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THE INDUCTION BY PROTONS OF ION CHANNELS THROUGH LIPID BILAYER MEMBRANES

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The appearance of ion channels was induced in phospholipid bilayers by acidification of the bulk solution on one side of the bilayer, by addition of HCl, acetic acid or by hydrolytic production of protons using purified acetylcholinesterase. Further acidification below an apparent critical pH range led to restoration of a low conductance state similar to that seen at neutral pH. Such experiments were performed with a heterogeneous soybean lecithin extract, with homogeneous synthetic diphytanoylphosphatidylcholine, and with a mixture of cholesterol and synthetic dioleoylphosphatidylcholine. It is proposed that the physical mechanism for this phenomenon involves fluctuations of lipid order induced by fluctuations in protonation of phospholipid head groups within a critical pH range; these, in turn, create conductive defects in the two-dimensional lattice of the lipid bilayer.

1. Introduction

Since their introduction, some 20 years ago, lipid bilayer membranes have served as valuable model systems [1] for studying the electrical properties and permeability of biological membranes. They are considered to correspond in many of their characteristics to the lipid bilayer leaflet which was the dominant component of the membrane in the Danielli-Davson model [2] and in the unit membrane of Robertson [3], and is still believed by most workers in the field to account for a substantial part of the area of various biological membranes [4]. Lipid bilayer membranes have been used in a variety of studies (for reviews see refs. 5 and 6) to investigate the pore-forming properties of various antibiotics [7–10] and, more recently, in reconstitution experiments in which channel-forming proteins [11–15] have been similarly incorporated into the bilayer.

Implicit in most of these studies has been the assumption that the lipid bilayer serves as an essentially inert matrix of high resistance and that the channels observed occur within the 'ionophore' or pore-forming protein [4,16] which has been introduced into the bilayer. However, there have been suggestions for a direct involvement of the lipid component in membrane permeability [17–19]. Furthermore, in recent reports, appearance of resolved ion channels [20] or 'current fluctuations' [21] was observed, in the vicinity of the phase transition temperature, through bilayers composed only of a synthetic phospholipid.

We have reported recently that the enzyme acetylcholinesterase (AChase), which is not believed to be a channel-forming protein, can induce the appearance of ion channels in lipid bilayers in the presence of its natural substrate acetylcholine [22], a prediction made earlier from theoretical considerations [18]. We suggested that the mechanism of channel induction might involve protonation of lipid head groups resulting from local pH

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changes produced by substrate hydrolysis [23], thereby leading to a change in the state of the bilayer [24]. In the following, we present direct evidence that, above a certain threshold for the proton concentration, stable lipid bilayers of very low conductivity are capable of increasing their conductivity stepwise, thus exhibiting resolved ion channels. Further acidification produces transition of the membrane to a new state of reduced permeability. The relevance of these observations to mechanisms and enzymatic regulation of ion transport through biological membranes will be discussed.

2. Materials and methods

2.1. Materials

KOH was obtained from Merck, acetic acid from Baker, and cholesterol from Serva. KCl, HCl, hexadecane, acetylcholine chloride, phenyl acetate, and type II-S soybean L-α-lecithin (a crude mixture which contained mainly phosphatidylcholine, phosphatidylethanolamine, and cholesterol) were purchased from Sigma. Synthetic diphytanoylphosphatidylcholine [25] was from Avanti; in this lipid, possible protein contamination was determined by amino acid analysis on a Biotronik amino acid analyser. The observed amino acid content was below 0.03 nmol per mg lipid, and glycine was the only amino acid observed. AChase was the 14 S + 18 S preparation purified from *Electrophorus electricus* [26].

2.2. Methodology

The experimental method is described in detail by Hanke (ref. 27; see also ref. 28). Electrolyte chambers of approx. 5 ml volume were made of Teflon and connected by a hole of 200 μm diameter across a Teflon disc cut 20 μm thick. The chambers were cleaned by immersion for 12–24 h in chloroform/methanol (1:1), followed by similar immersion in sulfochromic acid. They were then washed in bidistilled water, boiled for at least 1 h in 1 N KOH, and finally boiled again and copiously washed in bidistilled water. In addition, so as to avoid contamination artefacts, experiments using pure lipid bilayers were performed only with separated chambers which had not been used in experiments involving proteins or polypeptide antibiotics.

