FEBS Letters 580 (2006) 2677–2685

Binding of antibiotic amphotericin B to lipid membranes: A ^{1}H NMR study

Janina Gabrielska[a], Mariusz Gagoś[b,c], Jerzy Gubernator[d], Wiesław I. Gruszecki[c,*]

[a] Department of Physics and Biophysics, Agricultural University, Wrocław, Poland
[b] Department of Physics, Agricultural University, Lublin, Poland
[c] Department of Biophysics, Institute of Physics, Maria Curie-Skłodowska University, 20-031 Lublin, Poland
[d] Department of Lipids and Liposomes, Institute of Biochemistry and Molecular Biology, Wrocław University, Wrocław, Poland

Received 24 March 2006; revised 5 April 2006; accepted 5 April 2006

Available online 19 April 2006

Edited by Sandro Sonnino

Abstract The ^{1}H NMR technique was applied to study binding of AmB, an antifungal drug, to lipid membranes formed with egg yolk phosphatidylcholine. The analysis of ^{1}H NMR spectra of liposomes, containing also cholesterol and ergosterol (at 40 mol%), shows that AmB binds preferentially to the polar headgroups. Such a binding restricts molecular motion of the choline fragment in the hydrophilic region at the surface of liposomes but increases the segmental motional freedom in the hydrophobic core. The same effects are also observed in the sterol-containing membranes, except that the effect on the hydrophobic core was exclusively observed in the membranes containing ergosterol.

Keywords: Amphotericin B; Biomembranes; Polyene antibiotics; Lipid membranes; Ergosterol; Cholesterol

1. Introduction

Amphotericin B (AmB) is a polyene antibiotic frequently used in medical treatment of systemic fungal infections [1–3]. According to the general understanding, the pharmacological action of the drug is directly associated with ability to form pore-like structures across biomembranes, that considerably affect physiological ion transport [4–8]. It was supposed that AmB forms channels in lipid membranes that are permeable to monovalent ions, water, and small molecules [4,5,9–11]. The ability of AmB to form hydrophilic pores results directly from the amphiphilic molecular structure of the drug (see Fig. 1A). Selectivity of AmB toward lipid membranes of fungi is most probably related to the presence of ergosterol (instead of cholesterol present in mammal cells), that is supposed to participate in the formation of the membrane pores [5,12]. On the other hand, the ionic channel activity has also been observed in the lipid membrane system containing AmB without any sterol components [7]. Aggregation of AmB and pore formation in the ergosterol-free membranes is most probably a direct cause of the toxic side effects associated with medical treatment with the application of preparations based on AmB [2]. It has been proposed that not only formation of porous molecular structures, but also affection of the physical properties of the lipid bilayers brings about increased membrane permeability to ions [13]. This effect is expected to be particularly pronounced at low concentrations of AmB, promoting monomeric organization of the drug within the lipid phase [14,15]. On the other hand, owing to the amphiphilic nature of AmB its binding to the membrane hydrophobic core, in the monomeric form, is rather unfavourable for energetic reasons [16]. Formation of molecular dimers of AmB have been recently reported both in the organic solvents and in lipid membrane environment and this mechanism has been postulated to facilitate binding of the drug to the lipid membranes from water phase [15,17,18]. Recently, we have found that AmB bound to the liposomes formed with egg yolk phosphatidylcholine at 3 mol% drug with respect to lipid causes an increase in the rate of fast proton transfer across the lipid bilayer but the character of ion current suggests unspecific transport, not facilitated by a presence of ion channels [8]. Moreover, at very low concentrations of AmB (0.1 mol%) the proton flow has been considerably slowed down, that suggests a mode of binding of AmB to the membranes that increases the membrane penetration barrier to protons [8]. Such an effect has been interpreted in terms of hydrogen bonding between the horizontally oriented AmB and the polar groups of lipids in the headgroup region of the membrane, that make the membrane more compact and less permeable to ions [8]. Very recently, we have found using the linear dichroism-FTIR technique, that AmB which binds to the lipid membrane from the water phase is distributed among two fractions: one perpendicular to the membrane plane and one parallel to the membrane, affecting considerably the polar–non-polar interface of the lipid bilayer [19]. In most studies of organization of AmB-lipid membranes the drug was incorporated into the membrane system from an organic phase, during model lipid membrane preparation. In the present work we apply the ^{1}H NMR spectroscopy to study molecular mechanisms responsible for binding AmB to lipid membranes from the water phase.

2. Materials and methods

Egg yolk phosphatidylcholine (EYPC), cholesterol, ergosterol and synthetic, crystalline amphotericin B (AmB) were purchased from Sigma Chem. Co. (USA). Praseodymium chloride – $PrCl_3 \cdot 5H_2O$

*Corresponding author. Fax: +48 81 537 61 91.
E-mail address: wieslaw@tytan.umcs.lublin.pl (W.I. Gruszecki).

Abbreviations: AmB, amphotericin B; EYPC, egg yolk phosphatidylcholine; NMR, nuclear magnetic resonance

0014-5793/$32.00 © 2006 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/j.febslet.2006.04.021

