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MINI-REVIEW

Proton Conductance Through Phospholipid Bilayers: Water Wires or Weak Acids?

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Abstract

The proton/hydroxide (H^+/OH^-) permeability of phospholipid bilayer membranes at neutral pH is at least five orders of magnitude higher than the alkali or halide ion permeability, but the mechanism(s) of H^+/OH^- transport are unknown. This review describes the characteristics of H^+/OH^- permeability and conductance through several types of planar phospholipid bilayer membranes. At pH 7, the H^+/OH^- conductances ($G_{H/OH}$) range from 2–6 nS cm^{-2}, corresponding to net H^+/OH^- permeabilities of (0.4–1.7) $\times$ 10^{-5} cm sec^{-1}. Inhibitors of $G_{H/OH}$ include serum albumin, phloretin, glycerol, and low pH. Enhancers of $G_{H/OH}$ include chlorodecane, fatty acids, gramicidin, and voltages $>$ 80 mV. Water permeability and $G_{H/OH}$ are not correlated. The characteristics of $G_{H/OH}$ in fatty acid (weak acid) containing membranes are qualitatively similar to the controls in at least eight different respects. The characteristics of $G_{H/OH}$ in gramicidin (water wire) containing membranes are qualitatively different from the controls in at least four different respects. Thus, the simplest explanation for the data is that $G_{H/OH}$ in unmodified bilayers is due primarily to weakly acidic contaminants which act as proton carriers at physiological pH. However, at low pH or in the presence of inhibitors, a residual $G_{H/OH}$ remains which may be due to water wires, "hydrated defects," or other mechanisms.

Key Words: Proton conductance; proton permeability; phospholipid bilayer membrane; weak acid; water wire; fatty acid; gramicidin.

Introduction

Proton/hydroxide (H^+/OH^-) permeability of phospholipid bilayers at physiological pH is at least five orders of magnitude higher than alkali or

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halide ion permeability. The "anomalous" H^+/OH^- permeability was first described by Nichols and Deamer (1980), who suggested that chains of hydrogen-bonded water molecules (water wires) extend into the hydrophobic region of the membrane, providing a pathway for H^+/OH^- transport by proton jumping (Grotthuss conductance). Another possible explanation for the high H^+/OH^- permeability is that phospholipids contain weakly acidic contaminants which act as proton carriers (Gutknecht, 1984a, b). Either water wires or weak acids could provide an H^+/OH^- transport mechanism which is not available to other inorganic ions.

During the past six years, about twenty laboratories have studied the H^+/OH^- transport properties of phospholipid bilayers. In general, the original observations of Nichols and Deamer have been confirmed. However, the mechanism(s) of H^+/OH^- permeability remain unknown. The purpose of this review is to summarize the characteristics of H^+/OH^- conductance and permeability and evaluate the data in light of the water-wire and weak-acid models of H^+/OH^- transport.

Materials and Methods

Two types of lipid bilayers are being used for studies of H^+/OH^- transport, i.e., unilamellar vesicles and planar bilayers. Vesicle methods are described in accompanying articles by D. W. Deamer and D. S. Cafiso. Therefore, only planar bilayer methods will be described here.

The primary advantage of planar bilayers is that the investigator has convenient access to both sides of the membrane. This facilitates both electrical and tracer flux measurements. A disadvantage of planar bilayers is the presence of organic solvents in the membrane. However, this is not a major problem for most types of investigations. For example, the permeabilities of planar (Mueller–Rudin) bilayers to H^+/OH^- and water fall within the ranges observed for unilammeller vesicles (see, e.g., Fettiplace and Haydon, 1980; Gutknecht, 1984b). Unless otherwise indicated, the membranes described in this review were formed from approximately 2.5% solutions of phospholipids in *n*-decane.

The H^+/OH^- conductance of unmodified bilayers is very low and sometimes overshadowed by the conductances of other ions. Thus, many early studies overlooked the significant H^+/OH^- conductance which exists at neutral pH. In order to measure H^+/OH^- conductance ($G_{H/OH}$), one must minimize the conductances and diffusion potentials of ions other than H^+ and OH^-. A simple method is illustrated in Fig. 1. Weakly acidic and weakly basic buffers, e.g., Mes and Bistris, are mixed to give a pair of well-buffered solutions which have similar osmolarities, similar ionic strengths, and similar concentrations of all ions except H^+ and OH^-. The membrane is formed

