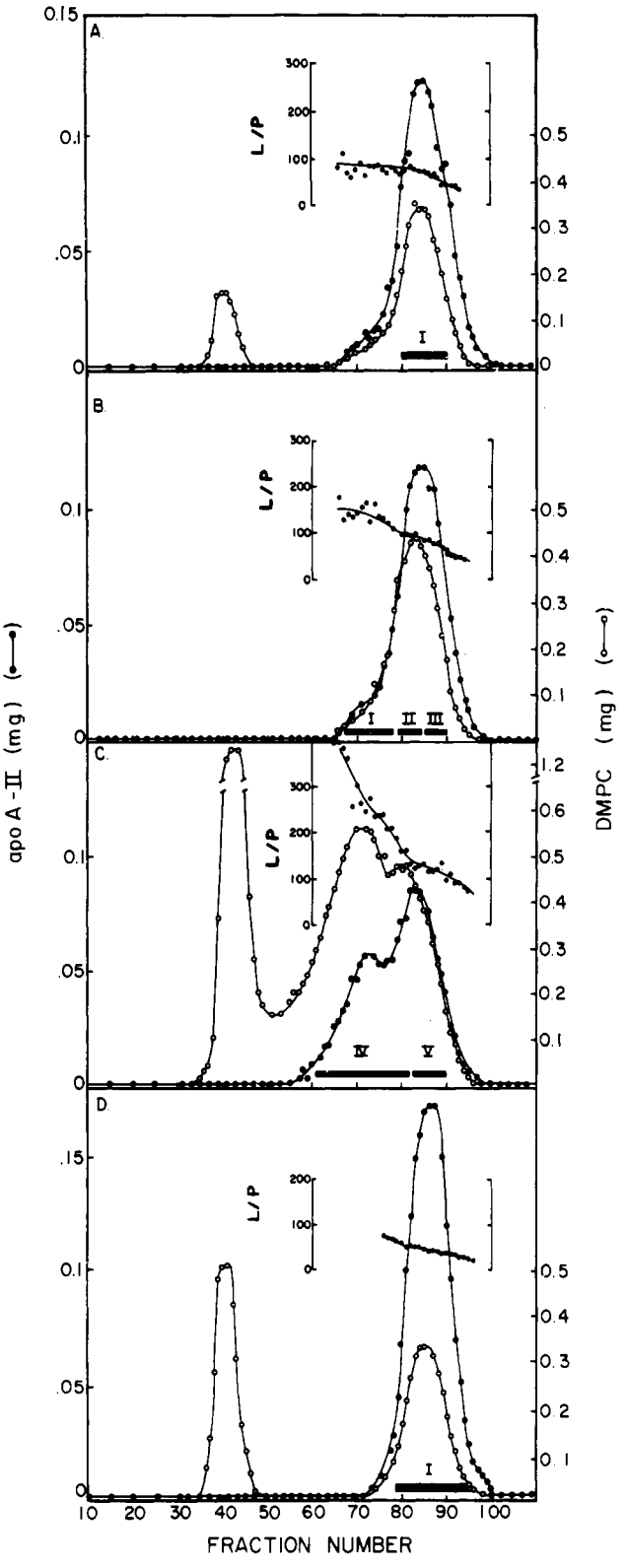

FIGURE 1: Elution profiles of DMPC–apoA-II complexes on Sepharose Cl-4B. (A) Complex formed by mixing 2 mg of apoA-II with 8 mg of DMPC in a total volume of 1 mL which was incubated (24 h) and chromatographed at 20 °C; (B and D) same as (A) except incubations and chromatography were conducted at 24 and 30 °C, respectively; (C) complex formed by mixing 2 mg of apoA-II and 24 mg of DMPC in a total volume of 1 mL which was incubated (24 h) and chromatographed at 24 °C. See text and Table I for description of the labeled zones. Insert shows composition across the peaks; L/P represents the lipid/protein molar ratio.

