TOWARD A MOLECULAR UNDERSTANDING OF EXCITABILITY

ALAMETHICIN IN BLACK LIPID FILMS

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Small molecules like alamethicin can produce conductances remarkably like those found in excitable biological membranes. For example, maximum sodium conductance and maximum potassium conductance in squid giant axons both increase *e*-fold for a change in potential of 4–5 mV just as the conductance induced by alamethicin does (Hodgkin and Huxley, 1952, for squid; Eisenberg et al., 1974, Mueller and Rudin, 1968, for alamethicin). We are thus justified to some extent in wondering if there is a fundamental similarity in the mechanism by which these different conductances are increased by voltage.

The molecules which produce strongly voltage-dependent conductances in excitable membranes must have interesting properties related to this specialized function. We are beginning to get data from a variety of sources which hint at the nature of some of these properties. This monograph will explore how the study of conductances induced in black lipid films by certain antibiotics contributes to our knowledge of possible molecular mechanisms of voltage-dependent conductance.

Studies of the conductance induced in black lipid films by alamethicin, a small peptide with molecular weight about 1700, serve as a good paradigm representative of the current state of knowledge and illustrate precisely the sorts of conclusions we can and cannot draw from such studies.

The conductance induced by alamethicin results from a statistical formation of conducting entities. Each entity, once formed has its own statistically fluctuating conductance properties (Eisenberg et al., 1974). At high conductance levels a given measurement of electrical properties reflects only some sort of average of the statistical properties of entity formation and of the fluctuation in conductance of each single entity. For convenience, we will call such a high level measurement a *macro-level measurement*. Kinetics measurements, response of current to a voltage pulse, and steady-state voltage-current curves are all examples of macro-level measurements. A particular example of a macro-level measurement is shown in Fig. 1. This is a voltage-current curve of a black lipid film which separates salt solutions of unequal concentration and to which alamethicin has been added. Note that the curve shows a pro-

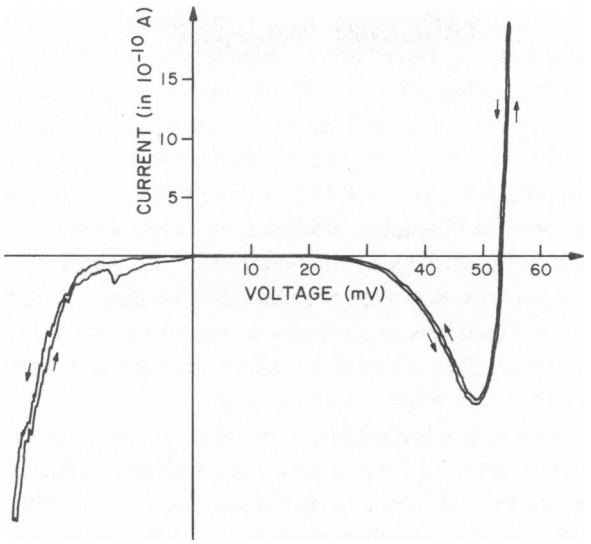

FIGURE 1 An example of a *macro-level measurement:* a voltage-current curve of a black lipid film formed from phosphatidyl ethanolamine (PE):decane (12 mg/ml) in a 100:1 KCl gradient (0.005 M KCl:0.5 M KCl and 9×10^{-6} g/ml alamethicin:6×10^{-7} g/ml alamethicin).

nounced negative resistance. Many models can be generated which explain not only this curve but additional macro-level data not discussed here.

If we make measurements at a much greater current sensitivity than for a macro-level measurement and with adequate time resolution, we find that the conductance increases in steps (Gordon and Haydon, 1972; Eisenberg et al., 1973). Fig. 2 shows an example of such a measurement. The voltage is clamped at a particular value and the resulting current fluctuations measured. We will call measurements which reveal such

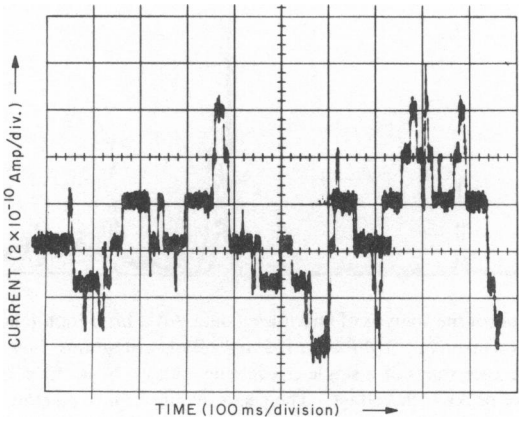

FIGURE 2 An example of a *micro-level measurement:* trace of alamethicin induced conductance fluctuations for a PE:decane film in 1 M NaCl and 10^{-7} g/ml alamethicin. The voltage is 145 mV.

