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Effect of cholesterol on bilayer location of the class A peptide Ac-18A-NH_2 as revealed by fluorescence resonance energy transfer

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Abstract An amphipathic class A peptide, Ac-18A-NH_2, has been employed in modeling the α-helical lipid-binding site of apolipoprotein A-I (apoA-I). To gain insight into the nature of protein–lipid interactions responsible for the ability of apoA-I to promote the efflux of intracellular cholesterol, the peptide disposition in model membranes composed of phosphatidylcholine (PC) and its mixture with cholesterol (Chol) has been characterized. By examining resonance energy transfer between the peptide Trp as a donor and anthrylvinyl-labeled PC as an acceptor it was found that Chol inclusion is conducive to shallower bilayer location of the Ac-18A-NH_2 α-helix. The limits for the Trp distance from the membrane center were estimated to be 1.5–1.7 nm (PC) and 1.9–2.1 nm (PC:Chol), indicating that in the PC bilayer the Trp resides at the level of the glycerol backbone and carbonyl groups while the region of the phosphocholine moieties is preferable for Trp location in the PC:Chol bilayer. These findings suggest that Chol can modulate the interactions between apoA-I and membrane lipids via reducing the depth of α-helix bilayer penetration.

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Abbreviations *apoA-I*: apolipoprotein A-I · *AV-PC*: anthrylvinyl-labeled phosphatidylcholine · *Chol*: cholesterol · *HDL*: high-density lipoproteins · *LUV*: large unilamellar vesicles · *PC*: phosphatidylcholine · *RET*: fluorescence resonance energy transfer

Introduction

Protein–lipid interactions are known to be involved in a wide variety of functionally important processes, among which cholesterol efflux promoted by apolipoprotein A-I (apoA-I), the main component of high-density lipoproteins (HDL), attracts particular interest (Oram and Yokoyama 1996; Yokoyama 1998).To explain the mechanisms of this process, two hypotheses have been proposed (Slotter et al. 1987; Johnson et al. 1991). The former hypothesis postulates the interactions between a specific sequence of apoA-I and membrane HDL receptors (Slotter et al. 1987), while the latter suggests that any α-helical segment of apoA-I can anchor an HDL particle to the plasma membrane, thereby modifying cholesterol packing and promoting its efflux (Johnson et al. 1991).

It is generally accepted that the lipid-binding site of apoA-I and other exchangeable lipoproteins contains class A amphipatic α-helices characterized by a specific charge distribution, with positively charged amino acid residues at the polar–nonpolar interface and negatively charged residues at the center of polar face (Lu et al. 2000; Maiorano and Davidson 2000). For this reason, one approach to elucidating the nature of apoA-I–lipid interactions is based on the examination of class A model peptides (Mishra and Palgunachari 1996; Palgunachari et al. 1996). Lipid-associating properties of these peptides are extensively studied in a number of aspects, including the thermodynamics of the binding

process (Anantharamaiah et al. 1985; Spuhler et al. 1994), the peptide effect on the structure of the lipid–water interface (Spuhler et al. 1994), the dependence of lipid affinity on the nature and topography of charged amino acid residues (Mishra et al. 1994; Lund-Katz et al. 1995), polypeptide chain helicity (Venkatachalapathi et al. 1993), arrangement of amphipathic helices (Mishra et al. 1995, 1998), structure and dynamics of peptide–lipid assemblies (Clayton and Sawyer 1999; Clayton and Sawyer 2000a) and peptide location and orientation at the lipid–water interface (Clayton and Sawyer 1999; Hristova et al. 1999).

