J Membrane Biol (2010) 237:21–30
DOI 10.1007/s00232-010-9303-0

A View of Hydrogen/Hydroxide Flux Across Lipid Membranes

J. Wylie Nichols · R. F. Abercrombie

Received: 30 June 2010 / Accepted: 27 August 2010 / Published online: 25 September 2010

Abstract A topic emerging roughly 30 years ago and engendering an incompletely resolved controversy is reviewed in this article: the relatively high permeability and pH independence associated with H^+/OH^- passive movements across lipid membranes. We summarize the expected characteristics of simple H^+/OH^- diffusion and those of a reaction between H^+ and OH^- being attracted from opposite surfaces and condensing in an interfacial zone of the membrane. An interfacial H^+/OH^- reaction mechanism gives the experimentally observed behavior of an H^+/OH^- flux that is independent of the pH measurement range. This mechanism assumes that H^+ and OH^- within the interfacial zone become electrostatically aligned on opposite sides of the hydrophobic membrane core. Electrostatic attraction and charge delocalization among a small cluster of water molecules surrounding the ions reduce the Born energy for H^+/OH^- insertion into lipid. This transmembrane condensation model predicts the magnitude of the experimentally determined H^+/OH^- flux, which is significantly greater than that of other monovalent ions. The consequences of an elevated H^+/OH^- permeability compared to other ions and the relative pH independence of this flux have consequences for understanding the chemical evolution of life.

Keywords Diffusion · Proton permeation · Charge delocalization · Water cluster

Introduction

The formation of a utilizable energy gradient of hydrogen ions across lipid membranes was a seminal development in the evolution of life on earth. Bacteria and yeast generate these gradients across their plasma membranes and utilize the stored energy to import nutrients and to export waste. The ability to maintain pH gradients is essential for the function of a variety of other cellular and organellar membranes including the gastric mucosa, lysosomes, chromaffin granules and synaptic vesicles. In most eukaryotes, mitochondria capture energy developed across the inner mitochondrial membrane to generate ATP, as Mitchell and Moyle (1967) described in their chemiosmotic hypothesis nearly half a century ago. To prevent short-circuiting in fruitless cycles of H^+ flux that would dissipate the energy, a low H^+ conductance becomes an absolute requirement. Indeed, Mitchell and Moyle measured the effective H^+ conductance in nonrespiring mitochondria to be 0.45 μmho/cm^2 at pH 7.2 and 25°C. In the report, they state the following: "This is the lowest natural membrane ion conductance known to us, the usual range being 10–1,000 μmhos/cm^2 (Maddy et al. 1966). Only in the artificial 'black lipid' membranes have ion conductances in the region of 0.1 μmho/cm^2 been recorded previously (Huang et al. 1964; Maddy et al. 1966)." They argued that the *flux* of H^+/OH^- equivalents across the phospholipid bilayer was only slightly greater than that of K^+ and Cl^-, the dominant ions used in the quoted experiments. As low conductance is necessary to maintain a H^+ electrochemical gradient, this measurement was critical in establishing the validity of the chemiosmotic hypothesis.

Mitchell and Moyle (1967) did not emphasize in this article that the H^+/OH^- *permeability* of the inner mitochondrial membrane, which can be calculated from the

J. W. Nichols · R. F. Abercrombie (✉)
Department of Physiology, Emory University School of Medicine, Atlanta, GA 30322-3110, USA
e-mail: ron@physio.emory.edu

measured conductance, was many orders of magnitude greater than that for K^+ and Cl^- ($\sim 10^{-4}$ cm s^{-1} for H^+/OH^- vs. $\sim$ 10^{-9} cm s^{-1} for other monovalent cations and anions). This observation does not bear on their conclusions concerning the chemiosmotic theory since physiological H^+/OH^- flux occurs at normal cytosolic concentrations of K^+, Cl^- and H^+. As flux is proportional to the product of permeability and concentration and as the H^+ concentration is much lower than that of K^+ or Cl^-, the H^+ flux is typically small. However, this observation raises the question of the mechanism by which a concentration of 10^{-7} M H^+ can produce the same flux, or current, as 10^{-1} M K^+. Nichols et al. addressed this issue directly (Nichols and Deamer 1980; Nichols et al. 1980) using fluorometric and pH titration techniques to record the decay of pH gradients across phosphatidylcholine liposomes. The calculated H^+/OH^- permeability coefficient for the liposome membrane was compared with that of Na^+ as measured by radioactive tracer flux in identically prepared liposomes. Similar to the H^+/OH^- permeability coefficient derived from Mitchell and Moyle's (1967) conductance measurements, the H^+/OH^- permeability of phosphatidylcholine liposomes was found to be approximately six orders of magnitude greater than that of Na^+ ($P_{H/OH} = \sim 10^{-4}$ cm s^{-1} vs. $P_{Na} = \sim 10^{-10}$cm s^{-1}). The large discrepancy between H^+/OH^- and Na^+ permeabilities implies fundamentally different mechanisms for their transport.

The observation that H^+/OH^- permeability was much larger than that of other monovalent cations and anions was initially challenged by two sets of experimental data. Nozaki and Tanford (1981) measured a slow rate of decay of pH gradients across liposome membranes and concluded that the H^+/OH^- permeability was, at most, 10^{-9} cm s^{-1}. When corrected for H^+ permeation via the neutral salts HCl and HNO_3, this was estimated to be in the range of 5×10^{-12}, a difference of eight orders of magnitude from the data of Nichols and Deamer (Nichols et al. 1980; Nozaki and Tanford 1981). It was later demonstrated, however, that in the absence of a sufficient permeation pathway for counter-ions, steady-state flux is limited by that of the counter-ion that is most permeant. When these experiments were repeated in the presence of K_2SO_4 and valinomycin to increase K^+ permeability, the calculated H^+/OH^- permeability coefficient was found to be in the range of 10^{-4} cm s^{-1} (see Fig. 1 from Deamer and Nichols 1983). These experiments reinforced the conclusion that the flux of H^+/OH^- equivalents must have been greater than that of K^+, Na^+ or Cl^- as H^+/OH^- dominated the diffusion potential even though their concentrations were many orders of magnitude smaller than those of other monovalent ions.

