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The Influence of Electric Charge of Aromatic Heptaene Macrolide Antibiotics on Their Activity on Biological and Lipidic Model Membranes

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SUMMARY

Natural aromatic heptaene macrolide antibiotics and their *N*-acyl and methyl ester derivatives, which differ mainly in their electric net charge, were compared for their efficiency in inducing yeast growth inhibition, red blood cell lysis, and increase in the ionic permeability of large unilamellar lipidic vesicles. Antifungal activity was found to decrease in the following order: neutral ≃ positively charged > negatively charged compounds. Hemolytic activity decreased in the order: neutral > negatively charged ≫ positively charged compounds. On lipidic model membranes, themselves either positively or negatively charged, electrostatic interaction was shown to have practically no influence on the efficiency of the differently charged antibiotics. On both biological and model systems, positively charged antibiotics consistently were found to be more active on ergosterol-containing than on cholesterol-containing membranes, and were therefore considered as potentially good candidates for specific antifungal agents.

INTRODUCTION

Aromatic heptaene macrolide antibiotics appear to be promising as antifungal agents. These antibiotics reversibly induce permeability only to monovalent cations in both microorganisms and animal cells (1–4). They are active on yeast at concentrations two to three orders of magnitude lower than active concentrations of nonaromatic heptaenes and members of other groups of polyene macrolides, such as amphotericin B and nystatin, most commonly used in therapy (5–8). Some aromatic heptaenes have a rather low hemolytic activity, which may be taken as an indication of low toxicity for animal cells (9).

This paper presents the results of a systematic comparison of the biological activity of native and modified aromatic heptaenes, tested on yeasts as a model of pathogenic fungal cells and on red blood cells as a model of animal host cells. Most of the known aromatic heptaenes have been included in this comparative study. The activity tests utilized were, for yeasts, the MIC,[3] and the H_{50} for hemolysis. As already observed for nonaromatic heptaene amphotericin B, whose amides (10) and esters (11) exhibit diminished hemolytic activity, it appears from this study of aromatic heptaenes that the absence of a carboxyl group, or its esterification, results in very low hemolytic activity and unchanged very high activity on yeasts. This observation led to the hypothesis that selective toxicity might be influenced by the electric charge of the antibiotic.

To check this hypothesis, experiments were carried out on LUVs. On this system, we studied the effect on sensitivity to polyenes of the membrane's electrical charge and the nature of its sterol, using the test of induced cationic permeability. It appears that within the limits in which comparison of the results for biological and model membranes is possible, the data obtained in the present model system account for the biological findings and seem to confirm that the electrical charge of aromatic polyenes is important for their preferential interaction with membranes containing ergosterol.

MATERIALS AND METHODS

Antibiotics and derivatives. Vacidin A and gedamycin were isolated from aureofacin (12), and candicin, mycoheptin, trichomycin, and can-

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[3] The abbreviations used are: MIC, minimal inhibitory concentration for yeast growth; H_{50}, concentration corresponding to 50% hemolysis; P_{50}, concentration for 50% vesicular proton release; LUV, large unilamellar lipidic vesicle; VME, vacidin methyl ester; GME, gedamycin methyl ester; NAV, *N*-*N*′-diacetyl vacidin; NAG, *N*′-acetyl gedamycin; NAP, *N*′-acetyl perimycin. NSP, *N*′-succinyl perimycin; FCCP, carbonylcyanid-*α*-trifluoromethoxyphenylhydrazone.

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dicidin D were isolated from their respective crude products (13). Countercurrent distribution in the chloroform/methanol/borate (2::3:1) buffer (pH 8.3) system was used in all cases. Perimycin A was isolated from its crude product according to the method of Kolodziejczyk *et al.* (14). VME and GME were prepared following the method of Bruzzeze *et al.* (15), slightly modified. NAV, NAG, NAP, and NSP were prepared according to the method of Schaffner and Borowski (16).

