Self-Association of Human Apolipoproteins A-I and A-II and Interactions of Apolipoprotein A-I with Bile Salts: Quasi-Elastic Light Scattering Studies†

Joanne M. Donovan,‡,§ George B. Benedek,‖ and Martin C. Carey*,‡,§

Department of Medicine, Harvard Medical School, Brigham and Women's Hospital, and Harvard Digestive Diseases Center, Boston, Massachusetts 02115, and Department of Physics and Center for Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received December 4, 1986; Revised Manuscript Received June 5, 1987

ABSTRACT: We employed quasi-elastic light scattering (QLS) to systematically study the aqueous self-association of human apolipoproteins A-I and A-II (apo A-I and apo A-II) and the interactions of apo A-I with common taurine-conjugated bile salts. Self-association of apo A-I was promoted by increases in apolipoprotein concentration (0.09–2.2 mg/mL) and ionic strength (0.15–2.0 M NaCl), inhibited by increases in temperature (5–50 °C) and guanidine hydrochloride concentration (0–2.0 M), and unaffected by hydrostatic pressures up to 500 atm. The mean hydrodynamic radius ($\bar{R}_h$) of apo A-I micelles ranged from 38 Å to a maximum asymptotic value of 68 Å. We examined several possible models of apo A-I self-association; the model that best fitted the $\bar{R}_h$ values assumed that apo A-I monomers first interacted at low concentrations to form dimers, which then further associated to form ring-shaped limiting octamers. Comparison of the temperature-dependent and ionic strength dependent free energy changes for the formation of octamers from apo A-I dimers suggested that hydrophobic forces strongly favored self-association and that electrostatic repulsive forces were only weakly counteractive. Apo A-II self-association was also promoted by increases in apolipoprotein concentration (0.2–1.8 mg/mL) and inhibited by increases in guanidine hydrochloride concentration (0–1.0 M) but was unaffected by variations in temperature (10–37 °C): the largest $\bar{R}_h$ values observed were consistent with limiting tetramers. As demonstrated by equilibrium dialysis, bile salts in concentrations below their critical micellar concentrations (cmc) bound to apo A-I micelles but had no effect upon apo A-I self-association, as inferred from constant $\bar{R}_h$ values. When bile salt concentrations exceeded their aqueous cmc values, a dissociation of apo A-I micelles resulted with the formation of mixed bile salt/apo A-I micelles. These studies support the concepts that apo A-I and apo A-II form small dimeric micelles at low concentrations that grow sharply to reach limiting sizes over a narrow concentration range. The influences of bile salt concentration and species upon these micelles have relevance to the plasma transport of bile salts in high-density lipoproteins and to the physical–chemical state of apo A-I and apo A-II molecules in native biles.

Apolipoproteins A-I and A-II (apo A-I and apo A-II)[1] solubilize phospholipids and cholesterol in blood in an analogous fashion to that of bile salts in bile (Small, 1977). Operationally defined as high-density lipoproteins (HDL), these apo A-I and apo A-II containing particles are believed to function, in part, in the transport of unesterified cholesterol from peripheral tissues to the liver [reviewed in Tall and Small (1980)]. Although bile salts are found in highest concentrations in bile and in proximal small intestine (approximately 100–300 and 3–10 mM, respectively), they are also present in smaller quantities in portal and systemic blood (approximately 10–75 and 1–5 μM, respectively) [reviewed in Carey (1982)]. Concentrations of apo A-I and apo A-II in peripheral blood average 1.0 and 0.3 mg/mL, respectively (Scanu et al., 1982) and in human gallbladder bile are 50–100-fold lower (Sewell et al., 1983). Despite the fact that self-association of apo A-I and apo A-II has been extensively studied by a variety of physical–chemical techniques [reviewed in Atkinson and Small (1986) and Scanu et al. (1982)], the results have disagreed, in part due to the use of techniques that, in themselves, can alter self-association (e.g., dilution during gel filtration and increased hydrostatic pressure during ultracentrifugation). Bile salts also self-associate to form micelles [Mazer et al., 1979; reviewed in Carey (1983, 1985)] and have been demonstrated to bind to apo A-I (Makino et al., 1974). Because these soluble amphiphilic molecules coexist in biologic fluids, their self- and heterointeractions as well as the structure of their complexes are physiologically important.

To understand the solution chemistry of apo A-I and apo A-II, we have sytematically studied the self-association patterns of apo A-I and apo A-II in aqueous solutions and have investigated their interactions with membrane lipid components (lecithin and cholesterol) of HDL, as well as with bile salts (Donovan, 1984).[2] In this paper, we report our findings with

† Supported in part by Grants DK 36588 and AM 34854 (M.C.C.) from the National Institutes of Health and by Grant DMR81-19295 from the National Science Foundation (G.B.B.). J.M.D. was supported in part by a Whitaker Health Sciences Fellowship.

* Address correspondence to this author at the Department of Medicine, Brigham and Women's Hospital.

‡ Brigham and Women's Hospital.

§ Harvard Digestive Diseases Center.

‖ Massachusetts Institute of Technology.

[1] Abbreviations: apo A-I, apolipoprotein A-I; apo A-II, apolipoprotein A-II; HDL, high-density lipoprotein; QLS, quasi-elastic light scattering; cmc, critical micellar concentration; TC, 3α,7α,12α-trihydroxy-5β-cholanoyltaurine (taurocholate); TUDC, 3α,7β-dihydroxy-5β-cholanoyltaurine (tauroursodeoxycholate); TDC, 3α,12α-dihydroxy-5β-cholanoyltaurine (taurodeoxycholate); DC, 3α,12α-dihydroxy-5β-cholanoate (deoxycholate); $\bar{R}_h$, mean hydrodynamic radius; R_h, hydrodynamic radius; DMPC, dimyristoylphosphatidylcholine; $\bar{I}$, mean scattered light intensity; $\bar{n}_w$, weight-average aggregation number; Ig, immunoglobulin; Tris, tris(hydroxymethyl)aminomethane; SDS, sodium dodecyl sulfate.

0006-2960/87/0426-8116$01.50/0

respect to pure apo A-I and apo A-II and apo A-I/bile salt systems. We employed quasi-elastic light scattering (QLS), a technique employed in recent studies of typical detergents (Missel et al., 1980) and bile salts and other biliary lipids (Mazer et al., 1979; Mazer & Carey, 1983) and in a few limited studies of model and native lipoproteins (Eigner et al., 1979; Kunitake et al., 1978; Morrisett et al., 1974). QLS allows the accurate measurement of particle sizes in solution without inducing perturbations in the systems under study. Our aims were to obtain direct information on the equilibrium sizes, structures, and thermodynamics of self-associated complexes (micelles) of apo A-I and apo A-II as functions of physical–chemical variables of physiological importance such as amphiphile concentration, ionic strength, temperature, and the presence of detergents such as bile salts and denaturants such as guanidine hydrochloride. In addition, we wished to construct a molecular and thermodynamic framework for further studies of the heteroassociation of apo A-I and apo A-II with membrane lipids such as occurs in nascent and mature serum HDL and in native bile.

Experimental Procedures

Materials

Holo-HDL was isolated from fresh citrate–dextrose human plasma (pooled from at least five donors, American Red Cross, Boston, MA) by the phosphotungstate precipitation method (Burstein & Scholnick, 1973). Upon further purification by double ultracentrifugation, HDL produced a single immunoprecipitation line against combined anti-human apo A-I and anti-human apo A-II antisera and no reaction against anti-human albumin and IgG antisera (Calbiochem-Behring, La Jolla, CA). Following delipidation with diethyl ether and ethanol (Scanu & Edelstein, 1971), the apo-HDL preparation was dried at 10 mTorr and dissolved in buffer (6 M urea, 0.01 M Tris, 0.001 M NaN_3, pH 8.6), and apo A-I and apo A-II were individually separated by gel chromatography employing Sephacryl S-200 (Pharmacia Fine Chemicals, Piscataway, NJ). Both apo A-I and apo A-II preparations were pure on 0.1% SDS–polyacrylamide gel electrophoresis (100-μg protein applications) (Weber & Osborn, 1975) and gave single immunoprecipitation lines against rabbit anti-human apo A-I or apo A-II sera, respectively (antisera courtesy of Dr. Angelo Scanu, Departments of Biochemistry and Medicine, University of Chicago Medical School, Chicago, IL). Phospholipid contamination of both apolipoproteins was less than 0.4% by weight as assayed by the choline oxidase method (Gurantz et al., 1981). Solutions of apo A-I and apo A-II were then dialyzed against multiple changes of buffer (0.15 M NaCl, 0.01 M Tris, 0.001 M NaN_3, pH 7.6) at 4 °C. Protein concentrations were determined spectrophotometrically by using published extinction coefficients (Gwynne et al., 1974, 1975a), as well as by the method of Lowry et al. (1951).

