J. Membrane Biol. 82, 105–112 (1984)

The Journal of
Membrane Biology

Proton/Hydroxide Conductance through Lipid Bilayer Membranes

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Summary. A simple method of measuring proton/hydroxide conductance ($G_{H/OH}$) through planar lipid bilayer membranes is described. First the total conductance (G_m) is measured electrically. Then the H^+/OH^- transference number ($T_{H/OH}$) is estimated from the diffusion potential (V_m) produced by a transmembrane pH gradient. The pH gradient is produced by a pair of buffered solutions which have identical concentrations of all ions except H^+ and OH^-. Thus, V_m is due entirely to H^+/OH^- diffusion and $G_{H/OH}$ can be calculated from the relations, $V_m = T_{H/OH}E_{H/OH}$ and $G_{H/OH} = T_{H/OH}G_m$, where $E_{H/OH}$ is the equilibrium potential for H^+ and OH^-. In bilayers made from bacterial phosphatidylethanolamine (PE) in *n*-decane, $G_{H/OH}$ is nearly independent of pH, ranging from about 10^{-9} S cm^{-2} at pH 1.6 to 10^{-8} S cm^{-2} at pH 10.5. Because $G_{H/OH}$ is nearly independent of pH, the calculated permeability coefficients to H^+ and/or OH^- are extremely pH dependent, which partly explains the wide range of values reported for phospholipid vesicles and biological membranes. $G_{H/OH}$ appears to be independent of the membrane surface charge, because titrating either the phosphate or the amino group of PE has little effect on $G_{H/OH}$. $G_{H/OH}$ is reduced about 10-fold when the water activity is reduced 33% by replacement with glycerol. Although the mechanism of H^+/OH^- conductance is not known, the relation between $G_{H/OH}$ and water activity suggests that several water molecules are involved in the H^+/OH^- transport process.

Key Words proton conductance · hydroxide conductance · lipid bilayer · membrane permeability · water

Introduction

Proton/hydroxide (H^+/OH^-) transport is important in most biological membranes, but the mechanisms of H^+/OH^- transport are poorly understood [6]. Recently, H^+/OH^- permeabilities of about 10^{-4} cm sec^{-1} were reported for several types of phospholipid vesicles [10, 12, 24, 30, 31, 39]. This value of $P_{H/OH}$ is almost as high as the water permeability [13, 39] and at least 10^6-fold higher than the Na^+ and Cl^- permeabilities [9, 30, 31]. Some biological membranes are also 10^6 times more permeable to H^+/OH^- than to Na^+ or K^+ [45]. Thus, phospholipid bilayers and biological membranes apparently possess pathway(s) for passive H^+/OH^- transport which is (are) not available to other inorganic ions [7, 10, 12, 30, 31, 36, 39].

Previous measurements of H^+/OH^- permeability through lipid bilayers have been generally limited to vesicle suspensions which provide sufficiently large membrane surface areas for the measurement of small H^+/OH^- fluxes at neutral pH. In this report I describe a simple method of measuring H^+/OH^- conductance through planar bilayers over a wide range of pH. My results confirm and extend the vesicle studies of Nichols and Deamer [10, 30, 31], Cafiso and Hubbell [7] and others [5, 8, 12, 24, 36, 39] who found surprisingly high H^+/OH^- permeabilities over the range of pH 5 to 9. The H^+/OH^- conductance through phosphatidylethanolamine-decane bilayers is nearly independent of pH and surface charge. However, $G_{H/OH}$ is inhibited by partially substituting glycerol for water in the membrane bathing solutions. Although the mechanism of H^+/OH^- conductance is unknown, the data suggest that several water molecules may be involved in the transport process.

Materials and Methods

Lipid bilayer membranes were formed from bacterial phosphatidylethanolamine (PE) in decane (20 mg/ml) by means of the brush technique of Mueller and Rudin [28]. Membranes were formed on a 2.0 mm^2 hole in a polyethylene partition which separated two magnetically stirred solutions of 1.1 ml each. In most experiments the front compartment was perfused continuously at a rate of 1 to 2 ml/min so that the ionic composition and pH could be changed during the course of an experiment. The temperature was 24 ± 1°C.

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Table. Buffer pairs for producing H^+/OH^- gradients across lipid bilayer membranes

pH range[a]	Acidic buffer and pK[b]	Basic buffer and pK[b]
5.2–7.2	MES (pK = 6.1)	Bis tris (pK = 6.4) or histidine (pK_2 = 6.0)
6.4–8.8	HEPES (pK = 7.4) or MOPS (pK = 7.2)	Tris (pK = 8.1)
7.6 – 9.8	AMPSO (pK = 9.2) or TAPS (pK = 8.4)	Bis-tris propane (pK_2 = 9.0)

[a] The useful pH range is within ±0.8 pH units of at least one buffer pK. In most experiments the pH difference across the membrane was 0.3 to 1.0 pH units.
[b] Most pK's require a small correction for ionic strength [34].

