Interaction of elaiophylin with model bilayer membrane

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Abstract. Elaiophylin is a new macrodiolide antibiotic, which is produced by the Streptomyces strains [1]. It displays biological activities against Gram-positive bacteria and fungi. The mode of action of this antibiotic has been attributed to an alteration of the membrane permeability. When this antibiotic is inserted into the bilayer membranes destabilization of the membrane and formation of ion-penetrable channels is observed. The macrodiolide antibiotic forms stable cation selective ion channels in synthetic lipid bilayer membranes. The aim of this work was to study the interactions of Elaiophylin with model bilayer membranes and to get information on the mechanical properties of lipid bilayers in presence of this antibiotic. Patch-clamp technique [2] were used in the study

1. Introduction

Ultrathin lipid membranes consist of lipid bilayers, self-assembled at the tips of patch pipettes from lipid monolayers. Monolayers are formed by spreading lipid in organic solvent onto the surface of electrolyte solutions [3].

Planar lipid bilayer is one of the unique electrophysiological techniques that is intended to study specific channel properties of the purified complexes in a well-controlled artificial environment [4]. The lipid bilayer enables study of functional activities of ion channels and monitoring ion channels behavior at the single molecule level in the model membranes.

Artificially created lipid vesicles mimic the cells and their membranes. The model bilayer membrane of lipid vesicles presents a simple model for investigation of membrane properties and can be formed from various lipids with controlled composition in controlled environment.

Elaiophylin also known as Azalomycin B, Gopalamicin or Salbomycin (figure 1) is a highly characteristic macrolide antibiotic that was first isolated from a culture of Streptomyces melanosporus [1]. It demonstrates antifungal activity, cytotoxicity against cancer cells and antibacterial activity aganst gram-positive bacteria [1,5,6]. Elaiophylin is thought to exert its antifungal activity by forming an ion-channel assembly [7]. The mode of action of Elaiophylin is attributed to an alteration of the membrane permeability and it is directly related to the effect of the drug on the lipid phase of membranes. The aim of the present study is to investigate the effect elaiophylin on the electric properties of lipid membranes formed from synthetic lipids.

In the present work the effect of Elaiophylin on the properties of lipid bilayers were studied with application of Voltage-clamp method.

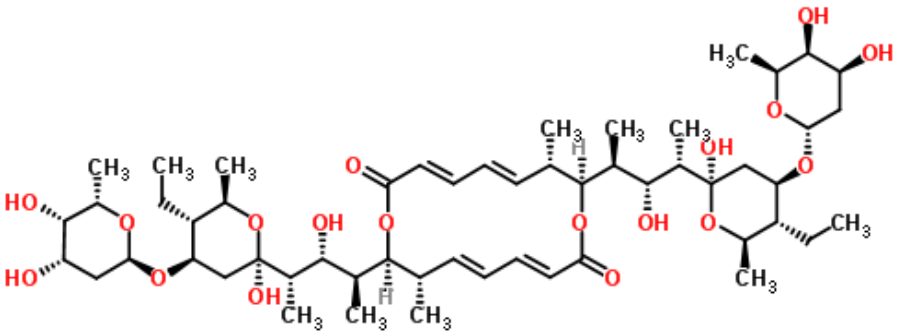

Figure 1. Chemical structure of Elaiophylin molecule.

2. Materials and methods

Soya bean phosphatidylcholine (L-α-Lecithin, Sigma, P5638), HEPES (buffer) and KCl (p.a.) were obtained from Sigma. Ethanol (p.a.) and n-hexane (p.a.) were purchased from Valerus Co. Elaiophylin were from Bioaustralis (BIA-E1028). All the chemicals were used without any further purification.

In the patch-clamp experiments lipid bilayers were self-assembled at the tips of patch pipettes, using the tip-dip patch clamp technique [2], from monolayers formed by spreading L-α-lecithin from soybean in n-hexane (10 mg/ml) onto the surface of electrolyte solution contained in Petri dishes (figure 2).

