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Nanosecond Rotational Motions of Apolipoprotein C-I in Solution and in Complexes with Dimyristoylphosphatidylcholine†

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ABSTRACT: Human apolipoprotein C-I (apo C-I) in solution, in monomeric and oligomeric form, and in micellar complexes with dimyristoylphosphatidylcholine (DMPC), below and above the phase transition temperature of DMPC, was investigated with steady-state and time-resolved fluorescence methods. The environment of the Trp residue of apo C-I, in each physical state, was evaluated from fluorescence spectra and their changes upon KI quenching. Rotational correlation times of Trp residues were obtained from fluorescence anisotropy decay measurements. Static fluorescence anisotropy was determined as a function of temperature for the Trp residues of apo C-I in all physical states and for diphenylhexatriene dissolved in apo C-I·DMPC complexes. It was found that the Trp residues of apo C-I in solution are exposed from 75 to 88% to the aqueous medium, depending on the state of self-association. On the other hand, the Trp residues in apo C-I·DMPC complexes are only 42–45% exposed to KI quenching through an environment distinct from water. Apolipoprotein C-I in all its physical forms had two rotational correlation times associated with Trp motions: a longer one dependent on the size and flexibility of the entire particle and a very short one in the range from 0.2 to 0.4 ns. The later correlation times correspond to local Trp residue motions. These Trp motions were not significantly affected by a transition from the gel to the liquid-crystalline state of the lipid in apo C-I·DMPC complexes, suggesting that there is no coupling between the local motions of lipids and those of Trp side chains of apo C-I.

The soluble apolipoproteins of the A and C classes, isolated from plasma lipoproteins, have variable amounts of α-helical structure in aqueous solutions. Their content of secondary structure usually increases upon self-association to various oligomeric forms, which depend on the nature and the concentration of the apolipoproteins (Osborne & Brewer, 1977; Morrisett et al., 1977). Human apo A-I, the major apolipoprotein of high density lipoproteins (HDL),[1] exists as an elongated monomer or as very asymmetrical oligomers in aqueous buffers (Barbeau et al., 1979) and exhibits a low free energy of denaturation—2.4 kcal/mol as compared to 7 kcal/mol for other proteins (Tall et al., 1976). Upon lipid binding, the α-helix content of the A and C apolipoproteins increases further, up to 70–80%, which is in the range of α-helical structure of the apolipoproteins in native HDL particles (Lux et al., 1972; Morrisett et al., 1977). The ability of these apolipoproteins to change dramatically their secondary structure, to denature very easily, and to exist in monomeric and oligomeric states in water as well as in micellar or vesicular complexes with phosphatidylcholines (Jonas et al., 1980; Patterson & Jonas, 1980b) or in spherical native lipoproteins suggests a remarkable degree of structural adaptability. This concept of structural adaptability of apolipoproteins is further supported by the observation that one type of apolipoprotein can exchange for another in native or synthetic lipoproteins, without changing significantly the overall properties of the particles (Lagocki & Scanu, 1980; Rosseneu et al., 1981).

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[1] Abbreviations: HDL, high-density plasma lipoproteins; apo A-I, major protein component of HDL; apo C-I, minor protein component of HDL and a relatively more important component of very low density lipoproteins; DMPC, dimyristoylphosphatidylcholine; apo C, apolipoproteins of the C class, which include human apo C-I, and animal apolipoproteins of low molecular weight isolated from HDL or from very low density lipoproteins; DPH, 1,6-diphenyl-1,3,5-hexatriene; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; Gdn·HCl, guanidine hydrochloride.

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In this study we investigate human apolipoprotein C-I (apo C-I) (M_r 6630 with a single Trp residue) (Osborne & Brewer, 1977) in attempts to establish to what extent the rotational motions of this protein are linked to its capacity for structural adaptation. We use steady-state and time-resolved fluorescence methods to observe the dynamics of apo C-I in the nanosecond time scale in monomeric and oligomeric forms in solution and in a micellar complex with lipid below and above the gel to liquid-crystalline phase transition of the lipid.

Experimental Procedures

Materials and Preparations. Apo C-I was isolated from delipidated human HDL (d = 1.063–1.21 g/mL) by DEAE chromatography in 6 M urea as described previously (Shore & Shore, 1969; Herbert et al., 1973). After lyophilization, the apolipoprotein was dissolved in 0.1 N acetic acid and was stored at −20 °C until use. The apo C-I solvent was changed to 0.01 M Tris-HCl, 0.1 M NaCl, 0.01% EDTA, and 10^{-3} M NaN_3, pH 8, or to 6 M guanidine hydrochloride in the Tris buffer, by dialysis at 5 °C. The apo C-I complexes with dimyristoylphosphatidylcholine (DMPC)[1] were prepared and stored in the same buffer. All solutions and buffers were made with glass redistilled water. After the experiments, the purity of the apo C-I was confirmed by $NaDodSO_4$–polyacrylamide electrophoresis in 10% gels. The concentration of the apolipoprotein was determined by using a percent extinction coefficient of 8.65×10^2 g^{-1} cm^2 at 280 nm (Osborne et al., 1977).

