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HYDROXYL ION PERMEABILITY OF LIPID BILAYER MEMBRANES

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The OH^- permeability of lipid bilayer (egg phosphatidylcholine-cholesterol) membranes was estimated from ionic transference numbers and membrane conductances at high pH. Membranes are slightly cation (Na^+) selective over the pH range of 6–10. However, at pH > 11, Na^+ and Cl^- conductances decrease and OH^- conductance increases so that the membrane becomes highly selective to OH^-. From the OH^- conductance we estimate the OH^- permeability coefficient to be $1.8 \cdot 10^{-9}$ cm $\cdot$ s^{-1}. The OH^- selectivity of lipid bilayers may contribute to the observed H^+/OH^- selectivity of some biological membrane at high pH.

The permeability to H^+ and OH^- is an important property of all cell and organelle membranes. Although $P_{H/OH}$ is usually assumed to be very low, recent studies on the fresh-water alga, *Chara,* have shown that at high pH the membrane conductance increases and becomes OH^- selective [1]. Although lipid bilayer membranes also show a conductance increase at high pH [2,3], the origin of the conductance increase has not been investigated. In this study we measured the electrical conductance and ionic selectivity of lipid bilayers in order to find out whether the ionic selectivity of the lipid bilayer might contribute to the observed H^+/OH^- selectivity of biological membranes at high pH.

Most lipids do not form stable bilayers at pH > 11. However, we found that mixtures of egg phosphatidylcholine plus cholesterol (1 : 1 mol ratio) in tetradecane form stable black films at NaOH concentrations up to 100 mM. We used tetradecane because capacitance measurements have shown that egg phosphatidylcholine-cholesterol-tetradecane bilayers contain very little hydrocarbon solvent [4]. Membranes were formed by brushing the lipid solution across a 1.8 mm^2 hole in a polyethylene partition which separated two aqueous solutions of 1.1 ml each. The rear compartment was covered with a Teflon plug to minimize CO_2 uptake by the alkaline solutions. The front solution was perfused continuously at a rate of about 1 ml/min.

The steady-state conductance was measured by imposing a small voltage across the membrane in series with a known resistance (voltage divider circuit). Membrane potentials were measured with a high impedance electrometer and two saturated KCl-calomel electrodes which made contact with the front and rear solutions. Unless otherwise specified, membrane conductance and ionic selectivity were measured at an ionic strength of 0.02–0.03. The temperature was 24 ± 1°C.

Over the pH range of 9.6 to 10.8, solutions were buffered with 5–10 mM HCO_3^-/CO_3^{2-}. Over the pH range 11.3 to 12.8, the solutions were buffered with NaOH. Egg phosphatidylcholine was obtained from Lipid Products (Surrey, U.K.), and cholesterol and tetradecane were obtained from Sigma Chemical Co. (St. Louis, MO).

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The membrane conductance (G_m) in unbuffered NaCl (pH 5–6) was $(1.2 \pm 0.4) \cdot 10^{-8}$ S · cm^{-2} (mean ± S.D.). By measuring the zero-current potentials produced by NaCl activity gradients [5,6], we found that the Na^+ transference number (T_{Na}) was about 0.6 and T_{Cl} was about 0.4. Both the total conductance and the slight cation selectivity are similar to results obtained by others [2,6,7]. From the specific ionic conductances the one-way fluxes and permeability coefficients (flux/activity) can be calculated [8]. This yields values of $P_{Na} = 8.5 \cdot 10^{-11}$ cm · s^{-1} and $P_{Cl} = 5.5 \cdot 10^{-11}$ cm · s^{-1}.

At pH 10.0–10.2, G_m was about 10-fold higher than the controls, i.e., $(2.2 \pm 1.7) \cdot 10^{-7}$ S · cm^{-2}. However, the relative ionic selectivity to Na^+ and Cl^- was similar to the controls. At a pH of 11.3 to 12.8, the membranes showed a marked change in ionic selectivity, becoming almost perfectly selective to OH^- (Fig. 1). At pH 12.2, G_m was $(1.1 \pm 0.3) \cdot 10^{-7}$ S · cm^{-2}, similar to G_m at pH 10. Thus, both G_{Na} and G_{Cl} must decrease and G_{OH} must increase at pH 12. Since $G_m \simeq G_{OH}$ at pH 12, we estimate that P_{OH} is $(1.8 \pm 0.5) \cdot 10^{-9}$ cm · s^{-1}.

Our results are qualitatively similar to those of Bisson and Walker [1], who found a decrease in cation conductance and an increase in the OH^- (or H^+) conductance of *Chara* membranes at high pH. Thus, the lipid bilayer selectivity may contribute to the observed OH^- (or H^+) selectivity of *Chara* at high pH. However, our value of G_{OH} for phosphatidylcholine-cholesterol bilayers is at least 100-fold lower than G_{OH} of *Chara*. Using egg phosphatidylcholine-decane bilayers we found a much larger conductance increase at high pH. However, the membranes were very unstable and would not thin at pH > 10.2.

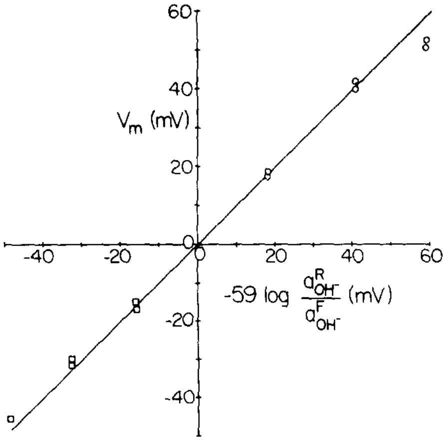

Fig. 1. OH^- selectivity of lipid bilayer membranes over the pH range of 11.2 to 13.1. The zero-current membrane voltage is plotted against the OH^- equilibrium potential (E_{OH}), calculated from the Nernst equation. The solid line has a slope of 1.0. The rear solution contained NaCl (4 mM) plus NaOH (20 mM). The front solution contained various concentrations of NaCl and NaOH. The circles indicate conditions under which $E_{Na} \simeq 0$ and $E_{Cl} \simeq -E_{OH}$. The squares indicate conditions under which $E_{Cl} \simeq 0$ and $E_{Na} \simeq -E_{OH}$. The sign of V_m is that of the rear solution relative to the front.

As pointed out by Bisson and Walker [1], a clear distinction between OH^- and H^+ selectivity is not possible because $E_{OH} = E_H$ under all the conditions shown in Fig. 1. However, previous studies by ourselves and others [2,6,9] have shown that at pH 1 the apparent H^+ permeability is very low, i.e., approx. 10^{-9} cm · s^{-1}, similar to our estimate of P_{OH}. Thus, if the apparent P_{OH} at pH 12 was actually due to H^+ diffusion, then P_H would have to increase from 10^{-9} cm · s^{-1} at pH 1 to >10 cm · s^{-1} at pH 12. Conversely, if the apparent P_H at pH 1 was actually due to OH^- diffusion, then P_{OH} would have to be >100 cm · s^{-1}. Such enormous absolute values and pH dependent changes in $P_{H/OH}$ seem very unlikely. Thus, we believe that the H^+/OH^- selectivity shown in Fig. 1 is, in fact, an OH^- selectivity.

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