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POTENTIAL-DEPENDENT FORMATION OF SINGLE CONDUCTING ION CHANNELS IN LIPID BILAYERS INDUCED BY THE POLYENE ANTIBIOTIC LEVORIN A_2

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The aromatic polyene antibiotic levorin A_2 forms ion channels permeable to monovalent cations, in lipid membranes containing cholesterol or ergosterol. Channel conductivity is in the range 0.3–0.5 pS. The channel has two main states: conducting (open) and nonconducting (closed). The potential-dependent formation of levorin A_2 channels is observed in lipid membranes. The system responsible for the ion-channel selectivity is localized on the hydrophilic side of the lactone ring of the polyene molecule.

The polyene antibiotic levorin A is produced by the microorganism *Streptomyces levoris* and was isolated for the first time in 1965 [1]. Levorin A enhances the permeability of lipid membranes to alkali metal cations [2]. The presence of cholesterol in membranes is a prerequisite for the antibiotic's efficacy [2]. The conductance of membranes increases with the third-fourth power of the antibiotic concentration [2]. Levorin A represents a mixture of substances with closely related physicochemical properties [3]. Levorin A_2 is the basic component of the antibiotic mixture levorin A. It belongs to the group of aromatic heptaenes. The chemical structure of this antibiotic has been established [8]. Comparison of chemical structures * shows that levorin A_2 differs somewhat from the polyene antibiotics amphotericin B, nystatin and mycoheptin. Apart from an amino sugar (mycosamine), it contains a positively charged aromatic ketone: *p*-aminoacetophenone. This paper deals with the properties of ion channels in the presence of levorin A_2.

Membranes were formed across a hole in a Teflon cell, 0.5 mm in diameter, from phosphatidylserine (10 mg · ml^{-1}) and cholesterol or ergosterol (1 mg · ml^{-1}) used in a weight ratio of 20 : 1 in an *n*-heptane solution. The current through the membrane was measured with a 'Keithley-301' electrometric amplifier. Levorin A_2 in dimethyl sulfoxide (10^{-3} M) was added in equal concentrations to both sides of the membrane, in salt solutions buffered with 10 mM potassium phosphate. All experiments were conducted at pH = 6.5 and at a temperature of 22 to 23°C.

Levorin A_2 does not increase the conductance of the membranes deprived of cholesterol. The measured relationship between the membrane conductance and concentration of levorin A_2 and cholesterol, suggests that complexes are present

* The structures of polyene antibiotics for which a complete chemical structure has been proposed can be found in the following references: amphotericin B [4,5], nystatin [6], mycoheptin [7], levorin A_2 [8].

in the membranes resulting from the interaction of the antibiotic with cholesterol. Such complex formation should have resulted in the membrane conductance increasing in discrete steps, just as in the case of nystatin, amphotericin B and mycoheptin [9].

The injection of antibiotic levorin A_2 in a concentration of $1 \cdot 10^{-8}$ M on the either side of membrane prepared from phosphatidylserine with cholesterol or ergosterol at ratio of 20 : 1 leads to a chaotic increase in the membrane current fluctuating with respect to the mean value. The current fluctuations may be due to the discrete functioning of individual conducting units, channels. Analysis of the current fluctuation indicates that the channel conductance lies in the range of 0.2–0.5 pS in 2 M KCl solutions at a membrane potential of 200 mV. The sharp changes in conductance of the membrane in the presence of levorin A_2 are shown in Fig. 1. The sharp changes in conductance attributed to the formation of ion channels each being in one of the two states: open (conducting) and short-duration close (nonconducting). The conductance of a channel is in the range 0.3–0.5 pS in 2 M KCl and 2 M KBr. The channels change over frequently from one state to the other. The mean lifetime of the two states was estimated from 100 transitions between two states using various sections of a single channel record. In Table I the lifetime of single channels in the open and closed state is shown. Comparison of lifetimes calculated from various sections