The electrostatic potential difference across the membranes was applied via the two bulk solutions using Ag/AgCl electrodes. Currents were measured by a low-noise amplifier designed by Sigworth and Neher [29]; maximal time resolution was 50 μs [30], but most data were filtered by a 0.3 kHz low-pass filter so as to reduce the electronic noise, and a vibration-free table helped to lower noise width of recorded currents to 0.3 pA or below. Data storage was on a Racal Store 4D high-frequency analog tape recorder. Scotch 212 LP tapes were erased on a Weircliffe Bulk Eraser before use. Current histograms were analysed on a Honeywell computer using a program developed by Dr. H.-W. Strube (III. Physikalisches Institut, University of Göttingen).

The preparation of the bilayer membranes followed standard methods [11,28,31]. The lipid to be used was dissolved (5 mg/ml) in hexane/ethanol (10:1). Usually, 20 μl of that solution were added to the electrolyte surface in each chamber. Bilayers then made from the two monolayers were controlled for resistance (above 10^{11} Ω) and for capacitive responses (around 10^{-9} F). HCl and acetic acid were added with a Hamilton syringe to the unstirred bulk solution only. pH values given are, therefore, estimates averaging over the whole electrolyte solution (approx. 4 ml volume in each chamber). Apparent conductivities are calculated from the observed membrane current, divided by the applied electrostatic potential difference ('voltage'). In experiments involving AChase, the enzyme was added as described previously [22], and substrates were added with a Hamilton syringe.

3. Results

In most of the experiments described below, bilayer membranes containing the appropriate phospholipid were built, as described in section 2, between two compartments containing unbuffered 1 M KCl (pH ≈ 6.5). The stability of the bilayers

was then observed, for at least 30 min, at the same applied voltage at which the bulk proton concentration was raised subsequently. Acid was added to one compartment only, and the voltage during the initial part of the experiment was usually +40 mV, so that protons in the acidified compartment would be attracted to the bilayer.

3.1. Soybean lecithin

The traces in fig. 1 are representative of the behavior of a membrane made from soybean lecithin, a crude lipid mixture, in unbuffered 1 M KCl, upon gradual lowering of the pH by stepwise addition of HCl. The membrane, of resting conductivity 2 pS (i.e., 5×10^{11} Ω) initially displayed no significant appearance of channels (fig. 1 at 0.3 μM H^+). After addition of the 9th aliquot of 1 μl of 0.1 N HCl (180 μM H^+, ↓) it started to display signs of opening and closing of small (≈ 6 pS) but long-lived channels, and this process was enhanced after the addition of the 10th and 11th aliquot (200 μM H^+). As can be seen, opening and closing of channels up to 20 pS apparent conductivity occurred, but the opening process predominated, so that by 1 h after addition of the 9th aliquot, the membrane was in a very high conductivity state, equivalent to several thousand pS (not shown). At this stage, the pH was further lowered drastically, this time by addition of 20 μl of 2 N HCl. The conductivity decreased almost immediately, and after further additions of 200 μl of 2 N HCl, returned to a basal conductivity level not significantly different from that observed at neutral pH. However, opening and closing of ion channels of well defined conductivity could also be observed (see at 90 mM H^+). The basal conductivity was not increased further by addition of more HCl (110 mM H^+, still at +40 mV).

As was to be expected, the ion currents were voltage dependent (see traces at 110 mM H^+), and the direction of current was reversed upon reversing the sign of the applied voltage (cf. also traces at 110 mM H^+ on an enlarged time scale in fig. 2).

