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Histograms of Conductance Fluctuations Induced by Alamethicin in Black Lipid Membranes

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SUMMARY

Histograms of membrane currents were obtained from alamethicin-induced conductances in black lipid membranes. One molar chloride salt solutions were used as electrolytes. At room temperature the histograms showed striking resemblances to binomial distributions, consistent with a theory that alamethicin pore conductance states may arise in parallel from sets of similar pores functioning independently. A ten-degree rise in temperature produced a new binomial distribution corresponding to an altered probability that an individual pore is in the open state.

INTRODUCTION

In recent years much attention has been focused on the mechanism of pore formation by the polypeptide antibiotic alamethicin in black lipid membranes (1,2,3,4,5). A primary motivation for these studies has been the voltage-dependent conductance of the modified black lipid membranes and a distinct resemblance to the conductance of potassium channels in axon membranes (1,6), but the observation of discrete conductance states in small (0.1 mm^2) modified black lipid membranes has stimulated separate investigations on the structure of alamethicin pores. It is fairly well established that the behavior seen during a burst of discrete conductance fluctuations is but weakly voltage-dependent. Thus the voltage-dependence of net membrane conductance probably lies in voltage-dependence of the likelihood of initial pore formation rather than in existing pore behavior. At present it is thought that pores may be structures which operate either like irises through

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a sequential set of diameters (2), or else that a pore is an array of similar, parallel channels (3). Evidence has been given in support of each model. In this work we present and discuss additional evidence which supports the parallel model.

MATERIALS AND METHODS

Black lipid membranes were formed between two electrolyte solutions, using methods and vessel design given by Eisenberg, et al (1). Membrane-forming solutions were either bacterial phosphatidyl ethanolamine (Supelco) or di-dihydrosterculoyl phosphatidyl choline (a gift of Dr. Will Veatch), made to 1% in n-decane (Aldrich).

Alamethicin was generously supplied by Dr. George Whitfield of the Upjohn Co. and was used without further purification. Aliquots of a 10^{-3} gram/ml solution in distilled H_2O were added to one of the solution chambers to give final concentrations between 10^{-7} and 10^{-6} gram/ml. Addition was always after membrane formation and blackening, and to one side only.

The electrolyte solutions were 1 molar salts, buffered to pH 7 with 5 mM tris in deionized distilled water. $MgCl_2$ and CsCl were purchased from Fisher; NaCl from Mallinkrodt, and $CaCl_2$, RbCl and KCl from Ventron-Alpha. $MgCl_2$, CsCl and NaCl were reagent grade; $CaCl_2$, RbCl and KCl were "ultrapure".

The electronic circuitry associated with measurement of membrane currents is essentially identical to that of Eisenberg, et al (1), except that a pre-amplifier with flat response from DC to 40 kHz was used to amplify signals from the high input impedance current amplifier, in order to optimize inputs into a SAICOR correlation and probability analyzer (Honeywell Instr.). Silver chloride electrodes were freshly coated for each experiment. For preliminary current-voltage measurements a function generator built around an Intersil 8038 integrated circuit was used for generating trans-membrane voltages. For recording current histograms a battery and resistive voltage divider were used as the voltage source.

Formation and existence of black lipid membranes were monitored first visually and then by capacitance, estimated from current voltage curves and the relation, $i_C = C(dV/dt)$. Based upon such capacitance measurements and visual observation of the blackened area, our membranes were typically 0.1 mm^2 in area with specific capacitance approximately 0.5 $\mu F/cm^2$ (1).

After the capacitance measurement alamethicin was added to one of the electrolyte chambers, and the solution was stirred by a small teflon-coated stir bar for two minutes, while the membrane voltage was maintained at zero. After a brief period, usually five to ten minutes, the voltage was raised slowly, positive on the side with alamethicin, until conductance fluctuations could be seen on a Tektronix 5103 oscilloscope monitoring the output of the current amplifier. The voltage was left at this level, and the recording of the histogram was begun. Data collection would halt either when a preset number of points (typically 32000) had been measured (usually at a rate of 2000/second), or when the membrane ruptured, in which case the number of data points could be estimated from elapsed time.

The current amplifier signal and the SAICOR probability density function output were displayed simultaneously on the Tektronix oscilloscope. This arrangement allowed us to be certain that fluctuations represented a stationary process by observing uniform growth of the histogram during the conductance fluctuations.