DMPC was purchased from Sigma Chemical Co.; it was shown to be pure by thin-layer chromatography on Eastman Kodak silica gel G plates developed in chloroform/methanol/water, 65:25:5 (v/v). Lipid phosphate was determined by the method of Chen et al. (1956). Single bilayer vesicles of DMPC were prepared from 10 mg/mL lipid dispersions in buffer by sonicating at temperatures above 20 °C for 1 h with intermittent cooling, using an instrument manufactured by Instruments Scientifiques, OSI France, at an amplitude of 8 μm peak-to-peak and a frequency of 21 kilocycles/s. Subsequently the preparations were centrifuged in a Beckman Model L5-75 ultracentrifuge at 40000 rpm, at 27 °C, for 1 h in order to remove titanium particles, residual large vesicles, and multilamellar liposomes. The vesicle preparations were used immediately in the preparation of apo C-I·DMPC complexes.

The complexes were made from apo C-I stock solutions of concentrations around 0.7 mg/mL, by adding enough DMPC vesicles to give a ratio of 3.6 g of DMPC/g of apo C-I. The mixture was incubated at 25 °C, with periodic stirring until its light scattering at 350 nm decreased to about 10% of that in a control vesicle sample. The reaction was complete in about 3–5 h. All the subsequent optical experiments were performed with these unfractionated complexes, which were stable for at least 2 months, stored at 5 °C, as judged by constant Trp fluorescence parameters and by a phase transition behavior characteristic of the complexes. The complexes were isolated by gel filtration on a (1.8 × 45 cm) Bio-Gel A-5m column and were characterized as previously described for apo A-I (Jonas et al., 1977; Jonas et al., 1980) and for bovine apo C[1] complexes with DMPC (Patterson & Jonas, 1980a,b).

Spectroscopic Methods. (1) CD Measurements. The CD measurements were performed with a Dichrographe II, CNRS Roussel Jouan instrument, using 1-mm cells and regulating temperature at 15 or 35 °C. The spectra were recorded between 260 and 200 nm, and a base-line trace was obtained for each solvent. Molar ellipticity values were calculated by using a mean residue weight of 116.3 obtained from the amino acid composition of apo C-I (Brown et al., 1969; Shulman et al., 1972). The empirical method of Greenfield & Fasman (1969) was used to estimate α-helix, β-sheet, and random coil contents in apo C-I. Contents of α helix were obtained, to a first approximation, from molar ellipticities at 208 nm by using the expression % α helix = $([\theta]_{208} - 4000)/29000$ (Greenfield & Fasman, 1969).

(2) Static fluorescence measurements were performed on a modified Jobin Yvon, Spectrofluo JY3C instrument interfaced to a Tektronix 4501 computer, plotter, and printer. The software for fluorescence intensity and anisotropy measurements was developed by Dr. J. C. Auchet, Centre de Biophysique Moléculaire, Orléans. Temperatures were regulated to ±0.2 °C by means of a refrigerated water bath. Fluorescence emission spectra, for the determination of quantum yields and for the KI quenching experiments, were recorded between 290 and 500 nm by exciting at 280 nm and using 4-nm bandwidth slits in the exciting and fluorescent light paths. Temperature was regulated at 15, 25, or 35 °C, as required. Apo C-I concentrations ranged from 0.68 down to 0.03 mg/mL in the static fluorescence measurements.

The fluorescence quenching experiments with KI were performed with apo C-I concentrations below 0.15 mg/mL, where the absorbance was less than 0.12 at 280 nm. The 5 M stock solution of KI (Prolabo, Rohne Poulenc) containing about 10^{-3} M $S_2O_3^{2-}$ (Prolabo, Rohne Poulenc) was prepared in the usual buffer; this stock solution was added to 2.0-mL samples of apo C-I by means of adjustable micropipets to give final concentrations from 0.025 to 0.37 M KI. Integrated fluorescence intensities were obtained from uncorrected fluorescence emission spectra.

Fluorescence quantum yields were determined by reference to a recrystallized, standard L-Trp (Fluka, A. G., Bucks, S. G.) solution in the usual pH 8 buffer, at 25 and 15 °C. The quantum yield of the L-Trp at 25 °C was taken to be 0.13 (Lehrer, 1971); the apo C-I quantum yields were calculated, as described previously (Privat et al., 1980), using uncorrected fluorescence emission spectra.

