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Human Apolipoprotein A-I Liberated from High-Density Lipoprotein without Denaturation†

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ABSTRACT: Apolipoprotein A-I (apoA-I) was liberated from human high-density lipoprotein (HDL) without exposure to organic solvents or chaotropic salts by the action of isolated insect hemolymph lipid transfer particle (LTP). LTP-catalyzed lipid redistribution results in transformation of HDL into larger, less dense particles accompanied by an overall decrease in HDL particle surface area:core volume ratio, giving rise to an excess of amphiphilic surface components. Preferential dissociation of apolipoprotein versus phospholipid and unesterified cholesterol from the particle surface results in apolipoprotein recovery in the bottom fraction following ultracentrifugation at a density = 1.23 g/mL. ApoA-I was then isolated from other contaminating HDL apolipoproteins by incubation with additional HDL in the absence of LTP, whereupon apolipoprotein A-II and the C apolipoproteins reassociate with the HDL surface by displacement of apoA-I. After a second density gradient ultracentrifugation, electrophoretically pure apoA-I was obtained. Sedimentation equilibrium experiments revealed that apoA-I isolated via this method exhibits a tendency to self-associate in an aqueous solution while its circular dichroism spectrum was indicative of a significant amount of α-helix. Both measurements are consistent with that observed on material prepared by denaturation/renaturation. The ability of apoA-I to activate lecithin:cholesterol acyltransferase was found to be similar to that of apoA-I isolated by conventional methods. The present results illustrate that LTP-mediated alteration in lipoprotein particle surface area leads to dissociation of substantial amounts of surface active apoprotein components, thus providing the opportunity to isolate apoA-I without the denaturation/renaturation steps common to all previous isolation procedures. As such, this reaction provides a unique model of the pool of apoA-I thought to function in vivo as a reservoir of apolipoprotein and as progenitor of nascent HDL-like particles.

As the major protein component of high-density lipoprotein (HDL),[1] apolipoprotein A-I (apoA-I) plays an important role in lipoprotein metabolism by stabilizing HDL particle structure and acting as an activator of lecithin:cholesterol acyltransferase (LCAT) (Eisenberg, 1984). Human apoA-I is a single polypeptide chain composed of 243 amino acids lacking cysteine (Brewer et al., 1978). The lipid binding domain of apoA-I is proposed to consist of multiple segments of amphiphilic α-helix 20–22 amino acids in length (Fitch, 1977; McLachlan, 1977). In human plasma, although the bulk of apoA-I is found

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[1] Abbreviations: apo, apolipoprotein; HDL, high-density lipoprotein; LTP, lipid transfer particle; LCAT, lecithin:cholesterol acyltransferase; CD, circular dichroism; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PC, phosphatidylcholine.

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associated with HDL particles, a distinct pool of free apoA-I is though to exist in equilibrium with lipoprotein-bound apoA-I (Pownall et al., 1978).

ApoA-I is conventionally isolated from HDL by extraction of particle lipids with organic solvents (Scanu & Edelstein, 1971) and solubilization of apolipoproteins in urea or guanidine hydrochloride, followed by one or more chromatographic steps (Scanu et al., 1969; Shore & Shore, 1969; Reynolds & Simon, 1974). These procedures require that the protein be denatured during isolation. The fact that apoA-I has been characterized after removal of denaturants may cause some skepticism about the physiological relevance of this data. In addition, it is known that prolonged exposure to 6–8 M urea during chromatographic steps can lead to carbamylation of amino groups resulting in artifactual heterogeneity (Gerding et al., 1971; Hagel et al., 1971). Nevertheless, it is generally assumed that, upon removal of chaotropic agents by exhaustive dialysis, apoA-I refolds to a conformation similar to that of the pool of free apoA-I in plasma.