RESULTS AND DISCUSSION

Alamethicin-modified membranes showed behavior similar to that described by other workers (1,2,3), in particular with respect to current-voltage

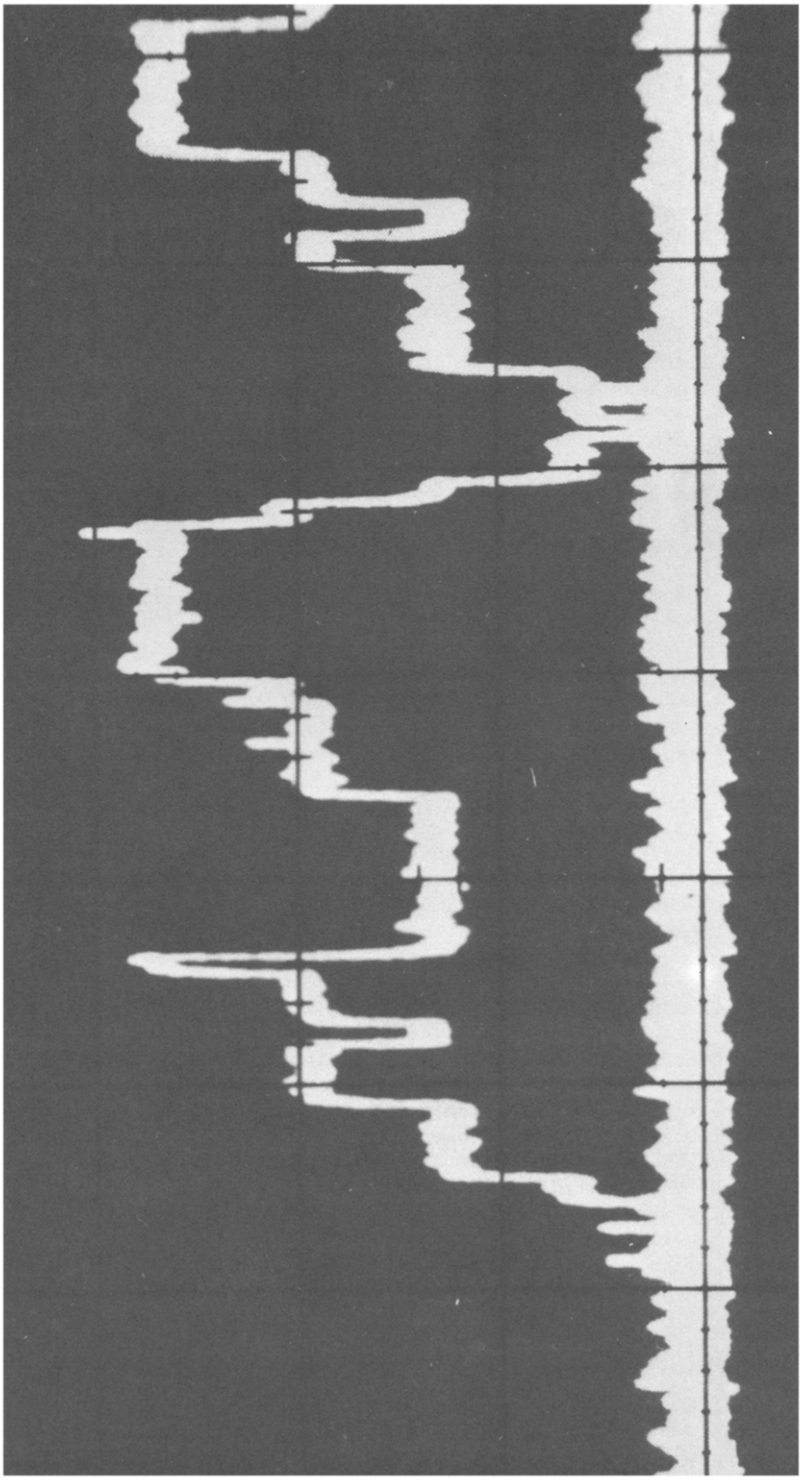


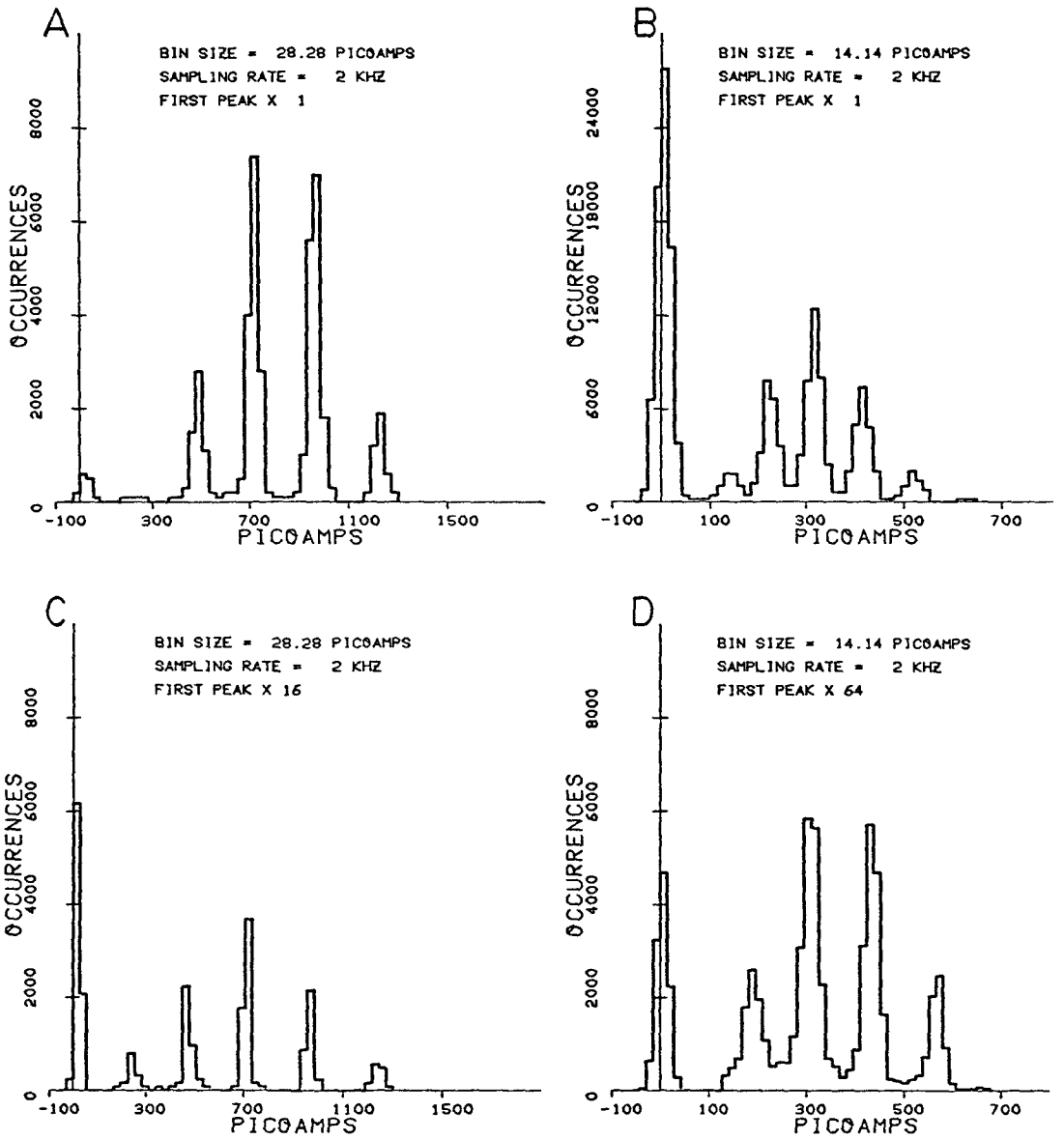
Figure 1. Oscilloscope trace of membrane current. The vertical scale is $2x10^{-10}$ amps per division; the horizontal scale is 20 milliseconds per division. Alamethicin concentration was $1.2x10^{-7}$gm/ml in 1 M NaCl on the positive side of a bacterial phosphatidyl ethanolamine membrane.

relations, occurrence of discrete conductance fluctuations, and some drift in absolute conductance (4), occurring occasionally as a sudden shift. (Data were recorded between such shifts.) Figure one shows an oscilloscope trace of a burst of conductance fluctuations very similar to those reported by other workers (1).

Figure one shows current, or conductance, dwelling in discrete states, including one which would be observed with an unmodified membrane. A sampling rate for histograms was chosen so that several current measurements could be made during the time in which conductance dwelt in a particular state. Since the bin size, or current range for one counting channel was one-half to one-fourth the spread of current background noise, a given conductance state could register counts in two or more adjacent bins. Thus a burst of conductance fluctuations like that of figure one gives rise to histograms like those of figures 2 - 4.

