Conformational properties of human and rat apolipoprotein A-IV

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Abstract Apolipoprotein A-IV has been isolated from four sources: human and rat lymph and plasma. Conformational properties of the rat and human apoA-IV in solution and denaturation changes induced by guanidine hydrochloride (Gnd • HCl) were studied using circular dichroic and fluorescence spectroscopy, and analytical sedimentation equilibrium ultracentrifugation. We have shown that both rat and human apoA-IV have similar secondary structure with negative maxima in the circular dichroic spectra at 222 nm and 207 nm. Furthermore, we have found no significant difference in the α-helical content of the apoA-IV from rat plasma (33%), rat lymph (37%), human plasma (35%), or human lymph (35%). Our denaturation studies with Gnd • HCl demonstrated reversibility and the fact that each apoA-IV had a tendency to self-associate in solution and the self-association could be disrupted by low concentrations of Gnd • HCl (≤0.4 M). Unfolding of the secondary structure of each apoA-IV occurred at higher concentrations of Gnd • HCl (midpoint ≤ 1.0 M). The apparent free energy of denaturation of the four apoA-IV proteins calculated from changes in the circular dichroic spectra upon addition of increasing concentrations of Gnd • HCl varied in a range from 3.0 to 4.2 kcal/mol. The fluorescence experiments revealed that apoA-IV from all sources had a maximum fluorescence emission at 342.5 nm, which shifted to the red region upon addition of increasing concentrations of Gnd • HCl. Fluorescence quenching of apoA-IV by neutral (acrylamide) and negatively charged (iodide ion) quenchers demonstrated the increased accessibility of the tryptophanyl residues under the denaturing conditions. We conclude that there are no significant differences in the solution properties of apoA-IV isolated from rat plasma, rat lymph, human plasma, or human lymph and that apoA-IV from both species is thermodynamically unstable in aqueous solution.—**Dvorin, E., W. W. Mantulin, M. F. Rohde, A. M. Gotto, Jr., H. J. Pownall, and B. C. Sherrill.** Conformational properties of human and rat apolipoprotein A-IV. *J. Lipid Res.* 1985. **26:** 38–46.

Supplementary key words HDL • circular dichroism • plasma • lymph • fluorescence spectroscopy • sedimentation equilibrium • ultracentrifugation

Apolipoprotein A-IV (apoA-IV), a polypeptide with a molecular weight of 46,000, has been identified in the plasma of rats, dogs, and humans (1–6). ApoA-IV is synthesized in the intestine (5, 7–9) and packaged onto intestinal chylomicrons before secretion into the lymph. In rats during lipolysis of the chylomicrons in the plasma to chylomicron remnants, apoA-IV is transferred predominantly to HDL (7, 8). In contrast, in humans during lipolysis of chylomicrons, the majority of apoA-IV transfers to the lipoprotein-free region located in the d > 1.21 g/ml fraction of plasma (4, 5). It is well known that apoproteins function to maintain lipoprotein structure and to regulate their metabolic activity. Loss of apoA-IV from plasma lipoproteins in humans as opposed to the transfer of apoA-IV to HDL in rats may account, in part, for differences in lipid metabolism between these two species. Knowledge of the physical chemical properties of rat and human apoA-IV will allow us to understand structure-function relationships in lipoprotions and will aid in determining the physiological role of apoA-IV. Our study has focused on the solution properties of rat and human apoA-IV and on the conformational changes induced by the guanidine hydrochloride (Gnd • HCl) denaturation process. For this purpose we have used circular dichroic and fluorescence spectroscopy and analytical sedimentation equilibrium ultracentrifugation.

EXPERIMENTAL PROCEDURES

Purification of apoA-IV

ApoA-IV was isolated from four different sources: rat plasma, rat mesenteric lymph, human plasma, and

Abbreviations: apoA-IV, apolipoprotein A-IV; HDL, high density lipoproteins; d, density; Gnd • HCl, guanidine hydrochloride; CD, circular dichroism; SDS, sodium dodecylsulfate; NAT, N-acetyl-l-tryptophan; O.D., optical density.

