Effect of Cholesterol on the Formation of Micellar Complexes between Bovine A-I Apolipoprotein and L-α-Dimyristoylphosphatidylethanolamine*

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The interaction of bovine A-I apolipoprotein with sonicated vesicles of dimyristoylphosphatidylethanolamine containing various molar ratios of cholesterol was investigated in this work. Complexes prepared at 37°C were isolated by gel filtration on Sepharose 4B columns. Stoichiometries were obtained and intrinsic fluorescence and circular dichroism spectral properties were determined for the purified complexes. Fluidity changes of the lipid domains were probed by measuring the fluorescence polarization of the fluorescent lipophilic probe diphenylhexatriene dissolved in the complexes.

Apoprotein A-I forms "small" breakdown complexes from vesicles of dimyristoylphosphatidylethanolamine containing up to 33 mol % cholesterol. These complexes are of the general size of high density serum lipoproteins and all have similar shapes. At cholesterol contents exceeding 37 mol %, there is no interaction of lipid vesicles with the apoprotein. It is likely that structural changes occur in the vesicles at this cholesterol content which prevent complex formation. All the complexes of apolipoprotein A-I with dimyristoylphosphatidylcholine and cholesterol contain the same molar ratio of phospholipid to protein (98:1), but they are enriched in phospholipid over cholesterol relative to the lipid ratios in the parent vesicles. These results, together with the observation that the protein spectral properties of complexes with or without cholesterol are very similar, strongly suggest that the lipid layer adjacent to the protein is exclusively phospholipid. The mobility of dimyristoylphosphatidylethanolamine in the complexes with apolipoprotein A-I is progressively and markedly restricted by the presence of cholesterol, indicating that cholesterol has a similar condensing effect in these complexes as in phospholipid bilayers.

Apoprotein A-I (apo A-I),† the major protein component of mammalian high density serum lipoproteins (HDL), is thought to determine the structure and perhaps the function of this lipoprotein class. For these reasons, the lipid binding properties of the pure apo A-I proteins have been the subject of numerous investigations in recent years (1). It is clear that the interaction of apo A-I with phospholipids is the primary interaction and that the other lipid classes only bind to apo A-I in the presence of phospholipids (2, 3). Also, it has been demonstrated that under appropriate conditions, apo A-I interacts with phosphatidylethanolamine (egg and dimyristoyl) to form well defined complexes that result from the breakdown of lipid vesicles (4–6). The complexes of dimyristoylphosphatidylethanolamine (DMPC) and apo A-I have a lipid to protein ratio of 2:1 by weight and a molecular weight of 235,000; they retain phospholipid bilayer properties (6) which suggest a structure consisting of a phospholipid bilayer disc ringed by protein (7–9).

The lipid requirements for complex formation of this type are not yet well understood, but they seem to involve critically the physical state of the lipid. Apparently, the liquid crystalline state is required for interaction (5) and the small single lamellar vesicles are preferred over multilamellar species or phospholipid dispersions on Celite. Since the effects of cholesterol on the fluidity and phase transition behavior of phospholipid bilayers are very well documented (10, 11), and its effects on vesicle hydrodynamic properties have been reported in detail (12, 13), we investigated in this work the effects of cholesterol incorporation into DMPC vesicles on the formation and properties of complexes with apo A-I. In addition to information on the lipid requirements for complex formation, we hoped to gain some insight into the structural role of cholesterol in native HDL.

