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Studies on Human Serum High Density Lipoproteins

SELF-ASSOCIATION OF APOLIPOPROTEIN A-I IN AQUEOUS SOLUTIONS*

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Human serum apolipoprotein A-I (apo-A-I), the major protein component of the human serum high density lipoproteins, was studied in aqueous solutions of differing ionic strengths and pH by the techniques of sedimentation equilibrium ultracentrifugation and frontal analysis gel chromatography. The ultracentrifugal studies indicate that apo-A-I is a self-associating system that is dependent upon protein concentration, but relatively independent of the nature of the medium. The apparent weight average molecular weights obtained from solutions of initial apo-A-I concentration between 0.2 and 0.9 mg/ml were in the range of 3.0 to 16.7 $\times$ 10^4 (monomer molecular weight = 28,014). Of the several models of self-association examined, that which gave the best theoretical fit was for the monomer-dimer-tetramer-octamer model. The self-association of apo-A-I in aqueous solutions was further documented by frontal analysis gel chromatography, which not only corroborated the ultracentrifugal results, but also indicated that the multiple species of apo-A-I in solution attain equilibrium rather rapidly.

Besides having intrinsic importance, these results indicate that the solution properties of apo-A-I must be established before ligand binding studies are conducted and interpreted.

Human serum apolipoprotein A-I (apo-A-I) is the major protein component of the class of human serum high density lipoproteins (HDL). Apo-A-I[1] represents a single polypeptide chain with a monomer molecular weight of 28,014 (2, 3). Its complete amino acid sequence has been reported recently (4). As with other apolipoproteins, the most important characteristic of apo-A-I is its ability to interact with lipids and to form soluble complexes with them. The precise nature of these interactions has not been determined and studies on the elucidation of this mechanism are now in progress in several laboratories (2, 3). In view of the rather scanty literature data, we found it necessary in approaching this problem to undertake a systematic investigation of the properties of apo-A-I in aqueous solutions. In this report, we will present the results of these studies, which were carried out by the techniques of sedimentation equilibrium ultracentrifugation and frontal analysis gel chromatography. A preliminary report on these findings has been published (1).

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[1] The abbreviations used are: HDL, high density lipoproteins; Apo-A-I, major apolipoprotein of apo-HDL.

MATERIALS AND METHODS

Isolation of Apo-A-I and Assessment of Purity—The methods used were essentially identical to those previously described (5, 6). Apo-A-I was isolated from delipidated HDL by column chromatography in Sephadex G-200, 8 M urea (5), followed by DEAE-ion exchange column chromatography in 6 M urea (6). The final preparation was dialyzed against 0.01 M NH_4HCO_3, lyophilized, and stored under nitrogen at $-10°$. Apo-A-I migrated as a single band by polyacrylamide gel electrophoresis in either 0.1% Na dodecyl-SO_4 or 8 M urea, regardless of the protein concentration (20 to 120 μg), and gave a single line of immunoprecipitation when tested against rabbit anti-apo-A-I antiserum. Whenever tested, it had an amino acid composition corresponding to that previously reported (2, 3).

Prior to use, the dry apo-A-I was solubilized in the given solvent and dialyzed in the cold against several changes of the solvent. Apo-A-I was studied under several conditions of pH and ionic strength, namely: 0.02 M EDTA, pH 8.6, μ = 0.12; 0.02 M EDTA, pH 10.1, μ = 0.15; 0.15 M KCl-0.01 M Tris, pH 8.6, μ = 0.16; and 0.01 M phosphate, pH 7.1, μ = 0.024. To prevent bacterial growth, we made all solutions 10^{-3} M in sodium azide. In some experiments, the protein was studied without lyophilization. Because the results were identical, most of the work was carried out on lyophilized specimens.

Ultracentrifugal Studies—Sedimentation equilibrium was studied in a Beckman model E analytical ultracentrifuge by the method of Richards *et al.* (7) with an initial overspeed period as described by Chervenka (8); the final speeds used for these studies ranged from 6,800 to 13,000 rpm. The initial concentrations, c_0, in fringes were determined from diffusion runs immediately following the completion of the sedimentation equilibrium experiment.

