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TRANSPORT OF PROTONS AND HYDROCHLORIC ACID THROUGH LIPID BILAYER MEMBRANES

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Summary

Transport of protons and hydrochloric acid through lipid bilayer membranes was studied by a combination of electrical conductance and pH electrode techniques. In the presence of large pH gradients, proton transport occurs primarily by diffusion of molecular HCl. The permeability of egg phosphatidylcholine/decane bilayers to HCl is about 3 $cm \cdot s^{-1}$, seven to nine orders of magnitude higher than the permeability to H^+, OH^- or Cl^-. The HCl permeability of phosphatidylserine or egg phosphatidylcholine/cholesterol (1 : 1) bilayers is about 50% lower than the permeability of egg phosphatidylcholine bilayers. Diffusion of molecular HCl may be an important process in tissues exposed to high HCl concentrations, e.g., gastric mucosa. However, at neutral pH the diffusion of molecular HCl is too slow to contribute significantly to net movements of H^+ or Cl^-.

Introduction

Acid/base and H^+/OH^- transport processes are important in many biological membranes and tissues, but the mechanisms of most of these processes are poorly understood. Recently Nichols et al. [1,2] found that the H^+/OH^- permeability of unilamellar egg phosphatidylcholine and phophatidylserine liposomes is greater than 10^{-4} $cm \cdot s^{-1}$. This H^+/OH^- permeability is almost as high as the permeability to water and is about six orders of magnitude higher than the Na^+ permeability. This high permeability suggests that H^+/OH^- transport occurs by a unique mechanism, possibly by rearrangements of hydrogen bonds along strands of water molecules extending into the membrane [1,2].

Electrical studies on planar bilayer membranes of various phospholipids indicate that the H^+ permeability is similar to or slightly higher than the Na^+ or K^+ permeability, but the absolute values of the H^+ conductance and permeability are very low [3—6]. For example, in (0.1 M) HCl, phosphatidylcholine bilayers have conductances ranging from $(0.02—2.0) \cdot 10^{-6}$ S · cm^{-2} [4—6]. Even if the entire conductance were due to H^+, then the H^+ permeability would be in the range of $(0.05—5) \cdot 10^{-9}$ cm · s^{-1}, 5—7 orders of magnitude lower than the H^+/OH^- permeability of liposomes [1,2]. Thus, we suspected that H^+/OH^- transport might occur by a nonconductive (electrically silent) mechanism. To investigate this possibility, we used a pH electrode as well as conductance measurements to estimate H^+/OH^- permeability of planar lipid bilayer membranes. Our study confirms that the conductive proton permeability of planar lipid bilayers is very low, but large proton fluxes are produced by diffusion of molecular HCl when large pH gradients exist across the membrane.

Materials and Methods

Lipid bilayers were formed by the brush technique [7] on a 1.8-mm^2 hole in a polyethylene partition which separated two aqueous solutions of 1 ml each. Both solutions were stirred magnetically and the front solution was perfused continuously with various solutions at a rate of about 1 ml/min. The rear solution was (50 mM) unbuffered NaCl and contained a small combination pH electrode (Markson, Model 583). Both front and rear solutions also contained calomel-KCl electrodes, which allowed measurements of the membrane voltage and conductance [8]. All solutions were equilibrated with argon, and an argon atmosphere was maintained above the solutions to minimize the absorption of CO_2. Phospholipids were obtained from Lipid Products (Surrey, U.K.). The temperature was 24 ± 1°C.

Results

Fig. 1 shows the rate of change of pH in the rear solution (50 mM NaCl) when the front solution is either NaCl (50 mM) or HCl (300 mM). The membrane is egg phosphatidylcholine in decane. The net H^+ flux is equal to the slope times the volume of the rear solution divided by the surface area of the membrane. Fig. 2 shows the net H^+ flux as a function of the molecular HCl activity gradient for several types of membranes. Since the relation between net H^+ flux and HCl activity is linear, we can estimate the HCl permeability coefficient (net flux/HCl activity gradient) to be 2.9 cm · s^{-1} for the egg phosphatidylcholine/decane membrane. The net flux is proportional to the first power of the molecular HCl concentration, which indicates that HCl monomers are diffusing through the membrane. The permeability for phosphatidylserine bilayers is about 1.5 cm · s^{-1} and the permeability of phosphatidylcholine/cholesterol (1 : 1) bilayers is about 1.6 cm · s^{-1} (Fig. 2). The main source of uncertainty in the permeability coefficients is the pK of HCl, which was assumed to be —6.1 [9,10]. However, values as low as —7 have been reported [10].