We have been studying the properties of a novel lipid transfer particle (LTP) discovered in insect hemolymph [see Ryan (1990) for a review]. LTP is a high molecular weight, very-high-density lipoprotein that is proposed to mediate the transfer of lipid between cell membrane and lipoprotein in vivo (Van Heusden & Law, 1989). Consistent with this postulated function is the capacity of LTP to catalyze vectorial net transfer of lipid mass among donor/acceptor lipid particles (Ryan et al., 1986, 1990a; Ando et al., 1990) which can result in dramatic alteration of their lipid content and composition. When human HDL is employed as a substrate in the absence of other lipid donor/acceptor particles, LTP induces a dramatic transformation of HDL into larger, less-dense lipid particles (Silver et al., 1990). As a result, there is a significant reduction in the overall ratio of particle surface area:core volume between substrate HDL and transformation product. Therefore, at equilibrium, surface components (phospholipid, unesterified cholesterol, apolipoprotein) in excess of those required to stabilize the product particles are present in the reaction mixture. As a result, significant quantities of apolipoprotein dissociate from the surface of the product lipoprotein particles and can be recovered as lipid-free apolipoprotein. Since apoA-I has less affinity for lipid surfaces than apoA-II or the C apolipoproteins (Rosseneu et al., 1976; Lagocki & Scanu, 1980), it preferentially dissociates. Thus, apoA-I liberated as a result of this reaction is representative of the pool of free apoA-I proposed to exist in vivo and function as a reservoir of apolipoprotein and as a precursor of lipoprotein species that serve as acceptors of cellular unesterified cholesterol in the reverse cholesterol transport pathway (Hara & Yokoyama, 1991).

Materials and Methods

ApoA-I and LCAT Purification. HDL was isolated from fresh human plasma between the density limits 1.063 and 1.21 g/mL by sequential flotation ultracentrifugation. LTP was isolated from hemolymph of the tobacco hornworm, *Manduca sexta*, as previously described (Ryan et al., 1990b). To prepare apoA-I, HDL was incubated with LTP (20 μg of LTP/mg of HDL protein) for 120 min in 0.10 M sodium phosphate, pH 7.0, 150 mM NaCl (phosphate-buffered saline; PBS) at 37 °C. After incubation, the sample was adjusted to 1.23 g/mL by the addition of solid KBr and subjected to density gradient ultracentrifugation for 16 h at 40 000 rpm in a Ti 70.1 rotor. The top yellow fraction, containing HDL transformation product and LTP, was removed with a syringe and the infranatant was collected from the bottom. Following dialysis, the infranatant was incubated with HDL (1 mg of HDL protein/10 mg of bottom fraction protein) for 60 min at 23 °C and then subjected to ultracentrifugation as described above. The bottom fraction was collected, dialyzed against buffer for storage at 4 °C or versus deionized H_2O, and lyophilized. For some experiments, apoA-I was isolated from human HDL fraction by delipidation and ion-exchange chromatography in 6 M urea as previously described (Scanu et al., 1969; Yokoyama et al., 1982). An aqueous solution of apoA-I isolated by this method was prepared from the lyophilized sample by dissolving it in 6 M guanidine hydrochloride and dialysis versus buffer.

LCAT was isolated from human plasma that was subjected to ultracentrifugation at a density of 1.21 g/mL. The clear middle fraction obtained from this centrifugation was dialyzed into 10 mM Tris, pH 7.4, 50 mM NaCl and subjected to Affi-Gel Blue (Bio-Rad) column chromatography where LCAT does not interact with the gel. LCAT-containing fractions were pooled and subjected to ion-exchange chromatography (DE-52; Whatman), and bound LCAT was eluted with an NaCl gradient from 75 to 200 mM. Following hydroxylapatite chromatography in 5 mM sodium phosphate, pH 7.0, where activity is recovered in the flow-through fraction, a purified LCAT preparation (2600 times versus plasma) was obtained that was not contaminated by apolipoproteins. The active fractions were pooled and concentrated 5-fold by ultrafiltration following the addition of bovine serum albumin (0.45%) and used for experiments.