Sodium salts of taurocholate (TC), tauroursodeoxycholate (TUDC), and taurodeoxycholate (TDC) (Calbiochem-Behring, La Jolla, CA) were purified (Norman, 1955; Pope, 1967), and each gave a single spot following thin-layer chromatography (200-μg applications) (Hofmann, 1962). Sodium [^{14}C]taurocholate (New England Nuclear, Boston, MA) had a specific activity of 50 mCi/mol and was greater than 98% radiochemically and chemically pure by thin-layer chromatography following plate scraping and scintillation counting (Beckman Instruments, Wakefield, MA). NaCl (Mallenckrodt, Paris, KY) was roasted for 3 h at 500 °C to oxidize and remove organic impurities. All other chemicals were of ACS quality or highest reagent grade purity. Pyrex glassware was alkali washed overnight in EtOH–2 M KOH (1:1 v/v), followed by 24-h acid washing in 1 M HNO_3, and rinsed thoroughly in filtered, deionized, and glass-distilled water.

Methods

(*a*) *Solutions.* Buffer solutions (0.01 M Tris, 0.001 M NaN_3, pH 7.6) with various apo A-I and apo A-II concentrations were prepared by mixing apolipoprotein solutions with different NaCl, guanidine hydrochloride, and/or bile salt concentrations, all in the same buffer. To sediment dust, solutions were centrifuged in acid-washed cylindrical scattering cells (6 × 50 mm, Kimble, Toledo, OH) at 10000*g* for between 10 min (for concentrated samples) and 15 h (for dilute samples).

(*b*) *Quasi-Elastic Light Scattering.* QLS measures fluctuations in light scattered from particles in solution, thus utilizing their Brownian motion to measure their diffusion coefficients. Values for the mean hydrodynamic radius ($\bar{R}_h$) were calculated from the relationship

$$\bar{R}_h = kT/6\pi\eta\bar{D} \quad (1)$$

where k is Boltzmann's constant, T the temperature (K), η the viscosity of the solvent, and $\bar{D}$ the mean diffusion coefficient. Details of the apparatus and data analysis methods have been described fully elsewhere (Missel et al., 1980; Mazer & Carey, 1983). Viscosities of guanidine hydrochloride solutions were obtained from the work of Kawahara and Tanford (1966). Each reported experimental result is the average of three to five $\bar{R}_h$ values derived from analysis of autocorrelation functions accumulated over 10 to 30 min. Temperature was thermostatically controlled to within 0.5 °C by a Peltier thermostatic device (Mazer et al., 1979). Samples were allowed to equilibrate at a given temperature for 15 min since longer equilibration times (up to 2 h) did not alter the measured $\bar{R}_h$ values. Polydispersity of particle size, which represents the width of a unimodal population distribution weighted by the particle weight of each species, was determined as described elsewhere (Mazer et al., 1979).

From measurements of the relative intensities of the scattered light angle (90° scattering angle) of apo A-I solutions, relative particle weights were obtained as follows. Mean scattered light intensity ($\bar{I}$) is defined as

$$\bar{I} = (I_a - I_0)/I_0 \quad (2)$$

where I_a and I_0 are the absolute intensities scattered from solutions of apo A-I and from the buffer alone, respectively. If a micelle is small compared to the wavelength of light, then the intensity of light scattered from each micelle is proportional to the square of the number of amphiphile molecules (n^2) in each micelle. Hence, the total intensity of scattered light ($\bar{I}$) from all micelles is proportional to $\sum n^2X_n$, where X_n is the mole fraction of micelles containing n amphiphile molecules. Since the weight-average aggregation number, $\bar{n}_w$, is defined as

$$\bar{n}_w = \sum n^2X_n/\sum nX_n \quad (3)$$

$\bar{n}_w$ is proportional to $\bar{I}/\sum nX_n$. The $\bar{R}_h$ value of a micellar solution is calculated from the hydrodynamic radius (R_h) of each species with n amphiphiles, $(R_h)_n$ (Missel et al., 1980), by

$$1/\bar{R}_h = \sum[n^2X_n/(R_h)_n]/\sum n^2X_n \quad (4)$$

Since $\bar{R}_h$ is weighted by the squares of the molecular weights,

[2] Two preliminary reports on this work have appeared (Donovan et al., 1983; Carey et al., 1985).

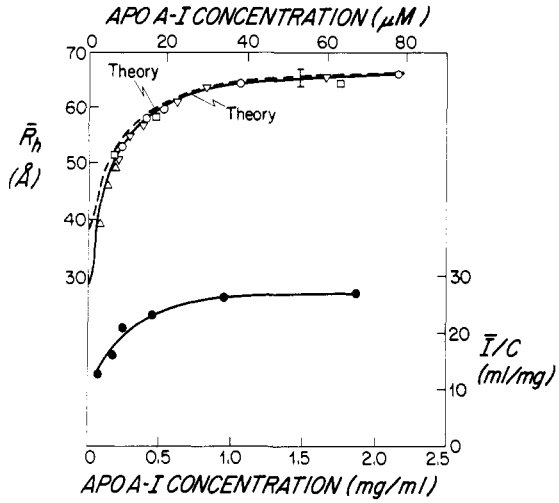

FIGURE 1: Concentration dependence of $\bar{R}_h$ and $\bar{I}/C$ values of apo A-I (0.15 M NaCl, 37 °C, 1 atm). Measurements of $\bar{R}_h$ values for four different apo A-I preparations are depicted by different symbols. Error bar denotes experimental error in $\bar{R}_h$ measurement (±2%). Solid and dashed lines are based on two theoretical models for $\bar{R}_h$ values that are described in the text and Appendix II.

$\bar{R}_h$ values predominantly reflect the larger particles present in solution.

(*c*) *Equilibrium Dialysis.* Equilibrium dialysis of apo A-I (1 mg/mL) and TC solutions (0.10–70 mM) was carried out at 22 °C in cells of 1-mL capacity that were separated by Spectrapor 2 dialysis membranes (Spectrum Medical Industries, Los Angeles, CA). In each case, a 1-mL solution of apo A-I (1 mg/mL) was dialyzed against a 1-mL solution of [^{14}C]TC in the same buffer (0.15 M NaCl, 0.01 M Tris, 0.001 M NaN_3, pH 7.6). To determine the time required for equilibration, 0.05-mL portions from both cells were withdrawn at several time intervals up to 72 h and the [^{14}C]TC concentrations were measured by scintillation counting. Since 99% of the [^{14}C]TC concentrations in each cell at 72 h was attained by 24 h, in all subsequent experiments, therefore, duplicate portions (0.1 mL) were withdrawn from each dialysis cell for counting at 24 and 48 h only. Measurements of $\bar{R}_h$ and $\bar{I}$ values of equilibrated apo A-I/TC and TC solutions were carried out at the end of these experiments as described under section b above.

(*d*) *High-Pressure Experiments.* The $\bar{R}_h$ values of a 1 mg/mL apo A-I solution were measured at 10 pressures between 1 and 500 atm at 37 °C in a high-pressure cell that is described fully elsewhere (Fisch & Benedek, 1986). Following each change of pressure, the apo A-I solution was equilibrated for between 2 and 15 h, during which several measurements of $\bar{R}_h$ were carried out.

RESULTS

Self-Association of Apo A-I. The dependence of $\bar{R}_h$ values (Å) on total apo A-I concentration (milligrams per milliliter and micromolar) at 37 °C is displayed in Figure 1. As apo A-I concentration was increased from 0.09 to 1.0 mg/mL, $\bar{R}_h$ values increased sharply and, above 1.0 mg/mL, approached an asymptotic value of approximately 68 Å. Because an apo A-I concentration of 0.09 mg/mL was the lowest concentration that gave a satisfactory autocorrelation function, the $\bar{R}_h$ value observed (38 Å) was significantly larger than that obtained for monomeric apo A-I by sedimentation equilibrium (28 Å) (Vitello & Scanu, 1976a; see also Appendix I). For four separate plasma preparations of apo A-I (different symbols, Figure 1), the data dispersion of $\bar{R}_h$ values was approximately the same as the experimental error (±2%). At an apo A-I concentration of 0.2 mg/mL ($\bar{R}_h$ value of 52 Å), the polydispersity of the micelles was approximately 50%, whereas at higher apo A-I concentrations (1.0–2.2 mg/mL, $\bar{R}_h$ values of 63–67 Å, respectively) polydispersities decreased to approximately 25%. The dashed and solid lines in Figure 1 were calculated from two theories of apo A-I self-association, to be described below. Also shown in Figure 1 is the magnitude of $\bar{I}/C$ (proportional to $\bar{n}_w$), which increased sharply with apo A-I concentration to reach a plateau value above an apo A-I concentration of 1 mg/mL.