A second challenge came from measurements of H^+/OH^- permeability across "black lipid" membranes. From the conductance of planar bilayers measured in 100 mM HCl (pH $\sim$ 1.0) or 4 mM NaOH (pH $\sim$ 11.6), Gutknecht and Walter (1981) calculated permeability to H^+ as 3×10^{-9} cm s^{-1} and that to OH^- as 4×10^{-9} cm s^{-1}, approximately five to six orders of magnitude less than had been measured in liposomes at pH 7 (Deamer and Nichols 1983) and only three or four times larger than the measurements of Nozaki and Tanford (1981).

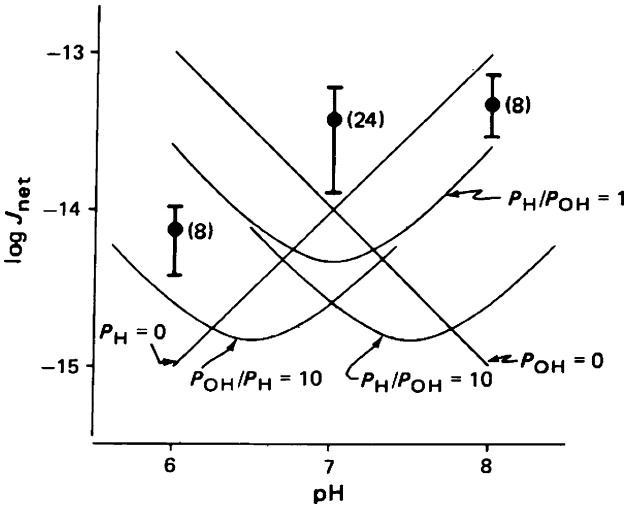

Fig. 1 pH dependence of net H^+/OH^- flux. *Lines* represent a few of the infinite possibilities for the dependence of the net H^+/OH^- flux on pH as predicted from Eqs. 1 (or 2) by assuming that H^+ and OH^- permeability remains constant. The absolute values for these coefficients were arbitrarily chosen such that the predicted minima would be in the same range as the measured fluxes. The mean (±SD in parentheses) net H^+/OH^- flux (*filled circle*) was calculated from $J_{net} = \frac{dpH_o}{dt}\frac{B_oV_o}{A}$, where B_o is the buffer capacity of the external solution, V_o is the external volume and A is the total surface area of the vesicles. dpH/dt was taken as the initial slope of the pH recording after an acid or base pulse of constant magnitude (approximately 0.06 pH unit) at each pH. The liposome solutions contained 2.0 mM 9:1 phosphatidylcholine/CTAB mixture. The internal and external solutions for the experiments performed at pH 7 contained 50 mM *N*-(2-acetamido)-2-aminoethanesulfonic acid (pK 6.9). For the experiments performed at pH 6 and 8,2-(*N*-morpholine)ethanesulfonic acid (pK 6.15) and *N*-tris(hydroxymethyl)methylglycine (pK 8.15), respectively, were substituted, in the same concentrations, for the buffer. (This graph was published in Nichols and Deamer 1980, Fig. 3, and is used with permission of the authors)

The resolution of this discrepancy had been anticipated (Nichols and Deamer 1980). In an effort to determine the relative permeabilities of H^+ and OH^-, Nichols and Deamer had measured the rate of pH collapse for a much smaller gradient ($\sim$0.1 pH) at pH 6, 7 and 8. For most ions, i.e., Na^+, K^+ and Cl^-, the permeability coefficient is relatively independent of, whereas conductance is generally proportional to, the concentration of the conducting ions. Therefore, if H^+/OH^- flux obeyed Fick's law and the permeability coefficients were independent of pH, the H^+/OH^- flux should be linearly dependent on their concentration gradients. If both H^+ and OH^- were equally permeant, the flux (for a fixed gradient) should achieve a minimum at pH 7. The pH of this minimum would vary with the ratio of the two permeabilities (Fig. 1). The measured fluxes were, however, found to be essentially independent of pH and not to follow the predictions of Fick's law.

The apparent discrepancy between the magnitudes of the H^+/OH^- permeability measurements made in liposomes vs. black lipid membranes (BLMs) was resolved only a few years later by Gutknecht (1984). It was found that the H^+/OH^- conductance of BLMs composed of phosphatidylethanolamine and decane varied only 10-fold (10^{-9}–10^{-8} S/cm^{-2}) over a pH range of ~2–10. Thus, the H^+/OH^- permeability coefficient as calculated from Fick's law must vary by many orders of magnitude over this range of measurement. At neutral pH, the apparent discrepancy between liposomes and BLMs is, therefore, reduced to only approximately a single order of magnitude as has been measured in numerous phospholipid vesicles of different compositions and sizes and using different techniques (Biegel and Gould 1981; Decoursey 2003; Elamrani and Blume 1983; Nichols and Deamer 1980; Nichols et al. 1980; Perkins and Cafiso 1986; Rossignol et al. 1982). Most of the discrepancy resulted from (1) extrapolating these measurements at extremes of pH to neutral pH values and (2) from the (relative) pH independence of H^+/OH^- conductance across lipid membranes. Much of the data can be reconciled by a lack of conformation of H^+/OH^- flux to assumptions regarding simple diffusion, upon which Fick's law is based.

