AUTOCATALYTIC TRANSPORT OF THE PEPTIDE ANTIBIOTICS SUZUKACILLIN AND ALAMETHICIN ACROSS LIPID MEMBRANES

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1. Introduction

Alamethicin, a peptide antibiotic, has been shown by Mueller and Rudin [1] to induce electrical excitability in artificial lipid membranes. This discovery prompted elaborate studies in many laboratories, which resulted in a detailed concept for the ionophoric action of alamethicin and several analogues ([2] covers the literature comprehensively). It was shown that alamethicin adsorbs to the membrane surface where monomers coexist with aggregates. Under the influence of an electrical potential certain aggregates form membrane spanning channels which are in a fast exchange with the monomer pool. Conclusive evidence for this mechanism was recently presented by Boheim and Kolb [2]. In most of these investigations both sides of the membrane contained equal amounts of peptide antibiotic. In the present study alamethicin or suzukacillin is added asymmetrically with respect to the lipid bilayer. This non-equilibrium induces a bistability of the membrane conductance. Direct evidence is given that this bimodal action refers to transitions between two modes of antibiotic flux across the membrane, a slow spontaneous flux and a flux of monomers via association with the channel aggregates. The latter process leads to a positive feedback mechanism (autocatalysis), which allows for a qualitative explanation of the main experimental findings.

2. Materials and methods

Suzukacillin (crude extract) was kindly provided by T. Ooka, Asahi Chemical Ind. Co., Japan [3]. The main component A, used in this study, was separated by preparative thin-layer chromatography on silica gel G. Alamethicin (R_F 30 fraction) was purchased from Microbiological Research Establishment, Porton Down, Salisbury. A further purification by thin-layer chromatography was necessary to obtain the R_F 30 fraction used. Dioleoylphosphatidylcholine was synthesized as described [4]. The formation of lipid bilayers and of Y-membranes from lipid monolayers, the manufacturing and pretreatment of the membrane frame and the measuring assembly were according to [5]. The control experiments mentioned in section 3 refer to: (a) other aperture pretreatment with hexadecane and squalene, (b) two other techniques to form the membranes: formation of black lipid membranes from 1% (w/v) lipids in *n*-decane and from vesicle-spread monolayers [4], and (c) the use of lecithin from bovine, egg and plant (Supelco Inc., Bellefonte, Pennsylvania) and of soybean lecithin (Sigma Chemical Co., St. Louis, Missouri).

3. Results

All results were obtained by adding suzukacillin or alamethicin to only one compartment of a membrane cell. The induced current was measured at constant voltage conditions. The sign of the electric potential always refers to the compartment to which no antibiotic had been added. The aqueous solutions were unbuffered and contained 0.1 M KCl. The results for suzukacillin and DOPC (dioleoyl-phosphatidyl-choline) bilayers at room temperature and

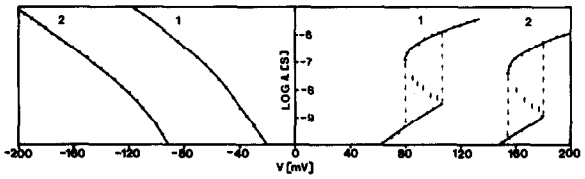

Fig.1. Steady state conductance–voltage relation for suzukacillin, added to only one compartment. The voltage sign refers to the antibiotic-free compartment. The two concentrations were $5 \cdot 10^{-7}$ g/ml (curves 1) and $1.5 \cdot 10^{-7}$ g/ml (curves 2).

membrane area of $2 \cdot 10^{-4}$ cm^2 were chosen for the presentation of data. Figure 1 shows the relation obtained between steady state conductance and voltage for two different suzukacillin concentrations. The conductance values for positive voltage lie at two branches well separated by a region of unstable states. Some details of the transitions between the two branches at the two thresholds are shown in fig.2. The current approaches a steady state level for each voltage value below (even just below) the threshold at 107 mV. At the threshold large current fluctuations occur, which eventually reach a critical amplitude after which the current increases with increasing rate towards its high steady state value. Further voltage changes result in a partial restoring of the instantaneous current changes. When the voltage was lowered beyond or to a second critical value (82 mV), the current decreased in a slow fashion to its low steady state level. The experimental points within the unstable region (double arrows in fig.1) have been determined at different conductance levels during the self-generative transition by reducing the voltage until the conductance was equally likely to decrease or increase continuously. These transitions occurred reliably and were well reproducible in their details. They were observed for all phosphatidylcholine lipids tested (cf. section 2) and for three different procedures to form the membrane (cf. section 2). At the high and the low steady state the same cation-chloride permeability ratios ($P_{Na}/P_{Cl} = 1.5$ and $P_K/P_{Cl} = 2.5$) were measured. Almost identical hysteresis loops in the conductance-voltage relations were found for alamethicin, only that the regenerative transition occurred several times more slowly. To investigate the molecular basis of these transitions, studies on recently developed 'branched membranes' [5], symbolically shown in fig.3, have been carried out. The electrical properties of two of the three membrane branches can be measured independently and simultaneously. Suzukacillin was again added to only