for obtaining reproducible complexes. The complexes were isolated on a water-jacketed column at the same respective temperatures. Figure 1 shows the elution profile for initial

Table I: Flotation Density of DMPC–ApoA-II Complexes[a]

fraction off Sepharose Cl-4B column[a]	temp of initial incubation (°C)	peak stoichiometry[b]	peak density (g/mL)
20I	20	75:1	1.132
24I	24	150:1	1.098
24II	24	95:1	1.132
24III	24	75:1	1.132
24IV	24	260:1	1.096
24V	24	120:1	1.118
30I	30	45:1	1.136

[a] The fractions are designated by where they eluted from a Sepharose Cl-4B column shown in Figure 1. [b] Peak stoichiometry is given as moles of lipid per mole of protein.

mixtures of apoA-II (2 mg/mL) and DMPC (8 mg/mL) at 20, 24, and 30 °C and for apoA-II (2 mg/mL) and DMPC (24 mg/mL) at 24 °C. In Figure 1A, the complex formed and isolated at 20 °C had a stoichiometry from 50 to 85 mol of DMPC per mol of apoA-II across the peak. The main peak was pooled and concentrated. This fraction, designated 20I, had an average stoichiometry of 75:1 (M/M). This value did not change if the lipid to protein ratio in the starting mixture was increased (Massey et al., 1980). The complex formed at 30 °C (Figure 1D) varied from 30 to 60 mol of lipid per mol of protein across the peak. The main peak was pooled and concentrated. This fraction, designated 30I, had an average stoichiometry of 45:1 (M/M). At 24 °C and a weight ratio of 4:1 lipid:protein, one major peak was formed (Figure 1B); however, at a weight ratio of 12:1 lipid:protein, a much larger complex was also formed (Figure 1C). At an initial concentration of 4:1 (w/w) Figure 1B), the average stoichiometry through the peak was 90:1 (M/M) and at 12:1 (w/w), the two peak fractions were about 260:1 (M/M) and 130:1 (M/M). As the steeper slope of the L/P vs. fraction number indicates, the larger complex was much more heterogeneous than the smaller one (Figure 1C, inset). Several fractions were pooled, concentrated, and characterized by density gradient ultracentrifugation. Figure 2 shows the density gradient behavior of the pooled fractions of apoA-II–DMPC complexes formed at different temperatures. The density gradient profiles also show that two complexes formed at 24 °C, one with a density of 1.098 g/mL seen in fractions 24I and 24IV (Figure 2D,G) and one with a density of 1.132 g/mL seen in fractions 24II, 24III, and 24V (Figure 2E,G,H). It should be noted that there is no free protein seen in the density gradient profiles, so all of apoA-II is fully associated with lipid. Table I summarizes the data from the density gradient ultracentrifugation. From the difference in stoichiometry, elution volume, and density there appears to be three isolatable DMPC–apoA-II complexes formed. The complex formed at 20 °C and the smaller of the two complexes formed at 24 °C (zone V of Figure 1C) have the same elution volume, density, and approximate stoichiometry and so are probably physically identical. A much larger, lipid-rich complex formed at 24 °C (zone IV of Figure 1C) and a smaller, protein-rich complex formed at 30 °C. The three types of complexes were further characterized. Fractions 24IV and 24V were rechromatographed separately and the peak fractions used for further characterization. The large, lipid-rich complex isolated for characterization varied from 210 to 260 mol of lipid per mol of protein across the peak.

The Stokes radii of the complexes were calculated by the gel filtration method of Ackers (1967) from their elution volumes and the column parameters determined from the elution volumes of several proteins of known size (Table II).