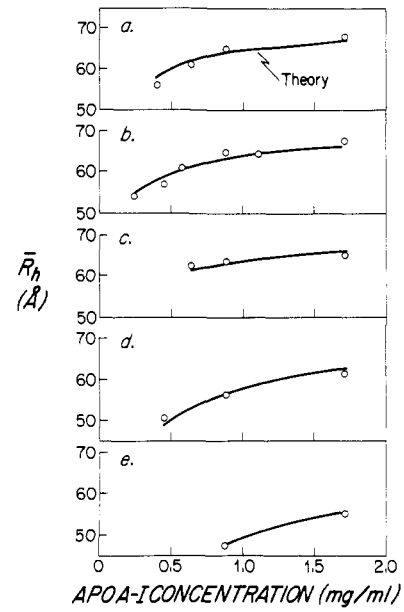

FIGURE 2: Influence of increases in temperature on the apo A-I concentration dependence of $\bar{R}_h$ values (0.15 M NaCl, 1 atm): (a) 10 °C; (b) 20 °C; (c) 30 °C; (d) 45 °C; (e) 50 °C. Solid curves are derived from theory as described in the text and Appendix II.

Figure 2 displays the experimental and theoretical (described below) temperature dependencies of $\bar{R}_h$ values as functions of apo A-I concentration up to 1.7 mg/mL. At all temperatures between 10 and 50 °C, $\bar{R}_h$ values increased monotonically with increases in apo A-I concentration. However, the maximum size observed was 68 Å at 10 °C but only 55 Å at 50 °C. For a single apo A-I concentration, $\bar{R}_h$ values were approximately constant at low temperatures, but these values decreased sharply as the temperature approached 50 °C. Within 15 min of each temperature change, $\bar{R}_h$ attained a new equilibrium value that returned to the initial $\bar{R}_h$ value upon cooling. At higher temperatures (55, 60, and 65 °C) (not displayed), $\bar{R}_h$ values of apo A-I solutions increased continuously over several hours, attaining $\bar{R}_h$ values of 100–200 Å. Following cooling to 37 °C and incubation for an additional 24 h, these $\bar{R}_h$ values continued to increase, consistent with irreversible denaturation of apo A-I.

Figure 3 displays the experimental $\bar{R}_h$ values as functions of increasing apo A-I concentrations in the presence of four added NaCl concentrations (0.15–2.0 M). While $\bar{R}_h$ values approached an asymptotic maximum of approximately 66 Å at the highest apo A-I concentrations, the rate of increase was less marked as the ionic strength increased. The increment in $\bar{R}_h$ values with added NaCl was most pronounced (~12–15%) at low apo A-I concentrations where smaller apo A-I micelles were present.

The effects of increasing guanidine hydrochloride concentration on the sizes of apo A-I micelles are displayed in Figure 4. When guanidine hydrochloride concentrations were increased from 0 to 2.0 M, $\bar{R}_h$ values of two apo A-I solutions (0.5 and 1.0 mg/mL) with initial $\bar{R}_h$ values of 58 and 63 Å

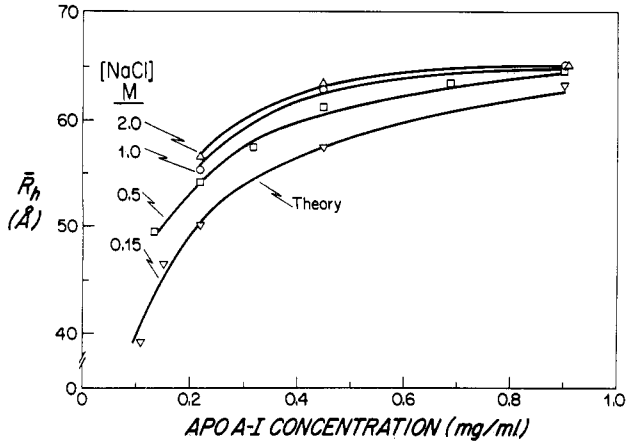

FIGURE 3: Dependence of R_h values on apo A-I concentration, in four added NaCl concentrations (37 °C, 1 atm). Solid curves are derived from theory (as described in the text and Appendix II).

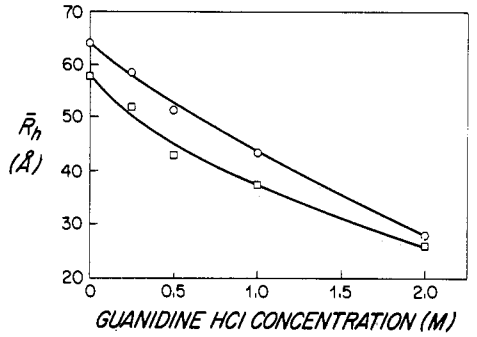

FIGURE 4: Dependence of R_h values for two apo A-I concentrations [(○) 1.0 mg/mL; (□) 0.5 mg/mL] on guanidine hydrochloride concentration (0.15 M NaCl, 37 °C, 1 atm).

decreased in a near linear fashion to approach a similar value (27 and 28 Å, respectively).

Over a range of hydrostatic pressures that varied from 1 to 500 atm, $\bar{R}_h$ values of a 1 mg/mL apo A-I solution (0.15 M NaCl, 37 °C) remained constant at 63 ± 2 Å (results not displayed). Furthermore, the size of the apo A-I micelles remained unchanged when the equilibration time at 500 atm was extended to 15 h. Following exposure to this hydrostatic pressure, $\bar{R}_h$ values of apo A-I solutions diluted from 1 mg/mL to 0.25 and 0.5 mg/mL were identical with the same concentrations of apo A-I equilibrated at 1 atm. These observations indicate that, within the pressure ranges studied, neither denaturation of apo A-I nor dissociation of apo A-I micelles occurred.

Bile Salt/Apo A-I Interactions. As displayed by the equilibrium dialysis results in Figure 5 (open circles and solid line), below the critical micellar concentration (cmc) of TC (lower limit approximately 3 mM, Figure 5),[3] less than 1 mol of TC was bound per mole of apo A-I. As TC concentrations were increased above the cmc, progressively more TC molecules became bound, and at the highest TC concentration (70 mM), 1 mol of apo A-I bound 27 mol of TC. As shown by the broken curve in Figure 5, published values (Makino et al., 1974) for equilibrium binding of the unconjugated bile salt deoxycholate (DC) by apo A-I display a parallel trend, with a steep increase in binding occurring at lower bile salt concentrations. In Figure 6, $\bar{R}_h$ values of apo A-I/TC solutions (1 mg/mL apo A-I), at 20 and 40 °C, are compared with the

[3] The apparent cmc of TC under the conditions of these experiments spans a concentration range from 3 to 10 mM depending, in part, upon the method of measurement (Carey, 1983).

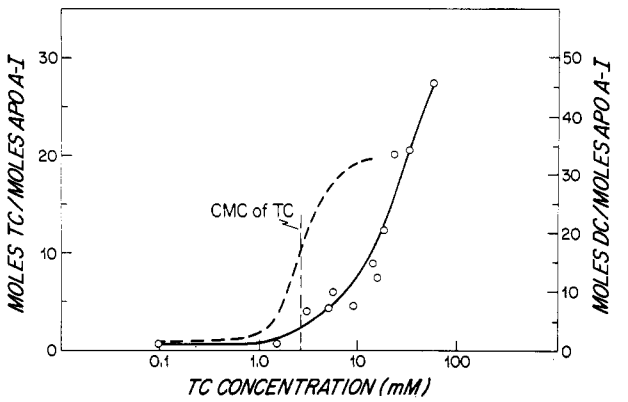

FIGURE 5: Influence of TC concentration on binding of TC to apo A-I by equilibrium dialysis (1 mg/mL apo A-I, 22 °C, 1 atm, 0.15 M NaCl). Dashed curve is taken from data of Makino et al. (1974) for equilibrium sodium deoxycholate (DC) binding to apo A-I (right ordinate). Vertical interrupted line denotes the lower limit for the cmc of TC (Carey, 1983).

