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A hydroxide ion carrier in planar phospholipid bilayer membranes: $(C_6F_5)_2Hg$ (dipentafluorophenylmercury)

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Although a number of molecules are known to function as current-carrying proton carriers across lipid bilayer membranes, no such hydroxide ion carriers have been found to date. We report that $(C_6F_5)_2Hg$, which can function as a chloride ion carrier, can also carry a hydroxide ion. In 100 mM Na_2SO_4 solutions, membranes treated with $(C_6F_5)_2Hg$ are almost ideally selective for H^+/OH^- between pH 6.0 and 9.5. Membrane conductance varies linearly with $[OH^-]$ over this pH range and with the square of the $(C_6F_5)_2Hg$ concentration. The presumed current-carrying species is the dimer $[(C_6F_5)_2Hg]_2OH^-$, which, along with the neutral molecule $(C_6F_5)_2Hg$, shuttles back and forth within the bilayer. In 0.2 M NaCl at pH 9.5, the OH^- and Cl^- conductances are approximately equal. Thus, the carrier displays an approximately 10^4-fold preference for OH^- over Cl^-.

Introduction

The generation of a proton conductance in lipid bilayer membranes is well known. In general, the agents used to generate this conductance are weak acids (HA), and the ion-conducting species is either A^- or HA_2^-. (See Ref. 1 for a review of proton transport across membranes.) These proton-transporting molecules also function as uncouplers of oxidative phosphorylation in mitochondria, a direct consequence of their ability to collapse the electrochemical potential gradient of protons across the inner mitochondrial membrane [1]. In addition, lipid bilayers possess a small endogenous proton conductance which has been attributed to trace amounts of fatty acid(s) contamination [2,3]. In each of these instances, the lipid bilayer has been made permeable to protons by agents that function as proton carriers, and in several cases, the kinetics of this carrier action have been extensively analyzed [1].

It is generally appreciated by the cognoscenti that, in theory, one cannot distinguish on thermodynamic grounds an electrochemical gradient of protons across a membrane in one direction from that of hydroxide ions in the other. Therefore, molecules which can shuttle protons across the inner mitochondrial membrane are not alone in their ability to uncouple oxidative phosphorylation; indeed a molecule capable of carrying hydroxide ions across a membrane will equally well abolish an existing electrochemical gradient of protons/hydroxide ions. To our knowledge, how-

Abbreviations: Mes, 4,-morpholineethanesulfonic acid; Hepps, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid; Ches, 2-(cyclohexylamino)ethanesulfonic acid; DPhPC, diphytanoylphosphatidylcholine.

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ever, there are no reports, with one possible exception [4], of a carrier capable of generating a hydroxide ion conductance in lipid bilayer membranes *. The present paper remedies this deficiency.

Materials and Methods

Planar phospholipid bilayer membranes were formed at room temperature by the brush technique of Mueller et al. [7] across a 1 mm diameter hole in a Teflon partition separating two Lucite compartments, each containing 3 ml of identical 100 mM Na_2SO_4 solutions. The solutions also contained 10 mM each of Mes (pK = 6.15), Hepps (pK = 8.0) and Ches (pK = 9.5), and were adjusted to the desired pH. All membranes were formed from a 3% solution of diphytanoylphosphatidylcholine (DPhPC) in *n*-decane. After the membranes were completely black, dipentafluorophenylmercury, $(C_6F_5)_2Hg$, (from stock ethanolic solutions of 0.02–2.0 mg/ml) was added to both compartments to concentrations ranging from 10 ng/ml to 1.5 μg/ml, and records were then taken. The pHs of the solutions were changed during the course of an experiment by additions of small volumes of 0.25 M H_2SO_4 and 1.0 M NaOH to one or both compartments; Cl^- was also added to one or both compartments by the additions of small volumes of 1 M NaCl. The pHs resulting from the H_2SO_4 and NaOH additions to the compartments were derived from a titration of the original solution. At the end of an experiment, the measured pH of the solution in the compartments was always found to be in good agreement with the predicted value. Both compartments were continuously stirred with magnetic fleas throughout the course of an experiment.

