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Apolipoprotein A-I Structure and Particle Stability

Alterations in high density lipoprotein (HDL) composition that occur in dyslipidemic states may modulate a number of events involved in cholesterol homeostasis. To elucidate the details of how HDL-core composition can affect the molecular structure of different kinds of HDL particles, the conformation and stability of apoA-I have been investigated in homogeneous recombinant HDL particles (LpA-I) containing palmitoyloleoyl phosphatidylcholine (POPC), triolein (TG), and/or cholesterol linoleate (CE). In a discoidal particle containing two molecules of apoA-I and 85 molecules of POPC, apoA-I exhibits an α-helix content of 70% and a free energy of stability of its α-helical segments ($\Delta G_\alpha$) of 2.2 kcal/mol. Inclusion of eight molecules of TG into the complex significantly reduces the α-helix content and stability of apoA-I, whereas inclusion of four molecules of CE into the complex has an opposite effect in that the α-helix content is significantly reduced and the stability of the remaining α-helical structural apoA-I is increased. Neutral lipids have a different effect on apoA-I conformation in spherical LpA-I particles. In a sonicated-spherical LpA-I particle containing two molecules of apoA-I and 70 molecules of POPC, apoA-I exhibits an α-helix content of about 60% and a $\Delta G_\alpha$ of 1.2 kcal/mol apoA-I. Inclusion of either 10 molecules of TG or six molecules of CE into such a particle increases both the α-helix content and stability of apoA-I. Increasing the CE/TG ratio in LpA-I particles that contain both neutral lipids enhances the stability of the α-helical segments. ApoA-I molecules tend to dissociate and cause particle instability when $\Delta G_\alpha$ for the lipid-bound α-helices is less than that for helices in the lipid-free state. The stabilities of both discoidal and spherical LpA-I particles are relatively low when the only neutral lipid present is TG but the particle stability is enhanced by the presence of CE molecules. Such dissociation of apoA-I molecules from LpA-I particles that have a low CE/TG ratio would be promoted in the hypertriglyceridemic state in vivo.

High density lipoproteins (HDL) comprise a heterogeneous class of particles that contain apolipoprotein A-I (apoA-I) (LpA-1) or apoA-I and A-II (LpA-I,A-II) as their primary protein constituents (1). The central role that HDL plays in cholesterol metabolism is thought to involve the transport of cholesterol from peripheral tissues to the liver (2). Several studies have suggested that the efficiency of HDL in mediating this flux may be impaired in hypertriglyceridemic patients and that this effect may be related to modifications in HDL composition and size (for review, see Ref. 3). Investigations in a variety of laboratories have shown that changes in HDL size and composition can lead to altered interactions between HDL and lecithin:cholesterol acyltransferase (4–6), cholesteryl ester transfer protein (7–9), and cell surfaces (10, 11). There is evidence that an increased number of small, neutral lipid-poor, HDL particles in hypertriglyceridemic patients may directly affect cholesterol transport by stimulating the production of cholesteryl ester within the HDL pool (12) and by promoting an enhanced transfer and potentially atherogenic accumulation of these lipids in apoB-containing lipoproteins (3, 13).

Alterations in HDL composition also give rise to specific changes in the conformation and charge of the primary protein of HDL, apoA-I (14–17), and it appears that these changes in the molecular properties of HDL closely correlate with the altered function of these lipoprotein particles (6, 14, 18, 19). While a substantial amount of information about the organization of apoA-I molecules in discoidal particles is now available (for review, see Refs. 20 and 21), very little information exists that describes the structure of apoA-I in spherical HDL particles. In recent studies with reconstituted HDL particles (LpA-I), we showed that the overall conformation of human apoA-I is significantly different in spherical particles containing a cholesteryl ester core than in discoidal complexes that do not contain a neutral lipid core (16, 17). These studies also showed that the surface charge and secondary structure of apoA-I are significantly different on spherical particles and appear to be modulated by changes in composition in a different manner than for apoA-I on discoidal LpA-I particles (17). In this study, we further show that the type and amount of neutral lipid in reconstituted discoidal and spherical LpA-I directly affects the surface charge and structural characteristics of the lipoprotein particles. The results indicate that cholesteryl ester and triglyceride have distinct effects on the physical properties of apoA-I that are specific to the kind, spherical or discoidal, of LpA-I particle. A decrease in the cholesteryl ester content in the LpA-I particles is associated with a reduction in the structural integrity of these lipoprotein structures. Such particles are very similar to the abnormal HDL particles found in the plasma of hypertriglyceridemic subjects (1, 3, 13), and it is probable that the unusual charge and structural characteristics of small, neutral lipid-poor, HDL give rise to differences in the functional properties of these lipoproteins.
EXPERIMENTAL PROCEDURES