The partial specific volume of apo-A-I, calculated from its amino acid composition (2, 3), was found to be 0.736 ml/g. Solvent densities were measured at 20° in a Mettler-Paar (Hightstown, N. J.) mechanical oscillator density meter.

Frontal Elution Column Chromatography—Frontal elution chromatography was performed at 20° in a Sephadex G-75 (Superfine) column (1 × 9 cm), equilibrated, and eluted in 0.02 M EDTA, pH 8.6. The flow rate of 6 ml/hour was kept constant by means of a Chromatronix (Berkeley, Calif.) surgeless, positive displacement pump.

The volume of the apoprotein solution applied to the column was greater than the void volume of the column, so that the concentration of protein in the plateau region of the elution profile equaled that of the sample. The apo-A-I solution was introduced by means of a Chromatronix sample injection valve, with no interruption of the flow. The elution patterns were monitored at 280 nm, and the integral elution profile was recorded directly (9). The column was calibrated against the following standards: xanthine oxidase, catalase, bovine serum albumin, ovalbumin, chymotrypsinogen, myoglobin, and ribonuclease. The exclusion volume, V_0, equaled the elution volume of blue dextran. The internal volume, V_i, was determined from the relation $V_i = V_{K_2CrO_4} - V_0$ (10). The elution volume, V_e, of each of the standards was used to determine the molecular sieve coefficient, σ, by the use of the expression $\sigma = (V_e - V_0)/V_i$. A linear relationship of the form $\sigma = -A \log M + B$ was followed, where A and B are constants and M is the molecular weight. For M values between 1.3 and 7×10^4, A was 0.448 and B was 2.229.

Other Analyses—Protein determinations were carried out by the Lowry method (11) with bovine serum albumin used as a standard. Based on the amino acid composition of apo-A-I, no correction factor had to be applied.

Reagents—All chemicals were reagent grade and were used without purification, except for urea which was recrystallized and deionized as described previously (6).

RESULTS

The graphs of the logarithm of the protein concentration in fringes (log c) *versus* the square of the distance from the center of rotation (r^2) showed pronounced curvature for solutions of apo-A-I in 0.02 M EDTA, pH 8.6. A typical plot is shown in Fig. 1. The slope of these plots is related to the apparent weight average molecular weight, $M_{w_{app}}$, according to the expression

$$M_{w_{app}} = \frac{2RT}{(1 - \bar{v}\rho)\omega^2} \frac{d \ln c}{d(r^2)} \quad (1)$$

where R is the gas constant, T is the absolute temperature, $\bar{v}$ is the partial specific volume of the solute, ρ is the density of the solution, and ω is the angular velocity.

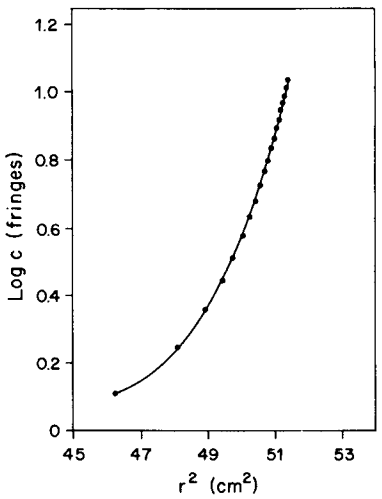


FIG. 1. Typical results obtained from sedimentation equilibrium experiments for apo-A-I. Initial concentration, 0.84 mg/ml; solvent, 0.02 M EDTA, pH 8.6; speed, 10,000 rpm; temperature 20°.

