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Effect of the modifications of ionizable groups of amphotericin B on its ability to form complexes with sterols in hydroalcoholic media

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Abstract

The interaction of amphotericin B and some of its semisynthetic derivatives with cholesterol and ergosterol has been tested in 1:4 (v/v) ethanol/water mixture by circular dichroism and absorption spectroscopy. The effect of the chemical modification of the 'ionizable head' of the antibiotic, the pH of the medium, and the sterol/antibiotic ratio has been studied. The results obtained show that in the presence of the sterols, amphotericin B forms several spectroscopically different species. A high extent of polyene–sterol interaction is observed for: (i) amphotericin B in neutral or acidic media, (ii) esters and amides at neutral or alkaline media, (iii) *N*-acyl derivatives only in acidic medium. The extent of interaction at neutral pH is highly correlated with the biological activity of compounds tested. The implication of these findings on the nature of the forces responsible for the antibiotic–sterol interaction is discussed.

Keywords: Antibiotic; Polyene macrolide antibiotic; Sterol; Membrane modeled medium; Amphotericin B–sterol interaction; Cell toxicity correlation

1. Introduction

Amphotericin B (AMB), a polyene macrolide antibiotic, is widely used in the clinical treatment of fungal infections, also in systemic ones. Pharmacological usefulness of the AMB is based on its higher toxicity to ergosterol-containing pathogenic microorganisms than to cholesterol-containing animal host cells [1–3]. It is generally assumed, although no direct experimental evidence is available, that the polyene macrolide antibiotics interact with membrane located sterol, which leads to the formation of multimolecular complex with consequent lethal impairment of membrane functions. For large macrolide ring antibiotics, like amphotericin B, it is postulated that this complex is organized in the form of aqueous channel spanning the membrane [4,5].

Interactions responsible for formation of polyene-sterol binary complex were proposed on the basis of results obtained in studies on the effect of the modification of polyene macrolides on their permeabilizing properties towards sterol-containing phospholipid vesicles [6]. A model of multimolecular, permeabilizing complex proposed by De Kruijff and Demel [5] was studied by molecular mechanics and its structure on molecular level was proposed [7]. A molecular explanation of the differential affinity of polyenes to cholesterol and ergosterol has been also proposed [8]. The usefulness of the theoretical findings for the rational drug design was confirmed by the development of polyene derivatives with improved selective toxicity among which amphotericin B *N′,N′*-dimethylaminopropylamide diaspartate (AMA) is the most promising [9,10].

The progress made in the development of polyene macrolide derivatives with improved pharmacological properties should be further stimulated when better knowledge on the molecular mechanisms of the interaction of these compounds with their membrane targets (sterols) will be available. Most of the current knowledge results from the studies with sterol-containing membranes (see [3]). The interaction of AMB with sterols has been also thoroughly investigated in phospholipid-free media, mimicking the dielectric constant of membranes, that is in water-alcohol mixtures [11–13]. Species formed with AMB and sterols in such homogenous media have spectroscopic properties very similar to those observed after interaction of AMB with sterol-containing vesicles [14]. However, any derivatives of AMB have been studied in such condition and nothing is known about influence of chemical modification on the antibiotic–sterol interaction in membrane-free systems. Therefore, we present here our studies on the effect of the

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modifications of properties of AMB ionizable functional groups (carboxyl and/or amino groups) on the direct affinity to ergosterol as well as cholesterol.

In present studies we used water/ethanol mixture as a medium for studied interactions. In these conditions the extent of interaction depends on the proportion of both solvents. The selected proportion of solvents was 4:1 water/ethanol (v/v). It enabled an optimal interaction between AMB and cholesterol as well as ergosterol. This simple, model system allowed us to examine direct affinity to sterols eliminating the influence of organization of the sterol molecules in the membrane as well as the interactions between antibiotic and constituents of the membranes other than sterol. Such simple medium allowed us also to test the interactions at different pH in order to determine the role of the ionic state of the examined polar groups and of the net charge of the polyene molecule. The results obtained in this polyene-sterol system are confronted with the molecular model of polyene-sterol binary complex [6] previously designed from studies on sterol containing phospholipid vesicles.

