Interaction of ApoA-II from Human High Density Lipoprotein with Lysolecithin

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The effects of lysolecithin and hexadecyltrimethylammonium bromide on the structure and stability of apoA-II from human high density lipoprotein have been evaluated by circular dichroism and fluorescence measurements. There is a profound enhancement in the stability of apoA-II to guanidinium hydrochloride denaturation when it forms a phospholipid complex with lysolecithin micelles. This complex is not only resistant to guanidinium hydrochloride denaturation, but it can be formed in a 6 M solution of this denaturant. The behavior of apoA-II in the native human high density protein is much closer to that of the lysolecithin apoA-II complex than to that of the free apoA-II.

The high density lipoprotein of human serum contains two major proteins (apoA-I and apoA-II) constituting about 90% of the protein content (1, 2). Although there are numerous studies in which attempts have been made to reconstitute the native lipoprotein from its lipid and protein components (2-6), there are relatively few studies on the interaction of the purified apoproteins with its component phospholipids. The interaction of apoA-I with the phospholipid, lysolecithin, has been reported recently (7-9). A similar study is now presented with apoA-II. Since apoA-II exists in a helical conformation in HDL and is devoid of structure in its free form, the effects of lipids on its structure should be instructive to our understanding of the properties of HDL.

It is, of course, important to evaluate the effects of the lipids present in HDL if we are to understand the nature of the interactions that occur in the native lipoprotein. Although present in much smaller amounts than the diacyl phosphatides, lysolecithin contains the same groups as lecithin but forms a soluble micelle (9, 10). It has been shown that lysolecithin facilitates the interaction of apoA-I with lecithin (7) to form protein lipid complexes of the same density as HDL. We have also investigated the effects of hexadecyltrimethylammonium bromide in order to compare the behavior of a phospholipid with a typical cationic detergent (11).

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1 The abbreviations used are: HDL, high density lipoprotein; C<sub>16</sub>NMe<sub>3</sub>+, C<sub>18</sub>NMe<sub>3</sub>+, tetra- and hexadecyltrimethylammonium bromide; CMC, critical micelle concentration; SDS, sodium dodecyl sulfate.

MATERIALS AND METHODS

The optical activity of the peptide chromophore has been used to follow changes in secondary structure. Fluorescence and polarization (12) of tyrosyl emission have been employed as indicators of tertiary structure. The procedures used to prepare and purify apoA-II were the same as described previously (13). The molecular properties of apoA-II are reported elsewhere (13).

Protein concentrations were determined by absorbance measurements at 280 nm using a value of 11,000 for apoA-II (13). Hexadecyltrimethylammonium bromide (J. T. Baker) was recrystallized twice from ethyl alcohol by addition of ether. Lysolecithin was synthetic l-palmitoyl-lysolecithin (Nutritional Biochemicals) and gave a single band on thin layer chromatography (silica gel, chloroform/methanol/water, 65:25:0) which co-migrated with a known standard.

Circular dichroism spectra were obtained with a Cary model spectropolarimeter. The mean residue ellipticity [θ] was calculated by:

\[ [\theta] = \frac{\theta_{MRW}}{I \times 100} \]

where \( \theta \) is the experimental ellipticity, MRW, the mean residue weight, is 113, \( I \) is the path length in centimeters, and \( c \) is the protein concentration in grams/ml.

Fluorescence measurements were made with a Hitachi Perkin-Elmer MPF III spectrophotometer. Hitachi polarizers were used for polarization measurements. The exciting wavelength was polarization at 280 nm, while the emission wavelength was at 305 nm for apoA-II. Polarization (P) was calculated by:

\[ P = \frac{I_{\perp} - G_{\perp}}{I_{\parallel} + G_{\parallel}} \]

where \( I_{\perp} \) is the fluorescence intensity, and the subscripts refer to the plane of polarization of the excitation and emission beams. The polarization values obtained on addition of lysolecithin, but not the detergents, were corrected for the increase in fluorescence intensity by the equation of Rawitch and Weber (14).

RESULTS

Interaction of Hexadecyltrimethylammonium Bromide

Water—apoA-II is an equilibrium mixture of monomer and dimer molecules (13). The CD spectrum of the dimer of apoA-II in the far ultraviolet is characterized by two extrema at 208 and 222 nm, which reflect its α-helical content (6, 13). We have followed the change in ellipticity at 220 nm, which is principally a measure of the α-helical residues (15, 16).