small discrete fluctuations in current *micro-level measurements*. We will discuss ways in which such data can be analyzed later. Eisenberg et al. (1973) have shown that, for alamethicin, the fluctuations like those shown in Fig. 2 come in packages (the "entities" of the preceding paragraph), and increasing the voltage increases the number of these conducting entities in the membrane. In the same way that many models could be generated to explain the macro-level data, so many models can be generated which can explain these results. But the micro-level data not only allow us to discard a number of models which can describe well the macro data, but suggest experiments at the micro-level which can narrow down the possibilities even more. The remainder of this discussion will try to indicate how this process works and to show how micro-level results can be used to obtain data relevant to understanding the molecular properties of alamethicin responsible for its ability to voltage gate.

Micro-level data probably reflect events at the molecular level (perhaps the aggregation of individual monomers to form a conducting channel (Bauman and Mueller, 1974) or the change in conformation of a cluster of molecules resulting in a step change in conductance. We should therefore require, at a minimum, that any model of alamethicin-induced conductance take into account micro-level data, which presumably measure directly some of the rate constants of the molecular processes involved. Models can also be tested by studying the effect of experimental manipulation on the micro-level results. Such experiments can give valuable clues about the nature of the molecular processes which are occurring.

As an example of how this can be done, let us consider a few limited ideas about molecular interaction which may be important in some voltage-dependent conductance mechanisms, and see how these ideas can be tested in the alamethicin black lipid film system using currently obtainable micro-level data.

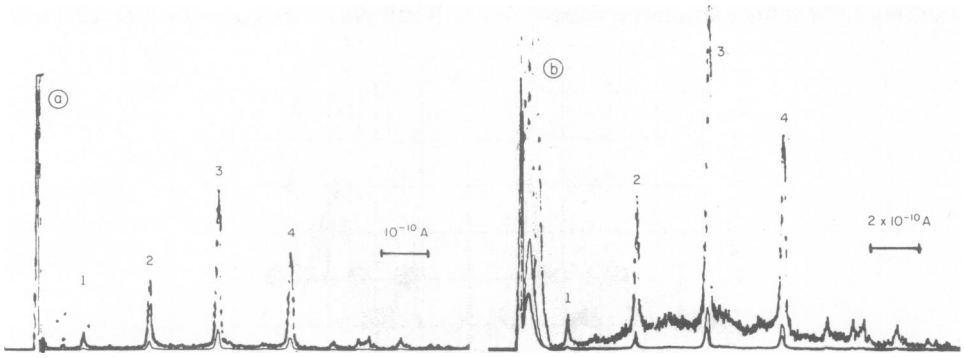

FIGURE 3 An example of the analysis of micro-level data: (*a*) a histogram taken in 1.0 M KCl at 99 mV. (*b*) a histogram taken in 1.0 M KCl at 155 mV. Both histograms show the relative probabilities of the conductance states of a single conducting entity. Note there is no change in the relative heights of the peaks with voltage. The peaks arising from alamethicin-induced conductance are labeled 1, 2, 3, and 4 for identification. The peak farthest to the left is at the conductance of the bare membrane. The ordinate is current and the abscissa is number of occurences.

One of the most revealing ways to analyze micro-level data of the sort shown in Fig. 2 is to construct a probability histogram. Such a histogram shows the relative probability of a given conductance level. Since for alamethicin-induced conductance at low conductance levels the conductance increases stepwise, a histogram under these conditions consists of a series of peaks, whose relative heights are proportional to the relative probability of the corresponding conductance. Such histograms are shown in Fig. 3 for two different experimental conditions. One way of testing various ideas of molecules interaction is to observe how the histogram changes under various experimental conditions. If the higher conductance states correspond to a movement of more gating charge[1] down the field, increasing the voltage should increase the probability of the higher levels and decrease those of the lower levels. Figs. 3 *a* and *b* show probability histograms at different voltage under otherwise identical conditions. The relative probabilities of the levels are unchanged. Boheim (1974), on the other hand, has found at low temperature that the relative probabilities of the steps are voltage dependent. The experimental situation is therefore unclear, and an attempt must be made to reconcile these two sets of data obtained under different conditions. Any proposed model should be able to do this.

