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The Properties and Clinical use of Polyene Antibiotics

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Abstract

Model membranes' similarity to the biological cell membrane and their simple structure with controlled composition and properties. They are formed from natural and synthetic lipids, and these membranes are an interesting object for scientific investigations. We studied the ultraviolet absorption spectrum of the complex formed by polyene antibiotics at different concentrations separately and stable with cholesterol. We also study the effects of the antibiotic concentration and channel-expression and the dynamics of channels, which are affected by pH in lipid membranes. We represent and define the behavior of such antibiotic-generated-channels over a wide range of pH values and ionic strength. Our information demonstrates the pH-induced modulation of AmB channels selectivity, stability of the open state, and conductance. This work might be of widespread importance for comprehending molecular aspects regarding fundamental properties of AmB, such as activity, toxicity, and formation of ion channels, and provide a more suitable familiarity. Learning mechanism help to develop a more unassailable AMB formulation for treatment.

Keywords: Amphotericin B ion channel, Sterol, Polyene antibiotic, Bilayer membrane, Membrane structure.

Introduction

The polyene antibiotics - nystatin and amphotericin B are known to increase the ion and nonelectrolyte permeability of sterol-containing biological and artificial membranes (Figure 1). Polyene antibiotics (PA) amphotericin B and nystatin are principally known as major antifungal drugs as well as one of the first model systems for trans-membrane ionic channel structures (Samedova et al., 2018). The effects of polyene antibiotics on thin lipid membranes are consistent with their action on

biological membranes. The biological effect of PA is the formation in the membranes of structural ion channels which permeable for ions and organic substrates. The PA have high affinity to biological membranes, which there are sterols of definite structure. Nystatin and amphotericin B create aqueous pores in thin lipid membranes; the effective radius of these pores is approximately 4 Å. There is a marked correlation between the permeability of a nystatin- or amphotericin B-treated membrane to water and small hydrophilic solutes and the permeability of the human red cell membrane to these same particles. Amphotericin B or nystatin may interact with membrane-bound sterols to produce multi molecular complexes which greatly enhance the permeability of such membranes for anions, and, to a lesser degree, cations. Although both nystatin and amphotericin B greatly increased the conductance of cholesterol-containing membranes, they also expanded cation conductance to a considerable degree.

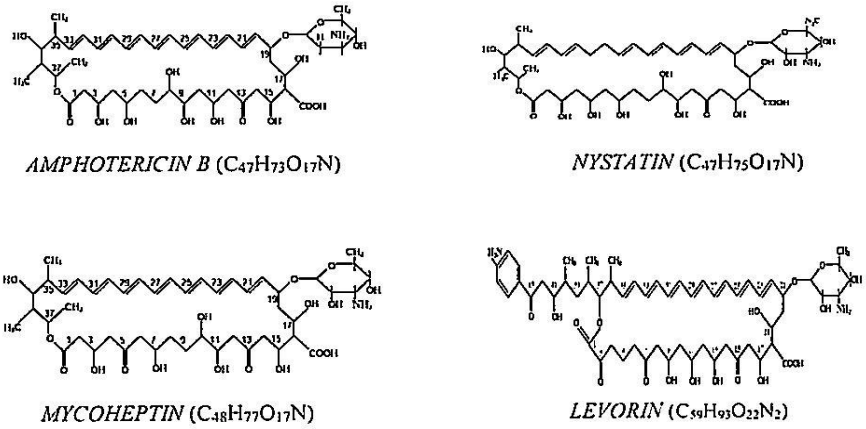

Figure 1. Chemical structure of the polyene antibiotics.

Additionally, the effect of amphotericin B on the electrical properties of the membranes occurred over a moderately narrow concentration range. In this scale, there was no detectable effect on membrane stability. Possibly the many hydroxyl groups in nystatin and amphotericin B are capable for anion selectivity. Nystatin and amphotericin B induce a cation-selective conductance when added to one side of a lipid bilayer membrane and an anion-selective conductance when added to both sides. The concentrations of antibiotic required for the one-sided action are comparable to those employed on plasma membranes and are considerably larger than those required for the two-sided action. The one-sided action of the polyene antibiotic amphotericin B on phospholipid bilayer membranes formed from phosphatidylcholines and sterols have been investigated. The properties of ion channels formed in membranes by polyene antibiotics with various chemical