The negative ellipticity of apoA-II increases with C<sub>16</sub>NMe<sub>3</sub>++ concentration and reaches a plateau value near 100 μM, a value which is less than the CMC, i.e., 795 μM (11). In contrast, the polarization of tyrosyl fluorescence remains constant under the same experimental conditions (Fig. 1). The difference between the ellipticity and polarization curves is explicable if
FIG. 1. The effect of C_{16}NMe_{3}^{+} on the ellipticity and polarization of apoA-II (7.4 μM). The excitation (polarized) and emission wavelengths were at 280 and 305 nm, respectively. pH = 7.4, 0.01 M phosphate, T = 25°.

FIG. 2. The effect of SDS on the ellipticity and polarization of apoA-II (7.4 μM). Conditions are the same as in Fig. 1.

the binding of C_{16}NMe_{3}^{+} dissociates apoA-II into monomer and induces the formation of α helical residues. The shift from dimer to monomer by dilution of a solution of apoA-II decreases the polarization (13), whereas folding the random chain monomer into an α helix would be expected to increase the polarization. Since the polarization remains essentially constant as the α helical content increases, the decrease in polarization from dissociation appears to be compensated for by the increase resulting from α helical formation.

A similar increase in ellipticity and a small decrease in polarization occur when SDS is added to an apoA-II solution (Fig. 2). Since the final negative ellipticity value is somewhat larger and the polarization is slightly smaller than that found with C_{16}NMe_{3}^{+}, apoA-II is folded a little differently in SDS solutions compared to C_{16}NMe_{3}^{+} solutions.

GdmCl (2.0 M) — It has been shown that apoA-II dissociates completely and loses most of its secondary and tertiary structure in GdmCl solutions above 1 M (13). It was of interest to determine whether C_{16}NMe_{3}^{+} could reverse the effects of GdmCl. The ellipticity, polarization, and fluorescence values of apoA-II change in parallel upon addition of C_{16}NMe_{3}^{+} (Fig. 3). It is of interest that in 2 M GdmCl, where the apoprotein is monomeric, the curves for all three measurements fit on the same line (when appropriately normalized) and are clearly sigmoidal. It should be noted that the initial polarization value (0.08) is as low as is found for several proteins, exhibiting only tyrosyl emission, unfolded in 6 M GdmCl in the presence of reducing agents (12). The initial ellipticity value also indicates that very little secondary structure is present in 2.0 M GdmCl. The effect of C_{16}NMe_{3}^{+} on the ellipticity and polarization of apoA-II, therefore, reveals an important recovery of secondary and tertiary structure.

Interaction with Lysolecithin

Water — Lysolecithin dissociates very slowly in water below its CMC value (8, 17). Since measurements were made immediately after addition of lysolecithin to solutions of apoA-II, the interaction therefore occurs with micelles of lysolecithin. The titration of apoA-II with lysolecithin resulted in increases in ellipticity, fluorescence, and polarization (Fig. 4). The polarization curve was slightly displaced, whereas the fluorescence curve was strongly displaced from the ellipticity curve. The difference between the fluorescence curves can be explained by a redistribution of bound apoA-II molecules (and a reduction in quantum yield between high and low ratios) from several at high to one at low apoA-II to lysolecithin ratios. Other explanations of the fluorescence data are, of course, possible but are difficult to evaluate since the properties of lysolecithin in water are still not well understood (9).

GdmCl (1.8 M) — We have reported recently that the addition of lysolecithin to a solution of apoA-I in 1.8 M GdmCl completely reverses the red shift in the tryptophanyl emission peak that occurs when the aqueous solution is made 1.8 M in GdmCl (8). The ellipticity of apoA-II is reduced to −2000 in 1.7 M GdmCl. Addition of lysolecithin (180 μM) results in a large increase in negative ellipticity, i.e. −18,500 (Table I). A simi-
lalar enhancement in ellipticity was obtained with C16NMe3+ in a 2.0 M GdmCl solution of apo-A-II (Fig. 3).

**Stability in GdmCl Solutions**

The relative stability of a protein may be assessed from the amount of a denaturant that is needed to unfold its native structure. GdmCl is the most common reagent used for this purpose since nearly all proteins are transformed into random coil polypeptide chains by 5 M GdmCl (18). The effects of GdmCl on the ellipticity of the free apo-A-II and its lipid complexes are shown in Fig. 5. The lipids were added first to a solution of apo-A-II. The lipid apo-A-II complexes were then added to GdmCl solutions. It is evident that apo-A-II is strongly stabilized by both C16NMe3+ and lysolecithin. The stabilization of apo-A-I by lysolecithin to GdmCl denaturation has been reported recently (7). The complexes of apo-A-II, i.e. with lysolecithin and C16NMe3+, are more resistant than the corresponding complexes of apo-A-I to GdmCl denaturation. Of the two lipid complexes of apo-A-II, that of lysolecithin is more refractory of GdmCl denaturation than that of C16NMe3+. The ellipticity of the latter complex is reduced in half by 5 M GdmCl whereas that of the former is only slightly reduced in 5 M GdmCl (Fig. 5).

