Physical Properties of Apolipoprotein A-I from the Chicken, *Gallus domesticus*†

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ABSTRACT: The amphipathic α-helices of exchangeable apolipoproteins (apo) function to simultaneously facilitate interaction with lipid surfaces and the aqueous environment. In contrast to mammalian apoA-I's, which self-associate in the absence of lipid, chicken apoA-I, which shares 66% sequence homology with human apoA-I, exists as a monomeric protein when dissociated from high-density lipoprotein (HDL). Sedimentation equilibrium studies conducted in the analytical ultracentrifuge yielded a weight-average molecular weight of 28 170. Corresponding sedimentation velocity and diffusion experiments gave rise to $s^0_{20,w}$ = 2.23 S and $D^0_{20,w} = 6.39 \times 10^{-7}$ cm^2/s. A translational frictional ratio (f/f_{min}) of 1.18 and an axial ratio of 4.0 were also determined from this data. The Stokes radius ($R_{s,sed}$ = 2.80 nm) and translational frictional ratio were subsequently used to calculate estimated molecular dimensions of 25.2 × 100.8 Å for chicken apoA-I. Circular dichroism (CD) studies revealed a highly α-helical structure predicted to be 74% by Provencher–Glöckner analysis. Denaturation studies performed on lipid-free apoA-I and monitored by CD revealed a midpoint of denaturation of 0.64 M guanidine hydrochloride. From plots of ΔG_{app} versus guanidine hydrochloride concentration, a $\Delta G_D^{H_2O}$ of 1.86 kcal/mol was determined. In other studies, a midpoint of temperature-induced denaturation for apoA-I of 57 °C was obtained. The effect of solvent pH on the secondary structure content of apoA-I revealed a significant loss of α-helix below pH 4.0 and above pH 10, suggesting that lipid-free apoA-I may by partially stabilized by the formation of intra- or interhelix salt bridges between oppositely charged amino acid side chains. Denaturation studies of apoA-I bound to the surface of HDL revealed a midpoint of guanidine hydrochloride induced denaturation of 3.25 M, indicating that considerable stability is conferred on this protein through interaction with lipid. The relative lipid binding affinities of chicken and human apoA-I's were examined in displacement studies employing a model lipid surface, insect low-density lipophorin. The data showed that chicken apoA-I has a slightly higher affinity for lipid surfaces than its human counterpart. The results are discussed in relation to the known structural properties of human apoA-I.

Apolipoprotein A-I (apoA-I)[1] plays a fundamental role in lipoprotein metabolism, functioning as the major apolipoprotein component of high-density lipoprotein (HDL) and the key metabolic activator of the plasma enzyme lecithin: cholesterol acyltransferase (LCAT; Eisenberg, 1984). In addition, apoA-I is thought to play an important role in the biogenesis of HDL particles that function as acceptors of peripheral cell associated free cholesterol in the reverse cholesterol transport pathway (Rothblat et al., 1992; Hara & Yokoyama, 1992). Purified human apoA-I has been extensively characterized and is known to exist as an amphipathic α-helical structure that self-associates in the purified, lipid-free state (Vitello & Scanu, 1976). Key questions regarding the physiological role of apoA-I pertain to the structural organization of the protein when bound to lipid surfaces and, by extension, the regions of the molecule responsible for interaction with LCAT.

Major advances in our understanding of the structure–function relationship of amphipathic apolipoproteins have come from the three-dimensional structure determination of insect apolipoprotein III (apoLp-III; Breiter et al., 1991) as well as from the N-terminal 22-kDa fragment of human apoE (Wilson et al., 1991). These proteins were found to possess similar molecular architectures in the form of up and down α-helical bundles. These bundles are organized such that their hydrophobic faces are oriented toward the center of the bundle while their hydrophilic faces are directed at the aqueous environment. In the case of apoLp-III, the protein is known to exist in lipid-bound and lipid-free states as a normal part of its metabolic function *in vivo*, where it reversibly associates with the surface of lipoproteins as a function of their lipid content (Ryan, 1990; Van der Horst, 1990). On the basis of the structure of lipid-free apoLp-III, models have been proposed to explain how the protein may interact with lipid surfaces (Breiter et al., 1991; Wang et al., 1992). Interestingly, intact apoE displays a strong tendency to self-associate in the absence of lipid. This self-association is mediated by its C-terminal domain, which also displays a high affinity for lipid. Removal of the C-terminal domain by *in vitro* treatment with thrombin results in a N-terminal fragment that is monomeric over a wide concentration range (Aggerbeck et al., 1988). An impediment to structural studies, including crystallization, of other major apolipoproteins is their self-association properties. This propensity may be directly related to lipid binding affinity or overall molecular architecture such that, in the monomeric lipid-free state, instability arises due to the presence of exposed hydrophobic surface. Indeed, this may explain why human apoA-I, apoA-II, intact apoE, apoA-IV, and apoC's have not been crystallized to date.

