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Structural organization of lipid phase and protein-lipid interface in apolipoprotein-phospholipid recombinants: influence of cholesterol

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Abstract

The complexes of individual human plasma apolipoproteins (apo) A-I, E and A-II with dipalmitoylphosphatidylcholine (DPPC) in the absence or in the presence of cholesterol (Chol) were prepared with initial DPPC/Chol/protein weight ratio as 3:0.15:1. ApoA-I/DPPC/Chol complexes with different protein content (initial DPPC/apoA-I weight ratios were changed from 10.5:1 to 2.6:1) but with a fixed initial DPPC/Chol weight ratio of 20:1 were also prepared. The complexes were isolated by gel-filtration and characterized by size and composition. ApoA-I- and apoA-II-complexes had the same size (80–84 Å) and the complexes became more heterogeneous upon Chol inclusion; apoE-complexes were larger (97–100 Å) and more homogeneous and Chol addition had no effect on their hydrodynamic properties. Chol seems to be excluded partially in the following manner for isolated complexes with different apo's: A-II > E > A-I. The possible existence of two lipid regions in the complexes differing in lipid dynamics – the lipid shell in the vicinity of apolipoprotein (boundary lipid) opposite to the remaining part of the lipid bilayer – has been studied by absorbance and fluorescence spectroscopy with *cis*-parinaric acid (*cis*-PA) and *trans*-parinaric acid (*trans*-PA) embedded into the complexes. Their application is based on a strong preference of *trans*-PA for solid lipid while *cis*-PA distributes more equally between co-existing fluid and solid lipid regions (Sklar et al. (1979) Biochemistry 18, 1707–1716). (1) For apoA-I-complexes, the partition of *cis*-PA between water and lipid phase at temperatures below and above the transition temperature of DPPC (T_t) was insensitive to Chol and temperature, while partition of *trans*-PA into the lipid phase of Chol-containing complex was increased at high temperature and decreased at low temperature. These results seem to be related to *trans*-PA redistribution between Chol-rich and protein-rich lipid domains, the latter being more disordered at $T < T_t$ and more immobilized at $T > T_t$ compared to the bulk bilayer; *cis*-PA localizes preferentially in boundary lipid. This hypothesis was directly confirmed by measurements of energy transfer between apoA-I tryptophanyls and probe molecules. (2) The relative response of *trans*-PA fluorescence intensity to temperature-induced phase transition of DPPC in apoA-I/DPPC/Chol complexes was decreased as a function of apolipoprotein content in a non-monotonic fashion with a transition midpoint at a mol ratio DPPC/A-I of 250:1, probably indicating two different modes of apolipoprotein/DPPC interaction in different sized complexes. (3) The comparative study of lipid dynamics in apoA-I-, apoE- and apoA-II-containing complexes with temperature response to

Abbreviations: apoA-I, apolipoprotein A-I; apoA-II, apolipoprotein A-II; apoE, apolipoprotein E; Chol, cholesterol; *cis*-PA, *cis*-parinaric acid; DPPC, dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; EM, electron microscopy; GGE, gradient gel electrophoresis; HDL, high density lipoproteins; rHDL, recombinant high density lipoproteins; *trans*-PA, *trans*-parinaric acid.

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phospholipid phase transition with fluorescence parameters such as intensity and anisotropy of *cis*-PA and *trans*-PA revealed the presence of boundary lipid in all three complexes without Chol. In contrast to apoA-I-containing complexes, in apoA-II/DPPC/Chol complexes, *trans*-PA seems to move preferentially into boundary lipid and *cis*-PA to distribute between two different regions probably as a result of more ordering action induced by apoA-II compared to apoA-I on the nearest phospholipid molecules in Chol-containing complexes; the apoE action on *trans*-PA and *cis*-PA distribution could be intermediate. Based on these results, the degree of Chol exclusion from the boundary lipid region for complexes with different apo's increasing in the order A-II > E > A-I can be suggested. Different Chol distributions between two lipid regions in the complexes seems not to be a function of complex size, but rather is an inherent property of the particular apolipoprotein molecule.

Keywords: Apolipoprotein; Protein-lipid interaction; Boundary lipid; *cis*-Parinaric acid; *trans*-Parinaric acid

1. Introduction

In the regulation of various interactions involved in HDL metabolism, the conformation of the major HDL protein – apoA-I [1] and particle charge [2] as well as both these parameters [3,4] could play a critical role. The use of recombinant particles consisting of apolipoprotein, phospholipid and cholesterol proved to be useful in physico-chemical studies [5]. There exists great uncertainty about the influence of Chol on the properties of rHDL with different apolipoproteins; the maximum size-dependent Chol inclusion remains to be solved [5] as well as the mode of Chol distribution between two lipid regions in the particle, namely, bulk lipid bilayer, closely resembling the pure lipid systems and boundary layer, lipid molecules with perturbed structure due to the interaction with protein molecule [6,7]. Tall and Lange [6] suggested the complete exclusion of Chol molecules from the boundary layer in apoA-I/DMPC/Chol recombinants, but Massey et al. [7] observed only Chol redistribution in favor of bulk bilayer. The direct influence of Chol on apoA-I conformation in rHDL has been suggested by Sparks et al. [8]. Cholesterol distribution could be one of the critical points involved in the reverse transport of Chol from cell to rHDL particle [1,9] as well as in the regulation of LCAT activity by apoA-I, apoE, apoA-II; distinct substrate properties of rHDL particles with these apolipoproteins in the LCAT reaction have been observed with the highest catalytic efficiency in the case of apoA-I-containing particles [10–12]. ApoE and apoA-IV can partially substitute for apoA-I at its deficiency [1].

In this study, the efficiency of complex formation between DPPC and individual apolipoproteins A-I, A-II, E in the absence and in the presence of Chol was studied. The lipid inhomogeneity in these complexes was visualized by the aid of fluorescent probes *cis*-parinaric acid and *trans*-parinaric acid whose spectral properties and application in membrane studies has been described in detail by Sklar et al. [13–16]. We used the different partition of these probes between different lipid domains: the *trans*-probe preferentially partitions into the phospholipid solid phase where it exhibits a strongly enhanced quantum yield, and with this probe by anisotropy measurements it is still possible to detect a few percent of the solid phase; the *cis*-PA distributes more equally between co-existing solid and fluid phospholipid phases [16]. By three independent approaches (1) the measurement of partition of probe molecules between water and lipid phase; (2) the study of energy transfer between apoA-I tryptophanyl residues and lipid-bound probe molecules; (3) the measurement of temperature response of probe fluorescence intensity and anisotropy to phospholipid phase transition – the existence of boundary lipid has been shown in apoA-I-containing rHDL. In these complexes, in comparison with the bulk bilayer, boundary lipid exists in a more disordered state at temperatures lower than the phospholipid transition temperature and in a more immobilized state at temperatures above the transition temperature; if Chol is added, it distributes between the two lipid regions. Boundary lipid also exists in apoE- and apoA-II-containing complexes, and the preference of Chol for the bulk bilayer is assumed for apoA-II/DPPC/Chol complexes based on the different distribution modes of *cis*- and *trans*-PA in the complexes with different apolipoproteins. ApoE possesses an action intermediate between apoA-I and apoA-II on Chol distribution.

2. Materials and methods

2.1. Materials

Dipalmitoylphosphatidylcholine and cholesterol were purchased from Sigma (MO, USA). ^{3}H-labelled cholic acid was purchased from NEN (MA, USA). Fluorescent probes *cis*- and *trans*-parinaric acids were purchased from Molecular Probes (OR, USA) and stored in the freezer in an ethanol solution (about 0.5 mM) until use. Apolipoproteins A-I and A-II were isolated from delipidated human plasma high density lipoproteins by ion-exchange chromatography in denaturing conditions [17]. Apolipoprotein E was purified from pooled human plasma very low density lipoproteins by gel filtration of delipidated VLDL proteins in denaturing conditions followed by affinity chromatography on heparin-Sepharose [18]. The purity of apolipoproteins was checked by SDS-PAGE [19] in a 8–25% precast gel (Pharmacia LKB Phast System) or in a 5–20% gel prepared in the laboratory. Generally, the purity was greater than 95%.