Changing salt concentration produces a very dramatic change in the probability histogram. Most investigators agree that single alamethicin molecules aggregate in some way to form conducting channels. We would like to know how this aggregation takes place: does the field push alamethicin monomers (or dimers) into the membrane and do these small units then aggregate to form multimers which correspond to the different conductance state? Or do alamethicin molecules aggregate to form essentially two-dimensional micelles on the surface of membrane, and does the field then push these aggregates into the membrane where they flutuate among various conformations corresponding to the discrete conductance changes? The salt concentration micro data gives tentative, but by no means certain, support to the two-dimensional micelle view.

Changing salt concentration can be though of as altering the electrostatic interaction by changing the amount of charge screening (i.e. altering the Debye length). At low salt, the electrostatic repulsion is stronger than at high salt. We know that alamethicin is charged (Meyer and Reusser, 1967; Payne et al., 1972) and consequently that the repulsion between aggregates should increase at low salt. Hence in the aggregation picture we would expect that decreasing the salt concentration should change the probability histogram and make the higher conductance state *less* probable. This is because a successful collision of a monomer with an aggregate becomes less probable by virtue of the increased electrostatic repulsion. In the micelle picture, the higher conductance states correspond to more open configurations. Hence the charges would be further apart and the coulomb interaction weaker for high conductance states. At low salt,

[1] "Gating charge" here means charge which moves in the membrane parallel to the electric field. It might be movement of a single charge, multiple charges, or the rotation of a dipole or dipoles. It should not be confused with ionic current.

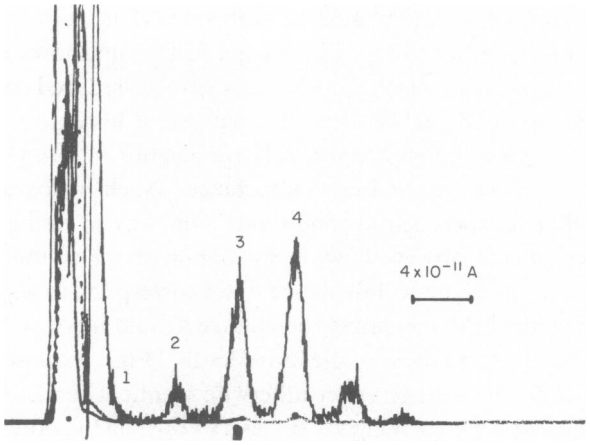

FIGURE 4 A distribution just like those of Fig. 3 except that the salt concentration has been lowered to 0.1 M KCl (taken at 158 mV). The numbers 1, 2, 3, and 4 identify peaks corresponding to those with the same numbers in Fig. 3. (The positions of the peaks are fixed by their conductances which vary in a known way with salt concentrations.) Note that the heights of the peaks corresponding to greater conductance have increased relative to those of low conductances. Peak 1 is not observable and peak 4 is now higher than peak 3. The confusion at the left results from the bare membrane peak being much higher than the other peaks. The position of its maximum is marked by a dot on the abscissa.

where the coulomb interaction is strong, the repulsion between charged molecules would thus tend to push the molecules into more open configurations, and the higher conductance states would become more probable than at high salt. Fig. 4 shows that this is indeed what happens: as the salt concentration is lowered, the higher conductances increase in probability. It thus seems possible that the micelle picture fits the experimental data better than the step-by-step aggregation picture.

The point of this discussion is that the data used to evaluate a model must include data at the micro-level when such data are available. If these data are not used, many multiparameter models will fit the micro-level data.

We have given only a few sketchy examples of how micro-level data can be used to discuss the nature of the molecular interactions responsible for the voltage-dependent conductance of alamethicin. Clearly much more complete and quantitative analyses can be made (see, for example, Boheim, 1974). In the mean time, the most direct data on molecular interaction is available in artificial membrane systems. In these systems we can examine at a molecular level *some* mechanisms of voltage-dependent conductance. We will have to wait and see if these mechanisms turn out to be similar to those in biological membranes. Probably we will have to know how to incorporate the relevant biological molecules into black lipid films before this question can be finally settled. The studies of artificial systems should tell us something about how to do this intelligently.

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