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human thoracic duct lymph. Rat plasma and lymph were obtained from male Sprague-Dawley animals as previously described (10, 11). Human thoracic duct lymph was collected from renal failure patients who were undergoing chronic lymph drainage prior to pretransplantation lymphocyte depletion. Total lipoproteins were isolated by centrifugal flotation of plasma or lymph adjusted to a density of 1.21 g/ml with solid KBr. All centrifugations were performed in a Beckman ultracentrifuge, model L5-65. Lymph samples were centrifuged in an SW 28 rotor at 25,000 rpm and 4 °C for 24 hr at d 1.21 g/ml. The floating lipoprotein cake was collected from the top of the tube with a spatula and resuspended in 0.9% NaCl. Plasma lipoproteins were separated by centrifugation at 55,000 rpm and 4 °C for 40 hr in a Ti60 rotor. The centrifugation tubes were sliced and the floating lipoproteins were collected. The isolated lipoproteins were dialyzed against 0.05 M ammonium bicarbonate prior to lyophilization. The rat and human lymph lipoproteins were delipidated in chloroform–methanol 3:1, washed with ether, and resuspended in 0.01 M sodium phosphate buffer, containing 1% sodium dodecylsulfate (SDS). To separate the apoproteins, 10% acrylamide preparative (20 × 17 × 0.5 cm) slab gels and a sodium phosphate buffer system were used (12). Electrophoresis was performed in Pharmacia apparatus GE - 2/4 LS at 15 °C for 24 hr. The edge loaded with low molecular weight standards (Pharmacia Fine Chemicals) was cut from the gel by a razor blade and stained with Coomassie brilliant blue R-250 in order to determine the position of the apoA-IV band. The apoA-IV region was cut from the gel, crushed in a 12-cc syringe barrel, and apoA-IV was eluted by mixing with 0.01 M phosphate buffer, pH 7.2, 0.01% SDS for 24 hr at 4 °C. Acrylamide was removed by low speed centrifugation, SDS was removed by dialysis in 0.05 M ammonium bicarbonate, and the protein was lyophilized. For isolation of human plasma apoA-IV, fresh plasma from The Methodist Hospital Blood Bank was used. The Intralipid apoprotein recovery from the plasma d 1.21 mg/l infranant procedure of Weinberg and Scanu (6) was used with the following modifications. After delipidation, the apoprotein mixture (mainly apoA-IV and apoA-I) was resuspended in 4 M Gnd • HCl, 0.05 M Tris HCl, pH 7.4, and applied to a Sephacryl 200 column (200 cm × 2.5 cm) which had been equilibrated with the same buffer. Fractions containing apoA-IV were collected and reapplied on an S-200 column (100 cm × 1.5 cm) for further fractionation. The purified apoA-IV fraction was dialyzed against 0.05 M ammonium bicarbonate and lyophilized. Purity of each of the isolated apoA-IV was ascertained by analytical SDS polyacrylamide slab gel electrophoresis and amino acid analyses.

Circular dichroism

Circular dichroic (CD) spectra of the purified apoA-IV samples were recorded on a calibrated Cary Model 61 spectropolarimeter at 25 °C in 0.1 M sodium phosphate buffer, pH 7.4, using a 0.1-cm cell and 1-cm cell. The molar ellipticity at a given wavelength was calculated from:

$$[\theta]_\lambda = \frac{\text{MRW } \theta_\lambda^{obs}}{10\ l\ c} \qquad \textit{Eq. 1)}$$

where c is protein concentration (g/ml) and l corresponds to the cell pathlength (cm). The mean residue weight, MRW, of apoA-IV was calculated from our amino acid analysis and was taken to be 111. The measured ellipticity angle in degrees, θ_λ^{obs}, at a given wavelength was read directly from the recorded spectra. The percentage of α-helix was calculated using the previously described procedure (13):

$$\%\ \alpha\text{-helix} = \frac{[\theta]_{222} + 3{,}000}{36{,}000 + 3{,}000} \times 100. \qquad \textit{Eq. 2)}$$

CD spectra were recorded in the range of wavelengths from 250 to 200 nm. Initial protein concentration used for this experiment was 500 μg/ml. For denaturation studies two concentrations of protein were used: 500 μg/ml and 50 μg/ml. The denaturation was done with guanidine hydrochloride (Gnd • HCl). Aliquots of 8 M solution of Gnd • HCl were added to apoA-IV and corrections for the concentration of the protein were made.

Fluorescence spectroscopy

All fluorescence experiments were conducted at 25 °C with excitation at 295 nm such that absorption would be almost entirely by the tryptophanyl groups. The emission spectra were recorded in the wavelength range from 300 to 450 nm. Protein solutions of 0.1 O.D. at 295 nm in 0.1 M phosphate buffer, pH 7.4, were used. For fluorescence quenching experiments, solutions of 8 M acrylamide or 5 M potassium iodide were added in aliquots to the protein. To prevent I_3^- formation, 10^{-4} M $Na_2S_2O_3$ was included in the 5 M KI solutions.

Analytical sedimentation equilibrium

Analytical sedimentation equilibrium was done in a Beckman Model E Ultracentrifuge as previously described (14), using the data collection methods of Inners, Tindall, and Aune (15). Samples initially contained 200 μg/ml of rat plasma apoA-IV in buffer alone or buffer plus 0.4 M Gnd • HCl. Conditions of the ultracentrifugation were 20,000 rpm, 22 °C, and the column height was 2.5 mm.

Analytical procedures

Protein concentration in solution was determined by the method of Lowry et al. (16). Amino acid analysis was done on a Beckman Amino Acid Analyzer Model 121 after hydrolysis in 6 M HCl in vacuo for 24 hr. Analytical gel electrophoresis was performed using 1% SDS, 10% polyacrylamide 8 × 8 cm slab gels. Each well of the slab was loaded with no more than 20 μg of protein. Tryptophan content was determined by the method of Edelhoch (17).