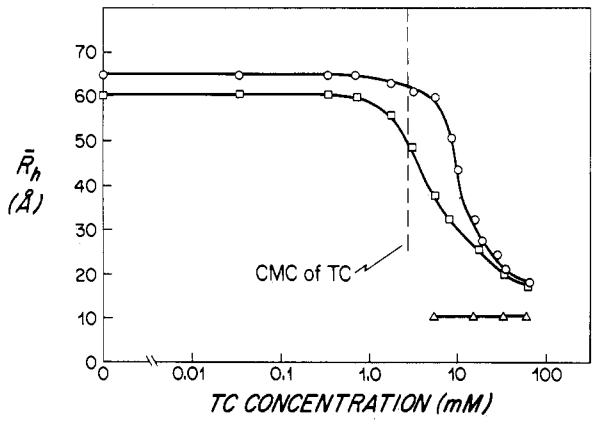

FIGURE 6: Dependence of $\bar{R}_h$ values of equilibrated TC and TC/apo A-I solutions on TC concentration in an equilibrium dialysis experiment (0.15 M NaCl, 1 atm): (○) 1 mg/mL apo A-I plus TC at 20 °C; (□) 1 mg/mL apo A-I plus TC at 40 °C; (△) TC concentrations in dialysate at 20 and 40 °C.

temperature-independent $\bar{R}_h$ value (11 Å) of pure TC solutions that had equilibrated with the apo A-I/TC system (Figure 6, open triangles). Below 1 mM TC (20 °C), $\bar{R}_h$ values of the apo A-I/TC solution remained constant at 63 Å, the value observed for apo A-I in the absence of TC (Figure 1). As the TC concentration exceeded its cmc value (lower limit of 3 mM, Figure 6), $\bar{R}_h$ values fell steeply to 18 Å. Intermediate $\bar{R}_h$ values between 63 (a simple[4] apo A-I micelle) and 11 Å (a simple TC micelle) represented a weighted average of simple TC micelles and mixed apo A-I/TC micelles, as was demonstrated by equilibrium dialysis. Because $\bar{I}$ values (see Methods) of equilibrated TC and apo A-I/TC solutions were approximately equal at 70 mM TC, we calculated that approximately 2 apo A-I molecules and 54 TC molecules were present in each apo A-I/TC mixed micelle at the highest TC concentration (Donovan, 1984).[5]

[4] Simple and mixed micelles refer to micelles containing one or more than one amphiphile species, respectively.

[5] The number of apo A-I molecules in each apo A-I/TC mixed micelles, $n_{apoA\text{-}I}$, was calculated from

$$\frac{\bar{I}_{TC}}{\bar{I}_{apoA\text{-}I}} = \frac{C_{TC}M_{TC}n_{TC}}{C_{TC}M_{TC}n_{TC} + C_{apoA\text{-}I}M_{apoA\text{-}I}n_{apoA\text{-}I}(1 + R_w)}$$

where $\bar{I}_{TC}$ and $\bar{I}_{apoA\text{-}I}$ are $\bar{I}$ values for the TC and apo A-I/TC solutions, respectively, C_{TC} and $C_{apoA\text{-}I}$ are the respective weight concentrations, n_{TC} is the aggregation number (5) of simple TC micelles (Small, 1971; Mazer et al., 1979), and R_w is the weight ratio of TC bound to apo A-I, as determined by equilibrium dialysis.

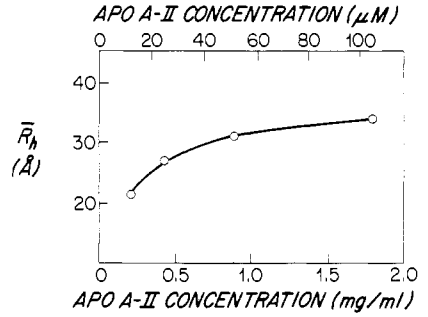

FIGURE 7: Dependence of $\bar{R}_h$ values on apo A-II concentration (0.15 M NaCl, 37 °C, 1 atm).

Other bile salts, taurodeoxycholate (TDC) and tauroursodeoxycholate (TUDC), that are more hydrophobic and hydrophilic than TC, respectively (Armstrong & Carey, 1982), showed a similar interaction with 1 mg/mL apo A-I solutions over the same bile salt concentration range (data not displayed). However, the bile salt concentrations at which $\bar{R}_h$ values began to decrease (as in Figure 6) differed for each bile salt and corresponded to the rank order of their aqueous cmc values: TDC < TUDC < TC (Carey et al., 1981).

Self-Association of Apo A-II. As depicted in Figure 7, $\bar{R}_h$ values increased from 21 to 34 Å as apo A-II concentration was increased from 0.2 to 1.8 mg/mL, leveling off toward the highest apo A-II concentrations. Within the range of the apo A-II concentrations investigated, $\bar{R}_h$ values were independent of temperature (10–37 °C). However, following the addition of 1.0 M guanidine hydrochloride, $\bar{R}_h$ values (0.15 M NaCl, 37 °C) of two apo A-II solutions (0.45–0.9 mg/mL) decreased from 27 and 32 Å, respectively, to a value of 21 Å (data not displayed).

Discussion

In this work, we have employed QLS to systematically investigate the concentration-dependent self-association of the two major apolipoproteins of human HDL as well as the heteroassociation of apo A-I with several common bile salts. While the QLS technique is a relatively noninvasive physical–chemical approach for obtaining direct information on particle sizes in the study of self- and heteroaggregating lipid and protein systems, the limitations and uncertainties of the methodology in relation to the present experiments require appreciation.

First, polymorphism of apo A-I and apo A-II may affect self-association, particularly in relation to charge. However, to minimize this potential effect, all apolipoprotein samples were prepared from pooled human sera, each from at least five donors. The minimal variation in $\bar{R}_h$ values observed between apo A-I preparations (±2%, Figure 1) suggests that polymorphism either was not of appreciable degree to affect apo A-I self-association or was constant between preparations. Further, it has been demonstrated (Brewer et al., 1983) that only small amounts of pro-apo A-I are present in human plasma, and no variations in charge or qualitative patterns have been found in studies of a large number of apo A-I isoproteins from different individuals (Zannis et al., 1980). Moreover, we show below in a thermodynamic analysis that electrostatic repulsive interactions only weakly counteract apo A-I self-association within the range of NaCl concentrations studied, and thereby a variation in isoprotein charge, if present, should not appreciably influence the overall results. Obviously, comparisons of the self-association patterns of rat apo A-I (25% pro-apo A-I; Ghiselli et al., 1983) and human apo A-I (<10% pro-apo A-I; Brewer et al., 1983) would be of considerable interest in this regard.

Other limitations apply to the QLS technique itself: A rigorous thermodynamic interpretation of our $\bar{R}_h$ measurements depends critically upon a knowledge of all the species present in solution and their individual hydrodynamic radii. Obviously, with the present state of the QLS "art", it was not possible to establish these parameters unequivocally. Owing to the very low signal-to-noise ratio at high dilution, monomeric apolipoprotein systems proved difficult, if not impossible, to study; moreover, the QLS technique itself is not capable of deconvoluting $\bar{R}_h$ values for different-sized particles where their respective hydrodynamic radii (R_h) are separated by less than an order of magnitude (Cohen, 1986). Therefore, in the present work, $\bar{R}_h$ represents a mean value that, in a QLS experiment, is biased by the scattering from larger particles. Despite these restrictions, we will show below that our experimental $\bar{R}_h$ values for apo A-I solutions are well fitted by monomer–dimer–octamer theory; clearly, further verification will have to await more refined approaches. Finally, with respect to micellar shape, the small sizes of the apolipoprotein micelles precluded our ability to obtain angular dissymmetry measurement at various scattering angles; hence, it was not possible to experimentally measure particle shape and axial ratios. Despite these reservations, we offer below a number of new insights into these important apolipoprotein systems.

Concentration Dependence of Apolipoprotein Self-Association. With varying experimental conditions of temperature and ionic strength, both $\bar{R}_h$ and $\bar{I}$ values of apo A-I solutions demonstrated asymptotic maxima with increases in apo A-I concentration. These results, together with a pronounced decrease in polydispersity (50 to 25%), suggested formation of a single population of maximum-sized micelles to which further association of apo A-I molecules was thermodynamically disadvantageous. To interpret the dependence of $\bar{R}_h$ values upon apo A-I concentration, we have examined several models of limited and indefinite apo A-I self-association, based on estimates of the hydrodynamic radii (R_h) for various n-mers (Appendix I). In models of isodesmic (constant incremental free energy change) self-association of monomers and dimers in various configurations (end-to-end or side-to-side), we predicted that $\bar{R}_h$ values would increase exponentially with concentration. In contrast, we showed that the association of monomers or dimers to form limiting hexamers, octamers, or even decamers reached asymptotic maxima. Appendix II presents the model that best fitted the present data (Figure 1, solid line). In this model, we assumed that monomers first associated to form dimers, which then further associated cooperatively to form limiting ring-shaped octamers with $\bar{R}_h$ values of approximately 60–70 Å (see Appendix I). We calculated the free energy changes for monomer–monomer and dimer–dimer self-association (ΔG_M and ΔG_D, respectively) at 37 °C and 0.15 M NaCl and obtained values of −39 and −7.43 kcal/mol, respectively (Appendix II), indicating that the association of monomers is much more strongly favored than that of dimers. It is of interest in this regard that the α-helicity of apo A-I, as inferred by circular dichroic spectroscopy, increases with increasing apolipoprotein concentration only up to a limit of 0.1 mg/mL (Osborne & Brewer, 1977). Since this is the concentration expected for apo A-I dimers (Figure 1, Appendix I), the present results clearly suggest that monomers dimerize with a marked increase in secondary structure but that further self-association occurs without appreciable intrinsic structural changes.

