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An effect of antibiotic amphotericin B on ion transport across model lipid membranes and tonoplast membranes

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Abstract

A pH sensitive fluorescence probe piranine trisulfonate, entrapped inside small unilamellar liposomes formed with egg yolk phosphatidylcholine, was applied to investigate effect of polyene antibiotic amphotericin B (AmB) on proton transport across lipid membranes. Time dependencies of fluorescence-monitored pH changes inside lipid vesicles, upon sudden acidification of the liposome suspension, were analyzed in terms of two-exponential kinetics. It appears that addition of AmB at 3 mol%, with respect to lipid, considerably increases the rate constant of the fast component of proton transport (a change from (60 to 149) $\times 10^{-3}$ s^{-1}) and decreases the rate constant of the slow component (a change from (11 to 5) $\times 10^{-3}$ s^{-1}). Incorporation of 0.1 mol% AmB results in the decrease of both parameters (to (33 and 2) $\times 10^{-3}$ s^{-1}, respectively). The increase in the rate of proton transfer across the lipid membrane is interpreted as related to the formation of membrane channels by AmB, at higher concentration of the drug or nonspecific destabilization of the membrane structure. At low concentrations, at which formation of molecular structures of AmB is not possible, the antibiotic molecules are oriented horizontally with respect to the plane of the membrane and act in making the membrane more compact and less permeable to ions. The presence of sterols (cholesterol, ergosterol and cholesterol dimer) in the lipid phase, in the concentration 3 mol% and lower, decreased the rate constants of proton transfer across the membranes but did not influence significantly the effect of AmB on the ion transport. The presence of AmB in the bathing solutions of tonoplast membranes isolated from *Conocephalum conicum* at the concentrations range 1×10^{-7} to 3.6×10^{-5} does not influence considerably the ion current, as monitored by means of the patch-clamp technique.

Keywords: Amphotericin B; Liposomes; Proton transport; Polyene antibiotics; Patch-clamp

1. Introduction

Opportunistic infections as a consequence of weakened immunity defenses, such as from AIDS or from many cancer therapies, have renewed the scientific studies on polyene antibiotic amphotericin B (AmB). This antibiotic is the most common antifungal agent used to treat deep-seated mycotic infections [1]. However, in spite of its broad spectrum of action pharmacological use of amphotericin B is limited by its human toxicity, especially nephrotoxicity [2].

Numerous studies carried out on both biological and model membranes using functional approaches have revealed that the action of amphotericin B causes changes in the permeability of membrane lipids [3,4]. This effect strongly depends on membrane state, its composition and amphotericin B concentration [5–7]. These results can be explained by the theory that biological action of amphotericin B is directly related with formation of molecular transmembrane channels, leading to uncontrolled leakage of monovalent ions and small molecules from the cell [8,9]. This disturbance in ionic distribution causes cell death. The hypothesis of hydrophilic pores is based on amphiphilic molecular structure of the drug but its precise structure and the mode of action is still under discussion [10–13].

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Formation of AmB membrane pores is supported from the results of molecular dynamic calculations [14,15] and scanning force microscopic analysis of AmB monomolecular layers [12,13]. On the other hand, an alternative mechanism of AmB activity, connected with unspecified interactions of drug with lipid membrane, leading to formation of defects at the antibiotic-lipid interphase is also proposed [16].

In the present work we study the effect of amphotericin B, at different concentrations, on proton transport across lipid membranes of unilamellar liposomes formed with EYPC as well as liposomes containing additionally incorporated different sterols: cholesterol, a sterol of the mammal cells, ergosterol, a sterol of the cells of fungi and cholesterol dimer, able to span a lipid bilayer. We also present an experiment on influence of amphotericin B alone and in combination with ergosterol on a natural biological membrane (vacuolar membrane–tonoplast isolated from the liverwort *Conocephalum conicum*). Currents flowing through the membrane were registered by the patch-clamp technique.

2. Materials and methods

2.1. Materials

Amphotericin B (AmB) was purchased from Sigma Chem. Co. (St. Louis, USA). AmB was dissolved and recrystallized from 2-propanol–water (4:6, v/v) and purified by means of HPLC directly before use. A Supelco PKB-100 column was applied (length 25 cm, internal diameter 4.6 mm) and the solvent mixture 2-propanol–water (4:6, v/v) was applied as a mobile phase.

Cholesterol and ergosterol were purchased from Sigma Chem. Co. (St. Louis, USA). Cholesterol was purified by recrystalization from *n*-hexane–methanol (4:1, v/v) before use. Dimer of cholesterol (see Fig. 1) was synthesized in the Institute of Chemistry of the University of Białystok as described elsewhere [17].