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[1] Abbreviations: apo, apolipoprotein; LCAT, lecithin:cholesterol acyl transferase; HDL, high-density lipoprotein; CD, circular dichroism; LDLp, low-density lipophorin.

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On the basis of chemical cross-linking experiments, it has been suggested that apoA-I isolated from chicken HDL is monomeric (Swaney & Greenfield, 1980). Others have suggested that chicken apoA-I has properties that are similar to those of mammalian apoE (Rajavashisth et al., 1987). Insofar as apoE is lacking in the avian species, it has been suggested that apoA-I may fulfill in birds the physiological roles performed by apoE in mammals. Sequence comparisons between chicken (Yang et al., 1987) and human apoA-I's (Brewer et al., 1978) reveal an overall 48% identity with an additional 18% conservative amino acid substitutions, yielding a 66% sequence similarity over the entire 240 amino acid sequence. Thus it is possible that structural information determined for chicken apoA-I may provide insight into the structure of mammalian apoA-I and, perhaps, its relationship to apoE. While a great deal of information is available on the tissue distribution of chicken apoA-I mRNA during development and its induction under various physiological conditions (Blue et al., 1982; Dawson et al., 1986), few studies have addressed the physical properties of this protein (Kruski & Scanu, 1976; Jackson et al., 1976). In the present study, we have characterized the biophysical properties of chicken apoA-I and compared its hydrodynamic and stability properties with those of its human counterpart. Our results indicate that, unlike mammalian apoA-I, lipid-free chicken apoA-I is monomeric, is less stable than human apoA-I, but displays a relatively higher lipid binding affinity.

MATERIALS AND METHODS

Isolation of High-Density Lipoprotein and Apolipoprotein A-I. Fresh chicken blood was obtained from a local poultry farm. Plasma was obtained by centrifugation, and HDL was purified by KBr sequential ultracentrifugation between the density limits 1.12 and 1.21 g/mL. Chicken apoA-I was isolated from HDL according to the procedure described by Wells et al. (1985) for insect apolipophorin III, dialyzed against H_2O, and lyophilized. The purity of the protein preparation was determined by SDS–PAGE to be >95%. Human apoA-I was isolated from fresh human plasma according to the method of Ryan et al. (1992).

Analytical Ultracentrifugation. Hydrodynamic experiments were carried out on a Beckman Model E analytical ultracentrifuge equipped with electronic speed control, an RITC temperature control system using a titanium rotor. The photoelectric scanner optical system was used for sedimentation equilibrium runs and lower concentration sedimentation velocity runs. The schlieren optical system was used for both the sedimentation coefficent and diffusion coefficient runs. A Gaertner microcomparator was employed for measurement of schlieren photographs.

Determination of molecular weights, sedimentation coefficients, and diffusion coefficients was performed according to the methodology described by Chervenka (1969). Samples were dialyzed for 48 h at 4 °C prior to running in the ultracentrifuge. All runs were performed at 20 °C. A partial specific volume of 0.743, calculated from the amino acid composition, was used for the molecular weight and sedimentation calculations in accordance with McCubbin et al. (1985).

For molecular weight determinations, 100 μL of sample solution was loaded into the right side of a double-sector, CFE sample cell equipped with sapphire windows. Dialysate (105 μL) was loaded into the left sector. Samples were run for a minimum of 48 h prior to taking equilibrium traces. Molecular weight calculations were carried out using an APL computer program. The ln Y versus r^2 data were fit to a second-order polynomial equation using least-squares techniques, and both point-average molecular weights and the apparent average molecular weight were calculated from the slope of the resulting plots.

Two degree, single-sector KelF sample cells with quartz windows were used for sedimentation velocity runs. The cell was filled with sample solution, and the rotor was accelerated to 60 000 rpm. The run timer was started when the speed reached 40 000 rpm, and photographs of the schlieren peak were taken at regular time intervals as the protein sedimented to the cell bottom. The S_{obs} was calculated from the slope of a plot of time versus the log of the distance of the peak from the center of the rotor.

Double-sector, capillary synthetic boundary, CFE sample cells with quartz windows were used for diffusion coefficient runs. Sample solution (150 μL) was loaded into the right sector, and the left sector was filled with dialysate. The rotor was accelerated to 6000 rpm, and the run timer was started when the dialysate began to layer onto the sample solution. Ten schlieren photographs were taken at 8-min intervals throughout the runs. The slope of a plot of the peak area squared versus time was used to calculate the apparent diffusion coefficient. Both the sedimentation and diffusion coefficients were corrected to standard conditions (H_2O at 20 °C) and extrapolated to infinite dilution.

