The Molecular Behavior of ApoA-I in Human High Density Lipoproteins

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SUMMARY

The properties of apoA-I in human high density lipoprotein (1.063 < d < 1.210 gm per ml) have been examined by fluorescence and difference absorption spectroscopy, while the behavior of the total complement of apoproteins has been evaluated by far ultraviolet circular dichroism. Marked increases in stability to temperature, pH, and guanidine hydrochloride were observed for apoA-I in the native particle as compared to the isolated state.

Although the chemical composition of normal human high density lipoprotein (HDL) is reasonably well known, the structural organization of the lipid and protein components remains to be elucidated. HDL is defined as those serum lipoproteins with densities between 1.063 and 1.210 g per ml; their molecular weights and protein content vary between 1.7 and 4.0 x 10^6 (1) and 45 to 55%, respectively (2). About 90% of the protein is accounted for by two proteins, apoA-I and apoA-II, while the remaining 10% is represented by several polypeptides present in very small amounts, i.e. apoC-I, apoC-II, apoC-III and apoD (3-5). On a weight basis apoA-I is three times more abundant than apoA-II (3).

ApoA-I contains a single polypeptide chain of molecular weight 25,000 to 28,000 (3, 6, 7) with 7 tyrosyl and 4 tryptophanyl residues (8). The amino acid sequence of the initial 39 residues (9) and the composition of the cyanogen bromide peptides have been reported (10). These studies indicated that the 4 tryptophanyl residues are located in the NH2-terminal half of the protein, while the tyrosyl residues are uniformly distributed over the entire chain.

The other major apoprotein, apoA-II, has a molecular weight of 17,380 and is composed of two identical polypeptide chains of 77 amino acid residues connected by a single disulfide bridge at position 6 in the sequence (11). ApoA-II contains 8 tyrosyl but no tryptophanyl residues.

Numerous studies, both enzymatic and chemical, indicate that specific groups in the two major proteins in human HDL are accessible to the solvent (12-15). It can be surmised therefore that these proteins are not completely embedded in lipid. Recent nuclear magnetic resonance studies with 31P suggest that the phosphate groups are also exposed to the solvent (16). The present report compares the properties of apoA-I in its free state with that in its lipoprotein form, i.e. in native HDL. The ability to observe certain properties of apoA-I in HDL in the presence of apoA-II is possible, since tryptophanyl residues are not present in apoA-II. The effects of temperature, pH, and Gdn-HCl on the structure of apoA-I in native HDL were investigated.

METHODS

Plasma for the isolation of HDL was collected from normal male volunteers by phlebotomy in 0.01% ethylenediaminetraacetic acid (EDTA). HDL was prepared by sequential preparative ultracentrifugation in KBr solutions and the 1.063- to 1.21-g per ml density fraction was isolated. The lipoprotein was found to be free of contaminants by paper electrophoresis and immunodiffusion against antibodies to serum LDL and albumin. The final HDL solution was dialyzed against 12 liters of 0.01 M ammonium bicarbonate, pH 8.5, stored at 4°C and used within 2 weeks. HDL was delipidated with chloroform-methanol (2:1, v/v) as previously described (17). The various apoproteins of HDL were separated by chromatography on Sephadex G-200 in 0.1 M Tris-HCl, 6 M urea, pH 8.5, according to the method of Scanu et al. (3). By this method four peaks were observed which included a small amount of aggregated protein in the void volume, apoA-I, apoA-II, and the C peptides. The relative amounts of the A and C apoproteins in native HDL were estimated by the Lowry method. ApoA-I, apoA-II, and the C peptides were found to represent 55 to 65%, 25 to 35%, and 5 to 8% of the protein, respectively. These results are in accord with those of Scanu (3). ApoC-III, apoC-I, and apoA-II contain 3, 1, and 2 tryptophanyl residues, respectively. Since apoA-II is devoid of tryptophan and apoA-I contains 4 residues, about 85% of the tryptophanyl residues in HDL will belong to apoA-I.