Sephadex G-100, egg yolk phosphatidylcholine (purity 99%, EYPC) and valinomycin were obtained from Sigma Chem. Co. (St. Louis, USA). The pH-sensitive dye 8-hydroxy-1,3,6-pyrenetrisulfonate (PTS) was purchased from Molecular Probes (Eugene, USA). Filters for liposome extrusion were from Millipore Corporation (Bedford, USA).

2.2. Plant material

Conocephalum conicum L. was grown in a greenhouse. Plants grew in flat pots filled with a soil from a natural habitat. Plants were watered every day with tap water. No additional illumination was applied during the growth period. The temperature was 23–25 °C in the day and 16–18 °C in the night. The humidity was kept between 60 and 80%.

2.3. Preparation of liposomes

Unilamellar liposomes were prepared from EYPC according to the procedure described previously [18]. Briefly, a test tube containing the vacuum-dried thin film of the lipid was filled with the solution of 0.1 M KCl, containing also pH-sensitive dye. The pH of solution was adjusted to 5.6. The final lipid concentration was 3.8 mg/ml. The concentration of PTS was 5×10^{-4} M. In the case of AmB-containing samples, the drug was introduced to the lipid solution in chloroform, before evaporation, in order to prepare homogenous EYPC-AmB film. The samples were vortexed in order to obtain homogeneous dispersion of large multilamellar vesicles and then sonicated 3×5 s with a 20 kHz sonicator with a titanium probe. This procedure yields formation of unilamellar vesicles as concluded from the ^{1}H NMR measurements of the liposome suspension supplemented with praseodymium ions [19,20]. To unify the size of lipid vesicles, the suspension was size calibrated with liposome extruder (Avestin Inc., Canada) using filters with 450 nm pores. The procedure yields formation of the liposomes of size 400 nm (>85%) as revealed by means of quasi elastic light scattering technique. Polidispersity index of the vesicles was determined as 0.188. In order to separate the dye that has not been incorporated into liposomes, gel chromatography was applied. The glass column (the internal diameter 17 mm and the length 11 cm) was filled with Sephadex G-100 gel. The 0.1 M KCl solution of the same pH level as inside of the vesicles was applied as a mobile phase. To identify the fraction of liposome solution combined detection of UV–vis absorption spectra containing also a light scattering component was applied. The fraction of free dye molecules that have not been entrapped inside liposomes was typically eluted 10–15 min after the fraction of lipid vesicles. A stability of liposomes was checked by absorption measurements in the UV–vis spectral region. AmB is an amphiphilic molecule and its solubility in water is extremely low. Owing to this fact this antibiotic binds to the membranes very efficiently. Our previous spectroscopic experiments show that while incorporated to the liposomes below 5 mol%, AmB remains exclusively attached to the lipid membranes and not present in the water phase [21,22].

2.4. Kinetic measurements

Ion transport across lipid membranes of liposomes was recorded fluorometrically using a Shimadzu RF-5001 PC spectrofluorometer. A magnetic microstirrer was used continuously during all the kinetic experiments. A 1 cm quartz cuvette was thermostated at 25 °C during all measurements. The emission of fluorescence was detected at 511 nm and the excitation was switched continuously between 403 and 455 nm (the main maxima of the excita-