Experiments were done under voltage-clamp conditions with a single pair of Ag/AgCl electrodes in 3 M KCl solutions coupled to the solutions in the compartments through 0.5 M Na_2SO_4 bridges in 3% agar. The membrane conductance (g) was calculated by taking the current (I) which flows in response to applied steps of voltage (V) and dividing by that voltage: $g = I/V$. The applied voltages and the current responses were displayed on a Narco physiograph chart recorder (Houston, TX). The conductance of the membrane prior to addition of $(C_6F_5)_2Hg$ was less than 50 pS. DPhPC was obtained from Avanti Polar Lipids (Birmingham, AL), and *n*-decane was from Aldrich Chemical Company (Milwaukee, WI). $(C_6F_5)_2Hg$ was a gift from Dr. Olaf Andersen (Cornell University Medical College), who had in turn received it from Dr. E.A. Liberman (Institute of Electrochemistry, Moscow). We have kept it stored as a 2 mg/ml ethanolic solution at −20 °C for approx. 15 years.

Results

Upon addition of $(C_6F_5)_2Hg$ in submicromolar amounts to both sides of a membrane, the mem-

TABLE I

POTENTIALS ($\Delta\Psi$) GENERATED BY ΔpHs ACROSS A $(C_6F_5)_2Hg$-TREATED MEMBRANE

ΔpH and $\Delta\Psi$ refer to $pH_2 - pH_1$ and $\Psi_2 - \Psi_1$, respectively. The data were obtained on a single DPhPC membrane separating identical solution of 100 mM Na_2SO_4, 10 mM Mes, 10 mM Hepps, and 10 mM Ches. The initial pH was 9.51. $(C_6F_5)_2Hg$ was added to the compartments on both sides of the membrane to a final concentration of 1.3 μg/ml, and the pHs of sides 1 and 2 were lowered or raised by the additions of small amounts of 0.25 M H_2SO_4 and 1.0 M NaOH, respectively. The data are presented in chronological order. The protocol consisted of changing the pH on one side (generally side 1) to establish a ΔpH, recording the resulting $\Delta\Psi$, and then changing the pH on the opposite side to abolish the ΔpH (and consequently the $\Delta\Psi$). Solutions were stirred continuously throughout the experiment.

$pH_{side\ 1}$	$pH_{side\ 2}$	ΔpH	$\Delta\Psi_{exp.}$ (mV)	$\Delta\Psi_{theory}$ (mV)
9.00	9.51	0.51	30.6	30.2
8.52	9.00	0.52	31.0	30.8
8.00	8.52	0.52	30.3	30.8
7.42	8.00	0.58	34.4	34.3
6.66	7.42	0.76	42.3	45.0
6.05	6.66	0.61	25.2	36.1
6.66	8.00	1.34	68.0	79.3
8.00	9.60	1.60	88.2	94.7

* Several organo-tin compounds have been shown to abolish the electrochemical gradient of protons/hydroxide ions across the inner mitochondrial membrane by functioning as hydroxide ion carriers [5]. These carriers, however, mediate an obligatory hydroxide–anion exchange [5]; such an exchange is electrically silent and therefore does not result in an increase of membrane conductance [6].

brane conductance rises within a minute to its steady-state value. With our approx. 3 Hz filtering, the current responses for small steps of voltage appear as steps of current, whereas for larger voltage pulses, some polarization is seen in the current responses (Fig. 1A); the steady-state $I-V$ characteristic is roughly linear up to 100 mV (Fig. 1B). Conductance increases linearly with $[OH^-]$ over the pH range 6.0–9.5 (Fig. 2) and is proportional to the square of the $(C_6F_5)_2Hg$ concentration (Fig. 3). The $(C_6F_5)_2Hg$-treated membrane is nearly ideally selective for H^+/OH^-, as evidenced by the almost Nernstian potentials developed in response to the imposition of ΔpHs across the membrane (Table I). Thus, the conductance induced by $(C_6F_5)_2Hg$ is a consequence of the membrane's permeability to either H^+ or OH^-.