The purified apoA-I was dialyzed free of salt and urea and was concentrated (Diaflo). Each preparation was found to be homogeneous by polyacrylamide disc gel electrophoresis (18) and NH2-terminal analysis by the Edman technique (19). Solutions of apoA-I were kept frozen in 0.002 M phosphate, at neutral pH.

Dansylated derivatives of apoA-I were prepared by adding a 5-μl aliquot of a 14 mM acetonitrile solution of dansyl chloride to 0.8 ml of a 50 μM solution of apoA-I in 0.10 M bicarbonate, pH 8.5, at 4°C with vigorous stirring (20). After completion of the reaction (approximately 30 min) the conjugated protein was separated from the hydrolyzed dansyl chloride by passing the solution over a column of Sephadex G-25 equilibrated with 0.01 M phosphate at pH 7.4. There were an average of 25 dansyl groups per mol of apoA-I.
apoA-I. This number of dansyl groups does not appear to affect significantly the structure of apoA-I, since no change was observed in the circular dichroism in the 200- to 240-nm region.

Circular dichroic spectra were measured with a Cary model 60 spectropolarimeter equipped with a Pockels cell and standardized with d-10-camphorsulfonic acid. The mean residue ellipticity of apoA-I was calculated by the equation:

$$\theta = \frac{\theta \times 115}{l \times 10^4}$$

where $\theta$ is the observed ellipticity, $l$ is the path length in cm, $C$ is the protein concentration in grams per ml, and 115 is the mean residue weight of apoA-I calculated from its amino acid analysis (8). Results of circular dichroic measurements on HDL are reported as observed ellipticity.

Fluorescence measurements were made in the Turner model 210 spectrofluorometer or Perkin-Elmer model MPF III fluorescence spectrophotometer, both equipped with thermostated cell holders. Polarization of fluorescence measurements were made using a Hitachi No. 018-0054 polarization accessory for the MPF III. All measurements of tryptophanyl polarization were made using polarized excitation at 280 nm and emission at 340 nm. All measurements of dansyl polarization were made using unpolarized excitation at 340 nm and emission at 426 nm.

Relaxation times ($\tau$) were calculated from the slope and intercept of a plot of $(1/P - 1/3)$ versus $T\eta/\eta$ according to the Perrin equation (20):

$$\left( \frac{1}{P} + \frac{1}{3} \right) = \left( \frac{1}{P_0} + \frac{1}{3} \right) \left( 1 + \frac{3\tau}{\rho} \right)$$

where $P = (I_{VV} - G_{VV})/(I_{VV} + G_{VV})$, $G = I_{VH}/I_{HH}$, $I$ is the fluorescence intensity, and the first and second subscripts refer to the plane of polarization of the excitation and emission beams, respectively ($V$, vertical; $H$, horizontal), and $\eta$ is the viscosity at temperature $T$. The relaxation time of a sphere (20) of molecular volume, $V$, is $\rho T V \eta V$ if $T$. Fluorescence lifetimes ($\tau$) were measured by the TRW (El Segundo, Calif.) nanosecond flash apparatus (21).

In all of the temperature studies solutions were allowed to reach thermal equilibrium by waiting 15 min after each temperature adjustment. No time effects were observed.

The $pH$ was measured by a Radiometer model 26 meter calibrated with Beckman buffers. $pH$ titrations were made by adding small aliquots of concentrated acid or base using a micrometer-driven Agla syringe. Corrections for dilution were made where necessary. All chemicals used were reagent grade, except guanidine hydrochloride which was Hoeco "synthesized extreme purity."

RESULTS

Properties of ApoA-I

Thermal Stability—The secondary structure of proteins may be estimated from their circular dichroic spectra in the far ultraviolet (22). The circular dichroic spectrum of apoA-I at neutral pH previously has been shown to contain two troughs at 208 and 222 nm (7, 23) which are characteristic of peptide groups in $\alpha$ helical conformation (22). The loss in secondary structure of apoA-I as a function of temperature has been followed by the change in ellipticity at 220 nm (Fig. 1). There is a small, linear decrease in negative ellipticity below $45^\circ$, followed by a larger decrease between 47 and $75^\circ$, indicative of a major configurational change with a transition temperature near $60^\circ$. The thermal curve observed by circular dichroism was reversible with cooling (Fig. 1).