Analytical Ultracentrifugation. ApoA-I samples were dialyzed against 10 mM Tris, pH 7.4, 100 mM KCl for 24 h at 4 °C prior to ultracentrifugation. A Beckman Model E analytical ultracentrifuge equipped with an electronic speed control system, an RTIC temperature control system, and a titanium rotor was used for all runs. Determination of molecular weights was made using the photoelectric scanner according to the method described by Chervenka (1970). Samples (120 μL) were loaded into a 12-mm double-sector charcoal-filled Epon cell equipped with sapphire windows. Runs were performed at 20 °C at speeds ranging from 12 000 to 20 000 rpm for a minimum of 48 h before equilibration photographs were taken. Molecular weight calculations were carried out using a computer program written in APL language. The ln Y versus r^2 data were fitted to a second-degree polynomial equation using the least-squares technique, and the point-average molecular weights were calculated from the slope of this equation.

Circular Dichroism. Circular dichroism (CD) was performed on a Jasco J-500C spectrapolarimeter with a DP500N data processor and a thermostated cell holder with a water bath at 25 °C. The cells were 0.05 cm. The instrument was calibrated with d(+)10-camphorsulfonic acid at 290 nm and with pantyllactone dissolved in water at 219 nm. Ten scans were performed on each sample in addition to the appropriate blanks. The data were plotted as mean residue weight ellipticity expressed in deg·cm^2/dmol versus wavelength in nanometers. The mean residue weight was taken as 117.0. The ellipticity versus wavelength data were analyzed by a computer program developed by Provencher and Glöckner (1981) which analyzes CD spectra as a sum of 16 proteins, the structures of which are known from X-ray crystallography. The input to the program was the mean residue ellipticities in 1-nm intervals from 190 to 240 nm.

LCAT Assay. Small unilamellar vesicles of egg phosphatidylcholine (PC)/cholesterol (4:1 mol/mol) (obtained from Avanti and Sigma, respectively) were prepared by sonication

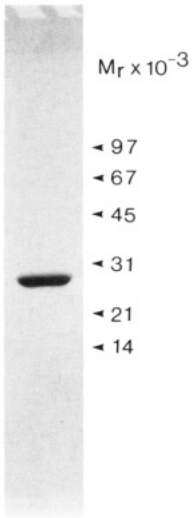

FIGURE 1: SDS–PAGE of affinity-isolated apoA-I. An 8–18% acrylamide gradient slab loaded with 25 μg of apoA-I was electrophoresed at 30 mA of constant current for 3.5 h. The position of molecular weight standards, including phosphorylase *b*, albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme, are shown.

and used as a substrate (Yokoyama et al., 1980). Assays mixtures (230 μL) contained 40 μL of the LCAT preparation, 100 nmol of PC, 25 nmol of unesterified cholesterol, 0.75 μCi of [7(n)-^{3}H]cholesterol (Amersham), 1.8 mg of bovine serum albumin, 40 nmol of β-mercaptoethanol, 7 nmol of EDTA, and a given concentration of human apoA-I. All solutions were prepared in 10 mM sodium phosphate pH 7.4, 150 mM NaCl. Incubations, conducted for 2.5 h at 37 °C, were terminated by the addition of 2.75 mL of chloroform/methanol (2:1) and extraction of the lipids. Lipid extracts were subjected to thin-layer chromatography on glass plates precoated with silica gel 60 and developed in hexane/diethyl ether/acetic acid (80:20:1). The cholesterol and cholesterol ester bands were scraped, and the radioactivity was counted in a Beckman LS 6000 TA liquid scintillation spectrometer.

Analytical Methods. SDS–PAGE was performed on 8–18% acrylamide gradient slabs electrophoresed at 30 mA for 3.5 h and stained with Coommassie Brilliant Blue. The content of choline-containing phospholipids and unesterified cholesterol in affinity-isolated apoA-I was determined by commercial enzyme-based colorimetric assays (Boehringer).