RESULTS

The apoA-IV isolated by the method described above showed one band on SDS polyacrylamide gel electrophoresis, which corresponded to a molecular weight of 46,000 (**Fig. 1**). Amino acid analyses of our preparations of apoA-IV from human lymph and human and rat plasma are consistent with the literature values as shown in **Table 1**.

Fig. 2 presents the circular dichroic spectra of apoA-IV isolated from rat plasma (panel A), rat lymph (panel B), human plasma (panel C), and human lymph (panel D). Also shown on Fig. 2 are the circular dichroic spectra of the same samples in 1.4 M Gnd • HCl. The circular dichroic spectra of native apoA-IV has two well-defined negative maxima at 222 nm and 207 nm wavelengths which correspond to α-helical protein structure. In the presence of 1.4 M Gnd • HCl, the latter spectra correspond to the denatured state of each apoA-IV as can be seen by the shape of the curves (18). Using equation 2 the percentage of α-helical structure in each apoA-IV was calculated to be 33% for rat plasma, 37% for rat lymph, and 35% for both human plasma and human lymph.

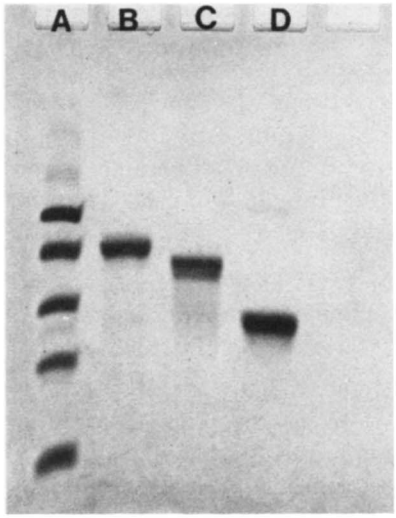

Fig. 1 Polyacrylamide gel electrophoresis of purified rat apolipoproteins. Lane A, low molecular weight standards; lane B, apoA-IV; lane C, apoE; and lane D, apoA-I.

TABLE 1. Amino-acid composition of human and rat apoA-IV expressed as a mol %

Amino Acid	Human			Rat	
	Lymph	Plasma	Plasma[a]	Lymph[b]	Plasma
Asp	11.1	14.1	9.1	10.6	13.7
Thr	3.8	4.5	4.0	5.0	4.8
Ser	5.6	6.4	5.0	5.9	5.9
Glu	24.5	20.2	22.9	16.5	21.9
Pro	4.1	3.2	3.0	4.1	3.2
Gly	4.5	7.2	5.2	6.5	6.5
Ala	5.7	6.8	8.2	5.7	7.0
Half-Cys	n.d.	n.d.	0.5	n.d.	n.d.
Val	5.2	4.9	5.2	5.8	5.8
Met	0.7	2.5	0.8	2.1	1.8
Iso	1.5	2.6	2.5	2.6	2.6
Leu	13.1	7.8	13.1	9.8	11.1
Tyr	1.4	2.0	1.9	1.0	1.5
Phe	2.2	2.5	2.6	2.9	2.4
Lys	6.8	4.5	6.9	6.0	6.5
His	1.4	3.1	2.0	1.4	1.6
Arg	6.6	4.7	6.4	3.8	4.3
Trp	2.0[c]	n.d.	n.d.	n.d.	2.0[c]

Each human value is the average of duplicate determinations; each rat value is the average of triplicate determinations; n.d., not determined.
[a]Values from reference 6.
[b]Values from reference 4.
[c]Values determined by the method of Edelhoch (17).

The percentage of change in the mean residue ellipticity, $\ominus_{222}$, of apoA-IV at 500 μg/ml upon addition of increasing concentrations of Gnd • HCl is shown in **Fig. 3**. The first transition midpoint is at 0.3 M Gnd • HCl for rat plasma (panel A) and lymph apoA-IV (panel B) and at 0.2 M Gnd • HCl for human lymph apoA-IV (panel C). This transition presumably corresponds to the disruption of protein self-association in solution. The second transition occurred at a higher concentration of Gnd • HCl. This larger change in percentage of $\ominus_{222}$ appeared at 1 M Gnd • HCl for rat plasma and lymph apoA-IV and at 0.8 M Gnd • HCl for the human lymph apoA-IV. At higher concentrations of Gnd • HCl, up to 4 M, no further changes at $\ominus_{222}$ were observed. Mean residue ellipticities, $\ominus_{222}$, of human plasma apoA-IV at 500 μg/ml (closed circles) and 50 μg/ml (open circles) as a function of increasing molarities of Gnd • HCl are shown in **Fig. 4**. In contrast to the higher concentration (500 μg/ml) where protein is aggregated, diluted apoA-IV (50 μg/ml) solution showed no transition at Gnd • HCl molarities between 0 and 0.4 M. Reversibility of the apoA-IV denaturation at all concentrations of Gnd • HCl (closed squares) is also shown in Fig. 4.