The compounds selected for this work comprise AMB derivatives in which carboxyl and/or amino groups have been modified to change the ionization state of the molecule. It has been suggested [6,9] that the proton-donor property of the protonated amino group and the proton-acceptor ability of the carboxyl anion are essential for the interaction of polyenes with sterol through the formation of two hydrogen bonds with sterol OH group.

The system of seven conjugated double-bonds, with its characteristic optical properties, present in the molecule of AMB allows us to use spectroscopic methods for monitoring the changes in the environment of the chromophore resulted from interactions with the sterol. Among those techniques electronic absorption and circular dichroism (CD) were found to be the most useful (see [3]). In electronic absorption spectra the high extinction coefficient of monomeric form of AMB allows to work at concentration as low as 10^{-7} M, i.e., at a concentration within the range of biological activity. CD spectra monitor the interaction of polyene chromophore with other compounds and also can be used for very low concentration of the antibiotic. Several types of species were detected by CD method when AMB was incubated with lipidic vesicles [14–16]. Both these techniques have been used in present study.

The compounds studied exhibit large differences in their biological activity. Correlations between the ability to direct interaction with sterols in our simple, model medium and toxicity against cells have been also analysed.

2. Materials and methods

Amphotericin B was from Squibb and Sons (Princetown, NJ, USA) and was used without further purification. All AMB derivatives used were obtained in our Laboratory [9]. The compounds to be tested were dissolved daily in dimethyl sulfoxide at a concentration of 10^{-3} M. Cholesterol and ergosterol were from Fluka and Merck, respectively, and both were purified by recrystallization twice from ethanol. Sterol stock solutions were prepared in $CHCl_3$ at a concentration of 10^{-2} M and stored during a few days in refrigerator. Sterol working solutions were prepared daily by mixing 1 part of stock solution with 9 parts of ethanol. Desired amount of antibiotic and sterol solutions were added to desired volume of ethanol and next mixed with four volumes of bidistilled water or buffer.

Spectroscopic measurements were done 1 h after mixing. After this period no changes of spectra were observed. In all experiments the concentration of AMB and its derivatives was the same and equal to $2 \cdot 10^{-6}$ M. Absorption spectra were recorded with Cary 219 (Varian) spectrophotometer and circular dichroism spectra with a Jobin-Yvon dichrograph Mark IV.

Buffers were obtained from 10 mM solutions of acetic acid (pH 3), Tris-HCl (pH 6), and triethanolamine (pH 10) adjusted by 0.1 M HCl to the desired pH.

3. Results

3.1. Interaction of AMB with sterols in water-ethanol mixture

In 1:4 (v/v) ethanol/water mixture the absorption spectrum of AMB at concentration $2.0 \cdot 10^{-6}$ M is typical for monomeric form of the antibiotic, i.e., four bands with decreasing intensity are observed at 409, 385, 365, and 347 nm. With increasing sterol/antibiotic ratio, R, the spectra are progressively modified: the bands are red shifted and proportions of bands intensities are changed (Fig. 1). At high sterol/antibiotic ratio ($R \geq 10$) the position of the bands are stabilized at 414, 388, 366, and 352 nm for cholesterol and at 416, 390, 368, and 354 nm for ergosterol. The fact that isosbestic points are not observed (Fig. 1) suggests that during AMB-sterol interaction at least two types of complexes, characterized by different absorption spectra, are formed. Because at 409 nm monomer exhibits very high ϵ value ($\epsilon = 1.7 \cdot 10^5$ M^{-1} cm^{-1}) but complexes exhibit low ϵ value ($\epsilon \approx 2 \cdot 10^4$ M^{-1} cm^{-1}), it is possible to estimate the percentage of free antibiotic with acceptable level of error ($\pm 5\%$). The results of such determination for increasing amount of cholesterol as well as of ergosterol are shown on Fig. 2. It is interesting to note that in this condition AMB has the same affinity to both sterols tested.