In summary, the flux of H^+/OH^- across phospholipid bilayers differs from that of other monovalent cations and anions in two significant ways. First, the flux is much faster than predicted for equivalent concentrations of other monovalent cations or anions at neutral pH and, second, the flux, or conductance, is not dependent upon the absolute concentrations of the H^+/OH^- ions within the solution. These differences suggest that such flux occurs by a unique mechanism. Here, we review and discuss the strengths and weaknesses of models that have been proposed to address these observations.

Theory/Calculation

Simple Linear Model

The simplest model to describe the flux of H^+ and OH^- across phospholipid bilayer membranes is one derived from Fick's law, which assumes that the net flux should be directly proportional to the difference in ion concentrations across the membrane. Thus,

$$J_{\text{net}} = \rho_{\text{H}}\left([\text{H}^+]_{\text{L}} - [\text{H}^+]_{\text{R}}\right) - \rho_{\text{OH}}\left([\text{OH}^-]_{\text{L}} - [\text{OH}^-]_{\text{R}}\right) \tag{1}$$

or expressed as a function of pH,

$$\begin{aligned} J_{\text{net}} = \rho_{\text{H}} &\cdot 10^{-\text{pH}_{\text{L}}}\left(1 - 10^{+\Delta\text{pH}}\right) \\ &- \rho_{\text{OH}} \cdot 10^{\text{pH}_{\text{L}}-14}\left(1 - 10^{-\Delta\text{pH}}\right) \end{aligned} \tag{2}$$

$[\text{H}^+]_{\text{L}}$, $[\text{OH}^-]_{\text{R}}$, etc., represent the hydronium and hydroxide concentrations on the left and right sides of the membrane, respectively; $[\text{H}^+] = 10^{-\text{pH}}$ and $\Delta\text{pH} = \text{pH}_{\text{L}} - \text{pH}_{\text{R}}$. For a given pH gradient, the net flux, would be determined by the H^+ and/or OH^- permeability coefficients (ρ_{H} and ρ_{OH}), which are dependent upon the ion partition into the bilayer and the rate of diffusion across it. According to Eq. 2, if the permeabilities of hydronium and hydroxide are equal ($\rho_{\text{H}} = \rho_{\text{OH}}$), J_{net} achieves a minimum at ($\text{pH}_{\text{L}} + \Delta\text{pH}/2$) = 7 and is steeply dependent on pH (Fig. 1).

Ion Partition into Lipid Membranes

All such models require a mechanism for the insertion of H^+/OH^- into the membrane. The partition of a "bare" H^+ (or OH^-) into a lipid has been estimated using the ratio of probabilities of residing in lipid vs. water. As a first approximation we use the self-energy of dehydration, or Born energy in lipid vs. water, E_{B}, defined as

$$\begin{aligned} E_{\text{B}} &= \frac{q_e^2}{4\pi \cdot 2 \cdot \in_0 \cdot r_0}\left\{\frac{1}{\in_{\text{L}}} - \frac{1}{\in_{\text{w}}}\right\} \\ &= \sim 2.8 \times 10^{-19}\,\text{joules} \sim 1.75\ \text{eV} \end{aligned} \tag{3}$$

This value 1.75 eV is determined for a ~2 Å "effective radius" of the ion, r_0 (hydronium or hydroxide ion). The electronic charge, q_e, is 1.6×10^{-19} coul; the dielectric constant of lipid, $\in_{\text{L}}$, is 2; $\in_{\text{w}} \sim 80$; and the permittivity of the vacuum, $\in_{\text{o}}$, is 8.9×10^{-12} coul^2/nt-m^2.

The ratio of probabilities in lipid vs. water ($P_{\text{L}}/P_{\text{W}}$, partition function) for this energy difference is, using $k_{\text{B}}T$, the Boltzmann constant times room temperature = 0.025 eV,

$$K^{\ddagger} = \frac{P_{\text{L}}}{P_{\text{w}}} = e^{-E_{\text{B}}/k_{\text{B}}T} = e^{-70} = 4 \times 10^{-31} \tag{4}$$

Eyring rate theory may be used to estimate the rate constant of ion partition into a bilayer. It will be assumed that each reaction center within the bilayer corresponds to a membrane surface area to be defined later.

$$\text{rate constant} = \frac{k_{\text{B}}T}{h}K^{\ddagger} \tag{5}$$

where $k_{\text{B}}T/h$ represents the universal constant for the transition state (frequency factor), $\sim 6 \times 10^{+12}\ \text{s}^{-1}$ at room temperature (Eyring 1935; Hill 1976).

Substituting for $K^{\ddagger} = \frac{P_{\text{L}}}{P_{\text{W}}} = 4 \times 10^{-31}$ predicts a rate constant for the transition state of $\sim 2 \times 10^{-18}\ \text{s}^{-1}$.

The rate constant for the transition state can be expressed as a permeability coefficient (ρ) using the following expression

$$\rho \sim \frac{k_B T}{h} \cdot K^{\ddagger} \cdot \frac{\text{reaction volume}}{\text{surface area}}, \tag{6}$$

where symbols have their previous meanings and the “reaction volume/surface area” refers to the hypothetical transition zone for the patch of membrane under consideration. As a starting point, we assume this ratio has an approximate value ranging from 1 Å to the membrane thickness, 30 Å. If we use 1–30 Å for the “reaction volume/surface area” ratio, the calculated value for the permeability coefficient, based on the “bare” hydronium/hydroxide penetration into lipid, is ~0.03 to 1×10^{-24} cm s^{-1}. This is many orders of magnitude smaller than experimentally measured permeabilities that range from 1.4×10^{-4} to 7×10^{-7} cm s^{-1}, depending on the composition of the phospholipid bilayer and the technique (Deamer 1987). The large discrepancy between the theoretical and experimental measurements essentially rules out H^+ permeation by the insertion of an isolated hydronium or hydroxide into the lipid bilayer.