2.2. Methods

The buffer used throughout this study was 50 mM Tris-HCl, pH 8.0, 0.15 M NaCl, 0.02% NaN_3.

2.2.1. Preparation and characterization of apo / DPPC recombinants with different apolipoproteins and apoA-I / DPPC / Chol complexes with different apolipoprotein content

Discoidal rHDL preparation involved a modification of a procedure originally described by Bonomo and Swaney [20]. 6–7 mg of DPPC in $CHCl_3$ was added to a 6-ml glass tube and dried under nitrogen and additionally with a vacuum pump. Cholesterol in a weight ratio DPPC:Chol = 20:1 was added in some cases before sample drying. Buffer was added to give a 8.2 mM DPPC concentration, the mixture was vortexed until the dry film dissolved completely, and sodium cholate was added from a 50 mM stock solution to a final concentration of 7.5 mM. The mixture was vortexed constantly with low heat (45–50°C) until completely clear. The apolipoprotein was added to give a protein:DPPC weight ratio of 1:3, and the final DPPC and cholate concentrations were 4.1 mM and 6 mM respectively. The mixture was incubated overnight at 39°C, diluted two-fold with buffer to decrease the cholate concentration below the critical micellar concentration, and detergent was removed from the emulsion by incubation with hydrated Bio-Beads (50 mg of particles/ml) for 3 h at 39°C followed by low-speed centrifugation to sediment Bio-Beads. The fresh portion of particles was then added, and the incubation was repeated. Protein and lipid recoveries were generally greater than 70%. The efficiency of cholate removal was greater than 99.5% as estimated from a control experiment by inclusion of a tracer quantity of [^{3}H]cholic acid into the initial mixture, i.e., complexes after Bio-Beads procedure contained less than 2 mol% cholate relative to DPPC content. After cholate removal, complexes were reisolated by gel-filtration on a Superose 6PG (1.0×48.5 cm) column with simultaneous measurements of optical density at 280 nm and tryptophan emission at 330 nm for apoA-I- and apoE-containing complexes or tyrosine emission at 305 nm for apoA-II-containing complexes. The Stokes radii R_s of the complexes were determined on the same column from the calibration curve $R_s = f(1/K_{av})$ obtained with standard proteins: thyroglobulin ($R_s = 85$Å), ferritin ($R_s = 61$Å), serum albumin ($R_s = 35.5$Å), ovalbumin ($R_s = 30.5$Å) and chymotrypsinogen A ($R_s = 20.9$Å) [5]; catalase was omitted from the original standard set [5] due to the large deviation from the calibration curve which seemed to become linear ($r^2 = 0.998$) in these conditions. The relative homogeneity of the complexes was estimated from the area under the elution profile normalized to the same height. The chemical composition of the isolated complexes was assayed as follows: DPPC and cholesterol were measured with commercial kits (Biomerieux, France, Boehringer Mannheim, Germany), and apolipoprotein content was determined by absorbance at 280 nm using known weight extinction coefficients: 11.5×10^2 $g^{-1}cm^2$ for apoA-I [21], 7.4×10^2 $g^{-1}cm^2$ for apoA-II [22]. The molar extinction coefficient 43376 $M^{-1}cm^{-1}$ for apoE was calculated by us based on the aromatic amino acid composition of apoE [23].

The same procedure was used to prepare four apoA-I/DPPC/Chol complexes with different apolipoprotein contents but trying to keep DPPC/Chol ratio constant. The initial DPPC/apoA-I weight ratios were 10.5, 6.6, 3.9 and 2.6. In this case, the apoA-I content was estimated by determination of phenylalanine by HPLC after protein hydrolysis [24]. To increase the separation, complexes were isolated on two identical Superose 6PG columns connected in series. For larger complexes, the elution profiles became more heterogeneous and, to isolate mainly one population of the particles, the profiles were deconvoluted by eye into 3–4 components. The tube material with absorbance/fluorescence values not smaller than one-half of the corresponding peak, was combined for subsequent procedures. The estimation of mean size of the complexes was performed in the usual manner.

2.2.2. Liposome preparation

Two aliquots of DPPC in chloroform, 5 mg each, to one of which cholesterol was added in DPPC/Chol weight ratio 20:1, were dried under nitrogen and in vacuo. 2 ml of buffer was added to each tube, and the mixture was sonicated 3 times, 7 min each, with the Branson sonifier equipped with the standard tip at 45°C under N_2, with the power setting 55W. The mixtures were centrifuged in 900-μl aliquots for 15 min, at 100 000 rpm, 22°C in an Optima TLX ultracentrifuge (Beckman) with a TLA 120.2 fixed angle rotor. After centrifugation, the upper 775 μl from each tube were removed and 1.55 ml of the sample was loaded onto a Sepharose CL-4B column. The peak, corresponding to single bilayer vesicles, was collected and concentrated with Centricon 100 (Amicon).

Mole fraction partition coefficients for *cis*-PA and *trans*-PA partition between lipid phase of liposome or complex and water phase were determined by absorption spectroscopy at 25°C and 50°C as described by Sklar et al. [16] for liposomes.

Fluorescence measurements were done on an Aminco SPF-500 spectrofluorometer equipped with a polarizer accessory and interfaced to a computer. A 0.3×1.0 cm quartz cuvette was placed in the thermostated chamber with long side parallel to the excitation beam. The temperature was maintained with 0.1°C accuracy and was measured constantly with a thermocouple placed immediately above the excitation beam. The efficiency of energy transfer E between apoA-I tryptophanyls as donors and parinaric acid probe molecules as acceptors is calculated from the reduction of the emission of the donor according to the relationship

$$E = 1 - Q_a/Q_o, \quad (1)$$

where Q_o refers to the unquenched quantum yield of the donor and Q_a refers to its quantum yield at a particular surface density of acceptor. Experimentally, apoA-I-containing complexes (200 μM as DPPC) in a total volume 400 μl were titrated at 25°C and at 50°C with increasing quantities of *cis*-PA and *trans*-PA added from concentrated ethanol solution. The final ethanol concentration at the end of the titration did not exceed 1%. The excitation wavelength was 280 nm, and quenching was calculated from the decrease of area under emission curve in the range 300–350 nm, where probe fluorescence is negligible.

To monitor temperature-induced phase transitions in the liposome and in the complexes, the sample in the cuvette (200 and 750 μM DPPC for complex and for liposome, respectively) with 4 μM *cis*- or *trans*-PA was placed into the cell chamber pre-heated to 55°C, and the temperature was gradually decreased to 25°C at a rate 0.7°/min while measuring fluorescence anisotropy at fixed time intervals. For both probes, excitation was at 320 nm with 0.5 nm excitation slit, and emission was recorded at 420 nm with 10 nm slit. Total intensity I is calculated as

$$I = I_{\parallel} + 2I_{\perp}, \quad (2)$$

$I_{\parallel}$ and $I_{\perp}$ refer to parallel and perpendicular orientation of polarizer and analyzer, respectively. With two parameters it is possible to make more precise conclusions about the probe microenvironment and its dynamics but it should be stressed that r parameter instead of I, is not an additive value and generally the registration of intensity changes to monitor the temperature-induced transitions is much more favorable [25]. The data were

treated as first order phase transition to obtain thermodynamic parameters (transition temperature T_t, the changes of enthalpy ΔH and entropy ΔS) and the ratio of the probe fluorescence intensities I_s/I_f and anisotropies r_s/r_f at the beginning and after the completion of the phase transition ('s', solid; 'f', fluid).

