EFFECTS OF HUMAN PLASMA APOLIPOPROTEINS C-I, C-II and C-III ON THE PHASE-TRANSITION OF SONICATED VESICLES OF DIPALMITOYLPHOSPHATIDYLCHOLINE

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1. Introduction

Human plasma chylomicrons and very low density lipoproteins contain 3 low M_r proteins called apolipoprotein C-I, C-II and C-III (review [1–3]). Apolipoprotein C-II plays an important role in the catabolism of triglyceride-rich lipoproteins by increasing the activity of lipoprotein lipase [4]; the metabolic roles of apoC-I and apoC-III are not firmly established. The C-apolipoproteins are thought to interact with phospholipids at the monolayer surface of the lipoproteins [3]. During catabolism of triglyceride-rich lipoprotein, the C-apolipoproteins, along with phospholipid and unesterified cholesterol, transfer to high density lipoproteins (HDL). It is not known whether the C-apolipoproteins alter the phospholipid structure of the lipoprotein so as to influence their metabolism. Here, we investigate the effect of the C-apolipoproteins on the molecular organization of phospholipids. Using fluorescently labeled sonicated vesicles of dipalmitoylphosphatidylcholine, we have determined the effects of the C-apolipoproteins on phospholipid structure.

2. Materials and methods

Dipalmitoylphosphatidylcholine (Sigma) gave a single species on a thin-layer plate of silica gel ($CHCl_3$/CH_3OH/H_2O, 65:25:4, by vol.). The fluorescent probes 2- and 12-(9-anthroyloxy)-stearic acid were obtained from Molecular Probes, Inc. The lipophilic probe 1,6-diphenyl-1,3,5-hexatriene was purchased from Sigma. Apolipoproteins C-I, C-II and C-III were purified from human plasma VLDL following lipid extraction, gel exclusion chromatography on Sephadex G-75 and DEAE ion-exchange chromatography in the presence of 8 M urea as in [5]. The phospholipid-binding fragment of apolipoprotein C-I (residues 1–38) and the non-lipid-binding fragment (39–57) were obtained by cyanogen bromide cleavage of the intact protein [6]. Concentrations and criteria for purity of apolipoproteins and peptide fragments were established by amino acid compositional analyses.

Unilamellar vesicles of dipalmitoylphosphatidylcholine were prepared by sonication (model W-225R, Heat Systems Ultrasonics, Inc.). Dipalmitoylphosphatidylcholine was sonicated above its phase-transition temperature at 10 mg lipid/ml standard buffer (10 mM Tris–HCl, 0.9% NaCl, 0.01% NaN_3, pH 7.4). Multilamellar structures were removed by centrifugation at 100 000 × g for 1 h at 15°C. Phospholipid was determined as in [7].

Lipid vesicles (200 μg/ml) were fluorescently labeled using a 1:200 probe:phospholipid molar ratio. The incorporation of 2- and 12-(9-anthroyloxy)-stearic acid into sonicated vesicles of dipalmitoylphosphatidylcholine was achieved by bath sonication (model 8846-50, Cole-Parmer) at 42°C for 20 min. The incorporation of the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene into the vesicles was monitored by the increase in fluorescence intensity as a function of time at 42°C [8]. Apolipoprotein–lipid complexes were prepared by adding vesicles of dipalmitoylphosphatidylcholine to a 1.2 μM solution of apolipoprotein in standard buffer at 42°C. The interaction of apolipoprotein and lipid was monitored by the increase in tryptophan fluorescence [3] whereas circular dichroism (CD) was used for the apolipoprotein C-I lipid-binding

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fragment [6]; phospholipid was added until there was no further change in fluorescence or CD. At this point, the [dipalmitoylphosphatidylcholine]/[peptide] ratios for apolipoproteins C-I, C-II and C-III and the apolipoprotein C-I lipid-binding fragment were 130, 250, 250 and 180, respectively.