Aureofacin, crude candicin, and candicidin were from Pharmaceutical Work Polfa (Tarchomin, Poland); crude mycoheptin was from the Leningrad Institute of Antibiotics (Union of Soviet Socialist Republics); crude trichomycin from Fujizawa Pharmaceutical Company (Japan); and crude perimycin from Lundbeck (Copenhagen, Denmark). Amphotericin B and nystatin were from E. R. Squibb and Sons (Princeton, N. J.) and were used without further purification.

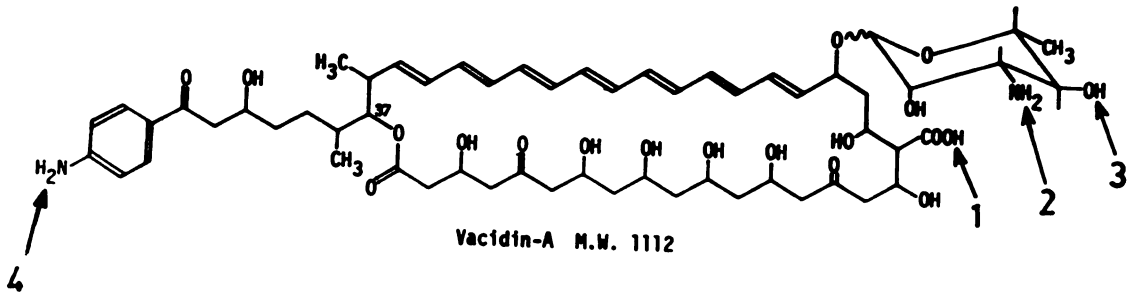
Molecular structures are fully elucidated only for vacidin A gedamycin, and perimycin A and their respective derivatives (VME, GME, NAV, NAG, NAP, NSP). All of these products can be considered natural or semisynthetic derivatives of the basic aromatic heptaene macrolide vacidin A (12, 13). Their molecular structures are shown in Fig. 1. The three natural antibiotics, vacidin A, gedamycin, and perimycin A, are characterized by the same planar structure. They have the same conformation as the heptaene chromophore, the same arrangement of keto and hydroxyl groups in the hydrophilic part of the macrolide ring, and an identical aliphatic chain connecting the aromatic residue to the ring. They differ by their polar substituents: the aromatic amino group is free in vacidin A, but is methylated in gedamycin and perimycin A, and the position of the amino group in the amino sugar is different (12–14). More important is the fact that perimycin A, unlike the two others, has a methyl group instead of a carboxyl group in position 18 (14). Therefore, at pH 7, vacidin A, gedamycin, and NAP can be considered as zwitterionic or neutral, in contrast to perimycin A, which bears a positive charge. Two other positively charged antibiotics, VME and GME, were obtained by esterification of the carboxyl group. On the other hand, the negatively charged derivatives, NAG and NSP, were obtained by acylation of the amino group of the amino sugar, and NAV was obtained by acylation of both aromatic amino and amino sugar groups. These structural differences between the antibiotics are tabulated in the *insert* of Fig. 1.

All of these compounds were used as freshly made solutions in dimethylformamide (1–5 mg/ml). These solutions can be kept at 0° in the dark for 1–3 days without any decrease in their activity or significant modification of their absorption spectrum.

Determination of biological activity. Hemolysis experiments were carried out on human erythrocytes obtained from citrated blood. Erythrocytes were separated from plasma and buffy coat by centrifugation (2000 × *g* for 15 min) and washed three times in isotonic choline chloride. Packed cells were then resuspended in 10 volumes of the same medium to form the stock red blood cell suspension. One volume of stock suspension was diluted in 25 volumes of 310 mM potassium chloride buffered at pH 7.4; the final cell concentration was about 2.10^7 cells/ml. A portion (2.5 ml) of this diluted suspension was incubated at 37° in a shaking water bath, and increasing amounts of the compounds tested were added. After 1 hr at 37°, the suspension was centrifuged and the supernatant absorbance was determined at 540 nm in a spectral colorimeter (Carl Zeiss, Iena). Complete lysis corresponds to an absorbance of 0.7. The concentration which gave 50% hemolysis (H_{50}) was graphically obtained from the dose-response curves. Three separate dose-response curves were established for each compound. The standard deviation was ±5%.