The range of $M_{w_{app}}$, obtained from several experiments at different initial concentrations of apo-A-I and speeds, varied from about 30,000 to 167,000. The observed steady increase of the slope, $d \ln c/d(r^2)$, and thus of the molecular weight of apo-A-I, could be assigned to self-association, to impurities of high molecular weight, or to irreversible aggregation. The second possibility appeared to be ruled out by the results of polyacrylamide gel electrophoresis in the presence of Na dodecyl-SO_4, which showed no high molecular weight components. The presence of an irreversible aggregate could also be ruled out by the observation that a decrease in protein concentration or ultracentrifugal speed, or both, was accompanied by changes in molecular weight distribution toward lower limiting values, indicating the existence of an equilibrium among the interacting species of apo-A-I in solution. Furthermore, the results were not influenced by the life history of the sample, particularly whether or not it had been subjected to lyophilization or concentration by dialysis. Thus it became apparent that the ultracentrifugal behavior observed with solutions of apo-A-I was due to self-association of the protein.

In order to determine whether the self-association of apo-A-I was influenced by the nature of the solvent, we also carried out sedimentation equilibrium studies on apo-A-I dissolved in 0.02 M EDTA, pH 10.1. No differences in the aggregation patterns were observed at this higher pH. Additional experiments were conducted in 0.01 M sodium phosphate (pH 7.1, μ = 0.024) and 0.15 M KCl/0.01 M Tris (pH 8.6, μ = 0.16). Again, the behavior of apo-A-I was similar to that observed in 0.02 M EDTA, pH 8.6.

Since most of the studies were carried out in 0.02 M EDTA, pH 8.6, the following treatment of the results pertains to this aqueous system. It should be noted that the monomer molecular weight of apo-A-I is 28,014 by sequence data (4), and that similar values can be obtained by several experimental methods in the presence of dissociating agents such as sodium dodecyl sulfate, urea, or guanidinium hydrochloride (2, 3).

The concentration dependence of the apparent weight average molecular weight, $M_{w_{app}}$, of apo-A-I in 0.02 M EDTA, pH 8.6, is shown in Fig. 2, where each point represents an average of at least three determinations. One of the characteristics of this plot is the very fast increase in $M_{w_{app}}$ as a function of concentration, indicating a strong self-association.

The treatment of self-associating systems requires several basic assumptions (12–15): (*a*) the refractive index increment, dn/dc, is the same for all associating species; (*b*) the partial specific volume, $\bar{v}$, of all associating species is equal; and (*c*) the logarithm of the activity coefficient, y_i, for each species is $\ln y_i = iBM_1c$, where i is the number of species in solution, B is the second virial coefficient, higher order terms in c are negligible, and M_1 is the molecular weight of the monomer.

The concentration dependence of the apparent weight average molecular weight is given by

$$\frac{M_1}{M_{w_{app}}} = \frac{M_1}{M_w} + BM_1c \quad (2)$$

where M_1 is the molecular weight of the monomer and M_w is the weight average molecular weight corrected for the nonideal term defined as

$$M_w = \sum c_i M_i / c. \quad (3)$$

The total concentration of an associating system can be expressed in terms of the sum of the concentrations of each species:

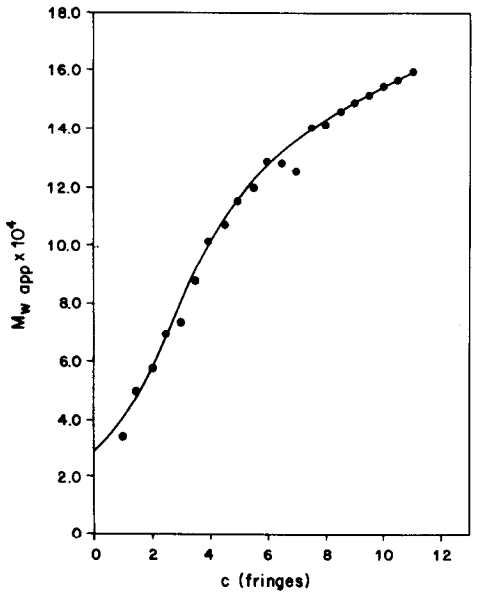


FIG. 2. Dependence of the apparent weight average molecular weight on concentration for apo A-I in 0.02 M EDTA, pH 8.6. *Filled circles* are experimental values. The *smooth curve* was obtained from the calculated values for the monomer-dimer-tetramer-octamer model (see text for details).

$$c = \sum c_i. \tag{4}$$

The concentration of the monomer is given by (12)

$$c_1 = \alpha \exp[-BM_1c] \tag{5}$$

where

$$\alpha = c \exp \int_0^c \left(\frac{M}{M_{w_{app}}} - 1\right) \frac{dc}{c}. \tag{6}$$

The value of α can be obtained by integrating a plot of $[(M_1/M_{w_{app}}) - 1]c$ *versus* c. The exponential term in equation (6) has been defined by Adams (13) as the weight fraction of the monomer, f_a.