Fluorescence measurements were conducted on a Perkin-Elmer MPF 44-A ratio recording thermoregulated spectrofluorimeter. Fluorescence polarization (P) was determined by the relation $P = (V_v - L_v)/(V_v + L_v)$, where V_v and L_v are the fluorescence intensities measured with polarizers parallel and perpendicular to the vertically polarized exciting beam, respectively. Fluorescence polarization values were corrected for the unequal transmission of polarization intensities due to dispersion at the emission monochrometer grating. Polarization studies were conducted with the Perkin-Elmer Polarization Accessory 063-0468. Excitation, emission wavelength pairs for 2-(9-anthroyloxy)-stearic acid, 12-(9-anthroyloxy)-stearic acid and 1,6-diphenyl-1,3,5-hexatriene were 365, 435 nm; 365, 435 nm; and 358, 425 nm, respectively. The thermotropic properties of probe-labeled vesicles in the presence and absence of apolipoproteins were quantified by measuring the fluorescence polarization at 0.5–1.0°C gradations. Solid lines through the experimental points were constructed by linear regressional analyses. For clarity, the experimental points of fig.1 and 2 are shown every 1–2°C.

3. Results

Sonicated vesicles of dipalmitoylphosphatidylcholine were labeled with either 2- or 12-(9-anthroyloxy)-stearic acids or 1,6-diphenyl-1,3,5-hexatriene, and the temperature dependence of the fluorescence polarization of these probe-labeled lipid vesicles was measured

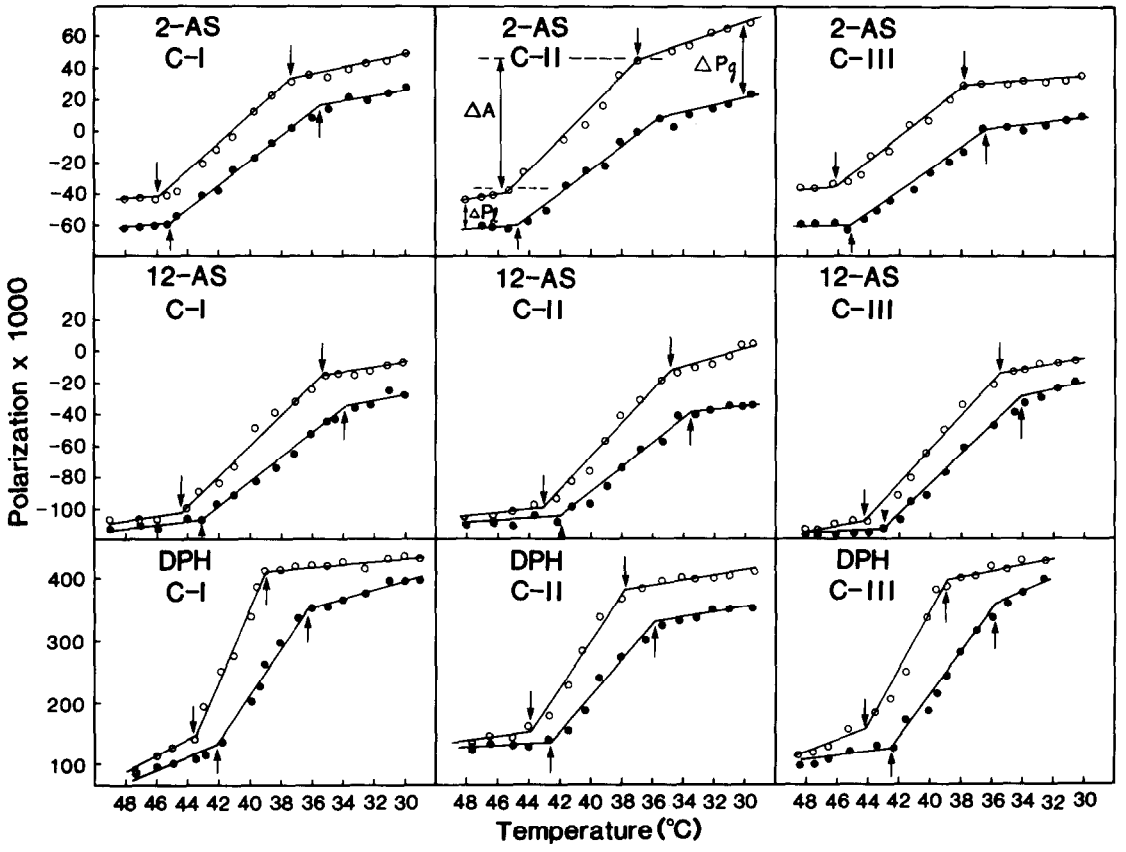

Fig.1. The fluorescence polarization of 2- and 12-(9-anthroyloxy)-stearic acids (2-AS and 12-AS, respectively) and 1,6-diphenyl-1,3,5-hexatriene (DPH) incorporated into sonicated vesicles of dipalmitoylphosphatidylcholine (DPPC) in the absence (•) and presence (○) of either apolipoprotein C-I, C-II or C-III. Apolipoprotein–lipid complexes were prepared as described in the text.