The antifungal activity was measured as the MIC on *Saccharomyces cerevisiae* (ATCC 9763). The MIC was determined by the serial-dilution method in liquid medium [1% bactopeptone (Difco) and 2% glucose in 0.5% NaCl] inoculated to an optical density of 0.02 at 660 nm (2.2×10^5 cells/ml). The extent of growth was monitored turbidimetrically with a photoelectric colorimeter (Carl Zeiss, Iena) at 660 nm after 24 hr of incubation at 28°.

Determination of lipidic vesicle permeabilization. L-α-Phosphatidylcholine was prepared from egg yolk according to the method of



Name of compounds	Subsistuent in position			
	1	2	3	4
Vacidin A	COOH	NH_2	OH	H
Gedamycin	COOH	NH_2	OH	CH_3
Perimycin A	CH_3	OH	NH_2	CH_3
Vacidin methyl ester (VME)	$COOCH_3$	NH_2	OH	H
Gedamycin methyl ester (GME)	$COOCH_3$	NH_2	OH	CH_3
N-N'diacetyl vacidin (NAV)	COOH	$NHCOCH_3$	OH	$COCH_3$
N'acetyl gedamycin (NAG)	COOH	$NHCOCH_3$	OH	CH_3
N'-acetyl perimycin (NAP)	CH_3	OH	$NHCOCH_3$	CH_3
N'succinyl perimycin (NSP)	CH_3	OH	$NHCO(CH_2)_2COO^-$	CH_3

FIG. 1. *Structure of vacidin A and its analogues and derivatives*

Patel and Sparrow (17); phosphatidic acid was enzymatically prepared from it. Stearylamine was from Sigma Chemical Company (St. Louis, Mo.). Cholesterol was from Fluka, and ergosterol from Sigma Chemical Company; both were purified by two crystallizations in ethanol. FCCP was from Boehringer.

Preparation of vesicles. LUVs were prepared according to the method of Szoka and Papahadjopoulos (18), using 40 μmoles of lipid mixture per milliliter of a 100 mM sodium phosphate/100 mM sodium sulfate solution (pH 5.50). The lipidic composition of negatively charged LUVs was L-α-phosphatidylcholine/phosphatidic acid/sterol (either cholesterol or ergosterol) in a molecular ratio of 80:10:10. After vesicle formation by reverse-phase evaporation, the vesicle suspension was filtered through polycarbonate porous membranes (Nucleopore Corporation, Pleasanton, Calif.), first using 1-μm and then 0.4-μm pore size.

For proton efflux determinations, 0.5 ml of filtered vesicles (6 μmoles of lipids) was diluted in 3.5 ml of 194 mM sodium sulfate solution in the titrating vessel of a pH-stat (Radiometer, Copenhagen). The vesicle suspension was then equilibrated at 20° under a nitrogen stream, and the pH was brought to 7.50. Ten microliters of a 1 mM FCCP solution in ethanol were then added. Subsequently, the desired amount of aromatic polyene was added as microliter amounts of its solution in dimethylformamide. The proton efflux was measured as the volume of a 5 mM NaOH solution in 194 mM sodium sulfate necessary to maintain pH 7.50.

RESULTS

Chemical Structure-Biological Activity Relationship

The results of the two biological tests, yeast MIC and red cell H_{50}, are presented in Table 1.

Antifungal activity. Comparison of MIC values indicates that all native aromatic heptaenes, as well as their methyl ester derivatives, are about 100 times more active than nonaromatic heptaenes or nystatin. On the other hand, the *N'*-actyl derivatives of aromatic heptaenes have a lower activity, comparable to that of nonaromatic heptaenes and nystatin.