structure of hydrophilic and hydrophobic chain of molecules are investigated. Small differences in a hydrophilic chain with the changed number of hydroxyl and carbonyl groups significantly influence on the size of conductivity and selectivity of the channel. The more number of double bounds in a hydrophobic part of polyene molecules leads to the higher biological activity of antibiotics. Measurement anion - cationic selectivity of the channels formed by polyenes showed that anionic selectivity, as well as conduction of channels, decreases among antibiotics: amphotericin B – nystatin. Research of physical and chemical properties of the single ion channels on the bilayer lipid membranes in the presence of polyene antibiotics makes possible to create theoretically reasonable recommendation to purposeful synthesis of new antibiotics with the known properties of molecule.

Materials and methods

Lipid membranes are to be used to study the effect of PA on membranes at the molecular level. Bimolecular lipid membranes (BLM) are made of phospholipids present in the brain tissues of large and small horned animals. It is one of the methods of reflecting the formation of lipid membranes by inserting phospholipids in the hollow part of a glass made of Teflon material. This method showed that PA is highly sensitive to sterols in the membranes. Polyenes interact with sterols to form molecular-sized ion channels in membranes, and the physicochemical properties of these channels have been studied by the patch-clamp method. (Samedova et al., 2018).

Membrane leaching of the antibiotic by the perfusion method and the reduction of membrane permeability has observed by the kinetic relaxation method. The purposeful synthesis of polyene antibiotics and the study of their physicochemical properties in membranes opens a wide way to define theoretical ways and create new antibiotics. All experiments were performed at a room temperature 23◦C. The stock AmB solution was obtained by dissolving AmB in DMSO. The conclusive pH was calibrated with a pH mini-electrode. Changes in pH were conducted by addition of small volumes of concentrated solutions of either HCl or KOH. The analysis was made using pClamp 8.2.

Results and disscussions

When amphotericin B enters one side of the membrane, it causes the formation of ion channels in the membrane (Figure 2.)

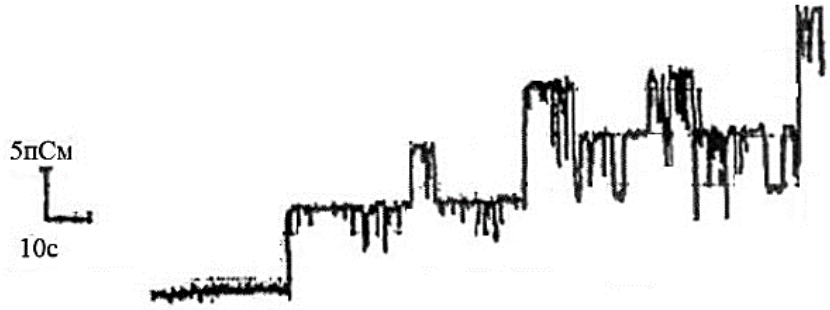

Figure 2. Dynamics of operation of single ion channels formed on one side.

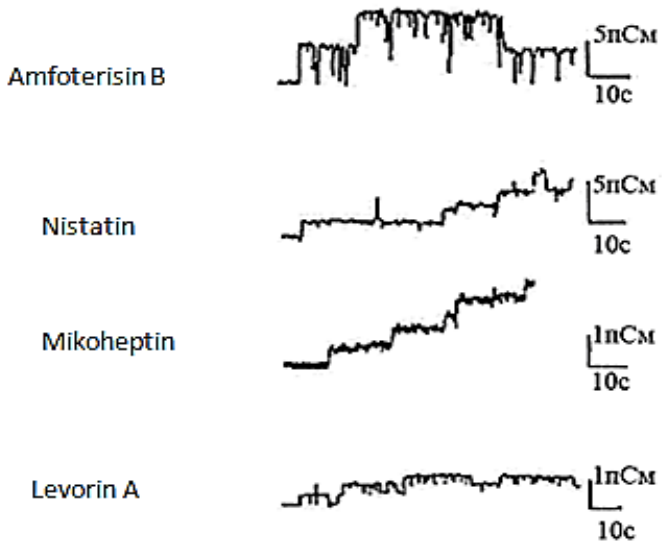

Figure 3. Single ion channels in lipid membranes in the presence of PA.