The Stokes radius ($R_{s,sed}$) was calculated from the extrapolated value of $s_{20,w}$ at infinite dilution ($s^0_{20,w}$) by the relationship (Tanford, 1961)

$$R_{s,sed} = M_r(1 - \bar{v}\rho)/(6N\pi\eta_0 s^0_{20,w}) \quad (1)$$

where M_r is the molecular weight, $\bar{v}$ is the partial specific volume, r is the solvent density, N is Avogadro's number, and η_0 is the solvent viscosity in poise.

The translational frictional ratio (f/f_{min}) was calculated from the experimental Stokes radius ($R_{s,sed}$) by using the relationship (Schachman, 1959)

$$f/f_{min} = R_s/R_0 = R_s/[3M_r\bar{v}/(4\pi N)]^{1/3} \quad (2)$$

where R_0 is the Stokes radius of the equivalent unhydrated sphere of molecular weight M_r and partial specific volume $\bar{v}$. The frictional ratio due to asymmetry (f/f_0) was calculated by separating the contribution of particle hydration (Oncley, 1941) according to

$$f/f_{min} = (f/f_0)[1 + \omega/\bar{v}\rho]^{1/3} \quad (3)$$

where ω is the degree of hydration expressed as grams of water bound per gram of protein. The degree of hydration was estimated as 0.44 g of H_2O/g of protein, employing the method of Kuntz and Kauzmann (1974), from the amino acid composition. The axial ratio for a prolate ellipsoid was generated from frictional ratio estimates by using tabulated data from Schachman (1959).

The longest semiaxis (a) for an ellipsoid model was evaluated from (Byers & Kay, 1982)

$$a = R_0(r_1^2 r_2)^{1/3} \quad (4)$$

where R_0 is the Stokes radius of the equivalent unhydrated sphere of molecular weight M_r and partial specific volume $\bar{v}$, r_1 is the ratio of the longest axis to the intermediate axis, and r_2 is the ratio of the intermediate axis to the shortest axis. For a prolate ellipsoid, r_1 is the axial ratio and r_2 is unity.

Circular Dichroism Experiments. Circular dichroism (CD) measurements were carried out on a Jasco J-270 spectro-

polarimeter (Jasco Inc., Easton, MD) interfaced to an Epson Equity 386/25 computer and controlled by Jasco software. The thermostated cell holder was maintained at 25 °C with a Lauda RMS circulating water bath (Lauda, Westbury, NY). The instrument was routinely calibrated with ammonium *d*-(+)-10-camphorsulfonate at 290.5 and 192 nm and with *d*-(−)-pantoyllactone at 219 nm. Each sample was scanned 10 times and noise reduction was applied to remove the high frequencies before calculating molar ellipticities. The voltage of the photomultiplier tube was kept below 500 V to prevent distortion of the CD spectrum. Cells used for the region below 250 nm were 0.01, 0.02, and 0.05 cm (calibrated for path length). Protein concentrations ranged from 0.70 to 1.5 mg/mL for the region below 250 nm, and a 1-cm microcell was employed for the 250–320-nm range. The mean residue weight for apoA-I was taken to be 116.520. Guanidine N–hydrochloride samples were prepared some 20 h before readings were taken in order to allow them to attain equilibrium prior to recording spectra. Urea samples were allowed 30 min before monitoring the ellipticity at 221 nm. CD spectra obtained in buffer alone as well as 50% TFE were recorded at 188–255 nm and analyzed for secondary structure using the Contin program version 1.0 of Provencher and Glöckner (1981). Stock protein concentrations were established in the analytical ultracentrifuge employing the Rayleigh interference optical system, assuming 41 fringes are equal to 10 mg/mL (Babul & Stellwagen, 1969).

Fluorescence Studies. Fluorescence spectra were obtained with a Perkin-Elmer MFB-44B spectrofluorimeter equipped with a DCSU-2 corrected spectra accessory which allows automatic background fluorescence correction. The temperature was maintained at 20 °C by circulating water from a Lauda RMS water bath through an attached thermostated cell holder. The samples were measured in a semimicro 1-cm cell, with a bandwidth of 5 nm used for both the excitation and emission monochromaters. The samples were excited at either 282 or 295 nm, and emission was monitored at 325 nm to record either the excitation or emission spectra with the blank mode set on the DSCU2.

Lipid Binding Studies. The relative lipid binding affinities of chicken versus human apoA-I's were studied using low-density lipophorin (LDLp) from the insect *Manduca sexta* as a model lipid surface as described by Liu et al. (1991). Chicken apoA-I was radiolabeled with *N*-succinimido [2,3-$^3H_2$]-propionate (102 Ci/mmol; Amersham). Radiolabeled apoprotein (2.5 mg), in the presence and absence of 2.5 mg of unlabeled chicken or human apoA-I, was incubated with 2.7 mg of LDLp protein for 1 h at 37 °C. Following incubation, the samples were subjected to density gradient ultracentrifugation according to Shapiro et al. (1984), and the specific activity of isolated LDLp (cpm/mg of protein) was determined by liquid scintillation counting and bicinchoninic acid protein assay (Smith et al., 1986).