The stability of the tertiary structure of apoA-I to thermal denaturation was investigated by several procedures which depend on properties of the tryptophanyl residues, i.e. absorption, fluorescence, and polarization. A negative difference absorption spectrum, representing the exposure of aromatic groups to the solvent, develops when apoA-I is heated above $\sim45^\circ$. The difference peaks at 291 and 283 nm, representing perturbations of the tryptophanyl and tyrosyl residues, respectively, show the same dependence on temperature. The temperature dependence of the structural transition of apoA-I (Fig. 2A) was in accord with that observed by circular dichroism. The total decrease in absorption for the transition ($\Delta A; \Delta A_{abs}$) is 6 and 8% for the 291- and 283-nm peaks, respectively.

The change in tryptophanyl absorption is also reflected in
The temperature dependence of fluorescence reveals a transition in the same range as observed for the absorption curve (Fig. 2B). For comparison the curve for the model compound, acetyl tryptophanamide, whose fluorescence decreases monotonically with increasing temperature, is also shown in Fig. 2B. The thermal denaturation is accompanied by a red shift of the fluorescence peak from 334 nm at 25° to 347 nm at 75°. The thermal quenching curve of apoA-I therefore represents a molecular transition which alters the interactions of the tryptophanyl residues. The fluorescence change induced by heating was reversible with cooling.

A method which affords direct information of the molecular form of apoA-I is that of polarization of fluorescence. If the size and shape of a protein remain fixed, the dependence of polarization on solution viscosity and temperature will obey the Perrin equation. The polarization of tryptophanyl fluorescence of apoA-I conforms to the Perrin equation below 45°; at higher temperatures the polarization values become too small and no longer fall on the same line (Fig. 3). It is evident from the smaller values above 45° that a structural change occurs which leads to a more randomly organized molecule wherein the tryptophanyl residues have much greater rotational freedom. The unfolding of the native structure was further verified by using an extrinsic probe, DNS, covalently coupled to apoA-I. A Perrin plot obtained by observing DNS polarization with increasing temperature showed similar changes to the tryptophanyl polarization curve (Fig. 3).

**Properties of HDL**

The influence of the constituents of HDL on the stability of apoA-I was evaluated from the properties of apoA-I in native HDL. The latter have been assessed by the same techniques used for the free apoprotein. The properties of HDL determined by techniques which depend on tryptophanyl absorption or emission will reflect essentially the behavior of apoA-I; in contrast, those that depend on the polypeptide backbone, i.e. circular dichroism, will reflect the behavior of all the proteins present in HDL.

**Thermal Stability**—The stability of the proteins in HDL to thermal denaturation was assessed by ellipticity measurements at 220 nm. A monotonic decrease in ellipticity of HDL was observed between 25 and 75° (Fig. 4) and represented a 45% loss in negative ellipticity. The initial value was recovered when the solution was cooled to 25°. This result is quite different from that observed with the isolated apoA-I where a transition was observed above ~45° and much less of the initial ellipticity remained at 75° (Fig. 1).

Further information on the stability of apoA-I in HDL was obtained from the thermal dependence of tryptophanyl fluorescence and polarization (Fig. 3). In contrast to the inflection in the curve of free apoA-I (Fig. 2), tryptophanyl fluorescence of HDL showed a monotonic decline between 14 and 84°, similar to the thermal quenching curve of acetyltryptophanamide (Fig. 5). In addition, the 325-nm emission peak of HDL did not change, indicating that the environment of the tryptophanyl residues was unaffected by the high temperatures.

The relaxation time of apoA-I in HDL can be evaluated from the Perrin equation when glycerol is used to alter the viscosity and the temperature is kept constant. The data shown in Fig. 6 were obtained when the glycerol concentration was increased to 61% (v/v) at 20°. The lifetimes were measured at 25° on each solution and varied from 3.9 ns in water to 4.3 ns in 61% glycerol. The lifetimes were corrected by 6% for the variation of emission intensity in water between 20 and 25°. A value of 150 ns was calculated for the relaxation time at 20° of apoA-I in HDL. When the HDL data of Fig. 3 are replotted (calculating the lifetimes from the value at 25° and the fluorescence intensi-
Temperature is 20°.