To analyze the properties of ion channels depending on the structure of the lactone ring of polyene molecules, studies were conducted in the form of comparing the properties of amphotericin B, nystatin, and mycoheptin channels in lipid membranes obtained from a mixture of phospholipids and cholesterol. (Figure 3). The polyene heptaene chain is the same for amphotericin B and mycoheptin, a double bond in the middle of the polyene chain in the nystatin molecule is hydrogenated (tetraene). The hydrophilic chain of the lactone ring is different for all three antibiotics: nystatin and amphotericin. The support of hydrophilic groups is the same, only the locations are different. In mycoheptin, in addition, one hydroxyl group is replaced by one carbonyl group. The amphotericin channel has the highest conductivity (6.5 pS). The nystatin channel has a lower conductivity (2 pS). The mycoheptin channel exhibits minimal

conductivity (0.5 pS). The permeability of the levorin channel (0.2-0.3 pS cm) is even lower.

One of the parameters characterizing the biological effectiveness of PA is the induced permeability level of antibiotics, the permeability level measured in BLM, and the constant time of antibiotic flushing. Studies of alkyl derivatives of amphotericin and levorin have shown that the biological activity of antibiotics decreases as the length of the alkyl chain increases. When a methamphosin molecule is inserted into one side of the membrane, discrete conductive single ion channels are formed, and methamphosine is the only derivative that increases the conductivity when it is on one side of the membrane.

We also examined at the single-channel level the effects of pH changes on the biophysical properties of AmB channels inserted in bilayer lipid membranes. Our information showed the pH-induced modulation of AmB channels open probability, and conductance.Although the ion selectivity did not change. The acidity greatly decreased the open probability of the channel. Also this information suggests that presumably an H+ on the regulatory site is available from the extracellular (cis) side of the channel where pH changes.(Figures 4 and 5)

The pH changes even changed the single-channel conductance of AmB oligomers. Our information indicates that the single channel conductance of AmB channels value is larger at pH 7 and pH 8, showing a drop at acid pH values. Furthermore, these data point out that probably an H+ on the regulatory site is available from the extracellular (cis) side of the channel where pH changes. The pH changes also altered the single-channel conductance of AmB oligomers. Our data show that the single channel conductance of AmB channels value is larger at pH 7 and pH 8, showing a drop at acid pH values (Figure 4).

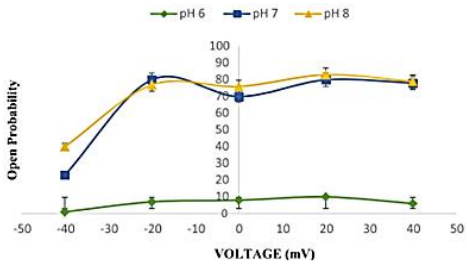

Fig. 4

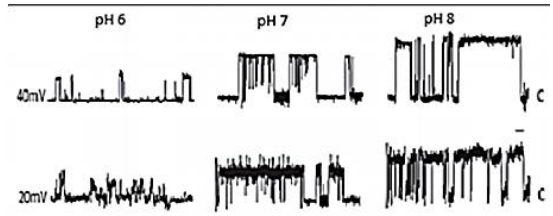

Fig. 5

Figure 4 and 5. Typical current recordings of single ion channels formed by amphotericin B in phosphorylcholine bilayer membranes measured at different potential and channel open probability at different voltages

The capacitance of the bilayers was smaller in a solution of lower pH than in a solution of medium pH. Since phosphorylcholine has a zwitterionic group, the polar groups would be positively charged at lower pH values, and this would lead to a mechanical constriction of the AmB pore displayed by a drop in its conductance at pH = 6.In contrary to open probability and conductance the reversal potential and ion selectivity did not change by pH. The reversalpotential analysis indicated that Amb in these pH was cation channel. At pH range from 6 to 8 the carboxyl group of AmB is deprotonated, so that the selectivity of channels is influenced mostly by the electric profile within the conductive pore, directing to a cation-selective behavior. We discovered that AmB channels become even more cationic selective in the acidic pH and can even change to anionic in the alkaline pH. In the finale, our data reveals that when pH changes from basic values (pH = 8) to acidic (pH = 6), the visible decrease happened in the conductance and open probability. This data is useful to sufficiently comprehend the mechanism of action of AmB and the toxic side effects manifestations.