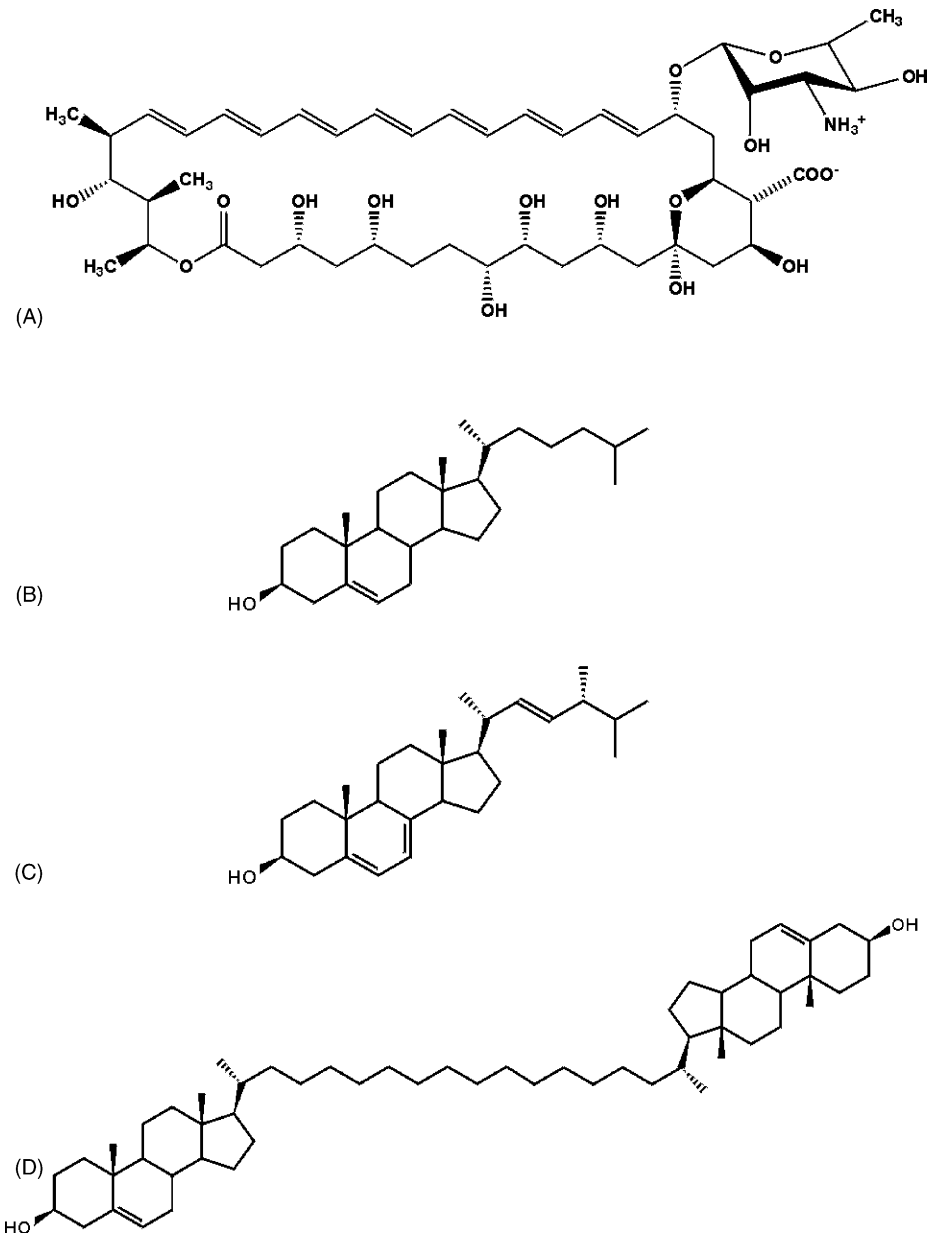

Fig. 1. Molecular structure of amphotericin B (A) and the sterols used in the study: cholesterol (B), ergosterol (C), and dimer of cholesterol (D).

tion spectrum bands of protonated and non-protonated forms of PTS).

In order to prevent a possible formation of an electrostatic barrier that may affect proton penetration into liposomes 2 μl of 0.1 M of the ethanolic solution of valinomycin (a K^+ ion carrier) was added to 2 ml of liposome suspension two minutes after the beginning of the recording. A gradient of proton concentration across the liposome membranes was generated by injection of 10 μl of HCl solution that created the pH change from 5.6 to 4.6 in the bulk phase of the liposome suspension. The pH change in this range can be followed by recording of fluorescence of PTS owing to the dependency of the absorption (fluorescence excitation) spectrum on proton concentration. Fig. 2 presents the calibration dependency and selected fluorescence excitation spectra corresponding to different pH values.

2.5. Isolation of vacuoles

Isolated vacuoles were obtained by a surgical method described by Trębacz and Schönknecht [23]. Briefly, fresh thalli were cut into squares of 5–8 mm. Then, the fragments were cut with a razor blade parallel to the main plane to expose one to three cell layers. The slices were plasmolysed in a medium containing: 100 mM $MgCl_2$, 15 mM Hepes/Tris, pH 7.2, 500 mM sorbitol. After 1 h incubation, tissue fragments were cut perpendicular to the main plane. Most of the protoplasts along the line of incision were destroyed, but a few emerged during a stepwise deplasmolysis from the cut cell walls. Lowering the osmolality of the perfusion solution to 300 mOsmol/kg by reducing the sorbitol content resulted in releasing vacuoles. Vacuoles origi-