RESULTS AND DISCUSSION

Characterization of Chicken apoA-I. A hallmark characteristic of apoA-I isolated from mammalian sources is a propensity to self-associate (Gwynne et al., 1974; Jonas, 1975; Teng et al., 1977). Human apoA-I, for example, is known to form oligomers ranging from dimers and octomers (Vitello & Scanu, 1976), and this property has been an impediment to hydrodynamic and crystallographic studies of the structure of apoA-I. From chemical cross-linking experiments, it has been proposed that, unlike its mammalian counterparts, chicken apoA-I is largely monomeric (Swaney & Greenfield,

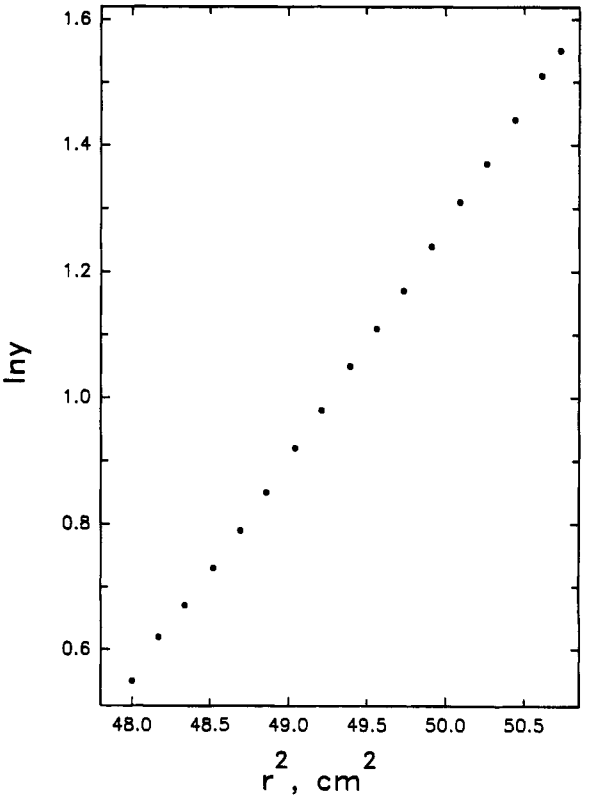

FIGURE 1: ln Y versus r^2 plot of chicken apoA-I. Sedimentation equilibrium was performed at 15 000 rpm at 20 °C in 10 mM Tris (pH 7.4) and 100 mM KCl. The protein concentration was 0.57 mg/mL. An apparent weight-average molecular weight of 28 170 was calculated from the slope of this plot.

1980). In sedimentation equilibrium studies of chicken apoA-I at 0.24 and 0.57 mg/mL, weight-average molecular weights of 30 240 and 27 720, respectively, were obtained (Figure 1). At both concentrations, plots of ln Y versus r^2 were linear. The molecular weight values obtained are in excellent agreement with the molecular weight of the protein determined by amino acid sequence analysis (27 965) and provide convincing evidence that, in contrast to mammalian apoA-I, isolated lipid-free chicken apoA-I is monomeric.

In an effort to obtain information about the size and shape of apoA-I, additional hydrodynamic experiments were performed in the analytical ultracentrifuge. In sedimentation velocity experiments, a single, symmetrical peak of sedimenting material was observed over the concentration range 0.48–7.5 mg/mL, yielding an $s^0_{20,w}$ of 2.23 S. In a similar manner, in diffusion experiments (Figure 2) there was no significant effect of apoA-I concentration over the range of 2.5–7.5 mg/mL, and a diffusion coefficient, $D^0_{20,w} = 6.39 \times 10^{-7}$ cm^2/s, was obtained. The symmetry of the schlieren patterns observed in sedimentation velocity and diffusion experiments provides additional evidence of the purity of the apoA-I employed in these studies.

Assuming a monomeric structure for apoA-I and using the molecular weight obtained from sedimentation equilibrium and the weight-average sedimentation coefficient, we calculated the translational frictional ratio f/f_{min} to be 1.18 (the level of hydration of chicken apoA-I was determined by the method of Kuntz and Kauzmann (1974) to be 0.44 g of H_2O/g of protein). Subsequently, an axial ratio for chicken apoA-I of 4.0 was determined. The shape of chicken apoA-I was further evaluated by determining its Stokes radius. A value of 2.80 nm was obtained from the sedimentation data and, together with the translational frictional ratio, indicates that