The profound effect of lysolecithin on the properties of apo-A-II was explored further by observing their combination in concentrated GdmCl solutions. In these experiments, lysolecithin was allowed to stand in the GdmCl solutions for 20 h before mixing with apo-A-II in order to ensure equilibration between monomer and micelle species. As seen in Fig. 6, the fluorescence and polarization of apo-A-II change in parallel with increasing lysolecithin concentration. Interaction with lysolecithin is observed in concentrations of GdmCl as high as 6 M. The curves for 3, 5, and 6 M GdmCl are sigmoidal and the stability of the lysolecithin apo-A-II complex to extremes of pH was also evaluated in order to determine whether charge interactions contribute significantly to its stability. When apo-A-II was titrated from neutrality, the optical activity decreased between pH 9.4 and 11.9 (Fig. 7). The stability of the lysolecithin apo-A-II complex to extremes of pH was also evaluated in order to determine whether charge interactions contribute significantly to its stability. When apo-A-II was titrated from neutrality, the optical activity decreased between pH 9.4 and 11.9 (Fig. 7). When the solution of apo-A-II at pH 11.9 was made 750 μM in lysolecithin, the ellipticity changed from -18.0 to 18.0 (Fig. 7). A small, further increase in negative ellipticity was observed after neutralization of the solution from pH 11.9 to 7.4. Further acidification to pH 1.9 had little additional effect. Since all the charged groups of apo-A-II except arginine will lose their positive charges between pH 9.4 and 11.9, the charge of apo-A-II and the phosphate of lysolecithin appears to have relatively little effect on the stability of the complex.

**DISCUSSION**

The most comprehensive studies on the binding of surfactant molecules to proteins have been made with serum albumin (19). The affinity of an homologous sorbic of surfactants depend, to a first approximation, on the length of their hydrocarbon chain (20). In some cases a limit is approached at longer chain lengths (21). The binding of these solutes shows that the lysolecithin apo-A-II complex to extremes of pH was also evaluated in order to determine whether charge interactions contribute significantly to its stability. When apo-A-II was titrated from neutrality, the optical activity decreased between pH 9.4 and 11.9 (Fig. 7). When the solution of apo-A-II at pH 11.9 was made 750 μM in lysolecithin, the ellipticity changed from -18.0 to 18.0 (Fig. 7). A small, further increase in negative ellipticity was observed after neutralization of the solution from pH 11.9 to 7.4. Further acidification to pH 1.9 had little additional effect. Since all the charged groups of apo-A-II except arginine will lose their protons between pH 9.4 and 11.9, the charge of apo-A-II and the phosphate of lysolecithin appears to have relatively little effect on the stability of the complex.

**TABLE I**

<table>
<thead>
<tr>
<th>Protein</th>
<th>GdmCl</th>
<th>C16NMe3+</th>
<th>Lysolecithin</th>
<th>[θ]_222 × 10^4</th>
<th>P_2</th>
</tr>
</thead>
<tbody>
<tr>
<td>μM</td>
<td>M</td>
<td>μM</td>
<td>μM</td>
<td></td>
<td></td>
</tr>
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<td>7.4</td>
<td>0</td>
<td>0</td>
<td>12.0</td>
<td>0.135</td>
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<td>-16.5</td>
<td>0.135</td>
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</tr>
<tr>
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<td>-16.0</td>
<td>0.150</td>
<td></td>
</tr>
<tr>
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<td>2.0</td>
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<td>-2.0</td>
<td>0.081</td>
<td></td>
</tr>
<tr>
<td>7.4</td>
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<td>750</td>
<td>-18.5</td>
<td>0.130</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>1.7</td>
<td>180</td>
<td>-18.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The stability of the lysolecithin apo-A-II complex to extremes of pH was also evaluated in order to determine whether charge interactions contribute significantly to its stability. When apo-A-II was titrated from neutrality, the optical activity decreased between pH 9.4 and 11.9 (Fig. 7). When the solution of apo-A-II at pH 11.9 was made 750 μM in lysolecithin, the ellipticity changed from -18.0 to 18.0 (Fig. 7). A small, further increase in negative ellipticity was observed after neutralization of the solution from pH 11.9 to 7.4. Further acidification to pH 1.9 had little additional effect. Since all the charged groups of apo-A-II except arginine will lose their protons between pH 9.4 and 11.9, the charge of apo-A-II and the phosphate of lysolecithin appears to have relatively little effect on the stability of the complex.