A greater frequency for transition to the lipid bilayer can be achieved, however, if H^+ and/or OH^- are allowed to partition into the lipid from the interfacial region by forming a water structure or cluster. This should be a consequence of the lattice structure of water surrounding a hydronium or hydroxide ion, which would distribute the ionic charge over a larger volume and thus reduce the Born energy required for partition into the lipid (Haines 2001; Liu and Ichiye 1999; Smith et al. 2006).

Making reasonable assumptions in calculations to follow allows an estimate of the size of a water cluster necessary to delocalize the charge on H^+ or OH^- sufficiently to obtain a permeability coefficient in the range of 5×10^{-6} cm s^{-1}. The required cluster radius will be ~4.6 Å. Thus, based on these calculations, the very high bilayer permeability to H^+ and/or OH^- can be explained if one assumes that the partitioning of the charges into the membrane is accompanied by surrounding water molecules that distribute the charge over a larger volume.

The transient formation of clusters or single-file alignments of water molecules that traverse the lipid bilayer have been suggested as alternative mechanisms for lowering the energy barrier for H^+ and/or OH^- permeation (Elamrani and Blume 1983; Jansen and Blume 1995; Nagle and Scott 1978; Nichols and Deamer 1980). Formation of a strand of hydrogen-bonded water molecules that traverse the lipid portion of the bilayer may allow a low-energy pathway to conduct H^+ and/or OH^- across the bilayer by proton hopping (referred to as “Grotthuss conduction” [de Grotthuss 1806]). Proton hopping allows for the movement of charge by the rearrangement of hydrogen bonds without requiring the transverse movement of H^+ or OH^- ions.

Although all of the mechanisms discussed above provide a low-energy transmembrane pathway that could account for the relatively high H^+/OH^- permeability, they do not account for the pH independence of the flux as demonstrated experimentally (Figs. 1, 2). These theoretical mechanisms would predict the observed pH independence of the flux if, for example, the formation of the water cluster/strand or the reorientation of the hydrogen bonds necessary for Grotthuss conduction became the rate-limiting step for the permeation event. However, if this were the case, the probability of H^+/OH^- transport from either side of the membrane would be independent of their solution concentrations and no net flux would be predicted even in the presence of a large gradient. Thus, we are led to consider a model in which H^+/OH^- flux occurs by the condensation of H^+ and OH^- from opposite sides.

Transmembrane Condensation Model

Condensation of H^+ and OH^- originating from opposite sides of the membrane as a mechanism to explain the pH independence of flux was originally proposed by Deamer and Nichols (1982) and subsequently addressed by others (Haines 2001; Marrink et al. 1996; Nagle 1987). The proposed transmembrane water condensation model provides a simple explanation both for how the flux of H^+ and OH^- becomes independent of the absolute range of the pH measurement and for the uniqueness of H^+/OH^- flux magnitude. The model assumes that net flux across a membrane is accomplished by condensations of H^+ and

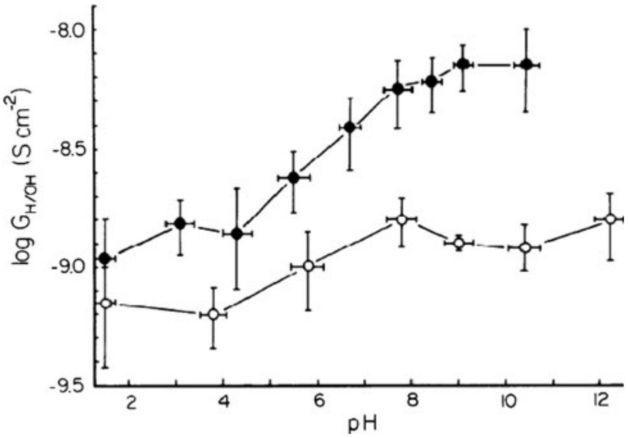

Fig. 2 Relation between H^+/OH^- conductance and pH for membranes formed from bacterial phosphatidylethanolamine (*filled circle*) or diphytanoyl phosphatidylcholine (*unfilled circle*). $G_{H/OH}$ was measured as described in the Materials and Methods section of Gutknecht (1987). *Horizontal bars* indicate the range of pH for each point, and *vertical bars* indicate standard deviations ($n = 4$–11 membranes at each point). Part of the statistical variation is due to approximately twofold differences among batches of lipids. (Used with permission of the publisher)

OH^- originating from opposite sides of the membrane. The following equation describes the consequence of this model in its simplest form (from Nagle 1987).

$$J_{\text{net}} = \left\{[H^+]_L \cdot [OH^-]_R - [H^+]_R \cdot [OH^-]_L\right\} f(\delta) \tag{7}$$

The symbol $f(\delta)$ represents a sum over all possible pathways of permeation and would be dependent on the membrane composition and thickness, δ, but independent of the H^+ and OH^- concentrations in solution.

Expressing this relationship for net flux, J_{net}, as a function of the pH gradient across the membrane (see Appendix), gives

$$J_{\text{net}} \approx -2 \cdot 10^{-14} f(\delta)\left\{2.3\Delta\text{pH} + \frac{1}{6}(2.3\Delta\text{pH})^3 + \cdots\right\} \text{ or}$$

$$J_{\text{net}} \approx J_o\left\{2.3\Delta\text{pH} + \frac{1}{6}(2.3\Delta\text{pH})^3 + \cdots\right\} \tag{8}$$

Note that the net flux is predicted to depend only on the magnitude of the pH gradient and not on the absolute value of pH in the bulk solutions. For small pH gradients, net flux would be a linear function of ΔpH. It would increase supralinearly as the gradient increases, assuming that the model applies for both small and large ΔpH. The feasibility of this model based on physical–chemical principles will be considered in the next section.