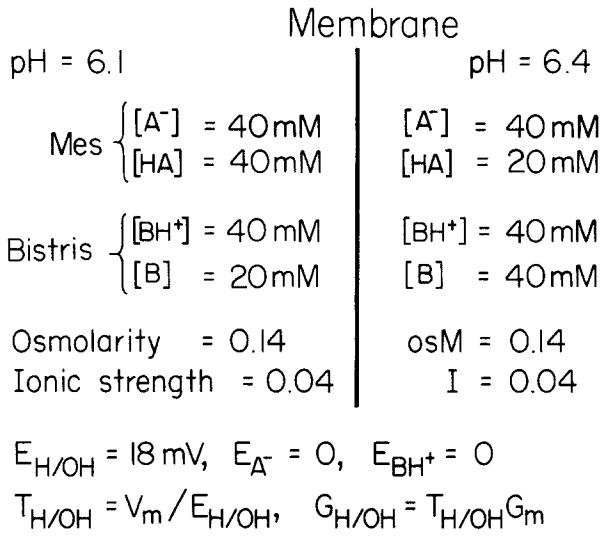

Fig. 1. An example of ionically and osmotically balanced buffer mixtures for producing a transmembrane H^+/OH^- gradient. A^- represents the Mes anion, and HA represents the zwitterionic species (pK = 6.1). B represents the Bistris free base, and BH^+ is the Bistris cation (pK = 6.4). Also shown are the equilibrium potentials for H^+/OH^-, A^-, and BH^+, and the equations for calculating $T_{H/OH}$ and $G_{H/OH}$ from V_m and G_m.

with the same solution on both sides. Then one side of the membrane is perfused with the second solution, creating the transmembrane pH gradient.

The advantage of using balanced buffer pairs, as shown in Fig. 1, is that the buffer ion equilibrium potentials (E_A and E_{BH}) are virtually zero (< 1 mV). Thus, the zero-current voltage (V_m) is due solely to H^+ and/or OH^- diffusion. Therefore, $G_{H/OH}$ (S cm^{-2}) can be easily estimated from the H^+/OH^- transference number ($T_{H/OH}$) and total membrane conductance (G_m), measured by standard electrical techniques.

At pH 7, H^+/OH^- conductance can be converted to H^+/OH^- "net" permeability ($P_{H/OH}$) by the relation, $P_{H/OH} = RTG_{H/OH}/F^2C_{H/OH}$, where R, T, and F have their usual meanings and $C_{H/OH}$ is the H^+ or OH^- concentration. Since the calculated permeabilities are extremely pH dependent, meaningful comparisons can be made only at the same pH, preferably at pH 7. For further details, see Gutknecht (1984b) and Deamer and Gutknecht (1986).

Predictions of the Weak-Acid and Water-Wire Models

Predictions of the weak-acid model are based primarily on the behavior of weakly acidic proton carriers, reviewed by McLaughlin and Dilger (1980).

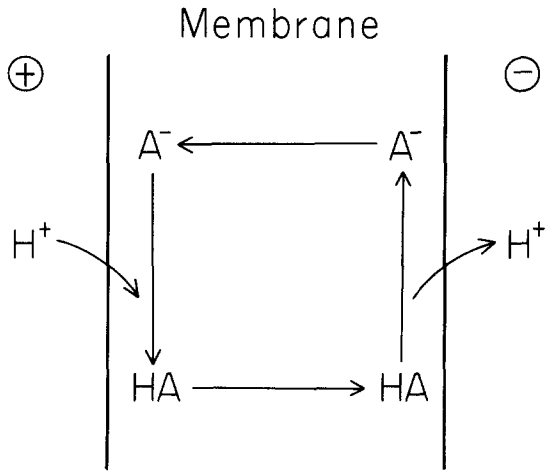

Fig. 2. A simple weak acid carrier model of proton transport through bilayers. Proton transport may be driven by either a voltage gradient, as shown here, or by a pH gradient. The pH of the aqueous solutions must be near or not too far above the pK. The model is similar to the A^- type proton carrier discussed by McLaughlin and Dilger (1980), except that in this case the carrier is confined to the membrane.

Predictions of the water-wire model are based primarily on the theoretical work of Nagle and Morowitz (1978) and J. F. Nagle (this volume).

The simplest (A^-/HA) type of weak-acid proton carrier is shown in Fig. 2. According to this model, protons cross the membrane in a nonionic (HA) form, but the carrier recycles in the membrane as an anion (A^-). Thus, proton transport may be driven by either a voltage gradient or a pH gradient. Furthermore, proton conductance shows an "anomalous" pH dependence, i.e., $G_{H/OH}$ increases as $[H^+]$ decreases because both HA and A^- are required for H^+ net transport. Third, as pH increases, $G_{H/OH}$ reaches a maximum and shows a plateau on the alkaline side of pK (see Fig. 5 in Kasianowicz *et al.*, 1987). The plateau is due to the normally higher permeability of HA compared to A^-. Fourth, $G_{H/OH}$ is proportional to the first power of the weak-acid concentration at constant pH. Fifth, if A^- translocation is rate limiting, then $G_{H/OH}$ will be influenced by agents which alter the membrane dipole potential and dielectric constant, e.g., phloretin and chlorodecane.

One difference between the classical weak-acid protonophores and the model shown in Fig. 2 is that protonophores are usually added to the aqueous phase and may cause A^- conductance at pH $\gg$ pK (LeBlanc, 1971). In contrast, the "endogenous protonophore" shown in Fig. 2 is confined to the membrane and cannot produce a steady-state A^- current between the two aqueous solutions.