Static fluorescence anisotropies of Trp in apo C-I and of 1,6-diphenyl-1,3,5-hexatriene (DPH)[1] (Aldrich Chemical Co.) dissolved in the apo C-I·DMPC complexes (about 500 DMPC/DPH, mol/mol) were measured with the Jobin-Yvon instrument by using excitation and emission polarizers, according to the method of Azumi & McGlynn (1962). In the fluorescence anisotropy mode the instrument is automated to measure fluorescence intensities sequentially and repetitively in all polarizer positions and to calculate anisotropies and standard errors. Excitation wavelengths were 285 and 362 nm for Trp and DPH, respectively. The emission wavelengths for Trp were chosen at the maximum fluorescence intensity for each apo C-I sample (337–355 nm); for DPH the emission wavelength was set at 428 nm. The excitation and emission bandwidths were 4 and 10 nm, respectively. Fluorescence anisotropy as a function of temperature was measured in the range from 10 to 40 °C.

(3) Fluorescence Decay Measurements. The fluorescence of the Trp residue of apo C-I in the various physical states and of DPH dissolved in apo C-I·DMPC complexes was excited by a short light pulse and was measured by using a photoelectron counting apparatus described earlier by Wahl et al. (1974). The excitation light pulse was generated by a free running flash lamp, containing hydrogen–neon mixtures and operating with a frequency of 10 kHz (J. C. Auchet and P. Wahl, unpublished results).

For the Trp measurements the excitation wavelength was selected through a Microphysic (Paris) interference filter at

296.5 nm (bandwidth = 4 nm, transmission = 10%), and the fluorescence was observed through a short-wavelength cutoff filter with 50% transmission at 320 nm (Schott, WG 320). Photons were detected by means of a Radiotechnique XP2020 photomultiplier. Counts were accumulated at a counting rate of 1–2% of the flash frequency in order to remain in the single photoelectron condition. Experiments were stopped after 5–10 h when the total counts in the fluorescence curve were about 10^6. The response function $g(t)$, which characterizes the true distribution of the flash intensity and the apparatus response function at the emission wavelength, was obtained with a solution of *p*-terphenyl in cyclohexane (τ = 0.96 ns) as described by Wahl et al. (1974). This procedure takes into account the wavelength dependence of the photomultiplier response. Measurements of $g(t)$ were performed before and after each observation on apo C-I solutions, permitting to test the stability of the response function over the long periods of data accumulation. Contributions of stray light were estimated by using DMPC vesicle solutions having a light scattering intensity equal to that of solutions containing apo C-I. When necessary, the stray light was subtracted from fluorescence curves.

For the DPH measurements, the fluorescence excitation wavelength (335 nm, bandwidth = 13 nm) was selected through a Bausch & Lomb 250-mm monochromator and the fluorescence emission through an MTO interference filter centered at 437 nm (bandwidth = 15 nm, transmission = 33%). In that particular case 1,1,4,4-tetraphenylbutadiene was used as reference compound (τ = 1.76 ns).

(*4*) *Fluorescence Decay Analysis.* The two polarized components $i_\parallel(t)$ and $i_\perp(t)$ of the transient fluorescence emission were measured. The fluorescence lifetime τ and the correlation times θ_i were determined as described earlier (Wahl, 1979; Ikkai et al., 1979) by a least-squares method outlined below. The experimental functions

$$s_e(t) = i_\parallel(t) + 2i_\perp(t)$$

$$d_e(t) = i_\parallel(t) - i_\perp(t)$$

$$r_e(t) = [i_\parallel(t) - i_\perp(t)]/[i_\parallel(t) + 2i_\perp(t)]$$

were fitted to the computed convolution:

$$s_c(t) = \int_0^t g(T)S(t - T)\,dT$$

$$r_c(t) = \int_0^t g(T)D(t - T)\,dT/s_c(t)$$

where

$$D(t) = R(t)S(t)$$

$$S(t) = \sum_n A_i \exp(-t/\tau_i)$$

$$R(t) = \sum_p \alpha_i \exp(-t/\theta_i)$$

The τ_i's are the fluorescence lifetimes and the θ_i's are the correlation times. The average lifetime was defined as

$$\langle\tau\rangle = (\sum_n A_i\tau_i)/\sum_n A_i$$

and the limiting anisotropy at zero time as

$$r_0 = \sum_p \alpha_i$$

The number of exponential terms n and p in $S(t)$ and $R(t)$, respectively, was the minimum number which was necessary to obtain satisfactory deviation functions and residuals (Wahl, 1979; Ikkai et al., 1979).

