EVALUATION OF SURFACE TENSION AND ION OCCUPANCY EFFECTS ON GRAMICIDIN A CHANNEL LIFETIME

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ABSTRACT The surface tension of glycerylmonooleate–hexadecane lipid bilayer membranes and the lifetime of gramicidin A channels were measured at various concentrations of the surrounding solutions. For HCl the surface tension is essentially constant at ~5 mN/m up to ~1 M, whereas the average lifetime increases ~40-fold. At higher concentrations the surface tension decreases markedly. For CsCl the surface tension is constant up to about 1 M then increases with salt level. The average lifetime in this case increases about sixfold. In both cases the lifetime levels off and even decreases at higher salt levels. The increase in lifetime observed with ion activity is therefore qualitatively different from, and not explained by, the established dependence of lifetime on membrane properties (Elliot, J. R., D. Needham, J. P. Dilger, and D. A. Haydon. 1983. *Biochim. Biophys. Acta.* 735:95–103). We have previously proposed that ion occupancy is a determinant of channel stability, and to test this hypothesis the voltage dependence of channel lifetime was measured in asymmetrical solutions. For the case of a potassium chloride solution on one side of the membrane and a hydrogen chloride solution on the other, the voltage dependence of the lifetime is asymmetrical. The asymmetry is such that when the electrical field is applied in the direction of the chemical gradient for each of the ions, the channel lifetime approaches, at increasing field strengths, that of a symmetrical solution of the respective ion. The voltage dependence of the surface tension, on the other hand, is negligible for the range of voltages used. These results, and the earlier findings that the order of the lifetimes for the alkali cations generally agree with the order of the permeability selectivity of the gramicidin A channel, support the hypothesis that ion occupancy is a major factor determining the lifetime of gramicidin A channels. The effects of multivalent blockers and osmotic agents were also tested. Ba^{2+}, La^{3+}, and Mg^{2+} decrease the lifetime and conductance markedly. Sucrose and urea increase the lifetime and decrease the conductance. The voltage dependence of the lifetime in symmetrical solutions was examined. Contrary to previous reports it was found that the lifetimes for K^+, Cs^+, and H^+ are voltage dependent. For 0.5 M HCl the lifetime decreases monotonically by ~60% at 150 mV, and for 0.5 M KCl the lifetime increases by ~60% at 200 mV. Below 10 mM there is no effect of voltage for H^+, K^+, and Cs^+. These effects of blockers, osmotic agents, and voltage on the lifetime, as well as the lack of effect of voltage at low salt levels, are consistent with the occupancy hypothesis.

INTRODUCTION

The valine gramicidin A pentadecapeptide forms transmembrane channels in bilayer and cell membranes by head-to-head dimerization (1–4), forming a single-stranded helical ($\beta^{6,3}$) pore with a diameter of ~0.4 nm. The permeability properties of the channel have been well characterized experimentally (5–8) using single channel and noise analysis techniques, and since it has a known structure it has served as a prototype for membrane channels in general and for single filing channel conduction in particular. (The channel is permeable to small single-valued cations, and for the alkali cations it obeys the Eisenman sequence number 1 [9] with channel permeabilities in the same order as the ionic mobilities.)

Theoretical modeling of channel permeability has focused on the Eyring rate-theory analysis (10–12) of the channel in terms of multiple barrier-site models. Recent theoretical advances using molecular dynamics (13–15) and quantum mechanical ab initio (16) calculations for simple gramicidin-like channels are consistent with the results of fitting the barrier models to experimental data. Independent x-ray (17), nuclear magnetic resonance (NMR), and circular dichroism-spectra measurements (3, 4, 12) support the simple barrier-site structure description of the channel. From these measurements it was concluded that each half of the channel has a site located ~0.25 nm from the channel opening with a barrier for channel entry at each end and a central barrier arising partially due to

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the low dielectric constant of the membrane. Also, there is evidence for the existence of an external site (11) at each end of the channel, extending into the solutions.

This paper examines the factors governing the lifetime properties of gramicidin A channels, an issue that has not been as extensively dealt with as have the permeability properties of the channel, mainly due to the inconvenience of the larger samples required to characterize the lifetime distribution (18) and earlier problems with the reproducibility of the measurements for certain experimental conditions (19, 20). Also, although the unit channel conductance is available from a single "noise" measurement, the average lifetime (hereafter called simply the lifetime) cannot, generally, be evaluated from a single experimental many-channel recording, but requires measurements at a number of gramicidin concentration levels (20).

The conclusions of these earlier studies were that the lifetime shows a tendency to increase with ion concentration (5, 18, 20, 21) and that the lifetime depends on the lipid composition (19,20). It was proposed that the lifetime depends on the thickness (22) and the surface tension of the membrane (19, 22, 23, 26).

In previous studies (18, 21), we have suggested that ion occupancy might be the important factor responsible for the increase in lifetime with salt concentration. We suggested there that a monomer occupied with an ion might be stabilized in a conformation favorable to the dimerized state and that a two-ion occupancy (one ion in each channel half) would further stabilize the open state.