Characteristics of H^+/OH^- Conductance and Permeability

A wide variety of chemical and physical agents have been found to alter H^+/OH^- conductance and permeability through bilayers. A partial list includes pH, serum albumin, phloretin, chlorodecane, temperature, glycerol, chloride, voltage, fatty acids, gramicidin A, deuterium oxide, and lipid oxidation and/or hydrolysis. In the discussion which follows, I will summarize the effects of these agents on $G_{H/OH}$, focusing attention on those agents and actions which may distinguish between weak-acid and water-wire mechanisms of H^+/OH^- transport.

pH Effects on $G_{H/OH}$

Figure 3 shows the effects of pH on $G_{H/OH}$ in planar (Mueller–Rudin) bilayers made from decane solutions of bacterial phosphatidylethanolamine (PE) or diphytanoyl phosphatidylcholine (PC). In both types of membranes, $G_{H/OH}$ is low (0.5–7 nS cm^{-2}) and moderately sensitive to pH, increasing 2- to 6-fold as pH increases from 2 to 8. At pH > 8, $G_{H/OH}$ appears to be fairly constant. The pH dependence of $G_{H/OH}$ shown in Fig. 3 is qualitatively similar to previous observations on egg PC vesicles. For example, Nichols and

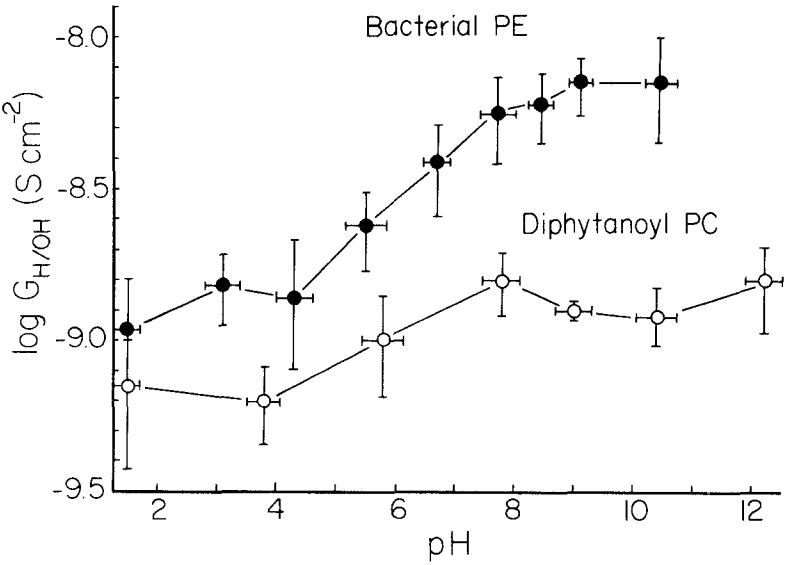

Fig. 3. Relation between H^+/OH^- conductance and pH in two types of lipid bilayer membranes. $G_{H/OH}$ was measured as described in Materials and Methods. Horizontal bars indicate the range of pH for each point, and vertical bars indicate standard deviations (n = at least four membranes at each point). Part of the statistical variation is due to a twofold variation among different batches of lipids.

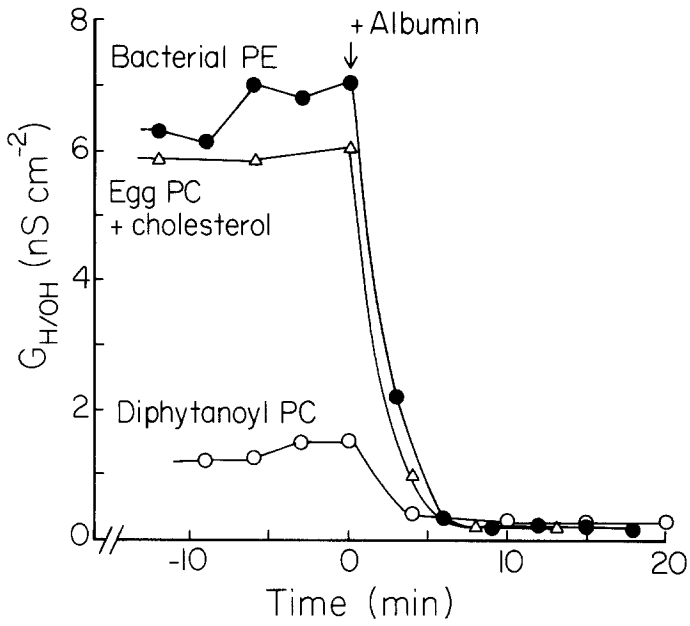

Fig. 4. Effects of bovine serum albumin on H^+/OH^- conductance in three types of lipid bilayer membranes. The serum albumin (fatty-acid free, Sigma No. A0281) was added to both sides of the membrane at 20 to 33 min after membrane formation. The time scale was normalized so that the three experiments could be plotted together. The albumin was injected into the rear compartment and perfused continuously through the front compartment. The solution pH's were 8.1 (rear) and 7.4 (front), buffered with Hepes plus Tris (I = 0.025, osmolarity = 0.08). The albumin concentration was 0.1–0.2 mg/ml (approximately 2 μM).