Analytical sedimentation equilibrium data are shown in **Fig. 5**. A striking difference is seen between the

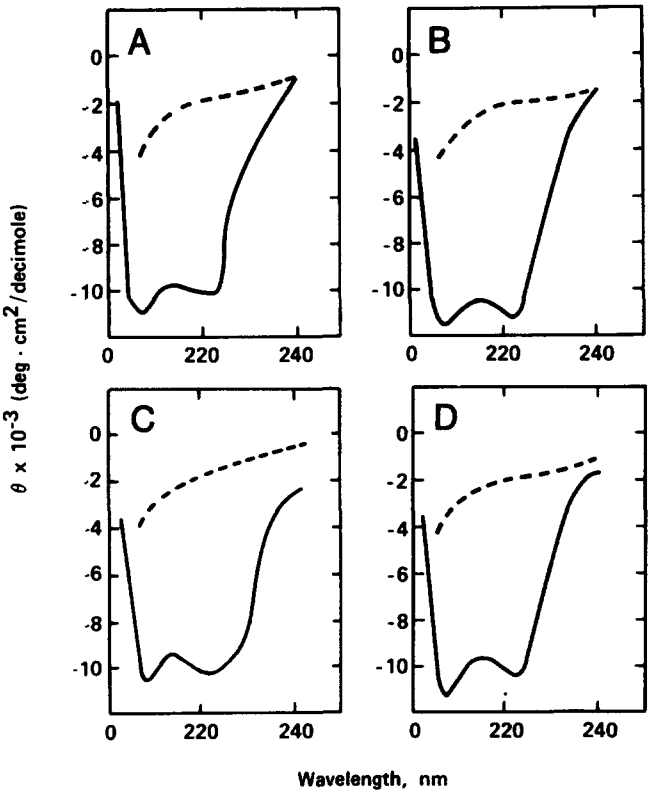

Fig. 2 Circular dichroic spectra of apoA-IV. Lower curves represent apoA-IV in 0.1 M phosphate buffer, pH 7.4; upper curves, apoA-IV in 1.4 M Gnd • HCl. Panel A, apoA-IV isolated from rat plasma; panel B, apoA-IV from rat lymph; panel C, apoA-IV from human plasma; panel D, apoA-IV from human lymph.

sample of rat plasma apoA-IV in the presence of 0.4 M Gnd • HCl and the same preparation in the absence of Gnd • HCl. The figure demonstrates that the protein in buffer alone exhibits curvature in the plot of the logarithm of concentration versus the square of the radial position. The indicated molecular weights were calculated from the slopes of the lines in the figure. The appropriate density of each solution was used in the calculation. The partial specific volume was calculated to be 0.722 from the known amino acid and carbohydrate composition (6). The limiting slopes of these lines represent the presence of sedimenting species between molecular weights of 90,000 to 182,000. Adding Gnd • HCl at the concentration of 0.4 M eliminates this curvature, causing a straight line which corresponds to a molecular weight of 46,000 for the monomeric state of the protein.

Using circular dichroism data it is possible to calculate the apparent free energy change (ΔG_D) for the denaturation of apoA-IV by the following equation:

$$\Delta G_D = -RT \ln K_D \qquad Eq.\ 3)$$

where

$$K_D = \frac{\Theta - \Theta_N}{\Theta_D - \Theta} \qquad Eq.\ 4)$$

R is the gas constant, T is absolute temperature in °K, and Θ is the molar ellipticity at any concentration of Gnd • HCl. Ellipticities of the native monomer and the denatured protein are Θ_N and Θ_D, respectively. Based on denaturation studies by circular dichroism and the analytical sedimentation equilibrium ultracentrifugation data, apoA-IV disassociates to a monomer in 0.4 M Gnd • HCl; thus, for the purposes of calculation, 0.4 M Gnd • HCl was taken as the concentration at which apoA-IV is completely in the monomeric state. Linear extrapolation of our experimental data to zero concentration of Gnd • HCl (**Fig. 6**) yields $\Delta G_D^{H_2O}$ values which estimate the stability of each apoA-IV. The