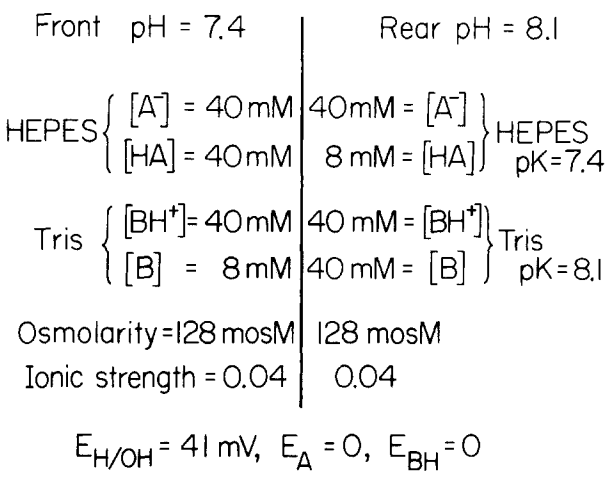

Fig. 1. An example of a "perfectly balanced" pair of buffered solutions for producing an H^+/OH^- gradient across a membrane. Acidic and basic buffers are mixed to produce two solutions which differ in pH but have identical osmolarities and identical ionic concentrations. Thus, V_m is due solely to H^+/OH^- diffusion. In this example, A^- represents the HEPES anion, *HA* represents the zwitterionic species (pK = 7.4), *B* represents Tris free base and BH^+ is the Tris cation (pK = 8.1). $E_{H/OH}$ is the H^+/OH^- equilibrium potential, calculated by the Nernst equation. *See* text for further details

Membrane conductances were measured by applying a small voltage pulse across the membrane in series with a known resistance. Membrane potentials were measured by means of a high impedance electrometer and two matched calomel-KCl electrodes which made contact with the front and rear solutions. Ionic transference numbers were estimated from the zero-current potentials produced by ionic gradients across the membrane [3].

In order to estimate the H^+/OH^- conductance, I wished to minimize the "background" conductances and diffusion potentials produced by all other ions. Therefore, I used fairly low ionic strengths (usually 0.02 to 0.04) and pairs of buffered solutions which had identical concentrations of all ions except H^+ and OH^-. In most experiments the solutions contained mixtures of polar anionic and cationic buffers, titrated against each other so that no inorganic ions were added (*see* Fig. 1 and Table). Under these conditions the zero-current (open circuit) potential (V_m) is given by the expression

$$V_m = T_H E_H + T_{OH} E_{OH} + T_{BH} E_{BH} + T_A E_A \quad (1)$$

where A is a buffer anion, BH is a buffer cation, T is the transference number, and E is the equilibrium potential, calculated from the Nernst equation. The transference number for the ith ion is defined as G_i/G_m, where G_i is the conductance of the ith ion and G_m is the total membrane conductance.

If there are no gradients of BH^+ or A^- across the membrane, i.e., if the only gradients are in nonionic or zwitterionic buffer species, then $E_{BH} = 0$ and $E_A = 0$, and these terms drop out of Eq. (1). Since T_H and T_{OH} cannot be measured separately, we define $T_{H/OH} \equiv T_H + T_{OH}$. Also, since $E_H = E_{OH}$, we define $E_{H/OH} \equiv E_H = E_{OH}$. Thus Eq. (1) becomes

$$V_m = T_{H/OH} E_{H/OH}. \quad (2)$$

The H^+/OH^- conductance ($G_{H/OH}$) is then obtained from the relation

$$G_{H/OH} = T_{H/OH} G_m. \quad (3)$$

If buffer ion conductances are negligible, then the slope of V_m *vs.* $E_{H/OH}$ will be 1.0, i.e., the membrane will behave electrically as a pH electrode. If buffer ion conductances are significant, then the slope of V_m *vs.* $E_{H/OH}$ will be less than 1.0, but $T_{H/OH}$ can still be estimated accurately if the slope is not too close to zero. In this study $T_{H/OH}$ ranged from 0.5 to 1.0, depending upon the pH and conductances of various buffer ions.