**DISCUSSION**

The behavior of the major apoprotein of human HDL, apoA-I, has been compared in its free state and in the native lipoprotein particle. Measurement of the properties of the tryptophanyl residues in HDL permits the study of apoA-I since about 85% of
these residues are found in apoA-I. This premise is supported experimentally by the agreement between the changes in tryptophanyl polarization and in ellipticity observed in the slower phase of the Gdn-HCl data (Fig. 8). The decrease in negative ellipticity must originate from apoA-I since it is the only protein present in high enough concentration in HDL to account for the 65% change in ellipticity. It is evident therefore that the change in tryptophanyl polarization arises from apoA-I and not the C peptides.

The denaturation of apoA-I with pH and Gdn-HCl has been previously reported (7). It also was found that apoA-I was monomeric at pH 7.4 up to concentrations of 20 μM. The previous and current results indicate that the structure of apoA-I is only stable in the pH range between 6.5 to 11.0, in concentrations of Gdn-HCl below ~1 M, and at temperatures below ~45°C. It is now evident that when apoA-I forms part of the native structure of HDL, it is stable between pH 2 and 12, to temperatures as high as 84°C and in Gdn-HCl concentrations below 2 M. Rather high concentrations of Gdn-HCl are required to affect the structure of apoA-I in its native lipoprotein environment. The stability of apoA-I to various denaturing conditions appears to be profoundly enhanced by the lipids (or proteins) present in HDL. The concentration of Gdn-HCl needed to denature apoA-I in HDL is approximately the same as needed for lysozyme (29). The stability of apoA-I in HDL therefore should be comparable to that of lysozyme. It should be noted that lysozyme is also stable to acid and alkali and high temperatures in aqueous solution (30, 31).

The changes in secondary structure of HDL, as measured by circular dichroism at 220 nm, are more complex than the effects observed by the fluorescence measurements. The ellipticity, of course, reflects the optical activity of the peptide chromophore in all the proteins present in HDL. The origin of the minor changes in circular dichroism in HDL with pH and temperature is not evident. It is possible that they do not represent a loss in α helical structure but result from an increase in rotational motion of some of the peptide groups, as has been suggested to explain the negative circular dichroic activity in the 220-nm region which is not associated with either α helical or β structure (32). Another possible source of the ellipticity changes is that they result from a loss in α helical structure in apoA-II since apoA-I is stable to pH and temperature in the ranges studied. The helical content of apoA-II is almost as large as in apoA-I (23). The magnitude of the changes observed is in approximate accord with the contribution that apoA-II makes to the circular dichroism of HDL, considering that it represents about 25% of the peptide groups in HDL. A third possibility is that the small decrease in ellipticity represents a loss in peptide structure in the COOH-terminal half of apoA-I since this part of the molecule is not monitored by the polarization measurements owing to the absence of tryptophanyl residues (10).

The origin of the ellipticity changes of HDL observed in Gdn-HCl is more readily assigned since the rate of the second and slower phase agrees very well with that seen by tryptophanyl polarization. The latter reaction can only represent the behavior of apoA-I since apoA-II is devoid of tryptophan. Since apoA-II is less stable than apoA-I to the other denaturing conditions,apoA-II may be responsible for the ellipticity changes observed in HDL with temperature and pH.