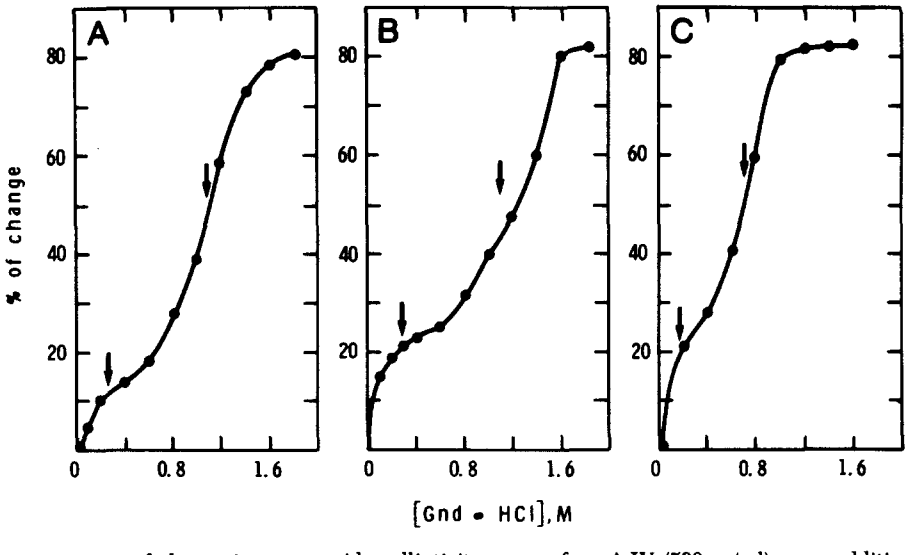

Fig. 3 Percent of change in mean residue ellipticity, Θ_{222}, of apoA-IV (500 μg/ml) upon addition of increasing concentrations of Gnd • HCl. Panel A, apoA-IV from rat plasma; panel B, apoA-IV from rat lymph; and panel C, apoA-IV from human lymph. The lower arrows indicate the transition midpoints for disaggregation and the upper arrows for unfolding of apoA-IV.

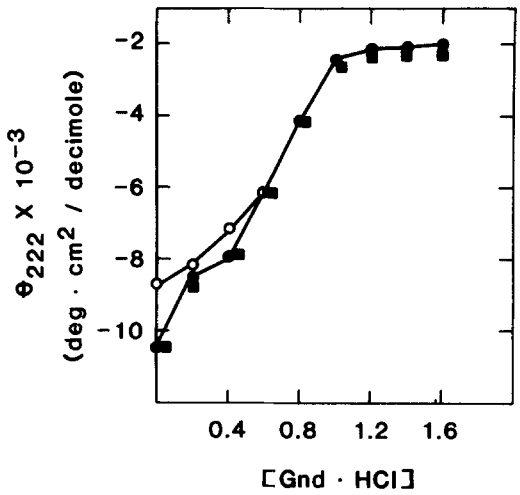

Fig. 4 Mean residue ellipticity, Θ_{222}, of human plasma apoA-IV (● - forward and ■ - reverse) at 500 μg/ml and 50 μg/ml (open circles) as a function of increasing Gnd · HCl concentrations.

$\Delta G_D^{H_2O}$ value for rat plasma apoA-IV (panel A) is 3.4 kcal/mol; for rat lymph apoA-IV (panel B), 3.0 kcal/mol; for human plasma apoA-IV (panel C), 2.7 kcal/mol; and 3.3 kcal/mol for human lymph apoA-IV (panel D).

According to Aune and Tanford (19) and Pace and Vanderburg (20, 21) the major driving force for denaturation of a protein is an increase in the number of Gnd • HCl binding sites in the unfolded state relative to those in the native state. This can be expressed by the following equation:

$$\Delta G_D = \Delta G_D^{H_2O} - \Delta n\ RT\ \ln(1 + km) \quad Eq.\ 5)$$

where Δn is the difference in the number of binding sites between the native and the denatured state, k is the average denaturant binding constant, and m is the molarity of the denaturant. This equation can be used also to calculate values of $\Delta G_D^{H_2O}$ for comparison with those values calculated by the linear extrapolation procedure. Taking k to be 0.8, $\Delta G_D^{H_2O}$ and Δn were calculated by least squares fit of ΔG_D as a function of m. The values for Δn for each apoA-IV were calculated to be 12.2 for rat plasma, 9.7 for rat lymph, 13.2 for human plasma, and 15 for human lymph. The $\Delta G_D^{H_2O}$ values calculated by this method were higher than those from the linear extrapolation, i.e., 4.2 kcal/mol for rat plasma apoA-IV, 3.7 kcal/mol for rat lymph apoA-IV, 3.6 kcal/mol for human plasma apoA-IV, and 4.2 kcal/mol for human lymph apoA-IV.

The fluorescence spectra of apoA-IV arises from the tryptophanyl residues. An uncorrected fluorescence maximum emission (λ_{max}) of 342.5 nm was registered for each apoA-IV. With addition of increasing concentrations of Gnd • HCl, the λ_{max} shifted toward the red region in an identical manner for each of the three apoA-IV and was 348 nm in 2 M Gnd • HCl. Fluorescence quenching experiments were performed with neutral (acrylamide) and charged (iodide ion) quenching agents on native and denatured apoA-IV. The decrease in fluorescence intensity caused by quenching agents can be treated quantitatively by the Stern-Volmer equation:

$$(I_0/I) = 1 + K_{SV}[Q] \quad Eq.\ 6)$$

where I_0 and I are the fluorescence intensities at the appropriate λ_{max} in the absence and presence of the quencher, [Q] is the concentration of the quencher and K_{SV} is the Stern-Volmer quenching constant. As shown in **Fig. 7** and **Table 2**, unfolded (in 2 M Gnd • HCl) rat plasma, rat lymph, and human lymph apoA-IV are more accessible to acrylamide than the native proteins. The results obtained with the negatively charged ion (iodide) shown in **Fig. 8** and Table 2 demonstrate that the native states of the apoproteins are not quenched as readily as by acrylamide, whereas in the unfolded state