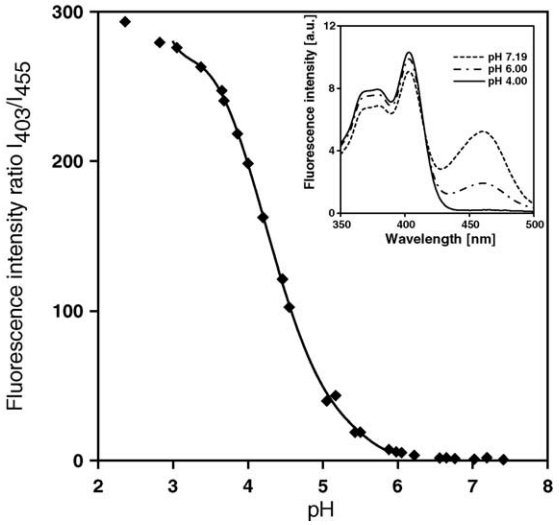

Fig. 2. The calibration curve of PTS: fluorescence intensity ratio: (Ex at 402 nm, Em at 511 nm/Ex at 455 nm, Em at 511 nm) corresponding to the main maxima of excitation spectrum bands of protonated and non-protonated form of PTS) as a function of pH. The inset presents the fluorescence excitation spectra of PTS (Em at 511 nm) for three different pH values indicated.

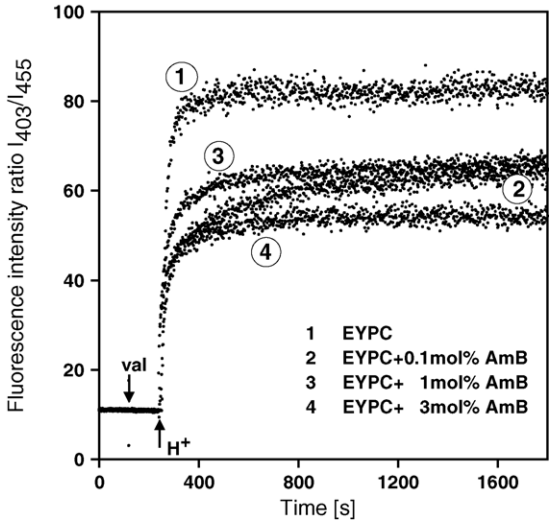

Fig. 3. Kinetics of fluorescence changes of PTS entrapped inside unilamellar liposomes formed with EYPC (1) or with EYPC containing additionally amphotericin B at different concentrations: 0.1 mol% (2), 1 mol% (3) and 3 mol% (4). The arrows indicate the moments of addition of valinomycin (val) and HCl (H^+) into the cuvette, that generated a proton gradient on the membrane (pH 5.6 → 4.6).

nated from large cells containing small chloroplast locating in a lower part of the thallus.

2.6. Patch-clamp experiments

Patch-clamp measurements were performed as described in [23] either in the ''whole-vacuole'' configuration, analogous to ''whole-cell'' or with excised patches in the ''cytoplasmic-side-out'' configuration with the cytoplasmic face of the vacuolar membrane facing the bath [24]. The convention of current and voltage signs was according to Bertl et al. [25].

3. Results

Fig. 3 presents exemplary time dependencies of fluorescence intensity ratio of PTS entrapped inside liposomes, recorded at 403 and 455 nm, monitoring the proton concentration changes. Experimental dependencies have been fitted with kinetic equation according to the formula:

$$1 - \frac{I - I_0}{I^{\mathrm{L}}_{\max}} = A\,\mathrm{e}^{-\alpha t} + B\,\mathrm{e}^{-\beta t} + C \quad (1)$$

where I is a I_{403}/I_{455} fluorescence intensity ratio corresponding to time t, I_0 the initial value of I corresponding to the moment of injection of HCl, $I^{\mathrm{L}}_{\max}$ a maximum value of I recorded in the case of the liposomes formed with the pure lipid ($I^{\mathrm{L}}_{\max}$ = 78.8, see Table 1), A and B the pre-exponential factors, α the rate constant of the fast proton transport component, β the rate constant of the slow phase of equilibration of proton exchange, and C is the free parameter which was applied to calculate a level of the steady-state proton concentration inside liposomes. Table 1 presents the values of both the kinetic parameters along with the pre-exponential factors and the steady-state pH level inside liposomes formed with pure EYPC and containing AmB as an additional component of the lipid phase, at different concentrations. As can be seen, the kinetics of the proton transport across the liposome membranes is different in the case of pure lipid as compared to the membranes modified with AmB. In the case of pure EYPC, more than

Table 1
Effect of amphotericin B on the kinetic parameters of proton transport into EYPC liposomes