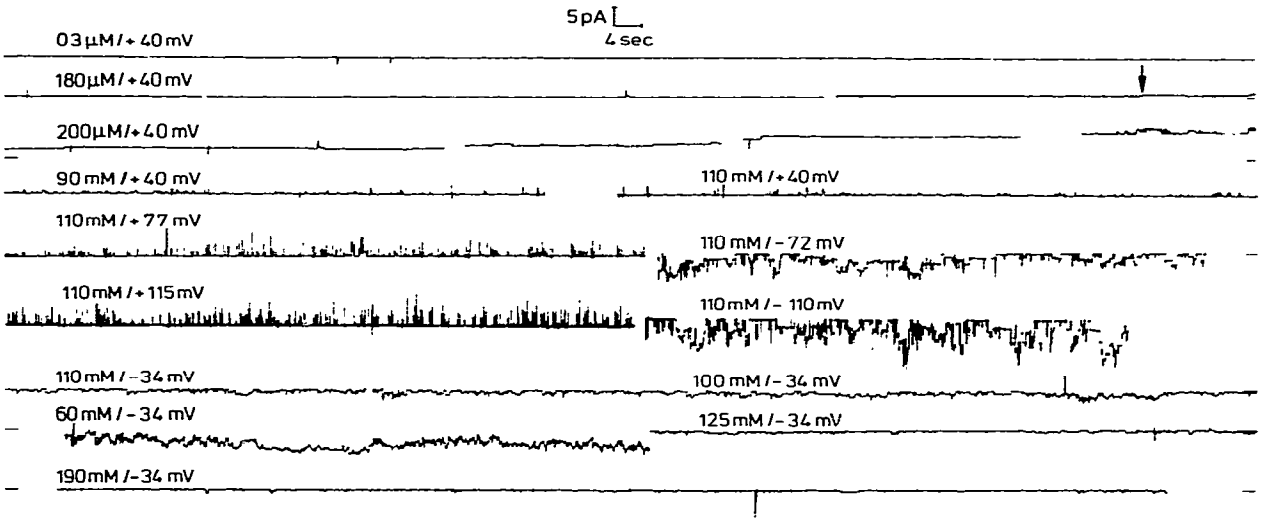

Fig. 1. pH-dependent appearance of ion channels in a soybean lecithin bilayer. The membrane was formed in unbuffered 1 M KCl. The bulk solution on one side was adjusted with aqueous HCl to the proton concentrations denoted. Back-titration was performed with aqueous KOH. The applied potential is denoted alongside the proton concentration. At positive applied potentials, protons in the acidified compartment are being attracted to the bilayer. The traces show the observed membrane current as a function of time. Initial membrane current is zero within experimental error (i.e., < 0.3 pA), and is indicated by horizontal bars at the origin of the current ordinate in each trace. At ↓, first appearance of resolved ion channels is observed.

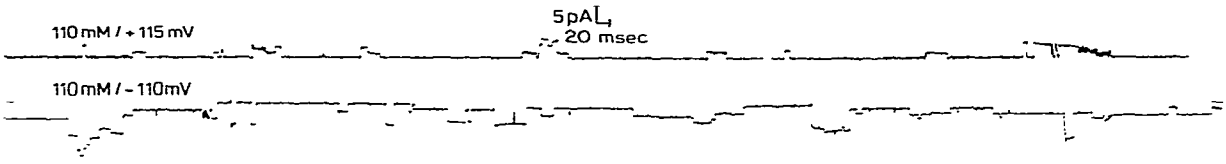

Fig. 2. Proton-induced ion channels in a soybean lecithin bilayer. Same experiment as fig. 1 showing traces with a 12.5-fold expanded time scale.

It is worth noting that in this experiment, the mean channel life times appeared much longer when the applied voltage was negative (reducing the expected pH gradient) than when it was positive (increasing the gradient).

As can be seen, at 110 mM H^+ and an applied potential of −34 mV, the membrane conductivity was again relatively low. However, on lowering the proton concentration by addition of 50 μl of 1 N KOH (to 100 mM H^-), so as to neutralize a substantial part of the HCl added, the membrane was brought back to a considerably more active state (see also at 60 mM H^-). That this enhanced activity was not due to some kind of irreversible damage, inflicted by the addition of KOH, was shown by addition of even more 2 N HCl so as not only to neutralize all the KOH, but also to lower the final pH to an estimated value of 0.5. As can be seen (fig. 1, traces at 125 and 190 mM H^+), this again quenches almost all membrane conductivity.

In most of the experiments performed, induction of channels was observed on addition of between 6 and 12 μl of 0.1 N HCl or acetic acid. Since the total volume of the bulk solution was approx. 4 ml, this would correspond to a bulk proton concentration of 150–300 μM (pH 3.9–3.3). The more acid pH, at which transition of the membrane to another state of low conductivity occurred, was less accurately determined, but seemed to be somewhat below pH 2; a pH in the bulk of below 1.5 was generally sufficient for obtaining reproducible quenching of the transitional high conductivity.

On lowering the pH from neutrality, the onset of high conductivity was gradual in most membranes and in small steps (fig. 1); however, in some cases the membrane either 'broke' (current above resolution) in the critical pH range, or jumped immediately to a very high, defined conductivity. In one case, where the lipid bilayer had 'opened' directly to a large conductivity of approx. 2000 pS, it was reversed to a lower conductivity state by back-titration with 0.1 N KOH, but this proved very difficult to achieve reproducibly.