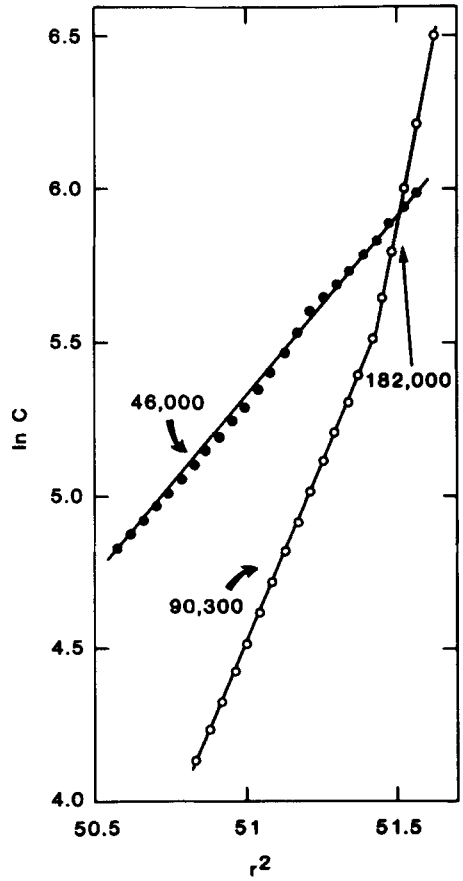

Fig. 5 Sedimentation equilibrium data presented as the logarithm of the concentration (A 280) versus the square of the radial position. Samples initially contained 200 μg/ml rat plasma apoA-IV in buffer alone (open circles) or buffer plus 0.4 M Gnd • HCl (closed circles). Ultracentrifugation conditions were: 20,000 rpm; 22°C; column height, 2.5 mm. The indicated molecular weights were calculated from the slopes of the lines as discussed in the Results section.

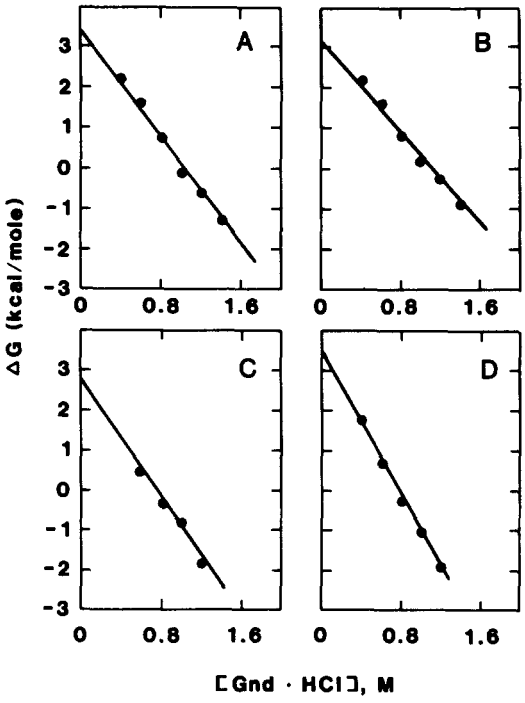

Fig. 6 The free energy of denaturation of apoA-IV determined from circular dichroic data. ApoA-IV isolated from rat plasma, panel A; from rat lymph, panel B; from human plasma, panel C; and from human lymph, panel D.

the quenching is similar. Apparent Stern-Volmer constants (K_{SV}) were calculated as a slope of the plots from Figs. 7 and 8 by using linear regression analysis. As a control and for normalization of the quenching data, N-acetyl-l-tryptophan (NAT) was used. Our experimentally determined K_{SV} for NAT quenched by acrylamide was 33, similar to the value of 26.6 reported by Eftink and Ghiron (22) and was 10.2 for quenching by the iodide ion. The values in parentheses in Table 2 are the ratio of K_{SV} of each apoprotein to K_{SV} of the standard solution of NAT.

DISCUSSION

Our circular dichroic data indicate that apoA-IV isolated from rat plasma, rat lymph, human plasma, and human lymph has a secondary structure with definite negative maxima in the spectra at 222 nm and 207 nm (Fig. 2). No significant difference in the α-helical content for rat plasma apoA-IV (33%), rat lymph apoA-IV (37%), or human plasma or human lymph apoA-IV (both 35%) was discernible. The denaturation studies with Gnd • HCl and the analytical sedimentation equilibrium data show that apoA-IV self-associates in solution and that the self-association can be disrupted by low concentrations (≤0.4 M) of Gnd • HCl (Figs. 3, 4, 5). The unfolding of apoA-IV occurs at higher concentrations of Gnd • HCl (≤1 M). Upon addition of increasing concentrations of Gnd • HCl, we calculated the apparent free energy of denaturation of apoA-IV using two different methods. Values of the free energy changes determined by linear extrapolation of our circular dichroic experimental data to 0 M Gnd • HCl are 3.3 kcal/mol for rat plasma apoA-IV, 3.0 kcal/mol for rat lymph apoA-IV, 2.7 kcal/mol for human plasma apoA-IV, and 3.3 kcal/mol for human lymph apoA-IV (Fig. 6), whereas the values calculated using equation 5 are 3.7 kcal/mol for rat lymph apoA-IV, 3.6 kcal/mol for human plasma apoA-IV, and 4.2 kcal/mol for both human lymph and rat plasma apoA-IV. These values are consistent with values for other apolipoproteins calculated in the same manner, i.e., 3.7 for apoA-I (23), 1.02 for apoA-II (24), and 2.8 for apoC-II (25) and are considerably lower than those values calculated with equation 5 by Pace and Vanderburg for globular proteins (21). They found the values of free energy of denaturation to be 9 kcal/mol for myoglobin and lysozyme, 11.4 kcal/mol for α-chymotrypsin, 16.3 kcal/mol for ribonuclease, and 22.3 for β-lactoglobulin. Comparison of all of the values