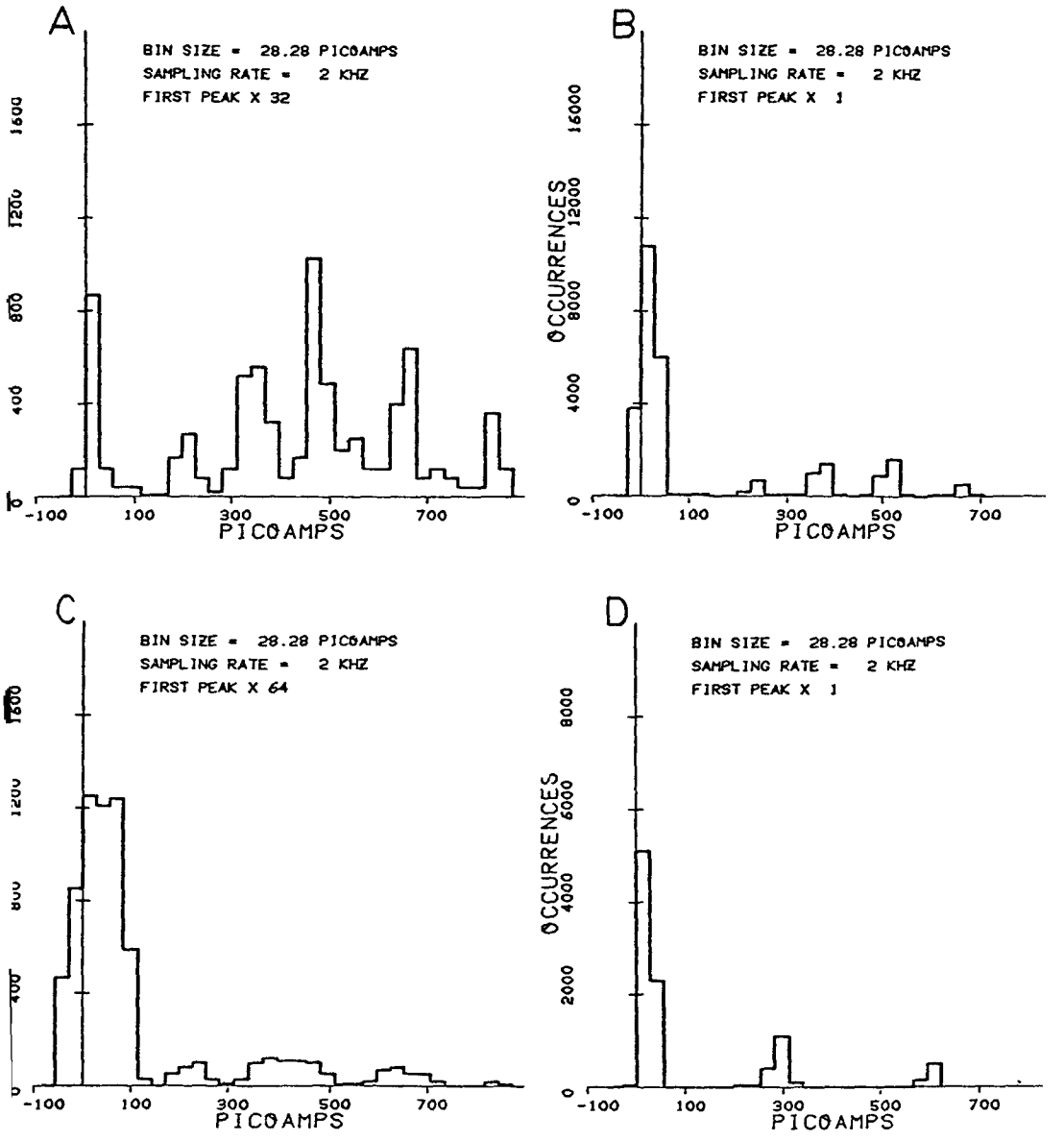
We have observed that many, but not all of our histograms show peak series whose areas correspond to ratios of the binomial distribution, i.e. 1:3:3:1 and 1:4:6:4:1. These patterns were most prominent when 1 M KCl was the electrolyte. Figures 2a - 2d are exemplary data obtained with KCl. We subsequently undertook a survey of the effects of substituting other cations, such as Cs, Na, Rb and Ca, and of substituting the membrane lipid with purely synthetic lecithin, di-dihydrosterculoyl phosphatidyl choline, and of raising temperature ten degrees. Figures 3a - 3d show sample histograms obtained with different cations, while figures 4a - 4c show the results of the lipid substitution and the temperature rise. These histograms represent our more interpretable data, rather than average data, which was often subject to the above-noted conductance shifts.

