The high density lipoproteins (HDL) in human plasma are classified on the basis of apolipoprotein composition into those containing apolipoprotein (apo) A-I but not apoA-II, (A-I)HDL, and those containing both apoA-I and apoA-II, (A-I/A-II)HDL. Cholesteryl ester transfer protein (CETP) transfers core lipids between HDL and other lipoproteins. It also remodels (A-I)HDL into large and small particles in a process that generates lipid-poor, pre-
β-migrating apoA-I. Lipid-poor apoA-I is the initial acceptor of cellular cholesterol and phospholipids in reverse cholesterol transport. The aim of this study is to determine whether lipid-poor apoA-I is also formed when (A-I/A-II)rHDL are remodeled by CETP. Spherical reconstituted HDL that were identical in size had comparable lipid/apolipoprotein ratios and either contained apoA-I only, (A-I)rHDL, or (A-I/A-II)rHDL were incubated for 0–24 h with CETP and Intralipid©. At 6 h, the apoA-I content of the (A-I)rHDL had decreased by 25% and there was a concomitant formation of lipid-poor apoA-I. By 24 h, all of the (A-I)rHDL were remodeled into large and small particles. CETP remodeled ~32% (A-I/A-II)rHDL into small but not large particles. Lipid-poor apoA-I did not dissociate from the (A-I/A-II)rHDL. The reasons for these differences were investigated. The binding of monoclonal antibodies to three epitopes in the C-terminal domain of apoA-I was decreased in (A-I/A-II)rHDL compared with (A-I)rHDL. When the (A-I/A-II)rHDL were incubated with Gdn-HCl at pH 8.0, the apoA-I unfolded by 15% compared with 100% for the apoA-I in (A-I)rHDL. When these incubations were repeated at pH 4.0 and 2.0, the apoA-I in the (A-I)rHDL and the (A-I/A-II)rHDL unfolded completely. These results are consistent with salt bridges between apoA-II and the C-terminal domain of apoA-I, enhancing the stability of apoA-I in (A-I/A-II)rHDL and possibly contributing to the reduced remodeling and absence of lipid poor apoA-I in the (A-I/A-II)rHDL incubations.

The cardioprotective properties of HDL© have been attributed to several mechanisms. These include their anti-inflammatory and anti-oxidant properties as well as their ability to participate in reverse cholesterol transport, the process whereby cholesterol from peripheral cells is transported to the liver for excretion. The first step of reverse cholesterol transport involves the efflux of cellular cholesterol and phospholipids to lipid-poor or lipid-free pre-β-migrating apolipoprotein (apo) A-I (1).

The HDL in human plasma are classified on the basis of apolipoprotein composition into those containing apoA-I but not apoA-II, (A-I)HDL, and those containing both apoA-I and apoA-II, (A-I/A-II)HDL. Recent work from this laboratory has established that substantial amounts of lipid-poor pre-β-migrating apoA-I are generated when either human plasma HDL or spherical reconstituted HDL (rHDL) containing apoA-I as the only apolipoprotein are remodeled by cholesteryl ester transfer protein (CETP) (3, 4).

The main aim of this study was to determine whether lipid-poor apoA-I is also formed when (A-I/A-II)HDL are remodeled by CETP. This is an issue of considerable importance given that (A-I/A-II)HDL comprise 50% or more of the HDL in normal human plasma and that under conditions of hypertriglycerideremia and in cases of low HDL states the level of (A-I/A-II)HDL rather than (A-I)HDL is affected (2, 5).

To address this question unequivocally, it is important to use HDL that are comparable in all respects with the exception of their apolipoprotein composition. The (A-I)HDL and (A-I/A-
II)HDL that are isolated from human plasma by immunoadherence chromatography do not fulfil these criteria because they contain several subpopulations of particles that vary in size (2). They also contain apolipoproteins other than apoA-I and apoA-II as well as multiple types of phospholipids (6, 7). These differences all have the potential to influence the CETP-mediated remodeling of HDL as well as lipid-poor apoA-I formation.