3.2. Synthetic lecithins

The results described above were obtained with a preparation of soybean lecithin, which is a crude mixture containing principally phosphatidylcholines, phosphatidylethanolamines and cholesterol, and which was found to contain approx. 5% amino acids as estimated by amino acid analysis of an acid hydrolysate (see section 2). In order to ascribe unequivocally the pH-dependent changes which we observed to changes in the permeability of the lipid bilayer itself, it was necessary to carry out similar experiments with pure lipids, controlled by the same criterion. For this purpose, further experiments were carried out with two different systems, with pure synthetic diphytanoylphosphatidylcholine, and with a mixture containing synthetic dioleoylphosphatidylcholine (90%, w/w) and cholesterol.

Membranes formed with diphytanoylphosphatidylcholine appeared to be rather more stable than those formed with either soybean lecithin or the dioleoylphosphatidylcholine/cholesterol mixture, and seemed to need both rather more acid and a higher applied voltage to initiate channel opening. Fig. 3, for 0.3 μM H^+, shows a trace from the resting state of approx. 5 pS conductivity. At 2.6 mM H^-, we see the membrane after 130 μl of 0.1 N HCl had been added; appearance of channels could be detected at an applied potential of +77 mV (a 5 pS step is seen at ↓). The frequency of

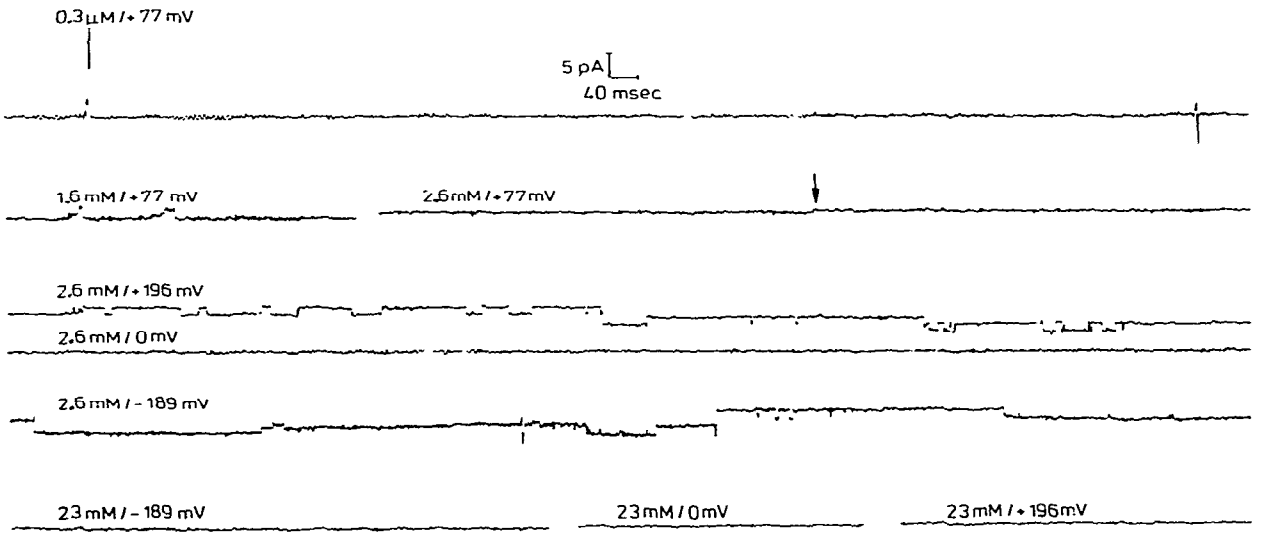

Fig. 3. pH-dependent appearance of ion channels in a synthetic diphytanoylphosphatidylcholine bilayer. The membrane was formed and titrated as in fig. 1. ↓ marks a 5 pS step. The zero current ordinate for the traces at 2.6 mM H^+/+196 mV and 2.6 mM H^+/−189 mV is given by the trace for 2.6 mM H^+/0 mV. The traces at 23 mM H^+ are all at zero current within experimental error.

appearance of channels increased when the applied voltage was raised to +196 mV or −189 mV. A conductivity of the order of 100 pS can be calculated using the reference trace 2.6 mM H^+/0 mV. Clearly, discrete opening and closing of ion channels is observed. However, in spite of the high voltages applied, the basal conductivity was reduced to below 3 pS when the proton concentration was raised further, to 23 mM, and even at −189 mV and +196 mV, channel opening was rather rare. This membrane could also be brought back to a state of high conductivity by back-titration with 1 N KOH (not shown). Similar observations were made with the dioleoylphosphatidylcholine/cholesterol mixture (not shown).