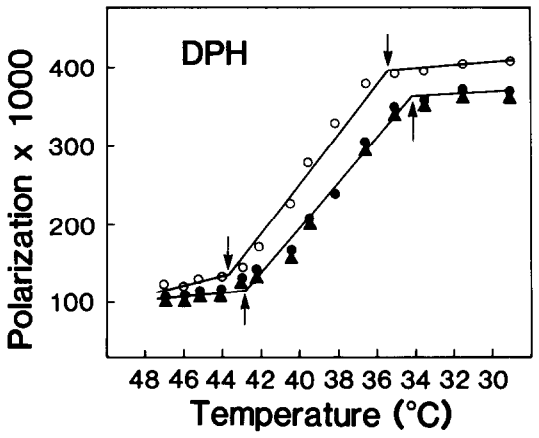

Fig.2. The fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene incorporated into sonicated vesicles of dipalmitoylphosphatidylcholine (•) and in the presence of either residues 1–38 of apolipoprotein C-I (○) or residues 39–57 (▲).

in the presence and absence of apolipoproteins C-I, C-II and C-III. Fig.1 shows that upon cooling, 2- and 12-(9-anthroyloxy)-stearic acids and 1,6-diphenyl-1,3,5-hexatriene report a sharp lipid phase-transition in the presence and absence of apolipoprotein. The effect of the C-apolipoproteins on these probe labeled vesicles was the following: to increase the fluorescence polarization over the investigated temperature range; to increase the phase-transition temperature by ~2°C; to shift the onset of gel-state formation to higher temperatures; and to increase the rate of gellation. These apoprotein-induced changes in fluorescence polarization are consistent with an increase in the rigidity of the phospholipid bilayer structure.

Fig.2 illustrates the temperature dependence of the fluorescence polarization of vesicles in the presence and absence of residues 1–38 of apolipoprotein C-I. In these experiments, 1,6-diphenyl-1,3,5-hexatriene was used since this probe exhibits the largest polarization change (fig.1). Residues 1–38 of apolipoprotein C-I exhibit the same general effects as the intact apolipoproteins upon the thermotropic properties of the lipid. In contrast, when the non-lipid binding fragment of apolipoprotein C-I (residues 39–57) was co-incubated with vesicles, no increase in polarization of the probe was observed over those values obtained for dipalmitoylphosphatidylcholine vesicles alone.

Table 1 summarizes the data of fig.1,2. These data

Table 1
Summary of physical parameters

Apoprotein	Probe	$\frac{\Delta A_p/\Delta T_p}{\Delta A/\Delta T}$ [a]	ΔP_l (48°C)	ΔP_g [b] (30°C)	ΔT_m (°C)[c]
C-I	2-(9-anthroyloxy)-Stearic acid	1.13	18	22	1.5
C-II	2-(9-anthroyloxy)-Stearic acid	1.42	18	42	2.0
C-III	2-(9-anthroyloxy)-Stearic acid	1.15	22	26	1.9
C-I	12-(9-anthroyloxy)-Stearic acid	1.33	2	21	1.5
C-II	12-(9-anthroyloxy)-Stearic acid	1.60	2	38	2.0
C-III	12-(9-anthroyloxy)-Stearic acid	1.30	2	17	2.0
C-I	1,6-Diphenyl-1,3,5-hexatriene	1.22	15	30	2.6
C-II	1,6-Diphenyl-1,3,5-hexatriene	1.51	15	55	2.2
C-III	1,6-Diphenyl-1,3,5-hexatriene	1.25	15	30	2.4
C-I (residues 1–38)	1,6-Diphenyl-1,3,5-hexatriene	1.23	13	27	1.5

[a] The term $\Delta A/\Delta T$ is a measure of the dynamic change in polarization amplitude in the region of the phase-transition; ΔA is the change in phase-transition amplitude as illustrated in fig.1 and ΔT represents the temperature interval in which ΔA occurs. $\Delta A_p/\Delta T_p$ and $\Delta A/\Delta T$ represent the rate of gelling of dipalmitoylphosphatidylcholine in the presence and absence of apolipoprotein, respectively. A ratio of these parameters >1.0 is a measure of the apoprotein-induced increase in gellation rate

