Biophysical Chemistry 13 (1981) 29–38
North-Holland Publishing Company

EVALUATION OF THE MIXED INTERACTION BETWEEN APOLIPOPROTEINS A-II AND C-I BY EQUILIBRIUM SEDIMENTATION [1]

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Received 7 March 1980

The mixed interaction between human apolipoproteins C-I and A-II, each of which self-associate in aqueous solution, has been evaluated by sedimentation equilibrium measurements. In order to simplify data analysis apoC-I and apoA-II were modified by treatment with 2-nitrophenylsulfenyl chloride and tetranitromethane respectively. The molecular properties of the resulting derivatives, S-apoC-I and N-apoA-II, each of which have appreciable extinction coefficients above 350 nm, were indistinguishable from the corresponding unmodified species. Sedimentation equilibrium data were obtained with mixtures of S-apoC-I and native apoA-II, N-apoA-II and native apoC-I, and native apoC-I and native apoA-II. Mixed complex formation was detected readily with all mixtures investigated. The combined results were most consistent with a single mixed oligomer containing 2 molecules of apoA-II and 4 molecules of apoC-I. The corresponding equilibrium constant was $31\,248 \pm 890$ (g/gm)^5.

1. Introduction

Plasma lipoproteins are large lipid-protein complexes that are involved in the transport and metabolism of endogenous and exogenous nonpolar lipids, primarily cholesterol (cholesterol esters) and triglycerides. Being held together by non-covalent interactions, the alipoprotein and lipid composition of plasma lipoproteins is sensitive to environment and serves as the hallmark for many abnormal metabolic states. Plasma lipoproteins have been separated classically on the basis of electrophoretic mobility or hydrated density. Each of the resulting fractions has been shown subsequently to be heterogeneous with respect to particle size and apolipoprotein composition and each of the nine apolipoproteins which have been described to date are integral components of several plasma lipoprotein classes [1–3]. In addition, the concentration and composition of plasma lipoproteins also changes with lipid metabolism, and thus the interrelationships between the structure and function of plasma lipoproteins, especially with respect to their apolipoproteins, are quite complex. In order to understand more fully the role of apolipoprotein–apolipoprotein and apolipoprotein–lipid interactions in the quaternary organization of plasma lipoproteins we have begun a systematic investigation of the mixed interactions between alipoproteins in aqueous solution (for a recent review see [3]). In the present report we describe the mixed interaction between apoA-II and apoC-I.

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2. Materials and methods

The isolation and purification schemes employed for human apoC-I and apoA-II have been described previously [4,5]. Protein concentrations were determined by absorbancy at 280 nm as measured on a Beckman Acta III spectrophotometer. The extinction coefficients $\epsilon_{280}^{1\,mg/ml} = 0.875$ and 0.632 for apoC-I and apoA-II, respectively, were determined by measuring the protein mass, by amino acid analysis, of a solution of known absorbancy. Glass redistilled water and reagent grade chemicals were used in the preparation of all solutions. A radiometer pH meter, type PHM 26, was used for pH measurements.

ApoA-II was nitrated with tetranitromethane using the method described by Sokolovsky, Riordan and Vallee [6] with minor modifications [7]. The resulting protein, designated NO_2-apoA-II, was fractionated on a 90 × 1.4 cm column of Sephadex G-150 (superfine) in buffer. The degree of nitration was determined spectrally by using an extinction coefficient of 2200 at 381 nm [8]. Protein concentrations were estimated by amino acid analysis or radial immunodiffusion. The molar extinction coefficient for NO_2-apoA-II used in this study was calculated to be 54 640 at 280 nm.

ApoC-I was sulfenylated with 2-nitrophenylsulfenyl chloride as described by Scoffone, Fontana and Rocchi [9]. The resulting derivative, S-apoC-I, was fractioned on a 90 × 1.4 cm column of Sephadex G-150 (superfine) resin. The degree of sulfenylation was determined by using a molar extinction coefficient of 4000 at 365 nm.

2.1. Sedimentation equilibrium

Sedimentation equilibrium experiments were performed in a Spinco Model E ultracentrifuge equipped with a photoelectric ultraviolet scanner and a temperature control system. Double sector cells with charcoal-filled epon centerpieces and quartz windows were centrifuged in an AN-G Titanium rotor. The temperature was maintained at 21°C and the layering fluid was omitted in each case. Initial protein concentrations were determined by absorbancy measurements at 280 nm prior to each run. An initial scan (λ = 280 to 381 nm) of each cell at time zero when the rotor reached the desired speed allowed the calculation of extinction coefficients for each run at several different wave lengths. Particular care was taken in the choice of the band width used for each cell, especially when obtaining data at the longer wavelengths. For instance, Beer's law was not obeyed at 381 nm when the slit of the monochromator was larger than 0.5 mm. The pen deflection was linear with optical density at 381 nm with slits below 0.5 mm. The data presented in this manuscript were obtained under conditions in which Beer's law was obeyed.

Equilibrium was monitored by comparing the concentration profiles at 4 hour intervals after a period of 24 to 72 hours. After equilibrium had been obtained at each rotor speed investigated, baseline determinations were made at each wavelength used by increasing the rotor speed to 44 000 rpm.