	$A \pm$ S.D.[a]	$\alpha \pm$ S.D. ($\times 10^3\ s^{-1}$)	$B \pm$ S.D.	$\beta \pm$ S.D. ($\times 10^3\ s^{-1}$)	$C \pm$ S.D.	$I_{\max} \pm$ S.D.	ΔpH $\pm$ S.D.	$\tau \pm$ S.D. (s)
EYPC	0.85 ± 0.09	60 ± 4	0.18 ± 0.06	11 ± 2	0.05 ± 0.05	78.8 ± 4.4	1.00 ± 0.04	15 ± 1
EYPC + 0.1 mol% AmB	0.50 ± 0.06	33 ± 3	0.28 ± 0.02	2 ± 1	0.23 ± 0.03	67.5 ± 3.1	0.86 ± 0.03	34 ± 3
EYPC + 1 mol% AmB	0.47 ± 0.03	59 ± 22	0.18 ± 0.03	4 ± 2	0.28 ± 0.03	65.6 ± 1.2	0.76 ± 0.02	15 ± 1
EYPC + 3 mol% AmB	0.37 ± 0.10	149 ± 31	0.19 ± 0.01	5 ± 2	0.45 ± 0.06	53.5 ± 1.0	0.64 ± 0.05	7 ± 2

Kinetic parameters α and β correspond to Eq. (1). A and B are the pre-exponential factors, $I_{\max}$ a steady-state level of the fluorescence intensity ratio of PTS excited at 403 and 455 nm (I_{403}/I_{455}) calculated on the basis of parameter C and ΔpH is a pH change inside liposomes corresponding to the steady-state conditions. τ denotes time corresponding to the I_{403}/I_{455} ratio equal to $I_{\max}/2$.

[a] The means and standard deviations were calculated from three to five measurements.

80% of proton transport, to equilibrate the gradient generated by the injection of HCl, is relatively fast and proceeds with the rate constant $\alpha = 60 \times 10^{-3}\ s^{-1}$ and less than 20% of the proton transfer is slower and is characterized by the rate constant $\beta = 11 \times 10^{-3}\ s^{-1}$. Incorporation of 3 mol% of AmB into the lipid phase increases considerably the rate constant of the fast proton transport component ($\alpha = 149 \times 10^{-3}\ s^{-1}$). Also time of half-increase of the fluorescence intensity ratio (τ, see Table 1) drops twice under such conditions. Such a result can be expected owing to the well-known ability of AmB to form pores across lipid membranes [8,9]. The increased membrane permeability, in the presence of AmB, has been also demonstrated by means of the spin label technique [6]. Surprisingly, incorporation of AmB into the liposomes at a relatively low concentration (0.1 mol%), results in the decrease in the rate constants of both the fast and the slow proton transport components. The parameter α drops almost twice and the parameter β by a factor of 5. Similar observation regarding liposome membrane permeability has been reported by Cohen [7]. Osmotic shrinkage of liposomes was enhanced at the presence of AmB in the medium at low concentrations but was blocked at high concentrations of the drug [7].

In order to compare an effect of amphotericin B on the proton transport across membranes formed with a pure lipid and the membranes containing sterols, the liposomes composed of EYPC and sterols (cholesterol, ergosterol and cholesterol dimers) were prepared and the kinetic measurements of proton transport across lipid membranes were carried out, as in the case of the membranes formed with pure lipid. The results are presented in Table 2. As may be seen, the presence of the sterols alone, within the lipid phase of the membranes, induces the marked decrease in the membrane permeability to ions, even at relatively low molar concentrations. Incorporation of 0.1 mol% cholesterol into the lipid phase decreases the rate constant of both the fast and the slow proton transport component across the membrane by a factor 2 and 3, respectively, as compared to the liposomes formed with pure EYPC (see Tables 1 and 2). The steady-state level of pH inside liposomes, following the acidification of the liposome suspension, does not undergo any essential change. It means that the presence of sterols affects only kinetic properties of the proton transport across the membranes. There are not any significant changes in the rate constants of the both proton transport components after the addition of 0.1 mol% AmB to the cholesterol-containing membranes. However, it can be observed that the incorporation of both amphotericin B and cholesterol has a slight effect in the increase in the rate of the fast proton transfer component, so that the parameter $\alpha = 23 \times 10^{-3}\ s^{-1}$ in the case of liposomes formed with EYPC and containing 0.1 mol% cholesterol and 0.1 mol% AmB rises up to a value $50 \times 10^{-3}\ s^{-1}$ as a result of the increase in the concentration of both AmB and cholesterol up to 3 mol% (see Table 2). On the other hand, the relative effect of amphotericin B on the proton transport across sterol-containing membranes is evidently not as strong as in the case of the membranes formed with the pure component. The slow component of the proton transport (β) remains practically unaffected by the presence of both additives at the same time (AmB and sterols, see Table 2). Three kind of sterols have been applied in the present study. Cholesterol, present in the cells of mammals, ergosterol, present in the cells of fungi and synthetic cholesterol dimers, that potentially may facilitate formation of double layer channels (DLC) of AmB, across a lipid bilayer, in contrast to a single layer channel (SLC) formed by the drug in a single lipid monolayer. Fig. 4 presents a comparison of the effects of AmB on the rate constants of the fast proton transfer components across the EYPC membranes containing additionally incorporated 3 mol% sterol (1.5 mol% in the case of cholesterol dimer) and 3 mol% AmB. As may be seen, there are no significant differences in the effects of all sterols examined.