EXPERIMENTAL PROCEDURES

Materials—Bovine apo A I was prepared by the methods described earlier (4, 14). Crystalline cholesterol and L-α-dimyristoylphosphatidylcholine (DMPC) were purchased from Sigma Chemical Co. DMPC was purified by passage through a silicic acid column, according to the procedure of Rouser et al. (15). 1H Cholesterol and 14C)DMPC were obtained from New England Nuclear and P. J. Cobert Associates, respectively. The purity of the lipids was assessed by thin layer chromatography on silica gel plates in petroleum ether:ethyl ether:diethyl ether:acetic acid, 90:10:1:1 (v/v) or cyclohexane:chloroform, 1:1 (v/v) for cholesterol and in chloroform:methanol:H2O, 65:25:5 (v/v) for DMPC. The plates were developed by dichromate/H2SO4 spray and charring or by phosphoric acid:H2O, 1:1 (v/v) spray and heating. Radiolabeled DMPC was detected by autoradiography and 14C cholesterol by scraping silica gel plate strips into Beckman Aquasol scintillation fluid and counting. By these methods, all the lipids were found pure, except 14C)DMPC, which was purified by chromatography and elution from thin layer plates under the same conditions as those described for analysis. At the end of the experiments, cholesterol and DMPC were extracted from complexes with apo A-I using chloroform:methanol, 2:1 (v/v) and were chromatographed again on thin layer plates using the same solvents as above. Significant degradation of lipids was not detected by these procedures. 1,6-Diphenyl-1,3,5-hexatriene was obtained from Aldrich Chemical Co.; it was used as a 10−3 m solution in tetrahydrofuran. All the experiments were performed using fresh solutions of all the lipid components.

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† The abbreviations used are: apo A-I, (apolipoprotein A-I) major protein component of mammalian HDL; HDL, high density lipoprotein; DMPC, L-α-dimyristoylphosphatidylcholine; Dns, covalent dansyl label (5-dimethylaminonaphthalene-1-sulfonyl).
formed in a 0.1 M Tris/HCl buffer, pH 8.0, 0.005% EDTA, and 10⁻³ m NaCl.

Preparations—Due-labeled apo A-I, used in rotational relaxation time determinations, was prepared as described previously (4, 16); the conjugates had 1.5 mol of Dns/mol of protein. Vesicles of DMPC and cholesterol were prepared by two methods. 1) sonication of radiolabeled DMPC (approximately 4 mg of DMPC/ml of buffer) with a Heat Systems-Ultrasonics, Inc. Sonifier, model W185, using a power setting of 3, for 15 min, under an atmosphere of N₂ at 30°C, addition of the appropriate amount of solid radiolabeled cholesterol, and resorption under the same conditions for 20 more min; 2) sonication of an appropriate mixture of radioactivity in a flask in sonicator, premixed in chloroform-methanol, 2:1 (v/v) and dried under N₂. The conditions in this vesicle preparation procedure were identical and the total sonication time was the same as in the first method. The lipid dispersions prepared by the above methods were subsequently centrifuged at 15,000 rpm, 28°C for 30 min, and the supernatant was used immediately for incubations with apo A-I. The lipid content of the preparations was determined by scintillation counting using a Beckman LS-100C instrument and a dioxane/naphthalene-based scintillation fluid. The specific activities for the lipids were in the range of 4 x 10⁶ cpm of ¹⁴C/µmol and 9 x 10⁶ cpm of ³H/µmol, and the efficiencies of counting were 98 and 57%, respectively. Up to 40 mol % cholesterol, the first sonication method produced relatively clear vesicle preparations and gave qualitatively similar results even when followed after with the second sonication procedure, containing cholesterol. At biopsy concentrations of cholesterol, lipids remained undispersed and were removed during centrifugation. The loss of lipid was of the order of 50%, but the original ratio of DMPC to cholesterol was preserved in the supernatant. These lipid preparations, including the supernatants with 49 mol % cholesterol, passed through a Sepharose 4B column, gave up to 80% of the lipid recovered at elution positions similar to pure DMPC vesicles (Rₑ= 1.56 to 1.68) (see Fig. 1). Rₑ is defined as the elution volume of the peak divided by the excluded volume of the column. The lipid preparations obtained by the second sonication method, however, gave clear dispersions only up to 30 mol % cholesterol; at higher cholesterol contents, the preparations were turbid and variable amounts of lipid were lost during centrifugation. The lipid ratio of DMPC to cholesterol was retained during all the steps of the preparation. Sepharose 4B column fractionation of these lipid dispersions gave elution profiles with the major vesicle peak at RF values between 1.36 and 1.45. Lipid recoveries from the column were around 70%.