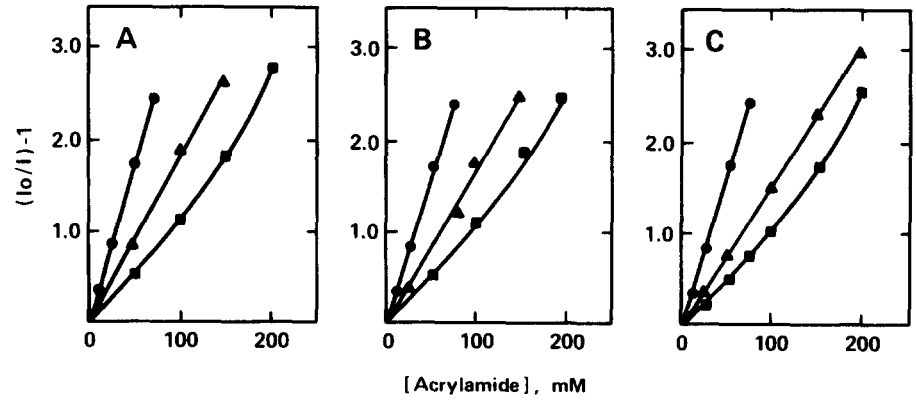

Fig. 7 Stern-Volmer quenching curves of apoA-IV by acrylamide. Panel A, rat plasma apoA-IV; panel B, rat lymph apoA-IV; panel C, human lymph apoA-IV. Standard solution of N-acetyl-l-tryptophan (●); apoA-IV in 0.1 M phosphate buffer (■); apoA-IV in 2 M Gnd • HCl (▲).

TABLE 2. Apparent Stern-Volmer constants of apoA-IV due to fluorescence quenching agents

Origin of ApoA-IV	[Gnd • HCl] (M)	K_{SV}, (M^{-1}) Quenching Agent: Acrylamide	Quenching Agent: Iodide Ion
Rat plasma	0	14.5 (0.44)	2.4 (0.23)
	2.0	17.5 (0.53)	4.3 (0.42)
Rat lymph	0	12.9 (0.39)	2.5 (0.24)
	2.0	16.3 (0.49)	4.2 (0.41)
Human lymph	0	12.4 (0.38)	2.9 (0.28)
	2.0	16.4 (0.50)	4.7 (0.46)

Values in parentheses are the normalized K_{SV} values, i.e., the ratio of each apoprotein K_{SV} relative to K_{SV} of the tryptophan standard, N-acetyl-l-tryptophan (NAT). The K_{SV} for NAT quenched by acrylamide is 33 and the K_{SV} for NAT quenched by iodide ion is 10.2.

indicates that apoA-IV, like other apolipoproteins, is thermodynamically unstable in aqueous solution.

The difference in the number of binding sites for Gnd • HCl between the native and unfolded state of each of the apoA-IV calculated by equation 5 was 10 for rat lymph, 12 for rat plasma, 13 for human plasma, and 15 for human lymph. The number of denaturant binding sites on a fully unfolded protein correlates with the sum of one-half of the number of peptide bonds plus the number of aromatic amino acids contained in the protein (21, 26). Based on amino acid analysis of both human and rat apoA-IV, the total number of binding sites would be approximately 230 to 232 in 1.4 M Gnd • HCl where apoA-IV is fully unfolded. Thus the majority of the denaturant binding sites are accessible in the native state and only a small increase in the number of accessible binding sites, i.e., 10 to 15, is required for full denaturation of native apoA-IV with no discernible significant difference between human or rat apoA-IV.

Fluorescence spectra of proteins are due to aromatic amino acids, predominantly the tryptophan residues. The fluorescence spectra of apoA-IV are predominantly due to one or more of the 6 tryptophans, although apoA-IV also contains 12 phenylalanines and 6 tyrosines. The fluorescence wavelength maximum (λ_{max}) for apoA-IV from rat plasma, rat lymph or human lymph was shifted to the red from 342.5 nm to 348 nm upon unfolding in 2 M Gnd • HCl. Our data indicate that in the native state some of the tryptophans are buried and, when the protein is unfolded (2 M Gnd • HCl), the tryptophans are more exposed to the aqueous environment resulting in the observed red shift (27).