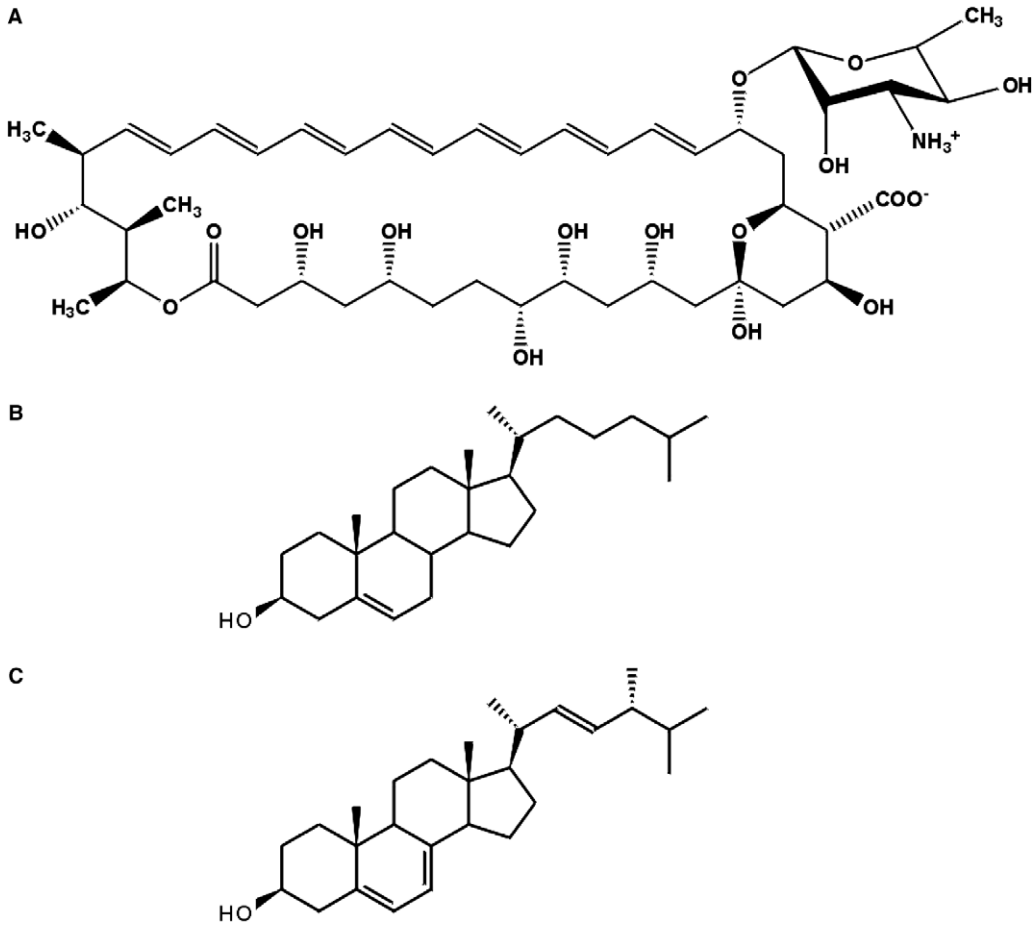

Fig. 1. Chemical structures of amphotericin B (A), cholesterol (B) and ergosterol (C).

was purchased from Aldrich Chem. Co. (Milwaukee, USA). Deuterium water (D_2O) and deuterated dimethylsulfoxid-d_6 (DMSO) were purchased from ARMAR Chem. (Switzerland). Chemicals were stored under argon in a deep-freezer.

Small unilamellar EYPC vesicles for nuclear magnetic resonance (NMR) measurements were prepared as described previously [20]. In brief, the EYPC stock solution has been prepared in chloroform. After removing solvent, the dry lipid film was hydrated with D_2O. The final concentration of lipid was 25 mg/ml. The suspensions were then sonicated under nitrogen for 30 min with a 20 kHz sonicator equipped with a titanium probe. During the sonication the samples were thermostated at 0 °C. The sonication was followed by the centrifugation for 5 min at $2000 \times g$ in order to remove possible titanium particles. NMR spectra were collected for samples of 0.5 ml of vesicle suspension supplemented with 4.09 mM $PrCl_3$ in 5-mm NMR tubes. Before addition of $PrCl_3$ to the liposome preparations liposome suspension was supplemented with AmB by injection of an appropriate amount of AmB solution in deuterated DMSO into 1 ml of the liposome suspension in D_2O and the samples were vortexed for 5 min. The final AmB concentration was 4.8 mol% with respect to lipid. The actual concentration of AmB bound to liposomes was certainly lower than that and it was reported recently that AmB incorporated to the unilamellar EYPC liposomes generally does not form transmembrane channels and affects the transmembrane flow of protons in a non-specific fashion [8]. On the other hand, a formation of single layer channels and also double layer channels may not be excluded. The apparent pH of D_2O (pD) was 7.5 (pD = pH + 0.4).

^1H NMR spectra were recorded on Bruker Avance DRX spectrometer. Three hundred megahertz ^1H NMR parameters were as follows: spectral window 6173 Hz; digital resolution 0.188 Hz; pulse width 4.5 μs (30° flip angle); acquisition and delay times were 2.65 s and of 1 s, respectively; acquisition temperature 25 °C.

Liposome size distribution was determined using photon correlation spectroscopy (PCS) on a Zetasizer 5000 instrument (Malvern Instruments Ltd., UK). Laser light of 635 nm scattered by the sample was amplified and then analyzed by a correlator to obtain a correlation function, whose shape depended on size of the liposomes in the sample. $PrCl_3$ was not present in the samples subjected to PCS measurements.

Electronic absorption spectra of liposome suspension was recorded with a double-beam UV–Vis spectrophotometer Carry 300 Bio from Varian equipped with a thermostated cuvette holder.

Experiments have been repeated from four to six times. The parameters representing analysis of NMR spectra are presented as an arithmetic mean ± S.D.

3. Results and discussion

Fig. 2 presents ^1H NMR spectrum of EYPC liposome suspension supplemented with $PrCl_3$. Addition of the praseodymium ions effects in the split of the ^1H NMR band corresponding to the $-N^+(CH_3)_3$ group (between 3.0 and 3.5 ppm), owing to the pseudocontact shifts produced by shift reagents from the group of lanthanides (e.g. Pr^{3+}) [21]. The resonance maximum shifted towards higher ppm values (lower magnetic field values) corresponds therefore to the lipid molecules forming the outer leaflet of the liposome membranes and the high-field maximum corresponds to the inner liposome surface. The proportion of these components, represented as a ratio of the area beneath the bands (S_o/S_i, outer-to-inner) determined as 1.56 for pure EYPC liposome suspension indi-

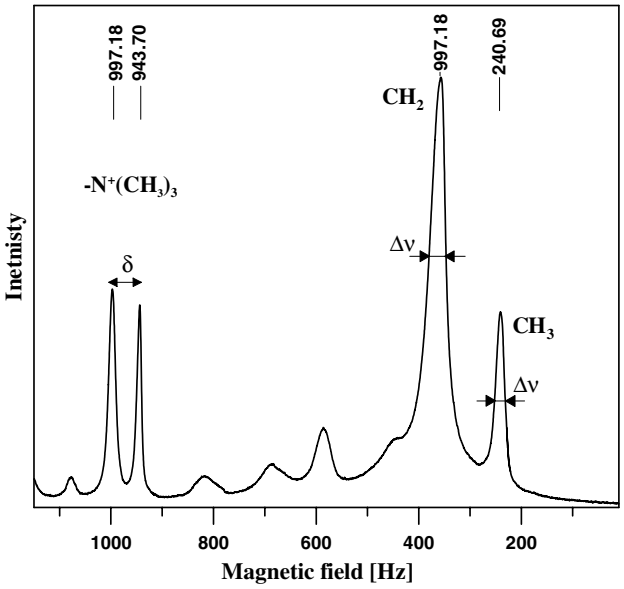

Fig. 2. ^{1}H NMR spectrum of EYPC liposome suspension supplemented with $PrCl_3$. Assignment of selected bands and spectroscopic parameters discussed in the text presented in the graph. Note the split of the resonance maximum corresponding to the $N^+(CH_3)_3$ choline polar headgroup of EYPC in liposomes, as discussed in the text.