Because of its amphipathic nature, a class A α-helix tends to reside in the interfacial bilayer region, orienting parallel with the membrane surface (Clayton and Sawyer 2000b). Such an orientation allows minimization of thermodynamically unfavorable contacts between polar amino acid residues and lipid acyl chains. In addition to general thermodynamic principles, helix positioning in a lipid bilayer may be governed by other factors, depending on the chemical nature of individual amino acid residues. Numerous studies provide evidence for specific interfacial interactions of some aromatic (Trp, Tyr) and positively charged (Lys, Arg) amino acids (Persson et al. 1998; Yau et al. 1998; De Planque et al. 1999). Both specific and nonspecific interactions between apoA-I α-helices and lipids may have essential structural and functional implications influencing the topographic features of the protein molecule, the depth of its membrane penetration, the extent of hydrophobic mismatch, the mechanisms of the protein conformational changes and structural reorganization of the lipid bilayer, etc. (Venkatachalapathi et al. 1993; Mishra et al. 1995, 1998). On the other hand, the helix membrane position can be modulated by changes in the structure and physicochemical properties of the lipid bilayer. The nature of these changes depends on a number of factors, particularly on the membrane cholesterol concentration. Despite considerable research effort, it is not yet fully understood to what extent the helix bilayer position is determined by the variations in cholesterol content. In the present study we made an attempt to approach this problem through examining the changes in the location of the class A model peptide Ac-18A-NH_2 on the inclusion of cholesterol (Chol) in the model membrane composed of phosphatidylcholine (PC). Ac-18A-NH_2 (Asp-Trp-Leu-Lys-Ala-Phe-Tyr-Asp-Lys-Val-Ala-Glu-Lys-Leu-Lys-Glu-Ala-Phe) is an amphipathic 18-residue peptide that mimics the lipid-binding site of exchangeable apolipoproteins, especially apoA-I (Anantharamaiah et al. 1985; Mishra and Palgunachari 1996; Palgunachari et al. 1996). As a parameter sensitive to the changes of the helix position within the membrane, the distance of the peptide Trp residue from the bilayer center has been chosen. For this purpose, the method of resonance energy transfer has been employed, involving the peptide Trp as a donor and the fluorophore of anthrylvinyl-labeled PC as an acceptor.

Materials and methods

Chemicals

Egg yolk PC was kindly provided by Asahi Kasei (Japan). The phospholipid purity assessed by thin layer chromatography exceeded 99.5%. The Ac-18A-NH_2 peptide was purchased from Takara Shuzo (Japan); HPLC, amino acid analysis and mass spectrometry indicated its purity above 98%. AV-PC (1-acyl-2-[12-(9-anthryl)-11-*trans*-dodecenoyl]-*sn*-glycerophosphocholine, 1-acyl: 16:0/18:0 = 3/1) was synthesized as described in detail elsewhere (Molotkovsky et al. 1979). All other chemicals were of special grade from Wako Pure Chemicals (Japan).

Preparation of lipid vesicles

Large unilamellar vesicles (LUV) were prepared from PC and its mixture with cholesterol (40 mol%) using the extrusion method. A thin lipid film prepared by evaporation of its chloroform solution was left under vacuum overnight to remove residual organic solvent, and then was hydrated with 10 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA. Lipid suspension subjected to five freeze–thaw cycles was extruded through a 100-nm pore size polycarbonate filter (Nucleopore). The phospholipid concentration was determined according to the procedure of Bartlett (1959). AV-PC was added to the initial PC or PC:Chol chloroform solutions; its bilayer concentration was determined spectrophotometrically, using the anthrylvinyl extinction coefficient of $\epsilon_{367} = 9\times10^3\ M^{-1}\ cm^{-1}$, after 10-fold dilution of the vesicle suspension with ethanol.

Fluorescence measurements

Fluorescence measurements were performed with a Hitachi F-4500 spectrofluorimeter using a 5-mm path-length cuvette. Peptide emission spectra were recorded at 25 °C with an excitation wavelength of 296 nm. Excitation and emission slit widths were set at 5 nm. The quantum yield of the peptide tryptophan (Q_D) was estimated using tryptophan solution in water as a standard [$Q = 0.14$ (Kirby and Steiner 1970)].

The fluorescence resonance energy transfer (RET) efficiency was estimated through determining the ratio of the Trp quantum yields in the acceptor-free (Q_D) and acceptor-containing (Q_{DA}) vesicles ($E = 1 - Q_{DA}/Q_D$). The measured fluorescence intensities of a donor were corrected for the inner filter and reabsorption effects using the following coefficients (Bulychev et al. 1988):

$$k = \frac{\left(1 - 10^{-A_0^{ex}}\right)\left(A_0^{ex} + A_a^{ex}\right)}{\left(1 - 10^{-\left(A_0^{ex} + A_a^{ex}\right)}\right)A_0^{ex}} \frac{\left(1 - 10^{-A_0^{em}}\right)\left(A_0^{em} + A_a^{em}\right)}{\left(1 - 10^{-\left(A_0^{em} + A_a^{em}\right)}\right)A_0^{em}} \quad (1)$$

where A_0^{ex} and A_0^{em} are the donor optical densities at the excitation and emission wavelengths in the absence of acceptor (in the neat PC or PC:Chol vesicles, $A_0^{ex} \approx 0.02$) and A_0^{ex} and A_0^{em} are the acceptor optical densities at the excitation and emission wavelengths, respectively.