Conductance increments from one state to the next can be calculated by dividing the current increment by the voltage. These appear to be strictly ohmic, i.e. independent of voltage providing that the components of the system (i.e., cation, lipid, etc.) are held constant. Conductance increments change



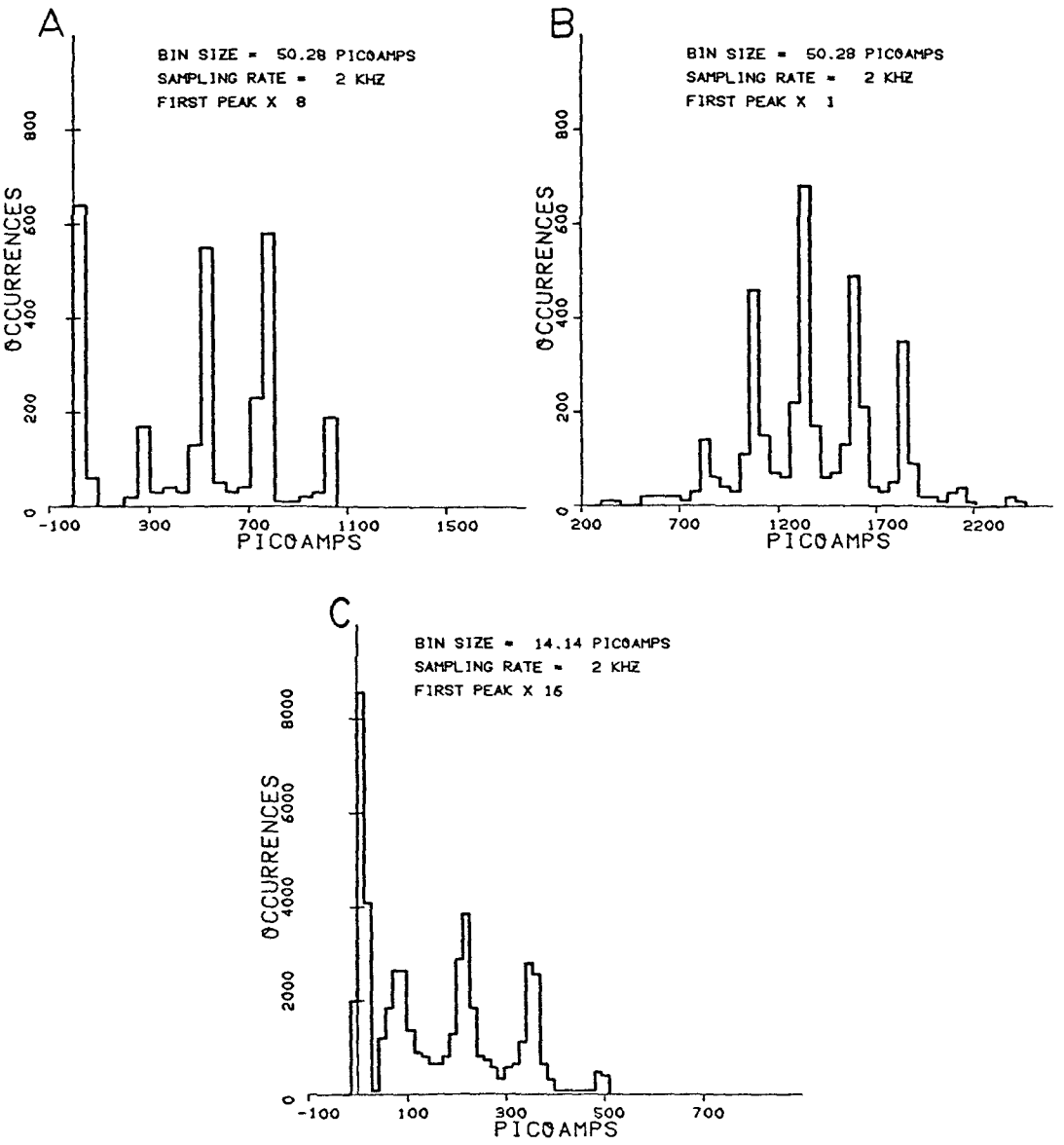
Figures 2a - 2d. Histograms of membrane current in presence of 1 M KCl. Membranes were each formed from bacterial PE; alamethicin concentrations on the electrically positive side were 2.4×10^{-7} gm/ml (a) and 7.5×10^{-7} gm/ml (b-d). Voltages were 137 mV in (a), 138 mV in (b), 48 mV in (c) and 35 mV in (d).

when new cations or lipids are introduced. Our values for these cation-depende increments are in fair agreement with those reported by Eisenberg, et al (1), using the same lipid source, bacterial phosphatidyl ethanolamine.