Deamer (1980) found an approximately 7-fold increase in H^+/OH^- net flux as pH increased from 6 to 8. Cafiso and Hubbell (1983) found an approximately 5-fold increase in $G_{H/OH}$ as pH increased from about 3.5 to 8.8. In both these studies, $G_{H/OH}$ tended to saturate at alkaline pH (see Fig. 3 in Gutknecht, 1984b).

The pH dependence of $G_{H/OH}$ is qualitatively consistent with a simple weak-acid model (see Figs. 1 and 5 of Kasianowicz *et al.*, 1987). However, the residual $G_{H/OH}$ at low pH is difficult to explain by the model shown in Fig. 2, which predicts no $G_{H/OH}$ when pH $\ll$ pK. Either one must postulate multiple weak acids with a range of pK's (Verkman and Ives, 1986), or else postulate a residual "background" $G_{H/OH}$ which is not related to weak acids acting as proton carriers.

Within the framework of the water-wire model, the pH dependence of $G_{H/OH}$ is more difficult to explain, because the mobility of H^+ is greater than OH^- in both water and ice (Eigen and De Maeyer, 1954; Nagle and Tristram-Nagle, 1983). However, the background $G_{H/OH}$, which may be independent

of pH (see below), can be explained by a water-wire mechanism, as pointed out by Nagle (this volume).

Serum Albumin Inhibits $G_{H/OH}$

Figure 4 shows that serum albumin (fatty-acid free) inhibits $G_{H/OH}$ in planar bilayers made from three different lipid mixtures. In each case, albumin reduces $G_{H/OH}$ to a background value in the range of 0.1–0.6 nS cm^{-2}, equivalent to a net H^+/OH^- permeability of (0.3–1.8) $\times$ 10^{-6} cm sec^{-1} at pH 7. As a "control" we also tested carbonic anhydrase (3 μM) but found no effect on $G_{H/OH}$ (data not shown).

The inhibition of $G_{H/OH}$ by albumin can be prevented by adding sodium octanoate (5 mM) to the aqueous solution prior to the addition of albumin (Gutknecht, 1987). Octanoate alone has no effect on $G_{H/OH}$ in phospholipid–decane bilayers. This is consistent with our previous studies on short-chain fatty acids, which showed that only the nonionic (HA) species permeate at a significant rate (Walter and Gutknecht, 1984).

Serum albumin is well known for its ability to reversibly bind amphiphilic molecules, especially long-chain fatty acids. For example, a reversible transfer of fatty acids between serum albumin and phospholipid bilayers was recently demonstrated by Hamilton and Cistola (1986). Thus, the inhibition of $G_{H/OH}$ by albumin suggests that albumin is removing a membrane component which causes $G_{H/OH}$. If so, then the ability of octanoate to block the albumin effect may reflect the saturation of binding sites with a competing ligand (Reynolds *et al.*, 1968).

Water Permeability and $G_{H/OH}$ Are Not Correlated

Figure 5 shows the relation between water permeability (measured with tritiated water) and $G_{H/OH}$ in several types of lipids under several different conditions which either enhance or inhibit $G_{H/OH}$. The lack of correlation between P_{H_2O} and $G_{H/OH}$ suggests that water and H^+/OH^- cross the membrane via separate pathways. (The effects of phloretin and chlorodecane will be discussed later.)

These results are consistent with the weak-acid model. For example, adding small amounts of long-chain fatty acids to the membrane increases $G_{H/OH}$ but does not affect P_{H_2O} (Gutknecht, 1987). On the other hand, the data are more difficult to explain by means of the water-wire model. According to the water-wire model, the unmodified bilayer would have to contain two independent "pools" of water, i.e., monomers involved in transmembrane solubility-diffusion, plus several percent of the membrane water in hydrogen-bonded chains of approximately 20 molecules each (see articles by Deamer and Nagle, this volume).