Materials

Trilinolenin, cholesterol, and cholesteryl linoleate were purchased from Sigma and 1-palmitoyl 2-oleoyl phosphatidylcholine was obtained from Avanti Polar Lipids (Birmingham, AL). All other reagents were analytical grade.

Methods

Isolation of HDL and Purification of Apoprotein A-I—HDL$_3$ was isolated from fresh plasma of normolipidemic donors by sequential ultracentrifugation (22). HDL$_3$ was delipidated (23), and purified apoA-I was isolated by either anion-exchange chromatography (24) on Q-Sepharose or gel filtration (25) on Sephacryl S-200. Prior to use, purified apoA-I was resuspended in 6 mM guanidine HCl and dialyzed extensively against the appropriate buffer.

Preparation of Recombinant HDL Complexes—Spherical recombinant HDL$_3$ complexes (apoHDL$_3$) were prepared by co-sonication of POPC, cholesteryl linoleate, triglyceride, and apoA-I using a technique similar to that originally described by Hirz and Scanu (26). POPC (3.2 mg) and other purified lipids in chloroform (see Table I for starting concentrations) were dried under nitrogen into a 12 × 75-mm test-tube, and 1.0 ml of Tris/saline, pH 8.0, was added. All sonications were performed in the 12 × 75-mm test tube suspended in a 15°C water bath and under nitrogen. The lipid-buffer solution was initially sonicated for 1 min using a Branson 450D sono tip with a 1/8-inch tapered microtip probe at a power control setting of 3 (manufacturer rated output of 40 watts) to disperse the lipids in the aqueous phase. This suspension was then incubated in a sealed tube for 30 min at 37°C and sonicated again for 5 min using a 95% duty cycle. ApoA-I (2 mg of a 1.4-mg of protein/ml Tris/saline solution, pH 8.0) was added to the lipid suspension, and the protein-lipid mixture was sonicated for 4 × 1 min (with maximal output of 40 watts and 90% duty cycle) punctuated by 1-min cooling periods. Discoidal recombinant HDL$_3$ were prepared by the cholate dispersion/Biobead removal technique as described previously (16). Discoidal LpA-I containing either cholesteryl ester or triglyceride were prepared similarly except that, after the initial incubation at 37°C, trilinolenin or cholesteryl linoleate was incorporated into the POPC-sodium cholate mixture by brief co-sonication for 3 × 30 s punctuated by 1-min cooling periods. ApoA-I was added to the mixture, and the preparation was incubated at 37°C and then with Bio-Beads as usual for the preparation of discoidal LpA-I. All LpA-I complexes were filtered through a 0.22-mm syringe tip filter and resolubilized by size exclusion chromatography on a Superose 6 column (16).

Determination of LpA-I Physical and Structural Characteristics—The protein-lipid homogeneity of apoA-I complexes were estimated by both electron microscopy and nondenaturing gradient gel electrophoresis as described before (27, 28). Complex hydrated densities were determined by density gradient ultracentrifugation using a discontinuous KBr gradient as described by Terpstra et al. (29). The number of molecules of apoA-I per particle was determined by apoprotein cross-linking with dimethyl suberimidate as described by Swany (30), and SDS-polyacrylamide gel electrophoresis was performed on 8–25% acrylamide gels using the Phast System (Pharmacia Biotech Inc.) to determine the extent of oligomer formation. Total and free cholesterol and phosphatidylcholine contents were determined enzymatically using Boehringer Mannheim (Indianapolis, IN) kits and the manufacturer’s suggested procedures. Phospholipids were also determined by phosphorus assay (31), and proteins were determined by the Lowry method as modified by Markwell et al. (32). Circular Dichroism—The average secondary structures of LpA-I apoA-I were monitored by CD spectroscopy on a Jasco J 410 spectropolarimeter calibrated with a 0.1% (w/w) 0–10-camphorsulfonic acid solution (17). CD spectra were measured at 24°C in a 0.1-cm path length quartz cell with a sample protein concentration of 67 mg/ml buffer. The percent $\alpha$-helix in apoA-I was calculated from the molar ellipticity at 222 nm using a mean residue weight of 113.3. The effect of GdnHCl concentration on the secondary structure of apoA-I in various LpA-I particles was monitored by the changes in molar ellipticity at 222 nm. All spectra of complex (33 mg protein/ml of buffer) were incubated with from 0–6 mM GdnHCl in 0.05 mM phosphate buffer, pH 7.2, for 72 h at 4°C. The free energy of unfolding of apoA-I on the surface of LpA-I complexes was calculated as described previously (17).