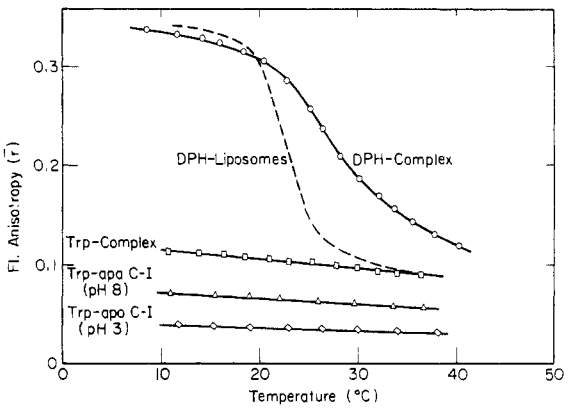

FIGURE 1: Static fluorescence anisotropy as a function of temperature: DPH in DMPC liposomes (---), DPH in apo C-I·DMPC complexes (○), Trp in apo C-I·DMPC complexes (□), Trp in apo C-I at pH 8.0 (△), and Trp in apo C-I at pH 3 (◇). The wavelengths of excitation were 362 and 285 nm for the DPH and Trp measurements, respectively. A molar excess of 500 DMPC/DPH was used.

Results and Discussion

Characterization of Apo C-I·DMPC Complexes. The complexes of apo C-I·DMPC isolated by Bio-Gel A-5m chromatography gave a single complex peak with a molar ratio of DMPC/apo C-I of 25/1 or a weight ratio of 2.7/1. There was no evidence of free apo C-I. The elution position from the gel filtration column, the lipid/protein weight ratio, and the phase transition behavior (see Figure 1) are essentially identical with those of discoidal apo A-I·DMPC or some of the bovine apo C·DMPC complexes, with molecular weights from 2×10^5 to 3×10^5, which are described in detail in the literature (Jonas et al., 1977, 1980; Patterson & Jonas, 1980b).

Spectroscopic Characterization of Apo C-I in Various Physical States. The spectroscopic results are summarized in Table I.

The content of α helix increases from the monomer to the oligomer to the DMPC bound state of apo C-I. The results in terms of molar ellipticities at 222 nm agree well with those reported by Osborne et al. (1977) for apo C-I in solution, Jackson et al. (1974) for apo C-I bound to single bilayer vesicles of egg phosphatidylcholine, and Morrisett et al. (1977) for apo C-I reaction mixtures with DMPC. For the apo C-I·DMPC complexes the experimentally determined α-helix content (74 and 77%) is close to that calculated by Jackson et al. (1974) for the amphipathic helix model of apo C-I (72%). It has been assumed in most lipid binding studies with apolipoproteins that the observed increases in molar ellipticity are the result of α-helix formation during interaction of apolipoprotein with lipid; however, at least in the case of apo C-I, it has been shown by Osborne et al. (1977) that maximal molar ellipticities can also be induced by protein–protein interactions. Thus, we cannot discount the possibility that protein–protein contacts in the complexes could be responsible for the observed α-helix contents.

The wavelengths of maximum fluorescence undergo a blue shift from the monomeric to the oligomeric to the lipid-bound state of apo C-I. The monomeric form has a wavelength of maximum fluorescence (353 nm), which approaches that of the Gdn·HCl denatured form (355 nm) and that of L-Trp in buffer (353 nm), indicating complete exposure of Trp residues to the aqueous buffer. The oligomer has a concentration-dependent wavelength of maximum fluorescence. At a con-

Table I: Spectral Properties of Apo C-I in Various Physical States

apo C-I state; conditions	molar ellipticity at 208 nm	molar ellipticity at 222 nm	α helix[a] (%)	max. fl. wavelength[b] (nm)	fl. quantum yield[b]	K_Q[c] (M^{-1})	Trp exposure[d] (%)	av fl. lifetime[e] (ns)	fl. anisotropy[f]
monomer; 0.1 N acetic acid, 15 °C[g]	13 800	10 800	34	353	0.066	22	88[j]	2.1	0.037
monomer; 6 M Gdn·HCl, 15 °C	3 500	1 100	4	355	0.082	3.5	100[j]		
oligomer; pH 8 buffer, 15 °C[h]	20 400	18 000	57	347	0.042	6.8 (11.1)[i]	75[j]	2.2	0.068
DMPC complex; pH 8 buffer, 15 °C	26 400	25 000	77	337	0.039	3.1	42[k]	1.2	0.108
DMPC complex; pH 8 buffer, 35 °C	25 500	24 000	74	338	0.032	2.6	45[k]	1.4	0.090