To circumvent these problems well defined, homogeneous preparations of spherical rHDL containing either apoA-I only, (A-I)rHDL, or apoA-I as well as apoA-II, (A-I/A-I)rHDL, were prepared. Because the (A-I)rHDL and (A-I/A-II)rHDL were identical in size and phospholipid composition and contained comparable proportions of lipids and apolipoproteins, variations in remodeling and lipid-poor apoA-I formation could be attributed unambiguously to their different apolipoprotein contents.

When the (A-I)rHDL and (A-I/A-II)rHDL were incubated with CETP and the phospholipid/triglyceride emulsion, Intralipid©, core lipid transfers were comparable for both of the rHDL preparations. However, the remodeling of the (A-I)rHDL was much greater than that of the (A-I/A-II)rHDL, and apoA-I dissociated only from the (A-I)rHDL. Additional investigations...
indicated that the reduced remodeling and absence of lipid-poor apoA-I in the (A-I/II)-rHDL incubations were the result of ionic interactions between the apoA-I and apoA-II on the (A-I/II)-rHDL surface.

EXPERIMENTAL PROCEDURES

Isolation of ApoA-I, ApoA-II, Lecithin:Cholesterol Acyltransferase (LCAT), and CETP—HDL were ultracentrifugally isolated from pooled samples of expired autologously donated human plasma (Gribbles Pathology, Adelaide, Australia) and delipidated as described previously (8, 9). The apoHDL were applied to a Q-Sepharose Fast Flow column (Amersham Biosciences) attached to an fast protein liquid chromatography system (Amersham Biosciences) (8). Following electrophoresis on a homogeneous 20% SDS-polyacrylamide PhastGel (Amersham Biosciences) and Coomassie Blue staining, the apoA-I and dimeric apoA-II appeared as single bands with respective molecular masses of 28.3 and 17.4 kDa.

LCAT was purified from pooled samples of autologously donated human plasma (10). The purified LCAT appeared as a single band on a silver-stained homogeneous 20% SDS-gels. Its activity was assessed using discoidal rHDL containing 1-palmitoyl-2-oleyl phosphatidylcholine (POPC) (Sigma), unesterified cholesterol (UC) (Sigma), tracer amounts of [1a,2a-3H]cholesterol ([3H]UC) (Amersham Biosciences), and apoA-I as the substrate (11). The assay was linear as long as <30% [3H]cholesterol was esterified. The LCAT preparations used in this study generated 1,627–2,350 nmol of cholesteryl esters (CE/nmol LCAT/h).

CETP was also purified from pooled samples of human plasma (12). Activity was determined as the transfer of [3H]-CE from [3H]-CE-HDL to ultracentrifugally isolated low density lipoproteins (LDL) (13, 14). The assay was linear as long as <30% of [3H]-CE was transferred. The CETP preparations used in this study generated 1,627–2,350 nmol of cholesteryl esters (CE/nmol LCAT/h).

Preparation of Spherical (A-I)rHDL and Spherical (A-I/A-II)rHDL—Discoidal rHDL containing POPC, UC, and either apoA-I or dimeric apoA-II were prepared by the cholate dialysis method (15). Mixtures of spherical (A-I)rHDL and spherical (A-I/A-II)rHDL were prepared by incubating discoidal (A-I)rHDL and discoidal (A-I/A-II)rHDL with LDL and LCAT (16). The resulting spherical HDL were isolated by ultracentrifugation (1.07 < d < 1.21 g/ml), dialyzed against 0.01 M Tris-buffered saline, pH 7.4, containing 0.15 M NaCl, 0.005% (w/v) EDTA-Na2, and 0.006% (w/v) NaN3, and applied to a column containing human apoA-II-specific polyclonal antibodies coupled to CNBr-activated Sepharose 4B (Amersham Biosciences) (16). The unbound spherical (A-I)rHDL were collected. The bound spherical (A-I/II)-rHDL were eluted from the column with 0.1 M acetic acid (pH 2.7). The (A-I/II)-rHDL were collected into tubes containing 1 M Tris, pH 11.0 (final concentration 0.1 M), dialyzed against Tris-buffered saline and concentrated 5-fold before use.