Statistical Methods—Standard deviation values reported (see Tables I–III) are maximum estimates determined from eight different measurements in representative experiments with different LpA-I particles.

| Table I | Particle composition POPC:CE:TG:apoA-I | EM shape$^a$ | Hydrodynamic diameter$^a$ | $\alpha$-Helix content$^b$ |
|---------|------------------------------------------|-------------|--------------------------|-------------------------|
| Initial | Final | nm | % | Initial | Final | nm | % |
| 100:0:0:2 | 85:0:0:2 | Disc | 9.0 | 70 |
| 100:1:2:2 | 90:6:0:2 | Disc | 9.0 | 61 |
| 100:1:2:2 | 90:6:0:2 | Disc | 9.0 | 54 |
| 100:0:2 | 70:0:0:2 | Sphere | 7.4 | 58 |
| 100:1:2:2 | 86:10:0:2 | Sphere | 7.5 | 64 |
| 100:1:2:2 | 84:6:0:2 | Sphere | 7.5 | 70 |
| 100:0:2 | 80:4:0:2 | Sphere | 7.5 | 68 |
| 100:1:2:2 | 102:12:2 | Sphere | 7.7 | 66 |

$^a$ POPC, CE (cholesteryl linoleate), TG (trilinolenin), and apoA-I molar composition before and after preparation and resolution; values are representative of three different preparations of LpA-I.

$^b$ Particle structural morphology determined by negative staining electron microscopy (EM) as described previously (17). Spherical particles prepared by sonication and discoidal complexes prepared by a modified cholate dispersion/Bio-Bead removal technique as described in the text.

RESULTS

Characterization of the Size and Composition of LpA-I Particles—Both spherical and discoidal reconstituted HDL particles (LpA-I) were prepared to contain POPC and apoA-I and also various amounts of triglyceride (trilinolenin) and/or cholesteryl ester (cholesteryl linoleate) (Table I). All recombinant complexes contain two molecules of apoA-I/particle (Table I) and exhibit only one band on nondenaturing gradient gels (Fig. 1). Particle size determination by negative staining electron microscopy gives similar sizes for spherical LpA-I complexes to that determined electrophoretically. Electron micrographs of sonicated preparations that contain a neutral lipid core reveal an overall spherical (circular) appearance that is similar to that observed for native spherical HDL particles (Fig. 2). Micrographs of LpA-I particles devoid of neutral lipids (final composition 70:2 mol/mol POPC:apoA-I; Table I) formed by sonication show a very similar “spherical” appearance; a similar result was reported by Kruski and Scanu (33) for sonicated mixtures of apoHDL$_3$ and egg PC. In contrast, in this study, electron micrographs of POPC:apoA-I particles containing some neutral lipids (<9 mol %) and prepared by cholate dispersion show a predominance of asymmetric, disc-shaped particles (major diameter, 9.0 nm) that form rouleaux of stacked discs (Fig. 2); it is well known that particles that contain only apoA-I and POPC are discoidal when prepared in the same manner (20, 21). In cholate-dispersion procedures, the addition and subsequent removal of bile salts allows the apoprotein to adopt a conformation that maintains a discoidal structure. The discoidal complexes of POPC and apoA-I used in this study are similar to those characterized previously (6, 14, 16–19). Sonication of POPC and apoA-I using the procedure described in this paper forms quite different structures that have no detectable asymmetry, are about 7.5 nm in diameter, and exhibit very similar structural properties to LpA-I that contain a neutral lipid core. Their electron micrographic appearance and electrophoretic migration patterns in both agarose and gradient acrylamide gels suggest that the 70:2 POPC:apoA-I LpA-I complexes produced by sonication are spherical or micellar-like structures. We cannot rule out the possibility that these structures may have some other morphology because there is insufficient resolution in the electron micrographs to determine any
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Asymmetry in the particles. However, since data from this study show that the physical properties of these sonicated LpA-I structures differ significantly from those of discs, but are very similar to previously characterized spherical LpA-I, it seems appropriate to refer to these structures as spherical as no other morphology could be justified.