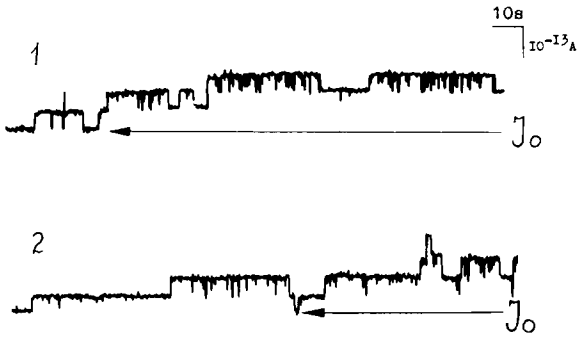

Fig. 1. Time variations of the membrane conductance at a membrane potential of 200 mV. Membrane solution, phosphatidylserine : ergosterol = 20 : 1. Levorin A_2 concentration: (1) in 2 M KCl, $5 \cdot 10^{-9}$ M; (2) in 2 M KBr, $1.5 \cdot 10^{-8}$ M.

TABLE I

LEVORIN A_2 CHANNEL PARAMETERS IN KCl AND KBr SOLUTIONS

	2 M KCl	2 M KBr
g (pico mho) [a]	0.4 ± 0.1	0.5 ± 0.1
Levorin A_2 (nM) [b]	2.5	10
τ_o (s) [c]	2.5 ± 0.2	5.2 ± 0.4
τ_c (s) [c]	3.5 ± 0.3	0.86 ± 0.05

[a] g, single-channel conductance measured with a membrane potential of 200 mV. Membrane solution, phosphatidylserine: ergosterol = 20: 1.

[b] Levorin A_2 concentration necessary to obtain a stationary record for a single channel.

[c] Average dwell time of a channel: τ_o, in the open state, and τ_c, in the closed state.

of the record showed that, within the limits of error, these were reproducible which proves that only one fluctuating unit was present. As the concentration of levorin A_2 in aqueous solutions is increased, one can observe formation of several channels (Fig. 1). In one of the records obtained in 2 M KBr solutions from 2 to 5 conductance levels of equal value were observed for a long period of time. The frequencies of short switches from the second, third and subsequent levels to the previous one is approximately proportional to the level numbers. It may only be the case if membrane consists of identical and independently functioning channels with two states.

Levorin A_2 exhibits a most interesting property, namely: potential-dependent forming channels in the membrane. Channel formation depends on the field magnitude and direction. For example, if, in the absence of an external field, levorin A_2 is added on one side of a membrane in a concentration of $1 \cdot 10^{-6}$ M, no increase in the membrane conductance is observed. In response to a constant +50 mV field applied to the membrane ('+' on the side of antibiotic), the conductance did not increase (Fig. 2). Only when the membrane potential is raised to +75 mV, a weak increase in conductance takes place. The rate of conductance increase depends on the magnitude of the applied field. With the current flowing in the opposite direction (100 to 150 mV), i.e. with '–' on the side of the antibiotic, the mem-

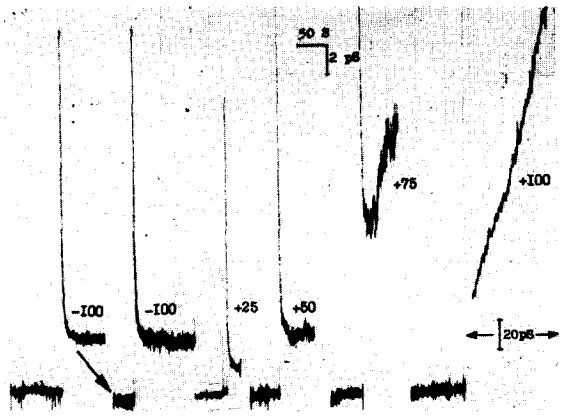

Fig. 2. Membrane conductance versus field magnitude and direction in 2 M KCl. The numbers stand for the membrane potential. The potential sign corresponds to a solution with antibiotics. The arrow indicates the instant at which levorin A_2 was added on one side of the membrane in a concentration of $1 \cdot 10^{-6}$ M. The conductance scale is given for a membrane potential of 100 mV and a feedback resistance of 10^{10} and 10^{10} ohm. Membrane solution, phosphatidylserine : ergosterol = 20 : 1.

brane conductance does not increase for 30 min more. It can be assumed that the molecule of levorin A_2 presents a dipole with a positive charge on *p*-aminoacetophenone which can orient itself along the direction of the field.