Quantitative Evaluation of the Transmembrane Condensation Model

We approach the relaxation of membrane pH gradients in five steps (Fig. 3): (1) appearance of an H^+ (or OH^-) in the interfacial zone (head group region); (2) coincident appearance of an OH^- (or H^+) within electrostatic range on the opposite side of the membrane; (3) electrostatic-induced migration within the interfacial zone to opposing positions across the low dielectric core; (4) rare penetration from either side into the hydrophobic core, overcoming the Born energy barrier; and (5) migration of the penetrating charge across the membrane to condense with its attracting counterpart in the interfacial zone of the opposite side. We will consider each step in order.

1. *Appearance of an H^+ (or OH^-) in the interfacial zone*. If we assume that 60% of the interfacial zone (head group region) is composed of water and the depth of this zone is 8 Å (Peitzsch et al. 1995), then for each square

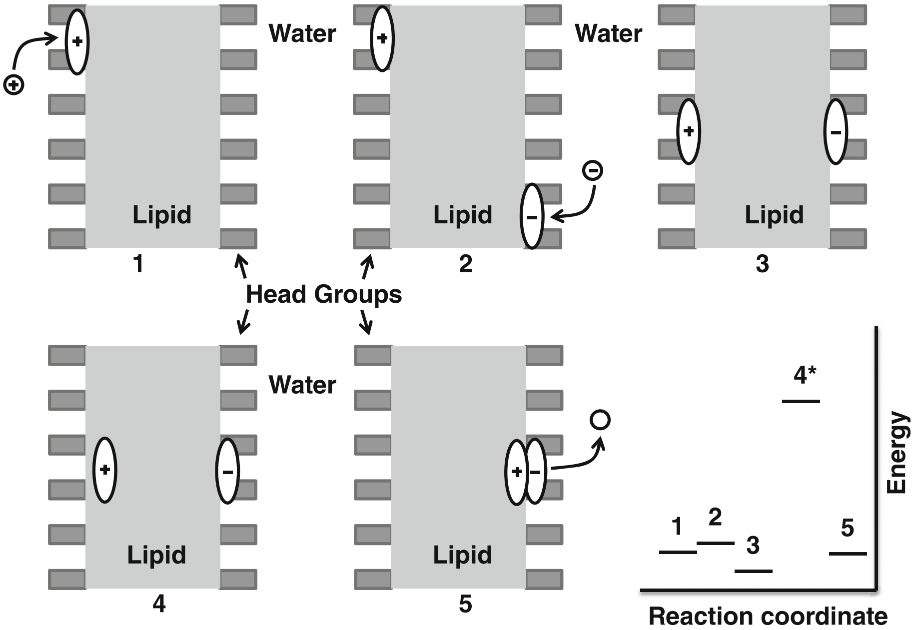

Fig. 3 Schematic representation of the transmembrane condensation model for H^+/OH^- flux across phospholipid membranes. *1–5* illustrate the five steps of the model as described in the text. (1) Appearance of an H^+ (or OH^-) in the interfacial zone (head group region). H^+ or OH^- diffuse into the water among the head groups. The charge is delocalized by Grotthuss conduction among 10–12 water molecules arranged parallel to the membrane surface. (2) Coincident appearance of an OH^- (or H^+) within electrostatic range on the opposite side of the membrane. (3) Electrostatic-induced migration within the interfacial zone to opposing positions across the low dielectric core. If an H^+ and an OH^- are present within the head group region on opposite sides of the membrane and are within electrostatic attraction distance, they diffuse laterally to directly opposing positions. (4) Occasional penetration from either side into the hydrophobic core, overcoming the Born energy barrier. (5) Migration of the penetrating charge across the membrane to condense with its attracting counterpart in the interfacial zone of the opposite side. Penetration of either charge into the hydrophobic zone results in migration across the membrane and then in condensation to form water. *Lower right* Hypothetical reaction coordinates and energy barriers for each step

centimeter of membrane surface area, there would be a volume of water equal to 4.8×10^{-8} cm^3 in one interfacial zone. At pH 7, there are 10^{-7} mol of H^+/l of water or 6×10^{13} H^+ ions/cm^3. Therefore, in 1 cm^2 of membrane surface area, there would be 3×10^6 free H^+ and/or OH^- in the interfacial zone residing among the phospholipid head groups. We assume that the H^+ and OH^- are free to exchange with the bulk solution and that the rate of their diffusion into this zone from the bulk is not rate-limiting to the permeation process; i.e., they are assumed to be in electrochemical equilibrium with their counterparts in bulk solution.

2. *Coincident appearance of an OH^- (or H^+) within electrostatic range on the opposite side of the membrane*. The second step in the condensation reaction is the coincident insertion of an H^+ into the interfacial zone on one side of the membrane and that of a OH^-on the opposite side within a distance that permits electrostatic attraction between the two. In order to determine the probability of this occurring, we first define the lateral dimension of a "domain" in the interfacial zone within which electrostatic attraction of oppositely charged ions would occur across the low dielectric membrane. We calculate this domain size by determining the distance at which the electrostatic attraction between the two oppositely charged ions falls below the randomizing effects of their kinetic energy (kT). We assume that H^+ and OH^- constitute most of the mobile charge residing in the interfacial zone. Dipolar potentials from the distributed orientations of water molecules are expected to decline at least as sharply as $1/r^2$; therefore, the electrostatic influence of orientated water dipoles would fall well below kT at distances greater than ~20 Å. Equation 9 can be used to estimate the diameter of a domain in which electrostatic attraction between H^+ and OH^- is greater than thermal dispersion energy, assuming these were fully immersed within a defined dielectric medium.

$$\frac{q_e^2}{4\pi \in\in_0 L} \geq kT, \tag{9}$$

Assuming that the dielectric coefficient of the lipid portion of the membrane ($\in$) is 2 at room temperature, L (the linear dimension used to estimate a domain area from which a second ion of opposite charge would be attracted) is ~280 Å, i.e ., ~10 times greater than the thickness of the hydrophobic core. (Here, we have assumed that the electrostatic effects of dipolar water molecules, symmetrically oriented around an H^+ or OH^-, would tend to cancel or diminish at larger distances.)