Inclusion of up to 9 mol % neutral lipids in the discoidal particle (Table I) has no detectable effect on the particle morphology or size. In this study, the amount of cholesteryl ester incorporated into the discoidal complex is very close to the reported solubility of cholesteryl ester in bilayer phases of egg lecithin (34). In contrast, considerably more triglyceride can be incorporated into a discoidal complex. The amount of triglyceride incorporated into the discoidal particle is substantially greater than the solubility expected (~3 mol %) for triglyceride in lecithin bilayers (35). This suggests that the physical characteristics of phospholipid in a discoidal complex differs from that in a typical liquid-crystalline bilayer because of the presence of apoA-I around the edge of the disc. It is possible that differences in the incorporation of cholesteryl ester and triglyceride in discoidal particles may be partially due to the previously reported ability of triglyceride to interact with phospholipid acyl chains to a greater extent than cholesteryl ester can (35, 36).

Similar to the situation with discs, inclusion of neutral lipids in sonicated complexes also has no significant effect on the particle size or spherical (circular) morphology evident in electron micrographs. These lipoprotein structures appear to be similar in size to small HDL3c particles (28). Similarly sized LpA-I complexes have been shown to be a major component of nascent HDL particles secreted by HepG2 cells (37) and also have been isolated from normolipidemic plasma (sm-LpA-I, Ref. 38) and from cholate dialysis preparations (rHDL1, Ref. 14). However, it should be noted that the reconstituted spherical LpA-I described here have different hydrated densities than HDL3 particles. The LpA-I prepared by sonication exhibit peak hydrated densities of 1.10–1.13 g/ml for neutral lipid-rich and -poor particles, respectively. This hydrated density estimated for the various reconstituted LpA-I is consistent with the lipid/protein ratio of these complexes. The protein content of the spherical LpA-I particles listed in Table I averages about 43% by weight, which is comparable with that observed for native HDL3 particles (1) but lower than for HDL3 particles (50–60% by weight).

Effect of Neutral Lipids on the Secondary Structure of apoA-I — Differences in apoA-I organization on spherical and discoidal LpA-I are evident in terms of the amount of amphipathic α-helical structure in apoA-I on the various structures. Sonicated (spherical) POPC:apoA-I particles devoid of neutral lipids exhibit a significantly reduced α-helix content in apoA-I as compared with a discoidal particle with a similar protein and lipid composition (Table I). Incorporation of cholesteryl ester or triglyceride into a discoidal particle leads to a significant reduction in the content of α-helix in apoA-I. In contrast, inclusion of up to 10 molecules of cholesteryl ester and/or triglyceride in the POPC:apoA-I spherical particle is accompanied by an increase in the content of α-helix in apoA-I; this increase in α-helix structure seems to be larger in complexes containing cholesteryl ester (Table I). Further increases in spherical LpA-I cholesteryl ester and triglyceride content appear to be associated with a reduction in the helical secondary structure of apoA-I (Table I).