A "perfectly balanced" pair of buffered solutions differ in pH but have identical ionic concentrations and identical osmolalities, e.g., the HEPES-Tris mixture shown in Fig. 1. In order to obtain perfectly balanced buffer pairs over the pH range of 5.2 to 9.8 I used the combinations of cationic and anionic buffers shown in the Table, mixing two concentrated buffer solutions to obtain the desired pH's and concentrations. Buffer pairs can be perfectly balanced if the high pH solution is at the pK of the cationic buffer, and the low pH solution is at the pK of the anionic buffer, as shown in Fig. 1. In order to vary the pH range while still maintaining ionic and osmotic balance, the pH's of both solutions must be changed by equal amounts in opposite directions. For example, the HEPES-Tris buffer pair shown in Fig. 1 can be perfectly balanced at pH's 7.4 and 8.1, 7.6 and 7.9, 7.1 and 8.4, etc. In this way $E_{H/OH}$ can be varied over a fairly wide range with a single buffer pair.

If an appropriate pair of polar buffers cannot be found for a desired pH range, then any weak acid or weak base can be titrated with a strong acid or strong base to produce a pair of "ionically balanced" solutions which differ in pH. For example, adding the same amount of NaOH to two different concentrations of weak acid (*HA*) will produce a pair of solutions which differ in pH but have identical concentrations of Na^+ and A^-. Thus, Eq. (2) can still be used to calculate $T_{H/OH}$. However, these two solutions will differ in osmolality because the *HA* concentrations differ. If necessary, osmotic balance can be achieved by adding a polar nonelectrolyte to the high pH (low *HA*) solution.

At very low pH the two solutions are "buffered" with H^+ but are not ionically balanced, i.e., $E_A \neq 0$. Thus, Eq. (2) cannot be used to calculate $T_{H/OH}$. However, $T_{H/OH}$ can be estimated by

measuring a "dilution potential" produced by two concentrations of strong acid, e.g., H_3PO_4. Under these conditions, $E_{H/OH} = -E_A$. Thus, rearranging Eq. (1), we get

$$T_{H/OH} = \frac{V_m + E_{H/OH}}{2E_{H/OH}}. \quad (4)$$

The same approach can be used for very high pH solutions (buffered with OH^-).

In addition to the buffer pairs listed in the Table, I used several weak acids titrated with NaOH or tetraethylammonium hydroxide to give ionically balanced solutions ($E_A = 0$, $E_{Na} = 0$) as described above. The weak acids were succinic acid (pK_1 = 4.1) or glutamic acid (pK_1 = 4.1) (pH range 3.6 to 4.6), and citric acid (pK_1 = 3.1, pH range 2.8 to 3.4). Over the pH range of 1.5 to 2.0 I measured dilution potentials with H_3PO_4 (pK_1 = 2.1) or HCl (pK = −6.1), and then calculated $T_{H/OH}$ by Eq. (4). At high pH I used lysine (pK_3 = 10.5) plus NaOH to give ionically balanced solutions over a pH range of 10.0 to 10.5. One potentially useful high pH buffer (CAPS, pK = 10.4) was rejected, because it increased the membrane conductance. Buffers having permeant nonionic forms, e.g., imidazole, were avoided also.

In order to convert conductance to permeability, I used the relation [21]

$$P_i = \frac{RTG_i}{Z_i^2F^2C_i} \quad (5)$$

where P_i and C_i are the permeability and concentration of the ith ion, Z_i is the ionic valence and R, T and F have their usual meanings.

Buffers were obtained from either Research Organics (Cleveland, Ohio) or Sigma Chemical Co. (St. Louis, Mo.). Bacterial phosphatidylethanolamine (PE) and diphytanoylphosphatidyl choline were obtained from Avanti Polar Lipids (Birmingham, Ala.). Decane (99+%) was obtained from Aldrich Chemical Co. (Milwaukee, Wis.) and was passed through an alumina column to remove polar impurities. Decane (99.9%) was also obtained from Wiley Organics (Columbus, Ohio) and was used as received. Similar results were obtained with both types of decane. Glycerol (99.5+%) was obtained from either Fisher or Aldrich. ^{14}C-labeled chloroform and acetic acid were obtained from New England Nuclear (Boston, Mass.). Water was deionized and then doubly distilled.

Results

Bacterial PE-decane bilayers behave electrically as fairly good pH electrodes (Fig. 2). Over the pH range of pH 7 to 11, the buffer ion conductances are negligible and $G_m \simeq G_{H/OH}$. As pH decreases from 7 to 1.6, $T_{H/OH}$ decreases gradually, i.e., the "background" buffer ion conductance becomes a significant fraction of G_m. At pH 1.6, $T_{H/OH}$ is about 0.5 and G_m is about 2×10^{-9} S cm^{-2}. Thus, background conductance is about 10^{-9} S cm^{-2}, which is near the lower limit of measurement in this system.