Theory

To quantitatively interpret the results of RET measurements, the model of energy transfer in two-dimensional systems proposed by Fung and Stryer (1978) has been employed. In the present study this model has been extended taking into account some specific features of the orientational dependence of RET in membranes. When donors and acceptors are randomly distributed in different planes separated by a distance d_a, the donor fluorescence decay can be represented as:

$$F(t) = F(0)\exp(-t/\tau_d)\exp\left(-C_a^s S(t)\right) \quad (2)$$

$$S(\lambda) = \int_{d_a}^{\infty}\left[1-\exp\left(-\lambda\left(\frac{R_0}{R}\right)^6\right)\right]2\pi R\,dR \quad (3)$$

where $F(0)$ is the initial fluorescence intensity, τ_d is the lifetime of the excited donor in the absence of acceptors, $\lambda = t/\tau_d$, R_0 is the Förster radius, and C_a^s is the concentration of acceptors per unit area that depends on molar concentrations of lipids accessible to acceptor (L_0) and bound acceptor (B):

$$C_a^s = \frac{B}{L_0\sum f_i A_{Li}} \quad (4)$$

where f_i and A_{Li} are the mole fractions and mean areas per molecule of lipid species constituting the membrane. The efficiency of the energy transfer is given by:

$$E = 1-\frac{Q_{DA}}{Q_D} = 1-\frac{1}{\tau_d}\int_0^{\infty}\frac{F(t)}{F(0)}dt = 1-\int_0^{\infty}\exp(-\lambda)\exp\left(-C_a^s S(\lambda)\right)d\lambda \quad (5)$$

The Förster radius is related to the donor quantum yield (Q_D) and the overlap between the donor emission ($F_D(\lambda)$) and acceptor absorption ($\epsilon_A(\lambda)$) spectra (Lakowicz 1999):

$$R_0 = 979\left(\kappa^2 n_r^{-4} Q_D J\right)^{1/6} \qquad J = \frac{\int_0^{\infty} F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda}{\int_0^{\infty} F_D(\lambda)d\lambda} \quad (6)$$

where n_r is the refractive index of the medium ($n_r = 1.37$), $\varkappa^2$ is an orientation factor defined as (Lakowicz 1999):

$$\kappa^2 = (\sin\theta_D\sin\theta_A\cos\phi - 2\cos\theta_D\cos\theta_A)^2 \quad (7)$$

where θ_D and θ_A are the angles between the donor emission (***D***) or acceptor absorption (***A***) transition moments and vector ***R*** joining the donor and acceptor, and ϕ is the dihedral angle between the planes (***D***, ***R***) and (***A***, ***R***). Equation (7) is applicable when the vectors ***D*** and ***A*** do not experience any reorientation during the transfer time. Alternatively, the dynamic average value of orientation factor ($\varkappa^2$) can be used in the R_0 calculation. When the donor emission and acceptor absorption transition moments are symmetrically distributed within the cones about certain axes $\boldsymbol{D}_x$ and $\boldsymbol{A}_x$, the value of $\varkappa^2$ is given by (Dale et al. 1979):

$$\begin{aligned}\langle\kappa^2\rangle = {} & (\sin\Theta_D\sin\Theta_A\cos\Phi - 2\cos\Theta_D\cos\Theta_A)^2\langle d_D^x\rangle\langle d_A^x\rangle \\ & + 1/3(1-\langle d_D^x\rangle) + 1/3(1-\langle d_A^x\rangle) \\ & + \cos^2\Theta_D\langle d_D^x\rangle(1-\langle d_A^x\rangle) + \cos^2\Theta_A\langle d_A^x\rangle(1-\langle d_D^x\rangle)\end{aligned} \quad (8)$$