Closer examination of the structure-activity relationships among the aromatic heptaene group indicates that some structural elements are essential for high activity on yeast and others are not. The latter comprises three elements: (a) the number and arrangement of hydroxyl and carbonyl groups in the hydrophilic part of the ring, since vacidin A, trichomycin B, and candicidin D—which differ in this respect—displayed similar activity; (b) the presence or absence of a methyl group in the aromatic amino group, since gedamycin (methylated) and vacidin A (nonmethylated; see Fig. 1) exhibited practically the same activity; and (c) the presence or absence of a carboxyl group in position C_{18}. The same activity was recorded for vacidin A and gedamycin and their respective methyl ester VME and GME, as well as for perimycin A, in which the carboxyl group is replaced by a methyl group in the native compound.

Essential for high antifungal activity is the presence of the ionizable amino group of the amino sugar. Acylation of this group always decreases this activity drastically, as shown by the comparison of vacidin A, gedamycin, and perimycin A with their respective derivatives (NAV, NAG, NAP, and NSP). On the other hand, the position of the amino group on the sugar does not seem important. The result obtained with perimycin A (amino group in position C'4) is not different from the results obtained with the other natural compounds which have the amino group in position C'3 (Fig. 1).

Hemolytic activity. First, the differences between the H_{50} of aromatic and nonaromatic polyenes were not as large (at most a 25-fold difference) as those observed in MIC. Larger differences in activity (50- to 100-fold) were observed among aromatic heptaenes themselves. Second, the structure-hemolytic activity relationships established for aromatic heptaenes were very different from those for MIC, and were complex: (a) The influence of the hydrophilic part of the ring cannot be disregarded, since at least candicidin D is 10 times less active than vacidin A, gedamycin, and trichomycin B, all of which displayed comparable activity. (b) Methylation of the aromatic amino group had a very slight effect, but it seemed to be magnified upon *N'*-acylation of the amino sugar. (c) *N*-Acylation of the amino sugar had various effects: The H_{50} values of NAV and NAG were much higher than the H_{50} of native polyenes, whereas the reverse was true of perimycin A. (d) The free carboxyl group in position C_{18} was essential for hemolytic activity. This group's esterification (VME, GME) or absence of esterification (perimycin A) resulted in very low hemolytic activity.

It appears that both the hemolytic and antifungal activities of aromatic heptaenes mainly depend upon the structure of their polar head groups (carboxyl in position C_{18} and amino sugar). On the basis of the state of ionization of these groups at physiological pH, aromatic heptaenes and their derivatives can be divided into three classes: Class a, electrically neutral, zwitterionic polyenes with free carboxyl and amino-groups (i.e., all natural polyenes except perimycin A); Class b, negatively charged polyenes, with a free carboxyl and substituted amino groups (i.e., NAV, NAG, and NSP); Class c, positively charged polyenes, having either no carboxyl group (perimycin A) or an esterified one (VME and GME). Note that NAP, although not zwitterionic, is electrically neutral and may be included in Class a, because it has an acetylated amino group, but no carboxyl group.

Class a polyenes exhibited the highest activity, both hemolytic and antifungal. All Class b polyenes had much lower antifungal activity than those in Class a, but he-

TABLE 1

Antifungal and nemolytic activities of polyene macrolide antibiotics

Compound	Activity	
	MIC	H_{50}
	μg/ml	μg/ml
Aromatic heptaenes and derivatives		
Vacidin A	0.002	0.2
VME	0.002	20
NAV	0.5	1.2
Gedamycin	0.001	0.4
GME	0.002	8.0
NAG	0.2	8.0
Perimycin A	0.001	20
NAP	0.01	9
NSP	0.1	4
Candicidin D	0.005	2
Trichomycin B	0.005	0.3
Nonaromatic heptaenes		
Amphotericin B	0.1	1.6
Mycoheptin	0.1	5
Candidin	0.2	1.6
Nystatin	0.25	15

molytic activity was affected by their negative charge in various ways. Class c polyenes displayed antifungal activity similar to that of Class a polyenes, and very low hemolytic activity.