A circular domain area with a diameter of 280 Å equals 6.2×10^{-12} cm^2. The product of this area and the interfacial H^+ or OH^- concentration of 3×10^6 free H^+ ions/cm^2 predicts a probability of 1.8×10^{-5} that an H^+ or OH^- would reside within the electrostatic attraction domain area of 6.2×10^{-12} cm^2. The probability of a coincident H^+ occurring on the left and an OH^- on the right within one domain is therefore $(1.8 \times 10^{-5})^2$. Since at pH 7 there is an equal probability of H^+/OH^- and OH^-/H^+ orientations across the membrane, the overall probability of an H^+ and OH^- entering the electrostatic attraction domain is $2 \times (1.8 \times 10^{-5})^2 = 6.5 \times 10^{-10}$.

3. *Electrostatic-induced migration within the interfacial zone to opposing positions across the low dielectric core*. Since the size of the interaction domain is defined by the distance for which the energy of electrostatic attraction is greater than that of thermal dispersion, the coincident presentation into the interfacial region of an H^+ and OH^- from opposite sides will induce the lateral migration of these charged species to oppose each other on opposite sides of the same domain. This migration may be accomplished by lateral diffusion of individual H^+ or OH^- in the interfacial zone or by the exchange of electrons via Grotthuss conduction between the interfacial zone water molecules (de Grotthuss 1806). Neither mechanism of diffusion within the interfacial water is expected to be rate-limiting to the overall process of transmembrane condensation.

4. *Rare penetration from either side into the hydrophobic core, overcoming the Born energy barrier.* If we assume an H^+/OH^- ion pair is electrically attracted to their positions directly across the lipid portion of the membrane interior, the maximum Born energy $\left(E'_B\right)$ for ion insertion under this arrangement may be given by an approximation shown below and based on Schnitzer and Lambrakis (1991, their Eq. 7; see also Parsegian 1969; Arakelian et al. 1993).

$$E'_B \approx \frac{q_e^2}{4\pi \in\in_0}\left(1 - \frac{r_0}{d}\right) \cdot \left(\frac{1}{2r_0} - \frac{1}{d}\right) \tag{10}$$

The symbol r_0 represents the water cluster radius around the ion, and d represents the thickness of the hydrophobic lipid interior. Here, the standard Born energy for lipid insertion of a particle of radius, r_0, is reduced by the finite thickness, d, of the low dielectric barrier and additionally by the electrostatic attraction of the opposing ions. A calculation for $r_0 = 4.6$ Å and $d = 30$ Å gives $E'_B = 0.44$eV, which is the energy barrier that is predicted to produce a permeability coefficient in the range measured (see below, Prediction of J_0 and ρ from the Condensation Model). Thus, distribution of the unitary charge of an H^+ or OH^- among a sufficient number of water molecules to produce a charge density equivalent to a spherical charge of radius 4.6 Å is sufficient to produce the rate of charge penetration into the hydrophobic region of the membrane necessary to account for the experimentally measured permeability. A sphere with a radius of 4.6 Å contains approximately 13 water molecules. Although a charged sphere of this size is unlikely to exist and penetrate the hydrophobic interior of the membrane as such, molecular modeling (Tepper and Voth 2005) provides evidence for the delocalization of positive charges among 9–12 hydrogen-bonded water molecules that lie parallel to the surface of the membrane. These hydrogen-bonded “transverse” water wires are stabilized by an excess charge and are likely to delocalize the excess charge of an H^+ or OH^- to the equivalent of a 4.6-Å sphere.

Assuming a “corrected” Born energy for insertion of H^+ or OH^- into the hydrophobic region, a maximum rate constant for this event can be calculated using the Eyring rate theory:

$$\text{rate constant} = \frac{k_B T}{h} e^{\left(-\frac{E'_B}{k_B T}\right)} \tag{11}$$

The frequency factor, $k_B T/h = 6 \times 10^{12}\, s^{-1}$, and E'_B represent the “corrected” Born energy required for insertion into the low dielectric (0.44 eV). Thus, the rate constant for the penetration of an H^+ or OH^- into the hydrophobic region from one side of the membrane is predicted to be $1.4 \times 10^5\ s^{-1}$.

5. *Migration of the penetrating charge across the membrane to condense with its attracting counterpart in the interfacial zone of the opposite side.* Penetration of either an H^+ or OH^- within the interaction domain from either side of the membrane would produce a condensation reaction with its oppositely charged counterpart on the other side. Once one of the charged species overcomes the Born energy barrier and gains access to the lipid interior of the membrane, the electrostatic attraction between it and the opposite charge on the opposing side of the membrane drives its diffusion (with accompanying water) across the membrane, resulting in condensation in the opposing interfacial zone. Once the opposing ions are aligned, only one high-energy penetration event is necessary to produce a condensation reaction. This may originate from either side and result in the permeation of an H^+ or OH^- across the full membrane.