Incubations of apo A-I or apo A-I-Dns (0.5 to 1 mg of protein/ml of reaction mixture) with a 200 to 250 molar excess of sonicated DMPC (plus or minus cholesterol) were carried out under N₂, in a shaking water bath, at 37°C. Two hours was the standard incubation time for all the samples. A few longer term incubations indicated that the reaction was complete after 2 h. Following incubation, immediate centrifugation, and variable amounts of lipid were lost during centrifugation. The lipid ratio of DMPC to cholesterol was retained during all the steps of the preparation. Sepharose 4B column fractionation of these lipid dispersions gave elution profiles with the major vesicle peak at Rₑ values between 1.36 and 1.45. Lipid recoveries from the column were around 70%.

Methods—Intrinsic fluorescence spectroscopy for apo A-I and its complexes with lipids were recorded with a Hitachi-Perkin-Elmer MFP-115 spectrophotometer at 295°C. Circular dichroism spectra were obtained on a Jasco J-40A automatic spectropolarimeter at 25°C. CD spectral data were analyzed for a helix content by the method of Greenfield and Fasman (20) at 208 nm. 

Fluorescence polarizations were measured with an SLM-Series 400 polarization instrument, while temperatures were regulated to ±0.1°C with a circulating water bath. Rotational relaxation times and Stokes radii for apo A-I-Dns and its complexes with lipids, were determined from fluorescence polarizations measured as a function of the ratio of absolute temperature (T) to the viscosity (η) of the solution. The ratio T/η was changed by adding sucrose to a sample at 25°C containing 10⁻⁴ m apo A-I-Dns (plus or minus lipid). The theory and applications of this method have been described previously in detail (4, 6, 16, 21). In these fluorescence polarization measurements, 340 nm exciting light was used, with 4 nm bandwidths and Corning glass 7-25 cut-off filters in the path of the emitted light. In experiments where diphenylhexatriene was used as the fluorescent probe of lipid domains, fluorescence polarization was measured as a function of temperature by exciting at 300 nm and using 4 nm bandwidths and Corning glass 3-74 filters. The fluorophore was added in a tetrahydrofuran solution by injecting microliter aliquots into 2.0 ml of stirred sample solution in fluorescence cuvette. The molar ratio of fluorophore to lipid in the samples was around 1:200 for the complexes. Prior to measurement, tetrahydrofuran was removed by flushing the solutions with N₂. Temperature changes in the samples were followed with a Fluke 2100A digital thermometer equipped with a copper/constantan probe that was immersed directly in the sample cell. Lipid fluidities in complexes with apo A-I, expressed as empirical microviscosity values, were determined by the method of Shinizky and co-workers (22-24). Previously, we employed the same techniques with serum lipoproteins and with model systems (5, 25).

Fluorescence lifetimes were measured in Dr. G. Weber's laboratory (University of Illinois, Urbana-Champaign) using the cross-correlation phase fluorometer described by Spencer and Weber (26) under identical conditions with fluorescein employed in the fluorescence polarization measurements with the Dns-labeled protein or with the diphenylhexatriene lipid probe.

RESULTS

Vesicles of DMPC and cholesterol, prepared by the two sonication methods, had remarkably different properties in terms of their interaction with apo A-I protein. At cholesterol contents above 15 mol %, the vesicles prepared from organic solvent mixtures of both lipids, after incubation with apo A-I solutions, did not show a distinct complex peak upon fractionation on the Sepharose 4B column. On the other hand, vesicles prepared by sonicating DMPC first and then introducing solid cholesterol and resorcinol gave distinct complexes with apo A-I protein at cholesterol contents up to 33 mol %. The apparent discrepancy in these results is due to the existence of two distinct metastable states for the DMPC cholesterol dispersions. The difference in the vesicles prepared by both methods is suggested by the elution position of the included lipid peak on the Sepharose 4B column; Rₑ = 1.56 to 1.68 for the resorcinol preparation, and Rₑ = 1.36 to 1.45 for the premixed preparation. The difference in behavior of these two preparations was not due to a heterogeneous distribution of DMPC and cholesterol, as is indicated by the constant ratio of DMPC to cholesterol across the elution profiles for both single-bilayer vesicle preparations (Fig. 1).