Apparent weight average molecular weights (M_w^{app}) were obtained in the following manner. The ln C versus radius squared data were fit to the following equation using a nonlinear least squares technique to obtain the least square values of A, B and C.

$$Y(x) = (A + Bx)e^{Cx} \tag{1}$$

where:

$$Y = \ln C, \quad x = r^2.$$

M_w^{app} versus concentration profiles were then generated analytically by using the following equation:

$$M_w^{app} = \frac{2RT}{\omega^2(1 - \bar{v}\rho)} [(B + AC + BCX)e^{Cx}], \tag{2}$$

where: R is the gas constant; T is the absolute temperature; ω is the angular velocity; $\bar{v}$ is the partial specific volume; and ρ is the solvent density.

Stoichiometry and equilibrium constants were evaluated as described in the appendix.

2.2. Circular dichroic measurements

Circular dichroic spectra were obtained with a Cary model 61 spectropolarimeter equipped with a pockel's cell and thermostatted cell. The mean residue ellipticity, $[\theta]$, at wavelength λ, was calculated by the following equation:

$$[\theta]_\lambda = \frac{(\theta)_{obs} \times 116}{10 \times l \times c}, \tag{3}$$

where: $(\theta)_{obs}$ is the observed ellipticity; 116 is the mean residue molecular weight of apoC-I (114 was used for apoA-II), l is the path length in cm and c is the protein concentration in gms/ml.

3. Results

Analysis of the mixed interaction of two self-associating systems by sedimentation equilibrium is simplified if the absorption spectra of the two systems are quite different from one another. We have presented the framework for this analysis previously [10] and have expanded the treatment of data in the Appendix of this manuscript. Briefly, if data are collected with the photoelectric scanner at a wavelength where only one of the self-associating systems has appreciable ab-

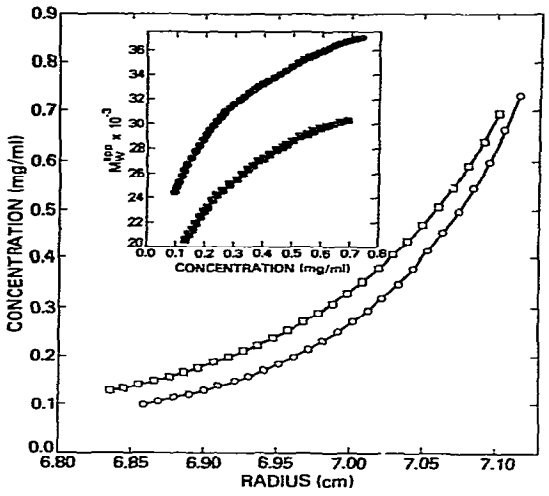

Fig. 1. Results of sedimentation equilibrium measurements of N-apoA-II (0.317 mg/ml) in the absence (□) and presence (○) of apoC-I (0.382 mg/ml) plotted as the absorbancy at 381 nm versus the distance from the center of rotation. The rotor speed was 18 000 rpm and the solution heights were 0.57 cm. The buffer used was 0.1 M potassium chloride, 0.01 M TRIS, 0.001 M sodium azide, pH 7.4 and the temperature was maintained at 21°C. The solid line represents the fit of the data to a model involving one mixed oligomer containing 2 molecules of apoA-II and 4 molecules of apoC-I. (See text for details.).

Inset: Plot of the weight average molecular weight of N-apoA-II in the absence (■) and presence (●) of apoc-I calculated from the data in fig. 1 as indicated in the Methods section.

sorption, then the resulting absorption versus radius profiles contain contributions only from those species that contain the absorbing protein. For instance, mixtures of apoA-II and apoC-I contain at least five species in solution, monomers and dimers of apoA-II [5] and monomers, dimers and tetramers of apoC-I [11], plus any mixed oligomers. If those species in solution that contain apoA-II could be monitored independently, then the number of terms in eq. (10) in the Appendix would be reduced from a minimum of six to a minimum of three terms; species containing only apoC-I, monomers, dimers and tetramers, would not contribute to the absorption profile.

We have modified the absorption profile of apoA-II by treatment with tetranitromethane. This procedure causes a "red shift" in the absorption profile due to nitration of tyrosine residues. The molecular properties of the resulting derivative, reported previously [12], closely resemble the native species. Briefly, 1) both apolipoproteins self-associate according to a monomer-dimer scheme; 2) both apolipoproteins undergo major concomitant changes in structure upon self-association, the secondary structure increasing with increasing protein concentration; 3) both apolipoproteins interact specifically with apoA-I in aqueous solution; and 4) both apolipoproteins interact with antibodies prepared against native apoA-II [12].

The results of a sedimentation equilibrium experiment with N-apoA-II and native apoC-I are presented in fig. 1. Since the data were collected at 381 nm, the isosbestic point for nitrated tyrosine, the presence of a mixed interaction between apoC-I and N-apoA-II is demonstrated readily. If mixed oligomers were not present in solution then the profiles of N-apoA-II at 381 nm in the presence and absence of apoC-I would be the same. The observed absorption profile is quite different and the apparent weight average molecular weight of N-apoA-II is increased in the presence of apoC-I.