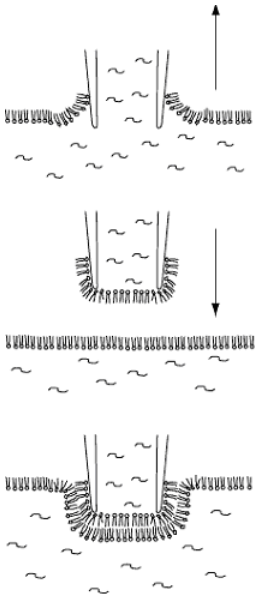

Figure 2. Scheme of lipid bilayer formation at the tip of patch pipette.

Patch pipettes (tip diameter 1-2 μm) and Petri dishes (10cm^2 area) were filled with aqueous solutions of KCl (1 M) buffered with HEPES (0.01 M) at pH 7. Only bilayers with seal resistances > 1GΩ were used. Elaiophylin, dissolved in ethanol, was added to the Petri dish to a final concentration of 5x10^{-7} g ml^{-1}. Single elaiophylin channel currents were monitored using a patch clamp amplifier Model 2400 (A-M Systems, Inc.). The currents were stored on a PC hard disc with 1 ms time resolution. Lipid bilayers were tested before and after introducing the ions with voltage ramps from -100 to +100 mV. The data

were analyzed using current-voltage surfaces' software. All measurements were performed at room temperature (~25ºC).

3. Results and discussion

3.1. Patch clamp technique

Elaiophylin forms long-lasting cation selective ion channels in planar lipid bilayer membranes prepared from soybean phosphatidylcholine.

As an explanation of the behaviour of elaiophylin in a lipid membrane, we are discussing, a barrel-like aggregation of several Elaiophylin molecules whereby a channel is formed. As shown in figure 3(a), the planar macrodiolide rings of several Elaiophylin molecules, as their lipophilic parts, could interact with the aliphatic hydrocarbon part of the lipid bilayer. As shown in figure 3(b) an ion-conducting pore spanning half of the bilayer thus, could be formed by self-assimilations of several elaiophylin molecules [8].

a) b)

Figure 3. Models for the elaiophylin channel in the lipid bilayer. (a) Cross-section through the membrane. (b) Suggested "double-pore" structures of the channel.

Membrane current was measured by the voltage-clamp method [2]. The principle of the method is to isolate a patch of membrane electrically from the external solution and to record current flowing into the patch. Single channel recording yields information about unitary conductance and kinetic behaviour of ionic channels. Ion channels form aqueous pores across the lipid bilayer and allow ions of appropriate size and charge to cross the membrane. The channels are "gated" and usually open transiently in response to a specific perturbation in the membrane, such as a change in membrane potential [9].

Figure 4 shows an example of channels recorded in presence of Elaiophylin in lecithin membranes. These recording demonstrate conductance levels, which are typical for elaiophylin channels opening. The membrane current started to increase within about 5 min after addition of the elaiophylin and reached a relatively stable level after approximately 10 min. Currents of single elaiophylin channels were recorded at 0.5 μg/ml of the antibiotic concentration.

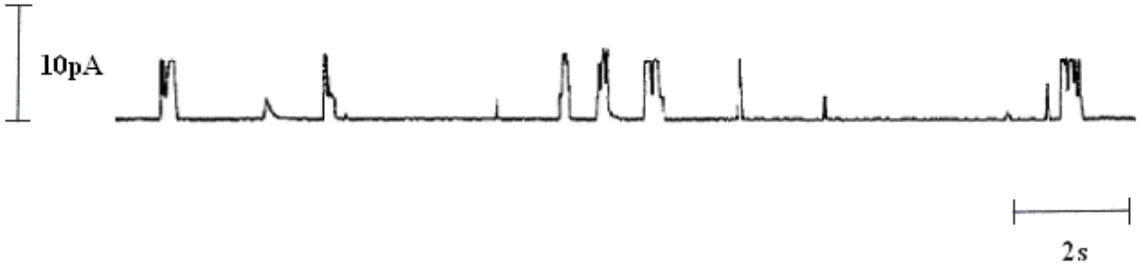

Figure 4. Traces of membrane current in presence of elaiophylin 0.5 μg/ml at the bilayer. Conditions: 1 M KCl, pH 7, membrane voltage—100 mV.

We observed that Elaiophylin has ionophoric activity and reduces the electrical resistance of the lipid membrane. The volt-ampere (I–V) characteristics were linear over a range of ± 100 mV. Multiple conductance states are seen only in the negative voltages' area (figure 5).