All the subsequent experiments were performed with the resorcinol vesicles and the results reported in this paper refer exclusively to complexes formed from apo A-I and DMPC-cholesterol vesicles prepared by the resorption method.

Typical Sepharose 4B elution profiles of incubation mixtures containing apo A-I and DMPC vesicles prepared with varying amounts of cholesterol, are shown in Figs. 2 and 3. In Fig. 2, the lipid vesicles originally contained 21.6 mol % cholesterol. Under these conditions, a complex of apo A-I DMPC-cholesterol eluted at the same position as apo A-I complexes with DMPC alone. At least 90% of the eluted protein could be found under the complex peak and the complex position was intermediate between vesicle and free protein elution positions, suggesting an intermediate size for these particles. Fig. 3 shows a similar experiment performed with DMPC vesicles containing initially 36.9 mol % cholesterol. Clearly, distinct complexes of the lipids and apo A-I did not form in this case. Over 80% of the eluted protein was recovered free under the peak around Fraction 52, indicating that there were no major adsorption products between the
Complexes of apo A-I with DMPC and Cholesterol

Fig. 1. Elution profiles on a Sepharose 4B column (2.2 × 43 cm) of [14C]DMPC plus [3H]cholesterol (CHOL) vesicles prepared by two distinct procedures. A, lipids premixed in organic solvent and dried under N2 were dispersed in buffer and were sonicated for 35 min at 40°C (see "Experimental Procedures" for details) and R, sonicated vesicles of [14C]DMPC were mixed with solid [3H]cholesterol and were sonicated under the same conditions as in A. The content of cholesterol was 21.6 mol % in both preparations. On the ordinate, R designates the molar ratio of DMPC/cholesterol across the main lipid elution peak. Recoveries of both lipids were around 80% from this column. V₃ and V₄ stand for excluded and total column volume, respectively.

lipid vesicles and apo A-I. Note that the unreacted lipid is predominantly in the single-bilayer vesicle form. Vesicles containing between 21.6 and 49.3 mol % cholesterol gave results similar to those shown in Fig. 2 or in Fig. 3. These results, including protein and lipid stoichiometries, are given in Table I. At and above 33 mol % cholesterol in the vesicles, the apo A-I protein no longer formed complexes with the lipids and was recovered essentially free from lipid. Up to 33 mol % cholesterol in the vesicles, complexes formed readily and exhibited the same DMPC to protein molar ratio as apo A-I complexes with DMPC alone (b) 95 ± 15. It is interesting to note that the cholesterol containing complexes have significantly higher molar ratios of DMPC to cholesterol than the parent vesicles. The remaining vesicles are, on the other hand, enriched in cholesterol. Apparently apo A-I selects, in some manner, phospholipids over cholesterol during the reaction with DMPC-cholesterol vesicles.

The properties of apo A-I·DMPC·cholesterol complexes are discussed below. The effect of cholesterol on the spectral properties of apo A-I was observed by fluorescence and circular dichroism spectroscopy on the pure complexes, isolated by gel filtration. Uncorrected intrinsic fluorescence spectra for samples adjusted to the same concentration of protein indicated an increase in fluorescence intensity of 40 to 50% in the complexes over pure apo A-I. Presence of cholesterol in the complexes caused only minor changes in the fluorescence intensity relative to apo A-I DMPC complexes. Wavelengths of maximum fluorescence were likewise unaffected by the presence of cholesterol in the complexes (327 ± 2 nm). Circular dichroism spectra indicated a similar 25 ± 5% increase in α helix content in all the complexes, from about 38% for the free apo A-I up to 74% for the complexes. Table II summarizes...