where Θ_D and Θ_A are the angles made by the axes $\boldsymbol{D}_x$ and $\boldsymbol{A}_x$ with vector ***R***, Φ is the angle between the planes containing the cone axes and vector ***R***, and $\langle d_D^x\rangle$ and $\langle d_A^x\rangle$ are so-called axial depolarization factors:

$$\left\langle d_{D,A}^x\right\rangle = 3/2\langle\cos^2\psi_{D,A}\rangle - 1/2 \quad (9)$$

where $\psi_{D,A}$ are the cone half-angles. These factors are related to the steady-state (r) and fundamental (r_0) anisotropies of the donor and acceptor (Dale et al. 1979):

$$d_{D,A}^x = \pm\left(r_{D,A}/r_{0D,A}\right)^{1/2} \quad (10)$$

Equation (5) is valid provided that the following conditions are met: (1) acceptors do not change their positions during the lifetime of the excited donor; (2) there is no homotransfer between the donors; (3) the Förster radius is the same for all donor–acceptor pairs. However, the last condition is not fulfilled when donor and acceptor planar arrays are located at different levels across the membrane. In this case there exists a set of Förster radii for multiple donor–acceptor pairs involved in energy transfer, since the orientation factor appears to be a function of the donor–acceptor separation (R). Particularly, for the most probable membrane orientation of $\boldsymbol{D}_x$ and $\boldsymbol{A}_x$, parallel to the bilayer normal, the angles Θ_D and Θ_A made by $\boldsymbol{D}_x$ and $\boldsymbol{A}_x$ with ***R*** are equal and depend on the distance between donor and acceptor ($\Theta_A = \Theta_D = \theta$, $\theta = f(R)$). Under these conditions, Eq. (7) can be rearranged to give (Davenport et al. 1985):

$$\begin{aligned}\langle\kappa^2(\theta)\rangle = {} & \langle d_D^x\rangle\langle d_A^x\rangle(3\cos^2\theta - 1)^2 + 1/3(1-\langle d_D^x\rangle) \\ & + 1/3(1-\langle d_A^x\rangle) + \cos^2\theta(\langle d_D^x\rangle - 2\langle d_D^x\rangle\langle d_A^x\rangle + \langle d_A^x\rangle)\end{aligned} \quad (11)$$

where $\cos^2\theta = (d_a/R)^2$ and d_a is the separation of the donor and acceptor planes. Thus, making the substitutions $R_0 = \left[\kappa^2(R)\right]^{1/6}R_0^r$ and $R_0^r = 979\left(n_r^{-4}Q_D J\right)^{1/6}$, Eq. (3) may be rewritten in the form:

$$S(t) = \int_{d_a}^{\infty}\left[1-\exp\left(-\lambda\kappa^2(R)\left(\frac{R_0^r}{R}\right)^6\right)\right]2\pi R\,dR \quad (12)$$

Analogously, one can describe energy transfer in the system containing one donor plane located at a distance d_c from the membrane center and two acceptor planes separated by a distance d_t (Fig. 1). Given that, for the outer acceptor plane, $d_a = |d_c - 0.5d_t|$, while for the inner plane, where $d_a = d_c - 0.5d_t$, the following relationships hold:

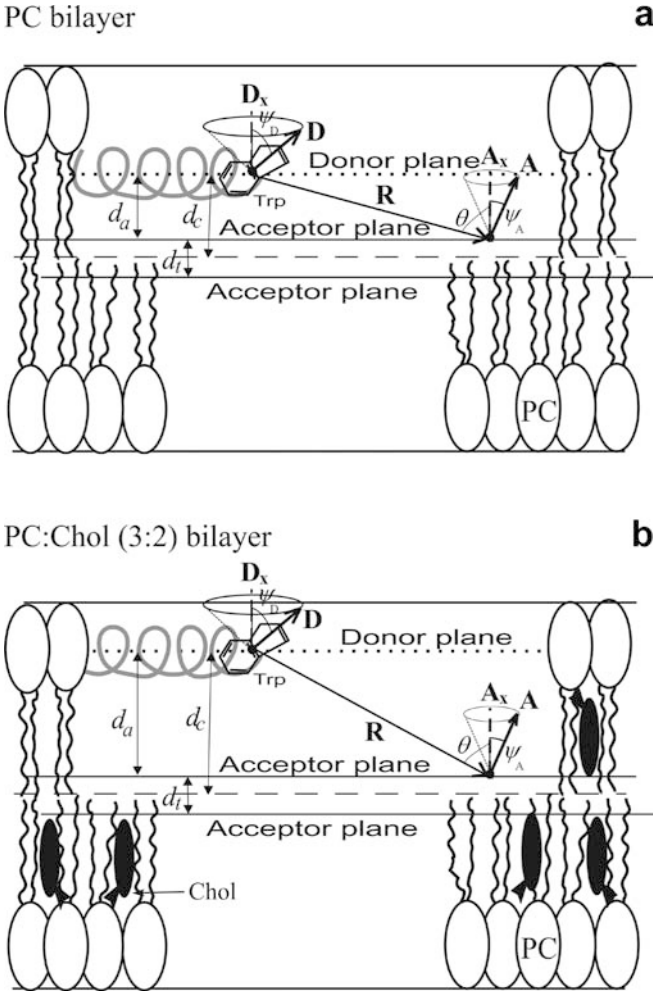

Fig. 1 Scheme for relative bilayer positions and angular relationships of donors (Ac-18A-NH_2 Trp residues) and acceptors (AV-PC) in PC (**a**) and PC:Chol (**b**) model membranes

$$F(t) = F(0)\exp(-t/\tau_0)\exp\left(-C_a^s[S_1(t) + S_2(t)]\right) \quad (13)$$

$$S_1(\lambda) = \int_{|d_c - 0.5d_t|}^{\infty} \left[1 - \exp\left(-\lambda\kappa_1^2(R)\left(\frac{R_0^r}{R}\right)^6\right)\right] 2\pi R\,dR \quad (14)$$

$$S_2(\lambda) = \int_{d_c + 0.5d_t}^{\infty} \left[1 - \exp\left(-\lambda\kappa_2^2(R)\left(\frac{R_0^r}{R}\right)^6\right)\right] 2\pi R\,dR \quad (15)$$

$$\kappa_{1,2}^2(R) = \langle d_D^x\rangle\langle d_A^x\rangle\left(3\left(\frac{d_c \mp 0.5d_t}{R}\right)^2 - 1\right) + \frac{1 - \langle d_D^x\rangle}{3} + \frac{1 - \langle d_A^x\rangle}{3} + \left(\frac{d_c \mp 0.5d_t}{R}\right)^2\left(\langle d_D^x\rangle - 2\langle d_D^x\rangle\langle d_A^x\rangle + \langle d_A^x\rangle\right) \quad (16)$$

where S_1 and S_2 are the quenching contributions describing energy transfer to the outer and inner acceptor planes, respectively. Ultimately, by substituting Eq. (13) in Eq. (5) one obtains:

$$E = 1 - \int_0^{\infty} \exp(-\lambda)\exp\left[-C_a^s(S_1(\lambda) + S_2(\lambda))\right] d\lambda \quad (17)$$

Relationships (13)–(17) are derived on the assumption that the donor and acceptor transition moments are distributed about the axes $\boldsymbol{D}_x$ and $\boldsymbol{A}_x$ which are parallel to the bilayer normal $\boldsymbol{N}$. If this is not the case, additional depolarization factors accounting for the deviations of $\boldsymbol{D}_x$ and $\boldsymbol{A}_x$ from $\boldsymbol{N}$ should be introduced: $d_{D,A}^a = \frac{3}{2}\cos^2\alpha_{D,A} - \frac{1}{2}$, where $\alpha_{D,A}$ are the angles made by $\boldsymbol{D}_x$ and $\boldsymbol{A}_x$ with $\boldsymbol{N}$. By applying Soleillet's theorem stating the multiplicativity of depolarization factors, Eq. (16) may be rewritten in a more general form:

$$\kappa_{1,2}^2(R) = d_D d_A\left(3\left(\frac{d_c \mp 0.5d_t}{R}\right)^2 - 1\right) + \frac{1 - d_D}{3} + \frac{1 - d_A}{3} + \left(\frac{d_c \mp 0.5d_t}{R}\right)^2(d_D - 2d_D d_A + d_A) \quad (18)$$

where $d_{D,A} = \left\langle d_{D,A}^x\right\rangle d_{D,A}^a$.