[a] Calculated by the method of Greenfield & Fasman (1969) using molar ellipticities at 208 nm. The remainder of the structure is random coil with the exception of about 6% and 16% β sheet in the acetic acid and Gdn·HCl samples, respectively. [b] Determined by using uncorrected fluorescence emission spectra. In the quantum yield determinations L-Trp at pH 8 was used as the standard with a quantum yield of 0.13 at 25 °C (Lehrer, 1971) and 0.16 at 15 °C. [c] Quenching constants (K_Q) were obtained from the initial slopes of Stern–Volmer plots ($F_0/F - 1$ vs. [KI]). Over the entire range of KI concentrations (0.025–0.37 M) all the plots were curved, except the one for Gdn·HCl which was linear. [d] Trp exposures were calculated from $F_0/\Delta F$ vs. 1/[KI] plots as described by Lehrer (1971). These modified plots were linear for low KI concentrations, suggesting two populations of chromophores, one exposed to I^- and another protected from it. [e] Average Trp fluorescence lifetimes averaged from two fluorescence decay experiments. [f] Measured using a 285-nm exciting wavelength. [g] According to Osborne et al. (1977) apo C-I is monomeric in 15% (v/v) acetic acid. [h] Under these conditions the spectral properties of apo C-I were found to be concentration dependent as already indicated by Osborne et al. (1977). The molar ellipticities, α helix, quantum yield, K_Q, and Trp exposure were obtained for 0.2 mg/mL apo C-I, whereas the maximum fluorescence wavelength, average fluorescence lifetime, and fluorescence anisotropy were measured at a 0.7 mg/mL apo C-I concentration. [i] Calculated $K_{Q,app}$ for completely exposed Trp residues to water. [j] Exposed to I^- quenching through water. [k] Exposed to I^- quenching through a medium other than water.

centration of 0.7 mg/mL this parameter is 347 nm; it increases with dilution to 351 nm (for 0.2 mg/mL) and extrapolates to 352 nm at zero concentration. The complexes with DMPC have the most blue-shifted fluorescence (337 nm), suggesting a displacement of the Trp residues to a more nonpolar environment, which is not, however, identical with that of a hydrocarbon. In dioxane the Trp fluorescence maximum is found at 329 nm (Konev, 1967), and various indole derivatives have wavelengths of maximum fluorescence from 295 to 320 nm in cyclohexane (Van Duuren, 1961). From published fluorescence wavelength maxima for phosphatidylcholine complexes with various apolipoproteins (Jonas et al., 1980; Morrisett et al., 1977; Patterson & Jonas, 1980a) which range from 328 to 338 nm, it is evident that the environment of Trp residues is diverse and cannot be determined only by the hydrophobic lipid interior of the particles.

The fluorescence quantum yield of apo C-I in acetic acid (pH 3) at 15 °C is 0.066, and it decreases to 0.042 at pH 8.0, 15 °C. This change may be due to quenching by adjacent amino acid residues, for example, a carboxylate group, as in the case of yeast 3-phosphoglycerate kinase (Privat et al., 1980). The quantum yield changes very little from the oligomeric to the DMPC-bound form of apo C-I suggesting either little change in the environment of the Trp residues (due to protein–protein contacts) or almost equally compensating quenching effects.

The details of the fluorescence quenching experiments will not be described here. For the purpose of this work we were mainly interested to show the progressive protection of Trp residues from I^- quenching, for the monomer to the oligomer to the DMPC-bound form of apo C-I. The Stern–Volmer quenching constant K_Q for apo C-I in Gdn·HCl does not represent the quenching of completely exposed Trp residues in water because the viscosity of the solution decreases I^- diffusion (Lehrer, 1971). However, from modified quenching plots [$F_0/\Delta F$ vs. 1/[KI] (Lehrer, 1971)] for the apo C-I at pH 8, one can obtain a $K_{Q,app}$ which corresponds to the completely exposed Trp residues—this constant is 11.1 M^{-1}, very near the K_Qs determined for free L-Trp (11.6) or *N*-acetyl-L-tryptophanamide (12.0) at neutral pH (Lehrer, 1971). By comparison with this $K_{Q,app}$ the K_Q for apo C-I in acetic acid (22.0 M^{-1}) is very high and is due, no doubt, to charge effects. The Trp residues in the oligomeric protein are somewhat protected from I^- quenching (K_Q = 6.8 M^{-1}) and are even less accessible to I^- in complexes with DMPC ($K_Q \sim 3$ M^{-1}). An interesting observation was made when the quenched fluorescence spectra (in 0.2 M KI) were subtracted from the initial spectra. For the apo C-I in acidic and neutral solution the difference fluorescence spectra had maxima at 355 and 352 nm, respectively. This indicates that the Trp residues initially quenched by I^- were exposed to water. In the complexes, however, the difference fluorescence spectra had maxima at 339 (at 15 °C) and 342 nm (at 35 °C), clearly indicating that I^- quenching had occurred through an environment other than water. In this connection, fluorescence quenching experiments on scatole (3-methylindole) dissolved in DMPC vesicles indicate that the chromophore can be completely quenched by I^- but with an efficiency of only about 20% that of quenching in water (A. Jonas and J. Privat, unpublished results). Thus indole chromophore locations in a DMPC bilayer can be accessible to I^- through the approach of the chromophore to the water–lipid interface and/or the penetration of I^- through the interface.