cates that the preparation is composed of small unilamellar vesicles with average external diameter of 52 nm (assuming the thickness of the EYPC bilayer as 5.2 nm). Determination of liposome size distribution with the application of the PCS technique confirms that the liposome preparation technique applied in this work yields formation of small vesicles characterized by the external diameter close to 50 nm (the maximum at 53 nm, see Fig. 3). The agreement of the liposome size determinations based on PCS and NMR technique shows also that Pr^{3+} ions do not penetrate into the lumen of liposomes. Addition of AmB to the liposome suspension after preparation does not affect the size of liposomes. This can be deduced from the analysis of both the S_o/S_i ratio (Fig. 4) and from the analysis of the PCS-based determinations (Fig. 3B). Fig. 3B shows the size distribution profile of the EYPC liposome suspension after injection of AmB. It can be seen that the main band corresponding to liposomes is only slightly affected. In addition, the distribution profile demonstrates the appearance of new structures characterized by relatively large diameters (above 320 nm). One possible interpretation of such large structures would be appearance of molecular aggregates of AmB in the water phase, not bound to the lipid membranes of liposomes. Fig. 5 presents the size distribution profile of the control sample prepared by injection of AmB solution into the pure water without lipid vesicles. The AmB molecules form wide range of molecular structures in the water phase, with the maximum fraction characterized by a diameter of 405 nm. In contrast to the liposome suspension, the sample in water does not contain structures larger than 1000 nm. Such a difference suggests strongly that those structures represent aggregated EYPC liposomes and that the aggregation process was induced by the presence of AmB. Both EYPC and AmB are zwitterions at the pH values applied in the experiments and it is highly probable that electrostatic interactions of AmB molecules with the surface of the lipid membrane is directly involved in binding of the drug to liposomes. The AmB molecules bound to the neighbouring liposomes are able to interact one to each other either by means of hydrogen bonds between the polar groups or by means of van der Waals interactions between the polyene chains of the molecules. Such interactions will give rise to liposome aggregation processes, as schematically depicted in Fig. 6. AmB-induced aggregation of phospholipid vesicles has been reported by Bolard and Cheron [22].

Pr^{3+} can potentially interact with the ionized carboxylic group of AmB. Such an interaction can be observed in the case of H^+ ions and results in the decrease in the aggregation level of AmB, observed in the water environment (Fig. 7). A short-wavelength spectral band, between 320 and 360 nm, attributed to the aggregated molecular structures of AmB [15,23,24] has clearly lower intensity at high proton concentration, in opposite to the 0–0 vibrational band of the main electronic transition of AmB in the monomeric form (406 nm, [17]). The analysis of the samples containing the same concentration of Pr^{3+} (Fig. 7) shows that praseodymium can also be involved in an electrostatic interaction with AmB, but to a much lesser extent. The difference can be directly related to the differences in ionic radii, 0.025 nm in the case of H^+ and 0.155 nm in the case of Pr^{3+}.

According to the theory, a pseudocontact shift δ is strongly dependent on geometric conditions and axial symmetry at the lipid–lanthanide binding site and will be produced only if the magnetic susceptibility of lanthanide–lipid complex is anisotropic [21]. Interestingly, the spectral shift δ of the ^{1}H NMR band corresponding to the $-N^+(CH_3)_3$ group in the outer surface of liposomes decreases in response to AmB binding (Fig. 4, lower panel), despite the fact that the S_o/S_i ratio is not affected (Fig. 4, upper panel). Such a result can be interpreted in terms of an affection of packing properties in the lipid headgroup region brought about by AmB, most probably by the hydrogen bond formation [19]. There are several experimental techniques that enable determination of size of lipid vesicles, including PCS, and therefore the discrimination between the inner and outer lipid polar headgroup regions of liposomes seems to be the main advantage of the approach based on introducing of Pr^{3+} ions into the liposome suspension.

The full width at half height of the band ($\Delta\nu$) reflects directly the motional freedom of the segment of the molecule corresponding to a particular resonance [20,25,26]. As can be seen from Fig. 8, binding of AmB to liposomes has an opposite effect on the motional freedom of the choline molecules located in the outer leaflet and the inner leaflet of the lipid bilayer. The decreased motional freedom in the outer lipid monolayer of liposomes, associated with the AmB can be explained by a direct binding of the antibiotic to the polar headgroup region [19]. The opposite effects of the drug on the inner and outer polar headgroup regions indicate also that AmB does not penetrate into the liposome lumen and that Pr^{3+} ions do not penetrate into the liposome lumen, even after the AmB binding to vesicles.

Interestingly, a binding of AmB to the outer surface of liposomes affects the lipid molecular packing properties, in the membrane, and provides more freedom for molecular motion in the hydrophobic core of the bilayer (higher fluidity), as demonstrated by the decrease in $\Delta\nu$ corresponding to the CH_2 and CH_3 groups (Fig. 9). This effect corresponds to the increased motional freedom in the inner polar headgroup region of liposomes, discussed above (Fig. 8). The AmB-induced fluidization