3.3. *Hydrolytic proton induction*

In our earlier publication [22], in which we described the induction of ion channels by the enzyme AChase in the presence of acetylcholine, we suggested that the mechanism might involve local proton production due to substrate hydrolysis, rather than a ligand-induced conformational change produced by enzyme-substrate complex formation. Phenyl acetate (PhA) is an electrostatically neutral ester which can actually be hydrolysed by AChase even faster than acetylcholine due to its higher k_{cat} value [32]. We have now demonstrated that PhA can also induce ion channel formation in bilayers containing AChase. Fig. 4 shows traces from such an experiment in which a high-conductivity state was induced in an AChase/soybean lecithin bilayer by stepwise addition of PhA. The resting state of the membrane after addition of AChase, but before addition of the substrate, represents the start of the experiment (0 μM PhA). Fig. 4, at 480 μM PhA, shows a trace obtained shortly after the addition of the last aliquot of a total of 130 μl of 20 mM PhA, at which the first significant appearance of channels was noted. Since PhA should release two protons per molecule hydrolysed, a threshold bulk proton concentration approaching 1 mM H^+ would be expected if all the substrate had been hydrolysed. Indeed, control assays suggest that hydrolysis should be completed within a few seconds after addition of substrate.

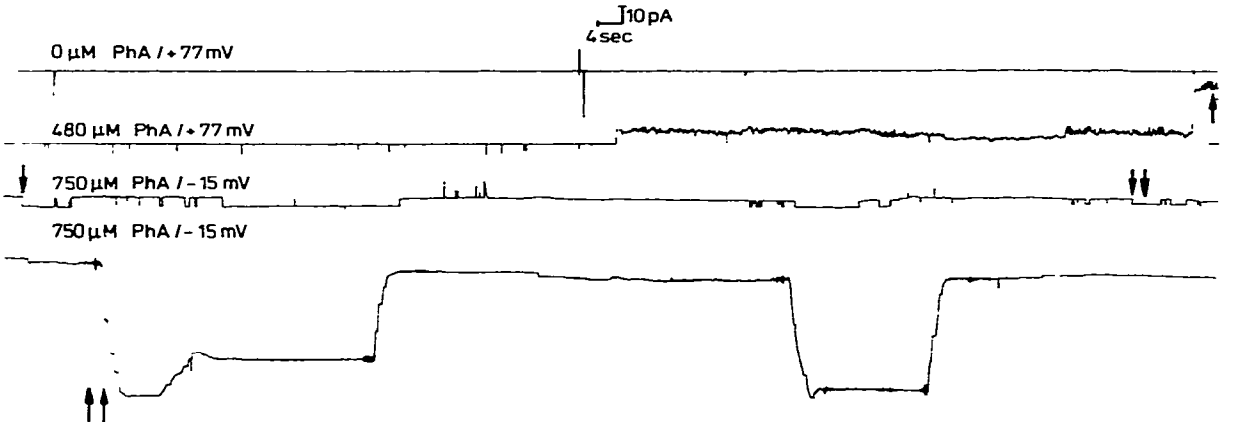

Fig. 4. Hydrolytic induction of ion channels in a soybean lecithin bilayer containing acetylcholinesterase by addition of phenyl acetate. The membrane was formed as in fig. 1 except that purified electric eel acetylcholinesterase was added to the aqueous phase in one compartment prior to bilayer formation. Titration was with aliquots of phenyl acetate (PhA) to the concentrations denoted. At ↑, a stationary state of 50 pS channels is seen, at ↓ a stationary state of 400 pS channels and at ↓ ↓ of 200 pS channels (cf. ref. 22). At ↑ ↑ appearance of approx. 1000 pS channels was observed. Further acidification quenched the high conductance reversibly (not shown).

Fig. 4, at 750 μM PhAc/− 15 mV, shows two selected traces from those obtained after addition of a further 60 μl of 20 mM PhAc. By this time (first trace), the mean conductivity of the membrane had increased to approx. 2000 pS. Nevertheless, the conductivity can clearly fluctuate between discrete conductivity levels superimposed on this high basal conductivity; the trace shows, in particular, the transition from a stationary state of 400 pS (↓) channels to another state of 200 pS channels (↓ ↓) which extended for minutes. But, note that earlier (at 480 μM PhAc, ↑), channels of approx. 50 pS also appeared reproducibly (cf. enlarged traces in fig. 5). Later (fig. 4, last trace), opening and closing of ion channels of calculated conductance of the order of 1000 pS was also observed (↑ ↑).