Spectroscopic Studies—The kinetics of the unfolding of apoA-I was assessed by incubating (A-I)rHDL and (A-I/A-II)rHDL (final apoA-I concentration 30 μg/ml) at 25 °C for 0–24 h with 4.0 M Gdn-HCl at pH 2.0, 4.0, and 8.0. The velocities of the unfolding were determined from 300–380-nm emission scans using an excitation wavelength of 295 nm. The excitation and emission bandwidths were 10 and 5 nm, respectively. Values represent the mean ± S.D. of triplicate determinations. The excitation wavelength was chosen so that the emission spectra reflected only the environment of the apolipoprotein Trp residues and excluded contributions from Tyr residues. As apoA-II does not contain Trp residues, the emission spectra of the (A-I/II)-rHDL therefore reported only on the unfolding of apoA-I.

Spherical rHDL phospholipid acyl chain and head group packing order was determined by measuring the steady state fluorescence polarization of samples, respectively, labeled with 1,6-diphenyl-1,3,5-hexatriene (DPh) and 1,4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluenesulfonate (TMA-DPh) (10). Lipid-water interface fluorescence was assessed by labeling spherical rHDL with 6-propionyl-2-(dimethylamino)-naphthalene (PRODAN) (10). Uncorrected fluorescence emission spectra of PRODAN-labeled rHDL were recorded from 390 to 600 nm using an excitation wavelength of 366 nm and respective excitation and emission bandwidths of 5 and 6 nm. Fluorescence intensities were measured at 440 and 490 nm. Polarization values and PRODAN fluorescence emission spectra were recorded at 5 °C interval from 5 to 50 °C. In all of the cases, the molar ratio of rHDL phospholipid/probe was 500:1. The final phospholipid concentration was 0.5 μM. Two-factor ANOVA with repeated measures was used to assess differences between data sets. Significance was set at p < 0.05.

Results

Physical Properties of Spherical (A-I)rHDL and (A-I/A-II)rHDL (Fig. 1)—The particle size distribution and composition of the spherical (A-I)rHDL and (A-I/A-II)rHDL are shown in Fig. 1. Both preparations were 9.7 nm in diameter, and they contained comparable proportions of lipids and apolipoproteins. The composition is similar to what has been reported for the (A-I)-HDL and (A-I/A-II)-HDL in human plasma (21). As judged by chemical cross-linking, the (A-I)rHDL contained three molecules of apoA-I/particle (data not shown). This is in agreement with previous reports from this laboratory (12). The composition of the (A-I/A-II)-rHDL is consistent with particles that contain two molecules of apoA-I and two molecules of dimeric apoA-II (16).

CETP-mediated Core Lipid Transfers between (A-I)rHDL and (A-I/A-II)rHDL and Intralipid (Fig. 2 and Table I)—The
ApoA-II Inhibits HDL Remodeling and Lipid-poor ApoA-I Formation

| TABLE I

Composition of (A-I)rHDL and (A-I/A-II)rHDL after incubation with Intralipid and CETP

(A-I)rHDL and (A-I/A-II)rHDL were mixed with Intralipid and incubated in the presence or absence of CETP. The final concentrations of rHDL cholesteryl esters and Intralipid triglycerides were 0.1 and 4.0 mM/liter, respectively. The samples without CETP were either maintained at 4 °C or incubated at 37 °C for 24 h. Samples containing CETP (final activity 2.7 units/ml) were incubated at 37 °C for 1, 3, 6, 12, or 24 h. The final volume was 2 ml. After incubation, the rHDL were isolated by ultracentrifugation (1.07 < d < 1.21 g/ml), and their composition was determined as described under “Experimental Procedures.” All of the measurements were made in triplicate, and the values varied by <10%. TG, triglycerides; PL, phospholipid.