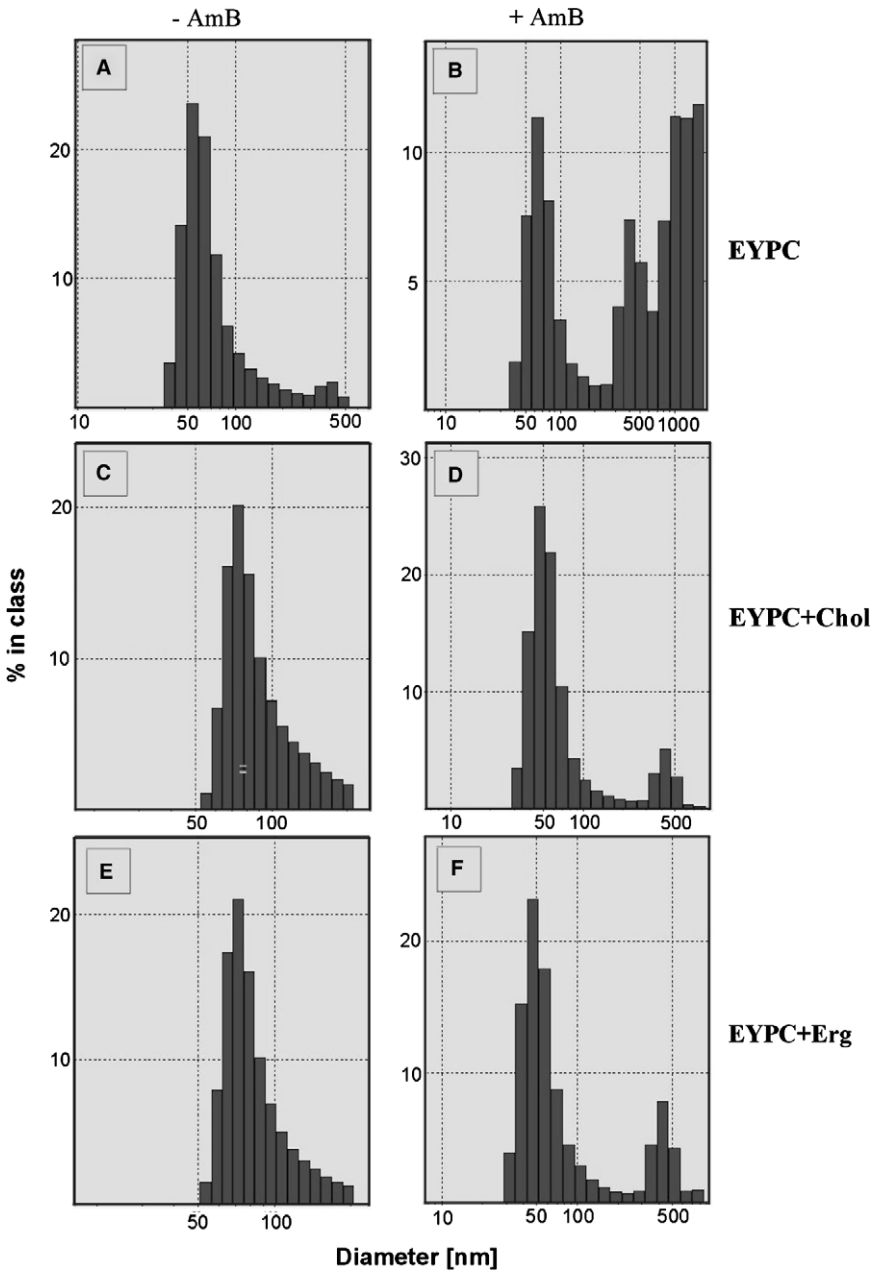

Fig. 3. Size dependency profiles of EYPC liposomes, prepared for ^{1}H NMR measurements, based on the photon correlation spectroscopy determination. Panels correspond to the liposome suspension formed with pure EYPC (A and B), EYPC with 40 mol% cholesterol (C and D) and EYPC with 40 mol% ergosterol (E and F). In three cases (B, D and F) liposome suspension was supplemented after preparation with AmB by injection of 15 μl of the solution in DMSO into 1 ml of the liposome suspension in D_2O followed by 5 min vortexing (final concentration 0.01 mg AmB/2 mg EYPC).

of the hydrophobic core itself, can potentially decrease the penetration barrier for ions and small molecules into the lipid bilayer and facilitate transmembrane ion transport. Such possibility has support from the results of recent studies on the proton permeability across the lipid membranes [8]. The results indicated that AmB present at relatively high concentrations in the lipid phase (above 1 mol%) increased the rate of transmembrane proton transport, but the proton flow observed was not typical of transmembrane ion channels [8].

Sterols are known as very important modifiers of dynamic and structural properties of lipid membranes and therefore the higher animal sterol – cholesterol, and the fungal sterol – ergosterol, have been a subject of numerous comparative research [27–32]. In general, ergosterol has been reported as more efficient than cholesterol, in ordering the hydrophobic core of the bilayers formed with saturated lipids [27–32] but the effect was opposite in the case of the membranes formed with an unsaturated lipid [31]. It was also reported that CH_2-segmental molecular order was higher in the case of dipalmitoylphosphatidylcholine membranes containing 40 mol% cholesterol than in the case of the same system containing the same fraction of ergosterol [29]. As can be seen

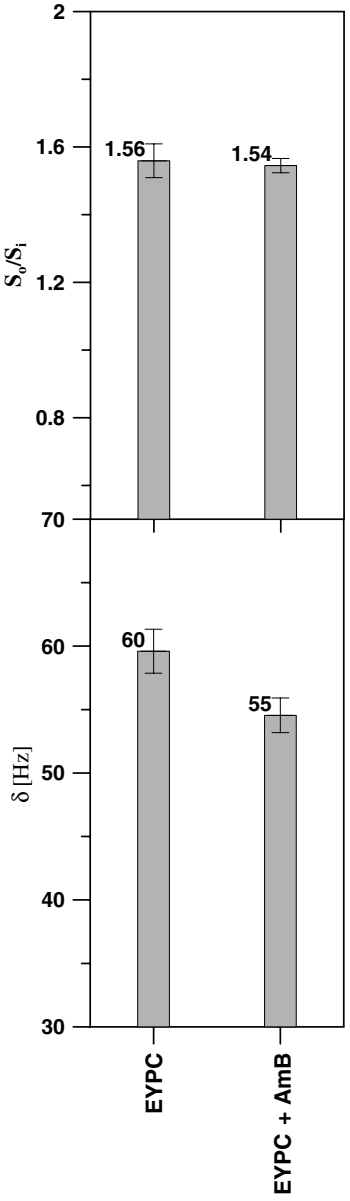

Fig. 4. Ratio (S_o/S_i) of the area beneath the downfield and upfield ^1H NMR resonance maxima corresponding to the $N^+(CH_3)_3$ group of EYPC in liposomes, split after addition of $PrCl_3$ (upper panel) and distance between the downfield and upfield maxima (δ, lower panel) corresponding to the liposomes formed with pure EYPC and the same sample after injection of AmB into the liposome suspension (indicated). The downfield and upfield resonance maxima are related to the outer and inner lipid monolayers of the liposome membranes, respectively.

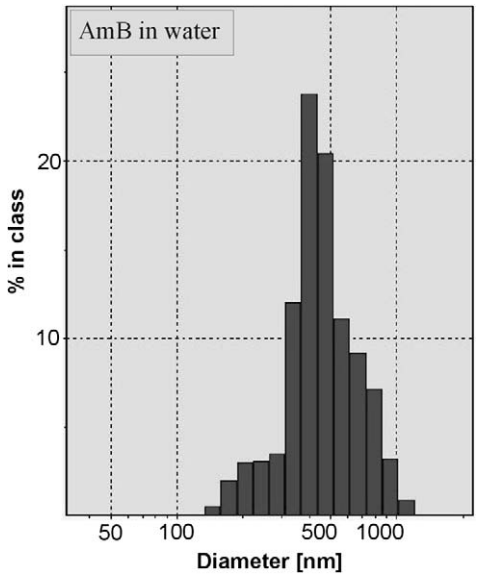

Fig. 5. Size dependency of the sample prepared by injection of 15 μl of the AmB solution in DMSO into 1 ml of D_2O followed by 5 min vortexing. Final AmB concentration 0.01 mg AmB/1 ml D_2O.