The average fluorescence lifetimes are low compared to that of L-Trp in water (2.8 ns) (Lehrer, 1971). Since there is no

Table II: Fluorescence Anisotropy Decay Results

apo C-I state; conditions	rotational correlation times (ns)	amplitudes	mean[a] anisotropy ⟨r⟩	limiting[b] anisotropy r_0	χ^2 [c]
monomer; 0.1 N	0.2 ± 0.8	0.12	0.06	0.21	1.23
acetic acid, 15 °C	3.6 ± 0.4	0.09			
oligomer; pH 8	0.2 ± 0.2	0.16	0.10	0.30	1.35
buffer, 15 °C	7.2 ± 0.2	0.14			
DMPC complex; pH	0.3 ± 0.3	0.18	0.17	0.33	1.11
8 buffer, 15 °C	44 ± 2	0.15			
DMPC complex; pH	0.4 ± 0.7	0.17	0.15	0.30	1.26
8 buffer, 35 °C	25 ± 2	0.13			

[a] Mean anisotropy from steady-state fluorescence measurements using excitation at 295 nm. [b] Limiting anisotropy at $t = 0$ is the sum of the amplitudes. [c] Fit of calculated and experimental decay curves, as described by Ikkai et al. (1979) and Wahl (1979).

direct correspondence between the quantum yields of all the apo C-I states with the corresponding lifetimes, static quenching effects must be involved.

The steady-state fluorescence anisotropy values of the Trp residue in apo C-I, at 15 °C, increase from monomer to oligomer to DMPC complexes, indicating a progressive slowing down of the overall rotational motions of this chromophore, an observation which is consistent with the increasing size of the particles. Since the limiting fluorescence anisotropy of Trp in the absence of Brownian motions for 285 nm excitation is 0.160, it is evident that some rapid depolarization must also occur in all the apo C-I states.

The relationship of lipid and Trp residue motions in apo C-I·DMPC complexes was examined by means of static fluorescence anisotropy measurements as a function of temperature (Figure 1).

The phase transition behavior of DMPC in the apo C-I·DMPC complexes (as reported by DPH fluorescence anisotropy) is essentially identical with that of apo A-I·DMPC complexes (Jonas et al., 1977, 1980) and is clearly distinct from the phase transition in DMPC liposomes. On the other hand, the temperature dependence of the fluorescence anisotropy of Trp residues in apo C-I·DMPC complexes (measured with 285 nm excitation) is linear and almost parallels the behavior of monomeric and oligomeric apo C-I in solution. Apparently, the mobility and order which change so dramatically in the lipid domains during the phase transition do not affect significantly the properties of the Trp residues in the protein domains.

Fluorescence and Anisotropy Decay of Apo C-I in the Various Physical States. Analysis of the fluorescence intensity decay data, $s(t)$, indicated that the decay was multiexponential in each case. The best fit (χ^2 from 1.8 to 4.3) was obtained for three Trp lifetimes: a very short one, in the range 0.2–0.6 ns contributing from 50 to 70%; an intermediate one, 2.5–3.3 ns contributing about 20–40%; and a long one, 6.0–7.5 ns with a contribution of less than 13%. Since the significance of these complex results is not clear, we only report in Table I the average lifetimes from two independent experiments. There are two possible reasons for the complex fluorescence decay behavior observed with apo C-I in acetic acid and pH 8 solutions and in complexes with DMPC below and above the phase transition temperature of the lipid. The first and most likely one is the existence of the Trp residues in diverse environments; the second one is dipolar relaxation in the nanosecond time scale, as reported by Lakowicz & Cherek (1979) for other proteins containing a single Trp residue. We attribute our observations to diverse Trp residue environments since in the case of apo C-I in acetic acid and in pH 8.0 buffer the chromophore is mostly exposed to water (see Table I), a solvent which relaxes very rapidly around the excited state of the aromatic residue at the temperatures of the experiment (15 and 35 °C). Moreover, apo C-I in acetic acid, even if it is present in the monomeric state (with a single Trp residue), probably has a flexible structure which is not unique. In the oligomeric form in solution and in DMPC complexes the Trp residues of apo C-I sense different environments perhaps as a result of nonequivalent structures and arrangements of monomers and due to local chromophore motions during the excited-state lifetime.