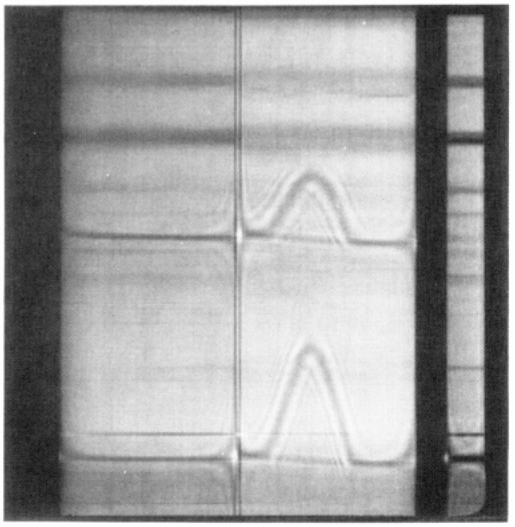

FIGURE 2: Diffusion schlieren pattern of chicken apoA-I. Centrifugation was performed at 6000 rpm and 20 °C in 10 mM Tris (pH 7.4) and 100 mM KCl. Two samples were run simultaneously by loading one sample, at a concentration of 2.5 mg/mL, into a sample cell containing a 1-deg positive wedge window. This sample corresponds to the upper image in the figure. The second sample, represented by the lower image, was run at a concentration of 4.41 mg/mL in a cell equipped with plane windows. The photograph shown was taken after 82 min.

apoA-I assumes a compact, globular structure with calculated molecular dimensions of 25.2 × 100.8 Å (Byers & Kay, 1982). These dimensions deviate from those of Edelstein and Scanu (1980) for human apoA-I in low concentrations of guanidine hydrochloride (26 × ~158 Å), which is proposed to dissociate apoA-1 oligomers without affecting the conformational shape of the protein. The self-association properties of human apoA-I have been shown to be ionic strength- and pressure-dependent phenomena (Formisano et al., 1978). Thus it is possible that chicken and human apoA-I's have similar solution structures, but that differences in amino acid sequence account for the self-association of human but not chicken apoA-I. Furthermore, given the overall estimated dimensions of chicken apoA-I, together with the fact that other monomeric globular apolipoproteins, such as apolipophorin III and the N-terminal domain of apoE, exist as up and down α-helical bundles (Breiter et al., 1991; Wilson et al., 1991), it is reasonable to speculate that chicken apoA-I may have a similar molecular architecture.

Spectroscopic Studies. UV absorption spectra of isolated apoA-I and intact chicken HDL revealed absorption maxima at 278.5 and 279.5 nm, respectively, corresponding to extinction coefficients of 0.76 and 0.40 OD/mg (based on an HDL particle composition comprised of 44% protein). In fluorescence studies, the spectra of apoA-I revealed an emission maximum at 325 nm (excitation = 282 nm), suggesting that the two Trp are shielded from the aqueous environment, in spite of the fact it does not self-associate. The corresponding spectrum for HDL reveals a further blue shift to 317 nm, indicative of further shielding of apoA-I Trp residues in the lipid-bound state.

Circular Dichroism Studies. As a basis for denaturation studies, and to evaluate the relative amount of secondary structure conformers of chicken apoA-I in the lipid-free and lipid-associated states, far-UV CD spectra were obtained. The CD spectrum of lipid-free apoA-I is indicative of a highly α-helical structure exhibiting negative troughs at 208 and 221 nm (Figure 3). Unlike the spectrum reported by Jackson et al. (1976), however, our data indicate similar values of negative ellipticity for the troughs at 208 and 222 nm. The

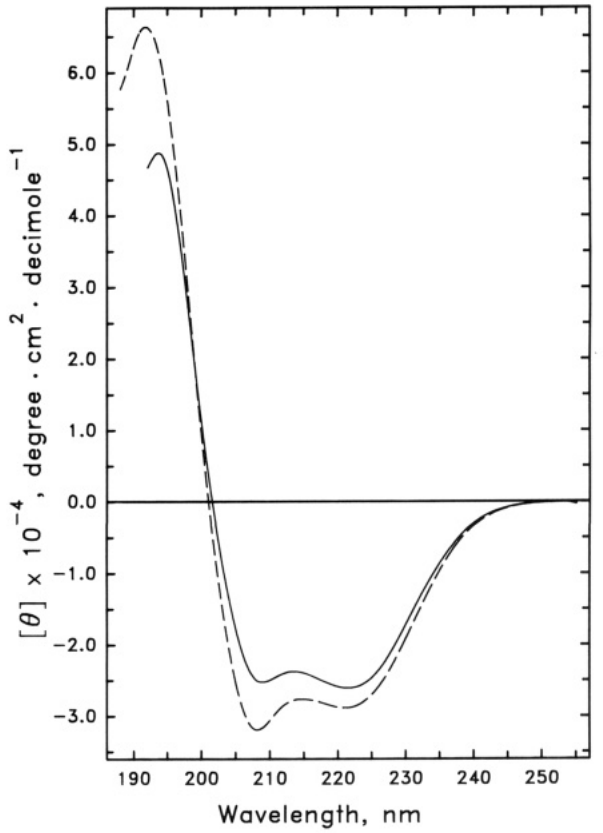

FIGURE 3: CD spectrum of chicken apoA-I. apoA-I samples were equilibrated in 50 mM Tris (pH 7.4) and 100 mM KCl and scanned from 188 to 255 nm: (—) apoA-I in buffer; (– – –) apoA-I in buffer containing 50% trifluoroethanol.

