BRIEF COMMUNICATION

ZWITTERIONIC WATER CHAINS AS H^+/OH^- TRANSPORTERS

I. R. Miller
Department of Membrane Research, The Weizmann Institute of Science, Rehovot, 76100 Israel

ABSTRACT Zwitterionic water chains spanning the hydrocarbon layer with an H^+ attached to one end and an OH^- to the other end are suggested to be H^+ and OH^- conductors. They explain higher H^+/OH^- fluxes and their very weak dependence on pH.

The permeability of H^+ or of OH^- around pH 7 is up six to seven orders of magnitude higher than the permeability of other monovalent ions (1–5). However, the fluxes of H^+ or OH^- depend little on pH or on the concentration of H^+ or of OH^- in this region. This means that the permeability decreases as the pH deviates from neutral pH to lower or higher pHs. Onsager (6) suggested that hydrogen-bonded water chains could serve as H^+/OH^- transfer channels. Such hydrogen-bonded proton conducting water chains connected to active sites of proteins were invoked in theoretical models of bioenergetic functions (7–10). According to Nicols et al., these hydrogen-bonded water chains do not translocate any other ions besides H^+ and OH^- (1, 2). The hydrogen-bonded water chains per se cannot explain the lack of dependence of the H^+ or OH^- fluxes on their concentrations (4, 5). I wish to suggest here existence of zwitterionic hydrogen-bonded water chains, with their concentration proportional to the product of the concentrations of H^+ on one side of the membrane, and of OH^- on the other side, and conversely, for the zwitterionic channels with opposite polarity. Their stationary concentration as well as their H^+/OH^- permeability are supposed to be higher than that of channels with only one charge (H^+ or OH^-) adsorbed on one side. This model just like any other model based on hydrogen-bonded water chains requires a sufficiently high concentration of water in the hydrocarbon layer.

WATER CONCENTRATION IN THE HYDROCARBON LAYER

The water concentration in the hydrocarbon region C_w^m of the lipid bilayer cannot be measured directly. We can estimate it from the water permeability P_w ($\sim 2 \cdot 10^{-3}$ cm/s) (11) and from its diffusion coefficient D_w^m in the hydrocarbon layer of thickness δ.

$$P_w = 18 C_w^m \frac{D_w^m}{\delta}. \tag{1}$$

D_w^m can be derived by the Stokes-Einstein equation from the measured microviscosities (12,13) ($n \approx 0.3$–0.7 poise). Taking 1.8 Å for the radius of the water molecule, one obtains for $D_w^m \approx 1.5$–$3 \cdot 10^7$ cm^2/s. This gives according to Eq. 1 for $P_w = 2 \cdot 10^{-3}$ cm/s and $\delta = 2.6 \cdot 10^{-7}$ cm a concentration of $1.2 \pm 0.4 \cdot 10^{-4}$ M/cm^3. Assuming for a mole of hydrocarbon chain a volume of 250 cm^3 this corresponds to a molar fraction of $3 \pm 1 \cdot 10^{-2}$. For water aggregates, the calculated diffusion coefficient would be lower and the concentration in the membrane hydrocarbon layer higher.

WATER CHAIN FORMATION

Hydrogen bonding is responsible for water chain formation with a binding constant determined by the negative exponential of the free energy of the bond formation. The negative value of the energy is highest for the sequential arrangement of the water molecules. It is appreciably lower in the case of branching when double acceptors or double donors are involved (14). We shall consider therefore only sequential, linear water chains. The hydrogen bonding increases because of charge transfer energy contribution, with the chain length up to $n = 5$ when it becomes constant.