![Figure 2](http://www.jbc.org/) of the reaction mixture of apo A-I and [14C]DMPC vesicles containing 21.6 mol % [3H]cholesterol (CHOL). Complexes elute around Fraction 44 and contain a molar ratio of 1:98:20 of protein:DMPC:cholesterol. V₀, V₃, and V₄ designate excluded column volume, free protein elution position, and total column volume, respectively. On the ordinate, R gives the molar ratio of DMPC to cholesterol across the elution peaks. Protein concentration was determined by the assay of Lowry et al. (17).

Fig. 3. Elution profiles on a Sepharose 4B column (2.2 × 43 cm) of the reaction mixture of apo A-I and [14C]DMPC vesicles containing 36.9 mol % [3H]cholesterol (CHOL). Free, single bilayer vesicles elute around Fraction 35 and free protein at Fraction 52. V₀ and V₄ stand for excluded column volume and total column volume, respectively. On the ordinate, R designates the molar ratio of DMPC/cholesterol across the main lipid peak. Protein concentrations were determined by the method of Lowry et al. (17).

![Figure 3](http://www.jbc.org/)

Table I

<table>
<thead>
<tr>
<th>Initial DMPC/cholesterol</th>
<th>Complexes</th>
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<tbody>
<tr>
<td>mol/mol</td>
<td>mol % cholesterol</td>
</tr>
<tr>
<td>No cholesterol</td>
<td>0</td>
</tr>
<tr>
<td>3.64</td>
<td>21.6</td>
</tr>
<tr>
<td>2.04</td>
<td>32.9</td>
</tr>
<tr>
<td>1.71</td>
<td>36.9</td>
</tr>
<tr>
<td>1.03</td>
<td>48.3</td>
</tr>
</tbody>
</table>

* Formation of complexes was judged by the presence of lipid protein complexes upon gel filtration on Sepharose 4B.
these spectral results and includes rotational relaxation times ($\tau$), fluorescence lifetimes ($\tau'$), and Stokes radii for apo A-I-DMS and its complexes with lipids. Rotational relaxation times (285 ± 30 ns) and Stokes radii (47.0 ± 2.0 Å) at 25°C are similar for all the complexes with or without cholesterol, indicating similar sizes and shapes.

Fluorescence polarization of diphenylhexatriene, as a function of temperature in apo A-I complexes with DMPC and cholesterol. Previously published results for pure DMPC vesicles (5) are included for comparison. Abbreviations as in Fig. 3.

**Fig. 4.** Fluorescence polarization of diphenylhexatriene, as a function of temperature in apo A-I complexes with DMPC and cholesterol. Previously published results for pure DMPC vesicles (5) are included for comparison. Abbreviations as in Fig. 3.

The A-I apolipoprotein readily forms complexes from DMPC vesicles containing up to 33 mol % cholesterol. At higher contents of cholesterol in the vesicles, no interaction can be detected. A possible reason for this observation is that the structural properties of DMPC-cholesterol vesicles may change at this content of cholesterol. Newman and Huang (13) have shown that sonicated egg lecithin vesicles containing up to 30 mol % cholesterol have similar hydrodynamic properties to pure lecithin vesicles, but at higher contents of cholesterol, they observed rapid changes in the physical properties of the vesicles which suggested a change from spherical particles to particles of “small but undefined asymmetry.”

The abrupt structural changes at 32 mol % cholesterol were attributed to a rearrangement of the lipids, with an increase in cholesterol content in the internal half of the bilayer and a displacement of lecithin with polyunsaturated chains to the outside of the bilayer (12). Experiments with L-a-dipalmitoyl-phosphatidylcholine vesicles containing various amounts of cholesterol indicated changes in head group packing between 10 to 20 mol % cholesterol, but no changes in the distribution of phospholipid between the two sides of the bilayer over the entire range of cholesterol contents (0 to 40 mol %) (12). The changes in head group packing for this phospholipid occur at a concentration of cholesterol that just exceeds the amount needed to condense all of the phospholipids in the vesicle without increasing the initial area of the vesicle. The structural changes taking place above this cholesterol content (19 mol %) have not been defined for this saturated phospholipid (12).