CD spectrum of chicken apoA-I was unaffected by protein concentrations over the 0.7–1.5 mg/mL range. When considered in light of the fact that known self-associating apolipoproteins display a concentration-dependent induction of α-helix (Gwynne et al., 1975; Stone & Reynolds, 1975; Yokoyama et al., 1985), this result provides further evidence that chicken apoA-I is monomeric. Provencher–Glöckner (Provencher & Glöckner, 1981) analysis of the spectrum revealed 74% α-helix. When spectra were recorded in 50% trifluoroethanol, there was a 26% induction of α-helix (Figure 3). Likewise, an increase in helix content was also observed in spectra of chicken HDL versus apoA-I alone, suggesting that association with lipid results in stabilization through increased helix content.

Denaturation Studies. In experiments designed to determine the relative stability of chicken apoA-I in its lipid-free and lipid-associated (as HDL) states, we examined the effect of a guanidine hydrochloride and urea on the negative ellipticity of apoA-I at 221 nm. Lipid-free apoA-I revealed a single denaturation event, with a transition midpoint of 0.64 M guanidine hydrochloride (Figure 4). On the assumption that the denaturation of apoA-I is a two-step process representing a reversible equilibrium between native (N) and denatured (D) states, the free energy of unfolding of apoA-I was determined from the guanidine hydrochloride experiments in accordance with eq 5 (Pace, 1986):

$$\Delta G_D = \Delta G_D^{H_2O} - m[\text{guanidine hydrochloride}] \quad (5)$$

where $\Delta G_D^{H_2O}$ is the free energy of unfolding in water and m is a constant that is proportional to the increase in degree of exposure of the protein upon denaturation. $\Delta G_D^{H_2O}$ was estimated by extrapolating the free energy of unfolding at each individual concentration of denaturant (ΔG_D) to zero

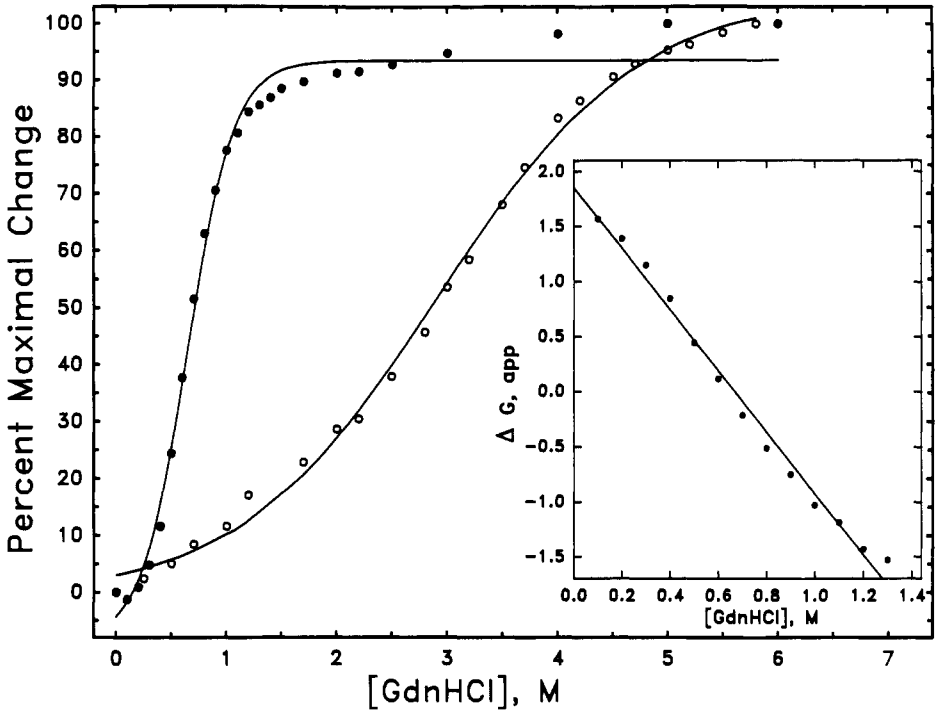

FIGURE 4: Effect of guanidine hydrochloride on the $[\theta]_{221}$ of lipid-free (●) and lipid-associated (○) chicken apoA-I. Guanidine hydrochloride was added to apoA-I in 50 mM Tris (pH 7.4) and 100 mM KCl, and at each concentration, the $[\theta]_{221}$ was determined. Inset: Plot of ΔG_{app} for lipid-free apoA-I (determined as described in the text) versus guanidine hydrochloride concentration.