We have analyzed these data as indicated in the Appendix according to eq. (4):

$$\xi(r) = C_{10}(m)\exp[A_{10}M_{10}(r^2 - m^2)] + K_{20}(m)C_{10}(m)^2\exp[A_{20}M_{20}(r^2 - m^2)] + \sum_{i=1}^{n}\sum_{j=1}^{m} K_{ij}(m)C_{10}(m)^i C_{01}(m)^j \times \left(\frac{iM_{10}\epsilon_{10}}{iM_{10} + jM_{01}}\right)\exp[A_{ij}M_{ij}(r^2 - m^2)]. \quad (4)$$

The sum of the squares of the deviations (δ^2) between experimental and calculated absorption versus radius profiles was determined for different mixed oligomer stoichiometries. All data were consistent with the presence of a single mixed oligomer. The variation of δ^2 with the number of apoA-II molecules in the mixed oligomer showed a sharp minimum at two molecules and the best fit of the data when varying the stoichiometry of apoC-I in the mixed oligomer was obtained with 4 molecules of apoC-I (fig. 2). The exact value of the equilibrium constant for mixed oligomer formation, K_{ij}, cannot be obtained from data at 381

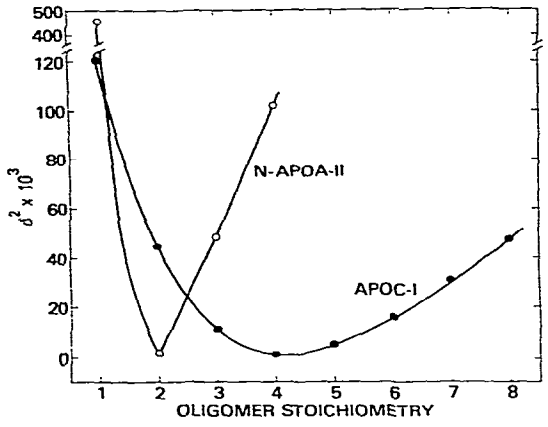

Fig. 2. Plot of the sum of the squares of the deviations between experimental and theoretical data as obtained by a simultaneous analysis of the absorption profiles at 280 nm and 381 nm of N-apoA-II and apoC-I, obtained under the conditions described in fig. 1, as a function of the stoichiometry of the mixed oligomer. (See text and Appendix for details).

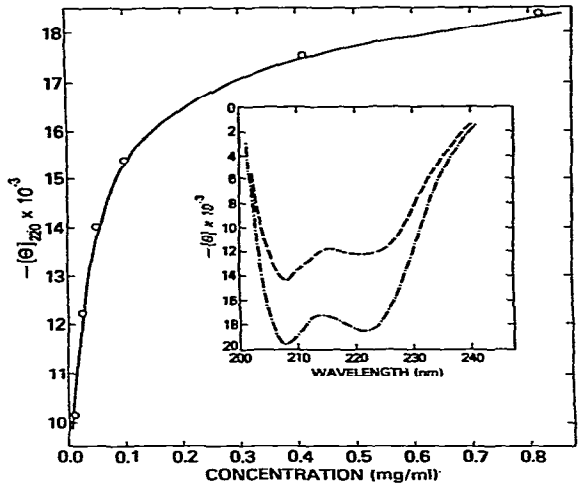

Fig. 3. Plot of the mean residue ellipticity at 220 nm of S-apoC-I as a function of protein concentration. The buffer used was 0.1 M potassium chloride, 0.001 M sodium azide, 0.01 M TRIS, pH 7.4 and the temperature was maintained at 24°C. The solid line represents the mean residue ellipticity at 220 nm of native apoC-I as a function protein concentration reported previously (4).

Inset: The mean residue ellipticity of S-apoC-I as a function of wavelength. The buffer was the same as that in fig. 2 and the protein concentrations were: 0.82 mg/ml (– . –) and 0.026 mg/ml (– –).

nm only; in the present case K_{24} cannot be separated mathematically from $C_{01}(m)^4$ in eq. (4). However, as discussed in detail in the Appendix, a simultaneous analysis of absorption profiles at 280 nm and 381 nm yields the corresponding equilibrium constant for mixed oligomer formation. The experimental data we have obtained for N-apoA-II and apoC-I is most consistent with the presence of a single mixed oligomer containing 2 molecules of apoA-II and 4 molecules of apoC-I, with $K_{24} = 179\,489 \pm 770$ (ℓ/gm)^5.

We have also analyzed the mixed interaction between apoA-II and apoC-I by using a derivative of apoC-I. Since apoC-I contains one tryptophan and no tyrosine or cystine residues, we have used sulfenylation instead of nitration for the modification of apoC-I. The resulting derivative had an absorption spectra that was "red shifted" well above 300 nm with a maximum at 365 nm. The molecular properties of sulfenylated apoC-I, S-apoC-I, were very similar to those reported previously for native apoC-I [4]. Both apolipoproteins self-associate according to a monomer–dimer–tetramer association scheme with major concomitant changes in secondary structure. The mean residue ellipticity of S-apoC-I decreases with decreasing protein concentration and is within experimental error of that obtained with the native apolipoprotein at all concentrations investigated (fig. 3). As was found previously for unmodified apoC-I, the shape of the circular dichroic spectra changes with decreasing protein concentration (Inset, fig. 3). Finally, S-apoC-I cross reacts with antibodies prepared against unmodified apoC-I.