On the basis of this classification, positively charged aromatic heptaenes were expected to be the most promising class from the point of view of selective toxicity. The selective antifungal toxicity of polyenes is ascribed to their ability to form complexes preferentially with ergosterol. This selective antifungal toxicity of polyenes is ascribed to their ability to form complexes preferentially with ergosterol. This selective toxicity is very often evaluated on the basis of the difference between MIC and H_{50}. However, the validity of conclusions drawn from comparing the results of two very different biological tests is questionable.

The high toxicity of positively charged aromatic polyenes observed here for yeast cells as compared with that of red cells might be ascribed to the effects of electrostatic interaction with the differently charged membranes of yeast and red cells. More interesting is the possibility that the electric charge of the polyene directly affects their ability to interact preferentially with a given sterol. To test these hypotheses, experiments were carried out on a model membrane, which enables comparative study of the effects of membrane charge and sterol.

Structure-Activity Relationship in Lipidic Vesicles

The permeability induced by aromatic heptaene macrolides and their derivatives in LUVs was measured by the proton-cation exchange method. In the vesicular suspension, a transmembrane ΔpH of about 2 units drives protons outward, whereas a corresponding gradient drives sodium ions inward. Since neither phosphate nor sulfate ions can cross the membrane, any cation movement can only take place by electroneutral exchange. When a specific proton carrier such as FCCP is added alone, it cannot promote any proton outflux, and another path must be provided for Na^+ to enter. Therefore, the permeability to cations induced by a given antibiotic on vesicles subjected to a pH gradient between the intravesicular and external media is conveniently and adequately measured by monitoring the proton flux occurring in exchange through FCCP, provided the latter path is not the limiting factor. This method has already been described in detail (19, 20).

In the presence of FCCP, and upon antibiotic addition, the proton flux develops in time until it reaches a plateau, which thereafter remains stable. Typical examples of this time course are given in Fig. 4, in which the proton flux (expressed as percentage of the total amount of proton titratable after vesicle disruption by Triton X-100 addition) is plotted versus time. The percentage of proton release at the plateau depends upon the antibiotic concentration. ^{31}P-NMR experiments, carried out as previously described (21), showed that, when this plateau was reached, two vesicle populations were clearly distinguishable: a population whose internal medium was in equilibrium with the external medium and a population whose internal pH remained identical with the initial pH of 5.50, and was therefore not permeabilized by the antibiotic. Consequently, the percentage of protons released measured at the plateau corresponded to the percentage of the total vesicle population being permeabilized. Dose-response curves were obtained by plotting the percentage of protons released at the plateau as a function of the antibiotic concentration, expressed in moles per mole of total vesicular lipids, as in Figs. 2 and 3. The concentration of antibiotic giving 50% proton release, P_{50}, was obtained from these dose-response curves and used as a measure of the efficiency of the antibiotic. These P_{50} values are given in Table 2.

TABLE 2

Efficiencies of aromatic hepataene macrolides on large unilamellar lipidic vesicles

Compounds	Electric net charge of "polar head"	P_{50}[a]		
		Cholesterol, 10 moles %		Ergosterol, 10 moles %
		+LUV	−LUV	−LuV
Vacidin A	None	0.4	0.2	0.08
NAV	Negative	0.4	0.3	1.0
VME	Positive	20	20	2.25
Gedamycin	None	—	0.15	0.12
NAG	Negative	—	3.0	2.0
GME	Positive	—	5.5	0.50
Perimycin A	Positive	—	2.5	0.4

[a] Concentration of compound for 50% proton release. In order to express P_{50} in moles of compound per mole of lipids, multiply by 10^{-3}; to express in moles of compound per liter of suspension, multiply by 1.2×10^{-6}. Standard deviation ±10%.

Effects of Membrane and Antibiotic Electric Charges

Table 2 gives the P_{50} values obtained on negatively and positively charged LUVs, both of which contained 10 moles % of cholesterol. In the case of vacidin A and its negatively and positively charged derivatives (NAV and VME), no significant difference was observed between the sensitivity of the two types of vesicles. It may thus be concluded that electrostatic effects do not play an important role in model membrane-polyene interaction.