From sedimentation equilibrium data it is possible to obtain the value of the apparent number average molecular weight $M_{n_{app}}$ (14), whose relation to the experimentally available $M_{w_{app}}$ is given by the relation

$$\int_0^c \frac{M_1}{M_{w_{app}}} dc = c\frac{M_1}{M_n} + \frac{BM_1c^2}{2} = \frac{cM_1}{M_{n_{app}}} \tag{7}$$

the value of $M_{n_{app}}$ being obtained from the area of the plot $M_1/M_{w_{app}}$ *versus* c and the number average molecular weight from the relation $M_n = c/\sum c_i/M_i$. Quantities of the form $M_1^n \sum c_i/M_i^n$ have been developed (13, 15) and are also used in the analysis of associating systems.

The concentration dependence of the apparent weight average molecular weight of apo-A-I in 0.02 M EDTA, pH 8.6, is shown in Fig. 2, which includes data obtained at several initial protein concentrations and at various ultracentrifugal speeds. Several theoretical models are outlined below.

Monomer-n-mer Associations—Associations of this type may be represented by $nP \rightleftarrows P_n$, where the association constants on a weight per volume basis are given by

$$K = \frac{c_n}{(c_1)^n}. \tag{8}$$

On the basis of the value of the apparent weight average molecular weight at the highest concentration, 167,000, the degree of association must be at least 6. We attempted to fit the data to several degrees of association, n = 6, 7, or 8. By proper combination of the relationships given, an equation was obtained in which the weight average molecular weight is expressed as a function of the concentration of the monomer, the equilibrium constant, and the nonideal term

$$\frac{1}{\frac{M_1}{cM_{w_{app}}} - BM_1} = \alpha \exp(-BM_1c) + nK_n[\alpha \exp(-BM_1c)]^n. \tag{9}$$

Of the two unknowns, K_n and BM_1, the former is eliminated if Equation 4 is written for two species, monomer and n-mer, and then combined with Equations 5 and 8 to give:

$$nc = (n-1)\alpha \exp(-BM_1c) + \frac{1}{\frac{M_1}{cM_{w_{app}}} - BM_1}. \tag{10}$$

By solving Equation 10 for BM_1, we found that, in all three cases (n = 6, 7, or 8), BM_1 decreased systematically as the protein concentration increased. The average values and their standard deviations were 1.2 ± 2.0 × 10^2 ml/g for n = 6, 1.6 ± 2.4 × 10^2 ml/g for n = 7, and 1.6 ± 2.8 × 10^2 ml/g for n = 8, indicating that BM_1 was equal to zero within experimental error in all three cases. The values of K_n were obtained from Equation 9.

We used a nonlinear least squares analysis to obtain the best fit of the experimental data to the given association model. The values of K_n and the sum of the squares of the deviations are given in Table I. In Fig. 3, the experimental values of $M_1/M_{w_{app}}$ are compared to those found by use of the calculated K_n. Table II lists the experimental and calculated values of $M_1/M_{w_{app}}$ for the monomer-heptamer system. From these results it is apparent that the aggregation of apo-A-I does not follow the models shown in Fig. 3 and may require intermediate steps in the association in order to fit the experimental results.