The monomeric form of AMB exhibits circular dichroism spectrum with four positive bands at 409 nm ($\Delta\epsilon =$ 12), 385 nm ($\Delta\epsilon = 10$), 365 nm ($\Delta\epsilon = 9$), and 347 nm ($\Delta\epsilon = 6$ M^{-1} cm^{-1}). When AMB is mixed with an increasing amount of sterol, in ethanol-water mixture, the

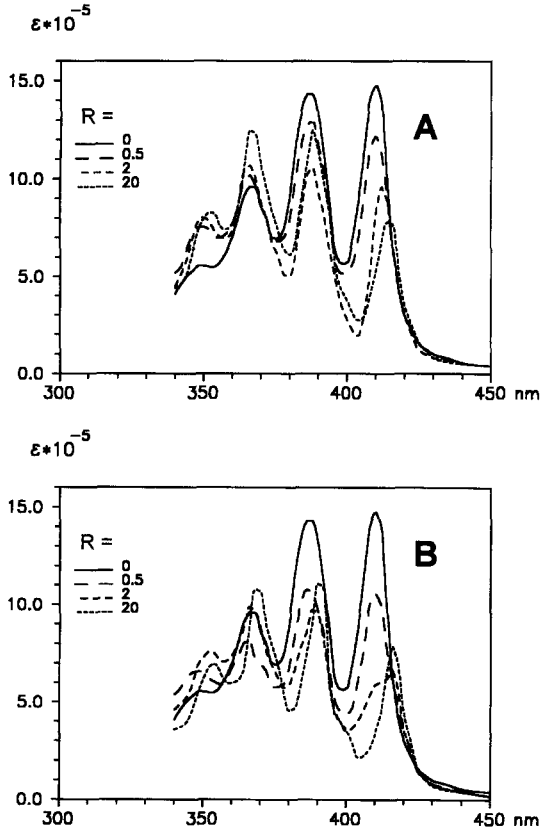


Fig. 1. Absorption spectra at pH 6 of $2 \cdot 10^{-6}$ M AMB in the presence of sterol in 1:4 ethanol/buffer mixture: (A) with cholesterol, (B) with ergosterol.

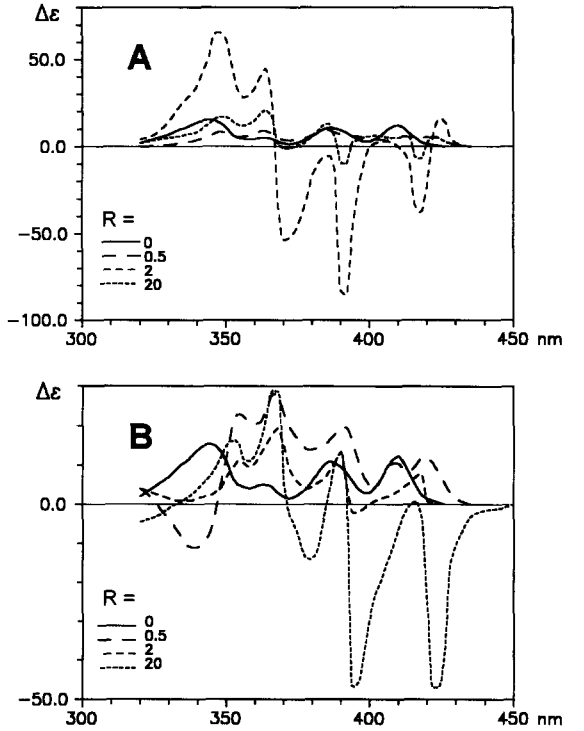


Fig. 3. Circular dichroism spectra at pH 6 of $2 \cdot 10^{-6}$ M AMB in the presence of sterol in 1:4 ethanol/buffer mixture: (A) with cholesterol, (B) with ergosterol.

circular dichroism spectrum is modified but in different manner for each sterols tested.

For cholesterol (Fig. 3A), three positive bands at 423, 364, and 348 nm as well as three negative bands at 418, 391, and 372 nm appear for R between 1 and 2 and then decrease.

For ergosterol (Fig. 3B), for R up to 1 spectra with four positive bands at 418, 390, 368, and 355 nm as well as a negative band at 340 nm are observed. When R increases new spectrum progressively appears with two positive bands at 368 and 353 nm as well as with three strong and sharp negative bands at 423, 395, and 379 nm.

Thus, evolution of circular dichroism spectra of AMB-sterol mixtures exhibits formation of several complexes. The circular dichroism spectra and probably the structures of these complexes depend on the sterol used and its proportion to the antibiotic.