The significance of the interaction of the occupant ions with the channel conformation was emphasized also in the early work on gramicidin, then in relation to the permeability properties of the channel (1). Allosteric stabilizing effects are also well known for enzymes in general (24, 25). Another possibility proposed by us and others (18, 20) is that the dimerized state is stabilized due to the electrostatic screening by one monomer on the ion in the other.

In this paper we examine the effects on bilayer surface tension and on channel lifetime of varying the ion concentration. The aim is to test whether surface tension alone (19, 22, 23, 26) predicts channel lifetime or whether ion occupancy is the major factor determining the concentration dependence of channel lifetime.

We also examine the effects of elevated osmotic pressure and of divalent blocking ions, since these may also be expected to influence channel occupancy and therefore channel lifetime.

EXPERIMENTAL METHODS

Surface Tension Measurements

The surface tension measurements were accomplished using a method similar to that described previously (19). The curvature of a black lipid membrane (BLM) subjected to a known pressure was observed and the surface tension calculated using Laplace's law (see Appendix). The experimental arrangement is shown in Fig. 1 and the details of the optical geometry in the Appendix (Fig. A1).

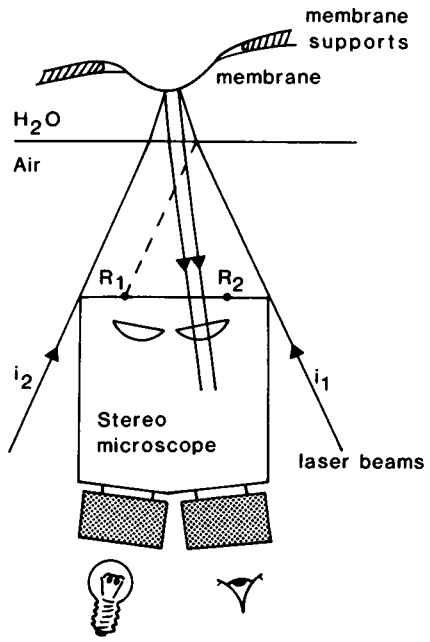

FIGURE 1. Experimental setup for measuring surface tension of bilayers. The laser beams il and i2 are directed (using mirrors not shown) to pass reference points on the edge of the microscope. The angle of inclination of the beams is then fixed by the focal distance of the microscope. The beams are adjusted to pass horizontally (the plane of the figure) and the glass chamber adjusted perpendicular to the microscope axis, both, by observing the lcoation of the reflections at *R*1 and *R*2. The spherical cap produced by the bulging membrane gives rise to two faint reflections, the distance between which, *DS*, is measures by viewing through the scale in the eyepiece. The radius of curvature, *r*, is given by $r = DS/[2 \sin (a/2)]$, where *a* is the angle to the normal of either of the beams (in the water). See Fig. A1 in the appendix.

The visibility, accuracy of measurement, and optical adjustments were facilitated using two (He-Ne) lasers. A stereo microscope was used with a fiber-optical illumination attached to one of the eyepieces. The inner chamber, made out of a polypropylene disposable syringe with a hole of 1-mm diam, was placed ~1 cm from the glass wall to provide space for applying lipid onto the chamber hole. Keeping the inner chamber away from the glass wall also minimizes reflections from the glass wall when observing the BLM.

Pressure was applied to the bilayer membrane by adding or retrieving solution from the outer chamber using a calibrated pipette. The laser beams were used to illuminate the membrane so that two faint point like reflections (dots), at a separation decreasing with the increasing curvature of the membrane, could be observed with the microscope. The use of laser beams enhanced the visibility of the optically black bilayer. The distance between the reflections was measured using an eyepiece equipped with a scale. The use of lasers was especially convenient for the geometrical adjustment of the optical system. Thus, the orientation of the beams was obtained by direct measurement using a protractor placed at the intersection of the beams, and the chamber orientation and the angle of incidence of the beams were adjusted by observing the position of the reflections *R*1 and *R*2 (see Fig. 1). The beams were directed (using adjustable mirrors not shown) so as to just pass by horizontal markings on the edge of the microscope, thus ensuring a precise and reproducible optical geometry for the experiments. The viewing direction with this arrangement is not symmetrical for the two beams (see Fig. 1) and not perpendicular to the the chamber wall. Nevertheless, due to the symmetries and small angles involved, the analysis is greatly simplified (see Appendix). The refractive index variations were small for the range of concentrations used.

The radius of curvature (r) of the BLM was measured as a function of the applied pressure and the surface tension was then obtained by plotting $1/r$ as a function of pressure (Fig. 2) and fitting a straight line to the data. In some cases this was done for both negative and positive pressures and a combination of both. As a check, the method was used to obtain the surface tension of the water/air and the water/octanol interfaces and the values obtained, 72.5 ± 3 and 8.5 ± 0.5 mN/m, respectively, are in excellent agreement with published values.