The patch-clamp technique has been applied to analyze the effect of AmB on ion transport across the natural

Table 2
Effect of sterols and amphotericin B on the kinetic parameters of proton transport into EYPC liposomes

Sterol	Concentration (mol%)		$A \pm$ S.D.[a]	$\alpha \pm$ S.D. ($\times 10^3\ s^{-1}$)	$B \pm$ S.D.	$\beta \pm$ S.D. ($\times 10^3\ s^{-1}$)	$C \pm$ S.D.	ΔpH $\pm$ S.D.	$\tau \pm$ S.D. (s)
	Sterol	AmB							
Cholesterol	0.1	0	0.44 ± 0.05	26 ± 5	0.35 ± 0.07	3.4 ± 0.9	0.19 ± 0.05	1.05 ± 0.06	70 ± 4
	3	0	0.58 ± 0.06	32 ± 4	0.27 ± 0.08	4.0 ± 0.1	0.09 ± 0.01	0.96 ± 0.07	29 ± 9
	0.1	0.1	0.43 ± 0.06	23 ± 7	0.30 ± 0.07	2.3 ± 0.4	0.20 ± 0.06	1.01 ± 0.18	75 ± 6
	1	1	0.56 ± 0.04	28 ± 5	0.32 ± 0.02	1.8 ± 0.1	0.14 ± 0.03	1.11 ± 0.01	46 ± 5
	3	3	0.44 ± 0.07	50 ± 7	0.14 ± 0.02	3.1 ± 0.7	0.41 ± 0.01	0.72 ± 0.03	19 ± 1
Ergosterol	3	0	0.46 ± 0.03	43 ± 10	0.20 ± 0.02	4.3 ± 0.5	0.30 ± 0.01	0.81 ± 0.02	22 ± 1
	3	3	0.40 ± 0.01	68 ± 6	0.09 ± 0.03	2.7 ± 1.5	0.49 ± 0.01	0.67 ± 0.02	13 ± 2
Cholesterol (dimers)	1.5	0	0.60 ± 0.01	45 ± 7	0.27 ± 0.01	4.0 ± 0.1	0.09 ± 0.03	0.76 ± 0.06	21 ± 1
	1.5	3	0.58 ± 0.08	61 ± 8	0.12 ± 0.03	3.9 ± 0.6	0.30 ± 0.09	0.88 ± 0.20	14 ± 2

Kinetic parameters α and β correspond to Eq. (1). A and B are the pre-exponential factors, I_{max} a steady-state level of the fluorescence intensity ratio of PTS excited at 403 and 455 nm (I_{403}/I_{455}) calculated on the basis of parameter C and ΔpH is a pH change inside liposomes corresponding to the steady-state conditions. τ denotes time corresponding to the I_{403}/I_{455} ratio equal to $I_{max}/2$.

[a] The means and standard deviations were calculated from three to six measurements.

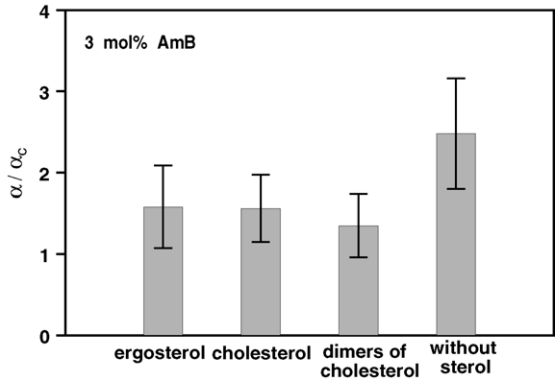

Fig. 4. Effect of AmB on EYPC liposome membranes containing different sterols: cholesterol, ergosterol, and dimer of cholesterol, presented as a ratio α/α_c, where α_c is a rate constant of the fast proton transport phase across lipid membranes with 3 mol% of sterol and α is the rate constant of the fast proton transport phase across lipid membranes with 3 mol% of sterol and 3 mol% of AmB. The last bar corresponds to the sterol concentration 0 mol% and represents the control measurements. In this case the parameter α_c represents the fast component of the proton transport across the pure lipid membranes and α, the membranes containing additionally 3 mol% AmB.

membrane–tonoplast isolated from the liverwort *C. conicum*. Isolated vacuoles were chosen because the tonoplast does not contain ergosterol. Moreover, they are much simpler objects than protoplasts surrounded by the plasmalemma, which allows better comparison of AmB effects with the other model systems. AmB was added to the bathing solutions at the concentrations in the range between 1×10^{-7} and 3.6×10^{-5} M. No significant enhancement in the ion channel activity has been observed in all six experiments (Fig. 5A). Also there has not been any effect of AmB observed when the drug was present in the bathing solution along with ergosterol with molar ratio AmB:ergosterol 1:2 (AmB concentration 1.12×10^{-5} M, two repetitions) (Fig. 5B). Such a result is most probably a demonstration of the fact that AmB at locally low concentrations would rather act in eliminating nonspecific ion transport than forming pore-like aggregated structures.