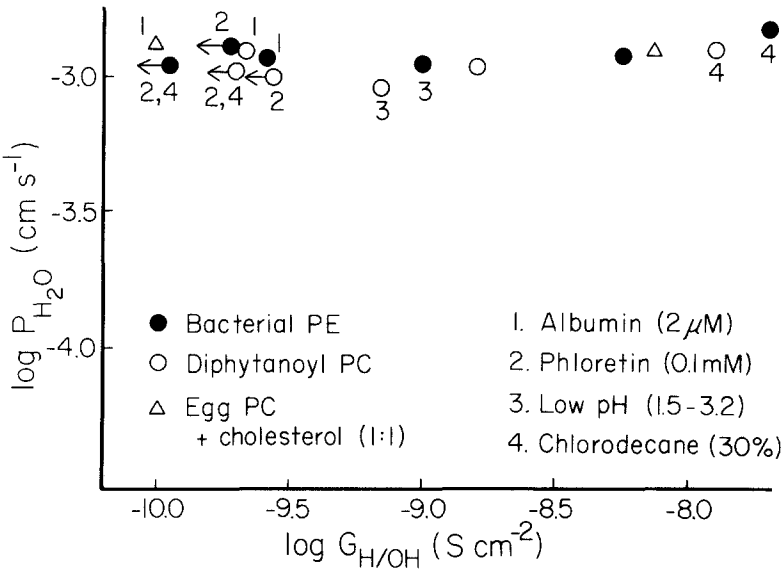

Fig. 5. Relation between water permeability and H^+/OH^- conductance in several types of membranes under several conditions. The lipid compositions are indicated by symbols, and the modifying agents are indicated by numbers. Leftward arrows indicate that the values of $G_{H/OH}$ are upper limits. Water permeabilities were measured with tritiated water and were corrected for the diffusional resistance of the unstirred layer which, in our system, has a thickness of 119 ± 20 μm (for further details, see Gutknecht, 1987).

Phloretin Inhibits $G_{H/OH}$

Phloretin inhibits $G_{H/OH}$ by at least 5-to 25-fold, depending upon the specific phospholipid (Fig. 5) (Gutknecht, 1987). The primary effect of phloretin on lipid bilayers is to decrease the membrane dipole potential, thus increasing cation conductance and decreasing anion conductance (Andersen *et al.*, 1976). Thus, the inhibition of $G_{H/OH}$ by phloretin suggests that the H^+/OH^- charge carrier is primarily anionic.

The inhibition of $G_{H/OH}$ by phloretin is consistent with the weak-acid model, because the rate-limiting step in proton transport is normally A^- (or HA_2^-) translocation. For example, phloretin inhibits proton conductance by weak acid protonophores, e.g., CCCP (Andersen *et al.*, 1976). On the other hand, the inhibition of $G_{H/OH}$ by phloretin is more difficult to explain by the water-wire model, because the mobility of H^+ is higher than OH^- in both water and ice.

Chlorodecane Increases $G_{H/OH}$

Addition of chlorodecane (30%, v/v) to the membrane-forming solution increases $G_{H/OH}$ by 3- to 8-fold, depending upon the lipid composition

(Fig. 5) (Gutknecht, 1987). The primary effect of chlorodecane is to increase the dielectric constant of the nonpolar region of the membrane, thus increasing the permeability to both cations and anions (Dilger *et al.*, 1979).

The chlorodecane effect on $G_{H/OH}$ is consistent with the weak-acid model, since A^- (or HA_2^-) translocation is normally rate limiting. However, the chlorodecane effect might also be consistent with the water-wire model, since substitution of chlorodecane for decane could conceivably increase the concentration of intramembrane water and thus increase the number of water wires. Note, however, that 30% chlorodecane does not significantly increase water permeability, and, furthermore, phloretin abolishes the increase in $G_{H/OH}$ which caused by chlorodecane (Fig. 5). Thus, even in the presence of chlorodecane, the primary H^+/OH^- charge carrier is apparently anionic.

D^+/OD^- *and* H^+/OH^- *Conductances Are Similar*

We also compared D^+/OD^- with H^+/OH^- conductance by substituting D_2O (96%) for H_2O in Hepes plus Tris buffers, pH 7.4–8.1. Within experimental error ($\pm 20\%$), we found no difference between $G_{H/OH}$ and $G_{D/OD}$ in diphytanoyl PC membranes. Similar results for egg PC vesicles were reported by Perkins and Cafiso (1986) and Deamer (this volume).

Fatty Acids Are Proton Carriers

In order to gain insights into the expected behavior of bilayers containing weak acid contaminants, we studied the effects of long-chain fatty acids on $G_{H/OH}$ (Gutknecht, 1987 and unpublished data). For example, addition of phytanic acid (a 20-carbon, branched-chain fatty acid) to the membrane-forming solution causes an increase in $G_{H/OH}$ which is proportional to the first power of the phytanic acid concentration at constant pH, as predicted by the model shown in Fig. 2. Furthermore, we have recorded eight similarities and no qualitative differences between the behavior of $G_{H/OH}$ in fatty acid-containing and unmodified bilayers. The similarities include inhibition of $G_{H/OH}$ by serum albumin, prevention of albumin inhibition by octanoate, inhibition by phloretin, inhibition by low pH, inhibition by glycerol, stimulation by chlorodecane, stimulation by membrane voltages $>$ 80 mV, and no effect of substituting D_2O for H_2O. These data provide circumstantial evidence for the existence of weak acids, possibly long-chain fatty acids, in unmodified membranes. Using the measured "flip-flop" rates of Storch and Kleinfeld (1986), we calculate that free fatty acid levels of 0.02–0.1 mol.% could produce H^+/OH^- conductances of 1.6 to 6 nS cm^{-2} (cf. Fig. 3). Another estimate, obtained by adding phytanic acid to diphytanoyl PC, yields an extrapolated value near 1% (Gutknecht, 1987).