The relationship between $G_{H/OH}$ and pH is shown in Fig. 3. As pH increases from 1.6 to 10.5, $G_{H/OH}$ increases gradually from about 10^{-9} to 10^{-8} S

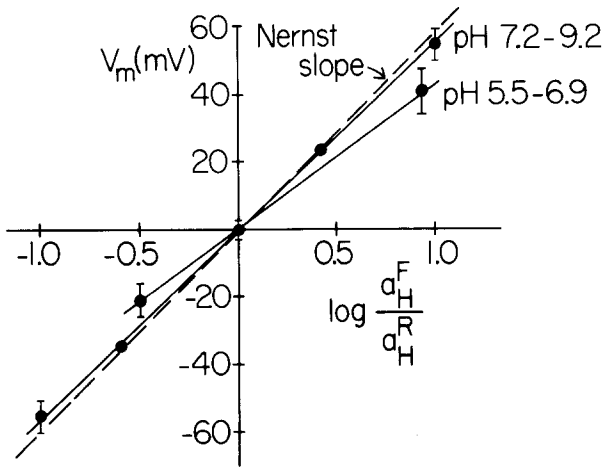

Fig. 2. Diffusion potentials produced by H^+/OH^- gradients across bacterial PE decane bilayers over the pH range of 7.2 to 9.2 and 5.5 to 6.9. HEPES and Tris buffers were used over the pH range 7.2 to 8.2, TAPS and Bis-tris propane were used over the pH range 8.2 to 9.2, and MES and Bis-tris were used over the pH range of 5.5 to 6.9. Dashed line is the Nernst slope (59 mV/pH unit). Error bars are standard deviations of 3 to 4 membranes

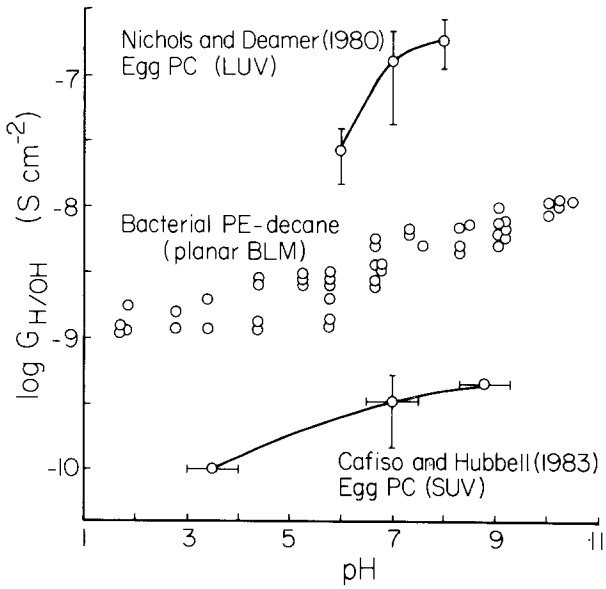

Fig. 3. Relation between H^+/OH^- conductance and pH in three types of phospholipid bilayers. In PE-decane membranes $G_{H/OH}$ was measured as described in Materials and Methods, and each point represents a single membrane. Cafiso and Hubbell [7] used sonicated egg PC to produce small unilamellar vesicles (SUV). They measured $G_{H/OH}$ by means of a hydrophobic cation (spin-labeled phosphonium). Nichols and Deamer [30] studied large unilamellar vesicles (LUV) (ether-injected lipids). They measured net H^+/OH^- fluxes with a pH electrode, and I have converted their fluxes to conductances using Eq. (5) and the relation, $J^{net}_{H/OH} = P_{H/OH}(\Delta C_H + \Delta C_{OH})$. Vertical error bars are standard deviations, and horizontal bars are pH ranges

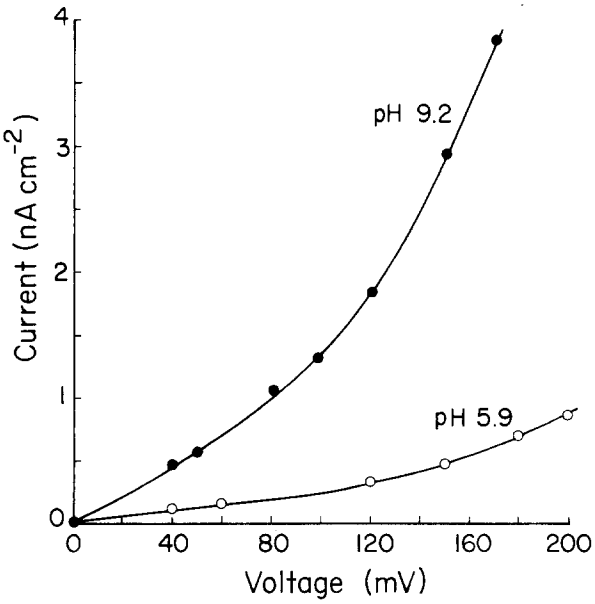

Fig. 4. Steady-state current-voltage curves for PE-decane bilayers at pH 9.2 and 5.9. The pH 9.2 solution contains TAPS plus Bis-tris propane (I = 0.04), and the pH 5.9 solution contains MES plus Bis-tris (I = 0.04)

cm^{-2}. However, compared to the enormous changes in H^+/OH^- concentrations, $G_{H/OH}$ is remarkably constant. These results are qualitatively consistent with those of Cafiso and Hubbell [7] and possibly also Nichols and Deamer [30], whose data are shown for comparison in Fig. 3.

