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The unit conductance channel of alamethicin

L.G.M. GORDON and D.A. HAYDON

Physiological Laboratory, University of Cambridge, Downing Street, Cambridge (Great Britain)

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SUMMARY

Some properties of the unit conductance channels of alamethicin are reported. The data suggest that, in lipid membranes, alamethicin forms relatively highly conducting pores, which occur in two-dimensional aggregates and interact with each other.

Alamethicin is a cyclic polypeptide antibiotic of molecular weight approx. 1700 and of known primary structure[1,2]. It has been shown that this substance produces interesting and unusual ion transport properties in lipid membranes[3]. Thus, an ion conductance is induced which may be either cationic or anionic according to the absence or presence, respectively, of certain basic molecules such as protamine or histone. The conductance may also increase sharply (roughly according to the sixth power) with increasing alamethicin concentration, electrolyte concentration and applied potential. If the alamethicin is placed initially on one side only of the membrane, asymmetric current–voltage curves are obtained. These observations have been confirmed by Chapman *et al.*[4].

In addition to its effects on artificial membranes, alamethicin has been shown by Pressman[5] and by Glynn and Hoffman (personal communication) to produce ion movements across certain biological membranes.

In their original paper, Mueller and Rudin[3] speculated on the mechanism of action of alamethicin and favoured the formation of pores by aggregates of about six molecules. Information concerning molecular mechanisms is very difficult to obtain in this type of system from observations of conductance at high levels and, if feasible, the examination of isolated conductance channels is much more helpful. This approach has been used for gramicidin A[6,7], and for the excitability inducing material (EIM) of Mueller and Rudin[8,9] Some success with this approach has now also been achieved for alamethicin, and the essential features of the results are described below.

The experimental techniques resembled those used for gramicidin A[6]. Important differences in the recording apparatus were necessary, however, as the current fluctuations produced by alamethicin can be very rapid. For this reason, traces were obtained initially by means of a tape recorder and were subsequently transposed to an ultraviolet recorder. A typical example of the current through the membrane, as a function of time, for a very low alamethicin concentration ($< 10^{-8}$ mole/l) is shown in Fig. 1. The time scale is much

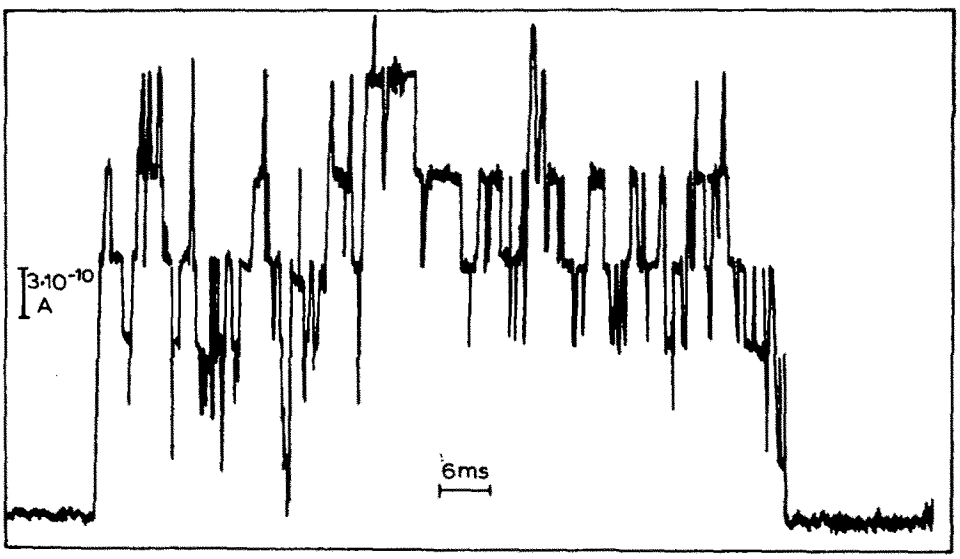

Fig. 1. Fluctuations in the current through a bilayer membrane, formed from glyceryl monooleate and *n*-hexadecane, in the presence of a very small amount of alamethicin. The aqueous phase was 2 M KCl and the applied potential 210 mV. The baseline at each end of the group of fluctuations corresponds to the conductance of the pure lipid membrane. (For clarity in reproduction the original record has been traced.)

smaller than for gramicidin but the currents are roughly one order of magnitude larger.

As can be seen, the current rises from zero, fluctuates violently for a period, and returns again to zero. A relatively long interval may then elapse before the process is repeated. The grouping of the fluctuations in this manner is a common phenomenon during conduction at low levels and the frequency of occurrence of the groups is dependent on the amount of alamethicin present, the electrolyte concentration and the applied potential. Higher frequencies are observed when the alamethicin is placed on the positive side of the membrane. At sufficiently high concentrations of the polypeptide, the groups occur frequently enough to overlap in time and, ultimately, give high average conductances in which fluctuations can no longer be resolved.