Effect of LpA-I Core Composition on the Net Charge of apoA-I — Discoidal and sonicated LpA-I particles exhibit different electrophoretic mobilities at pH 8.6 (Table II). Electrophoretic analysis shows that the negative surface charge varies on the spherical particles, whereas the discoidal particles have similar surface charge characteristics (Table II). Also, the net negative charge of apoA-I on the discoidal particles is somewhat larger than that for apoA-I on a spherical particle. The discoidal particles characterized in this and previous studies (17) exhibit an average molar valence of ~3.5e/mol of apoA-I, while the mean valence for the spherical particles is ~2.7e/mol of apoA-I. This reduced magnitude of negative charge on spherical LpA-I relative to discs is consistent with that observed in earlier studies (17), but the values in Table II are about one-third less than prior values for similar particles. This difference is due to a correction of a previous error in the calculation of valence; a recalibration of the molar valences for POPC:apoA-I reconstituted LpA-I (particles D1–D4 in Ref. 17) gives similar values to those reported here. Recalculation of the apoA-I valence on spherical LpA-I (particles 51 and 52 in Ref. 17) also gives rise to similar values to those obtained for the sonicated preparations in this study. However, it is of note that the different charge of apoA-I on the various sonicated spherical particles does not appear to be due to the presence of a neutral lipid core.
The sonicated LpA-I particle that lacks a neutral lipid core also exhibits a significantly reduced negative molar valence relative to that for discoidal particles (Table II). Addition of cholesteryl ester or triglyceride to the spherical LpA-I is associated with a small increase of about 0.2–0.4e in the magnitude of the negative charge on apoA-I. When cholesteryl ester and triglyceride are combined in a single LpA-I particle, however, the net charge on apoA-I is from 0.4 to 0.9e less negative than that for a particle containing only one neutral lipid (Table II). In contrast to the observations with the spherical particles, inclusion of cholesteryl ester and/or triglyceride in discoidal complexes has no effect on the net charge on apoA-I.

Isothermal Denaturation of apoA-I in LpA-I—Variations in the shape and core composition of LpA-I particles have significant effects on the denaturation of lipid-bound apoA-I by GdnHCl. Fig. 3 shows the GdnHCl denaturation curves for POPC:apoA-I LpA-I prepared by sonication or cholate-dispersion and the differences in estimated midpoints of denaturation

The insets of Fig. 4A illustrate that the unfolded apoA-I is resistant to denaturation. Analysis of the GdnHCl denaturation curves shows that, in contrast to the discoidal particles, changes in the ΔG° of apoA-I parallel ΔD20 values (Table III and Fig. 4, A and B, insets). It is evident from the insets of Fig. 4, A and B, that including or increasing the cholesteryl ester content in a spherical LpA-I particle corresponds to a significant increase in the ΔG°. Increasing the cholesteryl ester content in spherical LpA-I, therefore appears to increase the conformational stability of apoA-I. This observation seems to be contrary to a recent report by Rye et al. (39) showing that a small cholesteryl ester-poor particle is more stable to denaturation with GdnHCl than a larger cholesteryl ester-rich particle. Interpretation of this observation is, however, complex as changes in cholesteryl ester content of the different reconstituted complexes described were accompanied by major changes in all of the other lipid constituents. For example, the cholesteryl ester-poor particle in the study also had a significantly reduced free cholesterol content and an increased POPC:apoA-I ratio. Investigations in both this laboratory and that of Jonas and co-workers have shown that similar changes in LpA-I cholesterol (40) and phospholipid (17, 41) content also modulate the stability of apoA-I to denaturation.
TABLE III

Denaturation characteristics of LpA-I complexes

Significance of difference from the value for the 85:0:0 particle: *p < 0.05; **p < 0.01; ***p < 0.005. Significance of difference from the value for the 70:0:0 particle: *p < 0.05; **p < 0.01; ***p < 0.005.

| Complex   | D_{\theta}^C | \Delta G_0^C | \Delta n |
|-----------|--------------|--------------|---------|
| PC:CE:TG  | 2.9          | 2.2          | 5.7     |
| 85:0:0 (disc) | 3.0          | 1.8**        | 4.5     |
| 90:0:8 (disc) | 2.8          | 2.9**        | 7.1     |
| 86:4:0 (disc) | 2.7          | 2.5          | 7.3     |
| 70:0:0     | 2.1          | 1.2**        | 4.4     |
| 86:0:10    | 2.0          | 1.4          | 4.9     |
| 84:6:0     | 2.4          | 1.6**        | 4.9     |
| 80:4:6     | 2.6          | 1.6**        | 4.6     |
| 82:10:12   | 2.7          | 2.0***       | 5.6     |

a Midpoint of GdnHCl denaturation curve (Fig. 3, A and B) \( \pm 0.03 \) M (S.D.); values are representative of three different preparations of LpA-I.

b Free energy of denaturation at zero GdnHCl concentration \( \pm 0.2 \) kcal/mol (S.D.).

c GdnHCl bound during denaturation \( \pm 1.0 \) S.D.