The results of sedimentation equilibrium measurements, obtained by absorbancy measurements at 365 nm of S-apoC-I in the presence and absence of unmodified apoA-II, are given in fig. 4. The absorbancy profile at 365 nm is changed in the presence of unmodified apoA-II and the apparent weight average molecular weight of S-apoC-I is increased which is indicative of mixed oligomer formation. In this case, since only those species containing S-apoC-I contribute to data collected at 365 nm, eq. (10) in the Appendix reduces to:

$$\xi(r) = C_{01}(m)\exp[A_{01}M_{01}(r^2 - m^2)]$$

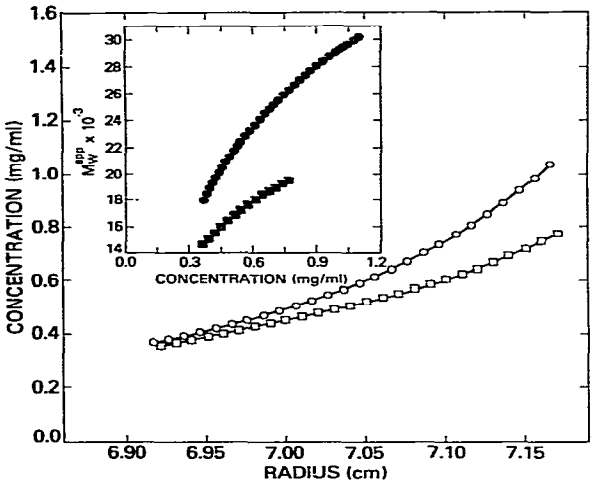

Fig. 4. Results of sedimentation equilibrium measurements of S-apoC-I (0.414 mg/ml) in the absence (□) and presence (○) of apoA-II (0.422 mg/ml) plotted as the absorbancy at 365 nm as a function of the distance from the center of rotation. The rotor speed was 15 000 rpm and the column heights were 0.57 cm. The buffer used 0.01 M potassium chloride, 0.01 M TRIS, 0.001 M sodium azide, pH 7.4 and the temperature was maintained at 21°C; The solid line represents the fit of the data to a model involving a mixed oligomer containing 2 molecules of apoA-II and 4 molecules of S-apoC-I. (See text for details.

Inset: Plot of the apparent weight average molecular weight of S-apoC-I in the absence (▪) and presence (•) of apoA-II calculated from the data in fig. 2 as indicated in the Methods section.

$$+ K_{02}(m)C_{01}(m)^2\exp[A_{02}M_{02}(r^2 - m^2)]$$

$$+ K_{04}(m)C_{01}(m)^4\exp[A_{04}M_{04}(r^2 - m^2)]$$

$$+ \sum_{i=1}^{n}\sum_{j=1}^{m} K_{ij}(m)C_{10}(m)^iC_{01}(m)^j$$

$$\times\left(\frac{iM_{01}\epsilon_{01}}{iM_{10} + jM_{01}}\right)\exp[A_{ij}M_{ij}(r^2 - m^2)]. \qquad (5)$$

We have analyzed the sedimentation equilibrium data for mixtures of S-apoC-I and native apoA-II as described above for nitrated apoA-II and native apoC-I. The δ^2 between experimental and theoretical absorption profiles as a function of the mixed oligomer

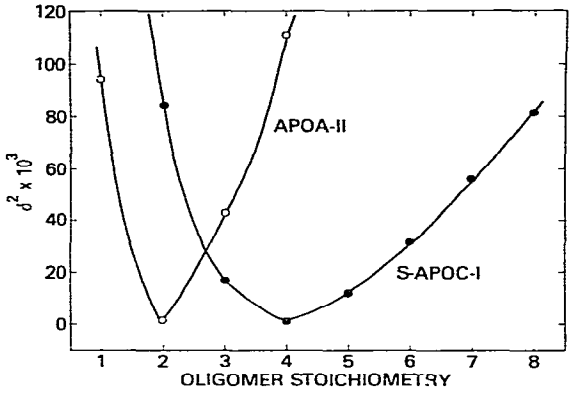

Fig. 5. Plot of the sum of the squares of the deviations between experimental and theoretical data as obtained by a simultaneous analysis of the absorption profiles at 280 nm and 365 nm of S-apoC-I and apoA-II, obtained under the conditions described in fig. 3, as a function of the stoichiometry of the mixed oligomer. (See text and Appendix for details).

stoichiometry is summarized in fig. 5. As was found for the mixture of nitrated apoA-II and native apoC-I, the equilibrium profile of mixtures of S-apoC-I and native apoA-II are most consistent with the presence of a single mixed oligomer containing 4 molecules of apoC-I and 2 molecules of apoA-II. The corresponding equilibrium constant for mixed oligomer formation was $K_{24} = 22\,977 \pm 50$ (ℓ/gm)^5.