Results

Presented in Fig. 2 are the energy transfer efficiencies measured for a series of vesicles in which the amount of AV-PC was varied relative to that of PC or PC and Chol. The surface concentration of acceptor was estimated from Eq. (4):

$$C_a^s = B/L_0(f_{PC}S_{PC} + f_{CH}S_{CH}) \quad (19)$$

where f_{PC} and f_{CH} are the mole fractions of PC and Chol, and S_{PC} and S_{CH} are the mean areas per lipid molecule. For the neat PC bilayer, S_{PC} was taken to be 0.65 nm^2, while for the PC:Chol model membrane experiencing the condensing effect of cholesterol the following values were used: $S_{PC} = 0.5$ nm^2 and $S_{CH} = 0.39$ nm^2 (Ivkov and Berestovsky 1981). Analysis of the experimental results in terms of the above model provided arguments in favor of the modulating effect of cholesterol on the position of the Ac-18A-NH$_2$ Trp residue in the lipid bilayer. These arguments have been obtained by comparing the Trp distances from the center of PC $\left(d_c^0\right)$ and PC:Chol $\left(d_c^c\right)$ bilayers. The parameters

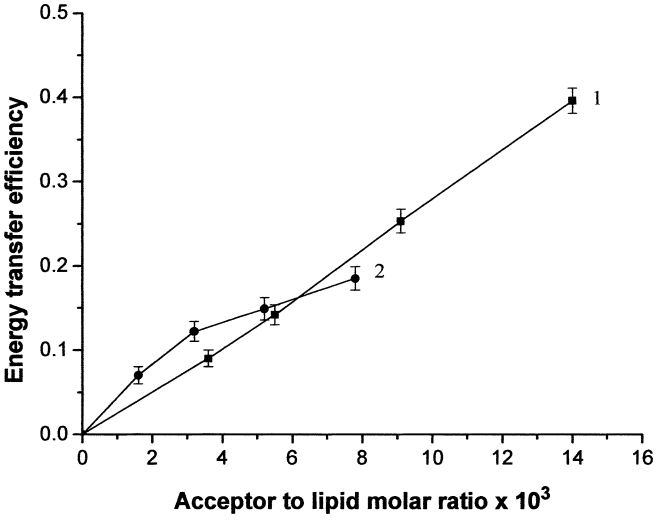

Fig. 2 Efficiency of energy transfer between Ac-18A-NH$_2$ Trp and AV-PC in PC (*1*) and PC:Chol (*2*) model membranes as a function of acceptor to lipid molar ratio

d_c^0 and d_c^c were optimized in the data fitting based on minimization of the function:

$$f = \frac{\sum_{i=1}^{n}(E_{ei} - E_{ci})^2}{n} \quad (20)$$

where n is the number of experimental points (i.e. the number of acceptor surface densities), E_e is the measured transfer efficiency, and E_c is the transfer efficiency calculated by numerical integration of Eqs. (13)–(17) using Förster radii of 2.23 nm (PC LUV) and 2.25 nm (PC:Chol LUV). The optimization procedure yielded f values not exceeding 6.3×10^{-4}.

To estimate the depolarization factors $\left\langle d_D^x\right\rangle$ and $\left\langle d_A^x\right\rangle$, anisotropy measurements were performed with the lipid-bound peptide [$\lambda_{ex} = 296$ nm, $\lambda_{em} = 333$ nm (PC), $\lambda_{em} = 345$ nm (PC:Chol)] and AV-PC ($\lambda_{ex} = 300$ nm, $\lambda_{em} = 430$ nm). The r_D and r_A values were found to be 0.16 and 0.06, respectively. The fundamental anisotropy of the anthrylvinyl fluorophore was taken to be 0.08 at $\lambda_{ex} = 300$ nm (Johansson et al. 1990). The tryptophan absorbance in the range 250–300 nm is determined by the two electronic transitions ^1L_a and ^1L_b, which have orthogonally oriented transition moments (Valeur and Weber 1977). The excitation wavelength of 296 nm used in our experiments predominantly populates the ^1L_a state of the fluorophore [$r_0 = 0.3$ (Lakowicz 1999)], whose transition moment lies in the plane of the indole ring (Albinsson et al. 1989). Therefore, the angle α_D may be regarded as characterizing the deviation of the normal to the plane of the Trp aromatic ring from the bilayer normal. ^{2}H NMR studies of the bilayer-located Trp analogs indicate that the indole ring has a considerable degree of motional freedom, being preferentially oriented parallel to the bilayer normal (Yau et al. 1998). Since the preferable orientation of the Ac-18A-NH$_2$ Trp residue is unknown, we found it reasonable to treat the

experimental data on the two limiting assumptions of $\alpha_D = 0$ (the plane of indole ring is parallel to the bilayer surface) and $\alpha_D = \pi/2$ (the plane of indole ring is perpendicular to the bilayer surface).