For the BLMs, however, a slow drift of the surface tension was frequently observed. This drift introduced errors in experiments where stable membranes were difficult to obtain. The membranes in these experiments would easily break under the application of hydrostatic pressure. Similar problems have been noted by others and are probably due to the particular method used in forming the membrane. Two to ten measurements (typically five) were made for each value, and the standard error of the mean was of the order of 1–2% throughout. Some experiments were repeated and the reproducibility for the stable membranes was within the experimental error of the method. The actual error is probably larger than this since, for unstable membranes, the surface tension was usually measured before a stable value was obtained.

For low salt levels the value obtained, 4.95 ± 0.15 mN/m (see Fig. 5), is in very good agreement with accurate determinations by Requena et al. (27). Using interferometric methods, they confirmed that the BLM tension is only slightly lower (order of 10^{-2} mN/m) than the expected tension of two bulk lipid/water interfaces. They report a value of 2.51 mN/m for the monoolein-hexadecane/water interfacial tension.

Channel Lifetime Measurements

Single channel recordings were carried out on synthetic black lipid bilayers of glycerylmonooleate (Nucheck or Sigma, 99%) *n*-hexadecane (Merck & Co. [Rahway, NJ] or Sigma Chemical Co. [St. Louis, MO] for gas chromatography) membranes (14 mg/ml) of an area varying between $1 \cdot 10^{-4}$ and $2 \cdot 510^{-3}$ cm^2 at temperatures between 19° and 22°C. For the experiments with symmetrical solutions, a concentric two-chamber system was used as described previously (6). For the asymmetric conditions, the same method was used for some of the experiments and, also, by a conventional two-chamber Teflon system to facilitate stirring close to the formed membrane. The separating hole in this case was made by melting a small hole (using a soldering iron modified to hold a thin needle) of ~0.25-mm diam in thin polyethene foil and the membranes formed using the Montal-Mueller technique (28). The salts used were of pro analysi grade and the water deionized from a Millipore system. Methanol, chloroform-methanol 2:1, and hexane were used for cleaning. The chambers were blown dry with nitrogen.

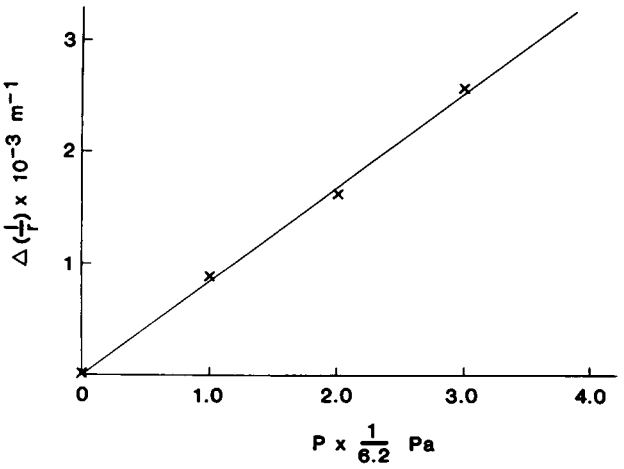

FIGURE 2. Plor of the change in $1/r$ (inverse radius of curvature) as a function of transmembrane pressure change *DP*. The pressure was changed in discrete steps by adding or withdrawing solution using a pipette. The *x*-axis markings represent steps of 0.2-ml additions to the outer chamber. From Laplace's law $P = 2\gamma/r$, the slope of the line gives the surface tension γ at 20°C, 4 M HCl, gmo-hexadecane.

The signals were filtered at 25 Hz and recorded on magnetic tape. The playback signal was digitized (12 bit samples at 0.1–1 ms and averaged over 20–100 ms to minimize 50-Hz noise). The signal was then preprocessed selecting high quality, stable, and noiseless portions, optionally high-pass filtered (digitally, for autocorrelation measurements). The lifetime was evaluated by measuring the intervals between openings and closings (transition times) of channels and then evaluating the average lifetime either directly or from the exponential decay of a modified survival plot (18). For the lowest conductivity recordings, the autocorrelation method was used. Recording and analysis was accomplished using a microcomputer (Cromemco sys. 3 Z80).

The transition-time method was preferred to the autocorrelation method because (*a*) the result is independent of the kinetics of the opening process (no contamination of correlation effects), (*b*) the method is insensitive to drift in the event activity (such as accumulation of gramicidin in the membrane or change in membrane area), and (*c*) the measurements could be accomplished with significantly shorter sample recordings than for the autocorrelation method (18). Below 10 mM, however, the autocorrelation method was employed because of the poor signal-to-noise ratio and the ensuing difficulties of event identification.

In Fig. 3 is reproduced a typical portion of a recording, showing the presence also of "miniature" channels recently described (29). In the evaluation of lifetime with the transition-time method, the minis were included in the evaluation, for consistency with the autocorrelation measurements. Although no systematic study of the effect of the distribution of unit-channel amplitudes was made (see however reference 18, Fig. 4), the frequence of minis was ~5% of the total number of channels and could not significantly influence the results.