In a few experiments I measured G_m in the presence of large pH gradients over a small voltage range of 0 ± 20 mV. Gradients of 2 to 3 pH units produced no significant increase in G_m. Gradients of 6 pH units (pH 9 *vs.* pH 3) produced approx. 10-fold increases in G_m, although the membranes were rather unstable under these conditions. In these asymmetrical pH experiments the solutions were not ionically balanced, so G_m represents the upper limit of $G_{H/OH}$.

Current-voltage curves were nearly linear below 80 mV but were always nonlinear at higher voltages (Fig. 4). Membranes often ruptured at voltages ranging from 120 to 180 mV and always ruptured near 200 mV, so the data at high voltages must be viewed with some caution. However, voltage-sensitive H^+/OH^- conductances similar to those shown in Fig. 4 have been observed previously in unmodified lipid bilayers [24], bilayers containing a weak-acid proton carrier [4] and also mitochondrial membranes [24]. One possible explanation for these current-voltage curves is that the charge carrier crosses a trapezoidal energy barrier in the membrane [4, 24].

In a few experiments I measured H^+/OH^- conductances through bilayers made of diphytanoyl phosphatidylcholine in decane (20 mg/ml). Results were qualitatively similar to those shown in Figs. 2 and 3, although the H^+/OH^- selectivity was not as good and $G_{H/OH}$ was slightly lower, at least over the pH range of 6.3 to 9.2. Cafiso and Hubbell [7] also found lower H^+/OH^- conductances with diphytanoyl PC vesicles, and they point out that the fully saturated hydrocarbons in this lipid make it non-autoxidizable. More extensive measurements with this and other types of lipids are in progress.

Cafiso and Hubbell [7] found that drying their egg PC for 15 hr under vacuum prior to sonication was necessary to produce vesicles having low H^+/OH^- conductances (Fig. 3). Thus, they suggested that residual chloroform might contribute to the high H^+/OH^- permeabilities observed by others [5, 8]. In order to test for a "chloroform effect," I compared vacuum dessicated lipids, supplied as a powder from Avanti, with lipids from standard stock solutions in chloroform, dried for about 5 min under argon. No differences were observed. To further test the persistency of chloroform as a contaminant in dried lipids, I added ^{14}C-labeled chloroform to chloroform stock solutions containing 4 mg of lipid. Then I dried the lipids in a small vial as if preparing for a normal bilayer experiment. After 3 min under a gentle stream of argon, 99.9% of the labeled chloroform was removed. However, when I did the same experiment with ^{14}C-labeled acetic acid, I found that several hours were required to remove 98% of the acid. These results suggest that the 15-hr vacuum treatment used by Cafiso and Hubbell [7] might be removing chloroform plus other volatile contaminants which have low vapor pressures and require long periods of vacuum for effective removal.

Nichols and Deamer [30, 31] initially suggested that H^+/OH^- fluxes occur by proton jumping through hydrogen-bonded chains of water molecules which extend into the bilayer. To find out whether $G_{H/OH}$ depends upon the water concentration, I formed bilayers in a series of glycerol-water mixtures, buffered with TAPS and Bis-tris propane over a pH range of 8.2 to 9.2. I chose a fairly high pH so that any inhibition of $G_{H/OH}$ could be easily observed. Figure 5 shows that $G_{H/OH}$ is very sensitive to the water activity over the range of about 44 to 100% water (vapor pressures of 16 to 23.7 mm Hg). Over this range the slope of log $G_{H/OH}$ versus log vapor pressure is about 8 (range 5 to 11), suggesting that several water molecules are involved in the H^+/OH^- conductance mechanism. At low water activities, (<16 mm Hg), $G_{H/OH}$ is low and approximately constant. Thus, the data suggest that two