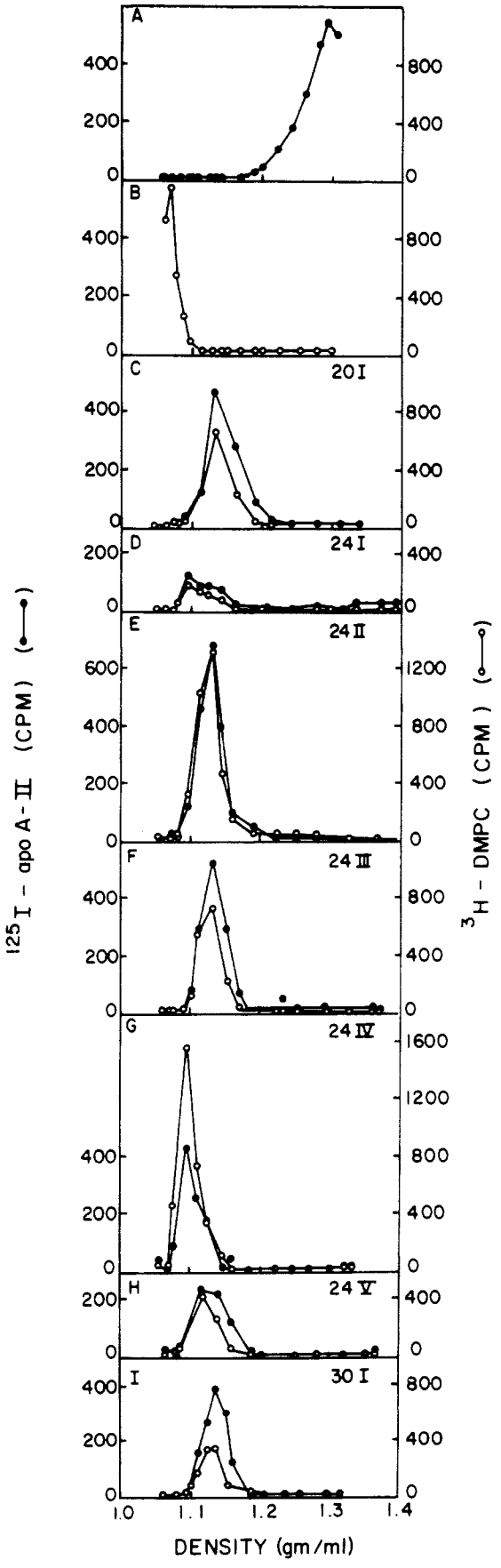

FIGURE 2: Isopycnic ultracentrifugation of DMPC–apoA-II complexes. (A) ApoA-II; (B) DMPC; (C–I) complexes isolated by chromatography according to the zones shown in Figure 1: (C) 20I; (D) 24I; (E) 24II; (F) 24III; (G) 24IV; (H) 24V; (I) 30I.

Table II: Stokes Radius Determination of DMPC–ApoA-II Complexes by Gel Filtration on Sepharose Cl-4B

eluted particle	elution volume (mL)	Stokes radius (A)
DMPC multibilayer vesicles	66[e]	
DMPC single-bilayer vesicles	109	125[a]
ferritin	128	78[b]
catalase	142	52[c]
cytochrome *c*	162	16.4[b]
[^{14}C]glucose	175[f]	
DMPC–apoA-II complex (245:1) formed at 24 °C	111	121 ± 5[d]
DMPC–apoA-II complex (78:1) formed at 24 °C	136	64 ± 5[d]
DMPC–apoA-II complex (75:1) formed at 20 °C	136	64 ± 5[d]
DMPC–apoA-II complex (44:1) formed at 30 °C	139	57 ± 5[d]

[a] Aune et al. (1977). [b] Siegel & Monty (1966). [c] Tanford et al. (1974). [d] Computed by the method of Ackers (1967) from the other elution volume and Stokes radius data given in this table. [e] V_0. [f] V_t.