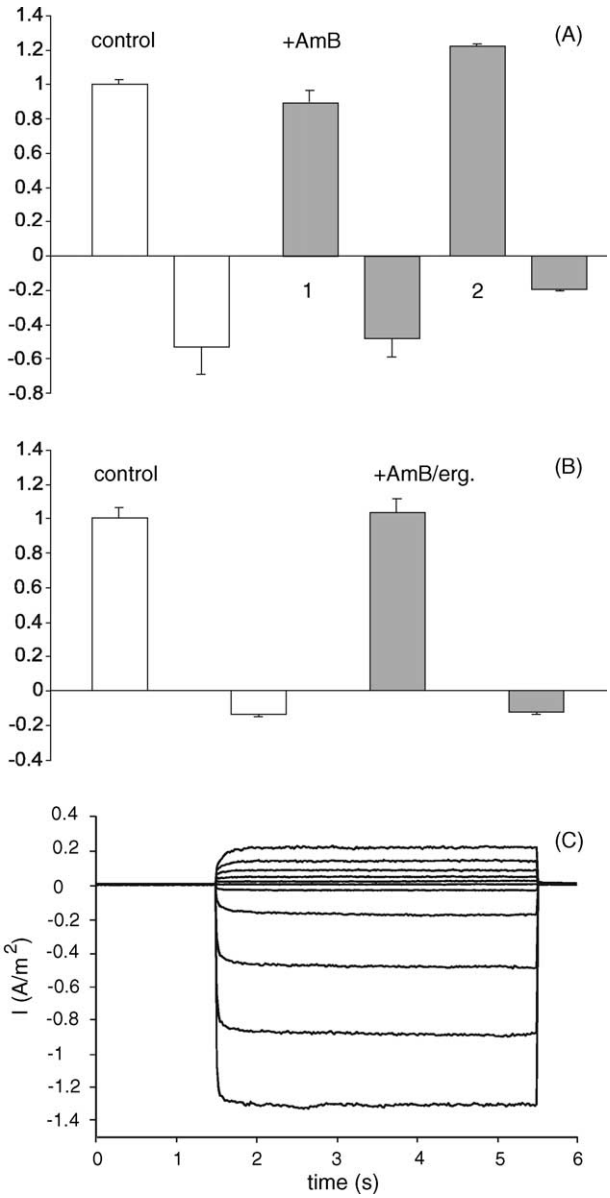

Fig. 5. Ion currents measured in the tonoplast of the liverwort *Conocephalum conicum* by a patch-clamp technique in a whole-vacuole mode. The average currents flowing at +100 and −100 mV command potentials were normalized to unity in control experiments (−100 mV). The patch-pipette contained 100 mM $MgCl_2$, 15 mM Hepes, pH 7.2, The bath solution has the same ion composition as the pipette one in the control and contained additionally (A) 1×10^{-7} M (1) and 3.6×10^{-5} M (2) amphotericin B, and (B) 1.12×10^{-5} M AmB and 2.24×10^{-5} M ergosterol. At negative command potentials anion currents flowing from the vacuole to the cytoplasmic side dominate, whereas at positive voltages vanishing slow vacuolar channels, SV permeable for cations flowing from the cytoplasm to the vacuole lumen are mainly active. (C) Exemplary traces of ion currents flowing through the tonoplast of *C. conicum*. Command voltages were stepped by 20 mV from −100 to +100 mV. The bath solution contained: 100 mM $MgCl_2$, 15 mM Hepes, pH 7.2 and 3.6×10^{-5} M amphotericin B.