The lifetime was corrected (to 20°C) for the variation of temperature (19–20°C) using a value of 20 kcal/mol for the activation energy (2, 5).

The accuracy and reproducibility of the lifetime measurements were of the order of those expected from the number of measured channel events (200–1,000). At least two membranes were used for the evaluation of each value of lifetime, but typically five membranes were formed in each experiment.

Some experiments were repeated, and for 0.14 M HCl, which is located on the steepest part of the zero-voltage lifetime curve and therefore expected to be a good candidate for checking the sensitivity to variations in experimental conditions, the lifetime was evaluated from three different experiments, giving an estimated error of the mean of 3.1% (927 events, 10 membranes). Additional checks of the method, such as evaluation of effects of noise and filters, have been reported elsewhere (18).

Fig. 4 shows a modified survival plot (18) of channel lifetime for 2 M HCl with a gmo/decane membrane. The lifetimes of the 445 channel events ($\tau = 2.75$ s) in a continuous recording of 65 min, which is of the order of $1{,}400 \cdot \tau$, shows no tendency of a biomodal distribution and therefore no evidence of a deterioration of the gramicidin channels even at this relatively high concentration of HCl.

The gmo/decane system was used for this check since the lifetime with this membrane is a factor of 10 lower than for the gmo/hexadecane

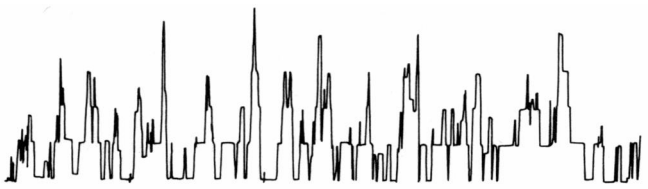

FIGURE 3. Recording of gramicidin A single channel events. 0.5 M KCl at 25 mV. The unit channel conductance is 35 pS. Rare occurrences of miniature channels are also seen.

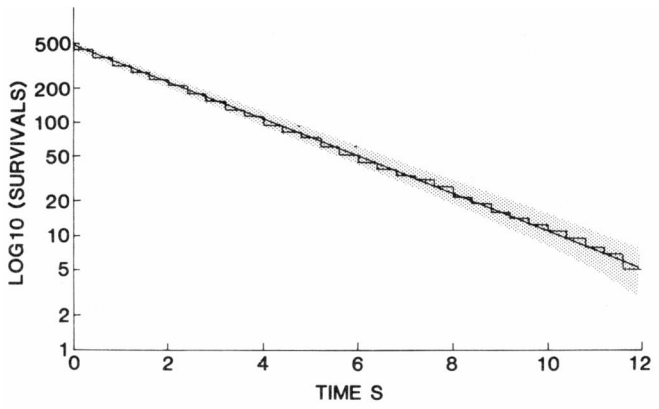

FIGURE 4. Survival plot of gramicidin channels. 2 M HCl and gmo/*n*-decane membrane. The bin (t_i, t_{i+1}) is proportional to the probability that channels will survive for at least t_i s. This plot is obtained from the multiple event record by a procedure described elsewhere (18). Total number of events is 445, lifetime = 2.75 s. 30 bins over 12 s are displayed. Total recording time (continuous recording) is 65 min = 1,444 · τ. The shading indicates 1 SD of the bin size. T = 21°C.

system, and therefore permits a high event activity (i.e., more channel openings in a fixed time without multiple events). This allows for a more sensitive evaluation of the lifetime distribution. We expect gramicidin in a gmo/decane membrane to be no less sensitive to low pH deterioration than it would be in a gmo/hexadecane membrane and, for the reasons explained above, the former system was preferred in order to test these effects. To obtain the same accuracy of the distribution of lifetime for the gmo/hexadecane system would require a continuous recording and a membrane stable for ~18 (!) h. Further, the shorter recording period with gmo/*n*-decane seems more appropriate since for extreme recording times one might obtain effects not seen in the more typical 1-h recordings.

The lack of effects at low pH is expected since gramicidin is extremely hydrophobic and since boiling in 6 M HCl for several hours is required to break peptide bonds. Also the activity coefficient of HCl rises sharply only above ~4 M.

RESULTS

Fig. 5 shows the effect on the surface tension of varying salt concentration. For comparison with earlier work, the surface tension has been plotted against lifetime, rather than as a function of concentration. For HCl it may be noted that the surface tension is only weakly dependent upon salt concentration, up to about molar concentrations. At higher concentrations the surface tension decreases markedly to ~60% of its value for distilled water. For CsCl the lifetime increases with surface tension. Electric potentials were applied to the membrane but no detectable changes in surface tension were observed for voltages up to 100 mV.

Fig. 6 shows the dependence of lifetime and unit channel conductance on the activity of HCl (pH), the effect of blockers, and the effects of various osmotic agents. The lifetime increases monotonically from ~1 s at low concentrations with a steep rise in the region of pH between 1 and 0 to very long lifetimes as compared with the lifetime in experiments with alkali cations, leveling off or possibly even decreasing at high concentrations. The channel conductance also saturates at high activities.