The increased resistance to thermal denaturation of the proteins in HDL reported in these studies is similar to the results obtained by Scanu et al. on the thermal denaturation of HDL and apo-HDL (33). Similar reports with LDL also have shown that the protein components are more resistant to denaturation when present in their lipoprotein form (34, 35). Studies of tryptophanyl polarization of HDL offer insight into the behavior and interactions of apoA-I in its native lipoprotein environment. The calculated relaxation times (ρ of HDL (mol wt = 400,000, 0 = 0.905 and mol wt = 175,000, 0 = 0.867) (1) are 446 and 186 ns, respectively, assuming that HDL is spherical. The relaxation time of apoA-I in HDL is comparable to the calculated values of HDL itself. The fluorescent chromophores covalently conjugated to globular proteins generally have relaxation times similar to that of the protein (20). The similarity in relaxation time between apoA-I in HDL and HDL suggests that either apoA-I is rigidly bound to HDL or if it is loosely bound, the high viscosity of the lipid in which it floats increases its apparent relaxation time significantly. It has been demonstrated that it is the microviscosity of the lipid micelles and not that of the aqueous solvent which controls the relaxation behavior of various fluorescent dyes bound to lipid micelles (36, 37). It is also possible that apoA-I in HDL contains a layer of tightly bound phospholipid which moves with it. It has been reported that certain membrane proteins, whose relationship to the membrane may be compared to that of apoA-I to HDL, are free to undergo translational (38–41) and even rotational motions (41) in the lipid bilayer of the membrane.

The molecular organization of the lipids and proteins in human HDL which imparts the unique stability to the intact lipoprotein particle is as yet unknown. It is evident, however, that the nonpolar environment afforded by the lipids in HDL enhances the helical quality of the polypeptide chains and endows the chains with greater resistance to denaturation, as evaluated by experiments employing different denaturing conditions.

The specific constituents which comprise the microenvironment and are responsible for the great increase in stability of apoA-I in HDL are not known. They may be polar phospholipids, nonpolar lipids, or other apoproteins alone or in combination. No information is currently available regarding interactions among isolated apoproteins, although considerable data exist on their lipid-combining capacity. Scanu et al. (4) have shown that apoA-I (Fraction III) and apoA-II (Fraction IV) when individually sonicated with the mixture of extracted HDL lipids will selectively combine with different proportions of the major lipid classes. Scanu and others have also shown that treatment of native HDL by diethyl ether (4), dehydration (42), or repeated ultracentrifugation (43) results in the selective loss of only apo-A-I, further indicating specificity in the microenvironment of the individual apoproteins. In addition, Scanu (4) found that incorporation of cholesterol and cholesterol esters into lipoprotein complexes with the HDL apoproteins requires phospholipids. These combined results suggest that native HDL contains complexes of apoproteins with a specific complement of lipid, most probably phospholipid, which comprise its microenvironment and that the remaining lipids are bound by lipid-lipid interactions to these complexes. That a similar situation exists in HDL is suggested by the work of Pollard and Chen (44) which showed that extraction of LDL with diethyl ether resulted in loss of up to 90% of the total cholesterol with very little loss of phospholipids or changes in protein conformation as detected by fluorescence or circular dichroism. Further studies are needed to clarify the specificity in molecular interactions prerequisite to the assembly of the individual apoproteins into different lipoprotein particles.