Nevertheless, the charge of the antibiotic molecule seems to be important for its activity on these cholesterol-containing vesicles, since the positively charged methyl esters of both vacidin A and gedamycin exhibited much lower activity than the parent compounds. On the other hand, the negatively charged NAV had an activity similar to that of vacidin A, whereas NAG had an activity as low as that of GME.

Effect of Sterol

Figures 2 and 3 show the dose-response curves obtained on negatively charged LUVs containing 10 moles % of either cholesterol or ergosterol, for vacidin A (Fig. 2A), NAV (Fig. 2B), perimycin A (Fig. 3A), and VME (Fig. 3B). The P_{50} values are given in Table 2. A comparison of the P_{50} values for the ergosterol- and cholesterol-containing membranes shows that a large difference in sensitivity is observed only for the three positively charged antibiotics, GME, VME, and perimycin A. These are 11, 8, and 6 times more efficient, respectively, on ergosterol-containing than on cholesterol-containing vesicles. The difference is much smaller for vacidin A, which is more efficient on ergosterol-containing membranes by a factor of 2.5. NAV is 3 times more efficient on choles-

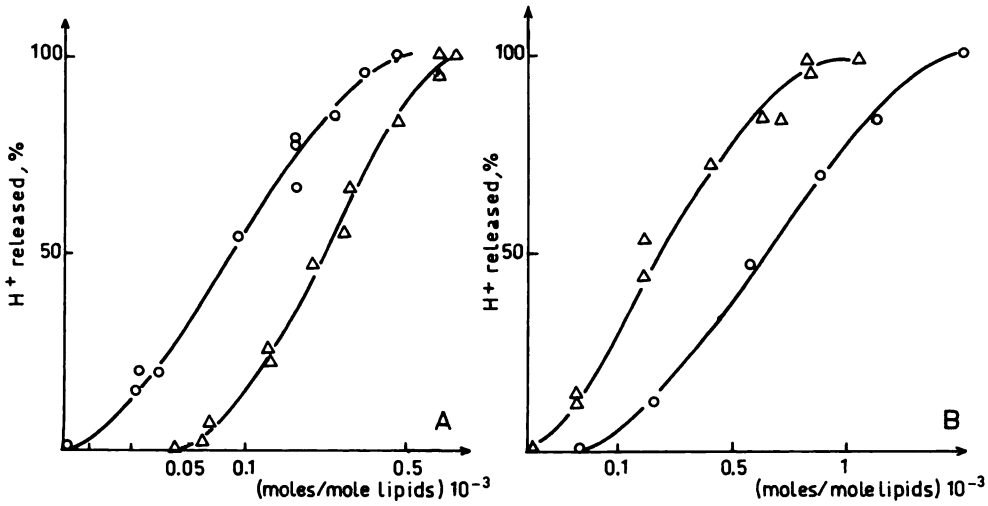


FIG. 2. *Dose-response curves obtained for vacidin A (A) and NAV (B) for vesicles containing 10 moles % cholesterol (△) and 10 moles % of ergosterol (○)*

terol-containing membranes, and the reverse seems to be true for NAG.

Kinetic Effects

The permeability induced by the positively charged compounds (perimycin A and both methyl esters) developed in time much more slowly than was the case of neutral or negatively charged antibiotics (vacidin, gedamycin, and their *N*-acetyl derivatives). In Fig. 4, the time courses of the proton efflux obtained under the action of gedamycin (Fig. 4A) and perimycin A (Fig. 4B) are given as examples of each group.

In the case of fast-acting compounds, such as gedamycin, permeability developed in a few minutes, whatever the concentration used. The latter affected mainly the percentage of protons released at the plateau. In the case of slow-acting compounds, such as perimycin, the proton flux developed quasilinearly over several hours. This slowness made it difficult to measure the plateau value precisely, especially in the low concentration range. This phenomenon seems to be independent of the activity of the compound, since, for instance, NAG, which has a low activity, belongs to the fast-acting group. This difference in kinetics did not depend on the nature of the sterol in the membrane. As shown in Fig. 5, the rapid permeabilization induced by vacidin A in both cholesterol and ergosterol membranes contrasts with the slow, quasilinear time course of the proton efflux obtained on both types of membrane with perimycin A.