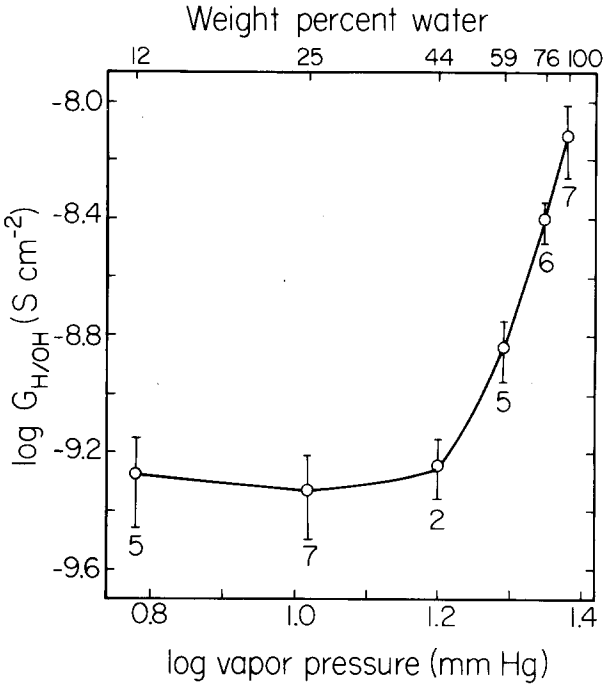

Fig. 5. H^+/OH^- conductance as a function of water activity (vapor pressure) in glycerol-water mixtures buffered with TAPS and Bis-tris propane (I = 0.04) over a pH range of 8.2 to 9.2. Vapor pressures of glycerol-water mixtures are from several sources, tabulated by Timmermans [41]. Error bars are standard deviations, and numbers indicate numbers of membranes

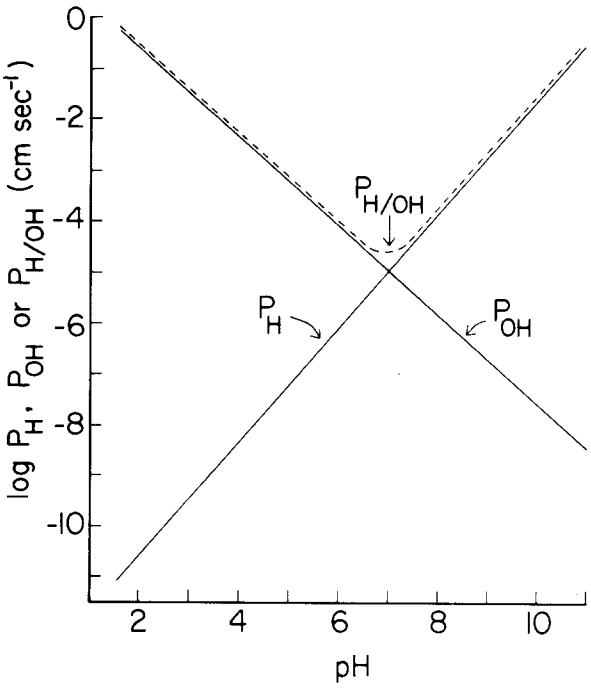

Fig. 6. Permeability coefficients for H^+, OH^- and H^+/OH^- as a function of pH. Permeabilities are calculated by Eq. (5), using the mean values of $G_{H/OH}$ for several different pH's in Fig. 3. The separate values of P_H and P_{OH} are calculated by assuming that either H^+ or OH^- is entirely responsible for $G_{H/OH}$. $P_{H/OH}$ is calculated as P_H plus P_{OH}

mechanisms may be involved in H^+/OH^- conductance, one of which is dependent upon the water concentration.

Discussion

This paper describes a simple method of measuring membrane H^+/OH^- conductances over a wide range of pH. The method is applicable to any planar or spherical membrane system in which the investigator has access to both sides of the membrane. My results confirm and extend several studies with phospholipid vesicles which found surprisingly high H^+/OH^- permeabilities in the physiological pH range [5, 7, 8, 10, 12, 24, 30, 31, 36, 39]. Thus, planar phospholipid bilayers, as well as vesicles, possess an H^+/OH^- transport mechanism which is not available to other inorganic ions.

The H^+/OH^- conductance through bacterial PE membranes is nearly independent of pH over the pH range of 1.6 to 10.5 (Fig. 3). Consequently, the H^+/OH^- permeability coefficient is extremely pH dependent, as shown in Fig. 6. The permeabilities to H^+, OH^- or H^+/OH^- were calculated by Eq. (5), which assumes that ions move independently through the membrane by simple, thermally activated diffusion [21]. If this assumption were correct, then $G_{H/OH}$ would be proportional to the H^+/OH^- concentration, and $P_{H/OH}$ would be independent of the H^+/OH^- concentration. Thus, Figs. 3 and 6 show clearly that H^+/OH^- are not crossing the membrane by simple diffusion, as suggested also by Cafiso and Hubbell [7]. Figure 6 also explains in part why large discrepancies exist among published values of P_H, P_{OH} and/or $P_{H/OH}$, which were obtained at pH's ranging from about 1 to 12 [7, 10, 17, 18, 36].