In all these experiments, the membrane voltage was clamped at zero. Due

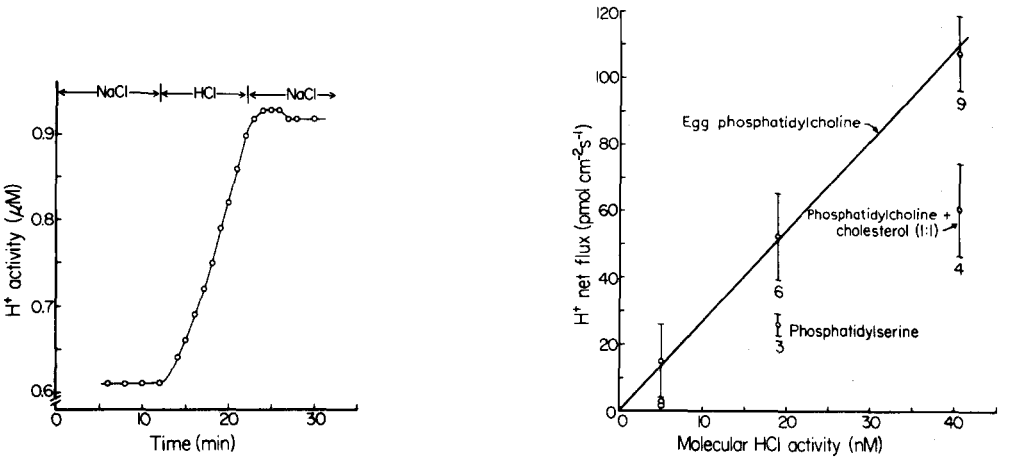

Fig. 1. Rate of change of H^+ activity in the rear solution when the front solution is changed from NaCl (50 mM) to HCl (300 mM) to NaCl (50 mM). The rear solution contains (50 mM) unbuffered NaCl and a pH electrode. The membrane is egg phosphatidylcholine in decane (35 mg/ml). The membrane potential is 0 mV.

Fig. 2. Net H^+ flux as a function of the HCl activity gradient across lipid bilayer membranes. The front solution contains (0.1—0.3 M) HCl and the rear solution contains (50 mM) unbuffered NaCl. The molecular HCl activity is calculated from the HCl concentration, using appropriate activity coefficients [28,33] and assuming a pK of —6.1 [9,10]. Membranes are formed from decane solutions of egg phosphatidylcholine (35 mg/ml), bovine phosphatidylserine (30 mg/ml) or egg phosphatidylcholine/cholesterol (1 : 1) (50 mg phosphatidylcholine and 25 mg cholesterol per ml). The vertical bars are standard deviations, and the numbers indicate the number of membranes.

to electrical noise from the pH meter, we measured the zero-potential currents in separate experiments. Under the conditions shown in Fig. 2, the net current flow was always less than 1% of the net H^+ flux. Thus, the net flux occurs by an electrically silent mechanism, i.e., diffusion of molecular HCl. Additional control experiments showed no significant H^+ flux produced by a NaCl gradient (300 mM front, 50 mM rear). We also observed no significant H^+ flux when the front solution was 200 mM H_2SO_4. Finally, we found no change in the net H^+ flux when $NaNO_3$ (50 mM) was substituted for NaCl in the rear solution.