With regard to the largest apo A-I aggregates observed in the present work, published studies employing sedimentation

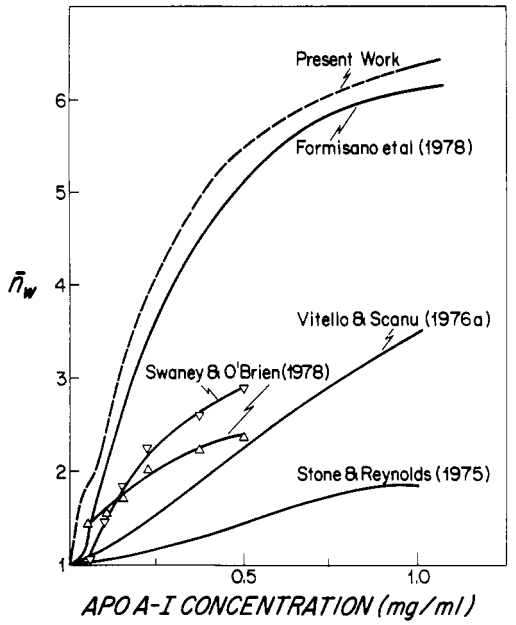

FIGURE 8: Literature values and the present work (all at 20 °C) for the dependence of $\bar{n}_w$ (weight-average aggregation number) on apo A-I concentrations: (▽) Swaney and O'Brien (1978) μ (added ionic strength) = 0.245; (△) Swaney and O'Brien (1978) μ = 0.045 ($\bar{n}_w$ values plotted); Vitello and Scanu (1976a) μ = 0.02; Stone and Reynolds (1975) μ = 0.045; Formisano et al. (1978) μ = 0.11 (curves of $\bar{n}_w$ values derived from sedimentation equilibrium constants); present work, μ = 0.16 (curve of $\bar{n}_w$ values derived from $\bar{R}_h$ values).

equilibrium for apo A-I concentrations of 0.1–1 mg/mL suggest that the limiting apo A-I aggregates are tetramers (Stone & Reynolds, 1975). By use of the same technique, higher apo A-I concentrations (greater than 1 mg/mL) have been shown to form limiting octamers (Vitello & Scanu, 1976a; Formisano et al., 1978). Other studies that employed viscometry (Barbeau et al., 1979) have suggested that apo A-I first dimerizes and then indefinitely self-associates, and diethylsuberimide cross-linking studies (Swaney & O'Brien, 1978) have suggested that apo A-I micelles grow to a maximum size of pentamers. In Figure 8 we compare the apo A-I concentration dependence of $\bar{n}_w$ calculated from the present data (see Appendix II) and $\bar{n}_w$ calculated from sedimentation equilibrium constants (Stone & Reynolds, 1975; Vitello & Scanu, 1976; Swaney & O'Brien, 1978; Formisano et al., 1978) for apolipoprotein concentrations up to 1 mg/mL. In the studies of Formisano et al. (1978), sedimentation equilibrium constants of apo A-I micelles were found to vary with rotor speed, and these authors concluded that hydrostatic pressures generated in the ultracentrifuge (up to 100 atm) favored apo A-I dissociation. Although $\bar{n}_w$ values derived from the present study showed good agreement with Formisano et al.'s (1978) extrapolations to zero rotor speed, our results showing the independence of apo A-I self-association on hydrostatic pressure (to 500 atm) do not directly support these authors' conclusions. It is likely that other perturbations inherent in the sedimentation equilibrium technique, most notably the differing sedimentation rates of counterions and self-associating apo A-I molecules, may alter the sedimentation profile in the direction of lower molecular weights. In agreement with the results of our high-pressure experiments, Mantulin and Pownall (1985), employing intrinsic fluorescence polarization of apo A-I, found minimal decreases in secondary structure of the apolipoprotein up to 500 atm; however, at higher hydrostatic pressures, changes in fluorescence polarization consistent with apo A-I denaturation were observed. Despite these reservations, it is clear that the present studies and those of Formisano et al. (1978) and Vitello and Scanu (1976a) support a monomer–dimer–octamer aggregation scheme for apo A-I self-association.

In contrast to the behavior of apo A-I, $\bar{R}_h$ values of apo A-II solutions did not reach an asymptotic value at the highest concentrations studied in the present work. The smallest $\bar{R}_h$ value observed (21 Å) did not change with added 1.0 M guanidine hydrochloride, suggesting that this value represented the size of apo A-II monomers, irrespective of whether they were in native or denatured conformations. Using the molecular weight and specific volume of apo A-II (Gwynne et al., 1975a), we have calculated that an apo A-II monomer is a prolate ellipsoid, with semimajor and semiminor axes of 36 and 11 Å, respectively (see Appendix I). We have shown that the largest $\bar{R}_h$ value of apo A-II observed (34 Å) approximates the size of an apo A-II trimer or tetramer (see Appendix I); however, published $\bar{n}_w$ values of apo A-II calculated from equilibrium constants at concentrations and ionic strengths similar to ours were consistent with dimers only (Gwynne et al., 1975a; Stone & Reynolds, 1975; Teng et al., 1978; Vitello & Scanu, 1976b). Nevertheless, self-association schemes for apo A-II derived from sedimentation equilibrium studies that extended to higher concentrations included monomer–dimer (Stone & Reynolds, 1975), monomer–dimer–trimer or indefinite (Vitello & Scanu, 1976b), and monomer–dimer–trimer or –tetramer schemes (Teng et al., 1978). The limiting sizes in the latter study are consistent with our results carried out at appreciably lower concentrations. As with apo A-I dimerization, circular dichroic spectroscopy (Gwynne et al., 1975a) was consistent with a marked increase in α-helicity of apo A-II upon self-association of monomers to form dimers. Thus, it appears that apo A-II self-association is quite similar to that of apo A-I, with initial dimerization accompanied by increased α-helicity, followed by further self-association without significant changes in the secondary structure of each dimeric unit.

Insights into the Molecular Structure of Apolipoprotein Micelles. Apo A-I monomers are currently believed to be composed of eight amphiphilic helices ~22 Å in length that are probably self-associated within the monomer (Atkinson & Small, 1986). The extensive α-helices of apo A-I constitute 55% of the molecule (Lux et al., 1972) and are relatively unstable with respect to denaturation with urea (Tall et al., 1976). Apo A-II monomers are composed of two identical polypeptide chains that are covalently linked within the molecule. These chains contain 35% α-helices (Lux et al., 1972) that are also easily denatured, for example, by ~1.5 M guanidine hydrochloride (Reynolds, 1976). In both apolipoproteins, the major structural changes that occur upon dimerization are reflected in increased α-helicity. These changes are presumably related to removal of nonpolar side chains of the amphiphilic helices from aqueous contact into a more hydrophobic environment.

To estimate the free energy changes for association of dimers (ΔG_D) to form octamers, we have conceptually simplified the monomer–dimer–octamer model by eliminating monomers since, at $\bar{R}_h$ values above 50 Å, this model (Figure 1, dashed curve) was essentially identical with the monomer–dimer–octamer model (Figure 1, solid curve). Whereas $\bar{R}_h$ values calculated from ΔG_D (Table I; Figures 2 and 3, solid curves) showed good agreement between theory and experiment, this is not the case with $\bar{R}_h$ values smaller than 50 Å (<0.2 mg/mL). Under these conditions, $\bar{R}_h$ values predicted by using the simplified model were generally larger than experimental sizes since monomers contributed significantly to the scattered

Table I: Values of ΔG_D for Apo A-I Self-Association at Various Temperatures and NaCl Concentrations

T (°C)	NaCl concn (M)	ΔG_D[a] (kcal/mol)
10	0.15	−6.78
20	0.15	−7.00
30	0.15	−7.21
37	0.15	−7.31[b]
45	0.15	−7.09
50	0.15	−6.89
37	0.50	−7.47
37	1.00	−7.56
37	2.00	−7.58

[a] ΔG_D is equal to one-fourth the free energy change involved in the association of four dimers to form an octamer. [b] This ΔG_D value is slightly different from that calculated from the monomer–dimer–octamer model as functions of the same conditions (see text).

Table II: Experimental and Theoretical Values of ΔG_{el}[a] for Apo A-I Self-Association

NaCl concn (M)	ΔG_D[b] (kcal/mol)	ΔG_{el} (theor) (kcal/mol)	$\Delta G_D - \Delta G_{D(2.0\ M\ NaCl)}$[c] (kcal/mol)
0.15	−7.31	+0.34	+0.27
0.50	−7.47	+0.08	+0.11
1.00	−7.56	+0.03	+0.02
2.00	−7.58	+0.01	+0.00

[a] Electrostatic component of ΔG_D value. [b] ΔG_D is one-fourth the free energy change involved in the association of four dimers of apo A-I to form an octamer. [c] Difference between electrostatic component of ΔG_D at the stated NaCl concentration and at 2.0 M NaCl.

light at lower apo A-I concentrations.