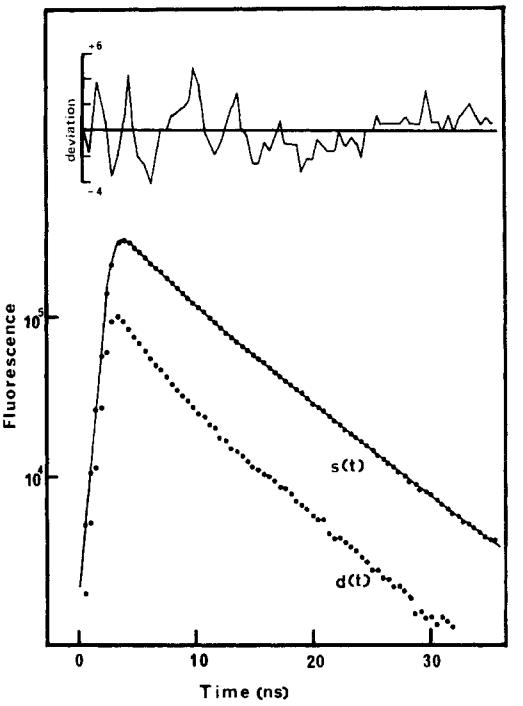

FIGURE 2: Fluorescence decays of apo C-I·DMPC complexes at 15 °C in pH 8 buffer. Excitation wavelength = 296.5 nm; bandwidth = 4 nm; emission wavelength = short-wavelength cutoff filter at 320 nm (Schott, WT 320). The solid line corresponds to the calculated convolution products and the points are related to experimental values. The inset is a representation of the deviation between convolution products and experimental values of $s(t)$; $s(t)$ and $d(t)$ are defined under Experimental Procedures.

In Figure 2, $s(t)$ and $d(t)$ are representative of the fluorescence decay measurements on apo C-I in all its physical states; such data were used to calculate the fluorescence anisotropy decay, shown in Figure 3. For all the apo C-I states the best fit of the fluorescence anisotropy results corresponded to two correlation times (θ). The calculated rotational correlation times, the corresponding amplitudes, the mean anisotropy values, ($\langle r \rangle$), limiting anisotropies (r_0), and fits (χ^2) are given in Table II.

For apo C-I in the monomeric state (in acetic acid) the mean anisotropy value was very low, suggesting a very flexible structure. The analysis of the anisotropy decay gives a larger

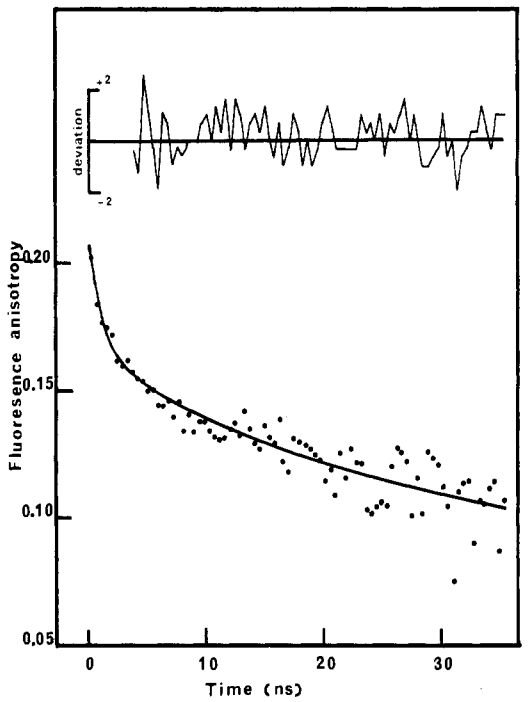

FIGURE 3: Fluorescence anisotropy decay of apo C-I·DMPC complexes at 15 °C in pH 8 buffer. The same experimental conditions apply as in Figure 2. The points represent experimental values, and the solid line represents the calculated curve. The inset is the deviation function relative to $r(t)$, which is defined under Experimental Procedures.

correlation time θ_2 (3.6 ± 0.4 ns) which is close to the value calculated for a spherical molecule containing 0.3 g of H_2O/g of protein (3.18 ns at 15 °C). The preexponential term associated with θ_2 is small, indicating a large contribution of internal motions. In addition to the motions characterized by θ_1 (0.2 ± 0.8 ns) there are still faster motions as suggested by the value of r_0 which is smaller than the r_0 values obtained in the other experiments with apo C-I. The indole moiety of the Trp residue must have an important local motion which is correlated with its almost complete exposure to the aqueous buffer (as discussed above).

Apo C-I in the pH 8 buffer had a concentration of about 0.7 mg/mL. At such a concentration, in neutral solution, Osborne et al. (1977) observed an apparent weight-average molecular weight of 13 000 which corresponds to a mixture of oligomeric forms. The observed correlation times (Table II) probably correspond to local motions of the Trp residues (0.2 ± 0.2 ns) and to overall particle rotations (7.2 ± 0.2 ns). For example, the equivalent hydrated sphere of a dimer of apo C-I has a calculated rotational correlation time of 6.41 ns at 15 °C.