The linear dependence of the net H^+ flux on the HCl activity gradient (Fig. 2) argues strongly for an H^+ rather than OH^- transport process. In these experiments, the net flux is approximately equal to the one-way flux, because the pH gradient is very large. Thus, if we were measuring a one-way OH^- flux from pH 7 to pH 1, we would expect the flux to be constant rather than proportional to the HCl activity in front. Furthermore, when the OH^- concentration in the rear is increased 10-fold, e.g., from pH 5.8 to 6.8, there is no change in the net H^+ flux from front to rear. Our attempts to measure directly the net OH^- flux at high pH were less successful because the membranes are very unstable in 100 mM NaOH. However, we observed no significant OH^- flux when the front solution contained 20 mM NaOH plus 80 mM NaCl. The smallest H^+/OH^- flux which can be measured by our pH electrode technique is about 5 pmol $\cdot$ $cm^{-2} \cdot s^{-1}$ [8]. Thus, the OH^- permeability under these conditions is less than $6 \cdot 10^{-7}$ cm $\cdot$ s^{-1}.

We also measured H^+/OH^- conductances in HCl and NaOH. In (100 mM) HCl, the conductance of egg phosphatidylcholine membranes ranged from 10^{-7} to 10^{-6} S · cm^{-2}, similar to values reported by others [4,5]. Similar results were obtained with membranes made from egg phosphatidylcholine/cholesterol (1 : 1) in tetradecane, which contain very little hydrocarbon solvent [17]. Stable black films are difficult to form at very high pH. However, using phosphatidylcholine/cholesterol (1 : 1) in tetradecane, we were able to measure conductances in (4 mM) NaOH plus (16 mM NaCl and obtained a value of (6 ± 2) · 10^{-8} S · cm^{-2}. In contrast to membranes in HCl, which showed little ionic selectivity, the membranes at high pH showed OH^- selectivity. For example, the zero-current potential produced by a 4-fold gradient of NaOH (16 mM as compared to 4 mM) was −33 mV, close to the OH^- equilibrium potential of −36 mV. Assuming for simplicity that all of the membrane conductance is due to OH^- diffusion, we can calculate the one-way OH^- flux from the relation, $J_{OH^-} = RTG_m/z^2F^2$, where R is the gas constant, T is the temperature, G_m is the conductance, z is the ionic valence and F is the Faraday constant [29]. This gives a value of about 10^{-14} mol · cm^{-2} · s^{-1}. Since the membrane potential is zero, we can estimate P_{OH^-} by dividing the one-way flux by the OH^- concentration (4 mM), which gives a value about $4 \cdot 10^{-9}$ cm · s^{-1}, about five orders of magnitude lower than the $P_{H/OH}$ of liposomes [1,2]. Using the same approach for the conductance in HCl (0.1 M), we obtained a maximum P_{H^+} of $3 \cdot 10^{-9}$ cm · s^{-1} for phosphatidylcholine or phosphatidylcholine/cholesterol (1 : 1), about five orders of magnitude lower than the $P_{H/OH}$ of liposomes [1,2]. The Na^+ permeability as determined from ionic transference numbers and the membrane conductance was $(0.3—2.7) \cdot 10^{-10}$ cm · s^{-1}, similar to the value obtained for liposomes [1].

Discussion

Our results indicate that molecular HCl is a highly permeant species which can account for large H^+ fluxes in the presence of HCl gradients. Thus, diffusion of molecular HCl may be an important transport process in tissues exposed to high HCl concentrations, e.g., gastric mucosa. This is the first measurement of the HCl permeability of lipid bilayer membranes; thus, no direct comparison can be made with the permeabilities of other membranes. However, the effects of membrane lipid composition on HCl permeability (Fig. 2) are consistent with previous studies on water and nonelectrolyte permeability. The permeabilities of phosphatidylserine and phosphatidylcholine/cholesterol (1 : 1) bilayers to water and small nonelectrolytes are 40—60% lower than the permeability of pure egg phosphatidylcholine bilayers [11,13]. The lower permeability of phosphatidylserine is probably due to a longer average chain length and fewer double bonds in the hydrocarbon chains [11]. The lower permeability of phosphatidylcholine/cholesterol bilayers is probably due to the closer molecular packing (decreased fluidity) [12] and a reduced partition coefficient for small nonpolar molecules in the hydrocarbon region [30].