By utilizing the Verwey–Overbeek theory of electrostatic interactions between two spherical double layers[6] (Mazer et al., 1979), we have estimated the relative magnitudes of the hydrophobic and electrostatic components of ΔG_D from the ionic strength effects (Figure 3). The electrostatic component of ΔG_D, denoted ΔG_{el}, was defined as the difference between ΔG_D at a given NaCl concentration and ΔG_D with complete shielding of charges. As shown in Table II, the calculated repulsive force of ΔG_{el} was +0.34 kcal/mol in 0.15 M NaCl, compared with a net attractive ΔG_D of −7.31 cal/mol. However, in the presence of 2.0 M NaCl, when ionic charges were essentially completely screened, ΔG_{el} became negligible (0.01 kcal/mol). Hence, $\Delta G_D - \Delta G_{D(2.0MNaCl)}$ (Table II) is similar to theoretical predictions of ΔG_{el} (Donovan, 1984). The small contribution of repulsive electrostatic forces to apo A-I self-association is demonstrated experimentally in Figure 3, where increases in ionic strength from 0.15 to 2.0 M NaCl only altered apo A-I micellar sizes by, at most, 12–15%, in agreement with Formisano et al. (1978) and Swaney and O'Brien (1978). Clearly, the much higher free energy change for association of monomers to dimers and the concomitant increase in α-helicity suggest that the hydrophobic surface area removed from aqueous contact is considerably larger upon the initial association of monomers than with further association of dimers. The predominance of hydrophobic forces in apo A-I self-association was clearly predicted earlier on the basis of the amphiphilic helical model of this apolipoprotein (Segrest et al., 1974). It is indeed likely that dimerization occurs between apolipoprotein surfaces that would otherwise be involved in lipid–protein interactions in native HDL, since similar increases in α-helicity have also been observed for the interactions of apo A-I with membrane lipids (Pownall et al., 1977).

Because apo A-I self-association was inhibited by increasing temperature (Swaney & O'Brien, 1978), the temperature dependence of ΔG_D (Table I) was used to calculate the thermodynamic quantities ΔH and ΔS with the Gibbs equation:

$$\Delta G_D/T = \Delta H/T - \Delta S \quad (5)$$

Between 10 and 30 °C, the dependence of ΔG_D/T on $1/T$ was linear (data not shown), giving values of −0.7 kcal/mol for ΔH and 21 cal/(mol·K) for ΔS. At low temperatures, therefore, the major driving force for apo A-I self-association is entropic, consistent with hydrophobic interactions. Above 37 °C, the dependence of $\Delta G_D/T$ on $1/T$ progressively decreased with increasing temperature, suggesting a more complex self-association process. In this regard, it is of interest that circular dichroic spectra of apo A-I as functions of temperature showed a progressive decrease in α-helicity between 20 and 80 °C (Gwynne et al., 1975b), and differential scanning calorimetry and ultraviolet difference spectroscopy have shown that apo A-I is progressively, but reversibly, denatured between 43 and 71 °C (Tall et al., 1976). However, in the present work we observed large increases in $\bar{R}_h$ values between 50 and 60 °C, suggesting that partial irreversible denaturation led to major changes in self-association properties. As pointed out earlier, QLS is extremely sensitive to small amounts of large molecular weight aggregates and therefore is capable of detecting a minute concentration of large particles formed from denatured proteins.

In contrast to the behavior of apo A-I, apo A-II self-association was independent of temperature between 10 and 37 °C, as has also been demonstrated by others (Teng et al., 1978). Whereas the α-helicity of apo A-I decreases continuously with temperature from 20 to 80 °C (Gwynne et al., 1975b), ultraviolet difference spectra of apo A-II solutions were consistent with a maximum of secondary structure at 30 °C (Gwynne et al., 1975a). Whereas changes in apo A-II secondary structure above and below 30 °C should favor dissociation, it is well-known that lower temperatures strengthen hydrophobic interactions and weaken electrostatic repulsive interactions. Apparently, both attractive and repulsive forces and alterations in secondary structure are balanced in the temperature range studied here (10–37 °C), and apo A-II self-association remains unaltered.

In comparing the influences of two potential denaturants, guanidine hydrochloride and bile salts, we note that high concentrations of both agents dissociated apo A-I micelles. Guanidine hydrochloride in high molar concentrations is a powerful denaturant of apo A-I (Swaney & O'Brien, 1978; Massey et al., 1981; Edelstein & Scanu, 1980); however, with progressive increases in guanidine hydrochloride concentrations from 0 to 3 M, the circular dichroic spectra of the molecules are consistent with two transitions in secondary structure (Osborne & Brewer, 1977; Edelstein & Scanu, 1980). These transitions are consistent with initial dissociation of apo A-I micelles (guanidine hydrochloride concentrations up to ~1.5 M) and then denaturation of apo A-I monomers to a random coil (guanidine hydrochloride concentrations of 2–3 M). In contrast, Makino et al. (1974) showed by circular dichroism that the secondary structure of apo A-I was largely preserved (<10% decrease in α-helicity) with binding of micellar concentrations of the bile salt deoxycholate (DC). We have shown (Figure 5) that TC, which is a more hydrophilic bile salt (Armstrong & Carey, 1982) with a higher cmc (Small, 1971), is less strongly associated with apo A-I then is DC (Makino et al., 1974). Our results demonstrating that high concen-

[6] This is a crude approximation, since apo A-I dimers are not spheres and do not have a symmetrical change distribution. We assumed that the net charge of an apo A-I dimer at pH 7.6 was −8 as inferred from potentiometric titrations (Heuck et al., 1983) and that its radius was 15 Å on the basis of its semiminor axis (see Appendix I).

trations of TC dissociated apo A-I micelles to dimers and not monomers strongly suggest that the secondary structure of apo A-I remained largely intact in micellar bile salt solutions. This is consistent with the observation that bile salt derivatives are capable of extracting and solubilizing hydrophobic proteins from biomembranes without denaturation (Hjelmeland, 1980). It appears, therefore, that the detergent-like bile salts interact with apolipoproteins predominantly as heteroassociating lipids, i.e., forming mixed micelles, rather than as denaturing agents, as occurs with guanidine hydrochloride.

Pathophysiological Correlations. We have shown in vitro that, in the absence of other lipids, apo A-I and apo A-II are extensively self-associated at concentrations found in serum (1 and 0.3 mg/mL, respectively; Scanu et al., 1982). Since essentially all serum apo A-I and apo A-II are associated with otherwise insoluble lipids, apolipoprotein–lipid heteroassociations are more favorable than self-association, as previously suggested (Tall et al., 1977). We have also shown that, at typical serum concentrations of apo A-I and bile salts, only a few molecules of bile salts bind to a molecule of apo A-I. This is consistent with the demonstration by photoaffinity labeling (Kramer et al., 1979) that bile salts bind principally to the phospholipids of native HDL.

In bile, where bile salt concentrations are 100–200 mM (Carey, 1982) and apo A-I concentrations are 0.01–0.02 mg/mL (Sewell et al., 1983), we predict that apo A-I should neither be self-associated nor appreciably affect the size of bile salt micelles. These two physiological detergent-like molecules have similarities. Above a critical concentration, both associate into primary micelles: bile salts into micelles with aggregation numbers of 3–10 (Small, 1971; Mazer et al., 1979) and apo A-I and apo A-II into dimers. These primary micelles further associate "indefinitely" for bile salts (Mazer et al., 1979), up to a limit of octamers for apo A-I and approximately tetramers for apo A-II. Both associations are driven primarily by hydrostatic forces, with only minimal repulsive electrostatic forces.

In summary, using the nonperturbing technique of QLS, we conclude the following. (1) Apo A-I and apo A-II self-associate with increasing concentrations in two steps: at very low concentrations, dimers are formed that, with further increases in concentration, self-associate to form octamers and tetramers, respectively. (2) The predominant driving force for monomer and dimer self-association is hydrophobic with only weak electrostatic repulsive forces. (3) In contrast to the strong denaturing and disaggregating properties of guanidine hydrochloride, bile salts, below their cmc values, have no influence upon A-I self-association but, above their cmc values, dissociate apo A-I octamers without denaturation to form bile salt/apo A-I mixed micelles.

ACKNOWLEDGMENTS

We thank Grace Ko for technical assistance and Rebecca Ankener for preparation of the manuscript. We are indebted to Dr. Michael Fisch for his assistance in performing high-pressure QLS experiments and to Dr. Angelo Scanu (Chicago) for providing rabbit antisera to human apo A-I and apo A-II.