Indefinite Self-association—This solute-solute interaction may be described by associations of the type

$$2P_1 \rightleftarrows P_2,\ K_2 = [P_2]/[P_1]^2;\ P_1 + P_2 \rightleftarrows P_3,\ K_{23} = [P_3]/[P_1][P_2];\ \text{etc.},$$

which indicate that the self-associations continue without limit. By setting the equilibrium constants in this system equal to each other and by the use of concentration units in grams per ml, we can, according to Adams *et al.* (13-16), define the intrinsic equilibrium constant, k, as 1000 K/M_1. The series

$$c = c_1(1 + 2kc_1 + 3k^2c_1^2 + 4k^4c_1^4 + \cdots) \tag{11}$$

represents the total protein concentration as a function of k. In the case where $kc_1 < 1$, Equation 11 becomes

$$c = c_1/(1 - kc_1)^2. \tag{12}$$

The relationship between the weight and number average molecular weights and the nonideal term is given by Equation 13

$$\frac{M_1}{M_{w_{app}}} = \frac{1}{\left[\frac{2}{\frac{M_1}{M_{w_{app}}} - \frac{BM_1c}{2}} - 1\right]} + BM_1c. \tag{13}$$

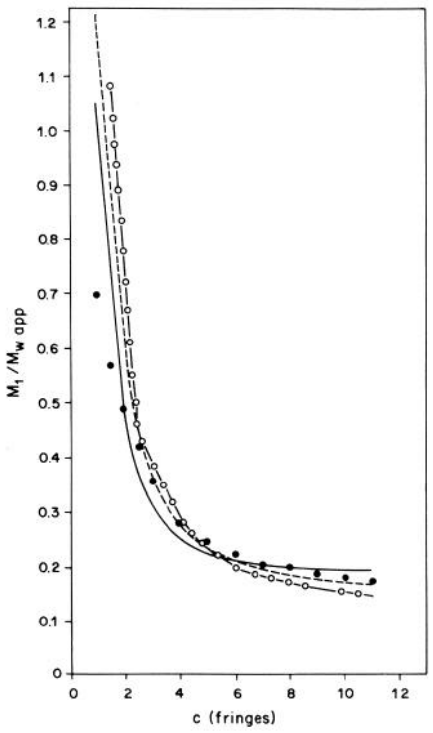
Solving Equation 13 for BM_1 at each protein concentration by successive approximation, we found the value of BM_1 for

TABLE I

Equilibrium constants calculated for different self-associating models (apo-A-1 in 0.02 M EDTA, pH 8.6, 20°)

K_n	$n = 1, 6$	$n = 1, 3, 6$	$n = 1, 2, 4, 6$	$n = 1, 7$	$n = 1, 8$	$n = 1, 2, 8$	$n = 1, 4, 8$	$n = 1, 2, 4, 8$	$n = 1, 2, 4, 6, 8$	$n = 1, 2, 3, 4, 5, 6, 7, 8$
$(M^{1-n})^a$										
K_2			$(1.6 \pm 0.5) \times 10^4$			$(4.0 \pm 0.1) \times 10^4$		$(3.3 \pm 0.2) \times 10^4$	$(3.3 \pm 0.2) \times 10^4$	$(3.3 \pm 0.2) \times 10^4$
K_3		$(8.4 \pm 3.4) \times 10^8$								$(0.0 \pm 0.4) \times 10^8$
K_4			$(0.001 \pm 0.2) \times 10^{12}$				$(1.5 \pm 0.2) \times 10^{14}$	$(3.2 \pm 0.6) \times 10^{13}$	$(3.2 \pm 1.0) \times 10^{13}$	$(3.2 \pm 0.9) \times 10^{13}$
K_5										$(0.0 \pm 0.2) \times 10^{18}$
K_6	$(9.2 \pm 0.3) \times 10^{23}$	$(8.4 \pm 0.3) \times 10^{23}$	$(8.7 \pm 0.3) \times 10^{23}$						$(0.0 \pm 0.3) \times 10^{23}$	$(0.0 \pm 0.2) \times 10^{23}$
K_7				$(4.9 \pm 0.4) \times 10^{28}$						$(0.0 \pm 0.1) \times 10^{28}$
K_8					$(9.8 \pm 0.4) \times 10^{33}$	$(2.2 \pm 0.03) \times 10^{33}$	$(1.6 \pm 0.08) \times 10^{33}$	$(2.0 \pm 0.03) \times 10^{33}$	$(2.0 \pm 0.05 \times 10^{33}$	$(2.0 \pm 0.06) \times 10^{33}$
$\sum \delta^{2b}$	5.701	4.916	3.928	9.035	12.717	0.206	1.736	0.072	0.072	0.077

[a] n designates a given n-mer (*i.e.* n = 1, 2, 4, 6, monomer, dimer, tetramer, hexamer).
[b] Sum of the squares of the deviations.