Results

Affinity Isolation of ApoA-I. When incubated with catalytic amounts of insect LTP, human HDL undergoes a dramatic transformation reaction which results in the formation of larger, less-dense product lipoproteins that shed apolipoproteins (Silver et al., 1990). In the case of HDL_3, up to 70% of the apolipoprotein dissociates from the particle surface. As such, this reaction is clearly distinct from ultracentrifugation-induced stripping of apoA-I from HDL in the presence of salt. Lipid-poor apoproteins obtained by LTP-mediated lipid transfer are comprised predominantly of apoA-I with lesser amounts of apoA-II and C apolipoproteins. On the basis of the differential affinity of these apolipoprotein components for lipid surfaces, it was possible to remove apoA-II as well as the C apolipoproteins by incubation with isolated HDL, resulting in a competition for the limiting available lipid surface. Under these conditions, apoA-II and C apolipoproteins present as minor contaminants displace apoA-I from the surface of HDL (Rosseneu et al., 1976; Lagocki & Scanu, 1980) and subsequently float with HDL when subjected to ultracentrifugation

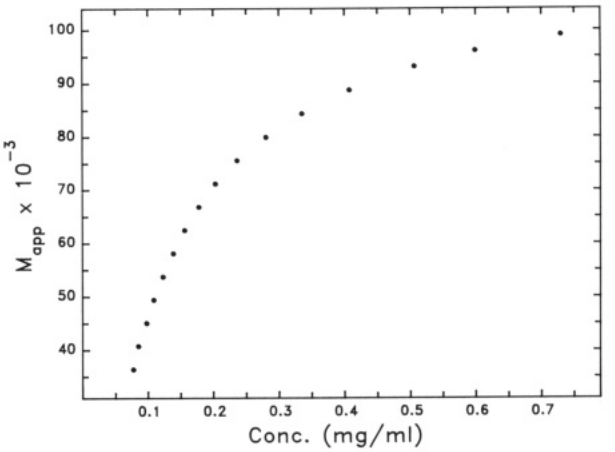

FIGURE 2: A plot of the apparent molecular weight (M_{app}) versus concentration for apoA-I prepared by the affinity method. The solvent system was 10 mM Tris and 100 mM KCl at pH 7.4. The centrifuge speed was 15000 rpm at 20 °C.

at a density = 1.23 g/mL. The resulting bottom fraction contains apoA-I as the sole protein component (Figure 1). Lipid analysis of isolated apoA-I revealed <1% by weight phospholipid and cholesterol as contaminants in our preparation indicating that under these conditions phospholipid and cholesterol moieties do not dissociate from the HDL particle surface and that among the apolipoproteins apoA-I does not compete effectively with apoA-II or C apolipoproteins for available surface. In contrast to all previous methods reported, isolation of apoA-I by this affinity technique does not employ denaturants or solvent extraction to remove lipid and denaturation/renaturation of the apolipoprotein is not required. Since it is not known what effect denaturation has on apoA-I, we characterized the apolipoprotein and compared its properties with apoA-I isolated by conventional techniques involving organic solvent extraction of HDL lipids and chromatography of resolubilized apolipoproteins in 6 M urea.

Hydrodynamic Properties of Affinity-Purified ApoA-I. Sedimentation equilibrium experiments were conducted to assess the self-association properties of affinity-isolated apoA-I. A plot of apparent molecular weight versus concentration (Figure 2) showed a range of molecular weights from 30000 to 100000 consistent with the presence of monomeric species as well as dimers and tetramers. These results are in agreement with the behavior of apoA-I isolated by conventional delipidation/denaturation methods (Vitello & Scanu,1976; Formisano et al., 1978; Yokoyama et al., 1982) and indicate that self-association of lipid-free apoA-I is an intrinsic property of the apolipoprotein rather than an artifact induced by denaturation/renaturation. The tendency to self-associate when not bound to lipid surfaces may provide a means whereby hydrophobic residues involved in interacting with lipid surfaces are shielded from the aqueous environment. From X-ray crystallography, it has been shown that helical segments in other water-soluble apolipoproteins form intramolecular bundles which shield hydrophobic residues from the aqueous environment when not bound to a lipid surface (Breiter et al., 1991; Wilson et al., 1991). Results with apoA-I, on the other hand, may suggest that a similar intramolecular arrangement of helical segments does not form, thereby inducing dimerization/oligomerization.