DISCUSSION

Two conclusions can be drawn from the results obtained on LUVs: first, electrostatic effects can be ruled out as a main cause of the difference in activity between differently charged polyenes, since the results obtained with the vacidin A, NAV, and VME series on positively and negatively charged vesicles are not significantly different; second, the data obtained on LUVs are in general

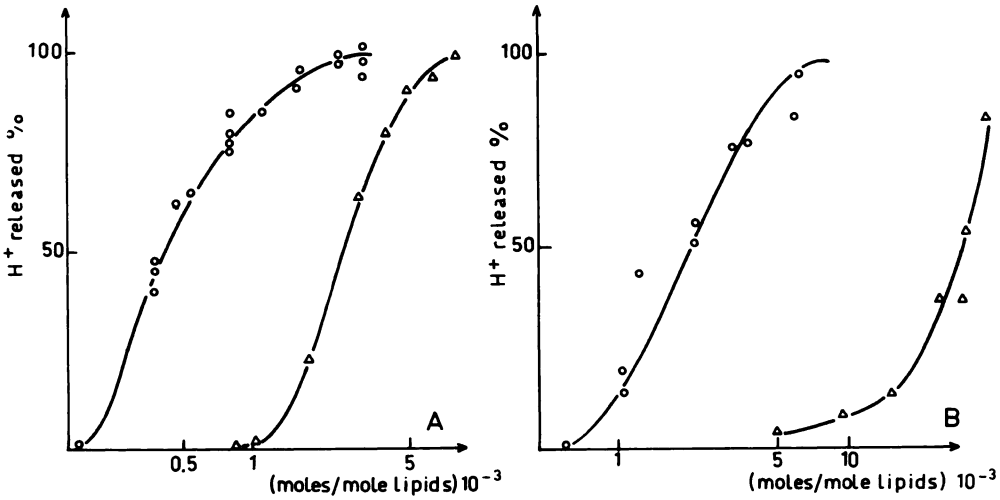


FIG. 3. *Dose-response curves obtained for perimycin A (A) and VME (B) on vesicles containing 10 moles % of cholesterol (△) and 10 moles % of ergosterol (○)*

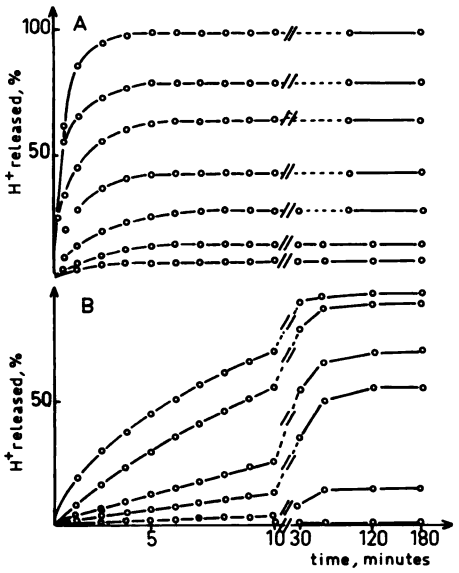


FIG. 4. *Time course of proton release by negatively charged vesicles containing 10 moles % of cholesterol induced by gedamycin A, concentrations ranging from 1.0 (top curve) to 0.01 (bottom curve) mmoles/mole of lipids (A) and by perimycin A, concentrations ranging from 20 (top curve) to 0.8 (bottom curve) mmoles/mole of lipids (B)*

consistent with the biological data. As in biological membranes, the positively charged compounds are more active on ergosterol-containing than on cholesterol-containing vesicles. The reverse is true for the negatively charged compounds. More detailed analysis of the data shows that the activity of the antibiotics in cholesterol vesicles is the same as that in red blood cells: the positively charged antibiotics, perimycin A, VME, and GME, have a very low activity as compared with that of vacidin A and gedamycin. Moreover, in both cases, NAV is more active than NAG.