For the sake of simplicity we shall assume here that the binding constant of a water molecule to a growing chain does not depend on the chain length. The hydrogen-bond formation energy in a dimer in a dielectric medium of 2.2 is ~4.2 kcal/mol or 7 kT (15). In a five-membered chain or longer it is ~9 kT (14). The energies of 7, 8, and 9 kT correspond to binding constants of $K_b = 1{,}100$, 3,000, and 8,000 when the calculation is done in molar fractions. The molar fraction of an n-membered chain X_{nw} containing $n - 1$ hydrogen bonds is

$$x_{nw} = K_b^{n-1} X_w^n. \tag{2}$$

The total molar fraction of water in monomeric units is

Biophys. J. © Biophysical Society · 0006–3495/87/09/497/04 $2.00
Volume 52 September 1987 497–500

given by

$$(X_w)_{\text{total}} = \Sigma n X_{nw} = \frac{X_w}{(1 - K_b X_w)^2}. \qquad (3)$$

The formed water chains will tend to orient normally to the hydrocarbon layer surface because of the polar boundaries and the alignment by the hydrocarbon chain. Since the O—O distance in a hydrogen-bonded water chain is 2.82 Å (14), we need a 9 to 10-membered water chain to span the hydrocarbon layer and the chain spanning the layer cannot be much longer than that. Any 10-membered chain or longer within the hydrocarbon layer can be considered to be in equilibrium with a constant K_s with chains of average length n_s spanning the layer. Thus the total molar fraction of the water in both types of configurations is

$$(X_w)_{\text{total}} = \frac{X_w}{(1 - K_b X_w)^2} + n_s X_w K_s \frac{(K_b X_w)^9}{1 - K_b X_w}. \qquad (4)$$

If we take $n_s = 10$ and $K_s = 1$, X_w calculated from Eq. 4 is not very different from that calculated by Eq. 3 and the fraction of the water molecules participating in the chains spanning the hydrocarbon layer varies between 0.23 and 0.27 when K_b varies from 1,100 to 8,000. If $K_s = 10$, the water fractions spanning the layer are 0.66 for $K_b = 1{,}100$, 0.72 for $K_b = 3{,}000$, and 0.79 for $K_b = 8{,}000$, whereas the respective values of X_w are ~10% lower than those calculated by Eq. 3. From this calculation and from the assessed water concentration, and taking into account the cross-sectional area of the water chain is 10 Å while that of a hydrocarbon chain is ~35 Å, we estimate the number of water chains to vary between $4 \cdot 10^{11}$ and 10^{12} per cm^2.

H^+/OH^- ADSORPTION BY WATER CHAINS

The spanning water chains can adsorb H^+ or OH^- at the polar boundary. Attachment of a charge at the end of a water chain augments the charge-transfer energy resulting from the mixing of molecular orbitals and induces an electron shift along the chain. The electron shift depicted in Fig. 1 forms a partial charge on the other side of the membrane which becomes an acceptor of the counter ion. Thus, if a proton is adsorbed on the chain on one end, a hydroxyl will tend to adsorb on the partly charged other end and vice versa. The fractions of the zwitterionic water chains of the two opposite polarities $X_{nw}{}^{+/-}$ and $X_{nw}{}^{-/+}$ will be then given by

$$X_{nw}{}^{+/-}/X_{nw} = K_z X^1_{H^+} X^2_{(OH)^-}$$

and

$$X_{nw}{}^{-/+}/X_{nw} = K_z X^2_{H^+} X^1_{(OH)^-}, \qquad (5)$$

where $X^{1,(2)}_{H^+(OH^-)}$ are the respective molar fractions of H^+ or OH^- in the solutions on the two sides of the membrane. K_z is probably many orders of magnitude lower than the association constant to water but sufficient to maintain a concentration of the zwitterionic water chains higher than those with only a single charge attached. We shall estimate the zwitterionic water-channel concentrations from the measured and from the calculated channel permeabilities P_z:

$$P_z = \frac{\overline{X}_z}{X^1_{H^+} - X^2_{H^+}} \frac{D_{H^+/OH^-}}{\delta} e^{-E_a/RT}, \qquad (6)$$

where $\overline{X}_z$ is the molar fraction of zwitterionic channels in the membrane, D_{H^+/OH^-} is the diffusion coefficient of H^+ or OH^- without any electrostatic barrier, and E_a is the activation energy given by the maximal electrostatic energy of a charge in the channel.