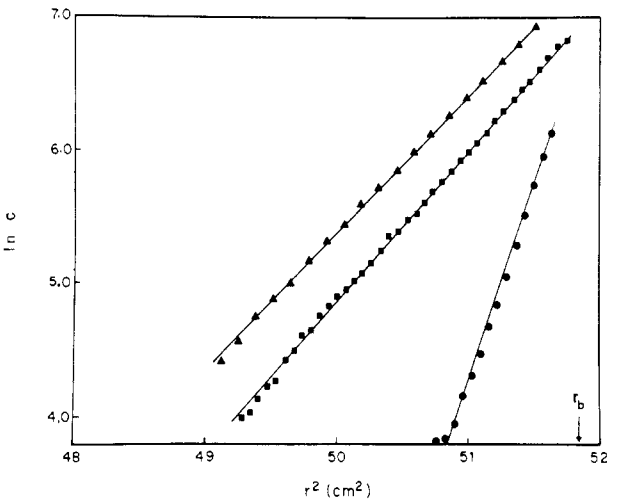

FIGURE 3: Plots of ln *C* vs. r^2 from sedimentation equilibrium experiments performed on apoA-II complexes with DMPC. The complexes, isolated by gel filtration, contained DMPC and apoA-II in respective molar ratios of 45:1 (▲), 75:1 (■), and 240:1 (●). Data were collected at 25 °C with respective rotor speeds of 12590, 14290, and 13410 rpm. The concentration, *C*, is expressed in μg/mL.

The DMPC–apoA-II complex formed at 20 °C and the smaller complex formed at 24 °C had identical Stokes radii (R_s = 64 ± 5 Å) and stoichiometries (75:1 mol of DMPC/mol of apoA-II). The complex formed at 30 °C was slightly smaller (R_s = 57 ± 5 Å) and had a decreased lipid to protein ratio (44:1 mol of DMPC/mol of apoA-II). The larger complex formed at 24 °C eluted slightly after DMPC single bilayer vesicles with R_s = 121 ± 5 Å.

We shall designate the three complexes as the 240:1 complex, formed at 24 °C, the 75:1 complex, formed both at 20 and 24 °C, and the 45:1 complex, formed at 30 °C; their designation is based on the different molar ratios of lipid to protein in each complex. The molecular weights of the isolated complexes were determined by sedimentation equilibrium ultracentrifugation. Each of the complexes was dialyzed extensively against buffer (0.01 M Tris, 0.001 M NaN_3, 0.001 M EDTA, and 0.01 M NaCl, pH 7.4), and the partial specific volume, $\bar{\nu}$, was calculated from density measurements. Figure 3 shows plots of ln *C* vs. r^2; the plots of the 45:1 and 75:1 complexes were nearly linear, suggesting that these two complexes are relatively homogeneous. In contrast, similar plots

Table III: Physical Properties of DMPC–ApoA-II Complexes

stoichiometry (mol of lipid/mol of protein) ± 5%[a]	240:1	75:1	45:1
(g of lipid/g of complex) ± 5%[a]	0.90	0.73	0.63
molecular weight (M_r)[b]	1 580 000	343 000	229 000
partial specific volume ($\bar{\nu}$) $(g/mL)^{-1}$	0.948[d]	0.914[c]	0.892[c]
complex density (g/mL)[e]	1.096	1.132	1.136
mol of protein/mol of complex[f]	8.6	5.3	4.8
mol of lipid/mol of complex[f]	2070	370	215
diffusion coefficient ($D_{20,w}$) (cm^2/s)[g]	1.78 ± 0.12 × 10^{-7}		
Stokes radius[h] (R_s) (Å)	118 ± 5		
Stokes radius[i] (R_s) (Å)	121 ± 5	65 ± 5	57 ± 5
Stokes radius[j] (R_0) (Å)	102	61	53
frictional coefficient (f/f_0)[k]	1.20		
average dimensions from electron microscopy (Å)[l]	500 × 55	250 × 62	175 × 60