Levorin A_2 induces in lipid membrane ideal selectivity for K^+. The zero current potential is 58 mV across membranes with a large number of channels. The system responsible for the ion-channel selectivity is most likely to be localized on the hydrophilic side of the polyene molecule.

In accordance with the molecular model of the polyene channel the carboxyl and mycosamine amino groups are situated at the membrane/water interface and the hydrophilic chain containing a few hydroxyl substituent lines the inner wall of the channel [10–12]. An ion channel is stabilized in the conducting state by an electrostatic interaction between the amino group of a molecule and the carboxyl group of an adjacent one in the channel [9]. The rupture of electrostatic interaction between the molecules in the channel (on account of the chemical modification or of pH shift) reduces the lifetime of the channel in the conducting state [9]. The polar groups determine the lifetime of the channel in this state, but not the selectivity and the conductance of the ion channels [9]. In Table II the comparative characteristics of the hydrophylic chain (up to the carboxyl group) of four molecules of the polyene antibiotics are given. The amphotericin B molecule contains six hydroxyl groups in the hydrophylic chain. Amphotericin B and nystatin have the same set of the hydrophylic groups but distinguish themselves by the location. They create in the membrane the same selectivity for Cl^-. In the molecule of mycoheptin a hydroxyl group is substituted by a carbonyl group and in the molecule of levorin A_2 two hydroxyl groups are absent. The substitution of a hydroxyl group by a carbonyl group reduces the

TABLE II

THE COMPARATIVE CHARACTERISTICS OF THE HYDROPHILIC CHAIN OF FOUR MOLECULES OF POLYENE ANTIBIOTICA

Zero current potentials (V_m) corresponding to a 10-fold change in the KCl concentration (2 M : 0.2 M) are also given. Membrane solution, phosphatidylserine: cholesterol = 20 : 1. Amphotericin B, $1 \cdot 10^{-7}$ M; nystatin, $5 \cdot 10^{-7}$ M; mycoheptin, $5 \cdot 10^{-7}$ M; levorin A_2, $1 \cdot 10^{-6}$ M.

No. of carbon atom	Amphotericin B V_M = −42 mV [a]	Nystatin V_M = −42 mV	Mycoheptin V_M = −9 mV	Levorin A_2 V_M = +58 mV
1	=O	=O	=O	=O
2				
3	–OH	–OH	–OH	=O
4				
5	–OH	–OH	=O	
6				
7		–OH	–OH	=O
8	–OH			
9	–OH			–OH
10		–OH	–OH	
11	–OH	–OH	–OH	–OH
12				
13	=O	=O	=O	–OH
14				
15	–OH	–OH	–OH	=O
16	–COOH	–COOH	–COOH	
17	–OH	–OH	–OH	–OH
18				–COOH

[a] Data taken from Refs. 13 and 14 for electrolyte concentrations of 10 and 100 mM KCl coincide with the data for 0.2 and 2 M KCl.

anion-cation selectivity. Moreover the absence of two hydroxyl groups in the hydrophilic chain of the molecule of levorin A_2 induces the cation selectivity.

As has been assumed earlier [9–12] anion selectivity is due to a positive potential induced by OH dipoles inside the channel. The hydroxyl groups produce the positive potential in the channel which enhances the anion affinity and reduces the cation affinity that results in anionic selectivity of the amphotericin B channel. The reduction in the number of hydroxyl groups in the hydrophilic chain of the molecules of polyene antibiotics (amphotericin B > mycoheptin > levorin A_2) changes in the selectivity of the channel from an anionic to a cationic character.

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