The high conductivity of this membrane was quenched by addition of fresh phospholipid to the surface of the bulk solution on both sides of the

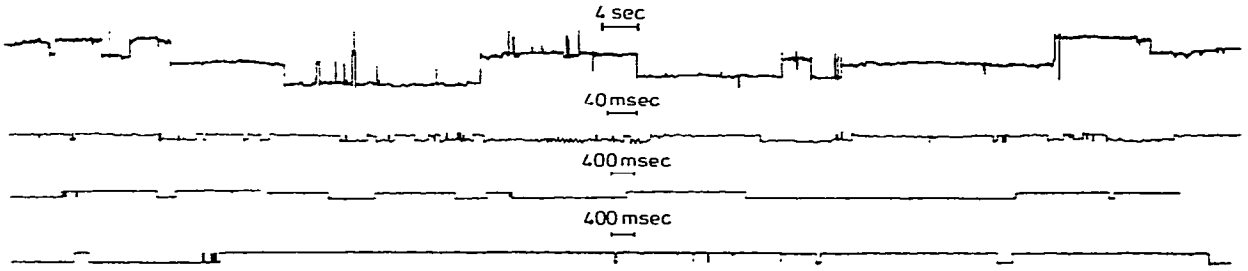

Fig. 5. Manifold ion-channel conductivities. Individual conductivity steps between 5 and 30 pS are visible in the first trace (taken from the same membrane as displayed in fig. 3, at 2.6 mM H^+/− 189 mV). However, calculated conductivities as low as 1 pS and as high as several thousand pS can also be resolved (not shown). Stationary states with unit conductivity steps of approx. 50, 200 and 400 pS are shown in the second, third, and fourth trace, respectively. The latter represent, on an expanded time scale, sections of traces from fig. 4.

bilayer, but later spontaneously reverted to a high-conductivity state which could, however, be abolished by addition of 2 N HCl and subsequently be reestablished by addition of Tris buffer (not shown).

4. Discussion

The results presented above show that ion channels can be induced in lipid bilayers by lowering the pH below a critical threshold value, and that on further lowering of the pH, the bilayer assumes a new low conductance state. Induction was presumably due to the increased proton concentration, since similar results were obtained with acetic acid and with HCl. Such results were obtained not only for the crude soybean phospholipid preparation, but also for a pure synthetic phosphatidylcholine alone, diphytanoylphosphatidylcholine, and for synthetic dioleoylphosphatidylcholine together with cholesterol. It thus seems reasonable to ascribe the appearance of conductance to a destabilization of the lattice structure of the phospholipids in the bilayer by fluctuations in protonation of the lipid head groups, and the subsequent decrease in conductance to a stabilization (presumably of a new lattice structure) by further protonation. Although the initiation of channel opening occurs at a bulk pH (≈ 3.5) which is somewhat above the estimated pK of the phosphate headgroup ($\approx$ pH 2 [33]), it should be taken into consideration that the applied membrane potential might lower the surface pH towards this pK, and that a very low degree of protonation might suffice to produce 'defects' which would account for the observed channel opening. It is worth noting that Papahadjopoulos [34] observed significant increases in surface potential of phosphatidylcholine monolayers in the pH range 3–4.

Detailed statistical analyses will be necessary in order to evaluate the distributions of magnitude and duration of the proton-induced ion channels. Also, experiments using different cations and anions will be needed so as to assess channel specificity. A number of points can, however, clearly be made at this early stage:

(1) The ion channels do not merely reflect a trivial increase in proton conductance due to lowering of the pH, since increased proton conductance, per se, would not explain the observed decrease in conductance on further lowering of the pH. Moreover, in high-conductance states, the channels should be conducting, at least in part, the major ionic species in the medium, either K^+ or Cl^- (presumably the former). This is clear, since the pH was lowered only on one side of the membrane and shown, by direct measurement in several experiments, to remain close to neutrality on the other side; yet, at a first approximation, membrane current was similar, but of opposite sign, when the sign of the applied potential was reversed. However, it is reasonable to postulate a significant conductance of protons through the same ion channels due to their high mobility [35].