[a] Lipid/protein weight and molar ratios determined by chemical analysis of complex isolated by gel filtration. [b] Molecular weight calculated from sedimentation equilibrium ultracentrifugation. [c] Partial specific volume determined by mechanical oscillator densimetry. [d] Partial specific volume approximated by weighted additivity of ν's for DMPC (0.972) and apoA-II (0.743). [e] Complex density as determined by density gradient ultracentrifugation. [f] Moles of protein and moles of lipid per mole of complex determined from molecular weight and the stoichiometry of the complex. [g] Translational diffusion coefficient determined by quasielectric light scattering. [h] Stokes radius calculated from the diffusion coefficient by using the Stokes–Einstein equation. [i] Stokes radius determined by gel filtration. See Table II. [j] Stokes radius calculated from the molecular weight of the complex (M_r), the partial specific volume of a complex ($\bar{\nu}$), and assuming the amount of hydration [δ = 0.75 g of H_2O/g of lipid: Aune et al. (1977)] by using eq 1. [k] Frictional coefficient calculated from eq 2. [l] Calculated from the dimensions of the average-sized particle as visualized by electron microscopy by assuming that the particles are a bilayer disk viewed from the edge.

of the 240:1 complex exhibit curvature suggestive of heterogeneity in composition and size. The partial specific volumes and the molecular weights of the three complexes are given in Table III. On the assumption that the particles are spherical and their hydration is known, hydrodynamic data can also yield their Stokes radii (R_0). We assumed a value for the hydration (δ) of 0.75 g of H_2O/g of complex which is that used by Aune et al. (1977) for apoC-III–DMPC complexes and the following equation (Tanford, 1961)

$$R_0 = [(3M_r/4\pi N)(\bar{\nu} + \delta\nu_0)]^{1/3} \quad (1)$$

where ν_0 is the partial specific volume of water. These values were slightly smaller but still in good agreement with R_s determined for gel filtration (Table III) for the 75:1 and the 45:1 complexes. However, for the 240:1 complex, R_0 from the molecular weight (R_0 = 102 Å) is much smaller than from gel filtration chromatography (R_s = 121 ± 5 Å). The Stokes radius for the 240:1 complex was also determined by quasielectric light scattering. A diffusion coefficient ($D_{20,w}$) of 1.78 ± 0.12 × 10^{-7} cm^2 s^{-1} and diameter of 236 ± 10 Å (R_s = 118 ± 5 Å) were determined. The value of R_s from this method agrees well with that from gel filtration chromatography. There are two possible reasons for the discrepancy between R_s from light-scattering (R_s = 118 ± 5 Å) and gel-filtration (R_s = 121 ± 5 Å) techniques and R_0 from the molecular weight (R_0 = 102 Å) using eq 1. First, the complex may be a spherical vesicle with an entrapped volume of buffer so that the mass of the particle would give a much smaller value for the Stokes radius of the particle as seen by light scattering and