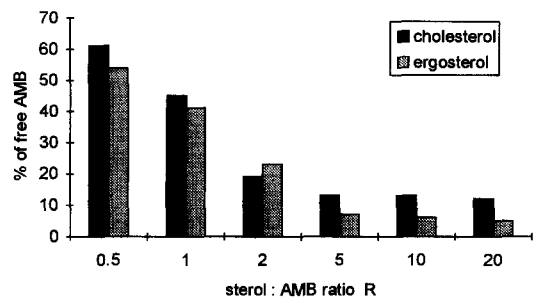


Fig. 2. The relationship between the amount of free AMB and sterol/antibiotic ratio, R, at pH 6.

3.2. *Influence of ionic state of the AMB on its interaction with sterols*

It was shown that ionic state of the 'ionizable head' of the AMB molecule, including carboxyl group at C-16 and amino group in amino sugar moiety, plays an important role in biological activity of this antibiotic [9]. We chose three values of pH for which the ionic states of these groups are different [17,18]. At pH 3 the dissociation of the carboxyl group is strongly inhibited and the molecule has a positive net charge. At pH 10 the amino group is not protonated and the molecule has a negative net charge. At pH 6 both groups are charged and the molecule has no net charge. The absorption as well as circular dichroism spectra of AMB at concentration $2.0 \cdot 10^{-6}$ M in 1:4 (v/v) mixtures of ethanol and 10 mM buffers of chosen pH are the same and no changes were observed during 2 h of incubation. The biological activity after such incubation is also practically the same (data not shown).

The sterol/AMB ratio $R = 5$ was used for testing the influence of pH on the interactions. For this ratio of cholesterol as well as of ergosterol about 90% of the antibiotic is bound and exhibits strong, characteristic spectra.

At pH 3 in the presence of sterols the extent of complexation was near the same as at neutral pH (80% for cholesterol and 97% for ergosterol). But the circular dichroism spectra depended on the sterol used. In the case of cholesterol the pattern of the spectra was similar to that at neutral pH. However, in the case of ergosterol the intensity as well as the shape of the spectrum are different: four positive bands at 417, 390, 368, and 352 nm with $\Delta\epsilon$ about 10 M^{-1} cm^{-1} as well as two negative bands at 422 and 396 nm with about -7 M^{-1} cm^{-1} are observed.

At pH 10, at which the amino group is no longer protonated, the extent of complexation was significantly lower than at neutral pH. In the case of cholesterol only about 10% of antibiotic was bound and circular dichroism spectrum was very similar to that of free AMB. In the presence of ergosterol about 80% of antibiotic was still bound. The circular dichroism spectrum was similar to that obtained at neutral pH for the same R but less intensive.

3.3. Influence of the chemical modification of the AMB ionizable polar groups in sterol–antibiotic interactions

The results obtained with native AMB at different pH were compared with those of AMB derivatives with substituted carboxyl and/or amino group (Table 1). For these compounds absorption and circular dichroism spectra were also recorded at the same conditions as for AMB.

In the 1:4 ethanol/water mixture without sterol all derivatives tested exhibited absorption spectra identical with parent antibiotic. These spectra are pH independent, similarly to the absorption spectrum of AMB. In the presence of sterol at $R = 5$ significant changes in the spectra were observed. The extent of the changes depended on the type of derivatives and pH, but the shapes of the spectra were very similar to those observed for AMB with adequate sterol. The degree of complexation calculated from absorption spectra are summarized on Fig. 4. One

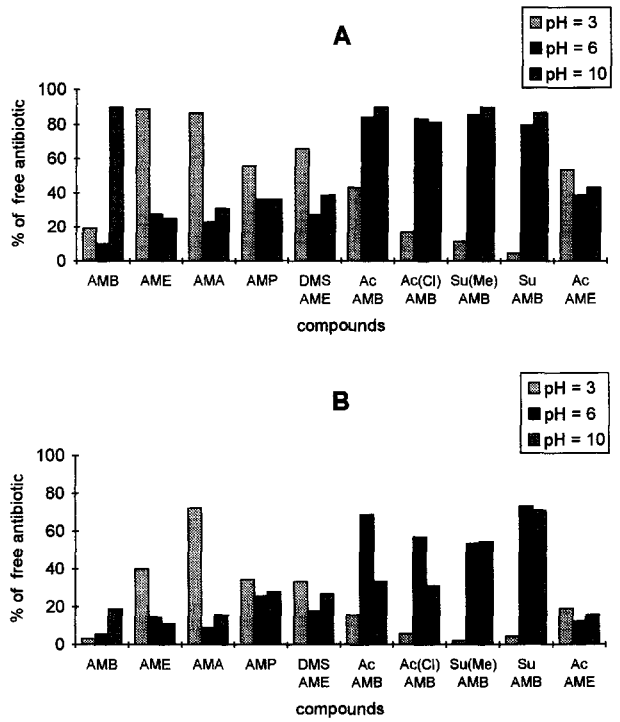
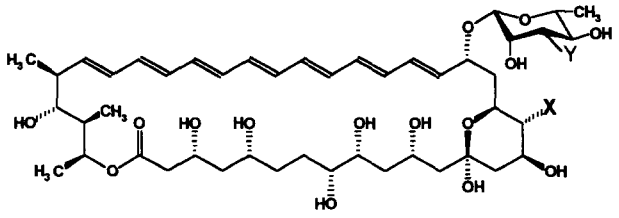