| rHDL | Additions | Incubation conditions | PL | UC | CE | TG | apoA-I | apoA-II |
|------|-----------|-----------------------|----|----|----|----|--------|---------|
| (A-I)rHDL | Intralipid, -CETP | 4 °C, 24 h | 32.8 | 0.4 | 17.9 | 0.9 | 48.0 | 0.0 |
| (A-I)rHDL | Intralipid, -CETP | 37 °C, 24 h | 34.6 | 0.5 | 17.4 | 0.9 | 46.5 | 0.0 |
| (A-I)rHDL | Intralipid, +CETP | 37 °C, 3 h | 34.6 | 1.4 | 7.2 | 10.2 | 46.6 | 0.0 |
| (A-I)rHDL | Intralipid, +CETP | 37 °C, 3 h | 37.4 | 0.8 | 2.8 | 12.6 | 46.3 | 0.0 |
| (A-I)rHDL | Intralipid, +CETP | 37 °C, 6 h | 39.4 | 0.5 | 2.5 | 10.3 | 47.3 | 0.0 |
| (A-I)rHDL | Intralipid, +CETP | 37 °C, 12 h | 40.4 | 0.3 | 2.7 | 8.4 | 48.2 | 0.0 |
| (A-I)rHDL | Intralipid, +CETP | 37 °C, 24 h | 40.7 | 0.3 | 2.8 | 5.6 | 50.6 | 0.0 |
| (A-I/A-II)rHDL | Intralipid, +CETP | 4 °C, 24 h | 34.8 | 0.3 | 14.5 | 0.0 | 30.6 | 19.5 |
| (A-I/A-II)rHDL | Intralipid, +CETP | 37 °C, 24 h | 35.5 | 0.3 | 14.1 | 0.0 | 30.3 | 19.7 |
| (A-I/A-II)rHDL | Intralipid, +CETP | 37 °C, 1 h | 34.8 | 0.7 | 4.2 | 8.6 | 32.0 | 19.7 |
| (A-I/A-II)rHDL | Intralipid, +CETP | 37 °C, 3 h | 38.7 | 0.2 | 1.7 | 7.9 | 31.8 | 19.8 |
| (A-I/A-II)rHDL | Intralipid, +CETP | 37 °C, 6 h | 38.4 | 0.0 | 1.7 | 7.0 | 32.6 | 20.2 |
| (A-I/A-II)rHDL | Intralipid, +CETP | 37 °C, 12 h | 38.2 | 0.0 | 1.8 | 5.6 | 33.5 | 20.8 |
| (A-I/A-II)rHDL | Intralipid, +CETP | 37 °C, 24 h | 36.5 | 0.0 | 2.0 | 3.7 | 36.3 | 21.5 |

(A-I)rHDL (closed symbols) and (A-I/A-II)rHDL (open symbols) were incubated for 0–24 h with CETP and Intralipid. The composition of the rHDL at each time point is shown in Table I. Transfers of cholesteryl esters and triglycerides between the rHDL and Intralipid are shown in Fig. 2. The rate and the magnitude of the core lipid transfers were comparable for both types of rHDL. By 6 h, most of the cholesteryl esters had been transferred from the rHDL to Intralipid. During the first 3 h of incubation, CETP transferred triglycerides from Intralipid to the rHDL. When the incubation was extended beyond 3 h, triglycerides were transferred from the rHDL back into the Intralipid. This progressively decreased the rHDL core lipid content and is in agreement with previous reports from this laboratory (4, 12). By 24 h, the core lipid content of the (A-I)rHDL and (A-I/A-II)rHDL had decreased by 55.3 and 60.7%, respectively (Table I).

**CETP-mediated Remodeling of (A-I)rHDL and (A-I/A-II)rHDL**

The (A-I)rHDL and (A-I/A-II)rHDL were incubated for 0–24 h with CETP and Intralipid and then isolated by ultracentrifugation. Non-denaturing gradient gel electrophoresis was used to assess changes in rHDL size (Fig. 3). By 24 h, ~78% (A-I)rHDL had been remodeled into small particles, 8.0 nm in diameter. The remaining (A-I)rHDL were converted into large particles (diameter 11.0 nm). Remodeling of the (A-I/A-II)rHDL was markedly reduced compared with the (A-I)rHDL. By 24 h, ~32% (A-I/A-II)rHDL were converted into small particles (diameter 8.0 nm). CETP did not remodel the (A-I/A-II)rHDL into large particles.