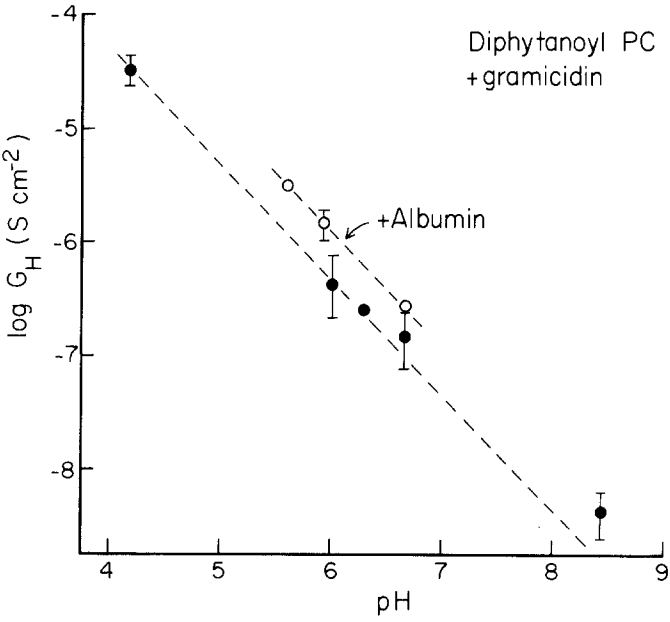

Fig. 6. Relation between H^+/OH^- conductance and pH in diphytanoyl PC bilayers containing gramicidin A (Sigma No. G5002). The gramicidin (10^{-10} M) was applied by continuous perfusion from a large reservoir. Due to a slow upward drift in $G_{H/OH}$, values were recorded 18–25 min after membrane formation. In a few experiments, serum albumin was also applied at a concentration of about 2 μM. Solutions were buffered as described in Fig. 1 and Gutknecht (1984b). The error bars are standard deviations of three membranes. The dashed lines are drawn with slopes of -1.0.

Gramicidin A Channels Contain Water Wires

Gramicidin A forms cation-selective channels which contain 6 to 9 water molecules arranged in single file (Rosenberg and Finkelstein, 1978; Levitt, 1984). The channels are highly permeable to protons, which traverse the channel by a proton jump mechanism (Myers and Haydon, 1972; Levitt *et al.*, 1978; Levitt, 1984). Thus, H^+/OH^- transport through the gramicidin A channel may provide insights into the behavior of water wires in unmodified bilayers. [For background information on the properties of gramicidin, see Deamer (this volume).]

Figure 6 shows that H^+ conductance through gramicidin channels is nearly proportional to the aqueous H^+ activity over the pH range of 4.2–8.5. A similar pattern is obtained by comparing the single-channel H^+ conductances at pH 2 (Hladky and Haydon, 1972; Neher *et al.*, 1978) and pH 7.5 (Krishnamoorthy, 1986). The pH dependence of G_H in gramicidin channels is consistent with a water-wire (H^+ hopping) mechanism. However, the

pattern differs dramatically from the pH dependence of $G_{H/OH}$ in unmodified bilayers (cf. Fig. 3).

In addition to the differing pH dependence, G_H through gramicidin channels differs from controls in three other respects, i.e., the effects of serum albumin, chlorodecane, and deuterium oxide. First, serum albumin causes a small increase in G_H (Fig. 6), probably by adsorbing to the membrane and imparting a negative surface charge which, in turn, increases the H^+ concentration at the membrane surface. Second, chlorodecane has no effect on gramicidin G_H (data not shown), probably because chlorodecane affects only nonpolar conductance pathways. Finally, D^+/OD^- conductance is reduced to about 40% of H^+/OH^- conductance through gramicidin channels (Deamer, this volume). These four qualitative differences lead to the conclusion that the mechanism of H^+ conductance through gramicidin channels is basically different from the primary mechanism of H^+/OH^- conductance in unmodified bilayers.

Behavior of Water Dissolved in Liquid Hydrocarbons

The water-wire hypothesis suggests the possibility that hydrogen-bonded aggregates of water molecules might exist in bulk hydrocarbon solvents. Conrad and Strauss (1985) investigated this possibility by measuring the vibrational spectra of water dissolved in liquid hydrocarbons. Their results indicate that water dissolved in alkanes and alkenes, e.g., decane and decene, is monomeric.

Effects of Lipid Composition on $G_{H/OH}$

$G_{H/OH}$ varies with lipid composition (Fig. 3, Nichols and Deamer, 1980; Perkins and Cafiso, 1986; and others), but the available data do not provide clear evidence for or against any specific mechanism of $G_{H/OH}$. Apparently, the lowest and the least pH-dependent $G_{H/OH}$ is observed in diphytanoyl PC (see Fig. 3 and Cafiso and Hubbell, 1983, but cf. Grzesiek and Dencher, 1986). This may reflect the high purity and/or absence of peroxidation in this fully saturated, synthetic PC. In egg PC the highest $G_{H/OH}$ is observed in vesicles made from the most highly unsaturated lipid fraction (Perkins and Cafiso, 1986).