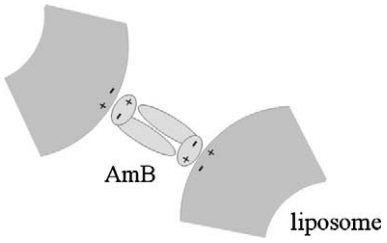

Fig. 6. Schematic model illustrating aggregation of EYPC liposomes induced by the molecules of membrane-bound AmB.

from Fig. 9 both sterols have very pronounced ordering effect in the acyl chain region of the membranes formed with EYPC. Interestingly, cholesterol appears to be more efficient in ordering the segmental molecular motion of the CH_2 groups, as in the case of the other studies [29], but ergosterol appeared to be more effective in ordering the CH_3 groups, in the centre of the bilayer. Molecular dynamics simulation study shows also possibility of interaction of polar sterol heads with the choline $N^+(CH_3)_3$ groups in the phosphatidylcholine membranes [28]. As can be seen from Fig. 8, incorporation of cholesterol and ergosterol into the liposomes formed with EYPC affects also the molecular motion of the $N^+(CH_3)_3$ headgroups. Both sterols increase motional freedom of the choline fragments in the outer membrane of liposomes. Such an effect is most probably directly associated with increased binding efficacy of AmB from water to the lipid phase, observed recently in the monomolecular layer experiments [19]. Interestingly, the facilitated AmB binding to liposomes is associated with considerable reduction of the formation of molecular aggregates in the water phase, as can be seen from the analysis of the PCS profiles (Figs. 3D and F). Electronic absorption spectra of the liposome suspension show that the molecular aggregates of AmB are also present in the case of the sterol-containing liposomes (Fig. 10), but most probably the molecular structures that give rise to hypsochromically shifted absorption spectra are smaller and remain associated with the surface of lipid vesicles. Interestingly, the molecular aggregates of AmB formed in the case of ergosterol-containing liposomes are qualitatively different from those formed in other cases (Fig. 10). The spectral band at 350 nm, diagnostic of molecular dimers [15,17] is dominant among the spectral forms of aggregated AmB in the ergosterol-containing liposomes.

Binding of AmB to the surface of the sterol-containing EYPC liposomes is also manifested by an increase of the

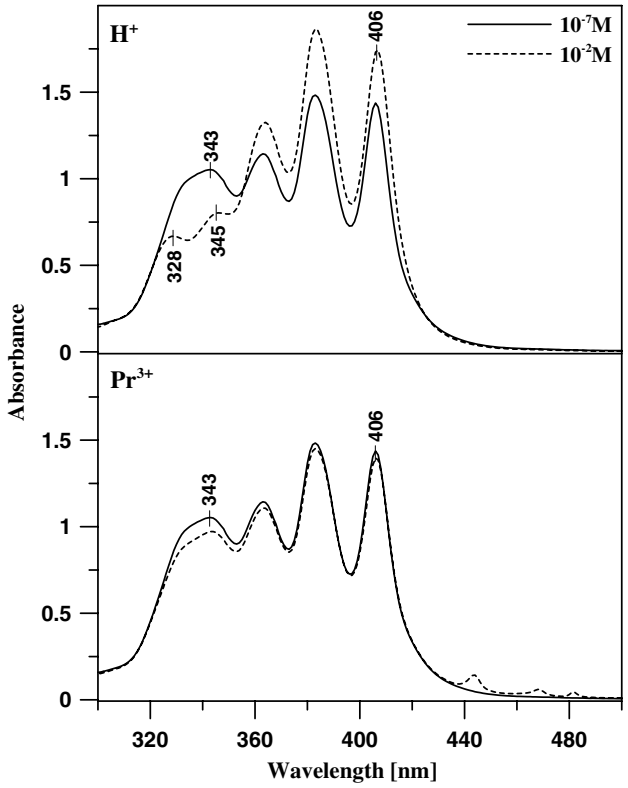

Fig. 7. Electronic absorption spectra of AmB dissolved in water containing H^+ or Pr^{3+} ions at the concentrations 10^{-2} and 10^{-7} M (in the case of H^+ ions pH of the solution was 2.0 and 7.0). The pH of the samples containing $PrCl_3$ was 7.0.

$\Delta\nu$ parameter corresponding to the $N^+(CH_3)_3$ groups (Fig. 8, upper panel). Interestingly, also in the case of the sterol-containing membranes, as in the case of pure EYPC membranes, binding of AmB to liposomes is associated with considerably decreased molecular order in the headgroup region of the inner lipid membrane (Fig. 8, lower panel). This effect is particularly pronounced in the case of the ergosterol-containing EYPC membranes and corresponds to the selective pharmacological action of AmB towards the fungal membranes containing ergosterol versus biomembranes containing cholesterol.

Binding of AmB to the sterol-containing liposomes has little or no effect on the hydrophobic core of the membranes (Fig. 9). This indicates the outer polar headgroup region as a main localization site of AmB and suggests that the effect on the inner polar surface observed, is rather a propagation of the disturbance of the membrane structural properties in the outer polar region [33]. The only exception is the motional freedom of the CH_3 groups in the case of ergosterol-containing membranes. In such a case, binding of AmB brings about fluidization in the centre of the membrane, manifested by the decrease in a value of the parameter $\Delta\nu$, to a level observed in the case of the pure EYPC liposomes (Fig. 9). Such an effect suggests the possibility of binding of AmB to the hydrophobic core of the membranes modified with ergosterol. The effect of AmB on the dynamics of the CH_2 groups, accompanying such a binding, is expected to be relatively small, owing to very strong ordering effect of the sterol. On the other hand, the effect of AmB is clearly pronounced in the centre of the hydrophobic core (see Fig. 9, CH_3 groups), in where the effect of sterols is limited. Differences in the effect of AmB on dynamic properties of the membranes containing ergosterol and cholesterol are also pronounced in the polar headgroup region of the outer leaflet of liposomes (Fig. 8).