It is also noteworthy that although osmotic and blocking

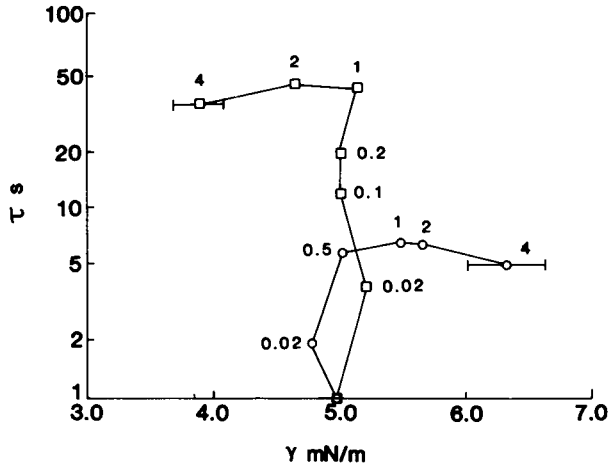

FIGURE 5. Plot of lifetime of gramicidin A channels against the surface tension of the gmo-hexadecane bilayer for H^+ (□) and Cs^+ (○). The numbers indicate salt-level in moles/liter. For HCl: 0.001, 0.002, 0.1, 0.2, 1,2, and 4 M.For CsCl 0.02, 0.5, 1,2, and 4 M. The value for distilled water coincides with the value for 1 mM (not indicated) H^+ 20°C. The bars indicate the SEM which is of the order of 5% throughout.

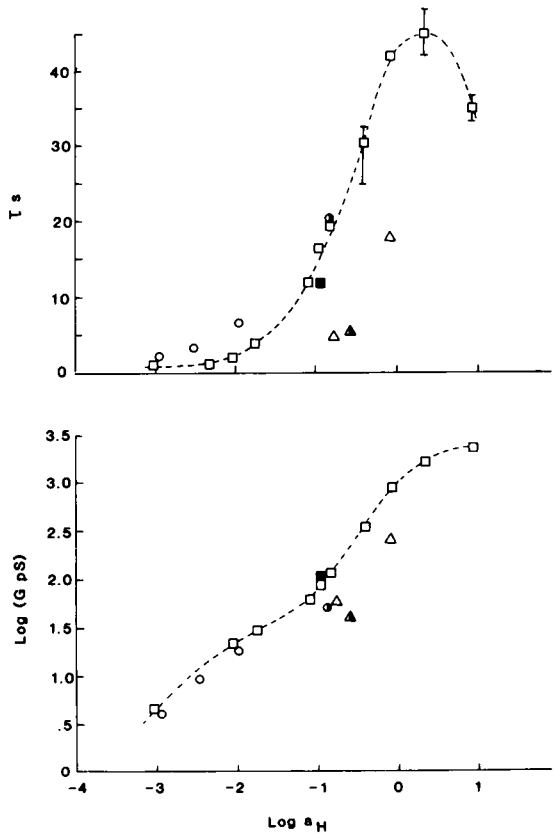

FIGURE 6. Dependence of (*a*) lifetime and (*b*) unit channel conductance of gramicidin A channels on pH for HCl. □ are HCl only. Also shown are the effects of osmotic agents and multivalent blockers. ○, urea; ◑, sucrose; △, Ba^{2+}; ▲, La^{3+}. ■ is H_2SO_4. The vertical bar for HCl at pH 0.5 indicates the extremum values obtained in this case, otherwise the bars indicate the SEM ($n > 3$) for the cases where the error was >5% and larger than the size of the symbol. Dashed curve is for visual aid only for the case of pure HCl. 20°C, gmo-hexadecane.

agents both decrease the ionic conductance, they have dissimmilar effects on the lifetime. Osmotic agents increase the lifetime, whereas blocking agents decrease the lifetime also when present at a high concentration (i.e., high osmotic level).

Fig. 7, *a–d* shows the voltage dependence of lifetime for H^+, K^+, Cs^+, and a typical result for a multivalent blocker, La^{3+}. The I-V curves shown are denoted with symbols (filled) corresponding to the τ-V curves.

Significant qualitative differences in both direction and magnitude are apparent. For K^+ the lifetime increases with voltage and for H^+ and Cs^+ the lifetime decreases with voltage. These results are different from earlier measurements which report little or no voltage dependence of gramicidin stability on voltage (2, 12, 19, 20). At 5 mM HCl (not shown) the variation of lifetime with the applied voltage was small and within the limits of the experimental errors.

The lifetime-voltage curves for HCl with Ba^{2+} and Mg^{2+} were similar to that of La^{3+}. The lifetime decreases with voltage evidently approaching a lower limit at high voltages. The conductance also saturates at high voltages in accord with results published previously for the effects of positively charged blockers on the I-V characteristic of alkali cations (30, 31).