Data were fitted by nonlinear least squares using the SAS package. Only the data with the asymptotic standard error not exceeding 7% of the estimate were taken into subsequent consideration. The verification of the achievement of the global minimum in the fitting procedure was done by wide variation of the initial parameters. Data were fitted to the equations:

$$I = \frac{I_{0s} + aT + \left(I_{0f} + bT\right) \cdot e^{\frac{-\Delta H^0_{sf} + T\Delta S^0_{sf}}{RT}}}{1 + e^{\frac{-\Delta H^0_{sf} + T\Delta S^0_{sf}}{RT}}}, \quad (3)$$

$$r = \frac{\left(r_{0s} + cT\right) \cdot \left(I_{0s} + aT\right) + \left(r_{0f} + dT\right) \cdot \left(I_{0f} + bT\right) \cdot \frac{e^{-\Delta H^0_{sf} + T\Delta S^0_{sf}}}{RT}}{I_{0s} + aT + \left(I_{0f} + bT\right) \cdot \frac{e^{-\Delta H^0_{sf} + T\Delta S^0_{sf}}}{RT}}, \quad (4)$$

where I and r are fluorescence intensity and anisotropy; $I_{0s}(r_{0s})$ and I_{0f} (r_{0f}) – the fluorescence intensities (anisotropies) of the probe in solid and fluid lipid at some reference temperature; $a(c)$ and $b(d)$ – the temperature dependence of the fluorescence (anisotropy) of the probe in solid and fluid lipid (i.e., baseline slope, assumed to be linear); ΔH^o_{sf} and ΔS^o_{sf} – the enthalpy and entropy changes for the (two-state) phase transition. The transition temperature, T_t, equals $\Delta H^o_{sf}/\Delta S^o_{sf}$.

3. Results

3.1. Complex preparation and characterization.

The data of a single representative chromatographic separation of the complexes apoA-I or apoE or apoA-II with DPPC with or without Chol (DPPC/Chol mol ratio 10:1 or 9.1 mol% Chol) are given in Fig. 1. Data about hydrodynamic dimensions of the complexes, their homogeneity and composition are summarized in Table 1. The initial DPPC/protein weight ratio was 3:1 and equal for all three proteins and corresponded to a mole ratio of 115:1 for apoA-I, 139:1 for apoE and 36:1 for monomeric apoA-II. Without Chol, the yield of the complexes

Table 1
The stoichiometry and the Stokes diameters of the complexes with different apolipoproteins (mean values ± S.E.M.) calculated from gel-filtration data on Superose 6PG column (1.0×48.5 cm)

Sample	DPPC/C/apo mol ratio [a]	Stokes diameter, Å	Width of the complex peak, ml [b]
A-I/DPPC	108:0:1	82 ± 2 (5)	3.87 ± 0.11 (5)
A-I/DPPC/C	105:9.2:1	79.5 ± 0.8 (3)	4.46 ± 0.10 (3)
E/DPPC	134:0:1	97 ± 2 (4)	3.55 ± 0.12 (3)
E/DPPC/C	132:9.8:1	100.5 ± 0.0 (2)	3.47 ± 0.04 (2)
A-II/DPPC	34:0:1	84 ± 2 (2)	4.06 (1)
A-II/DPPC/C	34:2.3:1	81 ± 3 (2)	4.66 (1)

The total number of runs for different preparations are indicated in brackets.
[a] Representative values from single preparation.
[b] The peak width measured at the baseline level is an indicator of the complex homogeneity.

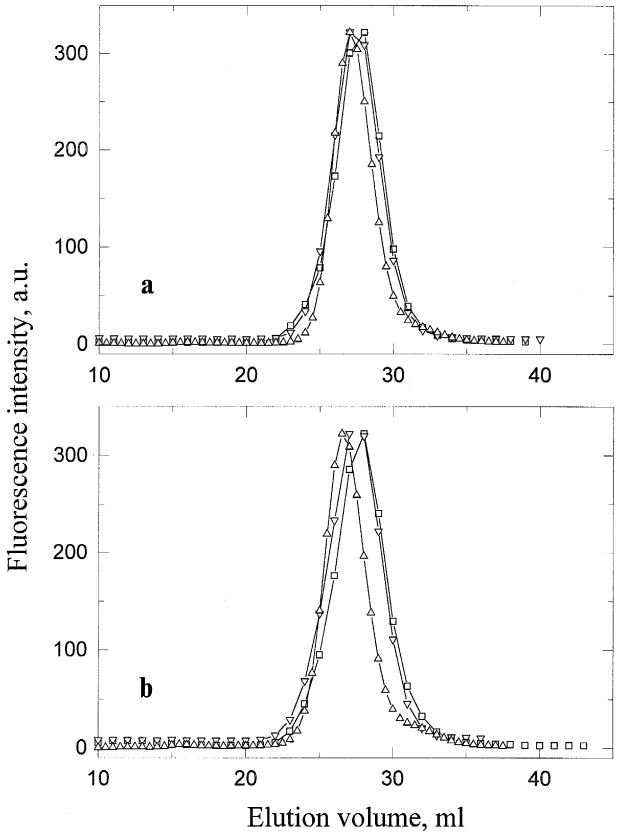

Fig. 1. Elution profiles of the apolipoprotein/lipid complexes on Superose column. a: complexes without Chol; b: Chol in a weight ratio DPPC/Chol = 20:1 was included in the initial mixtures. Profiles shown are for apoA-I(□)- apoA-II(▽)- and apoE(△)-containing complexes and were detected by protein fluorescence (excitation and emission wavelengths for apoA-I- and apoE-containing complexes were 295 and 330 nm, respectively, and 280 and 305 nm for apoA-II-containing complexes. The column (1.0×48.5 cm) was eluted at a flow rate 0.5 ml/min and 1-ml fractions were collected.

was near 100% as (1) there was no free protein and free lipid in elution profiles; (2) the initial DPPC/protein ratios were equal to the values in the complexes isolated. Chol inclusion in the complexes seems to be dependent on the specific apolipoprotein: Chol/DPPC mole ratios were 0.087, 0.074 and 0.068 for apoA-I-, apoE- and apoA-II-containing complexes, respectively, i.e., 22% decrease between the former and the latter complexes (Table 1). Chol inclusion did not change the mean values of Stokes diameters (80–84 Å) for these complexes but they became more heterogeneous. In comparison with these complexes, apoE/DPPC complexes were larger (97–100 Å) and more homogeneous, and Chol addition had no effect on their hydrodynamic properties.

3.2. cis-PA and trans-PA distribution between water and lipid phases

The partition coefficient of these probes is a sensitive indicator of the lipid dynamics, and it is necessary to know it to compare the concentration dependencies of efficiency of energy transfer. The partition coefficients *K* were determined both for liposomes and for apoA-I-complexes at 25° and at 50°, i.e., at temperatures below and above the DPPC transition temperature (Table 2). The values for partition of both probes between the liposome bilayer and the water phase agree well with the values obtained by Sklar et al. [14,16]. The inclusion of Chol in liposomes at 12:1 DPPC/Chol mol ratio resulted in (1) the increased partition of *cis*-PA into lipid phase and decreased partition for *trans*-PA at 25°C; (2) 3-fold increased partition of *trans*-PA at 50°C. These effects could

Table 2
Mol fraction lipid/water partition coefficients K for *cis*- and *trans*-parinaric acids measured at two temperatures (probe concentration was 4 μM)

Sample	Probe	K	
		25°C	50°C
DPPC liposome	c	3.1×10^5	n.d.
	t	5.6×10^6	*n.d.*
DPPC/C liposome	c	3.5×10^5	n.d.
	t	4.6×10^6	13.9×10^6
A-I/DPPC	c	1.4×10^6	1.1×10^6
	t	6.9×10^6	5.5×10^6
A-I/DPPC/C	c	9.3×10^5	1.4×10^6
	t	4.3×10^6	9.3×10^6

n.d., not determined.