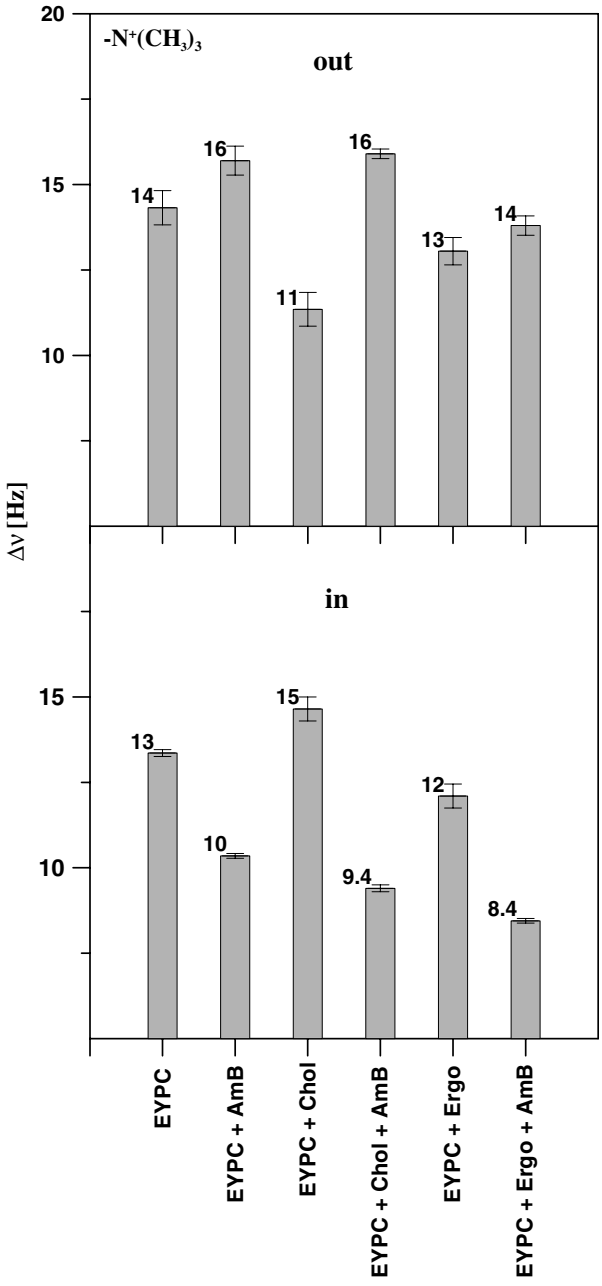

Fig. 8. Full width at a half height of the ^1H NMR resonance maximum corresponding to the $N^+(CH_3)_3$ groups located at the outer and inner surface of liposomes (indicated) as a function of the sample composition: EYPC – pure EYPC liposomes, EYPC + AmB – pure liposomes after injection of AmB, EYPC + Chol – liposomes composed of EYPC and 40 mol% cholesterol, EYPC + Chol + AmB – liposomes composed of EYPC and 40 mol% cholesterol after injection of AmB, EYPC + Erg – liposomes composed of EYPC and 40 mol% cholesterol, EYPC + Erg + AmB – liposomes composed of EYPC and 40 mol% ergosterol after injection of AmB.

The analysis of the ^1H NMR spectra of liposomes unequivocally indicate the polar headgroup region of the membrane as

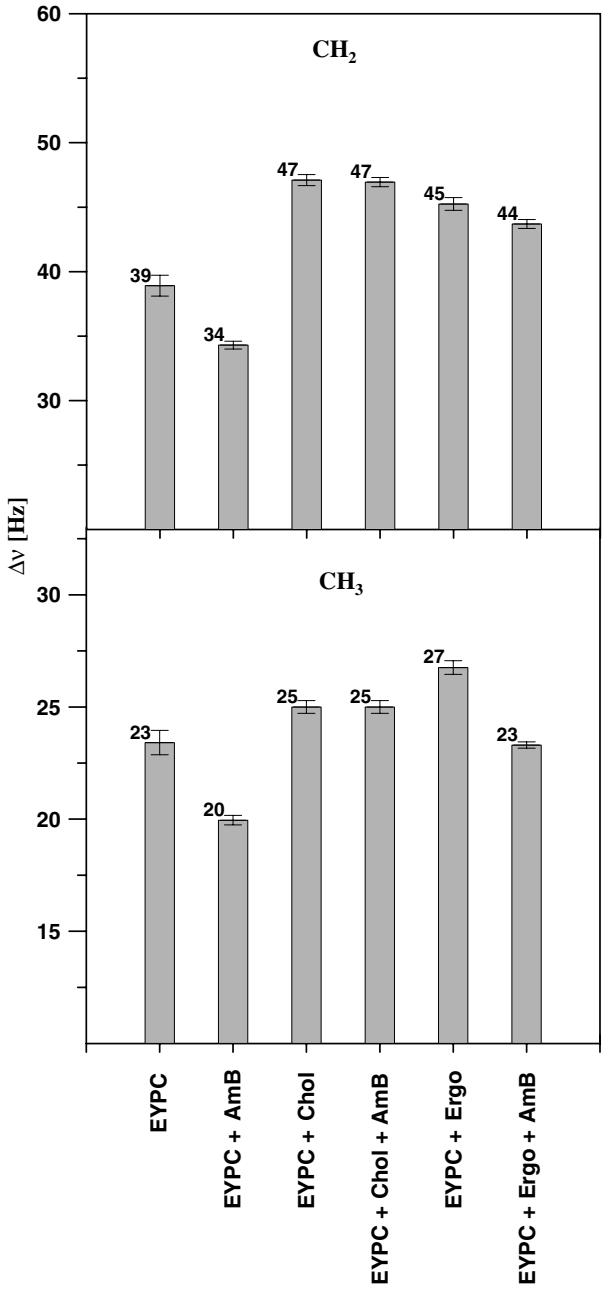

Fig. 9. Full width at a half intensity of the ^{1}H NMR resonance maximum corresponding to the CH_2 groups and CH_3 groups (as indicated) of alkyl chains of EYPC in liposomes as a function of composition of the sample. Legend is the same as for Fig. 8.