For a more reasonable understanding of how amphotericin molecules interact with the lipid membrane, we carried out electrophysiology investigations at the single-molecule level and aimed to quantify how pH differences of the buffer solution, which are predicted to change the ionization state of AmB's polar head, change the stability of AmB-lipids complexes, it is noticed that low pH values facilitate more prolonged residence times of the AmB channel in its open state, whereas at neutral and alkaline pH's rapid flickering occasions between open and closed forms ensue (Figure 6), (Pashazade, 2022).

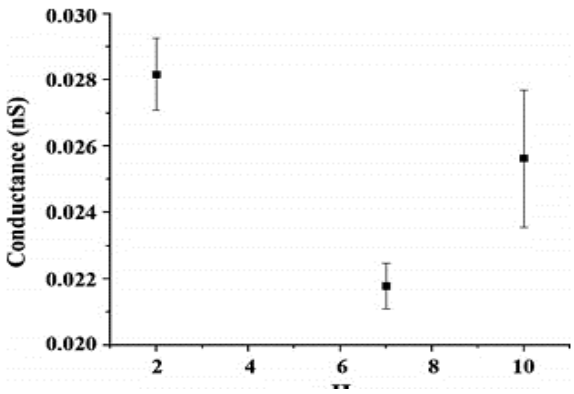

Figure 6. Typical current recordings of single ion channels formed by amphotericin B in phosphorylcholine bilayer membranes measured at different potential and pH dependence of the single-channel conductance of AmB oligomers.

The conductance for pH 7 and 8 were 500 and 560 pS. By reduction of cis pH to 6, the single-channel conductance decreased to 250 pS. As seen in this figure the reversal potential in the pH of 6, 7, and 8 were 35, 36, and 40 mV. The calculation of ionic selectivity founded on the middle values of related reversal potentials indicated a highly cation-selective channel. By changing the pH between 6-8 the ion selectivity did not significantly change. The effect of voltage on the channel activity was analyzed by measuring the channel open probability as a function of voltage in asymmetrical K+ conditions (200 mM K cis/50 mM K trans. As seen the channel open probability increased at potentials above −40 mV in the pH of 7 and 8. By reduction of cis pH to 6, the open probability significantly decreased in all voltages (Pashazade, 2022)

Clinical use of polyene antibiotics.

uses for polyenes that appear in the World Health Organization model list of essential medicines: 22nd list 2021. We then address their current clinical use and some important clinical advances. In its several formulations, AmB is used to treat coccidioidomycosis via intrathecal route, mucocutaneous leishmaniasis, invasive aspergillosis, blastomycosis, candidiasis, coccidioidomycosis, cryptococcal meningitis in patients with HIV infection, cryptococcosis, severe fungal infection of central nervous system, severe fungal infection of lung, histoplasmosis, histoplasmosis in patients with HIV infection, pulmonary cryptococcosis in patients with HIV infection, infection by Basidiobolus, mucormycosis, sporotrichosis, and urinary tract mycosis. Nys is used to treat candidal vulvovaginitis, candidiasis of skin, cutaneous and mucocutaneous infection, and non-esophageal gastrointestinal candidiasis. Natamycin is used to treat blepharitis, fungal conjunctivitis, and fungal keratitis. In addition to polyenes' use as antimycotics and antiparasitics, experimental trials of other therapeutic applications have been reported. (Pashazade, 2020)

Conclusion

In significance, we conclude that:

The high antifungal activity and low resistance incidence to polyene treatment are the main reasons why they are still being studied. Among polyene antibiotics, amphotericin B, levorin and nystatin have the strongest membrane effect. Amphotericin B channels are selective for anions, but levorin channels ideally transport cations through membranes. This difference depends on the number of carbonyl and carboxyl groups present in the hydrophilic chain of antibiotics.

Amphotericin B and its N-methyl derivative form unilateral conduction channels through the membrane and allow the transmembrane transfer of ions into the cell. It has been shown that the longer the aquifers of amphotericin B and levorin, the longer the molecules remain in the membrane.

In acidic solutions, the reversal potential of AmB channels carries a negative value, so that AmB channels become anion-selective When raising the pH value of the bathing solution AmB channels display a cationic selective behavior. AmB oligomers are mostly anionic-selective in low-pH solutions and cationic-selective at neutral and alkaline solutions. Acidity changes change the single-channel conductance of AmB oligomers. Extracellular acidity can reduce AmB activity.