Circular Dichroism Spectroscopy. It is known that urea or guanidine hydrochloride treatment employed in conventional methods of apoA-I isolation results in loss of secondary structure, and in this solvent apoA-I has little if any organized structure (Lux et al., 1972). Upon removal of urea or

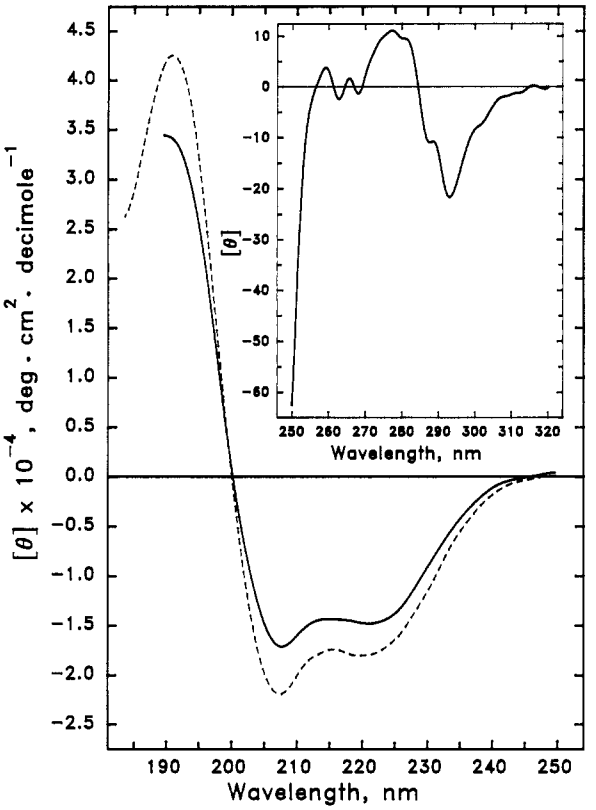

FIGURE 3: Circular dichroism spectra of apoA-I. Far-UV spectrum for apoA-I prepared by the affinity method, in 0.1 M KCl, 0.1 M Tris-HCl, pH 7.4 (—), and in 50% TFE, 0.05 M KCl, 0.05 M Tris-HCl, pH 7.4 (--). The inset represents the near-UV spectrum in 0.1 M KCl, 0.1 M Tris-HCl, pH 7.4.

guanidine, apoA-I refolds with the return of secondary structure with a helical content 20–30% lower than apoA-I bound to HDL. It has been postulated that these spectral differences may reflect changes in length, tightness, or orientation of the different helical segments of the polypeptide. On the other hand, these alterations could be due to incomplete or improper refolding of apoA-I upon removal of chaotropic agents. Thus, we compared the spectral properties of apoA-I prepared without denaturation with those reported earlier for apoA-I isolated by conventional methods. ApoA-I contains 4 tryptophans, 7 tyrosines, and 6 phenylalanines. The CD aromatic region, as shown in the inset in Figure 3, is very similar to an earlier study by Lux et al. (1972) on apoA-I prepared by a denaturation step. There are minima at 293, 268, and 262.8 nm, maxima at 281, 277.1, 268, and 259 nm, with a shoulder at 302 and 288.3 nm. The tryptophans will be responsible for all the peaks and troughs above 280 nm, while the 277.1-nm band is due to the tyrosine residues and the other bands in the lower wavelength region are due to the phenylalanine residues.