Slightly oxidized phospholipids display high values of $G_{H/OH}$ (Rossignol *et al.*, 1982; Cafiso and Hubbell, 1983). Lipid oxidation and/or hydrolysis produces a variety of weakly acidic products (Toyoshima and Thompson, 1975; Frankel, 1982 and personal communication). Commercial lecithins often contain free fatty acids at levels near 1% (Uhlendorf, 1984). Phosphatidylethanolamine sometimes contains negatively charged contaminants (see McLaughlin *et al.*, 1975). For example, a recent study on bacterial PE

bilayers revealed a surprisingly high surface charge of 0.5 $\mu C\,cm^{-2}$ (Chernomordik *et al.*, 1984), suggesting the presence of more than 1% fatty acids or other anionic impurities. Apparently, a variety of phospholipids contain small amounts of unidentified substances which titrate near pH 7 (Grzesiek and Dencher, 1986; Krishnamoorthy, 1986 and personal communication). The identity of these substances and their effects on $G_{H/OH}$ are unknown.

Effects of Voltage, Temperature, and Glycerol on $G_{H/OH}$

$G_{H/OH}$ is independent of membrane voltage at low voltages (< 80 mV, Cafiso and Hubbell, 1983) but increases at least 2-fold at voltages approaching 200 mV (Krishnamoorthy and Hinkle, 1984; Gutknecht, 1984b, 1987). Roughly similar current–voltage curves are also observed in bilayers containing fatty acids (Gutknecht, 1987), weak acid protonophores (Kasianowicz *et al.*, 1987), and, theoretically, in at least one water-wire model (Nagle, this volume). Thus, the available current–voltage (or conductance–voltage) data do not clearly distinguish between weak acids and water wires.

The temperature dependence of $G_{H/OH}$ yields apparent activation energies in the range of 13–22 $kcal\,mol^{-1}$ (Elamrani and Blume, 1983; Grzesiek and Dencher, 1986; Seigneuret and Rigaud, 1986; and others). However, these data do not distinguish between weak acids and water wires, because both mechanisms may show high activation energies. The absence of both H^+/OH^- and water permeability maxima at the phase transition temperature has been invoked as evidence for water wires (Elamrani and Blume, 1983). However, this is equally consistent with a proton carrier model, because hydrophobic ions also fail to show conductance maxima at the phase transition temperature (Krasne *et al.*, 1971; Boheim *et al.*, 1980; Stark and Awiszus, 1982). Finally, a correlation between lipid fluidity and H^+OH^- flux has been cited as evidence for a water-wire mechanism (Rossignol *et al.*, 1982), but this finding is equally compatible with a proton-carrier model.

Glycerol inhibition of $G_{H/OH}$ may also be consistent with both weak-acid and water-wire models (Gutknecht, 1984b). Substitution of glycerol for water reduces bulk water activity, reduces the fluidity of bilayer lipids (Surewicz, 1984), and reduces the membrane dipole potential (Cadenhead and Bean, 1972; S. A. Simon, personal communication). Thus, glycerol inhibits various types of carrier-mediated transports, including H^+ conductance mediated by long-chain fatty acids (Gutknecht, 1987) and 2-4-dinitrophenol (Kronick, 1977).

Effects of Chloride on H^+/OH^- *Transport*

Chloride promotes net proton transport through bilayers, especially at low pH (Gutknecht and Walter, 1981; Nozaki and Tanford, 1981). However,

at pH 7, Cl^- has either no effect (Nichols and Deamer, 1980) or causes a severalfold increase in net proton flux (Seigneuret and Rigaud, 1986; Grzesiek and Dencher, 1986). The Cl^--dependent H^+ flux may be identical to the H^+-dependent Cl^- flux studied by Toyoshima and Thompson (1975) and others.

At low pH the proton transport mechanism is diffusion of molecular HCl (Gutknecht and Walter, 1981). At neutral pH, a specific binding of HCl to phospholipids appears to be involved (Toyoshima and Thompson, 1975). In either case, the net HCl transport is nonconductive (electrically silent). Thus, the mechanism(s) are basically different from the conductive (electrogenic) H^+/OH^- transport mechanism(s) which we are considering in this review.

"*Background*" H^+/OH^- *Conductance*

As pointed out above, the H^+/OH^- conductance at low pH (Fig. 3) cannot easily be explained by weak acids acting as proton carriers. Furthermore, at pH 7 a residual or 'background" $G_{H/OH}$ remains in the presence of serum albumin and possibly also phloretin (Figs. 4 and 5 and unpublished data). Collectively, these data suggest the possibility of a background $G_{H/OH}$ in the range of 0.1–0.6 $nS\,cm^{-2}$ at pH 7, corresponding to a net H^+/OH^- permeability of (0.3–1.8) $\times$ 10^{-6} $cm\,sec^{-1}$. The background $G_{H/OH}$ may be independent of pH, and, if so, a water-wire model provides a plausible explanation (Nagle, this volume).