Unpublished observations.
REFERENCES

1. Hazelwood, R. N. (1958) J. Amer. Chem. Soc. 80, 2152-2156
2. Osclely, J. L. (1963) in Brain Lipids and Lipoproteins and The Leukodystrophies (Folch-Pi, J., and Bauers, H., eds) pp. 1-17, Elsevier, Amsterdam
3. Scanu, A., Toth, J., Edelstein, C., Koga, S., and Stiller, E. (1969) Biochemistry 8, 3309-3316
4. Scanu, A., Cump, E., Toth, J., Koga, S., Stiller, E., and Alberson, L. (1970) Biochemistry 9, 1327-1335
5. McConathy, W. J., and Alauovic, P. (1973) Conference on Serum Lipoproteins, Graz, Austria, Abstr. 1319
6. Edelstein, C., Lim, C. T., and Scanu, A. M. (1972) J. Biol. Chem. 247, 5842-5849
7. Gwynne, J., Brewer, B., Jr., and Edelchoh, H. (1974) J. Biol. Chem. 249, 2411-2416
8. Lux, S. E., and John, K. M. (1972) Biochim. Biophys. Acta 278, 266-270
9. Shore, B., and Shore, V. (1972) Proceedings of the European Society of Atherosclerosis, Masson et Cie, Paris
10. Jackson, R. L., Baker, H. N., David, J. S. K., and Gotto, A. M. (1972) Biochem. Biophys. Res. Commun. 49, 1444-1451
11. Brewer, H. B., Jr., Lux, S. E., Ronan, R., and John, K. M. (1972) Chemistry and Biology of Peptides, p. 705, Ann Arbor Science, Ann Arbor
12. Ashworth, L. A. E., and Green, C. (1963) Biochem. J. 89, 561-564
13. Camilo, C. (1968) Biochim. Biophys. Acta 175, 290-300
14. Scanu, A., Reader, W., and Edelstein, C. (1968) Biochim. Biophys. Acta 106, 32-45
15. Attaiaux-Jabrier, M., Pastier, D., Burdin, J., and Polononski, J. (1971) Protides Biol. Fluids Proc. Colloq. Bruges 19, 29-33
16. Asmann, G., Sokoloski, E. A., and Brewer, H. B., Jr. (1974) Proc. Natl. Acad. Sci. U. S. A. 71, 549-553
17. Lux, S. E., John, K. M., and Brewer, H. B., Jr. (1972) J. Biol. Chem. 247, 7510-7518
18. Rutter, R. A., and Small, P. A., Jr. (1966) Science 152, 1253-1255
19. Edman, P. (1970) in Protein Sequence Determination (Needleman, S. B., ed) p. 211, Springer Verlag, New York
20. Steiner, R. F., and Edelchoh, H. (1962) Chem. Rev. 62, 457-485
21. Chen, R. F., Vurek, G. G., and Alexander, N. (1967) Science 156, 949-951
22. Greenfield, N., and Fasman, G. D. (1969) Biochemistry 8, 4108-4116
23. Lux, S. E., Hirz, R., Shrager, R. I., and Gotto, A. M. (1972) J. Biol. Chem. 247, 2598-2606
24. Jonas, A. (1972) J. Biol. Chem. 247, 7773-7778
25. Steiner, R. F., and Edelchoh, H. (1963) Biochim. Biophys. Acta 69, 341-355
26. Edelchoh, H., and Lippolt, H. E. (1969) J. Biol. Chem. 244, 3876-3883
27. Edelchoh, H., Brand, L., and Wilcher, M. (1967) Biochemistry 6, 547-559
28. Tanford, C., Kawahara, K., and Lapanje, S. (1967) J. Amer. Chem. Soc. 89, 729-736
29. Aune, K. C., and Tanford, C. (1969) Biochemistry 8, 4579-4585
30. Edelchoh, H., and Steiner, R. F. (1962) Biochim. Biophys. Acta 60, 365-372
31. Steiner, R. F. (1964) Biochim. Biophys. Acta 79, 51-63
32. Fasman, G. D., Huyning, H., and Timasheff, S. N. (1970) Biochemistry 9, 3316-3324
33. Scanu, A. M. (1969) Biochim. Biophys. Acta 181, 268-274
34. Scanu, A., Poolard, H., Hirz, R., and Kothary, K. (1969) Proc. Natl. Acad. Sci. U. S. A. 62, 171-178
35. Dearborn, D. G., and Wetlauffer, D. B. (1969) Proc. Natl. Acad. Sci. U. S. A. 62, 175-185
36. Shinitzky, M., Deanaux, A. C., Gitler, C., and Weber, G. (1971) Biochimica 10, 9106-9113
37. Cogan, U., Shinitzky, M., Weber, G., and Nishida, T. (1973) Biochemistry 12, 512-528
38. Fryd, L. D., and Edidin, M. (1970) J. Cell Sci. 7, 319-335
39. Scandellia, C. J., Devaux, P., and McConnell, H. M. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 2056-2060
40. Foo, M., and Cone, R. A. (1974) Nature 247, 438-441
41. Cone, R. A. (1972) Nature New Biol. 236, 39-43
42. Nichols, A. V., Lux, S., Fonde, T., Gong, E., and Levy, R. I. (1972) Biochim. Biophys. Acta 270, 132-148
43. Scanu, A., and Granda, J. L. (1966) Biochemistry 5, 446-455
44. Pollard, H. B., and Chen, R. F. (1973) J. Supramol. Struct. 3, 177-183
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