4. Discussion

The results of the experiments presented in this work show two different effects of AmB on proton transport across the lipid membranes, in dependence on actual concentration of the drug with respect to lipid. Surprisingly, at low AmB concentration, the rate of the proton transfer was essentially decreased. Interestingly, according to the recent linear dichroism analysis [13,21,26], a certain fraction of AmB incorporated to lipid membranes does not penetrate the lipid phase and remains oriented horizontally with respect to the lipid membrane plane, presumably in the head-group region. It is possible that molecules of AmB present in the interfacial region of the membrane, decrease passive ion flux across the membrane, owing to the formation of network of hydrogen bonds between drug and lipid molecules (see also discussion in [21]). It is very likely that AmB incorporated to the lipid membranes, at relatively low concentrations, is not able to form molecular aggregates that can penetrate a membrane and form porous structures. The increase in AmB concentration causes the

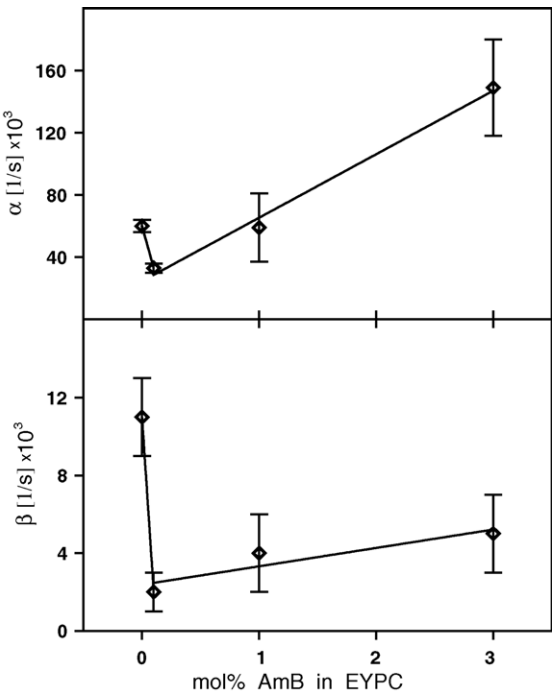

Fig. 6. Rate constants α and β (see Eq. (1) and Table 1) as a function of amphotericin B concentration in liposomes.

increase in the rate of the fast proton transport component (α) but the slow component (β) remains practically unchanged, as can be seen in Fig. 6. Such a result suggests that the slow component represents a nonspecific proton transport across the membranes, that is, preferentially blocked by AmB, even at very low concentration of the drug. In contrast, the fast kinetic component represents a relatively efficient proton transport, most probably owing to a disturbance of the membrane structure by amphiphilic AmB molecules but also possibly through the membrane-spanning pores, formed by AmB. As can be seen from Fig. 6 and Table 1, the steady-state level of pH inside liposomes, following the acidification of the medium depends also on the presence of AmB. The differences are small but reproducibly observed. One possibility is that the presence of AmB influences the activity of valinomycin, the K^+ ion carrier across the membrane applied in the present study, and formation of an electrostatic barrier across the membranes prevents proton diffusion into the liposomes. Another possibility is that AmB, owing to its amphoteric properties [27,28], acts locally as a buffer, at the lipid–water interface, although the pK values of both the carboxyl and the amine groups of AmB are far from the pH values applied in the experiments.

Another striking result is that none of the examined sterols increases the rate of the proton transfer across the membrane. On the contrary, the effect of AmB on ion transport across the sterol-containing membranes is not as much pronounced as in the case of the sterol-free membranes. Such a result strongly suggests that the effect of AmB consists in a disturbance of the structure of the lipid bilayer, expressed in modulation of the ion transfer, and that this effect of the drug is restricted in the membranes modified by the presence of sterol components. An important observation seems to be the lack of differences between the effects of cholesterol, a sterol of mammal cells, and ergosterol, a sterol of the cells of fungi, in the experimental system studied. It is therefore possible, that physiological differences in the pharmacological effect of AmB on cholesterol-containing membranes and on ergosterol-containing membranes are related to the affinity of the drug to the natural membranes (binding and/or incorporation) and not related to different ability of ergosterol and cholesterol to form two-component porous structures with AmB in the lipid membrane environment.

5. Conclusions

The results of the experiments presented in this work demonstrate without doubt that antibiotic amphotericin B interacts with lipid molecules forming the lipid membranes in a fashion that influences the membrane proton transport. The findings do not support the concept on the transmembrane pore formation by the drug molecules. On the contrary, the antibiotic incorporated to the lipid membrane system increased the barrier for transmembrane proton transport: the fast proton transport component was inhibited at low AmB concentrations (below 1 mol%) and the slow proton transport component was found to be inhibited in the entire AmB concentration range studied. Most probably, binding of the amphiphillic AmB molecules at the polar–nonpolar interface of the membrane is stabilized via formation of a hydrogen bound network which results in reinforcement of the membrane structure in the polar headgroup region and decreased membrane permeability to ions. Also, the kinetics of the transmembrane proton transport recorded are not typical of the ion transport via channels. The findings presented in this work suggest that the surface properties of lipid membranes and organization of polar headgroup region of the lipid membranes (also modified by sterols) may play a key role in both the pharmacological and the toxic side effects of amphotericin B.

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