(2) Although in high-conductance states of the membrane, the conductivity did not seem to be greatly affected by the magnitude and polarity of the applied voltage, the channels were clearly voltage dependent in the sense that, at stages of titration where only a few channels were opening and closing, events could often be prevented completely, in a reversible fashion, by decreasing the applied voltage. Since, in the experiments described, the applied potential during the initial part of the experiment was attracting protons to the bilayer in the acidified compartment, such a decrease in the applied potential should decrease the apparent proton gradient across the bilayer. Thus, at initiation, channel opening was controlled by the asymmetric protonation produced by the combined effects of acidification and the applied potential.

(3) Although a consistent correlation of channel amplitudes with the macroscopic state of the membrane cannot be presented, clearly resolved channels of various magnitudes could be perceived (figs. 4 and 5). Both at initiation and at later stages, channels of amplitude as small as 0.3 pA could be clearly resolved; thus, at high voltages channel conductivities below 2 pS could be calculated. It is worth noting that induction of 'mini'-channels of apparent conductivity as low as 5 pS (in 1 M KCl) has been observed in gramicidin-containing bilayers [36,37]. However, much larger channels also appeared through our membranes,

usually at later stages, and quite sharp distributions of magnitude often prevailed in a certain situation. Thus, in fig. 6A, which presents an amplitude histogram calculated from the trace shown in fig. 3, discrete conductance maxima are observed which exhibit an apparent multiplicity of 'unit' conductivity approx. 5 pS. However, if a region immediately following the trace in fig. 3 is included, which contains smaller channels, the histogram shown in fig. 6B is obtained, in which one of the maxima is split. It is worth emphasizing that, frequently, in membranes of high conductance, opening and closing of channels of a well defined magnitude (fig. 5) might occur over extended periods of time (e.g., on the scale of minutes). One way to explain such a phenomenon might be the transition of preexisting lattice defect(s) of high conductivity between two metastable states, a situation resembling, in its general features, that observed for alamethicin-induced channels [10].

As mentioned in section 1, current theories of ion-channel formation, whether in artificial bilayers or in native membranes, favor the notion of a polypeptide 'ionophore' providing the pore through which ion movement occurs. There is, however, no direct experimental evidence bearing on this issue in biological membranes. Furthermore, the observation of selectivity with respect to molecular weight or charge does not, in itself, imply an autonomous ionophore pore (cf. ref. 38).

Our experimental procedure, like that of other workers in the field, involves pretreatment of the hole with long-chain hydrocarbons, in our case hexadecane. Furthermore, small amounts of the volatile short-chain hexane solution from which the lipid monolayers were spread might also be incorporated into the hydrocarbon lattice of the bilayer. The participation of these adventitious hydrocarbon species in the phenomena we observed cannot be excluded. Nor can we exclude the possibility that the events we observe involve the annulus around the bilayer. If either the annulus or adventitious hydrocarbons are involved, however, it is only in the critical range demonstrated by our experiments.

Our results point to a direct involvement of the lipid bilayer in pore formation, by a mechanism in which protonation of phospholipid head groups leads to destabilization of the bilayer lattice. We have already cited evidence, based on single-channel conductance measurements, that resolved ion channels can be observed in pure lipid bilayers close to the phase transition temperature, and