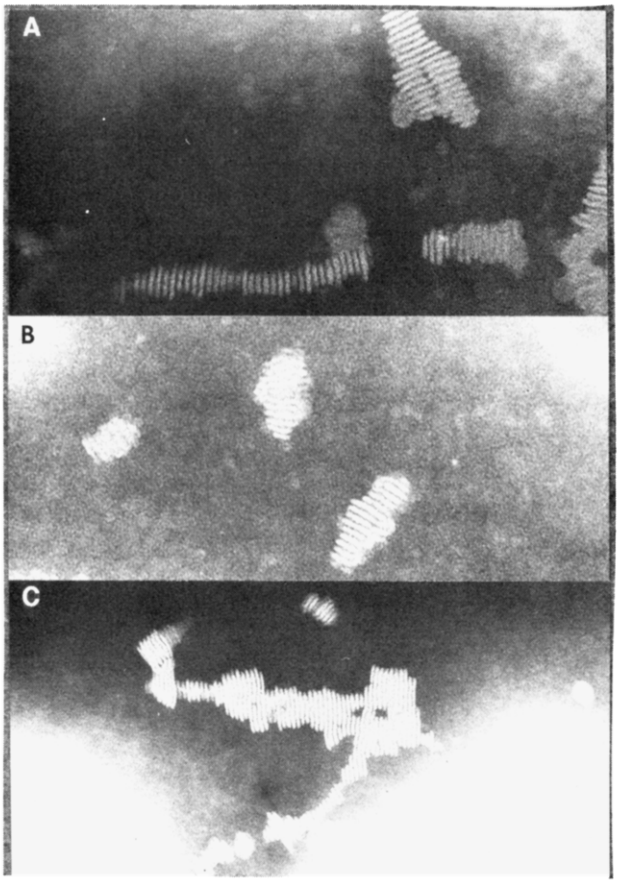

FIGURE 4: Electron micrographs of DMPC–apoA-II complexes obtained after negative staining with sodium phosphotungstate. (A) 45:1 (×198 000); (B) 75:1 (×198 000); (C) 240:1 (×132 000).

gel filtration. However, we were not able to trap a radioactive marker ([^{3}H]inulin) when this was added to the original buffer. Second, the particle may be asymmetric. Using the diffusion coefficient and R_0 calculated from the molecular weight in eq 1, we calculated the frictional coefficient (f/f_0) (Tanford, 1961)

$$f/f_0 = KT/(6\pi N D_{20,w} R_0) \quad (2)$$

From eq 2 we obtain a value of 1.20 for f/f_0. For either a prolate or oblate elipsoid, a value for f/f_0 gives an axial ratio (a/b) of 3.8. The axial ratios were determined graphically (Tanford, 1961).

Each of the isolated complexes of DMPC and apoA-II were examined by electron microscopy after negative staining with sodium phosphotungstate; these are shown in Figure 4. All appeared as rouleax of stacked disks whose shortest dimension was relatively constant and roughly corresponded to that of a bilayer (55–62 Å). The 240:1 complex appeared as stacked disks and not as a collapsed vesicle which would have the thickness of two bilayers. Therefore, the difference in R_0 from the molecular weight and R_s based on gel filtration and quasielastic light scattering must be due to the asymmetry of the particle. The long dimension was highly variable within a given complex and increased with the molecular weight (Table III). The hydrodynamic characteristics of each of these complexes determined by several physical methods are fairly invariant with respect to the technique used. Given the internal consistency of the physical data, the stacked disks may be artifactual as suggested by Morrisett et al. (1974) and Laggner et al. (1979), and these subunits may have little resemblance to the morphology of the complexes before their preparation for electron microscopy. It is still interesting that the complexes which differ drastically in stoichiometry and molecular

weight still behave the same as visualized by electron microscopy.

We found all three complexes to be stable over the time required (2–3 days) to conduct our physical studies. Even after 30 days at 4 °C the images observed by electron microscopy were virtually the same as those observed when the samples were examined immediately after preparation; moreover, the elution volumes of the fresh and 30-day-old complexes were identical and the chromatographic peaks corresponding to the complexes contained >85% of the phospholipid radioactivity.

It is difficult to speculate upon the physiologic significance of the different structures formed by apoA-II. The observation of different secondary structures of apoA-II in the 45:1 complex (Massey et al., 1980) may contribute to the distribution of apoA-II in plasma. Recognizing that DMPC is probably not a physiologically abundant lecithin, it is interesting to note that apoA-II cannot define the size of the complex formed because we isolate three different complexes. However, in plasma apoA-II appears mainly in the denser HDL subclass, HDL_3 (Kostner et al., 1974; Daniellsson et al., 1978; Albers et al., 1978). The presence of apoA-I or neutral lipids in HDL_3 must be important in determining the final three-dimensional structure of this lipoprotein. It is impossible to guess whether there are physiologic correlates for the 240:1, 75:1, and 45:1 complexes; presumably the different protein structure in these model particles would, in part, control the exchange or transfer of apoA-II or the formation of HDL precursors from the surface components of chylomicrons during their catabolism (Tall et al., 1979; Redgrave & Small, 1979). This view, however, awaits additional experimental support.

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