Fig. 4. The effect of pH and chemical modification on the extent of interaction for AMB at $2 \cdot 10^{-6}$ M with sterol/antibiotic ratio $R = 5$: (A) with cholesterol, (B) with ergosterol.

Table 1
Structure of compounds studied



No.	Abbreviation	X	Y
1	AMB	$-COO^-$	$-N^+H_3$
2	AME	$-COOCH_3$	$-N^+H_3$
3	AMA	$-CONH(CH_2)_3N^+H(CH_3)2$	$-N^+H_3$
4	AMP	$-CONH(CH_2)_2CH_3$	$-N^+H_3$
5	DMSAME	$-COOCH_3$	$-N^+(CH_3)_3$
6	AcAMB	$-COO^-$	$-NHCOCH_3$
7	Ac(Cl)AMB	$-COO^-$	$-NHCOCH_2Cl$
8	SuAMB	$-COO^-$	$-NHCO(CH_2)_2COO^-$
9	Su(Me)AMB	$-COO^-$	$-NHCO(CH_2)_2COOCH_3$
10	AcAME	$-COOCH_3$	$-NHCOCH_3$

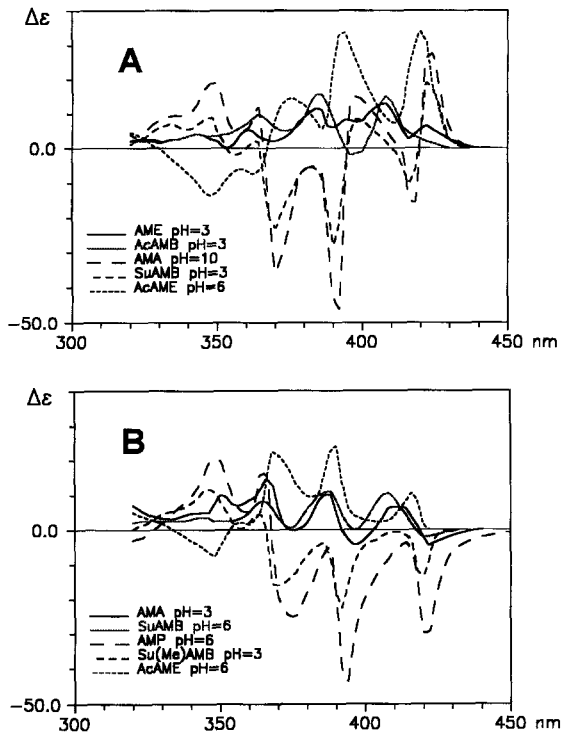


Fig. 5. Exemplary circular dichroism spectra of AMB derivatives at different pH in the presence of sterol: (A) cholesterol, (B) ergosterol.

can note that there do not exist simple relationships between the ionic state of nitrogen atom and ability of complexation with sterols. Moreover, each of the sterol tested has its own pattern of sensitivity on pH and polyene structural changes. However, it is possible to note some general rules:

(i) The interaction with ergosterol is less sensitive to the changes of ionic state of molecule than with cholesterol.
(ii) The compounds with substituted carboxyl group (AME, AMA, AMP) interact easily with both sterols in neutral and alkaline medium, but not in acidic, particularly with cholesterol.
(iii) The compounds in which the amino group of amino sugar was transformed to amide (AcAMB, Ac(Cl)AMB, SuAMB, Su(Me)AMB) interact easily with both sterols only in acidic medium.