The apoA-I content of the ultracentrifugally isolated (A-I)rHDL and (A-I/A-II)rHDL was also measured. During the first 6 h of incubation, the amount of apoA-I in the (A-I)rHDL decreased by 25% (data not shown). The apoA-I content of the (A-I)rHDL did not decrease further when the incubation was extended to 24 h. Incubation with CETP and Intralipid had no effect on the concentration of either apoA-I or apoA-II in the (A-I/A-II)rHDL (data not shown).

To determine whether the loss of apoA-I from the (A-I)rHDL could be explained in terms of the dissociation of lipid-poor apoA-I, aliquots of uncentrifuged incubation mixtures were subjected to non-denaturing gradient gel electrophoresis, transferred to nitrocellulose membranes, and immunoblotted for apoA-I (Fig. 4A). The (A-I/A-II)rHDL were also subjected to non-denaturing gradient gel electrophoresis and immunoblotted for apoA-I (Fig. 4B) and apoA-II (Fig. 4C). Lipid-free apoA-I and lipid-free apoA-II were applied to the gels as indicated. The distribution of apoA-I in the (A-I)rHDL was comparable with what is shown in Fig. 3 with small particles predominating at 24 h (Fig. 4A). Lipid-poor apoA-I was apparent at 6, 12, and 24 h. This coincided with the loss of apoA-I from the ultracentrifugally isolated rHDL.

The apoA-I in the (A-I/A-II)rHDL partitioned into small particles (Fig. 4B), whereas all of the apoA-II remained associated with the particles that did not change in size (Fig. 4C). Neither lipid-poor apoA-I nor lipid-poor apoA-II dissociated from the (A-I/A-II)rHDL (Fig. 4, B and C).

To determine whether the lipid-poor apoA-I that dissociated from the (A-I)rHDL was comparable in size and charge to pre-β apoA-I, the (A-I)rHDL that had been incubated for 24 h with Intralipid and CETP were subjected to two-dimensional gel
electrophoresis. (A-I/A-II)rHDL that had been incubated under the same conditions were also subjected to two-dimensional gel electrophoresis (Fig. 5A). Migration of the samples on the agarose gels is indicated in the figure. Lipid-free apoA-I was applied to the gradient gels as a control (Fig. 5B). The apoA-I that dissociated from the (A-I)rHDL (arrow) co-migrated with the lipid-free apoA-I, confirming its identity as pre-/H9252-migrating lipid-poor apoA-I. Pre-/H9252 apoA-I did not dissociate from the (A-I/A-II)rHDL.

Structural Studies (Figs. 6–8)—Additional physico-chemical studies were carried out to understand why the CETP-mediated remodeling of (A-I/A-II)rHDL was decreased relative to (A-I)rHDL and why CETP mediated the dissociation of lipid-poor apoA-I from (A-I)rHDL but not from (A-I/A-II)rHDL. One explanation for these results is that the high hydrophobicity of apoA-II reduces the lipid-water interfacial hydration of (A-I/A-II)rHDL, increases the penetration of the apoA-I α-helices into the (A-I/A-II)rHDL surface, and reduces the flexibility of the entire apoA-I molecule. As the number of lipid-associated apoA-I α-helices varies when rHDL change in size (22), it follows that anything that decreases the flexibility of apoA-I may compromise HDL size changes and inhibit the dissociation of lipid-poor apoA-I.

To determine whether the surface hydration of (A-I/A-II)rHDL was decreased relative to (A-I)rHDL, the preparations were labeled with PRODAN (Fig. 6). Comparison of the ratios of the fluorescence intensities of the labeled samples at 440 and 490 nm established that the (A-I/A-II) rHDL lipid-water interface (closed circles) was less hydrated than that of the (A-I)rHDL (open circles) (p < 0.001).