Prediction of J_0 and ρ from the Condensation Model

The frequency at which condensation of an H^+ and OH^- from opposite sides of the membrane occurs can be calculated by multiplying the probability of coincident residence of an H^+ and OH^- within the electrostatic attraction domain on opposite sides of the membrane (6.5×10^{-10}) by the rate constant for penetration of one of these into the hydrophobic region of the membrane ($1.4 \times 10^5\ s^{-1}$). Thus,

$$\begin{aligned}\text{condensation frequency} &= 2 \times 6.5 \times 10^{-10} \times \frac{kT}{h} e^{\left(-\frac{E'_B}{kT}\right)} \\ &= 1.8 \times 10^{-4} s^{-1}\end{aligned} \tag{12}$$

represents the condensation frequency for each domain, i.e., for an area of $6.2 \times 10^{-12}\ cm^2$. An additional factor of 2 is included because an equivalent condensation reaction can *initiate* from either side and proceed in either direction. Expressing the condensation frequency in terms of a 1-cm^2 unit area results in a value for J_0 of 2.8×10^7 condensation $cm^{-2}\ s^{-1}$, corresponding to the situation of pH 7 on both sides and $\Delta pH = 0$. When ΔpH is not zero, net movement must grow as $J_{net} = J_0(2.3\Delta pH)$, as given in Eq. 8. Assuming a symmetrical ΔpH near neutrality, the expression $J_{net} = \rho\Delta C$, where ΔC is the hydrogen ion concentration gradient in ions per cubic centimeter, predicts a permeability coefficient, $\rho = 5 \times 10^{-6} cm\ s^{-1}$, which is in the range of experimentally measured net H^+/OH^- permeability for small ΔpH values. These measurements range from 1.4×10^{-4} to 7×10^{-7} $cm\ s^{-1}$ depending on the structure of the phospholipid comprising the membrane and the method used (Deamer 1987; Decoursey 2003). Thus, the transmembrane condensation model predicts a net H^+/OH^- flux and permeability for small ΔpH values that is in the range of published values and is consistent with the observed pH independence of the net flux.

Confounding Possibilities

Other considerations might affect this theoretically calculated permeability. For example, buffer diffusion in an unstirred layer could enhance presentation of H^+ and OH^- to the membrane surface (Gutknecht and Tosteson 1973). Additionally, water clusters containing an H^+ or OH^- within the interfacial zone could result in disruption of the normal phospholipid molecular arrangement, reducing the energy required for insertion.

These calculations obviously require assumptions. To consider how they would be affected by changing some of these assumptions one could, for example, change the effective dielectric constant, within the membrane from 2 to 4. This would result in a reduction of the target area for condensation by a factor of 4. However, increasing the dielectric constant from 2 to 4 would reduce by half the energy barrier for ion penetration into the membrane. This alone would increase the frequency of membrane penetration by a factor of e^2, or 4.7. Thus, these two effects nearly, but not completely, cancel each other. The effective dielectric constant would depend on the behavior of the water molecules at the interior surface of the interfacial zone, as previously mentioned.

One also could consider that the 4.6-Å cluster may not be the appropriate size or that the theoretically predicted frequency factor of $6 \times 10^{12}\ s^{-1}$, based on $k_B T/h$, may not apply to the situation of a 4.6-Å cluster at the membrane surface. If the cluster or the frequency factor were smaller, penetration is reduced and the theoretically calculated permeability would be too low, increasing the discrepancy between the theoretical value and that determined experimentally.

Molecular Modeling of Water Condensation Mechanism

Marrink et al. (1996) have examined theoretically the class of water condensation models proposed by Nagle (1987). In one model a single water chain or "wire" forms within the membrane in which H^+ and OH^- ions are allowed to condense via mutual access. It was concluded that in order for condensation to dominate over single-ion transport (a condition for pH independence), such a model would require that $k_3K_w \gg k_2[H^+]$, where $K_w = 10^{-14}$, k_3 is the rate that H^+_{in} and OH^-_{out} condense in the proposed water chain "pore" and k_2 is the rate that a single hydronium ion would transit the entire membrane via the hypothesized "water chain" sequence. According to these assumptions, this inequality would never be satisfied because the probability of an H^+ and OH^- entering the water-chain pore simultaneously will always be less than the probability that one or the other will enter alone. However, for the model we propose there is no single low-energy pathway for an H^+ or OH^- to cross the membrane and once H^+ and OH^- penetrate the interfacial zone to the low dielectric portion of the hydrophobic core, this creates a larger effective domain for ion attraction. Such an arrangement of attraction to opposing positions effectively reduces the thermal energy needed for insertion into the hydrophobic core and greatly increases the condensation probability. The data require that condensation events must occur much more frequently than a single ion diffusing through the entire membrane thickness, here represented by $k_2[H^+]$. We propose that $k'_3K_w \gg k_2[H^+]$, where the rate constant k'_3 represents *all* condensation reactions (attraction between H^+ and OH^-) over a wider membrane area and including many possible pathways.

Evidence in Support of the Transient Pore Model

Theoretical descriptions of the solubility-diffusion model predict only modest dependence on bilayer thickness, while transient pore models predict an exponential relationship (Paula et al. 1996). In an effort to distinguish between the two mechanisms, Paula et al. measured the H^+/OH^- permeability of phospholipid membranes as a function of phospholipid chain length (i.e., membrane thickness). A plot of these data showed a nearly exponential decrease of H^+/OH^- flux as the acyl chain length of the membrane phospholipids increased from 14 to 20 carbons and a smaller rate of decrease for membranes composed of phospholipids with longer chains. The authors concluded that H^+/OH^- permeation occurred during the formation of transient water pores for membranes composed of the shorter-chain (14–20 carbons) phospholipids, whereas it occurred by simple diffusion for the thicker membranes. Although this interpretation is supported by the data, neither mechanism accounts for the pH independence of H^+/OH^- flux as discussed earlier. The transmembrane condensation model was not considered in their analysis, and although our theoretical description of this mechanism does not predict an exponential dependence on membrane thickness, unknown mechanisms not included in the model and governing the behavior of $f(\delta)$ in Eq. 7 might provide such a dependence. For example, hydrophobic stabilization and attraction among the hydrophobic tails of the longer-chain phospholipids could reduce the water cluster penetration events. None of the three models in their basic representations is consistent with both the dependence of H^+/OH^- flux on membrane thickness and its independence of pH. This discrepancy could be resolved by measuring the pH dependence of H^+/OH^- flux through membranes composed of short-chain phospholipids, i.e., with 14 carbon acyl chains. H^+/OH^- flux through water pores would be predicted to increase exponentially as the pH range of measurement is decreased (as shown in Decoursey 2003, Fig. 13), whereas flux via transmembrane condensation would remain constant over the pH measurement range.