Fig. 4. Effect of GdnHCl concentration on the molar ellipticity of apoA-I on spherical LpA-I. All fluids of spherical LpA-I (POPC: CE: TG molar ratios: panel A, \( \bullet \), 70:0:0; \( \square \), 86:0:10; \( \blacklozenge \), 84:6:0 and panel B, \( \triangle \), 70:0:0; \( \blacktriangle \), 80:4:6; \( \blacklozenge 62:10:12 \)) were incubated with 0–6 M GdnHCl, and CD spectra were measured as described in Fig. 3. Inset, linear regression plots are shown of the observed free energies of denaturation (\( \Delta G_0^C \)) against RTln(1 + ka) for spherical LpA-I.

Fig. 5. Densitometer profiles of LpA-I electrophoresed in 8–25% nondenaturing gradient gels. Profiles of discoidal (D) and spherical (S) LpA-I that were electrophoresed immediately after preparation (f) or after storage at 4 °C for one month (s) are shown. Stokes diameters were determined as described previously (16).

Discussion

Effect of Neutral Lipid on the Charge and Conformation of apoA-I in LpA-I Particles—Previous studies have identified a strong positive relationship between apoA-I surface charge and the free energy of stability of the \( \alpha \)-helical segments of apoA-I on discoidal particles (17, 40). In these investigations, it appeared that this relationship may also be valid for spherical particles (17); however, in the present study a much more detailed characterization has shown that a very different relationship exists for spherical LpA-I (Fig. 6). The surface charge on spherical particles appears to vary in a similar fashion to that seen for discoidal LpA-I particles. However, in contrast to discs, the conformational stability of apoA-I on spherical LpA-I appears to be lower and much less variable. Fig. 6 shows that the surface potential and \( \Delta G_0^C \) are strongly related for apoA-I on discoidal LpA-I (panel A, \( r = 0.93 \)), but are only weakly related for apoA-I on spherical complexes (panel B, \( r = 0.48 \)). This is consistent with our previous suggestion that apoA-I may maintain a more flexible organization on the spherical complex than on a disc (17). Small increases in the density of negative surface charge on a disc appear to increase charge repulsions, which disrupt the molecular integrity of apoA-I \( \alpha \)-helices and give rise to major changes in \( \alpha \)-helix content and stability. In contrast, the structural organization of apoA-I on a spherical complex can tolerate much greater changes in negative surface charge and does not exhibit the same magnitude of secondary structure disruption seen in the discoidal particles.

apoA-I structure appears to be extremely sensitive to the neutral lipid content of discoidal particles. Thus, addition of cholesteryl ester or triglyceride to a discoidal complex is associated with a reduction in the content of \( \alpha \)-helical segments in apoA-I by about 10–15% (Table I). The observed helix contents suggest that, in the presence of a neutral lipid, apoA-I maintains five to six 22-residue helical segments on the discoidal
LpA-I particle. It follows that a minimum of five to six helical segments are required to maintain the structural integrity of a discoidal complex that normally has about eight helical segments on its surface (14). Inclusion of neutral lipid in a discoidal complex results in a reduction in \( \alpha \)-helix content and stability but still leaves the complex more stable than a sonicated particle. This suggests that some of the apoA-I helical structure in a discoidal complex is not functioning to maintain the particle integrity. It is possible that this component of apoA-I helical structure is not localized at the edge of the disc but rather is associated with the phospholipid head groups on the face of the disc. This is consistent with previous studies that suggested that amphipathic helical peptides may be able to interact with both the edge and face of a discoidal complex (42).

Inclusion of cholesteryl ester or triglyceride in a sonicated POPC:apoA-I complex is also associated with changes in both the content and stability of the amphipathic \( \alpha \)-helical segments in apoA-I. However, in contrast to the situation with discoidal LpA-I, the inclusion of up to 12 molecules of neutral lipid in sonicated complexes is associated with a significant increase in the \( \alpha \)-helix content of apoA-I (Table I). The changes in secondary structure correspond to increased involvement of between 15 and 30 amino acids in the \( \alpha \)-helical structure of apoA-I. If it is assumed that the \( \alpha \)-helical structure of apoA-I is comprised of 22-residue \( \alpha \)-helix segments, the lowest \( \alpha \)-helical estimate (58%) for the sonicated POPC:apoA-I particle corresponds to six to seven such helices. The observed increase in \( \alpha \)-helicity associated with the presence of cholesteryl ester would correspond to an increase of approximately one, 22-residue, helical segment. Increases in the \( \alpha \)-helical content of apoA-I may also involve the formation of new short segments (17), and it appears that the formation of shorter helical segments is consistent with the smaller increase in \( \alpha \)-helix content in apoA-I that results when triglyceride is incorporated into the LpA-I complex.