concentration. ΔG_D in turn was calculated from the experimental data using the equation

$$e^{-\Delta G_D/RT} = ([\theta]_N - [\theta])/([\theta] - [\theta]_D) \quad (6)$$

where $[\theta]$ is the observed ellipticity at any particular guanidine hydrochloride concentration and $[\theta]_N$ and $[\theta]_D$ are the ellipticities of the native and denatured states, respectively. Extrapolation of a plot of guanidine hydrochloride concentration versus ΔG_{app} to zero concentration revealed $\Delta G_D^{H_2O}$ = 1.86 kcal/mol with an m value of 2.78 kcal/mol·M. Similar experiments were performed in which the effect of guanidine hydrochloride and urea on the intrinsic fluorescence of apoA-I tryptophan (excitation = 300 nm, emission = 325 nm) or tryptophan plus tyrosine (excitation = 282 nm, emission = 325 nm) residues was monitored. The results revealed a guanidine hydrochloride induced transition midpoint of 0.71 M and a urea-induced transition midpoint of 1.82 M when excited at 300 nm versus the corresponding values of 0.64 and 1.76 M when excited at 282 nm. The fluorescence data for guanidine hydrochloride denaturation are in general agreement with the results obtained by CD, yielding $\Delta G_D^{H_2O}$ = 2.97 (excitation = 282 nm) and 2.88 kcal/mol (excitation = 300 nm). When compared to human apoA-I it is apparent that lipid-free chicken apoA-I is more susceptible to denaturation and has a lower free energy of stabilization in H_2O than human apoA-I (transition midpoint = 1.08 M guanidine hydrochloride; $\Delta G_D^{H_2O}$ = 4.2 kcal/mol; Edelstein & Scanu, 1980). Thus it is possible to speculate that the additional stability displayed by human apoA-I may be attributable, in part, to its self-association properties.

Since apoA-I is the sole major apolipoprotein component of chicken HDL, we employed this lipoprotein in experiments to determine the stability of apoA-I when is its associated with a lipid surface. The CD spectrum of chicken HDL was essentially similar to that reported by Kruski and Scanu (1975), with the exception that the ellipticities at 208 and 222 nm were in the range of −27 000 deg·cm²/dmol and our data were extended to 185 nm, as opposed to theirs which were truncated at ~202 nm. The results revealed that, in the lipid-associated state, apoA-I has a midpoint of guanidine hydrochloride induced denaturation of ~3.0 M. Thus considerable stability is conferred to chicken apoA-I upon association with a lipid surface. This property is consistent with results obtained with human apoA-I complexed with dimyristoylphosphatidylcholine (Reijngoud & Phillips, 1982). The irreversibility of the denaturation event in this condition precluded determination of $\Delta G_D^{H_2O}$ in the lipid-bound state. The effect of increasing temperature on the stability of isolated apoA-I was monitored as a function of $-[\theta]_{221}$. Chicken apoA-I displayed a midpoint of temperature-induced denaturation of 57 °C, which is in the range of other apolipoproteins including human apoA-I ($T_m \approx 60$ °C; Tall et al., 1976) and insect apolipophorin III (52 °C; Ryan et al., 1993).

In other studies, we investigated the effect of pH on the stability of lipid-free apoA-I solutions. A plot of $[\theta]_{221}$ as a function of pH for apoA-I is shown in Figure 5. At pH values below 4 and above 10, a marked reduction in $-[\theta]_{221}$ was observed. In addition, a slight decrease in $-[\theta]_{221}$ occurs between pH 6.0 and 7.0. At pH values between 4.5 and 5.5 the protein is insoluble. Above pH 10, deprotonation of the 24 lysine residues present in apoA-I will result. In a similar manner, below pH 4.0, protonation of the side-chain carboxylic groups of glutamate (31 residues) and aspartate (15 residues) occurs. Combined, charged residues account for 37% of the amino acids present in apoA-I. The large number of oppositely charged residues which exist as nonoverlapping $i + 4$ pairs (13) suggests that side-chain charge–charge interactions may represent a significant stabilizing effect in chicken apoA-I (Marqusee & Baldwin, 1987), and pH-induced disruption of such interactions may result in significant loss of secondary structure. Support for this concept can be derived from the X-ray crystallograhpic results on the 22-kDa fragment of human apoE, which reveals the existence of intra- and interhelix salt bridges. Thus, it is possible that chicken apoA-I, as well as human apoA-I (Gwynne et al., 1974), may be stabilized in a similar manner.