Figure 3 also shows that quantitative discrepancies exist among published values of $G_{H/OH}$ or $P_{H/OH}$ even for identical phospholipids at the same pH [7, 30]. The reason(s) for the discrepancies are unknown, but my results suggest that the high values of $P_{H/OH}$ are not caused solely by traces of organic solvents in the phospholipids. My results suggest also that the presence of hydrocarbon solvent in the bilayer does not have a major effect on $G_{H/OH}$. My calculated value of $P_{H/OH}$ at pH 7 (approx. 3×10^{-5} cm sec^{-1}) (Fig. 6) is within an order of magnitude of most of the values reported for various types of phospholipid vesicles at neutral pH [9, 10, 24, 28]. A survey of the published permeabilities and vesicle

methodologies also suggests that the high H^+/OH^- permeabilities are not caused by any specific vesicle size, any specific method of membrane preparation, or any specific method of measuring H^+/OH^- fluxes. Furthermore, high H^+/OH^- permeabilities have now been observed in bilayers made from at least six different types of phospholipids or lipid mixtures.

Several mechanisms have been proposed to explain the high H^+/OH^- permeabilities of lipid bilayers. The most popular model is that of Nichols and Deamer [30, 31], who suggested that hydrogen-bonded chains (HBC) of water molecules extend into the hydrophobic region of the bilayer and provide a pathway for H^+/OH^- movements by proton jumping, similar to the Grotthuss conductance mechanism in water and ice [11, 29]. Several lines of evidence are consistent with this model. *First,* $G_{H/OH}$ is sensitive to the water activity, decreasing about 10-fold as water activity decreases 33% (Fig. 5). The maximum slope of log $G_{H/OH}$ *vs.* log vapor pressure is about 8, suggesting that several water molecules are involved in H^+/OH^- conductance. *Second,* the concentration of water in the hydrophobic region of the bilayer is high enough (approx. 10^{-2} M) that the surface density of transmembrane strands of water could be about 10^{10} cm^{-2} [13]. If all the water in the bilayer was arranged in transmembrane strands, and if these strands had the same conductivity as water or ice [11, 44], then the membrane conductance would be in the range of 10^{-7} to 10^{-6} S cm^{-2}, which is sufficient to account for all the observed conductance of a lipid bilayer (Fig. 3).

Although the HBC model is attractive, several lines of evidence argue against the idea of H^+/OH^- fluxes via linear aggregates of water molecules. *First,* $G_{H/OH}$ decreases as $[H^+]$ increases (Fig. 3), despite the fact that the mobility of H^+ is greater than OH^- in both water and ice [11]. *Second,* water dissolved in bulk hydrocarbon (decane or decene) is 100 ± 10% monomeric [M. Conrad and H.L. Strauss, *personal communication*]. Thus, if the interior of a bilayer is like wet hydrocarbon, as inferred from water permeability studies [1, 13, 35], then the existence of linear aggregates or clusters of hydrogen-bonded water molecules in the hydrophobic region seems unlikely.

An alternative to transmembrane HBC's is the possibility that "short" HBC's extend part way into the bilayer. The existence of short HBC's would allow H^+ and OH^- to enter the membrane from opposite sides, forming water whenever two oppositely charged HBC's made contact within the membrane (J.W. Nichols, *personal communication*). The H^+/OH^- conductance might then be independent of pH under symmetrical conditions, because the product, $[H^+]^{cis}[OH^-]^{trans}$, would always be constant. In contrast, asymmetrical pH would cause the product, $[H^+]^{cis}[OH^-]^{trans}$, to increase, and we would therefore expect $G_{H/OH}$ to increase. However, I observed no significant increase in G_m with a pH gradient of 2 or 3 units, and even gradients of 6 units (pH 3 *vs.* pH 9) caused only a 10-fold increase in G_m.

Another possible pathway for chains of hydrogen-bonded water molecules might be through transient "hydrated defects" in the bilayer structure [9]. Indirect evidence for polar pathways exists already. *First,* the intrinsic ionic conductance of unmodified bilayers is orders of magnitude higher than the value predicted from thermodynamic considerations [28, 40]. *Second,* bilayers made from zwitterionic phospholipids, PE and PC, are slightly cation selective ($G_{Na} > G_{Cl}$) at neutral pH [3, 20, 22], despite the large positive dipole potential in these bilayers [1, 2, 38]. If alkali and halide ions traversed the hydrophobic region, we would expect $G_{Cl} \gg G_{Na}$, analogous to the conductances of hydrophobic anions and cations, e.g., tetraphenylborate and tetraphenylarsonium [2, 38]. *Third,* the existence of hydrated defects could explain why the conductances of alkali/halide ions "feel" the surface charge [16, 22] rather than the dipole potential.