**Table III**

**Fluidity of lipid domains of apo A-I complexes with DMPC and cholesterol**

<table>
<thead>
<tr>
<th>Sample</th>
<th>DMPC/cholesterol</th>
<th>11°C</th>
<th>25°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mol/mol</td>
<td>$\tau'$</td>
<td>Viscosity</td>
<td>$\tau'$</td>
</tr>
<tr>
<td>Apo A-I-DMPC</td>
<td>4.90</td>
<td>8.70</td>
<td>15.4</td>
<td>4.19</td>
</tr>
<tr>
<td>Apo A-I-DMPC cholesterol</td>
<td>2.80</td>
<td>8.45</td>
<td>18.1</td>
<td>8.28</td>
</tr>
</tbody>
</table>

**Notes:**
- $\tau'$ Lifetimes for diphenylhexatriene were determined by the phase method.
- Viscosities were calculated from fluorescence polarization and lifetime data using the constants given previously (4, 22, 23). The units of viscosity are poise.
cules can occur (32%) is fairly close to the observed region where apo A-I fails to form complexes with the lipids (33 to 37 mol % cholesterol), the original structure of DMPC vesicles may be critical for complex formation. However, regardless of the exact nature of the physical changes in the vesicles, it is evident that apo A-I interactions strongly depend upon the physical state of the phospholipid. The importance of the physical state of the lipid for the interaction with apo A-I is further illustrated in this study by the very different behavior of DMPC and cholesterol vesicles of the same composition prepared by the two different sonication methods. The vesicles that were prepared from premixed lipids were larger and eluted with average $R_F$ values of 1.36 to 1.45 from the Sephadex 4B column, as opposed to the smaller, pure DMPC vesicles, or resonicated, cholesterol-containing vesicles, which had $R_F$ values of 1.56 to 1.68. Under the same experimental conditions, the larger vesicles did not interact with apo A-I when the smaller vesicles gave distinct complexes with the protein (up to 33 mol % cholesterol content). The difference between the properties of these vesicles must reside in their curvature and in the surface packing of the lipids. Heterogeneous distribution of DMPC and cholesterol among single bilayer vesicles of different sizes is discounted because of the constant DMPC to cholesterol ratio across the included vesicle peaks shown in Fig. 1. A distinct distribution of cholesterol between the inner and outer halves of the vesicle bilayer for both vesicle preparations may be suggested by the different mode of cholesterol introduction into each preparation; however, the flip-flop half-times for cholesterol in egg phosphatidylcholine vesicles are, at most, of the order of 30 min. Since our experiments take about 3 h from the sonication step to column fractionation, cholesterol may be expected to distribute uniformly throughout both preparations of vesicles. The possibility that solid cholesterol may be trapped inside the aqueous space of the vesicles prepared by the resonication method was considered and discarded for several reasons. Firstly, only 0.2% of the total sample volume is represented by the aqueous space within DMPC vesicles; therefore, if cholesterol did not partition into the bilayers but remained dispersed in water, only 0.2% of the total cholesterol would have appeared in the vesicles. This was obviously not the case. Secondly, vesicles prepared by both methods had very similar fluorescence polarization behavior observed by means of the diphenylhexatriene probe, i.e., the effect of cholesterol in both preparations was essentially identical on the mobility of DMPC acyl chains.

Other types of heterogeneity in lipid distribution within vesicles of phosphatidylcholine and cholesterol have been considered in the literature. Huang et al. (12) showed that in lecithin vesicles containing less than 32 mol % cholesterol, cholesterol molecules were evenly distributed (relative to lecithin) between the inner and outer halves of the bilayer. Above this content of cholesterol, there was an increase in concentration of lecithin at the outer surface and an increase of cholesterol on the inside. Similar experiments with dipalmitoylphosphatidylcholine indicated a uniform distribution of the phospholipid and cholesterol over the entire range of mixtures (0 to 40 mol % cholesterol). The distribution of cholesterol within each side of a bilayer has also been investigated. In mixtures of lecithin and cholesterol, where the mole per cent of cholesterol is less than 50, there may be a separation of clusters of pure phospholipid and clusters of cholesterol-phospholipid complexes in a 1:1 molar ratio (30, 31). This view, however, is not shared by all investigators; others believe that the distribution of the lipids in such mixtures is random (10, 32).