[b] The values of ΔP_l (48°C) and ΔP_g (30°C) represent the difference in polarization values (ΔP) of probe-labeled vesicles of dipalmitoylphosphatidylcholine in the presence and absence of apoprotein at 48°C and 30°C, respectively. The subscripts 'l' and 'g' refer to the assumed physical state of the lipid as either liquid-crystalline (48°C) or gel-crystalline (30°C), respectively, in the regions outside the transition interval as shown in fig.1

[c] These data represent the magnitude of the apoprotein-induced shift in the phase-transition temperature of the probe-labeled vesicles

illustrate the change in fluorescence polarization as a function of apolipoprotein, probe location in the bilayer and physical state of the lipid. In the phase-transition region, the incremental change in polarization amplitude per unit change in temperature ($\Delta A/\Delta T$) was greater in the presence than in the absence of apolipoproteins, i.e., the ratios $\Delta A_p/\Delta T_p$: $\Delta A/\Delta T > 1.0$. Thus, apolipoproteins appear to increase the rate of gellation of dipalmitoylphosphatidylcholine. The most pronounced effect of the apolipoproteins on lipid structure, as reported by each probe, was to increase the rigidity of the phospholipid. Moreover, 12-(9-anthroyloxy)-stearic acid reported greater rates of gellation than 2-(9-anthroyloxy)-stearic acid in the presence of apolipoproteins. Also, the magnitude of the transition amplitudes (ΔA_p) was smaller for 2-(9-anthroyloxy)-stearic acid than for 12-(9-anthroyloxy)-stearic acid. These effects in rate and magnitude are likely due to the smaller changes in microviscosity which occur near the glycerol head region of phosphatidylcholine relative to the center of the bilayer as the temperature is increased through the phase-transition region. Similar findings were reported in [9]. The apolipoproteins produced a change in polarization amplitude (ΔP) in the liquid crystalline (l) and gel-crystalline (g) states of the lipid. The ΔP_l for 2-(9-anthroyloxy)-stearic acid, measured at 48°C, is greater than the ΔP_l for 12-(9-anthroyloxy)-stearic acid; ΔP_g values, measured at 30°C, were approximately the same. These results suggest that apolipoproteins increase bilayer rigidity at the 2-position over the complete temperature range. At the 12-position, however, apolipoproteins increase bilayer rigidity more dramatically in the gel-state than in the liquid-crystalline state of the lipid. In addition, apolipoprotein C-II caused a larger increase in gellation rate in the phase-transition region ($\Delta A_p/\Delta T_p$) and a larger change in ΔP_g than apolipoprotein C-III, C-I or residues 1–38 of apolipoprotein C-I.

4. Discussion

These findings, showing that the C-apolipoproteins increase the rigidity of phospholipids, are consistent with other studies with different apoproteins and lipid systems [10–13]. In [10], 2-(9-anthroyloxy)-palmitic acid and 12-(9-anthroyloxy)-stearic acid were used to monitor the fluorescence polarization changes in sonicated dimyristoylphosphatidylcholine vesicles in the presence and absence of the apolipoproteins of porcine HDL. Their experiments showed a greater apoprotein-induced increase in fluorescence polarization in the liquid-crystalline state (30°C) at the 2-position as compared to the 12-position of the phospholipid acyl chain. Human apolipoproteins were also shown to increase the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene in vesicles of dimyristoylphosphatidylcholine throughout the phase-transition region [11,12].

C-Apolipoproteins increase the rigidity of dipalmitoylphosphatidylcholine at both the 2- and 12-positions of the bilayer. Residues 1–38 of apolipoprotein C-I, but not residues 39–57, have effects similar in magnitude on lipid fluidity to those induced by intact apolipoprotein C-I. In the liquid-crystalline state, however, the apolipoprotein-induced acyl chain crystallization of lipid molecules occurs more dramatically at the 2-position than the 12-position of the bilayer. These apolipoprotein-induced increases in bilayer rigidity are likely to be mediated through the lipid-binding portion of the apolipoproteins. It is apparent from the data in fig.1 and table 1 that apolipoprotein C-II causes the largest perturbations in lipid structure. Whether these apolipoprotein C-II effects are related to the role of apolipoprotein C-II in increasing the activity of lipoprotein lipase is unknown but under investigation.

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