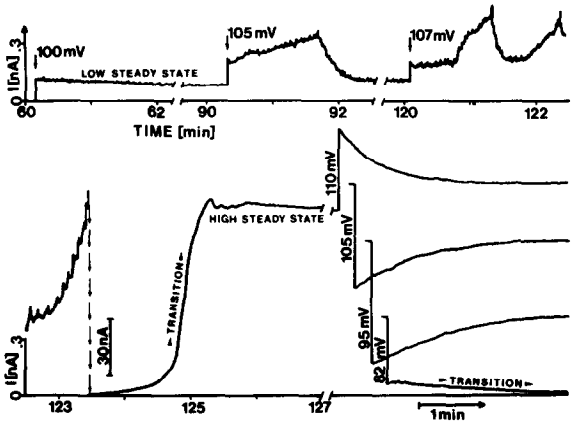

Fig.2. Time course of current close to and during the transitions shown in fig.1 (curve 1). Before the application of 100 mV the membrane had rested for 1 h at zero voltage.

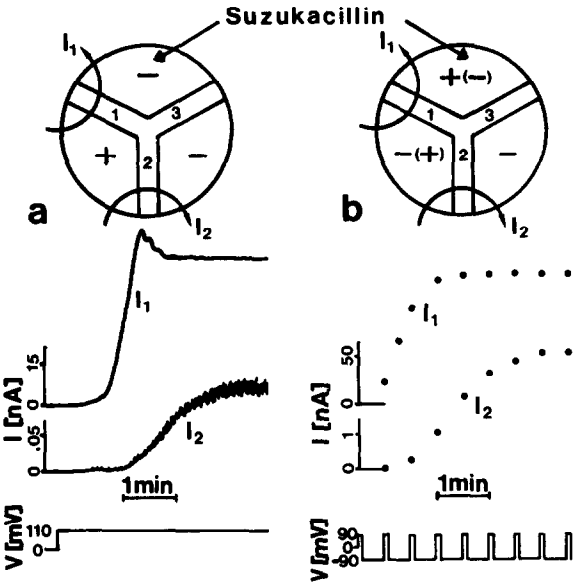

Fig.3. Transport of suzukacillin in Y-membranes for positive voltage (a) and negative voltage (b). The current values in (b) were measured during short voltage reversals (polarity in brackets). Suzukacillin concentration was $5 \cdot 10^{-7}$ g/ml. The area of each membrane wing was $1.1 \cdot 10^{-4}$ cm^2.

one compartment. In fig.3a a positive voltage is applied across membrane branch 1. The measured current I_1 shows the same regenerative transition as described before. Concomitantly, an increasing current I_2 is observed, which unambiguously demonstrates that the induction of channels for positive voltage needs the presence of antibiotic at the side of higher potential. Figure 3b shows that a transmembrane suzukacillin flux is also induced by a voltage of opposite polarity (−90 mV). The current values I_1 and I_2 were measured during intermediate short voltage reversals to +90 mV. For the qualitative discussion intended here three results of such experiments may be emphasized: (1) the high sigmoidicity of the current rise (fig.3b) indicates that, at least predominantly*, single antibiotic molecules (monomers) are crossing the membrane, (2) this monomer transport is dependent on the number of channels present and virtually independent of the membrane potential and the applied antibiotic concentration. (At different concentrations the value of the negative voltage in fig.3b was adjusted to give the same conductance; the I_1 and I_2 traces were about the same.) (3) Besides this monomer transport catalyzed by channels, a residual (spontaneous) monomer flux was measured in the absence of channels (the negative voltage in fig.3b was set to low values or to zero).