The circular dichroism spectra of derivatives studied without sterol are identical with that of AMB and are also pH independent. In the presence of sterol at $R = 5$ a large diversity of shapes as well as of bands intensities is observed.

For cholesterol (Fig. 5A) in the cases when absorption spectra indicated small interaction (AME, AMA, AMP, and DMSAME at pH 3 as well as AcAMB, Ac(Cl)AMB, Su(Me)AMB, and SuAMB at pH 6 and pH 10) the circular dichroism spectra were very similar to the spectrum of the monomeric form of AMB. In the other cases the circular dichroism spectra were similar to those observed for AMB at neutral pH. But the intensities of the bands varied from spectrum to spectrum. Only for AcAME completely different spectra were observed: at pH 3 and pH 6 three positive bands at 422, 395, and 375 nm as well as two negative bands at 364, and 348 nm, but at pH 10 four positive bands at 422, 397, 364, and 350 nm as well as three negative bands at 418, 391, and 370 nm.

For ergosterol (Fig. 5B) spectra similar to the spectrum of free AMB were observed for AMA at pH 3 and for SuAMB at pH 6 and pH 10. For AME, DMSAME, and AcAME spectra characterized by four positive bands at 418, 391, 370, and 357 nm as well as two negative bands at 348 and 335 nm were observed at all the pH tested. Only the intensities of the bands varied. For other cases the spectra look like a superposition of the spectrum of free AMB and the spectrum typical for AMB-ergosterol complex.

3.4. Correlation between biological activity and antibiotic–sterol interaction

As a measure of biological activity we used the concentration of compounds causing 50% hemolysis of human red blood cells (HC_{50}) as well as causing 50% growth inhibition of yeast *Saccharomyces cerevisiae* (IC_{50}). Data, expressed as negative logarithms, are from [9].

As a measure of antibiotic-sterol affinity we used the ratio, p, of antibiotic bound to sterol at ethanol/water mixture for sterol/antibiotic ratio equal to 5. These values are expressed as $\text{logit} = \log(p/(1-p))$, and named CA, and EA, for cholesterol and ergosterol, respectively.

The AcAME was not taken into consideration in this analysis, because its circular dichroism spectra indicated that complexes formed at neutral pH have probably a totally different structure.

The values for which correlation was determined are shown in Table 2. The calculated correlation coefficients between biological and physicochemical tests for the same

Table 2
Biological activity and sterol affinity of AMB and its derivatives

No.	Compound	Cholesterol		Ergosterol	
		pHC_{50}	CA	pIC_{50}	EA
1	AMB	−0.23	0.95	1.30	1.30
2	AME	−0.68	0.43	1.15	0.75
3	AMA	−0.81	0.55	1.10	1.00
4	AMP	−0.72	0.23	1.05	0.45
5	DMSAME	−0.90	0.43	1.10	0.66
6	AcAMB	−1.40	−0.72	0.46	−0.35
7	Ac(Cl)AMB	−1.51	−0.69	0.51	−0.10
8	SuAMB	−1.43	−0.58	0.40	−0.41
9	Su(OMe)AMB	−1.57	−0.75	0.43	−0.05
10	AcAME[a]	−2.06	0.19	0.26	0.87
	r [b]	0.967		0.957	

[a] This compound was not used in calculation of correlation coefficients (see text).
[b] Correlation coefficient.

sterol are also shown in this table. The coefficients are equal to 0.967 for pHC_{50} and CA, and 0.957 for pIC_{50} and EA. The high values of these coefficients indicate that the direct polyene–sterol interaction is essential for the biological activity of the polyene antibiotics.

4. Discussion

The changes of circular dichroism and absorption spectra of AMB in the presence of sterols in 1:4 (v/v) ethanol/water mixture for increasing sterol/antibiotic ratios, R, indicate (Figs. 1 and 3) that several different spectral species are formed.

The evolution of the AMB circular dichroism spectra in the presence of cholesterol suggests the formation of at least three species. The first one is observed for R between 0 and 1. The second one is detected for R between 1 and 20 and reaches maximal concentration for R about 2. The existence of the third one is only presumed for explaining the decrease of the intensity of the spectrum at high R.