The combined data with nitrated apoA-II and sulfenylated apoC-I leave little doubt that apoA-II and apoC-I interact in aqueous solution to form specific mixed oligomers. In addition, the stoichiometry of the mixed oligomer is well defined, consisting of 2 molecules of apoA-II and 4 molecules of apoC-I. The agreement between the data using modified apoA-II and modified apoC-I with respect to the equilibrium constant for mixed oligomer formation is, however, poor, 179 000 (ℓ/gm)^5 versus 22 700 (ℓ/gm)^5, respectively. It should be noted, however, that with a knowledge of the mixed oligomer stoichiometry, one can obtain reliable estimates of the mixed oligomer equilibrium constant by analysis of sedimentation equilibrium measurements of mixtures of unmodified apoA-II and apoC-I. In this case, eq. (10) reduces to:

$$\xi(r) = C_{10}(m)\epsilon_{10}\exp[A_{10}M_{10}(r^2 - m^2)]$$

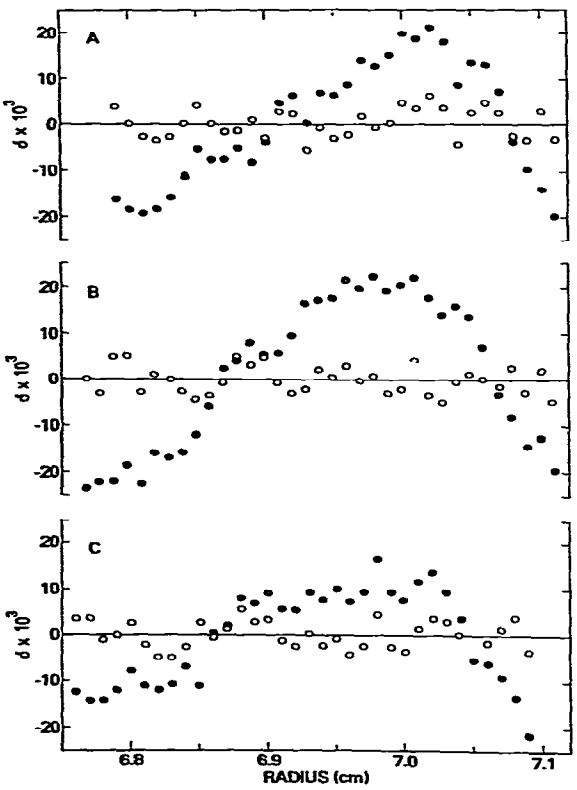

Fig. 6. Comparison of the sum of the squares of the deviations between experimental and theoretical absorbancy profiles at 280 nm for two different association schemes at three different mixtures of apoA-II and apoC-I. The filled symbols represent the best fit of the data to a model in which there are no mixed oligomers in solution. The open symbols represent the best fit for a model in which there is one mixed oligomer containing 4 molecules of apoC-I and two molecules of apoA-II. The initial concentrations of protein were: panel A, apoA-II = 0.16 mg/ml, apoC-I = 0.80 mg/ml; panel B, apoA-II = 0.32 mg/ml, apoC-I = 0.40 mg/ml; panel C, apoA-II = 0.48 mg/ml, apoC-I = 0.20 mg/ml. The buffer used was 0.1 M potassium chloride, 0.01 M TRIS, 0.001 M sodium azide, pH 7.4 and the temperature was maintained at 21°C. The rotor speed was 14,000 rpm and the column heights were 0.57 cm. (See text for details).

$$+K_{20}(m)C_{10}(m)^2\epsilon_{10}\exp[A_{20}M_{20}(r^2-m^2)]$$

$$+C_{01}(m)\epsilon_{01}\exp[A_{01}M_{01}(r^2-m^2)]$$

$$+K_{02}(m)C_{01}(m)^2\epsilon_{01}\exp[A_{02}M_{02}(r^2-m^2)]$$

$$+K_{04}(m)C_{01}(m)^4\epsilon_{01}\exp[A_{04}M_{04}(r^2-m^2)]$$

$$+K_{24}(m)C_{10}(m)^2C_{01}(m)^4$$

$$\times\left(\frac{M_{20}\epsilon_{10}+M_{04}\epsilon_{01}}{M_{20}+M_{04}}\right)\exp[A_{24}M_{24}(r^2-m^2)]. \quad (6)$$

The unknowns in eq. (6) are $K_{24}(m)$, $C_{10}(m)$ and $C_{01}(m)$. We have analyzed simultaneously the absorption profiles obtained at equilibrium at 280 nm from three different mixtures of apoA-II and apoC-I. The least squares analysis gave a value of K_{24} = 31 250 ± 890 (ℓ/gm)^5. As an illustration of the quality of the fit of the final model to the experimental data we have illustrated the deviation between calculated and experimental absorption versus radius profiles for all three cells in fig. 6. The deviation for all three mixtures of apoA-II and apoC-I was small and a random function of the distance from the center of rotation. We have also illustrated in fig. 6 the deviation between calculated and experimental data if K_{24} is assumed to equal zero. In this case the deviation is much larger and a non-random function of the distance from the center of rotation; thus, although eq. (6) contains many variables one cannot account for this data with self association alone.