In the case of ergosterol-containing membranes, the correlation between the activity in yeast and vesicles is not as good as the above correlation between cholesterol LUVs and red cell membranes; thus, in ergosterol vesicles, VME has the lowest activity of all of the compounds tested, and the differences between positively and negatively charged compounds are much smaller than those

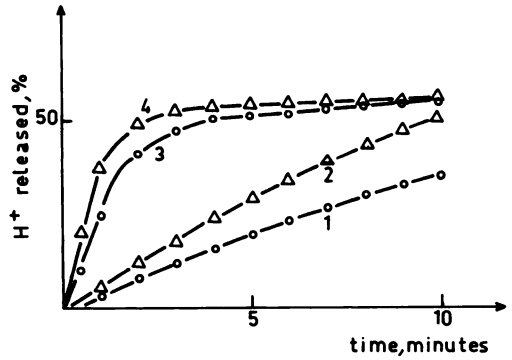


FIG. 5. *Time course of proton release induced by perimycin A (1 and 2) and by vacidin A (3 and 4) on vesicles containing 10 moles % of cholesterol (2 and 4) and 10 moles % of ergosterol (1 and 3)*

in yeast. However, some caution must be exercised when comparing experiments on biological and model membranes. Direct comparison of biological activity with activity on lipidic vesicles is possible for H_{50} determination but not for MIC determination. Although the toxic effects of polyene macrolide antibiotics on yeast as well as their lytic effects on red cells are the result of changes in membrane permeability (mainly to monovalent cations), the mechanisms of these two toxic effects are different. Inhibition of yeast growth is caused by metabolic imbalance related to the decrease in the intracellular potassium ion concentration (5), whereas hemolysis arises from an osmotic imbalance due to the ion flux and the effect of hemoglobin, which is a purely physicochemical effect correctly modeled in lipidic vesicles.

The other important parameter to be considered is time. The results in vesicles show that positively charged compounds act much more slowly than the others, this difference in kinetics being observed on both ergosterol and cholesterol vesicles. In the short time allowed in hemolytic experiments, this kinetic difference may be important when evaluating the efficiency of the compound. Such is not the case in MIC determination on yeast, since the cell remains in contact with the antibiotics for 24 hr, during which time both fast- and slow-acting compounds have enough time to develop their damage. The difference between the two does not appear in the final result.

The activity of polyenes seems to be associated with the formation of a hydrogen bond between the hydroxyl group of the sterol and the nitrogen atom of the amino sugar as proton acceptor. Simple acylation, which decreases the proton-acceptoring ability of this nitrogen atom, constantly decreases the polyene activity, and it has been shown (22) that aminoacylation maintains it. Therefore, it seems that the earlier suggestions of other authors (23) that the hydrogen bond is formed with the carboxyl group cannot be supported further.

As far as the chemical modifications for better selective toxicity are concerned, the important conclusion remains that only positively charged compounds exhibit clear selectivity for ergosterol-containing membranes. The selective toxicity apparently requires a specific conformation of the sugar moiety, suitable for differential hydrogen bond formation with hydroxyl group of ergosterol or cholesterol, respectively. The freedom of the amino sugar moiety to rotate seems to be very important for this differentiation, which is hindered in zwitterionic polyenes by electrostatic interaction with the carboxyl group. The interaction with the ergosterol molecule does not seem to be influenced by hindered or free rotation of amino sugar. On the other hand, a clear preference exists for interaction with the cholesterol of zwitterionic polyene molecule, in which the amino sugar is fixed in a particular conformation. This reasoning leads to the conclusion that it is the absence of the ionizable carboxyl group in position C_{18} rather than the net charge of the molecule which determines the selective toxicity. This might provide a clue for the further chemical modifications needed to improve the selective toxicity of this group of antifungal agents.

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