The background H^+/OH^- pathway could be through the hydrophobic (nonpolar) region or through "hydrated defects" in the bilayer (Deamer and Barchfield, 1984). The likelihood of ionic conductance through structural defects in bilayers is discussed by Smith *et al.* (1985). For illustration, consider the fact that at pH 2 the proton conductance of a single gramicidin A channel is 22–240 pS (Hladky and Haydon, 1972; Neher *et al.*, 1978). If we take the gramicidin channel to be a model of a hydrated defect, then in an average size planar bilayer (0.02 cm^2) a $G_{H/OH}$ of 1 $nS\,cm^{-2}$ could be caused by less than one hydrated defect containing a single water wire. Intuitively, one would be surprised *not* to find any residual H^+/OH^- conductance, especially at low pH.

However, at pH 7 most of $G_{H/OH}$ is sensitive to both dielectric constant (chlorodecane) and dipole potential (phloretin) (Fig. 5 and Gutknecht, 1987). Furthermore, the increase in $G_{H/OH}$ caused by chlorodecane is abolished by phloretin, suggesting that the charge carrier is primarily anionic and the pathway is primarily nonpolar. Thus, hydrated defects, if they exist, cannot explain the predominant $G_{H/OH}$ which, in our system, comprises about 65 to 98% of the total $G_{H/OH}$ at pH 7 (Figs. 4 and 5).

In other systems, the background $G_{H/OH}$ may comprise a larger fraction of the total $G_{H/OH}$. For example, small unilamellar vesicles studied by Cafiso and Hubbell (1983) and Perkins and Cafiso (1986) have a $G_{H/OH}$ corresponding to a net H^+/OH^- permeability of about 10^{-6} cm sec^{-1}, substantially lower than most published values. Although the pH dependence of $G_{H/OH}$ is qualitatively similar to that in Fig. 3, the H^+/OH^- current is not sensitive to phloretin and only moderately sensitive to pretreatment with serum albumin (D. S. Cafiso, personal communication). The relatively low $G_{H/OH}$ and insensitivity to inhibitors may reflect the exceptionally high purity of their phospholipids and, perhaps, the properties of a second, low-conductance pathway for H^+/OH^-.

Conclusions

We have summarized the characteristics of H^+/OH^- transport through phospholipid bilayers and evaluated the data in light of two models, i.e., the weak-acid contaminant model and the hydrogen-bonded water-wire model. The available data generally conform to the predictions of a simple weak-acid proton carrier model. Furthermore, comparing the behavior of $G_{H/OH}$ in unmodified and fatty acid-containing membranes reveals eight similarities and no qualitative differences. In contrast, comparing the behavior of $G_{H/OH}$ in unmodified and gramicidin-containing membranes reveals four qualitative differences. Thus, if water wires exist in unmodified membranes, their transport properties differ from the properties of water wires in gramicidin channels.

At pH 7, values of $P_{H/OH}$ ranging from about 10^{-7} to 10^{-3} cm sec^{-1} have been reported from about twenty different laboratories. From our review of the literature, it seems clear that at least part of this variation can be explained by variations in the levels of weak-acid contaminants in the phospholipids. Nevertheless, the weak-acid hypothesis cannot explain all of the observed H^+/OH^- conductance or permeability. At low pH, or in the presence of inhibitors, a background $G_{H/OH}$ remains which may be due to water wires, hydrated defects, or other mechanisms. Unfortunately, this background $G_{H/OH}$ is too low to study in conventional planar bilayers, and a more sensitive method is required, such as that developed by Cafiso and Hubbell (1983).

Whether weak acids or water wires are important in biological membranes remains to be seen. However, in at least some biological membranes P_H is orders of magnitude higher than P_{Na} (Wright *et al.*, 1984). In inner mitochondrial membranes $G_{H/OH}$ is about 400 nS cm^{-2} (Mitchell and Moyle, 1967), which converts to a $P_{H/OH}$ of about 10^{-3} cm sec^{-1} (Nichols and

Deamer, 1980). In renal brush border membranes the proton permeability is 5×10^{-3} cm sec^{-1}, and proton diffusion utilizes a lipid pathway which is independent of the pathway for water (Ives and Verkman, 1985). To the extent that biological membranes contain weak-acid "contaminants," e.g., free fatty acids, our bilayer results may help explain H^+/OH^- conductance and permeability. For example, "washing" photoreceptor membrane vesicles with bovine serum albumin reduces H^+/OH^- conductance by almost two orders of magnitude (D. M. Ojcius, personal communication), and exposure of gastric microsomes to serum albumin reduces H^+/OH^- permeability 2- to 3-fold (D. K. Hanzel and J. G. Forte, personal communication).

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