be a direct result of 'fluidizing' and 'freezing' action of Chol on the bilayer at gel and liquid crystalline states respectively [26] and preferential distribution of *trans*-probe into lipid regions enriched with Chol. Both in the absence and in the presence of Chol in the liposome, K values for *trans*-probe were more than one order of magnitude higher than for *cis*-probe and increased 3-fold for DPPC/Chol liposome at increasing temperature from 25°C to 50°C. For apoA-I/DPPC complexes the K values measured with both probes at two temperatures were higher than the corresponding values for liposome, and this increase, minimal for the *trans*-probe, was marked (4.5-fold) for *cis*-PA distribution into the lipid phase of the complex at 25° (Table 2), i.e., apolipoprotein induces local defects in the phospholipid packing with the accumulation of *cis*-PA in these regions. Chol inclusion in the complex at 11:1 DPPC/Chol mol ratio resulted in the decreased partition of *cis*-PA at 25°C, probably due to the more immobilizing action of Chol compared to apolipoprotein that coincides with the decreased incorporation of *trans*-PA into the apoA-I/DPPC/Chol complex compared to DPPC/Chol liposome at 50°C. However, the temperature-induced changes in opposite directions of K values for both probes obtained for the complexes with and without Chol respectively cannot be explained by the simple partition of the probe molecule into homogeneous lipid phase. The preferential location of *cis*-PA in the close vicinity to the apolipoprotein molecule in the complex without Chol at 50°C and preferential location of *trans*-PA in the Chol-containing complex in the DPPC/Chol region more ordered at high temperature compared to boundary lipid should be assumed. To visualize the spatial relationships in these systems, energy transfer study was done.

3.3. Energy transfer study

There is a spectral overlap between emission spectrum of apoA-I tryptophanyls and the absorbance spectra of *cis*-PA and *trans*-PA in the range 290–350 nm (Fig. 2a), i.e., these chromophores can form donor–acceptor pairs, and the distance between them can be determined by measuring the efficiency of energy transfer. With addition of increasing quantities of *trans*-PA (Fig. 2b) or *cis*-PA (data not shown) to the apoA-I/DPPC complex, fluorescence of apoA-I tryptophanyls was quenched and probe fluorescence was increased due to energy transfer. The concentration dependencies of energy transfer for both probes bound to apoA-I/DPPC or apoA-I/DPPC/Chol were measured at two temperatures (Fig. 3). In the absence of Chol at 25°C, these curves for both probes had sigmoidal character and energy transfer to *cis*-PA was much more efficient than to *trans*-PA (Fig. 3a), i.e., at low temperatures the boundary lipid is in a more disordered state compared to the bulk bilayer. At 50°C the energy transfer to *trans*-PA was increased and the energy transfer to *cis*-PA was decreased, i.e., boundary lipid, compared to the bulk bilayer, is more immobilized at high temperature. Inclusion of Chol in the complex did not change significantly the ratio between energy transfer to *cis*-PA at two temperatures, but dramatically altered this ratio for *trans*-PA (Fig. 3b). The large decrease of energy transfer to this probe at 50°C

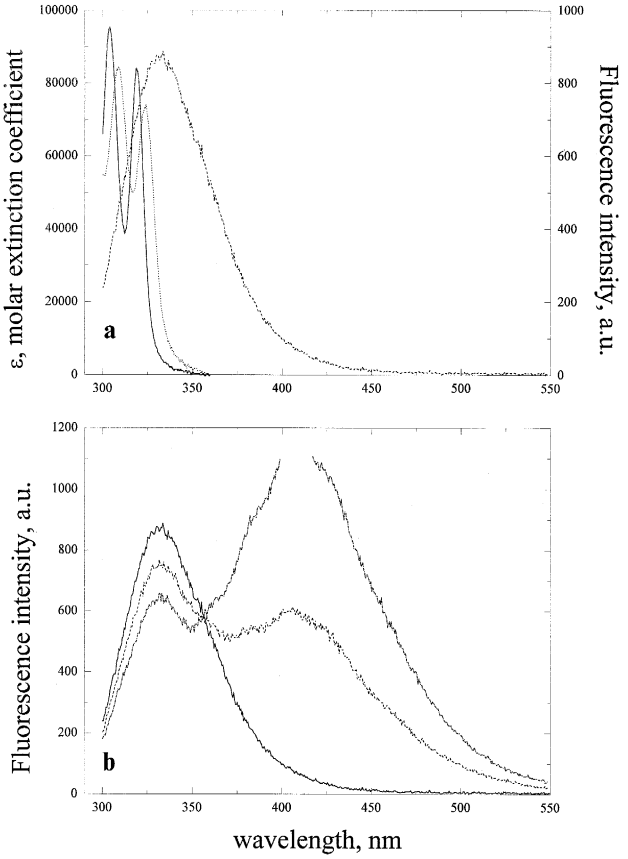

Fig. 2. The spectral overlap between apoA-I tryptophan emission and parinaric acid absorption bands (a) and quenching of apoA-I tryptophan fluorescence by *trans*-PA (b). a: (---) emission spectrum, (————) *trans*-PA absorbance spectrum, (· · ·) *cis*-PA absorbance spectrum. b: (————) 0 μM, (---) 1 μM and (– · –) 2 μM *trans*-PA were added to the apoA-I/DPPC complex, 200 μM as phospholipid, from stock solution in ethanol. Excitation was at 280 nm and the spectra were taken at 25°C.

is an indicator of probe redistribution from boundary lipid into the Chol-immobilized regions. At 25°C, the efficiency of energy transfer for *trans*-PA in apoA-I/DPPC/Chol complexes is higher than in apoA-I/DPPC complexes, which could be a consequence of more disordered Chol/DPPC regions compared to the boundary lipid.

3.4. Boundary lipid in apoA-I / DPPC / Chol complexes with different apolipoprotein content

Complexes with different apoA-I content but with the same initial DPPC/Chol ratio were prepared and isolated, and the magnitude of the transition was measured by *trans*-PA (Fig. 4). Probe/DPPC mol ratios were 1:200 and 1:50 for liposomes and for the complexes, respectively, i.e., according of partition coefficient values, more than 90% of the probe molecules are localized in the lipid phase. The complex composition as well as the data on transitions obtained with both probes by fluorescence intensity and anisotropy measurements are summarized in Tables 3 and 4. From the data in Fig. 4, it is clear that the transition magnitude in the complex relative to liposomes decreased in a non-monotonic fashion with increasing apoA-I content; the transition midpoint between the two components was at 250:1 DPPC/apoA-I mol ratio. The relative Chol content in the complexes isolated also decreased in an analogous fashion with the same transition as apoA-I content increased (Table 3).

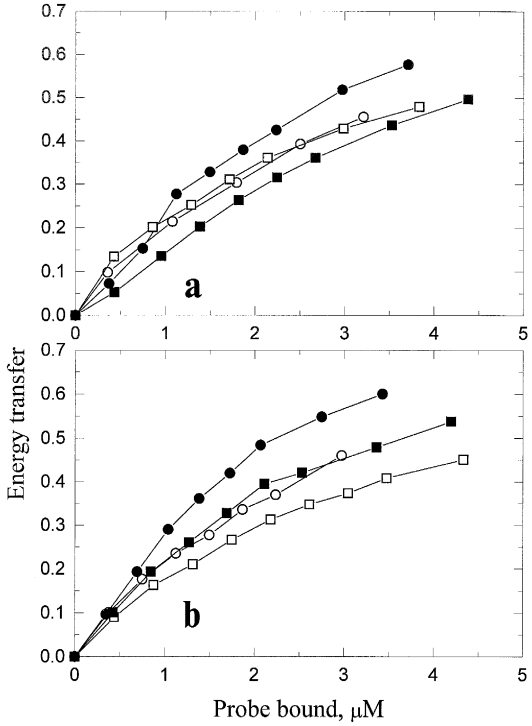

Fig. 3. Concentration dependence of energy transfer between apoA-I tryptophanyls and *cis*-PA or *trans*-PA in apoA-I/DPPC (a) and apoA-I/DPPC/Chol (b) complexes at 25°C (closed symbols) and 50°C (open symbols). The complexes (200 μM as DPPC) were titrated with increasing quantities of cis-PA (○,●) or trans-PA (□,■) added from stock solution in ethanol. The excitation was at 280 nm and the energy transfer was calculated from the decrease of area under protein emission curve in the range 300–350 nm where probe does not contribute. Bound probe concentration was calculated from the partition coefficient values determined for *cis*-PA and *trans*-PA at two temperatures.