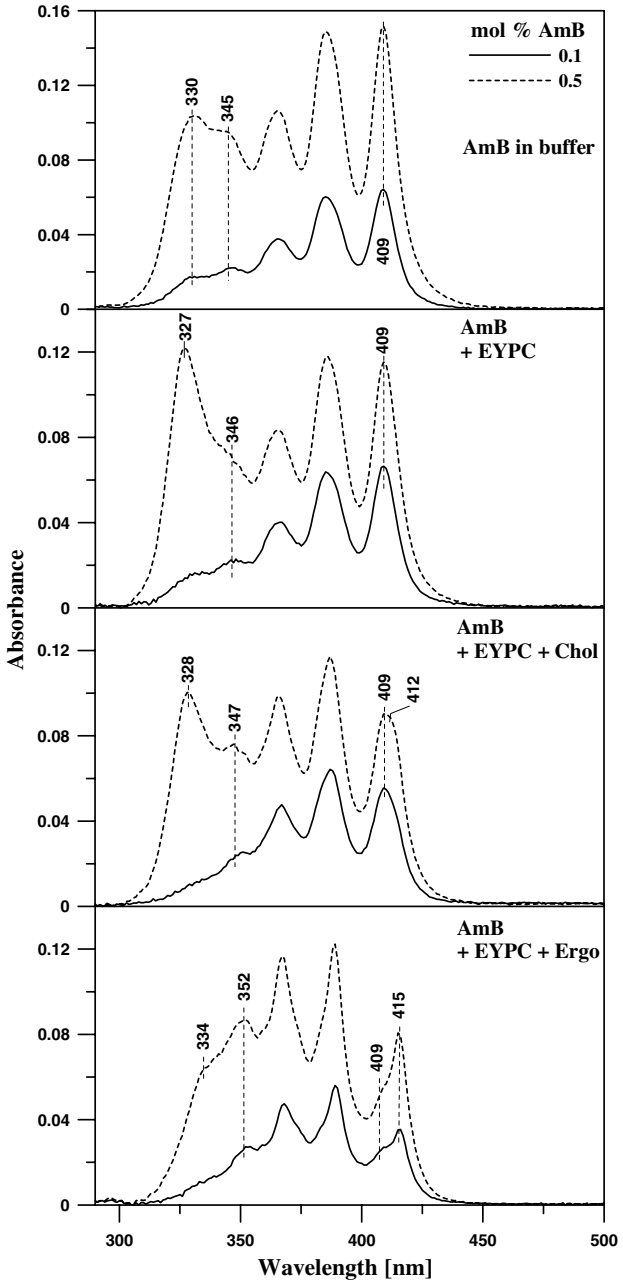

Fig. 10. Electronic absorption spectra of AmB injected to the EYPC unilamellar liposome suspension to final concentration 0.1 and 0.5 mol% with respect to lipid or injected to the same volume of buffer solution (indicated). The liposomes were prepared with pure EYPC or with EYPC containing 40 mol% ergosterol (Ergo) or cholesterol (Chole) as indicated. Lipid concentration in the liposome suspension was 0.1 mg/ml, the optical path 1 cm.

a place of localization of exogenously added AmB. It may not be excluded that penetration of amphiphilic molecules of AmB into the hydrophobic core is a source of decreased molecular order observed. On the other hand, aggregated structures of AmB formed in the hydrophobic membrane region, presumably hydrophilic pores [5,9,11,23], will expose the rigid polyene fragments to the lipid alkyl chains and therefore the ordering effect can be expected in such a case, as observed in the case of other polyenes e.g. carotenoid pigments [34–36]. It is therefore highly probable that AmB present in the water phase binds preferentially to the polar headgroup region of the lipid membranes and that such a binding affects also the hydrophobic core of the bilayer and in consequence influences the transmembrane ion transport. This mechanism would be similar to that one observed in the class of other membrane transporters which bind to the hydrophilic membrane layer and do not penetrate its hydrophobic core, e.g. a membrane transporter for tryptophan composed of RNA [37]. Both sterols examined, facilitate AmB binding to the lipid membranes as concluded from the analysis of the PCS profiles. Similar effects of AmB on the dynamic properties of the EYPC membranes were also

observed in the sterol-containing membranes, although certain differences have been observed among the systems modified with different sterols. The effect of AmB on the polar headgroup region is stronger in the case of the cholesterol-containing membranes than in the case of the ergosterol-containing membranes. On the other hand, the effect of AmB on the hydrophobic core was exclusively observed in the membranes containing ergosterol but not in the membranes containing cholesterol. The effect of AmB on the hydrophobic core of the ergosterol-containing membranes suggests also possibility of penetration of the drug into the membranes. This effect corresponds to the specificity of the drug towards fungal membranes enriched with ergosterol and can be attributed to an exceptional ability of ergosterol to promote formation of the liquid-ordered phase in the liquid-crystalline lipid membranes (lipid rafts) [30].

Acknowledgements: This work was supported by the Ministry of Education and Science of Poland from the budget funds for science in years 2004–2007 within the Research Project 2P05F04327.