The complexes of apo A-I with DMPC and cholesterol which were formed below 33 mol % cholesterol had a considerably higher molar ratio of DMPC to cholesterol than the original vesicles. This result implies that apo A-I selects DMPC over cholesterol in complex formation. How this selection occurs is not entirely clear. Since heterogeneity in cholesterol and DMPC distribution among vesicles of different sizes or between the inner and outer halves of the bilayer were discounted, it may be that apo A-I binds to vesicles in regions rich in DMPC (e.g. DMPC clusters) (30, 31) or that, during the binding process, apo A-I progressively displaces cholesterol from its vicinity. In addition, the data in Table II indicates that there are no changes in the structure of the complexes upon incorporation of cholesterol and that fluorescence and CD properties of the protein component remain unchanged. These results strongly suggest that the environment of the protein does not change in the presence of cholesterol and that DMPC remains directly associated with the protein in these complexes.

In contrast to the insignificant effect of cholesterol on protein structure and the overall structure of the complexes, the bulk of the lipids in the complexes undergoes marked changes in fluidity as the cholesterol content increases (Fig. 4 and Table III). Lipid fluidity, expressed as microviscosities at 25°C, increases from 4.19 poise for the apo A-I:DMPC complex up to 6.41 poise for the complex containing 2.80 mol of DMPC/mol of cholesterol. This decrease in fluidity is consistent with the restriction of lipid mobility that results from the condensing effect of cholesterol in phospholipid bilayers, and suggests a bilayer-like cooperative melting phenomenon. Compared to the fluidity of the original vesicles at 25°C, the complexes have microviscosities higher by 3 to 4 poise, probably due to the restricting effect of protein on molecular motions.

From the information available about saturated complexes of phosphatidylcholines with apo A-I (4-9, 33, 34), including chemical composition data, molecular weights, physical properties of lipids, and electron micrographs, models for the structure of these complexes can be constructed. Since apo A-I protein and phospholipids are surface components of native lipoproteins (1), the recombined complexes should retain this basic physical characteristic of its components. Under this assumption, there is only a limited number of models where the phospholipids and most of the protein can be placed on the surface. Within the restrictions imposed by the size and chemical composition of the complexes, these models are: 1) a phospholipid cylinder having a diameter equal to the length of two lipid molecules, where the highly curved surface is somehow stabilized by intercalated protein or 2) a phospholipid bilayer disc surrounded by a stabilizing ring of protein. In the first case, extensive protein contact with phospholipids, required to maintain the highly curved surface of the particles, would probably obliterate the phase transition behavior of the lipids and would give elongated, rather than discoid, electron micrograph patterns. The second model is clearly the preferred one and has been proposed by various investigators (8, 9, 33).

Assuming that such disc-like structures in fact exist, our results on the cholesterol incorporation into these complexes can be explained very easily. Since the overall size and shape of the complexes do not change perceptibly upon introduction of cholesterol, the disc-like structure would be retained and the cholesterol would be simply intercalated between DMPC molecules in the bilayer region of the structure, producing the usual condensing effect upon the phospholipids. If, in addition,
Complexes of apo A-I with DMPC and Cholesterol

cholesterol was excluded from the lipid layer adjacent to the protein during complex formation, then the increased content of DMPC over cholesterol in the complexes could be explained. The experimental observation that the spectral properties of the protein component are not affected by cholesterol incorporation argues in favor of a "boundary" lipid layer made up of DMPC.

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Effect of cholesterol on the formation of micellar complexes between bovine A-I apolipoprotein and L-alpha-dimyristoylphosphatidylcholine.
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