With these data and a knowledge of the association schemes and equilibrium constants for the self-association of apoA-II and apoC-I, one can predict the distribution of species for different mixtures of apoC-I and apoA-II. The distribution obtained is a complex function of not only the weight ratio of apoC-I to apoA-II, but also a function of the total concentration of protein (fig. 7). This results from a competition between homogeneous and mixed oligomer formation. It is stressed that this distribution corresponds to room temperature, atmospheric pressure and aqueous solutions in the buffer used in this study. A change in these parameters or the addition of other ligands, including other apolipoproteins, may cause a redistribution of oligomeric species.

4. Discussion

The solution properties of apolipoproteins have been the subject of numerous investigations [12–17] and

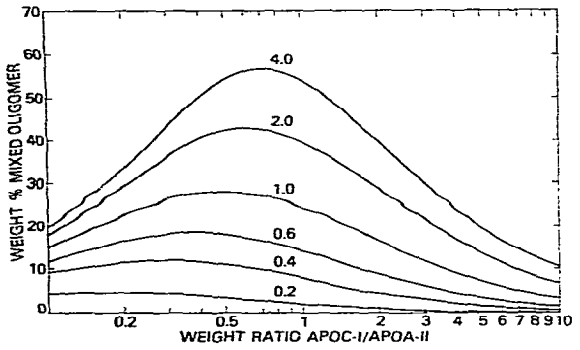

Fig. 7. Theoretical plot of the weight % of the mixed oligomer, $(apoA\text{-}II)_2(apoC\text{-}I)_4$, as a function of the weight ratio of apoC-I to apoA-II at several different concentrations of apoC-I (mg/ml) shown above each curve. The equilibrium constants used were: K_{20} = 8.52 (ℓ/gm), K_{02} = 17.3 (ℓ/gm), K_{04} = 1386 $(ℓ/gm)^3$, K_{24} = 31248 $(ℓ/gm)^5$.

have been reviewed recently [3]. Each of the apolipoproteins which have been well characterized self-associates at neutral pH with concomitant changes in secondary and tertiary structure. The monomeric forms of apoA-II and apoC-I, with molecular weights of 17 400 and 6630, respectively, are loosely folded, have a high degree of exposure of nonpolar residues to solvent and in many ways resemble random coils. Presumably their amino acid sequence is such that in their monomer forms there is little gain in free energy upon the sequestering of nonpolar groups from solvent. The shielding of nonpolar residues from solvent is accomplished for these systems by self-association. ApoA-II was shown previously to self-associate according to a monomer–dimer scheme, whereas the solution properties of apoC-I are consistent with a monomer–dimer–tetramer self-association scheme. Thus mixtures of apoA-II and apoC-I contain at least five species, monomers and dimers of apoA-II and monomers, dimers, and tetramers of apoC-I, plus any mixed oligomers, and a quantitative analysis of the stoichiometry and equilibrium constant for mixed interaction is quite complex. The analysis of this system was simplified by modification of the absorption spectrum of apoA-II by treatment with tetranitromethane. The behavior of the resulting derivative (N-apoA-II) in the Model E ultracentrifuge was monitored, in the presence and absence of apoC-I, by absorbancy measurements at 381 nm. Since apoC-I does not absorb uv radiation at 381 nm, only those species containing N-apoA-II contributed to the experimental data. Using this technique the presence of a mixed interaction between N-apoA-II and apoC-I was demonstrated readily; the combined results are most consistent with the presence of a single oligomeric species that contained 2 molecules of N-apoA-II and 4 molecules of apoC-I.

In order to rule out any artifacts due to a difference in the molecular properties of N-apoA-II and native apoA-II, this system was also analyzed using native apoA-II and a derivative of apoC-I (S-apoC-I) obtained by treatment with sulfenyl chloride. The molecular properties of S-apoC-I were indistinguishable from those of the native species. In this case the absorbancy profile in the Model E ultracentrifuge at 365 nm in the presence and absence of native apoA-II resulted only from those species that contained S-apoI. Again, mixed oligomer formation was demonstrated easily and the combined results were most consistent with a mixed oligomer stoichiometry of 2 molecules of apoA-II and 4 molecules of S-apoC-I.

Finally, with a knowledge of the mixed oligomer stoichiometry the corresponding equilibrium constant for mixed oligomer formation was evaluated from sedimentation equilibrium measurements of mixtures of the native, unmodified apolipoproteins. These combined data demonstrate experimentally that apoA-II and apoC-I specifically interact in aqueous solution to form a mixed oligomer containing two molecules of apoA-II and four molecules of apoC-I. We have shown previously that apoA-II and apoA-I, an apolipoprotein with a monomer molecular weight of 28 016 that self-associates according to a monomer–tetramer–octamer scheme, specifically interact to form a mixed oligomer containing equi-molar amounts of apoA-II and apoA-I. We are presently investigating the mixed interaction between apoC-I and apoA-I in aqueous solution. These studies will form the framework for the quantitative evaluation of the complex interrelationships between plasma lipoproteins.

Appendix
(by Luigi Servillo and James C. Osborne, Jr.)