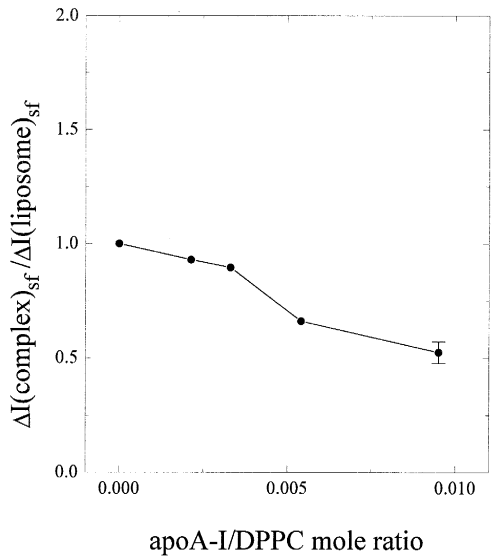

Fig. 4. The relative magnitude of temperature-induced phase transition in apoA-I/DPPC/Chol complexes as a function of apolipoprotein content. The results shown are the ratios of fluorescence intensity change of *trans*-PA as a result of DPPC phase transition from solid ('s') to fluid ('f') state in the complex and in the liposome.

Table 3
Thermodynamic parameters of temperature-induced transitions in cholesterol-containing liposomes and apoA-I complexes I-IV with different protein/lipid ratio detected by fluorescence intensity of *trans*-parinaric acid (means ± S.E.M., the number of separate experiments are indicated in the brackets)

Sample	ΔH, kcal/mol	ΔS, e.u.	T_t, °C	I_s/I_f
DPPC/C liposome, 12:1 mol ratio	137 ± 5 (4)	437 ± 15	40.3 ± 0.1	2.86 ± 0.12
A-I/DPPC/C (**I**, 312 Å), 1:469:54 mol ratio	130 (1)	411	42.0	2.60
A-I/DPPC/C (**II**, 192 Å), 1:302:35 mol ratio	109 ± 1 (2)	344 ± 2	42.7 ± 0.0	2.54 ± 0.04
A-I/DPPC/C (**III**, 144 Å), 1:185:20 mol ratio	96 (1)	302	42.9	2.32
A-I/DPPC/C (**IV**, 79.5 Å), 1:105:9.2 mol ratio	70 ± 0 (2)	222 ± 1	42.2 ± 0.0	2.19 ± 0.06

Stokes diameters of the complexes were calculated from gel-filtration data; the value for the complex **I** obtained by extrapolation of the calibration curve beyond the data for standard proteins has only approximate meaning.

3.5. Boundary lipid in complexes with different apolipoproteins

The temperature responses of fluorescence intensity and anisotropy of *cis*-PA and *trans*-PA embedded in the lipid phase of the complexes of apoA-I, apoE and apoA-II with DPPC and with or without Chol as a result of measurements with single preparations are given in Figs. 5 and 6, respectively. Data obtained with fluorescence intensity or anisotropy measurements with different complex preparations are summarized at Tables 5 and 6, respectively.

In DPPC liposomes without Chol, there was a sharp ($\Delta T = 2°$) temperature-induced phase transition with transition temperatures T_t 41.48°C and 40.82°C, calculated from fluorescence intensity and anisotropy of *trans*-PA. A less cooperative transition ($\Delta T = 5°$) shifted by 0.4–0.6°C in the low temperature region was detected by *cis*-PA. These data agreed well with the T_t values for DPPC obtained by DSC [26] and by fluorescence measurements with the same probes [14,16]. The formation of the complex with the apolipoprotein suppressed the amplitude of the transition detected by fluorescence intensity of both probes due to the decrease of quantum yield at temperatures both lower and higher than T_t. With *cis*-PA, there was no apolipoprotein

Table 4
Thermodynamic parameters of temperature-induced transitions in cholesterol-containing liposomes and apoA-I complexes I–IV with different protein/lipid ratio detected by anisotropy of fluorescence of *trans*-parinaric acid (means ± S.E.M., the number of separate experiments are indicated in the brackets)

Sample	ΔH, kcal/mol	ΔS, e.u.	T_t, °C	r_s/r_f
DPPC/C liposome, 12:1 mol ratio	170 ± 17 (4)	544 ± 53	39.2 ± 0.2	1.62 ± 0.03
A-I/DPPC/C (**I**), 1:469:54 mol ratio	119 (1)	380	40.5	1.61
A-I/DPPC/C (**II**), 1:302:35 mol ratio	91 ± 1 (2)	288 ± 2	41.1 ± 0.0	1.64 ± 0.01
A-I/DPPC/C (**III**), 1:185:20 mol ratio	99 (1)	314	41.0	1.36
A-I/DPPC/C (**IV**), 1:105:9.2 mol ratio	115 ± 27 (2)	368 ± 85	39.7 ± 0.4	1.13 ± 0.04

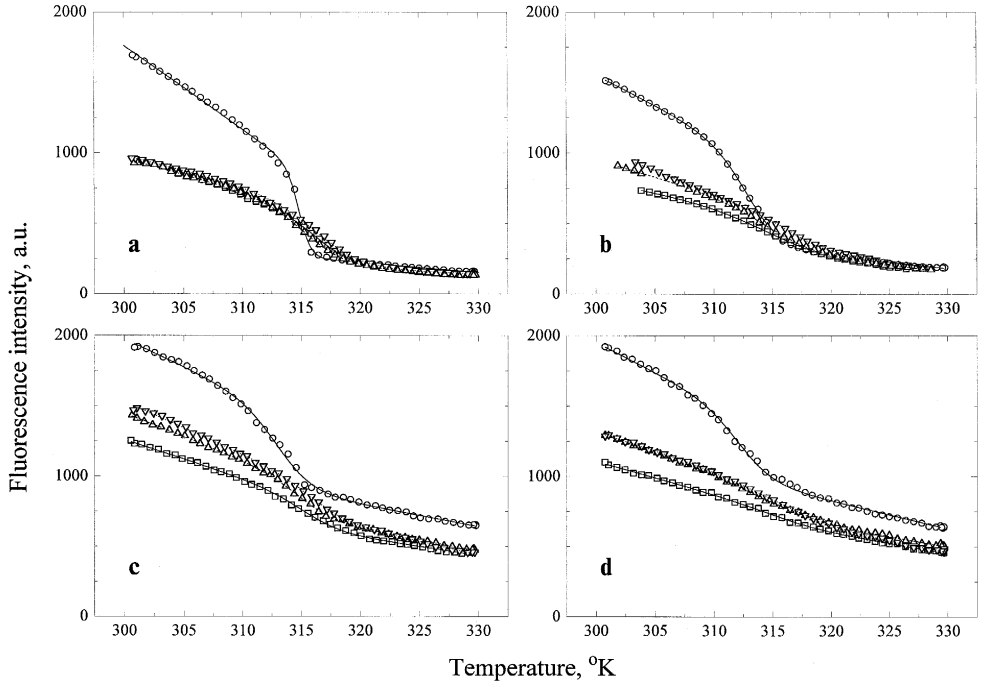

Fig. 5. Temperature responce of the fluorescence intensity of *trans*-PA (a,b) and *cis*-PA (c,d) in liposomes and in the complexes with different apo's in the absence (a,c) and in the presence of Chol (b,d) in preparations. Symbols denote to experimental data and the corresponding lines are the results of nonlinear curve fitting for the two-state phase transition model: (○ ——— ○) liposomes; (□ ——— □) apoA-I-complexes; (▽ · · · ▽) apoA-II-complexes; (△ – – – △) apoE-complexes. Excitation was at 320 nm and emission recorded at 420 nm for both probes with polarizer accessory was calculated as $I = I_{\parallel} + 2I_{\perp}$, $I_{\parallel}$ and $I_{\perp}$ refer to parallel and perpendicular orientation of polarizer and analyzer, respectively.