A major discrepancy exists between the H^+/OH^- permeabilities of liposome and planar bilayer membranes. Although our estimated HCl permeability is

very high, the concentration of molecular HCl in a physiological saline at neutral pH is only about 10^{-14} M. Therefore, the HCl flux we predict at neutral pH would be only about 10^{-17} mol · cm^{-2} · s^{-1}, about three orders of magnitude smaller than the H^+/OH^- fluxes observed by Nichols et al. [1,2]. Furthermore, the high H^+/OH^- permeability of liposomes does not require the presence of Cl^- [1,2]. Thus, diffusion of molecular HCl is not a plausible explanation for the high H^+/OH^- permeability of liposomes.

One difference between the two types of membranes is the presence of hydrocarbon solvent in the planar bilayers. However, the water and non-electrolyte permeabilities of solvent-containing bilayers, 'solvent-free' (Montal-Mueller) bilayers and liposomes are similar (within a factor of 5) [12,14—16, 19,31,32]. Furthermore, the addition of cholesterol to egg phosphatidylcholine greatly reduces the amount of hydrocarbon solvent in the bilayer interior [17]. If decane was inhibiting the proton permeability, we would expect an increase rather than a decrease in the H^+/OH^- permeability of phosphatidylcholine/cholesterol bilayers (Fig. 2). Thus, we do not believe that hydrocarbon solvent can account for the enormous discrepancy between the $P_{H/OH}$ of liposomes and planar bilayers.

The possible role of trace contaminants should perhaps be considered in the liposome experiments in which very small fluxes of H^+/OH^- are measured [1,2]. For example, the nonionic forms of small organic acids (lipid oxidation products?) have permeability coefficients ranging from about 0.01 to 1 cm · s^{-1} [14,16,18,19]. Thus, the presence of small amounts of organic acids could account for the observed H^+/OH^- fluxes of about 10^{-14} mol · cm^{-2} · s^{-1} [1,2]. Further work is obviously necessary to resolve this discrepancy. For example, it would seem desirable to measure the H^+/OH^- permeability of liposomes which contain no double bonds which are susceptible to peroxidation. Also, if the high $P_{H/OH}$ is due to a 'proton jump' along strands of water molecules [1,2], then $P_{H/OH}$ should be very sensitive to the water concentration in the membrane. This could be tested by conducting the liposome experiments in high concentrations of nonaqueous solvents such as glycerol. Finally, it would be interesting to study the $P_{H/OH}$ of liposomes over a wide range of pH in view of the surprising observation that either P_{H^+} or P_{OH^-} changes in response to changes in the aqueous pH [2].

Our results are also relevant to the long-standing problem of why the Cl^- permeability of lipid bilayers is orders of magnitude higher than the Na^+ or K^+ permeability [20—25]. Although molecular HCl diffusion has been proposed to explain the relatively high Cl^- permeability of lipid bilayers [21,23,26], others believe that a more complicated interaction of H^+ and Cl^- with membrane lipids is involved in the Cl^- transport process [20,24,25,27]. Our predicted HCl flux at neutral pH and 0.1 NaCl is 2—6 orders of magnitude smaller than the observed Cl^- fluxes in various types of lipid bilayers [20—26]. Thus, a simple diffusion of molecular HCl does not seem to be a plausible mechanism for the Cl^- selectivity of lipid bilayers, and a more complicated mechanism must be sought.

The permeability of other small acids and bases can also be measured by the pH electrode technique. For example, the permeability to nitric acid is easy to measure since its pK is fairly high (—1.3) and the nonionic form is relatively

abundant. Our preliminary experiments suggest that the HNO_3 permeability of phosphatidylcholine/decane membranes is about 10^{-3} cm · s^{-1}. We believe that the lipid bilayer-pH electrode system can provide permeability estimates for other small acids and bases which are biologically important but are difficult or impossible to study in biological membranes and tissues.

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