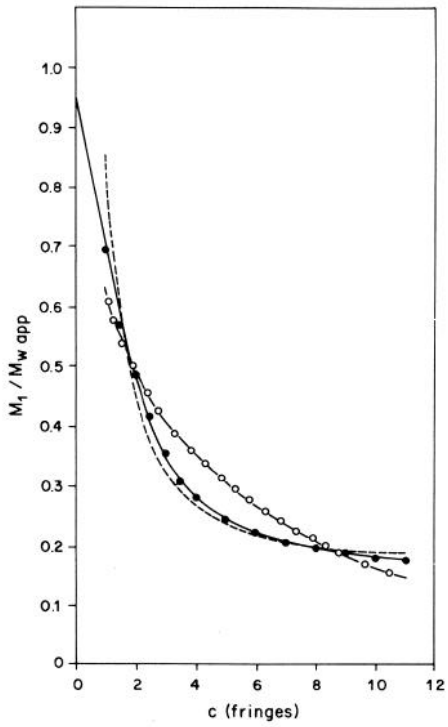


FIG. 3 (*left*). Experimental and calculated values of $M_1/M_{w_{app}}$. ●, experimental; ——, monomer-hexamer; – – –, monomer-heptamer; O——O, monomer-octamer.

FIG. 4 (*right*). Experimental and calculated values of $M_1/M_{w_{app}}$. ●, experimental; ——, monomer-dimer-tetramer-octamer; – – –, monomer-tetramer-octamer; O, definite self-association.

apo-A-I to be $-3.6 \pm 1.2 \times 10^{-1}$ ml/g. The corresponding value of k was evaluated according to Equation 13

$$\frac{M_1}{M_{w_{app}}} = 1 - kc_1 + \frac{BM_1c}{2} \qquad (14)$$

and found to be 13.85×10^2 ml/g. The ratio of $M_1/M_{w_{app}}$ for the indefinite self-association is calculated from

$$M_1/M_{w_{app}} = \frac{1 - kc_1}{1 + kc_1} + BM_1c. \qquad (15)$$

The results obtained show no correspondence between predicted and experimental values (Fig. 4). indicating that this model does not describe the experimental data.

Discrete Self-associations—Self-associations of the discrete type are represented by

$$2P_1 \rightleftarrows P_2;\ 3P_1 \rightleftarrows P_3 \cdots, \text{ etc.},$$

where the association constants on a weight per volume basis are given by $K_2 = (c_2)/(c_1)^2$; $K_3 = (c_3)/(c_1)^3$, etc. Adams (13, 14) has described in detail the methods for establishing the relationships among the equilibrium constants, the nonideal term, and the molecular weight of discrete self-associating systems. The procedure requires the elimination of each unknown until an equation is obtained having only one unknown, BM_1. The necessary steps are similar to those described for the monomer-n-mer associations, which are, in

TABLE II

Comparison of experimental values of $M_1/M_{w_{app}}$ with results from different self-associating models (Apo A-I in 0.02 M EDTA, pH 8.6)

c (fringes)	$M_1/M_{w_{app}}$ [a]			
	Experiments	$n = 1, 7$ [b]	$n = 1, 2, 8$ [b]	$n = 1, 2, 4, 8$ [b]
2	0.492	0.625	0.507	0.489
4	0.281	0.273	0.288	0.283
6	0.225	0.210	0.223	0.224
8	0.199	0.186	0.195	0.199
10	0.182	0.174	0.179	0.184
$\sum\delta^{2c}$		9.035	0.206	0.072

[a] $M_1/M_{w_{app}}$ calculated by use of K_n values in Table I (see text for details).