For interacting as well as non-interacting multicomponent systems the concentration of each species

in solution at equilibrium in the analytical ultracentrifuge is given by the following equation [18]:

$$C_i(r) = C_i(m)\exp[A_i M_i(r^2 - m^2)], \quad (1)$$

where: C_i is the concentration of species i in mg/ml (activity coefficients are assumed to be unity); r is the radial distance in cm from the center of rotation to the meniscus of the cell; M_i is the molecular weight of species i; and $A_i \equiv \omega^2(1 - \bar{v}\rho)/2RT$, where ω is the angular velocity in radians per second, R is the gas constant in ergs per degree per mole, T is the absolute temperature in degrees Kelvin, $\bar{v}$ is the partial specific volume in ml/gm of species i (which is assumed to be independent of pressure), and ρ is the solvent density in gm/ml.

For a self-associating system at equilibrium in the ultracentrifuge

$$K_i(m) = C_i(m)/C_1(m)^i, \quad (2)$$

or

$$C_i(m) = K_i(m)C_1(m)^i. \quad (3)$$

Combining eqs. (1) and (3) one obtains:

$$\sum_i C_i(r) = \sum_i K_i(m)C_1(m)^i \exp[A_i M_i(r^2 - m^2)], \quad (4)$$

where: $K_i(m)$ corresponds to the equilibrium constant at atmospheric pressure for the formation of species i, $C_1(m)$ is the concentration of the protomer at the meniscus, and $K_1 \equiv 1$.

For two self-associating systems that also exhibit mixed interactions eq. (4) becomes [19,20]:

$$\sum_{i=1}^{n} C_{i0}(r) + \sum_{j=1}^{n} C_{0j}(r) + \sum_{i=1}^{n}\sum_{j=1}^{m} C_{ij}(r)$$
$$= \sum_{i=1}^{n} K_{i0}(m)C_{10}(m)^i \exp[A_{i0}M_{i0}(r^2 - m^2)]$$
$$+ \sum_{j=1}^{n} K_{0j}(m)C_{01}(m)^j \exp[A_{0j}M_{0j}(r^2 - m^2)]$$
$$+ \sum_{i=1}^{n}\sum_{j=1}^{m} K_{ij}(m)C_{10}(m)^i C_{01}(m)^j$$
$$\times \exp[A_{ij}M_{ij}(r^2 - m^2)], \quad (5)$$

where: the subscripts $i0$ and $0j$ correspond to species due to the self-association of each system; the subscripts ij correspond to the mixed oligomers containing i protomers of the first and j protomers of the second self-associating system; $C_{10}(m)$ and $C_{01}(m)$ correspond to the concentration of the protomers at the meniscus; and K_{10} and $K_{01} \equiv 1$.

The primary data obtained from an ultracentrifuge equipped with a photoelectric ultraviolet scanner is the total absorbance of the solute as a function of the distance from the center of rotation. In terms of absorbancy, assuming that the extinction coefficient of each species is independent of radial position, eq. (4) becomes:

$$\xi(r) = \sum_i \xi_i(r) = \sum K_1(m)C_1(m)^i \epsilon_i$$
$$\times \exp[A_i M_i(r^2 - m^2)], \quad (6)$$

where: $\xi(r)$ and $\xi_i(r)$ correspond to the total absorbancy and the absorbancy of species i respectively, ϵ_i is the extinction coefficient of species i. Assuming that the extinction coefficient of the protomer does not change with oligomer formation, equation (6) becomes:

$$\xi(r) = \sum_i K_i(m)C_1(m)^i \epsilon_1 \exp[A_i M_i(r^2 - m^2)]. \quad (7)$$

For two self-associating systems showing mixed associations equation (5) in terms of absorbancy becomes:

$$\xi(r) = \sum_{i=1}^{n} \xi_{i0}(r) + \sum_{j=1}^{m} \xi_{0j}(r) + \sum_{i=1}^{n}\sum_{j=1}^{m} \xi_{ij}(r)$$
$$= \sum_{i=1}^{n} K_{i0}(m)C_{10}(m)^i \epsilon_{10} \exp[A_{i0}M_{i0}(r^2 - m^2)]$$
$$+ \sum_{j=1}^{n} K_{0j}(m)C_{01}(m)^j \epsilon_{01} \exp[A_{0j}M_{0j}(r^2 - m^2)]$$
$$+ \sum_{i=1}^{n}\sum_{j=1}^{m} K_{ij}(m)C_{10}(m)^i C_{01}(m)^j$$
$$\times \epsilon_{ij} \exp[A_{ij}M_{ij}(r^2 - m^2)], \quad (8)$$

where: ϵ_{10} and ϵ_{01} are the extinction coefficients of the protomers of the two interacting systems and ϵ_{ij} is the extinction coefficient of the ij mixed oligomer. In the present treatment we have assumed that the ex-

tinction coefficient of the protomers do not change upon self- or mixed association, therefore ϵ_{ij} is equal to the weight average of the two protomeric extinction coefficients as per the following equation [21]:

$$\epsilon_{ij} = \frac{iM_{10}\epsilon_{10} + jN_{01}\epsilon_{01}}{iM_{10} + jM_{01}},$$

where: M_{10} and M_{01} are the molecular weights of the protomeric species.