The far-UV spectrum for apoA-I prepared by the affinity method has minima at 221 and 208 nm with a peak at 190 nm, all indicative of a helix-containing protein, in agreement with earlier CD studies carried out on material prepared by denaturation/renaturation procedures (Scanu, 1965; Lux et al., 1972; Jackson et al., 1973; Yokoyama et al., 1982). The negative ellipticity at 221 nm in the present study and the earlier investigations is ~−15 000°, which results in comparable α-helical content estimates (50–55%). Provencher–Glöckner analysis of the spectrum in Figure 3 reveals 51% α-helix, 26% β-sheet, 19% β-turn, and 4% random coil. Trifluoroethanol, known to have a β-sheet- and α-helix-stabilizing effect by inducing formation of intramolecular hydrogen bonds and/or nonpolar interactions (Greff et al., 1976), induced substantial increases in the far-UV ellipticities: the $[\theta]_{190nm}$ value increases by 22%, the $[\theta]_{208nm}$ value increases by 28%, and the $[\theta]_{220nm}$ value increases by 24%. These changes, when analyzed by the Provencher–Glöckner program, reveal 69% α-helix, 18% β-sheet, 9% β-turn, and 4% remainder, resulting in the induction of some 20% additional helix in apoA-I upon the addition of trifluoroethanol.

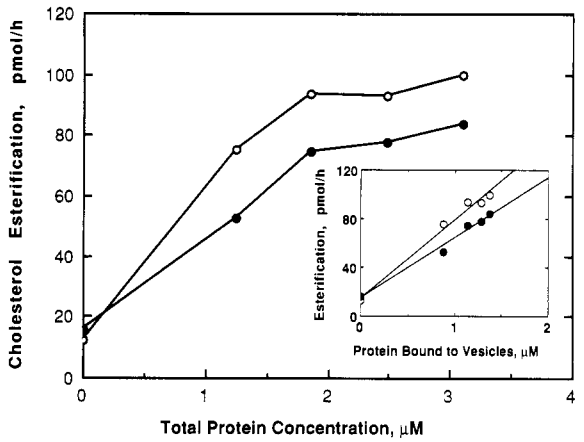

FIGURE 4: Activation of lecithin:cholesterol acyltransferase by human apoA-I on small unilamellar vesicles of PC/cholesterol (4:1 mol/mol) as a function of apoA-I concentration in the reaction mixture. Open symbols are the activation by apoA-I isolated by methods employing denaturation steps, and closed symbols represent activation by apoA-I isolated by the affinity method described in the present work. Details of the experimental conditions are described in Materials and Methods. The inset illustrates the same data plotted against the apoA-I bound to the substrate vesicles (calculated according to the equation of equilibrium binding using parameters measured in a previous study; Yokoyama et al., 1980). The straight lines represent least-squares linear regression of the data.

Activation of LCAT. The ability of affinity-isolated apoA-I to activate LCAT was determined by incubation of apoA-I with PC/cholesterol unilamellar vesicles and partially purified LCAT. The results showed (Figure 4) a concentration-dependent activation of LCAT that was similar to that of apoA-I isolated by conventional methods. As indicated in the inset, the rate of activation was proportional to the bound apoA-I, calculated using K_d (3.0×10^{-7} M) and maximum binding (3.7×10^{-3} mol of apoA-I/mol of PC) levels obtained from equilibrium binding experiments of the protein previously measured with this type of vesicle (Yokoyama et al., 1980). The data are consistent with previous studies of LCAT activation by apoA-I as well as synthetic model peptides (Yokoyama et al., 1980; Fukushima et al., 1980). These results suggest that apoA-I which dissociates from lipoprotein surfaces as a function of lipoprotein metabolism likely resides in plasma as a reserve capable of reassociation with lipid surfaces and LCAT activation. Furthermore, the fact that apoA-I isolated by denaturation/renaturation was similar to affinity-isolated apoA-I in these LCAT activation studies provides support for the concept that, following denaturation, apoA-I refolds to a conformation very similar to that of native lipid-free apoA-I.