In the presence of ergosterol the formation of at least two species can be observed. The first one is observed for R lower than 2. The second one is observed, with increasing intensity, for R higher than 2.

Spectral characteristics of these species are very similar to those observed in the presence of sterol-containing phospholipid vesicles [14–16,19], human plasma lipoproteins [20], and ghosts or vesicles of human red blood cells [19]. Earlier study of AMB–sterol interaction in 25% n-propanol [12,13] also showed high similarity between species formed in the presence of phospholipid vesicles and in aqueous solution in the presence of sterol.

The extent of interaction, as a function of pH, for AMB and its derivatives with cholesterol is summarized in Table 3, where AME and AcAMB are considered as representatives of derivatives with neutralized carboxyl and amino groups, respectively.

Herve et al. [6] have supposed that the formation of primary complex is the most important step in AMB mode of action. The primary complexes spontaneously aggregated to the channels. In this model the interaction between the amino group of AMB and 3-β-OH group of the sterol plays the crucial role. The interaction between carboxyl group (as well as ester or amide group) of AMB and hydroxyl group of sterol plays minor role only. Totally different point of view has been presented by Khutorsky [7]. On the basis of his theoretical calculations he has supposed that channels are formed mainly due to electrostatic interaction between positively charged amino group of one AMB molecule and carboxyl group of neighbouring AMB. Van der Waals and/or π–π electron interactions between AMB and sterol molecules play also important role in channel formation. Both these models have some weaknesses. In Khutorsky's model the role of sterol hydroxyl group is underestimated. Well known fact [5] that only sterols with hydroxyl group at position 3 and in β configuration can interact with AMB to increase membrane permeability suggests high degree of stereospecificity of the interaction. In primary complex model [6] the hydrogen bond between amino group and sterol hydroxyl group plays the important role of 'orienting force' for sterol molecule. On the other hand it is known, that derivatives with quaternalized nitrogen atom [9] which can not form hydrogen bond, but can interacts by electrostatic force are also biologically active. Also derivatives in which the protonable amino group is shifted by a few bonds (aminoacyl derivatives [21]) express full biological activity. The results obtained at pH 6, the significance of which was confirmed by their high correlation with biological activity, allow us to merge the molecular model of primary complex with Khutorsky's model of the channel. We postulate that for the stability of the permeabilizing complex three different kinds of forces are responsible:

(i) 'Binding forces' between hydrophobic parts of the antibiotic and sterol molecules (Van der Waals and π–π interactions).
(ii) 'Stabilizing forces' between positively charged amino group of one AMB molecule and free or substituted carboxyl group of neighbouring one. The nature of that forces is electrostatic: charge-charge for free, dissociated carboxyl group or charge-dipole for undissociated or substituted group.
(iii) 'Orienting forces' between sterol 3-β-OH group and unidentified place in antibiotic molecule. The most probably, highly stereospecific hydrogen bond is responsible for this interaction.

The decrease of affinity to sterol observed for AcAMB and other N-acyl derivatives is a result of the lack of protonated amino group. The same explains the decrease of AMB affinity to cholesterol at high pH. For AME and AcAMB at low and high pH some additional assumptions are necessary. For AME (and amides) at low pH at least partial protonation of carbonyl oxygen of esters and amides can be postulated. Such protonation is less probable in the case of undissociated carboxyl group. After protonation the electrostatic interactions vanish. For AcAMB at high pH we assume existence of electrostatic repulsive force between negatively charged carboxyl group and partial negative charge of free amino group. Such repulsive interaction is absent in the case of esters and amides.

As for the results concerning the interaction with ergos-

Table 3
The extent of interaction of AMB and its selected derivatives with cholesterol at different pH

Compound	% of bound antibiotic		
	pH 3	pH 6	pH 10
AMB	81	90	11
AME	13	73	75
AcAMB	57	16	11

terol (Fig. 4B), they indicate that the ionic state of ionizable groups is not so important as in the case of cholesterol and that some other forces play more important role. These forces in part counteract the repulsive forces, but do not help in ergosterol–polyene interaction when repulsive forces are not present. Comparison of the structure of both sterols may suggests two reasons for which AMB interacts more tightly with ergosterol than with cholesterol. Firstly, the additional double bounds present in the side chain and in ring B of ergosterol may interact with the system of conjugated double bonds present in the antibiotic molecule. Secondly, it has been demonstrated [8] that in minimum energy conformation a side chain of the ergosterol molecule is co-planar with a mean plane of its ring system while a side chain of cholesterol is out-of-plane. Thus, the Van der Waals interaction between the side chain of the ergosterol and lipophilic part of the antibiotic molecule could be more effective. This hypothesis can also explain the differences in relative affinity of AMB to both sterols observed at hydroalcoholic media of various concentration of aliphatic alcohols [11,12,22].