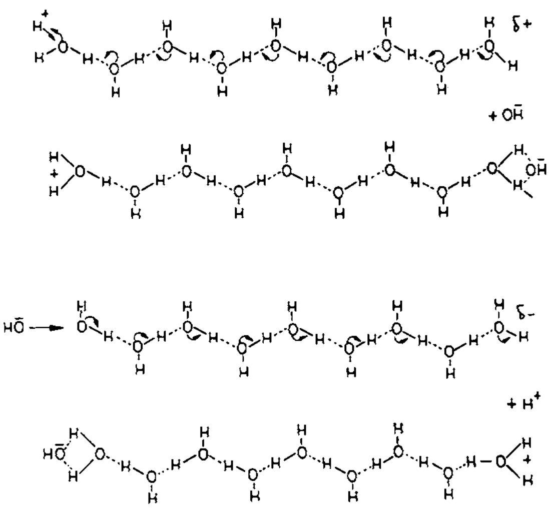

FIGURE 1 Charging process of linear water chains.

H^+/OH^- TRANSLOCATION ALONG WATER CHAINS

The energy profile of a charge passing a channel can be calculated numerically by summing up its interaction energies with the membrane surface charges, according to Levitt (16). Similar but higher energy values are obtained by an approximate equation which combines the energy required to transfer the charge into an infinite pore (17) and the energy of interaction with the membrane surface charge

$$\Delta E = \frac{e^2}{\epsilon_\omega a} P\left(\frac{\epsilon_\omega}{\epsilon_m}\right) - \frac{e^2}{2\epsilon_m}\left(\frac{1}{2d_1} + \frac{1}{2d_2}\right) \ln \frac{2\epsilon_\omega}{\epsilon_\omega + \epsilon_m}. \qquad (7)$$

$P(\epsilon_\omega/\epsilon_m)$ is ~0.17 for $\epsilon_\omega/\epsilon_m = 40$, d_1 and d_2 are the distances of the charge from side 1 and side 2 of the water channel $d_1 + d_2 = \delta$. In the case of a zwitterionic water channel the electrostatic interaction energy ΔE_{es} between the passing charge and the one on the receiving end of the channel is added. To compute this electrostatic interaction energy an effective dielectric constant ϵ_{ef} is assumed which is obtained from the relation:

$$\epsilon_{ef} = \frac{e^2}{2E_a}. \qquad (8)$$

Δe_{es} at a distance d from the charge on the receiving end of the channel then becomes

$$\Delta E_{es} = e^2 \left(\frac{1^d}{\delta \epsilon_{w\delta}} + \frac{dr}{\epsilon_{cf} r^2} \right). \tag{9}$$

In Fig. 2, *a* and *b* the energy profiles of a single charge passing a channel of radius 1.8 Å and dielectric constant 80, as obtained by numeric computation according to Levitt (16) and by the approximate Eq. 7, are presented. In the same figure a charge passing through a zwitterionic channel of the same size and dielectric constant is also given. Its energy in the channel is lowered by ΔE_{es} as obtained from Eqs. 8 and 9. The numeric computation according to Levitt yields an activation energy of 16.5 kT in an open channel and 14.3 kT in a zwitterionic channel. The approximate equations yield activation energies that are higher by ~20%. Assuming $D_{H^+/OH^-} = 5 \times 10^{-5}$ cm^2/s, $\delta = 2.6 \times 10^{-7}$ cm, and $\Delta E_a = 14.3$ kT, we obtain $D_{H^+/OH^-}/\delta e^{E_a/kT} \simeq 1.2 \times 10^{-4}$ which is nearly equal to the measured permeabilities. This means that $\bar{X}_z$ in Eq. 6 is about equal to the molar fraction of H$^+$ in water around pH 7 $\approx 2 \times 10^{-9}$. Taking into account that the cross-sectional area of a "liquid" hydrocarbon chain is ~33 Å^2, the number of zwitterionic chains per cm^2 is ~6×10^5 which is ~10^6 smaller than the total concentration of water chains. A higher activation energy, e.g., the one obtained by Eq. 7 (16.5 kT), and lower value for $D_{H/OH}$ would yield up to 10^3 times higher concentrations of zwitterionic chains. Accordingly, the estimated values of K_z (Eq. 5) would be 10^3–10^6 times smaller than the association constant of H$^+$ and OH$^-$ to water.