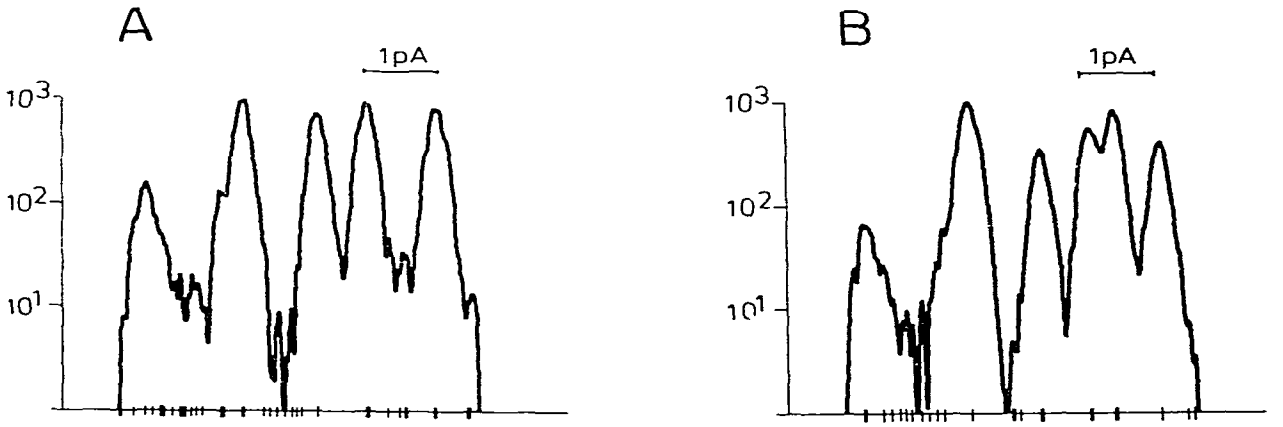

Fig. 6. Conductivity histograms of ion channels through a diphytanoylphosphatidylcholine bilayer. The abscissa shows membrane current on a linear scale. The histogram on the left (A) is computed from the trace at 2.6 mM H^+/+196 mV in fig. 3 and exhibits an apparent multiplicity of unit conductivity approx. 5 pS. However, if a region immediately following the trace in fig. 3 is included, which contains smaller channels, the histogram on the right (B) is obtained, in which one of the maxima is split.

earlier studies on liposomes also suggested an increased permeability near the phase transition temperature [39]. Recently, it has been shown by Antonov et al. [40] that sharp increases in conductance occur in bilayers constituted of various phosphatidic acids at phase transitions induced either by temperature or pH changes.

In principle, a change of any thermodynamic variable in the appropriate direction, assuming other variables to remain constant, could lead to induction of a critical state, and variations of such parameters as monolayer surface pressure [41] and applied voltage might have effects similar to those of pH and temperature. In a number of cases, we noted voltage induction of discrete conductance changes at neutral pH and, in one case, these conductance changes were abolished at a high HCl concentration. Yafuso et al. [42] earlier reported voltage-dependent stepwise conductance changes in bilayers constructed with oxidised cholesterol. Whether one modifies protonation by varying the applied voltage or the pH, one alters the lateral Coulombic interaction between the lipid head groups at the bilayer surface and should thus induce, in a critical range, considerable fluctuations of membrane area per hydrocarbon chain. As a consequence, defects might arise in the hexagonal lattice of the lipid bilayer. If unoccupied chain positions were to result from these defects (ref. 43a; for unoccupied positions in polymer diffusion cf. ref. 43b), aqueous channels of well defined conductivity might occur.

In the case of diphytanoylphosphatidylcholine, proton-induced ion channels were observed although no caloric phase transition temperature could be detected [44]; moreover, the heterogeneous soybean lecithin preparation employed in many of our experiments appeared devoid of a homogeneous phase transition temperature, as controlled by scanning calorimetry. It is thus possible that the degree of protonation of lipid head groups characterizes a transition of the bilayer accompanied by conductive defects, even under conditions of lipid heterogeneity which prevail in biological membranes.

In the experiments described in this paper, channel formation in artificial bilayers was achieved by lowering of the bulk pH. In the experiments which we reported earlier, qualitatively similar phenomena were evoked by addition of acetylcholine to bilayers containing AChase [22]. In the latter case, channel formation could be achieved by addition of amounts of acetylcholine, even the complete hydrolysis of which could not have lowered the bulk pH to the levels where we observed onset of channel formation in the experiments performed with lipid bilayers alone. Local pH changes at the membrane surface, produced by rapid substrate hydrolysis, might therefore be evoked for the acetylcholine-induced ion channels. It was, indeed, earlier shown that hydrolysis of acetylcholine by AChase in electric organ membrane fragments could produce pH changes of 2–3 units at the membrane surface [23], and more recently, that in artificial AChase enzyme membranes, such local pH changes could create a significant potential difference across the membrane [45]. In either case one is invoking a macroscopic pH change, whether in the bulk or adjacent to the membrane, in order to explain the phenomenon. However, it is also possible that protons produced by enzymic action of AChase could directly protonate lipid head groups in close proximity to the active site, even in cases where the bulk solution is buffered to more physiological pH values.

Other membrane-bound hydrolases such as ATPases might produce similar effects. Local proton production would be analogous to the localized proton circuit theory of oxidative phosphorylation [46], whereas protonation promoted by a macroscopic pH change would be analogous to the delocalised electrochemical proton gradient in the chemiosmotic theory (ref. 47; see also ref. 48).

Finally, our present experiments lend support to our earlier hypothesis that acetylcholine-induced channels in bilayers containing AChase are produced by a change in the state of the membrane produced by protonation of lipid head groups. It is worth noting that two groups have recently reported the presence of proton receptors in excitable cells [49,50]. In both cases rapid desensitization was observed. This should be taken into consideration in the evaluation of measurements in which ion conductance was studied in a steady-state situation with respect to pH (e.g., refs 51 and 52).

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