Regardless of whether the HBC mechanism operates in the hydrophobic region or in transient hydrated defects, the pH independence of H^+/OH^- conductance (Fig. 3) remains to be explained. This result might be explained if the rate-limiting step in H^+/OH^- transport were the rate of formation of HBC's rather than the rate of movement of ionic defects or turning defects within the HBC (J.F. Nagle, *personal communication*). This hypothesis could also explain why titration of either the phosphate (pK ≃ 4.1) [19] or the amino group (pK ≃ 7.8) [19] of the phosphatidylethanolamine has virtually no effect on $G_{H/OH}$ (Fig. 3). However, there is no obvious reason why the rate of formation of HBC's would be voltage sensitive, as shown in Fig. 4.

The primary alternative to a proton wire (HBC) model is a proton carrier model. Cafiso and Hubbell [7] showed that 21 mM chloroform causes a roughly fivefold increase in $G_{H/OH}$. This is quantitatively similar to the effect of chloroform on tetraphenylborate conductance, which is due primarily to an increase in the dielectric constant of the membrane [38]. Cafiso and Hubbell also showed that 2% oxidation of the double bonds in egg PC causes a 15-fold increase in $G_{H/OH}$. Lipid oxidation and/or hydrolysis produces a variety of weakly acidic products [14, 33, 42] which might act as H^+ carriers. By analogy with proton ionophores (uncouplers), we know that phospholipids which are >99.99% pure may still

contain functional amounts of proton carriers. For example, 3×10^{-9} M FCCP (carbonylcyanide *p*-trifluoromethoxyphenylhydrazone) at pH 7 can produce an H^+/OH^- conductance of about 10^{-7} S cm^{-2} [27], corresponding to an H^+/OH^- permeability of about 3×10^{-4} cm sec^{-1}. Based on the adsorption coefficient of FCCP into bilayers [4], I estimate that the mole ratio of FCCP to phospholipid under these conditions is $<10^{-4}$. A weakly acidic proton carrier could also explain the lower $G_{H/OH}$ in acidic solutions (Fig. 3), because the maximum $G_{H/OH}$ is expected to be near the pK of the carrier [27]. However, the steadily increasing $G_{H/OH}$ at alkaline pH (Fig. 3) argues against the weak acid model, because the predicted titration of the carrier at high pH is not observed. Thus, if $G_{H/OH}$ occurs by a carrier mechanism, then perhaps more than one carrier is involved, or the carrier is not titratable, or some other pH-dependent process, e.g., lipid hydrolysis [32, 33], occurs during the H^+/OH^- conductance measurements.

From the above discussion it should be clear that none of the current models for H^+/OH^- conductance through lipid bilayers is strongly supported by the available data. The most puzzling observation is that $G_{H/OH}$ is nearly independent of H^+/OH^- concentration over a very wide range of pH (Fig. 3). Additional work is needed to determine whether H^+/OH^- conductance occurs through the hydrophobic region or through transient hydrated defects. Additional work is also needed to establish the dependence of $G_{H/OH}$ on water activity (Fig. 5), because nonaqueous solvents can inhibit various types of transport processes [25]. Thus, elucidation of the mechanism(s) of H^+/OH^- transport remains a challenging problem for the future.

The mechanism(s) of H^+/OH^- transport may be biologically important, because most of the values of $P_{H/OH}$ reported for bilayers are within an order of magnitude of many biological membranes, e.g. toad bladder [15], red blood cells [23, 43], inner mitochondrial membranes, chloroplast thylakoid membranes, roots of higher plants, bacterial membranes and sarcoplasmic reticulum (reviewed by Maloney [26], Raven and Beardall [37] and Deamer and Nichols [10]). In some biological membranes, P_H/P_{Na} is approx. 10^6 [23, 43, 45], and P_H increases as pH increases [45], qualitatively similar to the bilayer data (Fig. 6). Furthermore, in a bacterial membrane system, $G_{H/OH}$ is independent of pH [26], again similar to the bilayer data (Fig. 3). However, the similarities between bilayers and biological membranes may be fortuitous, because H^+/OH^- diffusion through biological membranes may be via protein rather than lipid pathways. Additional work is needed to determine whether the special mechanism for H^+/OH^- transport through lipid bilayers is also an important mechanism for passive H^+/OH^- transport through biological membranes.

For valuable discussions and correspondence I thank Drs. David Cafiso, Morgan Conrad, David Deamer, David Hastings, John Nagle, Wylie Nichols, Sidney Simon and Anne Walter. This work was supported by National Institutes of Health grant GM 28844.

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Received 24 April 1984; revised 5 June 1984