Incorporation of neutral lipids in discoidal and spherical particles has distinct and different effects on the content and stability of the amphipathic \( \alpha \)-helix secondary structure of apoA-I. The study shows that variations in LpA-I triglyceride content have different effects on apoA-I stability in discoidal particles relative to that in spherical LpA-I particles. In contrast, an increase in LpA-I cholesteryl ester content is positively related with an increase in the free energy of stability of apoA-I \( \alpha \)-helices, in both discoidal and spherical particles (Fig. 6, A and B). The free energy of denaturation of apoA-I \( \alpha \)-helical structure (\( \Delta G_b \)) in spherical (○) and discoidal (●) LpA-I is plotted against the particle cholesteryl ester content (Table I).

7) Both cholesteryl ester and triglyceride reduce the \( \alpha \)-helix content of apoA-I in a disc, but while triglyceride reduces \( \alpha \)-helix stability on the disc, cholesteryl ester increases the helix stability. This may be the result of cholesteryl ester coming into close contact with apoA-I in a similar manner to that which has been proposed for cholesterol (40); inclusion of a small amount of cholesterol in a discoidal complex also gives rise to a reduction in \( \alpha \)-helix content with a concomitant increase in the helix stability. This implies that the steroid ring structure of either a cholesterol or cholesteryl ester molecule may interact with apoA-I and induce a conformational change. Incorporation of cholesteryl ester and triglyceride in sonicated, spherical LpA-I is associated with increases in the stability of apoA-I \( \alpha \)-helices. This stability is consistent with previous investigations (17) and suggests that it may be an increase in helix-helix interactions that stabilizes the secondary structure of apoA-I. Conversely, increasing the cholesteryl ester content to 10 molecules in an LpA-I complex also containing 12 molecules of triglyceride significantly reduces the amount of \( \alpha \)-helical structure in apoA-I, while at the same time increasing the helical stability. An increase in \( \alpha \)-helix stability that is concomitant with a reduction in helix content has also been observed when the cholesterol content of a reconstituted LpA-I particle is increased (40). It appears that both cholesterol and cholesteryl ester may compete with apoA-I for “hydrophobic solvation” by the phospholipid acyl chains. In this manner, these lipids may be able to displace apoA-I from the phospholipid interface and promote a reorganization of the remaining helical segments into a more stable conformation. This novel conformation is associated with an increased stability of apoA-I \( \alpha \)-helices and appears to be concomitant with an increase in the structural integrity of the lipoprotein complex.

Effect of LpA-I Neutral Lipid on LpA-I Particle Stability—Since the binding of apoA-I to lipid is mediated by its amphipathic \( \alpha \)-helices, the various effects of neutral lipids on helix stability described above might be expected to affect LpA-I particle integrity. If the amphipathic \( \alpha \)-helices are unstable, then the apoA-I molecules will tend to dissociate and destabilize the LpA-I particle. The dissociation of apoA-I from an LpA-I complex has been shown to occur when cholesterol is included in a discoidal LpA-I complex (40) and also when the POPC:apoA-I ratios are decreased (17). Such spontaneous dissociation of apoA-I is also evident from Figs. 1 and 5. It follows that the free energy of binding (\( \Delta G_b \)) of apoA-I to the particle
surface is no longer negative. The free energies of denaturation of apoA-I listed in Table III are consistent with this concept. Thus, assuming that the apoA-I molecules dissociated from the LpA-I surface by addition of GdnHCl adopt a random coil conformation in the aqueous phase, it can be shown that the difference between the free energy of denaturation of apoA-I ($\Delta G_{D}$) in the lipid-free and lipid-bound states ($\Delta G_{D}^{\text{free}} - \Delta G_{D}^{\text{bound}}$) is approximately equal to $G_{b}$ (G(bound/negative) − G(free/negative)). Since $\Delta G_{D}^{\text{free}}$ for apoA-I is measured as 2.4 kcal/mol when lipid-free protein is denatured by GdnHCl (17), it follows that $\Delta G_{D}$ is positive (i.e. spontaneous dissociation of apoA-I from LpA-I particles is thermodynamically favored) when $\Delta G_{D}^{\text{bound}}$ is < 2.4 kcal/mol.