Fluorescence quenching experiments with acrylamide for native apoA-IV (Fig. 7) show positive curvature. Although it is difficult to interpret uniquely fluorescence quenching data determined with multi-tryptophan proteins, qualitatively the upward curving plots of fluorescence quenching are either due to static quenching (27) or to nonlinear effects (28). Our linear plots, in the case of the denatured protein, suggest that in the unfolded state all of the tryptophans are probably accessible to the quencher (26). This result also can be visualized by comparing the normalized Stern-Volmer constants in Table 2. When each apoA-IV is in its native state (0 M Gnd • HCl), acrylamide quenches on average 40% of the fluorescence relative to the tryptophan standard, whereas in the unfolded state (2 M Gnd • HCl) acrylamide quenches 51% of the fluorescence relative to the standard. Thus, no significant increase in tryptophan accessibility to acrylamide is evident upon unfolding of the protein.

The fluorescence quenching results with the negatively charged ion, iodide, are shown in Fig. 8 and Table 2. They are the same for each of the apoA-IV. The negative curvature of the Stern-Volmer plots for native apoA-IV arises from the selective quenching of certain tryptophanyl residues in this multi-tryptophan protein. In the unfolded state (2 M Gnd • HCl) the fluorescent residues of apoA-IV are equally accessible to both the neutral acrylamide and the charged iodide ion. In contrast, with the native apoA-IV the iodide quenching relative to the acrylamide quenching is dif-

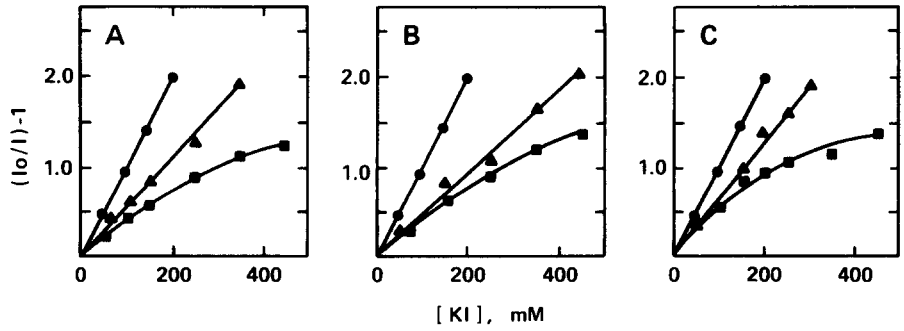

Fig. 8 Stern-Volmer quenching curves of apoA-IV by potassium iodide. Panel A, rat plasma apoA-IV; panel B, rat lymph apoA-IV; panel C, human lymph apoA-IV. Standard solution of N-acetyl-l-tryptophan (●); apoA-IV in 0.1 M phosphate buffer (■); apoA-IV in 2 M Gnd • HCl (▲).

ferent (Table 2, normalized Stern-Volmer constants). The negative iodide ion has approximately 63% relative quenching efficiency when compared with acrylamide. These results suggest that, although the fluorescent residues of the native apoA-IV are accessible to the uncharged acrylamide, they are not accessible to the same degree to the negatively charged iodide ion. This is a reasonable conclusion because apoA-IV like other apolipoproteins probably has amphipathic helix characteristics (29). Thus, some or all of the tryptophans may be buried in a hydrophobic region surrounded by a charged hydrophilic surface, which prevents the charged iodide ion access to the fluorescent tryptophans.

In conclusion, as shown by circular dichroism, analytical sedimentation equilibrium, and fluorescence experiments, no significant differences in the solution properties of the apoA-IV isolated from rat plasma, rat lymph, human plasma, or human lymph were found. Thus the physical characteristics of the apolipoprotein itself will not explain the divergent plasma distribution of apoA-IV in rats and humans. With differences in the apoA-IV itself being excluded as an explanation, other possibilities must be considered to resolve the differing distribution of apoA-IV between plasma lipoprotein and lipoprotein-free regions in the two species. These possibilities include: *1*) apoA-IV having preferential lipid-binding properties, thereby implicating the differing lipid composition of rat and human HDL; *2*) apoA-IV possessing differential protein-protein interactions with other apoproteins such that the differing apoprotein composition of the human and rat HDL might contribute; *3*) preferential binding of apoA-IV to HDL_2 as opposed to HDL_3 such that the rat whose plasma contains predominantly HDL_2 would retain apoA-IV on its lipoproteins rather than being lipoprotein-free as in humans (whose plasma is predominantly HDL_3); or *4*) the rate of clearance of HDL from plasma which differs considerably between rat and human may be contributory. If the interaction of apoA-IV with the lipoprotein particle is not very strong, then in humans there is a greater probability for apoA-IV to be displaced from HDL into the plasma compartment than in rat. Currently these studies are underway to determine the source of the divergent plasma distribution of apoA-IV between rat and human.

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