specifity in the suppression of transition, while with *trans*-PA the suppression was maximal for apoA-I/DPPC and minimal for apoA-II/DPPC complexes; apoE-containing complexes were characterized by an intermediate suppression. The protein-lipid interactions resulted in a more disordered state of lipid bilayer at $T < T_t$ and more ordered at temperatures above the transition temperature as evidenced by the *r* measurements with both probes (Fig. 6); however, the disordering effect of the protein was much more evident with *trans*-PA. Generally, r_s/r_f changes were parallel to I_s/I_f changes. The direct effect of decreased quantum yield on the observed increase in *r* values at $T > T_t$, however, cannot be excluded. It should be stressed that in liposomes the values of fluorescence anisotropy for *cis*-PA were lower than for *trans*-PA over the whole temperature range studied, which coincides with the data of Sklar et al. [16] and could be an indicator of the existence of a solid domain(s) even at the temperatures above T_t. In the complexes, the transition was broadened and shifted to higher temperatures in comparison with pure phospholipid. This shift 1–2°C, detected by *cis*-PA and depending on the *I* or *r* registration, was approximately equal for all three apo/DPPC complexes. With *trans*-PA fluorescence intensity, the shift in the transition temperature was greater for apoA-I/DPPC complexes (5°C) than for apoE/DPPC (1.2°C) or apoA-II/DPPC (2°C) complexes; the analogous shifts were detected by *trans*-PA fluorescence anisotropy. Enthalpy and entropy changes for the DPPC transition in liposomes detected by *trans*-PA were larger than the corresponding values for *cis*-PA. For apoA-I/DPPC, apoE/DPPC, apoA-II/DPPC complexes, ΔH and ΔS values for the transition, detected by fluorescence intensity of *cis*-PA, were at the mean level 51%, 67% and 64%, respectively, of the values for liposome. For *trans*-PA, these values were 32%, 29% and 24%. Qualitatively, the changes of the same magnitude were obtained with *r* measurements for both probes (Table 6).

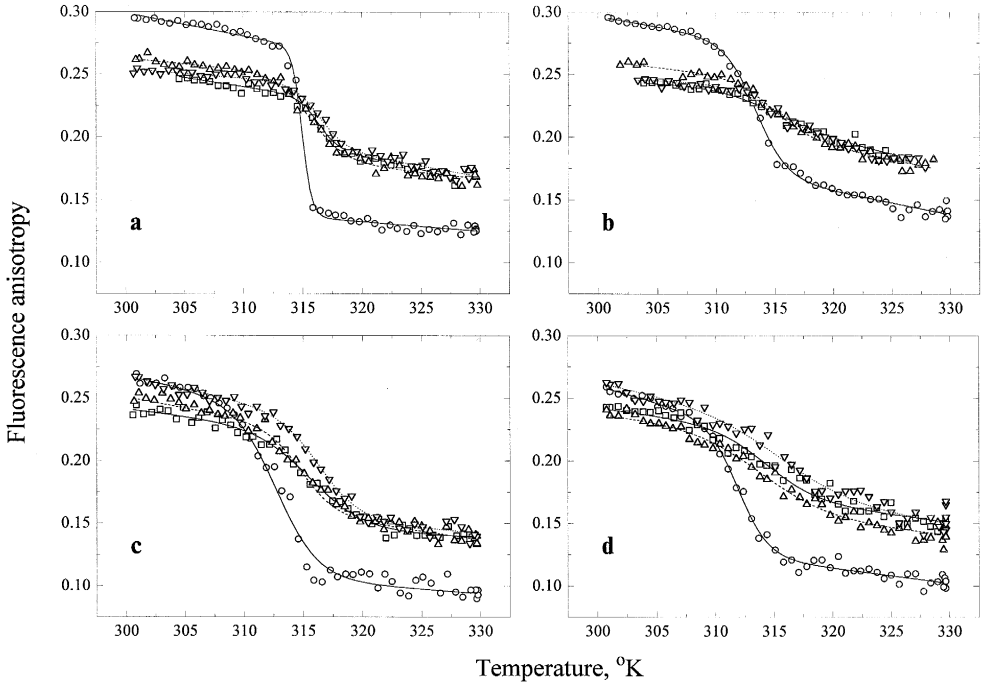

Fig. 6. Temperature responce of the fluorescence anisotropy of *trans*-PA (a,b) and *cis*-PA (c,d) in liposomes and in the complexes with different apo's in the absence (a,c) and in the presence of Chol (b,d) in preparations. Fluorescence anisotropy was calculated as $r = (I_{\|}\text{-}I_{\perp}) / (I_{\|} + 2I_{\perp})$. For experimental details, see legend to Fig. 5.

Inclusion of Chol in liposomes resulted in (1) a decrease of transition amplitude (more prominent with *trans*-PA (Fig. 5)); (2) a decrease of the transition temperature by 1.2°C and (3) a decrease of enthalpy and entropy changes calculated from I and r responses for both probes. The transition became more broadened, as

Table 5
Thermodynamic parameters of temperature-induced transitions in liposomes and in the complexes with different apolipoproteins detected by fluorescence intensity of *cis*- and *trans*-parinaric acids (means ± S.E.M., the number of separate experiments are indicated in brackets)

Sample	Probe	ΔH, kcal/mol	ΔS, e.u.	T_t, °C	I_s / I_f
DPPC liposome	c (5)	191 ± 27	608 ± 87	41.1 ± 0.3	1.58 ± 0.06
	t (2)	*378 ± 8*	*1203 ± 22*	*41.5 ± 0.2*	*3.51 ± 0.08*
DPPC/C liposome	c (4)	105 ± 6	334 ± 20	39.8 ± 0.3	1.54 ± 0.07
	t (4)	*137 ± 5*	*437 ± 15*	*40.3 ± 0.1*	*2.86 ± 0.12*
A-I/DPPC	c (2)	97 ± 10	307 ± 31	43.2 ± 0.1	1.42 ± 0.003
	t (2)	*120 ± 6*	*375 ± 21*	*46.4 ± 1.8*	*2.66 ± 0.20*
A-I/DPPC/C	c (2)	69 ± 11	218 ± 34	43.2 ± 0.1	1.32 ± 0.14
	t (2)	*70 ± 0*	*222 ± 1*	*42.2 ± 0.0*	*2.19 ± 0.06*
E/DPPC	c (1)	127	404	42.4	1.34
	t (1)	*110*	*347*	*42.7*	*3.04*
E/DPPC/C	c (1)	63	201	42.0	1.28
	t (1)	*84*	*265*	*42.4*	*2.07*
A-II/DPPC	c (3)	122 ± 5	387 ± 16	43.3 ± 0.1	1.35 ± 0.09
	t (2)	*92 ± 5*	*292 ± 17*	*43.5 ± 0.2*	*3.74 ± 0.15*
A-II/DPPC/C	c (2)	78 ± 13	249 ± 43	41.3 ± 1.6	1.36 ± 0.03
	t (2)	*137 ± 2*	*432 ± 6*	*43.8 ± 0.5*	*1.54 ± 0.14*

Table 6
Thermodynamic parameters of temperature-induced transitions in liposomes and in the complexes with different apolipoproteins detected by anisotropy of fluorescence of *cis*- and *trans*-parinaric acids (means ± S.E.M., the number of separate experiments are indicated in brackets)