Figures 3a - 3d. Histograms of membrane current in presence of various chloride salts. Membranes were formed from bacterial phosphatidyl ethanolamine. Salts, alamethicin concentrations and voltages were as follows: (a) 7.5×10^{-7} gm/ml alamethicin, 1M NaCl, 150 mV; (b) 2.4×10^{-7} gm/ml alamethicin, 1M CsCl, 85 mV; (c) 2.4×10^{-7} gm/ml alamethicin, 1M RbCl, 128 mV; (d) 1.2×10^{-7} gm/ml alamethicin, 1M $CaCl_2$, 224 mV.

The significance of the fit of histograms to binomial distributions is in the support such data give to a model of parallel pores. Given sets of several similar pores, each with probability p of being open, probability theory would



Figures 4a - 4c. Histograms of membrane current in presence of 1 M KCl. Membranes were formed from di-dihydrosterculoyl phosphatidyl choline (a and b) or bacterial PE (c). Figures a and b were recorded at room temperature (23°C) while figure 3 was recorded at 33°C. Alamethicin concentrations and voltages were as follows: (a) 2.4×10^{-7} gm/ml alamethicin; 180 mV; (b) 2.4×10^{-7} gm/ml, 170 mV; (c) 7.5×10^{-7} gm/ml, 72 mV.

predict histograms like those shown, assuming the pores fluctuated from closed to open at random, and independently. It cannot be claimed that the data rule out any other models, such as the iris, or barrel stave model, described in

detail by Boheim (2). Rather, we have found that the binomial distribution gives the better fit with our data. (Statistical methods for fitting binomial distributions to the histograms are given in (7).)

It has been reported previously that different numbers of conductance states may be realized in different conductance experiments. In using the binomial distribution for interpretation of histograms, the number of conductance states should be one more than the number of pores. Thus, figure 2a would represent a group of three pores, while figure 2b would represent a group of four pores. Figures 3d and 4b represent unusual cases, namely of a group of one pore only and a group of eight pores.

It is probably coincidental that distributions obtained at room temperature are described by the specific binomial coefficients, 1:3:3:1, etc., because these represent equal probabilities of an individual pore in the group being open or closed. We reasoned that the above mentioned probability p should be a ratio of the opening and closing transition probabilities and hence rate constants for the same. Thus, $p/(1-p)$ may represent an equilibrium constant which should vary with temperature when there is an enthalpic contribution to the energy difference between the closed and open states. Figure 4c suggests that the open state may have lower enthalpy. If the probability p is changed to 0.40 (probability of being open), the resulting binomial distribution calculated for three pores gives a good fit. The same value of p gave a good fit with other distributions of four conductance states as well at the same temperature noted in the legend for figure 4c (data not shown). The effect of a rise in temperature is to shift the open/closed equilibrium towards closed. The enthalpic interpretation follows from this and from LeChatlier's principle.

Although our findings of interpretability by the binomial distribution are encouraging, some features of the histogram are less easily interpretable. First, the cause of the variations in numbers of pores fluctuating during the recording of one histogram is not known. We have seen this vary from one (fig. 3d) to eight (fig. 4d). Second, the conductance which corresponds to zero

open pores in a group is quite distinct from the conductance of an unmodified membrane. The conductance of such a state could be due to a core, or central opening in a group of pores (such a model is proposed by Gordon and Haydon (3)), or perhaps due to a local alteration in membrane structure upon pore formation. Finally, the conductance increment grows distinctly larger among the higher conductance states, as has been noted in previous studies (1,2,3). We have calculated on that basis of a hydrodynamic model of alamethicin pores (8) that the barrel stave model would predict even greater divergence than is observed here and elsewhere (1,2,3). Some of the divergence of conductance increments at higher levels could be accounted for on the bases of the hydrodynamic model and the parallel pore model by assuming that individual pores were contiguous with a central core.

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