**FIG. 5.** Effect of GdmCl on the ellipticity of apo-A-II and complexes of apo-A-II with C16NMe3+ (1.9 μM apo-A-II/130 μM C16NMe3+) and lysolecithin (2.8 μM apo-A-II/450 μM lysolecithin). The curve for free apo-A-II is taken from Reference 13; pH 7.4, 0.01 M phosphate, T = 25°.
Interaction of ApoA-II with Lysolecithin

FIG. 6. Effect of lysolecithin on the fluorescence and polarization of apoA-II (2.0 μM) in GdmCl. Polarization values (○, ▽, and ▼) and fluorescence values (■, △, and ▼) in 3, 5, and 6 M GdmCl, respectively. The fluorescence increased by about 150%, while the polarization increased from 0.085 to 0.160 at each level of GdmCl. pH 7.4, 0.01 M phosphate, T = 25°C.

only a small dependence on temperature and therefore the free energy changes are largely determined by the entropy changes (21, 22). The dependence of the free energy change on the length of the hydrocarbon chain and the large entropy changes closely resemble the behavior of the surfactants themselves when they form micelles and therefore indicate that the binding process is essentially hydrophobic in origin (23). Very similar thermodynamic behavior is found in the free energy and entropy changes of micellization of SDS and decyltrimethylammonium bromide (24). Stone and Reynolds (25) have discussed the importance of the hydrophobic interactions to the binding of surfactants to apoA-I.

Reynolds et al. have recently reported the binding parameters of several charged surfactants (SDS, C12NMe3+, deoxycholate) to apoA-II (26, 27). They have shown that binding is a two-step process, i.e. independent site followed by cooperative binding. In the cases of SDS and C18NMe3+, analysis of the former shows about four sites for SDS and ten for C18NMe3+ with affinity constants near 10^4 M^-1 for both ligands. At higher surfactant concentration, i.e. above 10^-4 M, a much larger number of detergent molecules are bound (~1.1 to 1.4 g/g of apoA-II) in a cooperative reaction which results in the dissociation of apoA-II into its monomeric form (26, 27). This type of binding curve can originate either from a cooperative process of ligand binding or from a ligand-induced protein association (28). Since apoA-II (and reduced apoA-II (29)) is monomeric in native HDL, when compared to their behavior in their free, i.e. delipidated, form (33).

The stabilization of apoA-II to GdmCl denaturation by lysolecithin has been recently reported (7). In the latter, synthetic complex slightly less than 5 M GdmCl was sufficient to completely unfold the helical structure of apoA-I. In the native lipoprotein, the rate of unfolding of apoA-I becomes significant near ~3 GdmCl, but still has a half-life of about 15 min in 5.9 M GdmCl. The apoA-II complex with lysolecithin is more resistant to GdmCl denaturation than the apoA-I complex. In fact, a complex of apoA-II with lysolecithin can be formed in 6 M GdmCl. Since apoA-II in the free state is completely unfolded in 1 M GdmCl (see Fig. 5), the stabilization afforded by lysolecithin is profound. It should be noted that apoA-II in HDL appears to lose its helical content at a lower concentration of GdmCl than apoA-I. Evidently the interactions of apoA-II in HDL are somewhat different than those encountered with lysolecithin. It is clear, however, that surfactants and especially lysolecithin transform the two apoproteins from molecular forms of limited stability into two lipid-protein complexes which are more stable than most native proteins, at least by the criterion of GdmCl denaturation (34).

The behavior of apoA-II toward C18NMe3+ and lysolecithin is typical of a polypeptide with little or no stable structure. Surfactants have been shown to increase the secondary structure of low molecular weight polypeptide hormones, glucagon (35, 36), calcitonin,4 hormone fragments (37), higher molecular

4 G. Malan, unpublished experiments.
weight "proteins" which are largely devoid of structure, i.e. casein (39), histones (38, 50), and charged synthetic polypeptides (40-42). The stable globular proteins are, however, less predictable in their interactions with surfactants, since the optical activity increases in some cases and decreases in others (43). In general, the surfactants are less effective than the polar organic solvents in transforming random peptide groups to α helical or β structures (15, 34, 36, 44-46). Both unfold the native structure by disrupting nonpolar, hydrophobic interactions and then enhance hydrogen bonded structures by reducing the total concentration of water (44, 47). Organic solvents, however, change the local environment of the peptide groups more profoundly than surfactants and appear to lead to a greater degree of peptide hydrogen bonding in most cases. It has been suggested by Mattice et al. (43) that SDS acts by combining with the basic amino acids and enhancing their ability to propagate a helix. Chou and Fasman have suggested that leucyl residues in a polypeptide chain play a similar role in initiating helix formation when polypeptides fold into their native conformations (48, 49).

REFERENCES
42. Satake, I., and Yang, J. T. (1975) Biopolymers 14, 1841-1846
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