TIME CONSTANTS OF THE DIFFERENT STEPS IN THE MODEL

The model involves three steps after the water chain formation (*a*) H$^+$/OH$^-$ adsorption on one side of the chain, (*b*) OH$^-$ or H$^+$ adsorption on its other side, and (*c*) H$^+$/OH$^-$ translocation along the chain. The stability time constant of a water chain can be identified with the time required to break a hydrogen bond around the middle of the chain and to transfer a part of the broken chain beyond a hydrocarbon chain to break the continuity of the water chain. This distance corresponds to the sum of the diameters of a water molecule and the hydrocarbon chain which is ~d = 8 Å. From here,

$$\tau = \frac{d^2}{2D} e^{E/kT} \approx 10^{-4}\ \text{s}.$$

This time is six to seven orders of magnitude higher than the relaxation times measured in hydrogen-bonded system by nuclear magnetic resonance (18) or mention scattering (19) where the transitions are between equivalent states. H$^+$ and OH$^-$ adsorption is diffusion controlled and at pH 7 the adsorption times of H$^+$ and of OH$^-$ are ~10 – 5 and 2 · 10^{-5}, respectively, which is within the stability time of the water chain. On the other hand the time required for a proton to reach the middle of a 25-Å-thick hydrocarbon layer with a diffusion coefficient of 10^{-5} cm^2 s^{-1} and an activation energy of 14.3 kr, is ~25 · 10^{-3} s. This is considerably longer than the chain stability time of 10^{-4} s. According to this, only a fraction of the chain charging H$^+$'s and OH$^-$'s are translocated. However, attached charges strengthen the hydrogen bonds (14) and may increase the chain stability for more than 10^{-4} s.

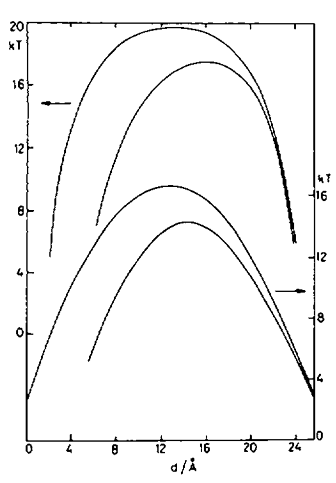

FIGURE 2 Energy profile of a H$^+$(OH$^-$) charge passing along a water chain of a radius of 1.8 Å spanning the hydrocarbon layer. The upper two curves (*left ordinate*) were calculated by the approximate Eq. 7. The lower two curves (*right ordinate*) were computed numerically according to Levitt. The lower curves in each group were calculated for zwitterionic channel taking into account the electrostatic interaction between the passing charge and the charge at the receiving end.

CONCLUSIONS

We can conclude that zwitterionic water channels can exist and they can explain the H$^+$/OH$^-$ permeability at neutral pHs and the independence of the H$^+$/OH$^-$ fluxes on pH. They may be also responsible for proton transfer between active sites of membrane proteins (7,10). The next objective should be to look for experimental evidence for the existence of these chains to support or to disprove this model. This is a very difficult task since the water channels are in equilibrium with bulk water and can exist only in the presence of a large excess of water. One could possibly design a nonequilibrium experiment in which the external water would be displaced before affecting the water chains within the hydrocarbon core which could be then detected by a spectroscopic method.