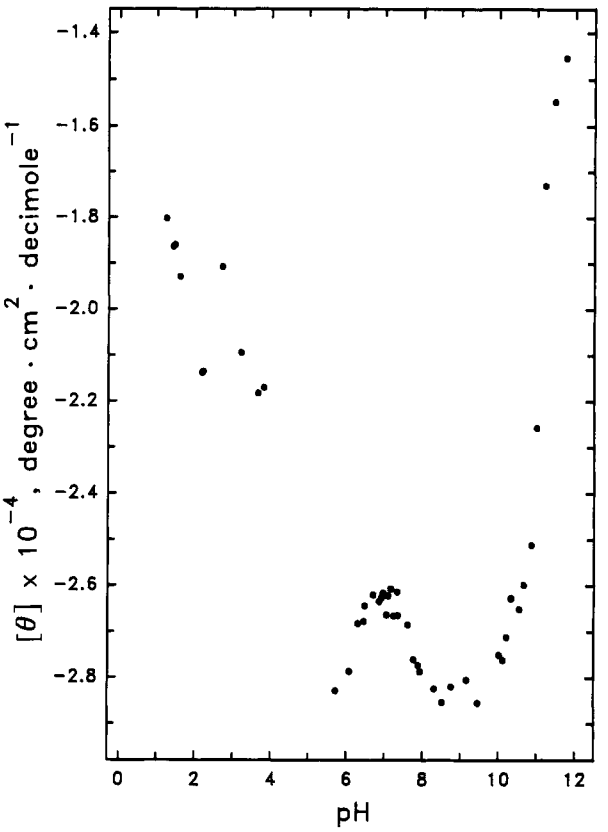

FIGURE 5: Effect of pH on the ellipticity of chicken apoA-I at 221 nm. A sample of chicken apoA-I was adjusted with respect to pH over the range 1.5–11.7, and CD scans were obtained at various intervals.

Lipid Binding Studies. To determine whether the differences in self-association and stability properties between chicken and human apoA-I are manifest in their respective lipid binding affinities, displacement studies were conducted using insect LDLp as a model lipid surface. In preliminary experiments [and in accordance with the data of Liu et al. (1991)], it was found that radiolabeled chicken apoA-I was recovered exclusively in the bottom fraction following density gradient ultracentrifugation, consistent with its lack of associated lipid. When incubated with insect LDLp (particle density = 1.03 g/mL; Ryan et al., 1986), however, radioactivity was recovered at a density corresponding to that of LDLp (specific activity = 11 700) cpm/mg of protein), indicating displacement of the resident apolipophorin III (Liu et al., 1991). Subsequently, competition experiments were performed by incubating ^{3}H-labeled and unlabeled apoproteins with LDLp. When ^{3}H-labeled chicken apoA-I was incubated with an equal mass of unlabeled chicken apoA-I, the LDLp specific activity was 7330 cpm/mg of protein. When a corresponding amount of unlabeled human apoA-I was employed in lieu of unlabeled chicken apoA-I, however, the LDLp specific activity was 8900 cpm/mg. On the basis of their relative abilities to decrease the specific activity of LDLp, we conclude that these two apoproteins have roughly similar affinities for the surface of LDLp, with that of chicken apoA-I being slightly higher. Furthermore, results consistent with this conclusion were obtained when ^{3}H-labeled human apoA-I and unlabeled chicken and human apoA-I were employed in displacement experiments with LDLp (data not shown). If one apolipoprotein possessed significantly higher affinity, then it would be expected that nearly complete displacement would result. Such is the case between human apoA-I and apoA-II (Lagocki & Scanu, 1980) and between human apoA-I and insect apoLp-III (Liu et al., 1991). The small apparent differences observed between chicken and human apoA-I's in the present study may be attributable to intrinsic lipid binding affinities or, alternatively, their respective stabilities in a lipid-free state, which may be influenced by self-association in the case of human apoA-I.

In summary, the present data indicate that chicken apoA-I possesses properties that are unique compared to those of apoA-I from mammalian sources. Foremost is its stable existence in a lipid-free state as a monomeric protein. The high α-helix content of the protein as well as its relative ease of denaturation by chaotropic reagents, temperature, and pH suggest that chicken apoA-I is susceptible to environmental conditions. By contrast, when associated with a lipid surface, considerable stability is conferred on this protein. It may be that, upon interaction with lipid, chicken apoA-I undergoes a conformational change to expose the hydrophobic regions of its structure. Given the high content of α-helix in its structure, chicken apoA-I may adopt a helical bundle structure such as that observed from insect apolipophorin III (Breiter et al., 1991) and the N-terminal fragment of human apoE (Wilson et al., 1991). We suggest that the lack of self-association and stability properties of chicken apoA-I make this protein an ideal candidate for crystallographic studies.

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