Up to now it is not known what type of species, primary complex or multimolecular channel, is responsible for the CD and UV spectra observed when AMB interacts with sterol. On the basis of the results presented in this paper the problem could not be solved. But high correlation (Table 2) observed between extent of antibiotic–sterol interaction in the proposed model experimental condition at pH 6 and biological activity shows that the critical step of interaction has been detected and that the direct interaction occurring in our model system is also important at the level of whole cells. It would suggest also that the interaction with other constituents of the biological membranes, like proteins, lipoproteins or lipids, observed in other models [3], does not determine the biological activity of AMB and its derivatives.

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References

[1] Medoff, G., Brajtburg, J., Kobayashi, G.S. and Bolard, J. (1983) Annu. Rev. Pharmacol. Toxicol. 23, 303–311.
[2] Thomas, A.H. (1986) J. Antimicrob. Chemother. 17, 269–279.
[3] Bolard, J. (1986) Biochim. Biophys. Acta 864, 257–304.
[4] Finkelstein, A. and Holz, R.B. (1973) in Membranes, Vol. 2 (Eisenman, G., ed.), pp. 377–408, Marcel Dekker, New York.
[5] De Kruijff, B. and Demel, R.A. (1974) Biochim. Biophys. Acta 339, 57–70.
[6] Herve, M., Debouzy, J.C., Borowski, E., Cybulska, B. and Gary-Bobo, C.M. (1989) Biochim. Biophys. Acta 980, 261–272.
[7] Khutorsky, V.E. (1992) Biochim. Biophys. Acta 1108, 123–127.
[8] Bagiński, M., Tempczyk, A. and Borowski, E. (1989) Eur. Biophys. J. 17, 159–166.
[9] Cheron, M., Cybulska, B., Mazerski, J., Grzybowska, J., Czerwiński, A. and Borowski, E. (1988) Biochem. Pharmacol. 37, 827–836.
[10] Zaremba, M., Borowski, J., Zaremba, K., Borowski, E. and Grzybowska, J. (1988) Proceedings of the 8th International Symposium on Future Trends in Chemoterapy, Tirrenia, 28–30 March 1988, p. 54.
[11] Gruda, I., Nadeau, P., Brajtburg, J. and Medoff, G. (1980) Biochim. Biophys. Acta 602, 260–268.
[12] Gruda, I. and Bolard, J. (1987) Can. J. Biochem. Cell. Biol. 65, 234–239.
[13] Gruda, I. and Dussault, N. (1988) Can. J. Biochem. Cell. Biol. 66, 127–134.
[14] Vertut-Croquin, A., Bolard, J., Chabbert, M. and Gary-Bobo, C.M. (1983) Biochemistry 22, 2939–2944.
[15] Bolard, J., Seigneuret, M. and Boudet, G. (1980) Biochim. Biophys. Acta 599, 280–293.
[16] Bolard, J. and Cheron, M. (1982) Can. J. Biochem. 60, 782–789.
[17] Travkin, O.V., Rodichev, A.G. and Komarov, E.V. (1981) Antibiotiki 1981, 427–431.
[18] Mazerski, J., Grzybowska, J. and Borowski, E. (1990) Eur. Biophys. J. 18, 159–164.
[19] Szponarski, W. and Bolard, J. (1987) Biochim. Biophys. Acta 897, 229–237.
[20] Brajtburg, J., Elberg, S., Bolard, J., Kobayashi, G.S., Levy, R.A., Ostlund, R.E., Schlessinger, D. and Medoff, G. (1984) J. Infect. Dis. 149, 986–997.
[21] Kim Wright, J.J., Albarella, J.A., Krepski, L.R. and Loebenberg, D. (1982) J. Antibiot. 35, 911–914.
[22] Readio, J.D. and Bittman, R. (1982) Biochim. Biophys. Acta 685, 219–224.