[b] n designates the presence of a given n-mer (*i.e.* $n = 1, 2, 8$ represents the monomer-dimer-octamer self-association).

[c] Sum of the squares of the deviations.

fact, a special case of the discrete association. Since the values of BM_1 were zero for the monomer-n-mer associations, we assumed ideality in our calculations and attempted to fit the data in terms of molecular associations. For the monomer to octamer step associations, the apparent weight average molecular weight is related to the total protein concentration, the monomer concentration, and the equilibrium constants by the equation

$$\frac{cM_{w_{app}}}{M_1} = c_1 + 2c_1^2K_2 + 3c_1^3K_3 + 4c_1^4K_4 + 5c_1^5K_5 + 6c_1^6K_6 + 7c_1^7K_7 + 8c_1^8K_8. \quad (16)$$

As noted above, Equation 16 may be reduced to represent different types of associations if some of the equilibrium constants are set equal to zero. In addition to the interactions represented by Equation 16, the data for apo-A-I were analyzed in terms of: (*a*) sequential dimerizations ($K_3 = K_5 = K_7 = 0$ and $K_3 = K_5 = K_7 = K_8 = 0$); (*b*) formation of the monomer-dimer-tetramer-octamer; (*d*) sequential tetramerization ($K_2 = K_3 = K_5 = K_6 = K_7 = 0$); (*d*) trimer formation ($K_2 = K_4 = K_5 = 0$); and (*e*) monomer-dimer-octamer association.

Using the appropriate form of Equation 16 for each of the models listed above, we analyzed the data by means of a nonlinear least squares fitting procedure which provided the best values for the equilibrium constants when the increase in molecular weight was evaluated as a function of concentration. By this procedure we also obtained the sum of the squares of the deviations, $\sum\delta^2$, between the calculated and experimental values of $M_1/M_{w_{app}}$. A summary of the equilibrium constants for all of the models described is given in Table I. It should be noted that the smallest value of $\sum\delta^2$ was found for the monomer-dimer-tetramer-octamer model because, in the consecutive dimerizations, the calculated value of K_6 is zero. Table II summarizes the calculated and experimental values of $M_1/M_{w_{app}}$ at several protein concentrations. Included in this table are the values for the best fitting model, the monomer-dimer-tetramer-octamer. Further comparison of the fits obtained for some of the models is given in Fig. 4.

Frontal Analysis Gel Filtration Chromatography—This technique was utilized as an independent method of analyzing the self-association of apo-A-I.

The elution profile for apo-A-I in 0.02 M EDTA (pH 8.6), c_0 = 0.94 mg/ml, is shown in Fig. 5*A*. The profile is characterized by

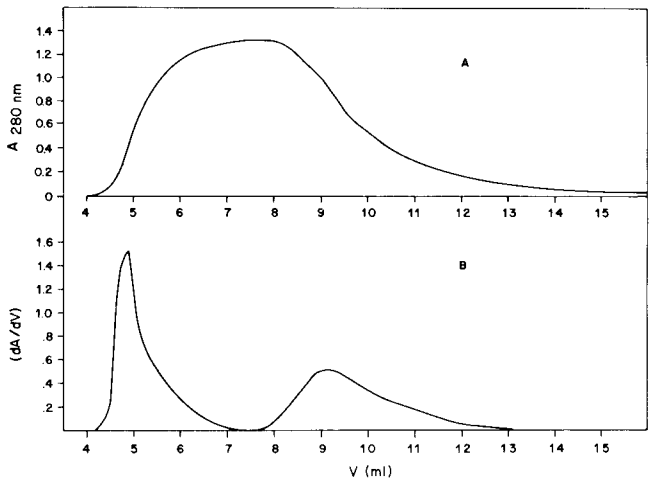


FIG. 5. *A*, elution profile for apo-A-I in 0.02 M EDTA, pH 8.6; concentration, 0.92 mg/ml; temperature, 20°. *B*, differential elution profile for apo-A-I. Conditions as in *A*.

unimodal leading and trailing edges, the former being characteristic of relatively fast associations, the latter representing a system in which several associating species are present (16). The unimodal nature of these boundaries is clearly shown in the differential elution profile at this concentration (Fig. 5*B*). These results confirm the presence of more than two species in solution and indicate that the equilibrium among species is attained rapidly compared to the time of passage through the column as shown by the steep increase of the leading edge.