Discussion

Insect hemolymph LTP is a remarkable catalyst that can induce redistribution of lipoprotein-associated lipid via facilitated vectorial net lipid transfer. It is thought that LTP functions to facilitate establishment of an equilibrium distribution of the lipid components of substrate lipid particles. In addition, when present, exchange or transfer of low molecular weight, water-soluble apolipoproteins may occur (Ryan et al., 1990c). A consequence of this redistribution, however,

is potential alteration of the surface area:core volume ratio in the substrate particles versus the products. When this ratio decreases, excess surface components are free to dissociate from the lipoprotein surface. Such a reaction has been observed to occur when human HDL is incubated with catalytic amounts of LTP. We have used this LTP-induced diminution of available surface to isolate water-soluble, lipid-free HDL apolipoproteins. We further suggest that this reaction represents an in vitro model of a genral phenomenon in lipoprotein metabolism whereby alterations in lipoprotein composition induced by enzymes such as hepatic lipase, lipoprotein lipase, or LCAT result in apolipoprotein dissociation/association to maintain a stable particle structure. To our knowledge, this is the first confirmed report of adaptation of this phenomenon to liberation of lipid-free apolipoproteins. We have found that apoA-I can be selectively isolated in a lipid-free, water-soluble form by incubation of dissociated apoproteins with a limiting quantity of HDL. Since apoA-I has a lower affinity for lipid surfaces than either apoA-II or the C apolipoproteins (Rosseneu et al., 1976; Lagocki & Scanu, 1980), these latter apoproteins effectively compete for available lipid surface on the added HDL and displace apoA-I. Subsequent flotation of the HDL results in recovery of apoA-I in the bottom fraction. While at the present time broad application of this procedure is limited by the availability of large amounts of LTP, an attractive feature of this reaction is that, unlike all previous isolation procedures, apoA-I denaturation is not required in this isolation scheme. Thus, we are confident that its conformation in solution accurately reflects that of the pool of lipid-free apoA-I postulated to exist in vivo (Eisenberg, 1984; Pownall et al., 1978).

An important property of apoA-I that could result from denaturation/renaturation is a tendency to self-associate into multimeric structures. When we examined the behavior of affinity-purified apoA-I in sedimentation equilibrium experiments, evidence of self-association was obtained suggesting that this property is not an artifact of denaturation/renaturation but is rather an intrinsic characteristic of lipid-free apoA-I. It is likely that this behavior may reflect a tendency of the hydrophobic regions of a given apoA-I to interact with the hydrophobic region of a second apoA-I, thereby shielding these residues from the aqueous environment. In contrast to this scenario is the proposed folding of water-soluble apolipoproteins which are not prone to self-association, such as insect apolipophorin III and the N-terminal region of human apoE (Kawooya et al., 1986; Aggerbeck et al., 1988), into helical bundles whereby hydrophobic residues face inward and hydrophilic residues face outward (Breiter et al., 1991; Wilson et al., 1991). This structural motif implies that the apolipoprotein unfolds to expose its hydrophobic residues upon binding to a lipid surface. Whether apoA-I may possess a similar structural motif will require additional structural information.