Sample	Probe	ΔH, kcal/mol	ΔS, e.u.	T_t, °C	r_s/r_f
DPPC liposome	c (5)	268 ± 66	855 ± 211	40.2 ± 0.2	2.14 ± 0.04
	t (2)	*394 ± 57*	*1253 ± 180*	*40.8 ± 0.3*	*1.98 ± 0.01*
DPPC/C liposome	c (4)	136 ± 6	437 ± 20	38.5 ± 0.2	1.86 ± 0.02
	t (4)	*170 ± 17*	*544 ± 53*	*39.2 ± 0.2*	*1.62 ± 0.03*
A-I/DPPC	c (2)	100 ± 21	317 ± 67	42.3 ± 0.3	1.51 ± 0.13
	t (2)	*205 ± 20*	*647 ± 68*	*44.7 ± 1.7*	*1.19 ± 0.00*
A-I/DPPC/C	c (2)	69 ± 35	220 ± 108	37.1 ± 4.5	8.51 ± 5.16
	t (2)	*115 ± 27*	*368 ± 85*	*39.7 ± 0.4*	*1.13 ± 0.04*
E/DPPC	c (1)	98	311	41.1	1.46
	t (1)	*131*	*416*	*40.8*	*1.37*
E/DPPC/C	c (1)	41	131	41.6	1.80
	t (1)	*117*	*375*	*40.3*	*1.21*
A-II/DPPC	c (3)	120 ± 23	379 ± 72	42.7 ± 0.1	1.45 ± 0.07
	t (2)	*155 ± 11*	*493 ± 34*	*41.6 ± 0.0*	*1.31 ± 0.04*
A-II/DPPC/C	c (2)	50 ± 7	158 ± 24	44.9 ± 2.1	2.07 ± 0.49
	t (2)	*59 ± 12*	*187 ± 37*	*42.3 ± 0.5*	*1.46 ± 0.15*

was evidenced by *I* and *r* measurements with *trans*-PA. The disordering (at $T < T_t$) and immobilizing ($T > T_t$) effects of Chol (Fig. 6) seems to be analogous to the effect of apolipoprotein addition. In tertiary apo/DPPC/Chol complexes in comparison with apo/DPPC counterparts the magnitude of the transition detected by *cis*-PA fluorescence intensity decreased most profoundly for apoA-I- and did not change for apoA-II-containing complexes; the transition temperature was also lower, but the direction of the changes was quite opposite to the I_s/I_f changes. For *trans*-PA, the changes in amplitude and in the transition temperature were opposite to changes in these parameters detected by *cis*-PA, namely, I_s/I_f decreased most significantly for apoA-II/DPPC/Chol, and T_t did not change for this complex (Table 5). Despite the opposite changes of T_t and ΔH or ΔS values induced by Chol and apolipoprotein, respectively (Tables 3 and 5), there was a strong linear dependence ($r^2 = 0.999$) both for *cis*-PA and *trans*-PA between ΔH and ΔS for the whole range of pairs. The slope values considered as 'isokinetic' temperatures, corresponded to 41.2 and 41.0 for *cis*-PA and *trans*-PA, respectively. The linear dependence obtained seems to verify the meaning of thermodynamic parameters derived from fitting procedure and, secondly, to suggest the enthalpy-entropy compensation of temperature-induced DPPC transition in different structures.

4. Discussion

In this study the complexes of apolipoprotein A-I, E, A-II with DPPC and with or without Chol were obtained by a cholate removal procedure. ApoA-I- and apoA-II-containing complexes had the same dimensions and Chol inclusion did not change the mean Stokes diameters but increased heterogeneity; inclusion of Chol in larger apoE-complexes increased their size (Table 1). These data are consistent with the observations of increased heterogeneity of apoA-I- [27] and apoE-containing complexes [28] upon Chol incorporation. The estimations of the dimensions of small discoidal complexes of apoA-I with various phospholipids measured by EM [4,8], non-denaturing GGE [8] and gel filtration [5] with an accuracy of 5–15 Å fluctuate widely with the difference as much as 20% [3]. Generally, EM values are higher [3] and data are within the range 93–123 Å [4,8]. In our study, the dimensions of apoA-I-containing complexes estimated from gel filtration analysis (80–84 Å) coincide

with these obtained by other methods within experimental error. It was shown in a separate study [29], that for the complexes with apoE prepared in an analogous manner, the complex dimensions calculated from EM and GGE data (148 Å and 116 Å respectively) coincided well with the literature data and the position of maximum in the elution profile of these complexes on the same column was indistinguishable from that for the complexes used in the present study with the Stokes diameter values 95–100 Å. The high degree of homogeneity of our preparations seen by gel filtration (Table 1) also agrees with the appearance of one discrete band upon nondenaturing GGG of apoA-I-containing complexes prepared by the same procedure [4]. For complexes with different apolipoproteins, the relative Chol incorporation progressively falls in the order A-I > E > A-II (Table 1). The conclusion that complexes with apoA-II contain less Chol compared to complexes with apoA-I can be drawn from data obtained by Jonas et al. [22]. To study lipid dynamics in the bulk bilayer and in the boundary lipid, two fluorescent probes that partition differently between coexisting lipid regions were used: the partition coefficient of *trans*-PA is 3-fold higher for solid compared to fluid lipid regions, while the concentration of *cis*-PA in the fluid region is 2-fold higher as measured by Sklar et al. [16]. To describe the partition of the probe in the mixture of phospholipids with phase separation upon cooling, these authors created a phase diagram obtained from fluorescence responses of the probe with variation of the ratio solid/fluid phospholipid [16]. The analogous approach has been developed to describe the partition of perylene between liquid crystalline and crystalline regions of vesicle membranes formed from binary mixtures of several lipids [30,31]. We do not know the ratio between boundary and bulk lipid; rather, we are interested in a qualitative description of the distribution of *cis*-PA and *trans*-PA between the two lipid domains in the complexes with different apolipoproteins and the influence of Chol on this distribution. For apoA-I-containing complexes, three different sets of measurements were done: (1) the measurement of the partition coefficient; (2) the study of the relative distances between the tryptophan residues of apoA-I and probe molecules by non-radiative energy transfer; (3) the measurement of the amplitude of the response of fluorescence intensity and anisotropy to a temperature-induced phase transition of DPPC in the complexes. We describe the probe behavior; however, other explanations cannot be excluded.

4.1. The partition of the probes in apoA-I-containing complexes

The incorporation of apoA-I produces structural inhomogeneity in the lipid bilayer and results in the increased partition of *cis*-PA into the lipid phase at 25°C (Table 2). At this temperature, boundary lipid can exist in a more disordered conformation compared to the DPPC molecules in the bulk bilayer (the fluidizing action of protein) with a concomitant decrease of the *r* value (Fig. 6). At 50°C the protein, like cholesterol, immobilizes the nearest lipid molecules and this immobilization resulted in the increased incorporation of *trans*-PA into the complex and increased anisotropy values (Fig. 6). The joint action of apolipoprotein and Chol molecules on incorporation of *trans*-PA at 50°C resulted in a partition coefficient value intermediate between those for isolated actions of apoA-I and Chol molecules on the probe incorporation (Table 2). At this temperature, *trans*-PA redistributes between two lipid domains, immobilized either by apolipoprotein or by cholesterol molecules and this redistribution caused a decrease of the efficiency of energy transfer (Fig. 3). At 25°C the existence of two disordered lipid regions ('cholesterol-rich' and 'protein-rich') and which could interact with each other, resulted in the decreased incorporation of *trans*-PA.