The values of $\Delta G_{D}^{\text{bound}}$ listed in Table III suggest that the presence of eight molecules of triglyceride in the discoidal LpA-I complex ($\Delta G_{D} = 1.8$ kcal/mol) induces apoA-I dissociation, whereas the presence of four molecules of cholesteryl ester ($\Delta G_{D} = 2.9$ kcal/mol) stabilizes the particle. The presence of a mixture of cholesteryl ester and triglyceride molecules also stabilizes the particle. Consistent with this, the discoidal particles that contain cholesteryl ester are stable upon storage at 4°C, whereas those containing triglyceride are not (Fig. 5). In contrast, the spherical LpA-I particles that contain cholesteryl ester and/or triglyceride are thermodynamically unstable ($\Delta G_{D}^{\text{bound}}$ values are in the range 1.4–2.0 kcal/mol, Table III). While this maximum change in free energy, for apoA-I on the cholesteryl ester and triglyceride containing sphere, is less than that observed for apoA-I in a lipid-free state, no substantial apoA-I dissociation is evident even after storage for 1 month (data not shown). This suggests that kinetic effects are involved in maintaining the structural integrity of small spherical LpA-I. However, when the cholesteryl ester content of the spherical LpA-I particle falls below five molecules/mole of apoA-I these lipoprotein particles tend to fuse and shed apoA-I (Fig. 5). The triglyceride-containing particles are somewhat less stable than the cholesteryl ester-containing particles as expected from the $\Delta G_{D}^{\text{bound}}$ values (Table III).

Results from this study have shown that LpA-I with a low thermodynamic stability tend to become more heterogeneous with increased storage time (cf. Figs. 1 and 5). This heterogeneity appears to be concomitant with the formation of particles containing three molecules of apoA-I from complexes that have only two molecules initially. Therefore, it appears that unstable LpA-I particles containing two molecules of apoA-I can reorganize and form more thermodynamically-stable complexes and, in the process, liberate a molecule of lipid-poor apoA-I (Fig. 8). Although this process appears to occur on a relatively slow time scale in vitro, the rate of the process may be enhanced by the actions of different plasma proteins. A recent report by Nishida and co-workers (43) suggests that the remodeling of HDL by phospholipid transfer protein may stimulate the formation of larger HDL particles from 8.3 to 10.7 nm and the concomitant liberation of apoA-I. Other investigations have also shown that changes in the neutral lipid core of HDL can also result in the dissociation of apoA-I. Thus, studies in the laboratory of Barter and co-workers (44) have shown that hydrolysis of HDL-surface and core lipids by hepatic lipase and neutral lipid transfer by cholesteryl ester transfer protein (39) can promote the structural destabilization of HDL and the liberation of lipid-poor apoA-I.

Physiological Significance—Investigations in a variety of different laboratories have shown that the plasma concentration of a lipid-poor or free apoA-I (also referred to as pre-$\beta_1$ HDL) is significantly elevated in a number of different hyperlipidemic states (45, 46). Individuals with low HDL-cholesterol levels appear to have abnormal LpA-I particles that are unusually small and depleted in cholesteryl ester (13, 47). Results from some studies suggest that apoA-I may be able to readily dissociate from these lipoprotein complexes because it is very loosely bound. A recent study suggests that apoA-I on hypoalphapolipoproteinemic HDL particles may readily dissociate from the lipoprotein complex, and this lipid-poor apoA-I may be more rapidly cleared by the kidney (47). It was proposed that this increased propensity of apoA-I to dissociate from HDL and be cleared from the plasma may give rise to the observed increased fractional catabolic rates for apoA-I and the low plasma apoA-I levels in patients with low HDL-cholesterol levels. Changes in plasma HDL levels in these patients may therefore be directly related to the thermodynamic stability of HDL particles. If the dissociation of apoA-I from HDL occurs chronically, such as proposed in dyslipidemic patients (47), large amounts of apoA-I may be cleared by glomerular filtration, and this may give rise to reduced plasma apoA-I and HDL levels and the concomitant increased risk for the development of coronary artery disease.

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