For the apo C-I·DMPC complexes, at both temperatures, we observe a short correlation time (0.3–0.4 ns) due to very fast motions and a longer one (44 ± 2 and 25 ± 2 ns at 15 and 35 °C, respectively) which represents an average of a very long correlation time due to the whole complex and of a shorter correlation time associated with protein motions. The rotational correlation time for the entire particle has been obtained separately by measuring the fluorescence anisotropy decay of DPH in the apo C-I·DMPC complexes (A. Jonas and J. Privat, unpublished results). The measured correlation time of 136 ± 13 ns compares reasonably well with that calculated for an oblate ellipsoid (axial ratio 3/1) of M_r 2 × 10^5, density = 1.112 g/mL, and 0.3 g of hydration H_2O/g of complex, θ = 166 ns. As already indicated by the static measurements shown in Figure 1 (285 nm excitation), the mean anisotropy $\langle r \rangle$ (0.17 and 0.15 at 15 and 35 °C, respectively, with 295 nm excitation) is not influenced by the lipid dynamics. The respective amplitudes of the two correlation times of $R(t)$ remain constant, i.e., the contribution of each type of motion is the same at both sides of the thermal lipid transition. Static and transient measurements are thus in good agreement. Additional information can be obtained from the analysis of the transient measurements. Particularly, the ratio of the long correlation times at 35 and 15 °C (0.57) follows closely the decrease of the ratio η/T of the buffer solution (0.59). This result indicates that the longer correlation time corresponds to motions that are directly influenced by the viscosity of the aqueous environment of the complex.

It is interesting to note that in all its physical forms, apo C-I has very rapid rotational motions of the order of a few hundred picoseconds, probably involving the local movement of the Trp residue. Rotational Trp motions of a similar magnitude were observed by Munro et al. (1979) for some proteins having a single Trp residue, including the myelin basic protein, human serum albumin at 43 °C, and azurin from *Pseudomonas aeruginosa*. All these proteins are water soluble but differ from apo C-I in having well-defined tertiary structures, which probably contain hydrophobic pockets where rapid Trp residue motions can occur. Apolipoprotein C-I and other apolipoproteins of the A and C classes do not have unique three-dimensional structures; rather they appear to have flexible and adaptable structures which change dramatically upon oligomerization or lipid binding. The spectral heterogeneity and high mobility of the Trp residue in all the physical states of apo C-I, examined in this work, agree with such structural characteristics.

The physiological function of apo C-I has not yet been elucidated; only a weak activator role for the enzyme lecithin–cholesterol acyltransferase has been demonstrated in vitro (Soutar et al., 1975). Together with other apolipoproteins, apo C-I is exchanged between lipoproteins during the lipolysis cycle of triglyceride-rich lipoproteins (Havel, 1980). Whatever its function, apo C-I exists in solution and in lipid bound forms in vivo and probably undergoes similar structural rearrangements to those observed in vitro.

Our observation that very rapid Trp residue motions persist in apo C-I complexes with DMPC, below the phase transition temperature of the lipid, indicates that the bulk behavior of the lipid has little or no effect on the local motions of Trp residues. This suggests the presence of a boundary lipid layer, disordered by the adjacent protein and providing similar space for Trp residue motions to the hydrophobic interior of some proteins (Munro et al., 1979). The boundary lipid layer is known not to participate in the phase transitions of the lipid in micellar complexes of apo A-I and DMPC (Tall et al., 1977).

Conclusions

(1) The changes in apo C-I structure going from the monomeric to the oligomeric to the DMPC-bound forms are marked and qualitatively similar to the structural changes reported by other workers with the same (Osborne et al., 1977; Jackson et al., 1974) or with other apolipoproteins (Jonas et al., 1980; Patterson & Jonas, 1980a). (2) Trp residues in monomeric and oligomeric apo C-I forms in solution are very exposed to water (88 and 75% exposure, respectively) even in the presence of relatively high contents of α-helical structure (34 and 57%, respectively). (3) In the apo C-I·DMPC complexes the Trp residues are not exposed to water, but they are not completely immersed in the hydrophobic lipid domains; they contact other portions of the protein and are partially

accessible to a rather large ion such as I^-. (4) In all the physical forms of apo C-I, including the monomer, the Trp residues are exposed to heterogeneous environments suggesting the absence of a unique tertiary and quaternary structure of apo C-I. (5) The lipid phase transition has no effect on the mean fluorescence anisotropy of the Trp residues of apo C-I in DMPC complexes. (6) Subnanosecond rotational motions of the Trp residues and slower motions corresponding, at least in part, to whole particle rotations were detected in all the physical forms of apo C-I. (7) In the apo C-I·DMPC complexes the amplitudes and rotational correlation times of the fast and slow rotational motions of Trp are equivalent on both sides of the thermal lipid transition, suggesting that the phase state of the lipid has no effect on these Trp motions.

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