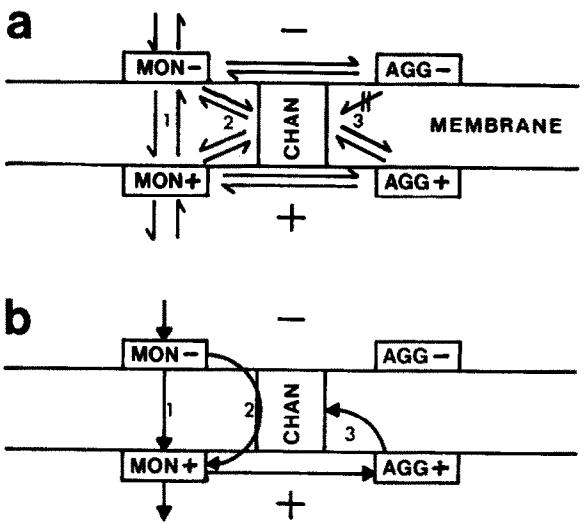

Fig.4. (a) Schematic drawing of the action of suzukacillin in the membrane which illustrates the main conclusions: Channel formation occurs only from aggregates at the positive side (3, see also footnote in the section 3). The monomers at both surfaces exchange either spontaneously or via association with the channel aggregates, symbolized by reactions 1 and 2. (b) The flow diagram showing the antibiotic flux across the membrane (1 and 2) and the feedback flux 3.

4. Discussion

The results obtained on Y-membranes are expressed in fig.4a in terms of three supplements to the otherwise well established reaction scheme.** The induction of channels for positive voltage (higher potential at the side initially free of antibiotic) always involved a transport of the antibiotic molecules across the membrane. Thus, channels apparently do not form from the side of lower potential (reaction 3 in fig.4a) as it was suggested by Gordon and Haydon [6]. The slow spontaneous monomer exchange between the two membrane surfaces (reaction 1) was enhanced by the induction of channels. As one mechanism for this catalysis one may consider diffusion through the aqueous channels. However, the observed current rise times are much shorter than the time for the necessary readsorption to the membrane (≈ 6 min). It also would be difficult to explain the observed restoring mode at the high steady state, the different transport rates found for suzukacillin and alamethicin and the absence of conductance for positive voltage reported for bacterial phosphatidylethanolamine membranes [7]. There seems only one alternative left: the channel aggregate not only exchanges monomers with the positive side, as is well established [2], but also with the opposite side (reaction 2). The occurrence of instabilities and of a regenerative transition as in figs.1 and 2 are intrinsic features of the thus supplemented model, as is best

* The time course and single channel analysis of the current I_2 in fig.3b for different voltage pulse sequences gave evidence for a transmembrane flux not only of monomers but also of a small number of channel aggregates; this aspect has been omitted here, because it does not interfere with the conclusions drawn

** The conclusions drawn are independent of the widely unknown position and conformation of the adsorbed antibiotic. Monomers and aggregates in fig.4 could equally well be drawn to enter the membrane to some extent

seen from fig.4b. The low steady state of conductance is determined by the spontaneous flux 1. With the induction of channels, their concentration will reach a critical value at which the monomer flux 2 via channels creates a monomer excess sufficient for the formation of additional channels (feedback flux 3). This cycle between flux 2 and 3 accelerates steeply (regenerative transition) and only slows down when a fast exchange equilibrium between the populations at the two interfaces is approached (high steady state). The partial restoring of the current levels in fig.2 is readily explained by a voltage dependence of this exchange equilibrium. Upon a reduction of the channel concentration by voltage decrease below a second critical value, the desorption loss from the positive side will exceed flux 2 and the concentration continuously decreases to its low steady state value. It may be added without proof that numeric predictions from the proposed model, based on the evaluation of the transport constants by the use of Y-membranes, are in quantitative agreement with experimental results like those in fig.1 (H. Schindler, unpublished results). The observed effect can be classified as a nonequilibrium transition between a membrane state of low permeability for both ions and the peptide antibiotics and a high permeability state. The transitions have been induced by voltage changes. However, from the concentration dependence in fig.1 it is apparent that transitions could equally well be generated by a change of antibiotic concentration at constant voltage. For certain rates of antibiotic addition to the negative side a stationary oscillation between the two states of antibiotic permeability is expected. It is intriguing to ask whether this regulation is involved in the release of such antibiotics by microorganisms.

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