APPENDIX

(I) With the reasonable assumption that the water of hydration is typically 0.3–0.4 g (H_2O) per gram of protein[7] (Cantor & Schimmel, 1980), the R_h values of spheres with a given molecular weight, defined as $(R_h)_s$, were estimated from the apolipoprotein molecular volumes (V): $(R_h)_s = (3V/4\pi)^{1/3}$. With values of 0.74 and 0.75 mL/g for the specific volumes of apo A-I and apo A-II, respectively (Gwynne et al., 1974, 1975a), the calculated $(R_h)_s$ values were 22 and 19 Å, respectively. For an ellipsoidal particle, because of orientational averaging, R_h is larger than $(R_h)_s$, and therefore the $R_h/(R_h)_s$ ratio defines f, the Perrin shape factor. The R_h value of an apo A-I monomer (28 Å) was calculated from the Svedburg equation and limiting values of the sedimentation coefficients (Vitello & Scanu, 1976a), as well as from gel filtration measurements (Edelstein & Scanu, 1980). Thus, the Perrin shape factor (f) is approximately 1.3, and an apo A-I monomer in solution is a prolate ellipsoid with an axial ratio of 6, possessing a semimajor axis of 75 Å and a semiminor axis of 12.5 Å (Barbeau et al., 1979). In similar fashion, an apo A-II monomer ($\bar{R}_h$ value 21 Å, Figure 7) has an axial ratio of 3 and, therefore, its semimajor axis is 36 Å and its semiminor axis is 11 Å. Modeling an apo A-I dimer as a prolate ellipsoid with an $\bar{R}_h$ value of 38 Å (derived by gel filtration; Morrisett et al., 1977) yields a semimajor axis of 95 Å and a semiminor axis of 15 Å.

Similarly, employing the molecular weight of apolipoprotein n-mers, together with their specific volumes (Gwynne et al., 1974, 1975a), we have calculated molecular volumes for oligomers larger than dimers and, thereby, $(R_h)_s$ values. To obtain $\bar{R}_h$ values for each oligomer, $(R_h)_s$ values were multiplied by an estimated value of f for a given ellipsoidal axial ratio (Cantor & Schimmel, 1980). In contrast, we modeled the ring-shaped apo A-I octamer as a toroid (Allison et al., 1980), and the resulting R_h values were approximately 60–70 Å.

(II) In the simplest model of apo A-I self-association, we assumed that four dimers associated to form an octamer, without forming any intermediate species. In the formation of a toroidal ring there are four interactions between dimers; hence, we defined $4\Delta G_D$ as the free energy change for the self-association of four dimers to form an octamer. The chemical equilibrium between dimers and octamers was then expressed as

$$X_8 = X_2^4 \exp(-4\Delta G_D/RT) \quad (6)$$

where R is the universal gas constant and T is the absolute temperature. To simplify notation, we defined a mole fraction of apo A-I molecules, X_A, as

$$X_A = \exp(4\Delta G_D/3RT) \quad (7)$$

The equilibrium relationship of dimers and octamers was then expressed as

$$X_8/X_A = (X_2/X_A)^4 \quad (8)$$

By mass conservation, the total concentration of monomers (X) must be a function of X_2:

$$X/X_A = 2(X_2/X_A) + 8(X_2/X_A)^4 \quad (9)$$

Using previously stated definitions (see Methods) and employing eq 9, we expressed $\bar{R}_h$ and $\bar{n}_w$ values in terms of X_2/X_A and, hence, X/X_A. We calculated X_A values for measured $\bar{R}_h$ values in 0.15 M NaCl at 37 °C, and these were randomly scattered about a mean value of 1.35×10^{-7} (dimensionless units). Employing this mean X_A value (i.e., at one temperature and ionic strength), we calculated the theoretical dependence of $\bar{R}_h$ on apo A-I concentration, which is displayed by the solid

[7] This value for the water of hydration is based upon studies of aqueous solutions of globular proteins (Cantor & Schimmel, 1980). While we assumed a similar value for apo A-I and apo A-II in the theoretical calculations that follow, a 50% error would change R_h values by only 1–2 Å.

curve in Figure 1. In such a cooperative model (Donovan, 1984), the presence of intermediates, i.e., tetramers and hexamers, did not affect the calculated values for $\bar{R}_h$ and $\bar{n}_w$.

In the more complex model of apo A-I self-association, we assumed an initial association of monomers to form dimers which, in turn, self-associated to form ring-shaped octamers with free energy changes of ΔG_M and ΔG_D, respectively. Since we expected that both of these values would be similarly affected by changes in physical–chemical properties (NaCl concentration, temperature, and guanidine hydrochloride concentration), we assumed that ΔG_D is related to ΔG_M by a proportionality factor, F:

$$\Delta G_M = F\Delta G_D \quad (10)$$

Because a knowledge of F was required to calculate ΔG_M (see text), we expressed $\bar{R}_h$ and $\bar{n}_w$ values as functions of X_2/X_A and calculated, for several theoretical values of F, the curvilinear dependencies of R_h on X/X_A. By minimizing the sums of the squares of the relative deviations of X/X_A curves, for experimental and theoretical $\bar{R}_h$ values, we obtained an F value of 5.3 (0.15 M NaCl, 37 °C).

REFERENCES

Allison, S. A., Easterly, R. A., & Teller, D. C. (1980) *Biopolymers 19*, 1475–1489.

Armstrong, M. J., & Carey, M. C. (1982) *J. Lipid Res. 23*, 70–80.

Atkinson, D., & Small, D. M. (1986) *Annu. Rev. Biophys. Biophys. Chem. 15*, 403–456.

Barbeau, D. L., Jonas, A., Teng, T., & Scanu, A. M. (1979) *Biochemistry 18*, 362–369.

Brewer, H. B., Jr., Fairwell, T., Kay, L., Meng, M., Ronan, R., Law, S., & Light, J. A. (1983) *Biochem. Biophys. Res. Commun. 113*, 626–632.

Burstein, M., & Scholnick, H. R. (1973) *Adv. Lipid Res. 11*, 68–108.

Cantor, C. R., & Schimmel, P. R. (1980) *Biophysical Chemistry: Techniques for the Study of Biological Structure and Function*, pp 549–569, Freeman, San Francisco.

Carey, M. C. (1982) in *The Liver: Biology and Pathobiology* (Arias, I. M., Popper, H., Schacter, D., & Shafritz, D., Eds.) pp 429–465, Raven, New York.

Carey, M. C. (1983) in *Bile Acids in Gastroenterology* (Barbara, L., Dowling, R. H., Hofmann, A. F., & Roda, E., Eds.) pp 19–56, MTP, Lancaster, U.K.

Carey, M. C. (1985) in *Sterols and Bile Acids* (Danielsson, H., & Sjövall, J., Eds.) pp 345–403, Elsevier, Amsterdam.

Carey, M. C., Montet, J.-C., Phillips, M. C., Armstrong, M. J., & Mazer, N. A. (1981) *Biochemistry 20*, 3637–3648.

Carey, M. C., Benedek, G. B., & Donovan, J. M. (1985) in *Recent Advances in Bile Acid Research* (Barbara, L., Dowling, R. H., Hofmann, A. F., & Roda, E., Eds.) pp 183–189, Raven, New York.

Cohen, D. E. (1986) Ph.D. Dissertation, Harvard University, Cambridge, MA.

Donovan, J. M. (1984) Ph.D. Dissertation, Massachusetts Institute of Technology, Cambridge, MA.

Donovan, J. M., Benedek, G. B., & Carey, M. C. (1983) *Clin. Res. 31*, 475A.

Edelstein, C., & Scanu, A. M. (1980) *J. Biol. Chem. 255*, 5747–5754.

Eigner, D., Schurz, J., Jurgens, G., & Holasek, A. (1979) *FEBS Lett. 106*, 165–168.

Fisch, M. R., & Benedek, G. B. (1986) *J. Chem. Phys. 85*, 553–558.

Formisano, S., Brewer, H. B., Jr., & Osborne, J. C., Jr. (1978) *J. Biol. Chem. 253*, 354–360.

Ghiselli, G., Bradler, W. A., Gotto, A. M., Jr., & Sherrill, B. C. (1983) *Biochem. Biophys. Res. Commun. 116*, 704–711.

Gurantz, D., Laker, M. F., & Hofmann, A. F. (1981) *J. Lipid. Res. 33*, 373–376.

Gwynne, J., Brewer, B., Jr., & Edelhoch, H. (1974) *J. Biol. Chem. 249*, 2411–2416.

Gwynne, J., Palumbo, G., Osborne, J. C., Jr., Brewer, H. B., Jr., & Edelhoch, H. (1975a) *Arch. Biochem. Biophys. 170*, 204–212.

Gwynne, J., Brewer, H. B., Jr., & Edelhoch, H. (1975b) *J. Biol. Chem. 250*, 2269–2274.

Heuck, C. C., Daerr, W., Haberbosch, W., Horn, D., & Luddecke, E. (1983) *J. Biol. Chem. 258*, 8317–8322.

Hjelmeland, L. M. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 6368–6370.

Hofmann, A. F. (1962) *J. Lipid Res. 3*, 127–129.

Kawahara, K., & Tanford, C. (1966) *J. Biol. Chem. 241*, 3228–3232.

Kramer, W., Buscher, H.-P., Gerok, W., & Kurz, G. (1979) *Eur. J. Biochem. 102*, 1–9.