In apoA-I/DPPC complex compared to DPPC liposome, the fluorescence quantum yield of both probes decreased at temperatures below and above the transition temperature and T_t values shifted to high temperatures. The incorporation of Chol into DPPC liposomes increased the fluorescence intensity of *trans*-PA at temperatures below and above the transition temperature with a concomitant decrease in the I_s/I_f ratio and a profound decrease of transition temperature (Table 5), i.e., Chol/DPPC regions are more disordered compared to the bulk bilayer. For the tertiary apoA-I/DPPC/Chol complex, the T_t value was intermediate between the values obtained for DPPC/Chol liposomes and for the apoA-I/DPPC complex, and quantum yields for *trans*-PA fluorescence at temperatures below and above T_t were higher than the corresponding values for the complex

without Chol (data not shown). These responses of *trans*-PA fluorescence to temperature-induced phase transition(s) additionally support the suggestion about *trans*-PA distribution between boundary layer and DPPC/Chol domains made from measurement of partition coefficients and studies of energy transfer. As the apolipoprotein content in apoA-I/DPPC/Chol complex increased, the T_t values increased also (Tables 3 and 4), probably due to the decreasing of distance between protein molecules [32]. At a DPPC/apoA-I mole ratio smaller than 250, there is a lower Chol incorporation into complex (Table 1), and Chol molecules are partially excluded from the boundary lipid with the increased suppression of the transition detected by *trans*-PA fluorescence (Fig. 4, Table 3). The structural transition in apoA-I/DPPC/Chol complexes differing in size could be suggested from data obtained by Jonas and McHugh [33], who mentioned the big difference in fluorescent properties of tryptophan residues of apoA-I in large (200 Å) and small (110 Å) complexes. For *cis*-PA fluorescence, Chol incorporation in DPPC liposomes resulted in the quantum yield increased at $T > T_t$ and decreased at $T < T_t$ without any change in T_t value (Table 5). For the tertiary apoA-I/DPPC/Chol complex, the T_t value calculated from the *cis*-PA fluorescence response was exactly the same as for the apoA-I/DPPC complex (Table 5) and the fluorescence intensity dramatically decreased at $T < T_t$. It can be concluded that for the apoA-I/DPPC/Chol complex, *cis*-PA is localized mainly in the boundary lipid and *trans*-PA is distributed between boundary lipid and the bulk bilayer. The relative exclusion of *trans*-PA from the lipid region in contact with the M13 coat protein has been suggested by Kimelman et al. [34].

4.2. The influence of Chol on the boundary lipid in the complexes with apoE and with apoA-II

For apoA-I/DPPC/Chol, apoE/DPPC/Chol and apoA-II/DPPC/Chol complexes, the complex with apoA-II had a highest suppression of temperature-induced response of fluorescence intensity of *trans*-PA and a minimal suppression of *cis*-PA response (Fig. 5, Table 5). We suggest a different mode of probe distribution in the complexes with different apolipoproteins: in an apoA-I/DPPC/Chol complex, *cis*-PA localizes mainly in boundary lipid and *trans*-PA distributes between boundary lipid and bulk bilayer, but in apoA-II/DPPC/Chol complex *cis*-PA distributes between two lipid regions and *trans*-PA localizes preferentially in the boundary lipid. For apoE-containing rHDL, the probe distribution was characterized by an intermediate situation (Table 5). This different mode of probe distribution seems to be related to differential exclusion of Chol from boundary lipid in the complexes with different apolipoproteins (Table 1). In this case, the interaction of apoA-II with phospholipid may be more tighter compared to the interaction between apoA-I and phospholipid and Chol did not influence the former interaction as has been observed experimentally [17]. The different mode of interaction of apoA-I and apoA-II with DPPC and the different Chol content in these complexes could result in different substrate properties of these rHDL in the LCAT reaction. A 20-fold decrease of enzyme activity on the complexes with apoA-II, in comparison with the complexes with apoA-I, has been observed [22]. The data obtained in this study agree well with the conclusion that Chol has a direct influence on apoA-I conformation [8] and contradict the suggestion made by Tall and Lange [6] about Chol exclusion from boundary lipid in apoA-containing complexes. At the same time, the different Chol distributions between the two lipid regions, obtained for rHDL with different apolipoproteins seem not to depend on complex dimensions but rather on the apolipoprotein structure. The suggestion made by us about the mode of Chol distribution in these complexes needs an additional, more direct experimental confirmation, and experiments to localize fluorescent Chol analogues in rHDL with different apolipoproteins are now in progress.

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References

[1] Fielding, C.J. and Fielding, P.E. (1995) J. Lipid Res. 36, 211–228.
[2] Jonas, A., Covinsky, K.E. and Sweeny, S.A. (1985) Biochemistry 24, 3508–3513.
[3] Sparks, D.L., Phillips, M.C. and Lund-Katz, S. (1992) J. Biol. Chem. 267, 25830–25838.
[4] Sparks, D.L., Lund-Katz, S. and Phillips, M.C. (1992) J. Biol. Chem. 267, 25839–25847.
[5] Jonas, A. (1986) Methods Enzymol. 128, 553–582.
[6] Tall, A.R. and Lange, Y. (1978) Biochim. Biophys. Acta 513, 185–197.
[7] Massey, J.B., She, H.S., Gotto, A.M., Jr. and Pownall, H.J. (1985) Biochemistry 24, 7110–7116.
[8] Sparks, D.L., Davidson, W.S., Lund-Katz, S. and Phillips, M.C. (1993) J. Biol. Chem. 268, 23250–23257.
[9] Johnson, W.J., Mahlberg, F.H., Rothblat, G.H. and Phillips, M.C. (1991) Biochim. Biophys. Acta 1085, 273–298.
[10] Jonas, A., Steinmetz, A. and Churgay, L. (1993) J. Biol. Chem. 268, 1596–1602.
[11] Zorich, N., Jonas, A. and Pownall, H.J. (1985) J. Biol. Chem. 260, 8831–8837.
[12] Bolin, D.J. and Jonas, A. (1994) J. Biol. Chem. 269, 7429–7434.
[13] Sklar, L.A., Hudson, B.S., Petersen, M., Diamond, J. (1977) Biochemistry 16, 813–819.
[14] Sklar, L.A., Hudson, B.S., Simoni, R.D. (1977) Biochemistry 16, 819–828.
[15] Sklar, L.A., Miljanich, G.P., Bursten, S.L. and Dratz, E.A. (1979) J. Biol. Chem. 254, 9583–9591.
[16] Sklar, L.A., Miljanich, G.P. and Dratz, E.A. (1979) Biochemistry 18, 1707–1716.
[17] Van Tornout, P., Vercaemst, R., Lievens, M.J., Caster, H., Rosseneu, M. and Assmann, G. (1980) Biochim. Biophys. Acta 601, 509–523.
[18] Dergunov, A.D., Aniskovich, L.P. and Shuvaev, V.V. (1990) Bull. Exp. Biol. Med. 110, 894–898.
[19] Laemmli, U.K. (1970) Nature 227, 680–685.
[20] Bonomo, E.A. and Swaney, J.B. (1988) J. Lipid Res. 29, 380–384.
[21] Gwynne, J., Brewer, B., Jr. and Edelhoch, H. (1974) J. Biol. Chem. 249, 2411–2416.
[22] Jonas, A., Sweeny, S.A. and Herbert, P.N. (1984) J. Biol. Chem. 259, 6369–6375.
[23] Wesgraber, K.H., Rall, S.C., Jr. and Mahley, R.W. (1981) J. Biol. Chem. 256, 9077–9083.
[24] Brasseur, R., De Meutter, J., Vanloo, B., Goormaghtigh, E., Ruysschaert, J.M. and Rosseneu, M. (1990) Biochim. Biophys. Acta 1043, 245–252.
[25] Eftink (1994) Biophys. J. 66, 482–501.
[26] McMullen, T.P.W., Lewis, R.N.A.H. and McElhaney, R.N. (1993) Biochemistry 32, 516–522.
[27] Matz, C.E. and Jonas, A. (1982) J. Biol. Chem. 257, 4535–4540.
[28] Gong, E.L., Nichols, A.V., Weisgraber, K.H., Forte, T.M., Shore, V.G. and Blanche, P.J. (1989) Biochim. Biophys. Acta 1006, 317–328.
[29] De Pauw M., Vanloo B., Dergunov A., Devreese A.-M., Baert J., Brasseur R. and Rosseneu, M. (1997) Biochemistry (Russia) 62, 196–209.
[30] Yguerabide, J. and Foster, M.C. (1979) J. Membrane Biol. 45, 109–123.
[31] Foster, M.C. and Yguerabide, J. (1979) J. Membrane Biol. 45, 125–146.
[32] Owicki, J.C. and McConnell, H.M. (1979) Proc. Natl. Acad. Sci. USA 76, 4750–4754.
[33] Jonas, A. and McHugh, H.T. (1984) Biochim. Biophys. Acta 794, 361–372.
[34] Kimelman, D., Tecoma, E.S., Wolber, P.K., Hudson, B.S., Wickner, W.T. and Simoni, R.D. (1979) Biochemistry 18, 5874–5880.