Fig. 8 shows the dependence of the lifetime and single channel current on voltage for a bilayer with 1 M HCl on one side and 1 M KCl on the other. Positive voltages here correspond to the HCl side being positive with respect to the KCl side. The I-V curve for this asymmetric situation is

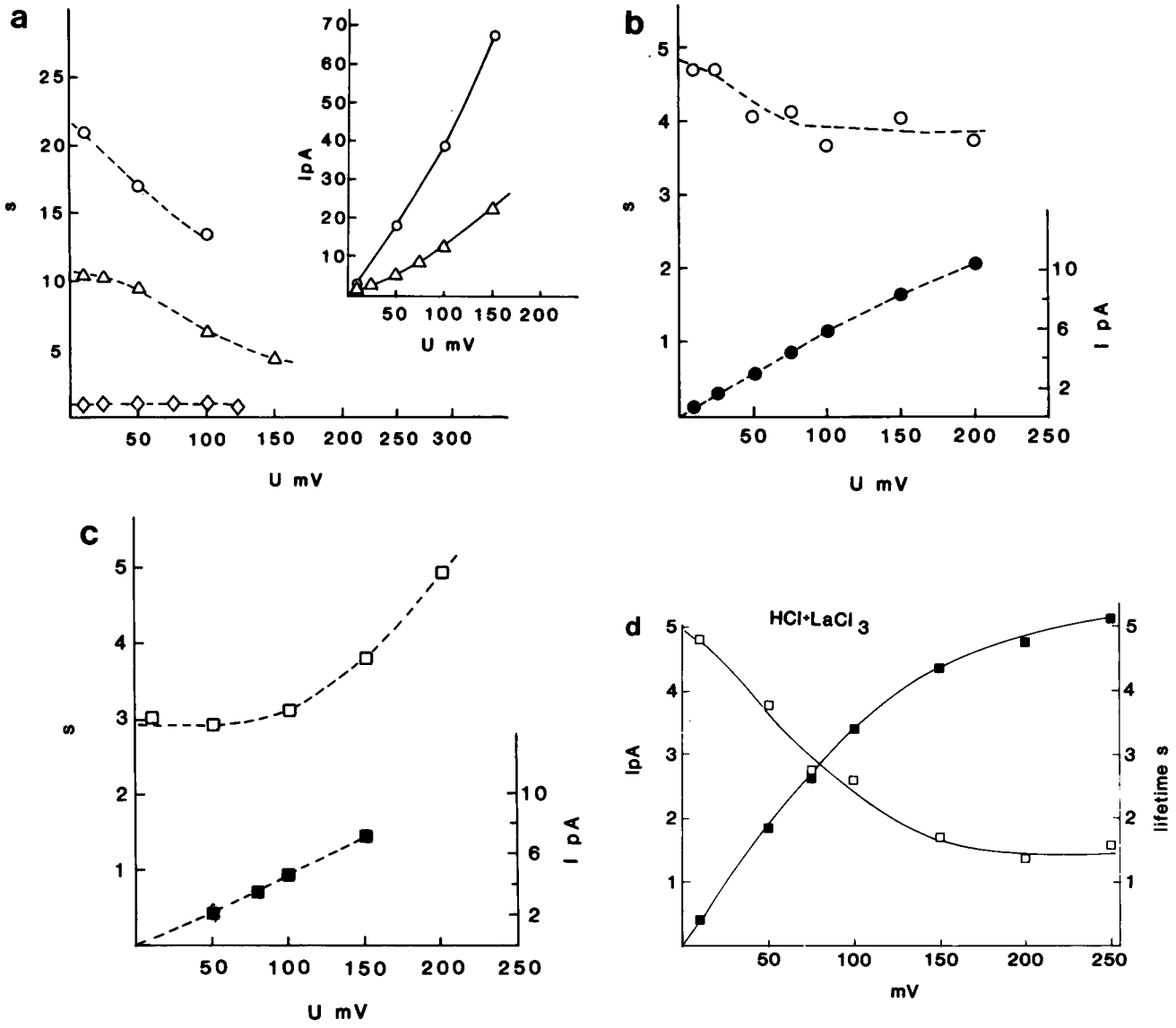

FIGURE 7. (*a–d*) The voltage dependence of lifetime for various conditions of electroylte composition. (*a*) ○, 0.5, M HCl pH 0.45, 21.5°C; △, 0.5 M H_2SO_4 + 20 mM HCl pH 0.96; ◇, 5 mM HCl. (*b*) 0.5 M CsCl. (*c*) 0.5 M KCl. (*d*) HCl + 0.9 M $LaCl_3$ pH 0.8. The I-V curves shown are denoted with symbols (filled) corresponding to the τ-V curves. The curves are for visual aid only.