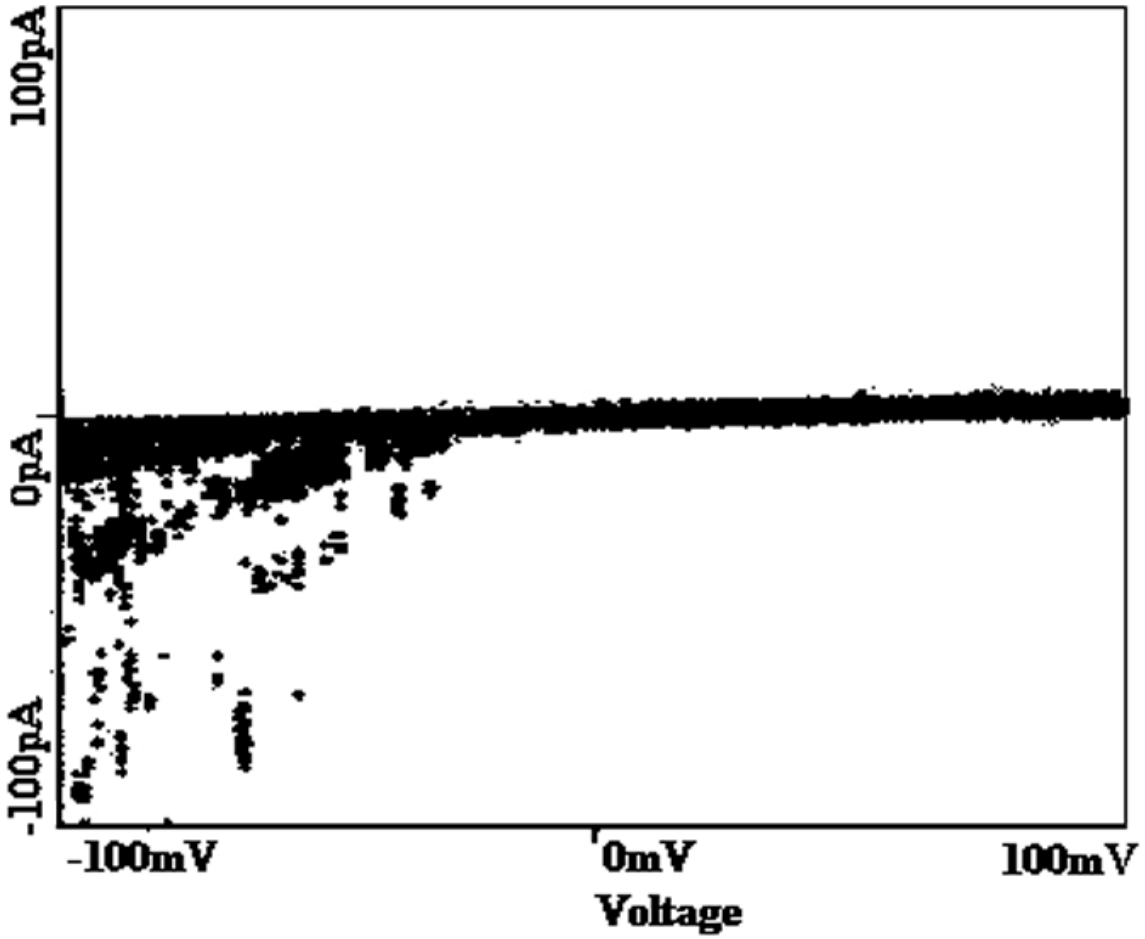

Figure 5. Elaiophylin channels' I-V surfaces in a patch clamped membrane after adding Elaiophylin to the bath (ramp. ±100mV; bath 1M KCl; pH 7.

4. Conclusions

Elaiophylin forms cation selective ion channels in model bilayer membranes with millisecond dwell times. The antibiotic has ionophoric activity and reduces the electrical resistance of the lipid membrane. The obtained volt-ampere characteristics were linear over a range of ± 100 mV.

The experiments in this work provide further evidence in support of the widely held view that Elaiophylin's primary mechanism of killing fungal and cancer cells is a result of formation of ion-permeable channels [5,6].

References

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