The value of α_A was taken to be $\pi/2$, since, according to the ^{1}H NMR data, the anthrylvinyl moiety of AV-PC that is attached to the terminal methyl groups of acyl chains adopts a preferential orientation parallel to the bilayer normal (Molotkovsky et al. 1981).

Discussion

According to the aforementioned hypotheses accounting for the role of apoA-I in cholesterol efflux, the interactions between the apolipoprotein α-helical segment and the lipid bilayer result in the membrane disruption being conducive to the desorption of cholesterol (Slotter et al. 1987; Johnson et al. 1991; Sviridov et al. 1996). Since the magnitude of these effects is likely to depend on the depth of α-helix insertion in the membrane interior, it seemed of importance to ascertain how the extent of bilayer penetration could be modulated by the variations in cholesterol content. In the present study, this question has been addressed using the class A model peptide Ac-18A-NH_2. Resonance energy transfer measurements have allowed us to monitor the changes in the bilayer location of the peptide Trp residue on the inclusion of cholesterol in PC bilayer. In evaluating the Trp position relative to the membrane center, we have made an attempt to circumvent the main limitation of the RET method originating from the uncertainty in the orientation factor value. Accurate determination of this parameter is hardly a feasible task, both from theoretical and experimental aspects. For this reason, the Förster radius is commonly calculated using the dynamically averaged κ^2 value (0.67), that is valid for isotropic orientational distributions of the donor and acceptor transition dipoles (Wu and Brand 1992). However, any fixed κ^2 value is inappropriate when RET takes place between donors and acceptors residing at different depths within the membrane. In this case, an orientation factor must be involved as a distance-dependent parameter at the integration over the R step in the theoretical calculation of RET efficiency (Eqs. 13, 14, 15, 16, 17). Taking into account this peculiarity of RET in membranes, we have obtained more accurate estimates of the Trp bilayer position as compared to those reported previously (Egashira et al. 2002).

As seen in Table 1, the Trp distance from the center of the PC bilayer ranges between 1.5 nm ($\alpha_D = \pi/2$) and 1.7 nm ($\alpha_D = 0$). Taking into consideration the time-averaged transbilayer distributions of the lipid structural groups (Wiener and White 1992), it can be assumed that Trp is located in the vicinity of the carbonyl groups ($d_c = 1.4$–1.5 nm) or glycerol backbone ($d_c = 1.7$–1.8 nm). This finding is in accordance with the numerous evidence for the predominant positioning of the Trp residue in the interfacial bilayer region composed of phosphocholine, glycerol, and acyl carbonyl moieties and upper acyl chain carbons (Persson et al. 1998; Yau et al. 1998; Braun and Heijne 1999; De Planque et al. 1999). The preference of tryptophan for the membrane interface is thought to originate from its ability in hydrogen bonding, dipolar, and cation–π interactions with the interfacially located lipid groups (De Planque et al. 1999; Yau et al. 1998).

Table 1 Limiting values of the Ac-18A-NH_2 Trp distance from the midplane of PC (d_c^0, nm) and PC:Chol (d_c^c, nm) model membranes

Membrane composition	$\alpha_D = 0$	$\alpha_D = \pi/2$
PC	1.7	1.5
PC:Chol (3:2, mol:mol)	2.1	1.9