Kunitake, S. T., Loh, E., Schumaker, V. N., Ma, S. K., Knobler, C. M., Kane, J. P., & Hamilton, R. L. (1978) *Biochemistry 17*, 1936–1942.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Lux, S. E., Hirz, R., Shrager, R. I., & Gotto, A. M., Jr. (1972) *J. Biol. Chem. 247*, 2598–2607.

Makino, S., Tanford, C., & Reynolds, J. A. (1974) *J. Biol. Chem. 249*, 7379–7382.

Mantulin, W. W., & Pownall, H. J. (1985) *Biochim. Biophys. Acta 836*, 215–221.

Massey, J. B., Gotto, A. M., Jr., & Pownall, H. J. (1981) *Biochem. Biophys. Res. Commun. 99*, 466–474.

Mazer, N. A., & Carey, M. C. (1983) *Biochemistry 22*, 426–442.

Mazer, N. A., Carey, M. C., Kwasnick, R. F., & Benedek, G. B. (1979) *Biochemistry 18*, 3064–3075.

Missel, P. J., Mazer, N. A., Benedek, G. B., Young, C. Y., & Carey, M. C. (1980) *J. Phys. Chem. 84*, 1044–1057.

Morrisett, J. D., Gallagher, J. G., Aune, K. C., & Gotto, A. M., Jr. (1974) *Biochemistry 13*, 4765–4771.

Morrisett, J. D., Jackson, R. L., & Gotto, A. M., Jr. (1977) *Biochim. Biophys. Acta 472*, 93–133.

Norman, A. (1955) *Ark. Kemi 8*, 331–342.

Osborne, J. C., Jr., & Brewer, H. B., Jr. (1977) *Adv. Protein Chem. 31*, 252–337.

Pope, J. L. (1967) *J. Lipid Res. 8*, 146–147.

Pownall, H. J., Hsu, F. J., Rosseneu, M., Peeters, H., Gotto, A. M., & Jackson, R. L. (1977) *Biochim. Biophys. Acta 488*, 190–197.

Reynolds, J. A. (1976) *J. Biol. Chem. 251*, 6013–6015.

Scanu, A. M., & Edelstein, C. (1971) *Anal. Biochem. 44*, 576–588.

Scanu, A. M., Edelstein, C., & Shen, B. W. (1982) in *Lipid–Protein Interactions* (Jost, P. C., & Griffith, O. H., Eds.) pp 259–316, Wiley, New York.

Segrest, J. P., Jackson, R. L., Morrisett, J. D., & Gotto, A. M., Jr. (1974) *FEBS Lett. 38*, 247–253.

Sewell, R. B., Mao, S. J. T., Kawamoto, T., & LaRusso, N. F. (1983) *J. Lipid Res. 24*, 391–401.

Small, D. M. (1971) in *The Bile Acids* (Nair, P. P., & Kritchevsky, D., Eds.) Vol. 1, pp 249–356, Plenum, New York.

Small, D. M. (1977) in *Liver and Bile* (Falk Symposium 23) (Bianchi, L., Gerok. W., & Sickinger, K., Eds.) pp 89–100, MTP, Lancaster, U.K.
Stone, W. L., & Reynolds, J. A. (1975) *J. Biol. Chem. 250*, 8045–8048.
Swaney, J. B., & O'Brien, K. (1978) *J. Biol. Chem. 253*, 7069–7077.
Tall, A. R., & Small, D. M. (1980) *Adv. Lipid Res. 17*, 1–51.
Tall, A. R., Shipley, G. G., & Small, D. M. (1976) *J. Biol. Chem. 251*, 3749–3755.
Tall, A. R., Small, D. M., Deckelbaum, R. J., & Shipley, G. G. (1977) *J. Biol. Chem. 252*, 4701–4711.
Teng, T., Barbeau, D. L., & Scanu, A. M. (1978) *Biochemistry 17*, 17–21.
Vitello, L. B., & Scanu, A. M. (1976a) *J. Biol. Chem. 251*, 1131–1136.
Vitello, L. B., & Scanu, A. M. (1976b) *Biochemistry 15*, 1161–1165.
Weber, K., & Osborn, M. (1975) in *The Proteins* (Neurath, H., & Hill, R. L., Eds.) pp 180–224, Academic, New York.
Zannis, V. I., Breslow, J. L., & Katz, A. J. (1980) *J. Biol. Chem. 255*, 8612–8617.

Formation of Mixed Micelles and Vesicles of Human Apolipoproteins A-I and A-II with Synthetic and Natural Lecithins and the Bile Salt Sodium Taurocholate: Quasi-Elastic Light Scattering Studies†

Joanne M. Donovan,‡,§ George B. Benedek,‖ and Martin C. Carey*,‡,§

Department of Medicine, Harvard Medical School, Brigham and Women's Hospital, and Harvard Digestive Diseases Center, Boston, Massachusetts 02115, and Department of Physics and Center for Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received December 4, 1986; Revised Manuscript Received June 5, 1987

ABSTRACT: We employed quasi-elastic light scattering to systematically study the interactions of human apolipoproteins A-I and A-II (apo A-I and apo A-II) with synthetic and natural lecithins, analogues of the major membrane lipids found in high-density lipoproteins (HDL). Equilibrium values of the mean hydrodynamic radius ($\bar{R}_h$) of systems with varying concentrations of dimyristoylphosphatidylcholine (DMPC) to apolipoprotein showed that as the percentage of DMPC was increased, three distinct regions were observed. At low DMPC to apolipoprotein ratios, $\bar{R}_h$ values either increased, remained constant, or decreased, depending upon the total apolipoprotein concentration, which influenced the size of pure apolipoprotein micelles [Donovan, J. M., Benedek, G. B., & Carey, M. C. (1987) *Biochemistry* (preceding paper in this issue)]. When the percentage of DMPC approached the micellar phase boundary, $\bar{R}_h$ values uniformly diverged (50 to ~150 Å); with the percentage of DMPC in excess of the micellar phase boundary, large $\bar{R}_h$ values (200–300 Å) were observed that were consistent with unilamellar apo A-I/DMPC or apo A-II/DMPC vesicles. Decreases in total solute concentration (1.0–0.25 mg/mL) and/or elevations in temperature (25–37 °C) shifted micellar phase limits to lower percentages of DMPC in the case of both apolipoproteins. Although apo A-I interacted spontaneously with DMPC at 25 °C, it was necessary to dilute mixed micellar solutions of sodium taurocholate (TC) and egg yolk phosphatidylcholine (EYPC) with apo A-I solutions to form apo A-I/EYPC mixed micelles. Despite the presence of submicellar concentrations of TC (below 3 mM, the lower limit of its critical micellar concentration), $\bar{R}_h$ values of apo A-I/EYPC mixed micelles were similar to those observed for the apo A-I/DMPC system. Dilution of micellar TC/EYPC solutions with low concentrations of apo A-I (0.001–0.10 mg/mL) influenced the width of the mixed micellar zone, the kinetics of micelle-to-vesicle transitions, and the size of metastable vesicles. Dilution with higher apo A-I concentrations (0.05 and 0.1 mg/mL) resulted in the transformation of bile salt rich EYPC micelles into apo A-I rich EYPC micelles with an intervening zone of metastable vesicles. The micelle-to-vesicle transition was abolished by dilution with an apo A-I concentration of 0.5 mg/mL, suggesting that bile salts and apo A-I can directly interchange in micellar solubilization of EYPC. Our results suggest that submicellar bile salt concentrations should not appreciably influence the size or structure of native HDL, whereas very low concentrations of apo A-I (and, presumably, apo A-II) may be important in determining the size, equilibria, and metastability of micelles and vesicles in native biles.

High-density lipoproteins (HDL)[1] play a crucial role in cholesterol transport in blood [reviewed in Tall and Small (1980)]. The important properties of this class of lipoproteins are, in part, determined by their major apolipoproteins, apolipoprotein A-I (apo A-I) and apolipoprotein A-II (apo A-II). Precursors of native HDL (nascent HDL) are synthesized in

† Supported in part by Grants DK 36588 and AM 34854 (M.C.C.) from the National Institutes of Health and by Grant DMR81-19295 from the National Science Foundation (G.B.B.). J.M.D. was supported in part by a Whitaker Health Sciences Fellowship.

* Address correspondence to this author at the Department of Medicine, Brigham and Women's Hospital.
‡ Brigham and Women's Hospital.
§ Harvard Digestive Diseases Center.
‖ Massachusetts Institute of Technology.

[1] Abbreviations: apo A-I, apolipoprotein A-I; apo A-II, apolipoprotein A-II; HDL, high-density lipoproteins; QLS, quasi-elastic light scattering; TC, 3α,7α,12α-trihydroxy-5β-cholanoyltaurine (taurocholate); DMPC, dimyristoylphosphatidylcholine; EYPC, egg yolk phosphatidylcholine; cmc, critical micellar concentration; IMC, intermicellar concentration; $\bar{R}_h$, mean hydrodynamic radius; Tris, tris(hydroxymethyl)aminomethane.

0006-2960/87/0426-8125$01.50/0