Conclusions

The magnitude and pH independence of H^+/OH^- flux across lipid membranes may be explained semiquantitatively by transmembrane condensation reactions. Flux magnitude arguments support a hypothesis that electrostatic attraction between an H^+ and OH^- in the head group regions from opposite sides of the membrane aligns the charges in direct opposition across the membrane and reduces the energy required for penetration across the hydrophobic core. The presence of an H^+ or OH^- tends to stabilize coherent water structures in the plane of the interfacial zone, delocalizing the charge and enhancing the rate of H^+/OH^- penetration into the lipid. This predicts a magnitude of H^+/OH^- flux within the range of measured values, which are five or six orders of magnitude greater than for Na^+ and Cl^-.

One consequence of the transmembrane condensation model and the resultant high H^+/OH^- permeability relative to other cations and anions is that the H^+/OH^- gradient would dominate the production of a diffusion potential across the membrane. A central problem in understanding the chemical evolution of life is to explain how energy was captured and maintained across primitive membranes. The production of pH gradients by physical or chemical events would generate a diffusion potential that would dissipate at the rate of flux by slower counter-ions. This would allow the pH gradient to coexist with an electrical gradient for some time. Both forms of energy (chemical and electrical) could be used to accumulate substrates required for metabolism and reproduction.

The finding, predicted by the transmembrane condensation model, that the high H^+/OH^- flux is independent of the pH range also would have been a beneficial occurrence for the evolution of life. Thus, production of diffusion potentials would be more uniform over a greater range of environmental pH values. The metabolic costs of accumulating substrates by this mechanism would also have been stabilized over a greater pH range. This unique behavior of protons and hydroxides, i.e., their ability to flux by a mechanism of condensation of H^+/OH^- ions, originating from opposite sides of a lipid membrane, may partly explain the universality of proton gradients in the capturing of energy in biology.

Acknowledgement We thank Drs. David Deamer and Otto Froehlich for excellent discussions and suggestions during the preparation of this article.

Appendix

The Water-Associated Proton/Hydroxide Transfer Model

This is treated as a general case of the H^+/OH^- "water-wire" condensation reaction (suggested by Deamer and Nichols (1982) and Nichols (Gutknecht 1984) and expanded by Nagle and Marrink (Marrink et al. 1996; Nagle 1987)) and considers net flux as a result of H^+ and OH^- from opposite membrane surfaces interacting within the membrane and yielding an expression (Nagle 1987) of the form

$$J_{\text{net}} = f(\delta)\left\{\left([H^+]_L[OH^-]_R\right) - \left([H^+]_R[OH^-]_L\right)\right\}, \qquad (A.1)$$

where $f(\delta)$ is a function of membrane thickness and composition and the number of available pathways. The net H^+/OH^- flux of Eq. A.1 is pH-independent, as was stated by Nagle (1987) and is reproduced below.

Using $[OH^-]_R \cdot [H^+]_R = [OH^-]_L\ [H^+]_L = 10^{-14}$ and $pH = -\log_{10}[H^+]$, $[OH^-] = 10^{-(14\text{-pH})}$, $10^x = e^{2.3x}$, etc., Eq. A.1 takes the form

$$J_{\text{net}} = f(\delta)\cdot 10^{-14}\left\{e^{-2.3\Delta pH} - e^{+2.3\Delta pH}\right\}, \qquad (A.2)$$

where $\Delta pH = pH_L - pH_R$. Rewriting the exponential expression as a hyperbolic sine function gives

$$J_{\text{net}} = f(\delta)\cdot 2\cdot 10^{-14}\{\sinh(-2.3\cdot\Delta pH)\} \qquad (A.3)$$

The hyperbolic sine term within the brackets of this expression can be expanded as a series giving the following:

$$\begin{aligned} J_{\text{net}} &= J_o\cdot\sinh[-2.3\cdot\Delta pH] \\ &= -J_o\left\{2.3\cdot\Delta pH + \frac{1}{6}(2.3\cdot\Delta pH)^3 \right. \\ &\quad \left. + \frac{1}{120}(2.3\cdot\Delta pH)^5 + \cdots\right\}. \end{aligned} \qquad (A.4)$$

The salient point is that the expressions representing net flux, Eqs. A.3 and A.4, are independent of the absolute value of pH and only depend on an energy gradient, ΔpH, across the membrane.

Inclusion of Membrane Voltage

One method of including voltage dependence in this formulation is by allowing the concentrations of charged particles within the membrane, relative to their concentrations at the surface, to be determined by the Boltzmann energy distribution. The net flux would then be represented as

$$J_{\text{net}} = \left\{[H^+]_L\cdot[OH^-]_R\cdot e^{\Delta v} - [H^+]_R\cdot[OH^-]_L\cdot e^{-\Delta v}\right\}f(\delta), \qquad (A.5)$$

where $\Delta v = (V_L - V_R)\cdot e/k_BT$. Using similar procedures, as previously given in Eqs. A.2 and A.3, yields

$$J_{\text{net}} = f(\delta)\cdot 10^{-14}\left\{e^{(-2.3\Delta pH+\Delta v)} - e^{(+2.3\Delta pH+\Delta v)}\right\} \qquad (A.6)$$

Setting $\Delta v = 0$, Eq. A.6 reverts to Eq. A.3. Setting the ΔpH to zero simplifies this expression to

$$J_{\text{net}} = f(\delta)\cdot 2\cdot 10^{-14}\sinh(\Delta v) \quad \left([H^+]_L = [H^+]_R\right). \qquad (A.7)$$

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