Inclusion of 40 mol% cholesterol in the PC LUV has been found to decrease the depth of Ac-18A-NH_2 bilayer penetration: the Trp distance from the membrane center has been estimated to be 1.9–2.1 nm (Table 1). This range of d_c values corresponds to the Trp location near to the phosphocholine moiety of the lipid molecules. Among factors that could be responsible for the observed effect, the most essential seems to be cholesterol-induced structural reorganization of the lipid–water interface. In the lipid bilayer the amphiphilic cholesterol molecule is thought to adopt a quasi-perpendicular orientation to the membrane surface, with the 3β-hydroxy group being located in the interfacial region and an apolar moiety composed of the tetracyclic ring and the isooctyl side chain embedded in the hydrophobic core. To date, the consequences of cholesterol inclusion in phospholipid bilayer are rather well characterized. For the liquid-crystalline lipid phase, the main consequences include: (1) an increase in the separation of the phospholipid headgroups (Levine 1972; Yeagle et al. 1977); (2) increased freedom of motion of the phosphocholine moiety (McLaughlin et al. 1975); (3) enhanced headgroup hydration (Ho et al. 1995); (4) reduced content of the acyl chain *gauche* conformations (Straume and Litman 1987); and (5) tighter lateral packing of the lipid molecules (condensing effect) (Demel and de Kruijff 1976). Modification of the physical properties of the lipid bilayer is considered as a predominant mechanism underlying cholesterol influence on the mode of the peptide–lipid interactions. This mechanism was demonstrated to be involved in the reduction of the peptide ability to membrane permeabilization observed for melittin (Monette et al. 1993), GALA (Nicol et al. 1996), and the N-terminal peptide of the influenza virus hemagglutinin (Duzgunes and Shavnin 1992). It was assumed that cholesterol prevents deep peptide penetration in the bilayer interior by increasing the acyl chain packing density (Duzgunes and Shavnin 1992; Monette et al. 1993). Solid-state ^{2}H and ^{31}P NMR studies revealed complete inhibition of the MLV-to-LUV transformation in the system melittin–DPPC upon

inclusion of 30 mol% cholesterol (Pott and Dufourc 1995). This effect was explained by the mutual compensation of the changes in the intrinsic monolayer curvature caused by melittin and cholesterol. In this regard it seems of importance to note that the concept of membrane monolayer curvature strain is now frequently used in interpreting the effects observed in the formation of peptide–lipid complexes (Epand et al. 1995). The nature of these effects is believed to be dependent on the depth of the α-helix insertion into the membrane that, in turn, is determined to a great extent by the size of the hydrophilic and hydrophobic helix faces, the charge distribution, and the position of the amphiphilic amino acid residues with significant hydrophobicity such as cationic lysine and arginine or neutral tryptophan and isoleucine (Epand et al. 1995). Class A amphipathic helices represented in this study by Ac-18A-NH_2 are characterized by a high lipid affinity, which is explained in terms of the snorkel hypothesis (Segrest et al. 1990). According to this hypothesis, hydrophobic moieties of interfacially located Lys and Arg considerably increase the total hydrophobicity of the apolar face determining the depth of helix penetration in the hydrocarbon core. Given the possible snorkeling of Lys and Arg toward the polar helix surface, it has been assumed that the cross-sectional shape of the class A amphipathic helix is a wedge with a polar base and hydrophobic apex (Tytler et al. 1993). It is assumed that this structural peculiarity renders the class A α-helix capable of promoting positive curvature strain in the membrane monolayer (Epand et al. 1995). Such an ability has been reported also for the Ac-18A-NH_2 peptide (Polozov et al. 1997).

Thus, taking into account the above considerations, cholesterol-induced reduction of the depth of Ac-18A-NH_2 penetration in the lipid bilayer can be interpreted in terms of the curvature modulation. Cholesterol, increasing the separation of the phospholipid headgroups and causing tighter packing of the acyl chains, could affect the curvature of the lipid–water interface in such a manner that the thermodynamically favorable location of the wedge-shaped amphipathic α-helix becomes less buried. This phenomenon is likely to have important functional implications, contributing to the apoA-I ability to promote cholesterol efflux from the plasma membrane. Several studies provide evidence for the shallower location of apoA-I in cholesterol-enriched membranes (Saito et al. 1997; Lecompte et al. 1998). Based on fluorescence and ^{13}C NMR data, we have previously assumed that the effect of cholesterol on the separation of PC headgroups modulates the lipid-binding behavior of apoA-I. More specifically, increased headgroup spacing causes apoA-I to reside closer to the bilayer surface. The results of the present study obtained with the amphipathic peptide Ac-18A-NH_2, whose lipid-associating properties are expected to be similar to those of apoA-I, provide additional arguments in favor of such a viewpoint.

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