The near- and far-UV CD spectra for apoA-I prepared by the affinity method is qualitatively similar to that of apoA-I prepared by the denaturation/renaturation procedure reported in earlier studies cited above. Provencher–Glöckner analysis reveals some 50% α-helix in the affinity-purified material, comparable to the value observed for denatured/renatured preparations, as well as the presence of some 26% β-structure and 19% β-turn. Trifluoroethanol induces some 20% additional α-helix at the expense of β-structure in affinity-prepared apoA-I, an effect paralleling the observed induction of 20–30% α-helix in the denatured/renatured material by the addition of phospholipid and cholesteryl ester (Lux et al., 1972). However, it is to be recognized that these figures may be misleading in light of recent X-ray crystallographic studies on functionally related apolipoproteins, locust apolipophorin III, and a 22-kDa fragment of apoE. Apolipophorin III has an overall molecular architecture of five long α-helices connected by short loops with some 84% of the residues in the α-helical conformation (Breiter et al., 1991), and the receptor binding domain of human apo-E is a four-helix bundle, with 62% of its residues in the α-helical conformation (Wilson et al., 1991). Of relevance to the CD analysis is the fact that a major limitation of the Provencher-Glöckner program, or any other current method used in the analysis of the various conformations of a protein from its CD data, is the choice of the reference proteins in the data base that will represent adequately the protein to be analyzed. Of the 16 reference proteins used in the Provencher–Glöckner analysis, only 2 (myoglobin and lactate dehydrogenase) have substantial amounts of helix. Most certainly with apolipoproteins, the data base should be expanded to include those whose crystal structures have been determined, particularly in view of their high helical content.

The ability of affinity-purified apoA-I to activate LCAT was very similar to that of apoA-I that had been previously denatured/renatured, and the levels of activation for both preparations were proportional to the apoA-I bound to the substrate vesicles (Yokoyama et al., 1980; Fukushima et al., 1980). This result indicates that denaturation/renaturation of apoA-I does not compromise its ability to activate this enzyme and indicates that its interaction with the substrate lipids is not significantly different from that of the affinity-isolated apoA-I.

Lipid transfer protein in mammalian blood plasma catalyzes random and nondirectional exchange reaction of the lipoprotein core lipids, mainly cholesteryl ester and triacylglycerol, and does not seem to catalyze net lipid transfer between lipoprotein subfractions (Morton & Zilversmit, 1983; Nishikawa et al., 1988). However, Barter and colleagues recently reported that, in the presence of free fatty acid, human plasma lipid transfer protein catalyzes net lipid movement from HDL to low-density lipoprotein (Newnham & Barter, 1990; Barter et al., 1990a,b) or conversion of HDL to large particles leaving a very small lipoprotein complex (Barter et al., 1990b; Lagrost & Barter, 1991). Furthermore, these authors have suggested the possible generation of lipid-free apolipoproteins from HDL by this reaction (Barter, 1991). Thus, this reaction may be analogous to that catalyzed by insect LTP which results in apoA-I liberation from the surface of HDL. Such a pool of free apolipoprotein may also function physiologically by interacting with, and accepting lipid from, peripheral cells to generate HDL-like particles with concomitant reduction of intracellularly accumulated cholesteryl ester (Hara & Yokoyama, 1991). Since the K_m value of this reaction is very low (i.e., 1/400 of plasma apoA-I), it is entirely possible that lipid-free apolipoprotein can be generated in processes similar to those described in this paper or by Barter and colleagues and thereby play an key role in the first step of the reverse cholesterol transport pathway.

In summary, we have shown that alteration of the surface area:core volume ratio of lipoproteins can be used as an effective method to prepare lipid-free apolipoproteins that may be further purified on the basis of their relative affinities for lipid surfaces. Whereas the affinity of certain apolipoproteins for lipid surfaces has been used in isolation procedures for many years (apoA-IV binding to intralipid; Weinberg & Scanu, 1983), the present method offers the first example of affinity-based dissociation of apolipoprotein from lipoprotein

surfaces due to a decrease in available lipid surface. Thus, apoA-I prepared by this method is suitable for use in lipoprotein reconstitution, LCAT assays, and physical characterization. Furthermore, it is possible that apoA-I isolated by this method may be more amenable to crystallization, thereby permitting further structural characterization. The results also provide validation of the assumption that denaturation steps commonly employed in apolipoprotein isolation are completely reversible.

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