Combining eqs. (8) and (9) one obtains the following general equation which is applicable to the mixed interaction between any two self-associating systems:

$$\xi(r) = \sum_{i=1}^{n} K_{i0}(m)C_{10}(m)^i\epsilon_{10}\exp[A_{i0}M_{i0}(r^2 - m^2)] + \sum_{j=1}^{m} K_{0j}(m)C_{0j}(m)^j\epsilon_{01}\exp[A_{0j}M_{0j}(r^2 - m^2)] + \sum_{i=1}^{n}\sum_{j=1}^{m} K_{ij}(m)C_{10}(m)^iC_{01}(m)^j \times \left(\frac{iM_{10}\epsilon_{10} + jM_{01}\epsilon_{01}}{iM_{10} + jM_{01}}\right)\exp[A_{ij}M_{ij}(r^2 - m^2)]. \quad (10)$$

In theory, with a knowledge of the equilibrium constants for self-association (K_{i0} and K_{0j}), one can determine the stoichiometry and equilibrium constants for the mixed interaction between two self-associating systems by a least squares analysis of sedimentation equilibrium data according to eq. (10). The uniqueness of the resulting parameters is however dependent critically upon the total number of species in solution. For instance, in the present study there are at least five species in solution, i.e., monomers and dimers of apoA-II and monomers, dimers, and tetramers of apoC-I, plus any mixed oligomers. The absorption profile at equilibrium in the ultracentrifuge is a composite of all species in solution, as given by eq. (10), and a least squares analysis in this case does not yield unique values of mixed oligomer stoichiometry and equilibrium constants. The analysis is simplified greatly when the two self-associating proteins have sufficiently different absorption spectra [10]. If data are obtained under conditions in which only one of the two proteins contributes to the absorption profile, then the number of terms in equation (10) can be reduced significantly. In the present study we have modified the absorption spectra of apoA-II by nitration with tetranitromethane. The molecular properties of this derivative, N-apoA-II, are not significantly different from those of the native species; however, the absorption spectrum is "red shifted" by approximately 100 nm. The equilibrium distribution of N-apoA-II in the presence and absence of native apoC-I was obtained by absorbancy measurements at 381 nm, the isosbestic point for nitrated tyrosine. Since these data contain contributions only from those species in solution that contain N-apoA-II, eq. (10) reduces to:

$$\xi(r) = C_{10}(m)\epsilon_{10}\exp[A_{10}M_{10}(r^2 - m^2)] + K_{12}(m)C_{10}(m)^2\epsilon_{10}\exp[A_{20}M_{20}(r^2 - m^2)] + \sum_{i=1}^{n}\sum_{j=1}^{m} K_{ij}(m)C_{10}(m)^iC_{01}(m)^j \times \left(\frac{iM_{10}\epsilon_{10}}{iM_{10} + jM_{01}}\right)\exp[A_{ij}M_{ij}(r^2 - m^2)]. \quad (11)$$

Since apoC-I does not absorb at 381 nm one cannot separate K_{ij} and $C_{01}(m)^j$ in the above equation with data obtained only at 381 nm. A simultaneous analysis of data obtained at 381 nm and 280 nm using eqs. (10) and (11) yields the stoichiometry and corresponding equilibrium constant. The data with N-apoA-II and apoC-I were most consistent with a single mixed oligomer containing two molecules of N-apoA-II and 4 molecules of apoC-I with $K_{24} = 179\,489 \pm 770$ (ℓ/gm)^5.

We have also investigated this system with native apoA-II and labeled apoC-I. Since apoC-I contains no tyrosine and only one tryptophan residue we have modified this apolipoprotein by sulfenylation with sulfenyl chloride. As was found previously for nitrated apoA-II, the molecular properties of sulfenylated apoC-I were quite similar to the native species except that the absorption spectrum was "red shifted" (see text for details). The distribution at equilibrium of s-apoC-I in the presence and absence of apoA-II was obtained by absorbancy measurements at 365 nm. Since only those species containing s-apoC-I contribute to the data at 365 nm eq. (10) reduces to:

$$\xi(r) = C_{01}(m)\epsilon_{01}\exp[A_{01}M_{01}(r^2 - m^2)] + K_{02}(m)C_{01}(m)^2\epsilon_{01}\exp[A_{02}M_{02}(r^2 - m^2)]$$

$$+ K_{04}(m)C_{01}(m)^4\epsilon_{01}\exp[A_{04}M_{04}(r^2 - m^2)]$$

$$+\sum_{i=1}^{n}\sum_{j=1}^{m} K_{ij}(m)C_{10}(m)^iC_{01}(m)^j$$

$$\times\left(\frac{iM_{10}\epsilon_{01}}{iM_{10} + jM_{01}}\right)\exp[A_{ij}M_{ij}(r^2 - m^2)]. \qquad (12)$$

As was found for nitrated apoA-II and native apoC-I, summarized above, a simultaneous analysis of data obtained at 280 nm and 365 nm was most consistent with a single mixed oligomer containing 2 molecules of apoA-II and 4 molecules of s-apoC-I with K_{24} = 22 977 ± 50 (ℓ/gm)^5.

Finally, with a knowledge of the stoichiometry of the mixed interaction, we analyzed the profiles obtained with several different mixtures of native apoA-II and native apoC-I according to eq. [10]. The resulting value of K_{24} was 31 248 ± 890 (ℓ/gm)^5.

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