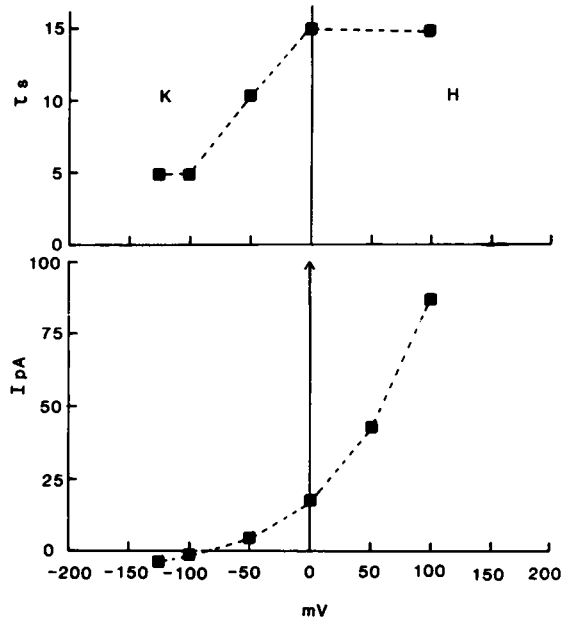

FIGURE 8. Lifetime (*upper*) and current (*lower*) as a function of voltage for a gramicidin channel with 1 M HCl on one side and 1 M KCl on the other. The K^+ side is taken as reference (zero) for the applied voltage. The lifetime is a factor of three lower for negative voltages than for positive voltages. This is interpreted as an effect on the change in ion occupancy (see main text). The channel also shows significant rectification. 20°C, gmo-hexadecane.

markedly asymmetric, concave to the *y*-axis, and the small signal conductance (dI/dV) approaches the conductance of the single ion case for the positive and negative extremes of voltages. More remarkable is that also the lifetime behavior is asymmetric being a factor of three larger for positive than for negative voltages. Similar results were obtained for 0.5 M solutions of HCl and KCl.

DISCUSSION

Unexpectedly in view of earlier work (19, 22, 23, 26), we find that the surface tension shows no correlation to the variation of lifetime with concentration. From the results presented in Fig. 5, we conclude that at moderate salt levels the lifetime changes with no changes in surface tension.

For HCl, up to about molar concentrations, the surface tension remains constant whereas the lifetime increases. At higher concentrations the lifetime "levels off" and the surface tension decreases. This result (lack of correlation between surface tension and lifetime) does not, however, directly contradict results reported elsewhere since, in those cases, the lipid composition was the variable of interest, and the conditions of salt level and voltage were kept constant. Thus, Neher et al. (19) found that increasing the solvent chain length decreases the surface tension and increases the lifetime. Changing the polar headgroup of the lipid with a constant chain length showed a similar correlation between lifetime and surface tension. Haydon et al. (22) have considered the effect of thickness and Elliot et al. (26) extended this hypothesis and showed that the effects of thickness and tension could be incorporated in a general model for the interaction of the lipid with the gramicidin A channel stability.

Our results are not in agreement, however, with the conclusion that the thickness and surface tension are the only relevant parameters for the prediction of lifetime, since the lifetime may change by a factor of 50 (e.g., for H^+ see Figs. 5 and 6) with a corresponding insignificant change in surface tension, or as is the case for Cs^+, the lifetime may increase with a simultaneous increase in surface tension in complete disagreement with the postulated prediction. Also, the lifetime for H^+ and K^+ is voltage dependent, whereas the surface tension is not (19, 27).

The lifetime for K^+ is seen to increase and that for H^+ to decrease with voltage (Fig. 7). The marked dependence of lifetime with voltage we report here is different from earlier reports where little or no voltage dependence of gramicidin channel stability was observed (2, 12, 19, 20). This difference is probably due to the earlier difficulties, noted in the introduction, in obtaining accurate results for the lifetime of gramicidin channels.

The results of the experiments with asymmetrical solutions support the hypothesis that the lifetime dependence on concentration is related to the variation in ion occupancy with concentration. In these experiments, when applying the electric field in the direction of the H^+ gradient (and thus against the K^+ gradient), one expects to approach, in the limit of high voltages, a situation where the channel state becomes independent of the cation composition of the "downhill" electrolyte. The lifetime therefore approaches the long lifetimes of symmetric HCl solutions for increasing voltages. Similarly for the opposite polarity, the shorter lifetime of K^+ occupancy is observed.

The sequence of the lifetime magnitudes of the alkali cations (18) generally agrees with the order of the single-channel conductances, the Eisenman sequence number 1 (9). These results and the much smaller effects on lifetime obtained by osmotic agents (Fig. 6) indicate that the increase in lifetime is not due to the increased osmotic pressure of the solutions.

In a following paper (32), we will show that the concentration and voltage dependence of the channel lifetime is explained by a quantitative model based on the occupancy hypothesis (18, 21). According to this hypothesis, the open state of the channel is stabilized by an ion occupying an internal site in the channel. The occupancy may increase or decrease with the applied electrical field depending on the relative effects of the field on the transition rates across the energy barriers seen by an ion passing through the channel, which explains the difference in lifetime behavior observed for K^+ and H^+.

With the occupancy hypothesis, the lack of a field effect on the lifetime at low salt levels is intuitively clear, since at a low ion occupancies it is expected that the channel

dissociation rate is negligibly affected by the occasional stabilization by an occupant ion, whatever the species.