Within a group of fluctuations the current tends to take certain well-defined values. The different values, however, occur with different probabilities and is not entirely clear from Fig. 1 that some of the higher and lower levels exist. The whole range of levels is more readily demonstrated by the superposition of many transitions (occurring over an appreciable period) in a small recording space. This may be achieved by the use of a storage oscilloscope and yields the type of result shown in Fig. 2. The intensities of the lines

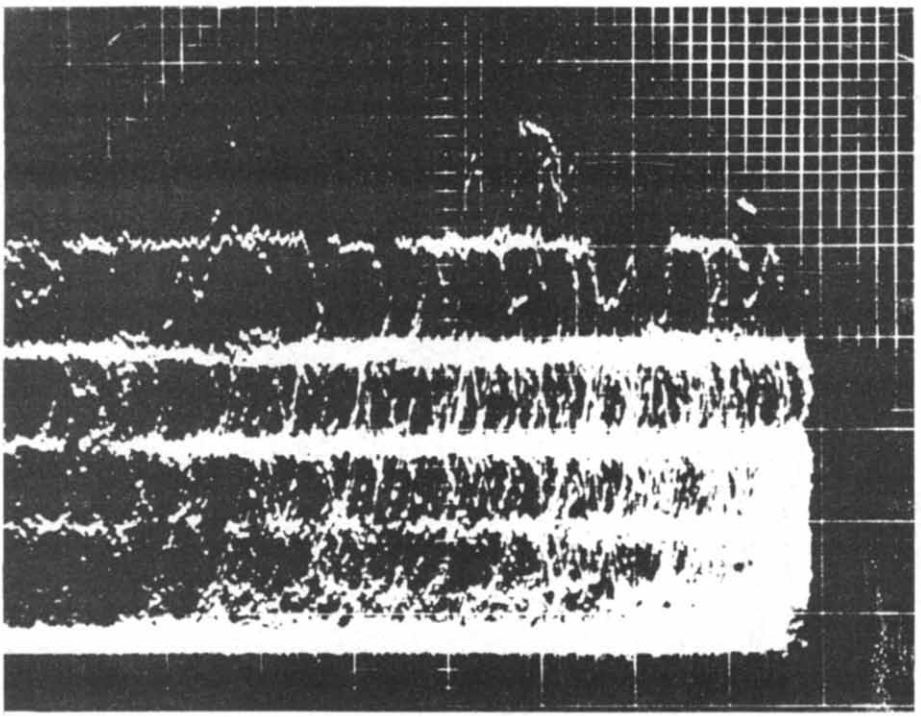

Fig. 2. Current fluctuations for the same system as in Fig. 1, recorded over a considerable period on the screen of a storage oscilloscope. The trace was triggered on the right hand side of the record by the leading edge of a current transition. (1 large division $\equiv 5 \cdot 10^{-10}$ A.)

represent the probabilities of finding the various levels. In this instance the third and fourth levels have the highest probability. Although, on careful inspection of several records, some very faint levels may be found which are equally spaced, the more obvious levels diverge as the current rises. Thus, in 2 M KCl and for 210 mV applied, the current may take the values ($\times 10^{-10}$) 3.6, 7.8, 13.3, 19.3, 26.2 and 33.0 A. For each level the current–voltage relationship curves somewhat towards the current axis, but for NaCl and KCl at 1–2 moles/l the integral conductances are approximately proportional to the electrolyte concentration. As found for gramicidin, the conductances of the respective levels do not vary when, by means described elsewhere[10,11], the thickness of the membrane is changed. Although precise data cannot be quoted, the channels exhibited poor ion selectivity, a conclusion which is consistent with the comments of Mueller and Rudin[3] and Chapman *et al.*[4]. At macroscopic levels of conductance, the systems behaved in much the same way as reported previously[3,4].

The results presented above clarify to some extent the mechanism of action of alamethicin. That pores are formed through the membrane seems almost certain. Not only is the unit channel conductance independent of the thickness of the membrane, but the ion fluxes through the channel are too large to be accounted for convincingly by a carrier process. Thus, if the membrane is 30 Å in thickness and the polypeptide carries one ion,

the diffusion coefficient of the carrier in the membrane would have to be approx. $\gtrsim 1.5 \cdot 10^{-5}$ cm^2/sec in order to produce the observed fluxes. Even for ions moving through a pore this value, which is comparable to that for K^+ and Cl^- in aqueous solution, is unexpectedly high and suggests that the pore may be rather wide. An alternative possibility, however, is that the pore may be relatively narrow and the diffusion coefficient less than that given above, but that these factors are compensated by the length of the pore being less than 30 Å. The poor ion selectivity suggests that the pore may be wide. That the pore should be either wide or short, or both wide and short, is consistent with the fact noted by Mueller and Rudin that in the presence of the positively charged macromolecules protamine, spermine and histone the selectivity is changed from predominantly cationic to anionic. For a narrow channel (in which the main ion binding sites could be well away from the entrances) it is difficult to imagine how this could occur except by means of a major conformational change.

Other notable features of the results are the non-additivity of the channel conductances, the grouping in time of the fluctuations and the inequality of the line intensities in Fig. 2. All *three* effects suggest that cooperation occurs between individual channels. The absence in Fig. 2 of integral multiples of the low conductance levels, implies that groups of fluctuations, such as in Fig. 1, arise from one assembly of interacting channels. The lack of additivity of the conductance and the fact that the third or fourth levels of conductance seem more probable than others are presumably consequences of this interaction. As a mutual enhancement of the individual channel conductances occurs, it is possible that surface charge effects are responsible. Thus if, when it became conducting, each alamethicin molecule made an additional contribution to the surface charge, and the ionic atmospheres of these charges overlapped, the effective ion concentration adjacent to each channel would depend on the number of open channels. The conductance of two juxtaposed channels would then be more than twice that of a single channel, that of three channels would be more than 3/2 times that of two channels, and so on. A limit to this cooperativity would probably be reached for about seven channels. On the present evidence, however, the results could also arise from an increase in the size of a channel on the opening of adjacent channels.

To conclude, it appears that alamethicin may form two-dimensional aggregates on the surface of a membrane, and that under an applied potential each of these yields clusters of short or wide (or perhaps both) pores situated closely adjacent to each other, the total conductance of which is greater than the sum of the individuals.

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