References

[1] Hartsel, S.C., Hatch, C. and Ayenew, W. (1993) How does amphotericin B work? Studies on model membrane systems. J. Liposome Res. 3, 377–408.
[2] Hartsel, S. and Bolard, J. (1996) Amphotericin B: new life for an old drug. Trends Pharmacol. Sci. 17, 445–449.
[3] Sternberg, T.M., Wright, E.T. and Oura, M. (1956) A new antifungal antibiotic, amphotericin B. Antibiotics Ann. 3, 566–573.
[4] De Kruijff, B. and Demel, R.A. (1974) Polyene antibiotic–sterol interaction in membranes of Acholeplasma Laidlawii cells and lecithin liposomes; III Molecular structure of the polyene antibiotic–cholesterol complex. Biochim. Biophys. Acta 339, 57–70.
[5] De Kruijff, B., Gerritsen, W.J., Oerlemans, A., Demel, R.A. and van Deenen, L.L. (1974) Polyene antibiotic–sterol interactions in membranes of Acholeplasma laidlawii cells and lecithin liposomes. I. Specificity of the membrane permeability changes induced by the polyene antibiotics. Biochim. Biophys. Acta 339, 30–43.
[6] Hartsel, S.C., Benz, S.K., Peterson, R.P. and Whyte, B.S. (1991) Potasium-selective amphotericin B channels are pre-dominant in vesicles regardless of sidedness. Biochemistry 30, 77–82.
[7] Cotero, B.V., Rebolledo-Antunez, S. and Ortega-Blake, I. (1998) On the role of sterol in the formation of the amphotericin B channel. Biochim. Biophys. Acta 1375, 43–51.
[8] Herec, M., Dziubinska, H., Trebacz, K., Morzycki, J.W. and Gruszecki, W.I. (2005) An effect of antibiotic amphotericin B on ion transport across model lipid membranes and tonoplast membranes. Biochem. Pharmacol. 70, 668–675.
[9] Gruszecki, W.I., Gagos, M. and Kernen, P. (2002) Polyene antibiotic amphotericin B in monomolecular layers: spectrophotometric and scanning force microscopic analysis. FEBS Lett. 524, 92–96.
[10] Khutorsky, V. (1996) Ion coordination in the amphotericin B channel. Biophys. J. 71, 2984–2995.
[11] Baginski, M., Resat, H. and McCammon, J.A. (1997) Molecular properties of amphotericin B membrane channel: a molecular dynamics simulation. Mol. Pharmacol. 52, 560–570.
[12] Baginski, M., Resat, H. and Borowski, E. (2002) Comparative molecular dynamics simulations of amphotericin B–cholesterol/ergosterol membrane channels. Biochim. Biophys. Acta 1567, 63–78.
[13] Wojtowicz, K., Gruszecki, W.I., Walicka, M. and Barwicz, J. (1998) Effect of amphotericin B on dipalmitoylphosphatidylcholine membranes: calorimetry, ultrasound absorption and monolayer technique studies. Biochim. Biophys. Acta 1373, 220–226.
[14] Gagos, M., Koper, R. and Gruszecki, W.I. (2001) Spectrophotometric analysis of organisation of dipalmitoylphosphatylcholine bilayers containing the polyene antibiotic amphotericin B. Biochim. Biophys. Acta 1511, 90–98.
[15] Gruszecki, W.I., Gagos, M., Herec, M. and Kernen, P. (2003) Organization of antibiotic amphotericin B in model lipid membranes. A mini review. Cell. Mol. Biol. Lett. 8, 161–170.
[16] Sternal, K., Czub, J. and Baginski, M. (2004) Molecular aspects of the interaction between amphotericin B and a phospholipid bilayer: molecular dynamics studies. J. Mol. Model. (Online) 10, 223–232.
[17] Gruszecki, W.I., Gagos, M. and Herec, M. (2003) Dimers of polyene antibiotic amphotericin B detected by means of fluorescence spectroscopy: molecular organization in solution and in lipid membranes. J. Photochem. Photobiol. B: Biol. 69, 49–57.
[18] Gruszecki, W.I. and Herec, M. (2003) Dimers of polyene antibiotic amphotericin B. J. Photochem. Photobiol. B: Biol. 72, 103–105.
[19] Gagos, M., Gabrielska, J., Dalla Serra, M. and Gruszecki, W.I. (2005) Binding of antibiotic amphotericin B to lipid membranes: monomolecular layer technique and linear dichroism-FTIR studies. Mol. Memb. Biol. 22, 433–442.
[20] Gabrielska, J. and Gruszecki, W.I. (1996) Zeaxanthin (dihydroxy-beta-carotene) but not beta-carotene rigidifies lipid membranes: a ^{1}H-NMR study of carotenoid-egg phosphatidylcholine liposomes. Biochim. Biophys. Acta 1285, 167–174.
[21] Hunt, G.R.A. and Tipping, L.R.H. (1980) Trans-bilayer pseudocontact shifts produced by lanthanide ions in the ^{1}H- and ^{31}P-NMR spectra of phospholipids, vesicular membranes and their temperature variation. J. Inorg. Biochem. 12, 17–36.
[22] Bolard, J. and Cheron, M. (1982) Association of the polyene antibiotic amphotericin B with phospholipid vesicles: perturbation by temperature changes. Can. J. Biochem. 60, 782–789.
[23] Barwicz, J., Gruszecki, W.I. and Gruda, I. (1993) Spontaneous organization of amphotericin B in aqueous medium. J. Colloid Interf. Sci. 158, 71–76.
[24] Bolard, J., Legrand, P., Heitz, F. and Cybulska, B. (1991) One-sided action of amphotericin B on cholesterol-containing membranes is determined by its self-association in the medium. Biochemistry 30, 5707–5715.
[25] Jezowska, I., Wolak, A., Gruszecki, W.I. and Strzalka, K. (1994) Effect of beta-carotene on structural and dynamic properties of model phosphatidylcholine membranes.II. A 31P-NMR and 13C-NMR study. Biochim. Biophys. Acta 1194, 143–148.
[26] Fenske, D.B. (1993) Structural and motional properties of vesicles as revealed by nuclear magnetic resonance. Chem. Phys. Lipids 64, 143–162.
[27] Bernsdorff, C. and Winter, R. (2003) Differential properties of the sterols cholesterol, ergosterol, beta-sitosterol, *trans*-7-dehydrocholesterol, stigmasterol and lanosterol on DPPC bilayer order. J. Phys. Chem. B 107, 10658–10664.
[28] Czub, J. and Baginski, M. (2006) Comparative molecular dynamics study of lipid membranes containing cholesterol and ergosterol. Biophys. J. 90, 2368–2382.
[29] Endress, E., Bayerl, S., Prechtel, K., Maier, C., Merkel, R. and Bayerl, T.M. (2002) The effect of cholesterol, lanosterol, and ergosterol on lecithin bilayer mechanical properties at molecular and microscopic dimensions: a solid-state NMR and micropipet study. Langmuir 18, 3293–3299.
[30] Hsueh, Y.W., Gilbert, K., Trandum, C., Zuckermann, M. and Thewalt, J. (2005) The effect of ergosterol on dipalmitoylphosphatidylcholine bilayers: a deuterium NMR and calorimetric study. Biophys. J. 88, 1799–1808.
[31] Urbina, J.A., Pekerar, S., Le, H.B., Patterson, J., Montez, B. and Oldfield, E. (1995) Molecular order and dynamics of phosphatidylcholine bilayer membranes in the presence of cholesterol, ergosterol and lanosterol: a comparative study using 2H-, 13C- and 31P-NMR spectroscopy. Biochim. Biophys. Acta 1238, 163–176.
[32] Xu, X.L., Bittman, R., Duportail, G., Heissler, D., Vilcheze, C. and London, E. (2001) Effect of the structure of natural sterols and sphingolipids on the formation of ordered sphingolipid/sterol domains (rafts). J. Biol. Chem. 276, 33540–33546.

[33] May, S. (2000) Theories on structural perturbations of lipid bilayers. Curr. Opp. Coll. Interf. Sci 5, 244–249.
[34] Gruszecki, W.I. (1999) Carotenoids in membranes in: The Photochemistry of Carotenoids (Frank, H.A., Young, A.J., Britton, G. and Cogdell, R.J., Eds.), pp. 363–379, Kluwer Academic Publ., Dordrecht.
[35] Gruszecki, W.I. (2004) Carotenoid orientation: role in membrane stabilization in: Carotenoids in Health and Disease (Krinsky, H., Mayne, S.T. and Sies, H., Eds.), pp. 151–163, Marcel Dekker, New York.
[36] Gruszecki, W.I. and Strzalka, K. (2005) Carotenoids as modulators of membrane physical properties. Biochim. Biophys. Acta 1740, 108–115.
[37] Janas, T., Janas, T. and Yarus, M. (2004) A membrane transporter for tryptophan composed of RNA. RNA 10, 1541–1549.