To determine whether this increased the penetration of apoA-I α-helices into the (A-I/A-II)rHDL surface, the phospholipid acyl chain and headgroup packing order of DPH- and TMA-DPH-labeled (A-I)rHDL and (A-I/A-II)rHDL was determined. These results showed that the packing order of the (A-I)rHDL and (A-I/A-II)rHDL phospholipids was comparable (data not shown). This established that the reduced remodeling of the (A-I/A-II)rHDL was not attributed to increased penetration of apoA-I α-helices into the particle surface.

We next investigated whether the reduced remodeling and absence of lipid-poor apoA-I in the (A-I/A-II)rHDL incubations could be explained in terms of interactions between apoA-I and apoA-II. Surface plasmon resonance was used to compare the binding of a panel of seven well defined epitope-specific apoA-I monoclonal antibodies to (A-I)rHDL and (A-I/A-II)rHDL (Fig. 7). The low affinities of mAbs AI-1.2, AI-11, and AI-115.1 for their epitopes in the N-terminal and central domains of apoA-I have been reported previously (17). Because the affinities of these mAbs for their epitopes in apoA-I were comparable for (A-I)rHDL and (A-I/A-II)rHDL, it follows that apoA-II does not affect the organization of, or interact with, any of these apoA-I domains in (A-I/A-II)rHDL. This was also the case for the epitope in the central domain of apoA-I to which mAb AI-17 bound. In sharp contrast, apoA-II did affect the affinity of mAbs AI-17.1, AI-187.1, and AI-141.7 for their epitopes in apoA-I. These antibodies, which recognize epitopes spanning three α-helices and a proline-punctuated β-turn in the C-terminal domain of apoA-I (17), had a higher affinity for (A-I)rHDL than for (A-I/A-II)rHDL. This is consistent with the epitopes located...
in residues 178–200, 187–210, and 220–242 of apoA-I being less exposed in (A-I/A-II)rHDL than in (A-I)rHDL. It also raises the possibility of a direct interaction, possibly involving salt-bridge formation, between the apoA-II and the C-terminal domain of apoA-I on the surface of (A-I/A-II)rHDL.

As salt-bridge formation enhances the stability of apolipoproteins (23), it follows that the apoA-I in (A-I/A-II)rHDL should be resistant to unfolding compared with the apoA-I in (A-I)rHDL. To determine whether this was the case, the (A-I/A-II)rHDL and (A-I)rHDL were incubated for 0–24 h with Gdn-HCl at pH 8.0 and the kinetics of the unfolding of apoA-I was assessed. As detailed under “Experimental Procedures,” the conditions were such that the unfolding of the apoA-I, but not the apoA-II, in the (A-I/A-II)rHDL was measured. A control sample of lipid-free apoA-I (open triangles) unfolded immediately and completely when incubated with Gdn-HCl (Fig. 8). The apoA-I in the (A-I)rHDL was also completely unfolded by 24 h (closed circles). This was not the case for the apoA-I in the (A-I/A-II)rHDL (open circles), which had unfolded by only 15% after 24 h of incubation with Gdn-HCl.

To determine whether this result could be explained in terms of the formation of salt bridges between the apoA-I and the apoA-II on the (A-I/A-II)rHDL surface, the incubations with Gdn-HCl were repeated at pH 4.0 and 2.0 (Fig. 8). Under these conditions, acidic residues in the apolipoproteins are protonated, salt bridges are disrupted, and the unfolding of apoA-I should be enhanced. The results showed that relative to what was observed at pH 8.0, the unfolding of the apoA-I in the (A-I/A-II)rHDL was markedly enhanced at pH 4.0 and 2.0. It is also noteworthy that the rate of unfolding of apoA-I in (A-I/A-II)rHDL and (A-I)rHDL, as well as the wavelength of maximum fluorescence in the absence of Gdn-HCl, decreased as the pH of the incubation mixture decreased. This is consistent with the protonation of acidic residues altering the global structure of the apoA-I in (A-I)rHDL and (A-I/A-II)rHDL.