DISCUSSION

Our studies have shown that, in aqueous solutions, apo-A-I self-associates under a variety of conditions of pH and ionic strength. In the range of protein concentrations used, the solute-solute interactions may best be described as an ideal discrete self-association of monomer-dimer-tetramer-octamer. Our data, obtained mainly by sedimentation equilibrium, are supported by the results of frontal analysis gel filtration chromatography, which indicate that equilibrium among the aggregating species is maintained during elution through the column. In addition, the relative insensitivity of the self-association of apo-A-I to changes in pH and ionic strength indicates that the interactions among the monomeric units are primarily nonionic. Our results are in keeping with a recent report by Stone and Reynolds (17), but do not support the observations of Gwynne *et al.* (18) who found that solutions of apo-A-I were monomeric at concentrations in the range of those used in this work. At this time we are not able to reconcile this discrepancy. It is of interest to point out that apo-A-II, the second major apolipoprotein of HDL, also self-associates in aqueous solution (26).

A comparison of the associating characteristics of apo-A-I and apo-A-II (26), under identical conditions, showed that the equilibrium constant for the formation of the dimer (K_2) is 1 order of magnitude greater for apo-A-I, indicating that the solute-solute interactions are stronger for the latter apoprotein.

The properties of A-I to self-associate offer a possible explanation for the difficulty in obtaining complete relipidation of apo-A-I with the high density lipoprotein lipids under the usual conditions of reconstitution[2] (1), and also suggest

[2] M. C. Ritter and A. M. Scanu, unpublished results.

that apo-A-I has to be in its monomeric form (protein concentration $\leq 10^{-7}$ M (1, 16)) to bind lipids readily. In this regard, it is of interest to note that apo-A-II, in contrast to apo-A-I, has no such a requirement and binds lipids even at concentrations at which dimerization of the protein occurs.[2, 3]

The role which the self-association of apo-A-I and apo-A-II play in determining the overall structure of human HDL cannot be assessed from the present data, or from those available in the literature. It has been reported that, in their lipid-free form, these apoproteins form a 1:1 molar complex at specific concentrations (19). Studies in our laboratory have shown that the amount of apo-A-I recovered as a protein·lipid complex increases when mixtures of apo-A-I and apo-A-II are present in solution.[2] Before further conclusions are drawn, however, the capability of C-peptides to form protein protein complexes with apo-A-I or apo-A-II, or both, must be assessed.

Furthermore, even though studies on binding of apo-HDL or its individual apoproteins with HDL lipids (20–22) or other ligands (19) have been reported, our current findings indicate that it is necessary to determine the solution properties of these apoproteins before such interactions are examined and interpreted. Studies of the properties of apo-A-I from animal species other than man, may also provide further insights into the mechanism of lipid binding and the overall structure of HDL. Recently, Jonas (23, 24) reported on the solution properties of bovine apo-A-I. The observations derived from studies on fluorescence polarization and ultracentrifugal techniques indicate that bovine apo-A-I forms stable tetramers within a wide range of pH and ionic strength. In accord with the interpretation of our results the author considered the possibility that the strong protein-protein interactions ($K_a < 10^{-7}$ M) could compete with the capacity of bovine apo-A-I to associate with lipids.

Barbeau and Scanu (25) have conducted studies on apo-A-I isolated from the serum of rhesus monkey (*Macaca mulatta*). In contrast to human and bovine apo-A-I, this apoprotein was found to be monomeric over a wide range of concentrations. Perhaps more significantly, rhesus apo-A-I bound more lipids than human apo-A-I under comparable conditions of relipidation.

From the above it becomes apparent that the state of association of a protein in solution plays a major role in determining its capacity to bind lipids or other ligands *in vitro*. According to our present work, apo-A-I is a good example of this.

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