Similarly, channel blockers are expected to lower the ionic occupancy (by decreasing the entry rate in the direction of the applied field without a corresponding decrease of exit rate on the "far field" side) and this also bears out qualitatively (see Figs. 6 and 7).

The effects of the elevated osmotic pressure are (Fig. 6) to increase the lifetime and to decrease the conductivity. Although the mechanism of this effect is unclear, it is possible to explain such effects through the influence on the channel binding constants. This interaction may result due to the changed interactions between the permeant ion, the ethanolamine ends, and the aqueous medium. Long range effects of the EA-ends on ion binding in a vacuum channel have been discussed by Etchebest and Pullman (33). The osmotic pressure may also have direct effects on the channel structure (8). Whatever the mechanism, the finding that osmotic pressure increases lifetime is consistent with a change in binding that increases the ion occupancy of the channel.

One of us, in a study (34) of gramicidin flickers (brief closures) similar to flickers of physiological channels, recently suggested that effects of surface tension and ion occupancy on the "loss of conduction" of the gramicidin channel may be of general significance also for more complex biological channels. It is suggested there that the surface tension modulates the conformational changes involved in channel gating independently of the detailed mechanism involved since protein conformational changes generally involve lipid rearrangement and thereby area changes and hence directly affect the transition rates (19, 22).

Since the membrane protein interact with the surrounding lipid, it is expected that the thickness and surface tension of the membrane have direct effects on the structure of the channel. Elliot et al. (26) have shown in detail how the thickness and surface tension of a "dimpled" lipid membranes interact with the breaking up process of the gramicidin dimer and modeled the effect of these on the lifetime.

We conclude that surface tension is of secondary importance in predicting the concentration and voltage dependence of gramicidin A channel lifetime and that these latter dependences may plausably arise from the variation of channel ion occupancy. In particular, the behavior of the lifetime in asymmetrical solutions approaching the lifetime for the "downhill" ion as the electrical field is "switched" across the membrane supports the occupancy hypothesis. We suggest therefore that the mechanism of ionic stabilization/destabilization of specific channel conformations may be of importance for predicting the "gating kinetics" of gramicidin A and also of biological channels in general.

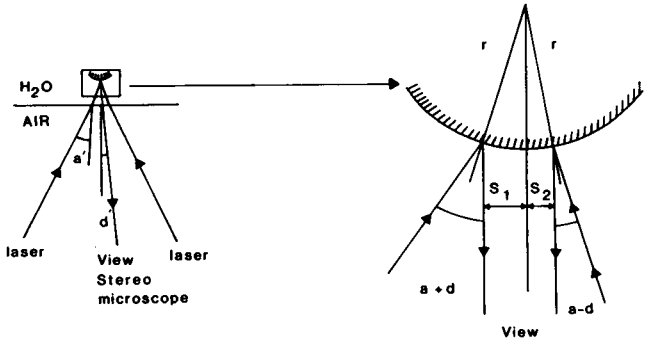

FIGURE A1. Schematic representation of Fig. 1 showing the optical geometry of the experimental arrangement of Fig. 1. a is the angle which the laser beam makes with the normal to the glass chamber in the water. d is the corresponding angle of the rays reflected from the biyaler in the direction of the viewer. a' and d' are the corresponding angles in air. $S1$ + $S2$ is the distance between the observed point reflections (see main text).

APPENDIX

Determination of the Radius of Curvature of Bilayer

According to Laplace's law, $P = 2\gamma/r$, where P is the transmembrane pressure applied, γ is the surface tension, and r the radius of curvature.

The optical arrangement for measuring the curvature is shown in Figs. 1 and A1.

The laser beams and the ray from the bilayer in the viewing direction (viewing ray) are all refracted by the transition from air to (via glass) water. The beams are however, by arrangement, equally inclined to the glass chamber and the angles these make with the viewing direction (in the water) and therefore $a + d$ and $a - d$, respectively, where a and d are the angles of the laser beams and the viewing ray to the glass normal in the water.

From the figure one therefore obtains

$$S1 = r \sin [(a + d)/2)] \text{ and } S2 = r \sin [(a - d)/2)]. \quad \text{(A1)}$$

The distance (DS) between the lumination reflecitons is therefore

$$DS = S1 + S2 = r \sin [(a + d/2)] + r \sin [(a - d)]$$
$$= r\, 2 \sin (a/2) \cos (d/2). \quad \text{(A2)}$$

a and d are obtained from the angles (a', d') the incident and viewing rays in air make with the normal to the glass, since

$$\sin (a')/\sin (a) = \sin (d') \sin (d)$$
$$= \text{refractive index} = n. \quad \text{(A3)}$$

a' is easily measured and d' is half the angle between the left and right viewing directions of the stereo microscope, obtained from the manufacturer.

For our experimental conditions ($d' = 6$), $\cos (d/2) = 0.9952$. Thus within 0.5%, $r = DS/2 \sin (a/2)$.

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