DISCUSSION

Lipid-poor pre-β-migrating apoA-I is the initial acceptor of cellular cholesterol in the first step of the reverse cholesterol transport pathway (1). Recent work from this laboratory has established that significant amounts of lipid-poor apoA-I dissociate from (A-I)rHDL when they are remodeled by CETP (3, 4). A key aim of this study was to determine whether lipid-poor apoA-I is also generated when (A-I/A-II)rHDL are remodeled by CETP. Given that (A-I/A-II)rHDL comprises ~50% total HDL in normal human plasma (2) and that the level of this HDL subclass is subject to variation in hypertriglyceridemic subjects and in individuals with low HDL levels (5), this is an issue of considerable significance.

Current evidence as to whether lipid-poor apoA-I is formed during the CETP-mediated remodeling of (A-I/A-II)rHDL is inconclusive. Whereas some investigators (24) have reported that lipid-poor apoA-I is generated when (A-I/A-II)rHDL are isolated from human plasma by immunoaffinity chromatography and incubated with CETP and LDL, others (25) have found this not to be the case. This discrepancy highlights the problems that arise when the HDL from human plasma are used to address questions of this type. Plasma HDL contain apolipoproteins other than apoA-I and apoA-II (6), and their phospholipid composition varies widely (7, 26). Recent work from this laboratory has shown that phospholipids regulate both the CETP-mediated remodeling of HDL as well as the dissociation of lipid-poor apoA-I (4). In addition, apolipoproteins other than apoA-I and apoA-II have been reported to influence CETP-mediated core lipid transfers (27). Thus, it is hardly surprising that contradictory results were obtained when native HDL were used in the earlier studies.

These problems were circumvented in this work by using well-characterized, homogeneous preparations of spherical (A-I)rHDL and (A-I/A-II)rHDL. The rHDL were identical in size and phospholipid composition and contained either apoA-I or apoA-I as well as apoA-II as the only apolipoproteins. Whereas CETP mediated comparable transfers of core lipids between the rHDL preparations and Intralipid, the remodeling of the (A-I/A-II)rHDL was decreased compared with the (A-I)rHDL. Lipid-poor apoA-I dissociated from the (A-I)rHDL but not from the (A-I/A-II)rHDL.
We initially attempted to explain these results in terms of the high hydrophobicity of apoA-II (28). The rationale for this approach was that apoA-II decreases the hydration of the (A-I/A-II)rHDL lipid-water interface and increases the partitioning of apoA-I 22-residue and two 11-residue α-helices available for association with lipid (29). During the remodeling of (A-I)rHDL, the number of α-helices in contact with lipid decreases as the particles get smaller and increases as the particles get larger (22, 30). Increased partitioning of apoA-I α-helices into the rHDL surface could reduce this flexibility, decrease the size changes that occur during remodeling, and inhibit the dissociation of lipid-poor apoA-I.

The polarity-sensitive probe, PRODAN, was used to establish that the surface hydration of (A-I/A-II)rHDL was reduced relative to (A-I)rHDL (Fig. 6). To determine whether this was accompanied by increased penetration of apoA-I α-helices into the (A-I/A-II)rHDL surface, the packing order of the (A-I)rHDL and (A-I/A-II)rHDL phospholipid acyl chains and headgroups was compared. The results of these experiments established that there was no difference between the (A-I/A-II)rHDL and (A-I)rHDL in terms of phospholipid acyl chain or headgroup packing. Therefore, it was concluded that apoA-I α-helices partition to the same extent into the surface of (A-I)rHDL and (A-I/A-II)rHDL and that the differences in the remodeling of (A-I)rHDL and (A-I/A-II)rHDL cannot be explained by variations in surface hydration.