A simpler and a more realistic approach would be the determination of the ratio of H$^+$/OH$^-$ to ordinary ionic permeability below the phase transition of the lipid membrane. The ordinary ionic permeability is by orders of magnitude lower below than above the phase transition of the lipid layer (20). The H$^+$/OH$^-$ permeability according to the suggested model should not be that much lower below the phase transition. Even though this experiment does not provide anything definitely, it could add support or cast doubt on the model.

Received for publication 24 November 1986 and in final form 25 March 1987.

REFERENCES

1. Nichols, J. W., M. W. Hill, A. D. Bangham, and D. W. Deamer. 1980. Measurement of net proton-hydroxyl permeability of large unilamellar liposomes with fluorescent pH probe, 9-aminoacridine. *Biochim. Biophys. Acta.* 596:393–403.
2. Deamer, D. W., and J. W. Nichols. 1983. Proton-hydroxide permeability of liposomes. *Proc. Natl. Acad. Sci. USA.* 80:165–168.
3. Cafiso, D. S., and W. L. Hubbell. 1983. Electrogenic H^+/OH^- movement across phospholipid vesicles measured by spin-labeled hydrophobic ions. *Biophys. J.* 44:49–57.
4. Gutknecht, J. 1984. Proton/hydroxide conductance through lipid bilayer membranes. *J. Membr. Biol.* 82:105–112.
5. Verkman, A. S., and H. E. Ives. 1986. Anomalous driving force for renal brush border H^+/OH^- transport characterized by 6-carboxyfluorescein. *Biochemistry.* 25:2876–2882.
6. Onsager, L. 1969. The motion of ions: principles and concepts. *Science (Wash. DC).* 166:1359–1364.
7. Eisenbach, M., and S. R. Caplan. 1979. Current Topics in Membranes and Transport. Vol. 12. F. Bonner and A. Kleinzeller, editors. Academic Press Inc., New York. 168–248.
8. Nagle, J. F., M. Mille, and H. J. Morowitz. 1980. *J. Chem. Phys.* 72:3959–3971.
9. Hobbs, A. S., and R. W. Albers. 1980. *Annu. Rev. Biophys. Bioeng.* 9:259–291.
10. Nagle, J. F., and S. Tristram-Nagle. 1983. Hydrogen-bonded chain mechanisms for proton conduction and proton pumping. *J. Membr. Biol.* 74:1–14.
11. Fettiplace, R., and D. A. Haydon. 1980. Water permeability of lipid membranes. *Physiol. Rev.* 60:510–550.
12. Shinitzky, M., and M. Inbar. 1976. Microviscosity parameters and protein mobility in biological membranes. *Biochim. Biophys. Acta.* 433:133–149.
13. Shimshick, E. J., and H. M. McConnell. 1973. Lateral phase separation in phospholipid membranes. *Biochemistry.* 12:2351–2360.
14. Schuster, P. 1975. Structure of Water and of Aqueous Solutions. E.A.P. Luck, editor. Verlag Chemie und Physik Verlag. 141–167.
15. Moelwyn-Huges, E. A. 1957. Physical Chemistry. Pergamon Press Inc., Elmsford, NY. 1002.
16. Levitt, D. G. 1978. Electrostatic calculations for an ion channel. I. Energy and potential profiles and interactions between ions. *Biophys. J.* 22:209–219.
17. Parsegian, V. A. 1975. *Ann. NY Acad. Sci.* 264:161–174.
18. Hertz, H. G., and M. D. Zeidler. 1976. The Hydrogen Bond. Vol. III. P. Schuster, G. Zundel, and C. Sandorty, editors. North-Holland Publishing Co., Amsterdam. 1027–1061.
19. Janik, J. A. 1976. The Hydrogen Bond. Vol. III. P. Schuster, G. Zundel, and C. Sandorty, editors. North-Holland Publishing Co., Amsterdam. 891–936.
20. Miller, I. R. 1985. Permeability of vescular phospholipid bilayer membranes to thallium and its facilitation. *Biochim. Biophys. Acta.* 817:199–207.