References

Asandei, A, & Luchian, T. (2008). Ion selectivity, transport properties and dynamics of amphotericin B channels studied over a wide range of acidity changes. Colloids Surf B Biointerfaces, 67:99-106.

Belkherroubi-Sari, L., Boucherit Otmani, Z., Chéron, M., Boucherit, K., Benyoucef, M., & Belbraouet, S. (2008). Modulation of the polyene antibiotic amphotericin B selective toxicity by pH change of the stock solutions. Afr J Microbiol Res; 2:242-6.

Bolard, J., Legrand, P., Heitz, F., & Cybulska, B. (1991). One-side action of amphotericin B on cholesterol-containing membranes is determined by its self-association in the medium, Biochemistry, 30: 5707.

Cavassin, F.B., Luiz, Bau´-Carneiro, J., Vilas-Boas. R.R., & Queiroz-Telles, F. (2021). Sixty years of Amphotericin B: An Overview of the Main Antifungal Agent Used to Treat Invasive Fungal Infections. Infect. Dis. Ther., p.1-33.

Chiriac, R., & Luchian, T. (2007). pH modulation of transport properties of alamethicin oligomers inserted in zwitterionic-based artificial lipid membranes, Biophys. Chem. 130-139.

Cotero, B.V., Rebolledo-Antúnez, S., & Ortega-Blake, I. (1998). On the role of sterol in the formation of the amphotericin B channel. Biochim Biophys Acta; 1375:43-51.

Huang, W., Zhang, Z., Han, X., Tang, J., Wang, J., Dong, S., & Wang, E. (2002). Ion channel behavior of amphotericin B in sterol free and cholesterol or ergosterol containing supported phosphatidylcholine bilayer model membranes investigated by electrochemistry, Biophys. J. 83: 3245.

Jakl, M., Straka, M., Jaklová Dytrtová, J., & Roithová, J. (2014). Formation and stability of calcium complexes of dimethyl sulfoxide in water. International Journal of Mass Spectrometry, Vol. 360, p. 8-14.

Kamiński, D.M. (2014). Recent progress in the study of the interactions of amphotericin B with cholesterol and ergosterol in lipid environments. European Biophysics Journal, *43 (*10-11), p. 453-467.

Lee, Y., Pincus, Ph.A., & Hyeon, Ch. (2016). Effects of Dimethyl Sulfoxide on Surface Water near Phospholipid Bilayers. Biophysical Journal. 111 (11), p. 2481-2491.

Pashazade T.C. (2022). Study of physical and chemical properties of ion channels created by polyene antibiotics in lipid membranes. Advances in Biology & Earth Sciences, 7(2); 1 - 7.

Pashazade, T. C. (2018). Some experiment on biomolecular lipid membranes. Symposium dedicated to the 120th anniversary of the Institute of Botany of ANAS and Academician VI Ulyanishev of the Azerbaijan Botanical Society. December 25, p.82.

Pashazade, T. C. (2019). The action of nystatin and amphotericin B on the thin lipid membranes. Action of nystatin and amphotericin B on lipid bilayer membranes. VI Biophysical Congress, Russia, Sochi. Vol 1, p. 178-179.

Pashazade, T. C. (2020). Pharmacology of amphotericin B. Eurasian Scientific Congress. I-st International Scientific and Practical Conference, Barcelona, Spain, p. 33-38.

Pashazade, T.C., & Gasimov, X.M. (2021). Investigation of the interaction of polyene antibiotics with cholesterol, Journal of Life Sciences & Biomedicine, 3(76), No 1, p. 77-83.

Pinisetty, R., Alapati, R.V., & Devireddy, A. (2012). Molecular Dynamics Study of DMPC Lipid Bilayers Interacting with Dimethyl sulfoxide–Water Mixtures. The Journal of Membrane Biology, 245 (12), p. 807-814.

Samedova, A. A., Tagi-zade, T.P., & Kasumov, Kh.M. (2018). Dependence of ion channel properties formed by polyene antibiotics molecules on the lactone ring structure. J Bioorganic Chemistry. Vol 44, p. 337–345.