The possibility that the differences in the remodeling of (A-I)rHDL and (A-I/A-II)rHDL were attributed solely to the presence or absence of apoA-II in the particles was also explored. ApoA-II contains three α-helices that have a high affinity for lipid and are likely to remain lipid-associated during HDL remodeling (28, 31). Therefore, it follows that apoA-II-containing HDL may preferentially sustain structural reorganizations rather than change in size when they are remodeled by plasma factors. This is consistent with the results in Figs. 2 and 3 where the interaction of (A-I/A-II)rHDL with CETP resulted in only a modest decrease in particle size despite a 60% reduction in their core lipid content.

The results of the surface plasmon resonance studies, which showed that the binding affinity of three antibodies to their epitopes in the C-terminal domain of apoA-I was reduced in (A-I/A-II)rHDL compared with (A-I)rHDL, also raised the possibility that the attenuated remodeling of (A-I/A-II)rHDL may be attributed to (i) apoA-II displacing the C-terminal domain of apoA-I from the (A-I/A-II)rHDL surface, (ii) the C-terminal domain of apoA-I penetrating further into the (A-I/A-II)rHDL surface than into the (A-I)rHDL surface, or (iii) stabilization of the apoA-I in the (A-I/A-II)rHDL by salt-bridge formation.

The likelihood of apoA-II displacing the C-terminal domain of apoA-I from the (A-I/A-II)rHDL surface is low. This region of apoA-I is important for lipid association (32, 33), and its displacement from the rHDL surface by apoA-II should enhance not inhibit lipid-poor apoA-I formation. Moreover, as discussed above, the surface plasmon resonance results cannot be explained in terms of increased penetration of apoA-I α-helices into the (A-I/A-II)rHDL surface.

Therefore, we explored the possibility that the reduced re-
apoA-II and apoA-I. Interhelical salt-bridge formation has been reported for apoA-I, apoA-IV, and α-helical peptides (23, 34) as well as between α-helices in unrelated proteins (35, 36). As salt bridges have been reported to enhance protein stability (23), it was important to determine whether this was the case for the apoA-I in the (A-I)rHDL. This was achieved by comparing the unfolding of the apoA-I in (A-I)rHDL and (A-I/A-II)rHDL (Fig. 8). The results of this experiment, which showed that the apoA-I in (A-I/A-II)rHDL is resistant to unfolding compared with the apoA-I in (A-I)rHDL, indicated that the apoA-I in (A-I/A-II)rHDL may be stabilized by salt-bridge formation.

This possibility was explored further by comparing the unfolding of apoA-I in (A-I)rHDL and (A-I/A-II)rHDL at pH 2.0 and 4.0. Under these conditions, acidic residues in the apolioproteins are protonated and the ionic interactions that are responsible for salt-bridge formation are disrupted. Thus, if salt bridges are responsible for the inhibiting the unfolding of apoA-I in (A-I/A-II)rHDL, it follows that the unfolding should increase as the pH is reduced. The results in Fig. 8, which show that this is indeed the case, provide strong evidence that the apoA-I in (A-I/A-II)rHDL is stabilized by salt bridges.

When this result is considered in light of the surface plasmon resonance data in Fig. 7, which show that apoA-II only affects the conformation of the C-terminal domain of apoA-I, it follows that the salt bridges are likely to be confined to apoA-II and the C-terminal domain of apoA-I. This gives an insight as to the conformation of the C-terminal domain of apoA-I, it follows from the resonance data in Fig. 7, which show that apoA-II only affects the conformation of the C-terminal domain of apoA-I in (A-I/A-II)rHDL. This was achieved by comparing the unfolding of apoA-I in (A-I)rHDL and (A-I/A-II)rHDL at pH 2.0 and 4.0. Under these conditions, acidic residues in the apolioproteins are protonated and the ionic interactions that are responsible for salt-bridge formation are disrupted. Thus, if salt bridges are responsible for the inhibiting the unfolding of apoA-I in (A-I/A-II)rHDL, it follows that the unfolding should increase as the pH is reduced. The results in Fig. 8, which show that this is indeed the case, provide strong evidence that the apoA-I in (A-I/A-II)rHDL is stabilized by salt bridges.
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