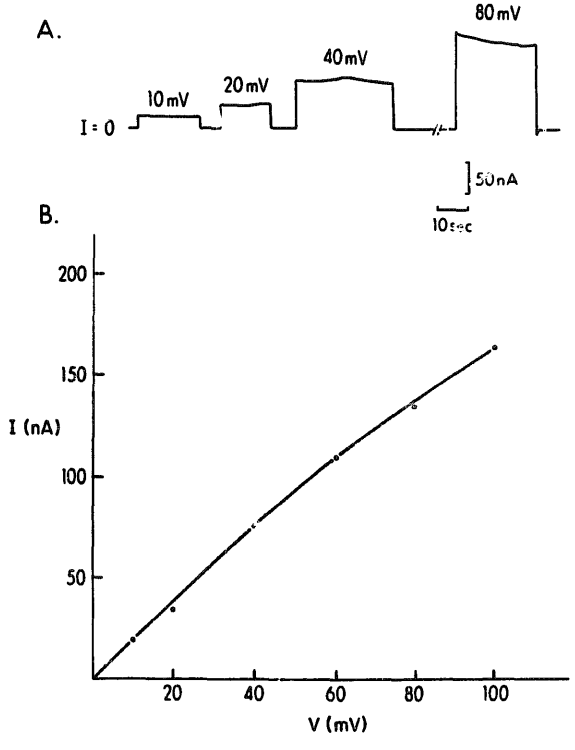

Fig. 1. The effect of $(C_6F_5)_2Hg$ on membrane conductance. A DPhPC black lipid membrane was formed across a 1 mm diameter hole in a Teflon partition separating identical solutions of 100 mM Na_2SO_4, 10 mM Mes, 10 mM Hepps and 10 mM Ches (pH 9.5). The membrane conductance was approx. 50 pS. $(C_6F_5)_2Hg$ was added to the solutions on both sides of the membrane to a final concentration of 1.5 μg/ml, and the solutions were stirred continuously throughout the experiment. The records shown in A, taken about 5 min after the addition of $(C_6F_5)_2Hg$, are filtered at 3 Hz. (B) The steady-state I vs. V plot was constructed from records such as those in A.

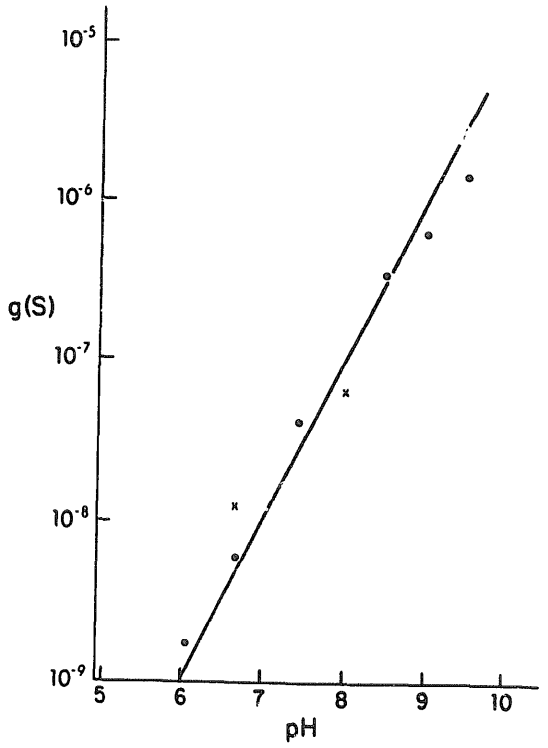

Fig. 2. Steady-state conductance (g) of a $(C_6F_5)_2Hg$-treated membrane as a function of pH. The initial conditions were the same as those in Fig. 1, and $(C_6F_5)_2Hg$ was added to the solutions on both sides of the membrane to a final concentration of 1.3 μg/ml. The pH was symmetrically lowered by small additions of 0.25 M H_2SO_4 (●) and then subsequently raised by small additions of 1 M NaOH (x). The line is drawn with the slope, $\Delta\log(g)/\Delta$pH, equal to 1.0. The conductance at each pH was determined from the ΔI in response to a ΔV of 10 mV. Solutions were stirred continuously throughout the experiment.

Prior to the present work, it was known that $(C_6F_5)_2Hg$ functions as a Cl^- carrier across lipid bilayer membranes in 0.1–1.0 M NaCl or KCl solutions at pHs of approx. 5.5 (Ref. 8 and unpublished observations), the presumed ion-carrying species being $[(C_6F_5)_2Hg]_2Cl^-$. This fact, the known ability of mercury compounds to bind anions, and the increase in conductance with pH (Fig. 2), clearly argue that the H^+/OH^- selectivity of the membranes in our present experiments is a consequence of an OH^--induced permeability (not an H^+-induced permeability), with the presumed ion-carrying species being $[(C_6F_5)_2Hg]_2OH^-$. We have found, moreover, that the carrier much prefers OH^- to Cl^-. For example, at pH 9.5 with 0.01

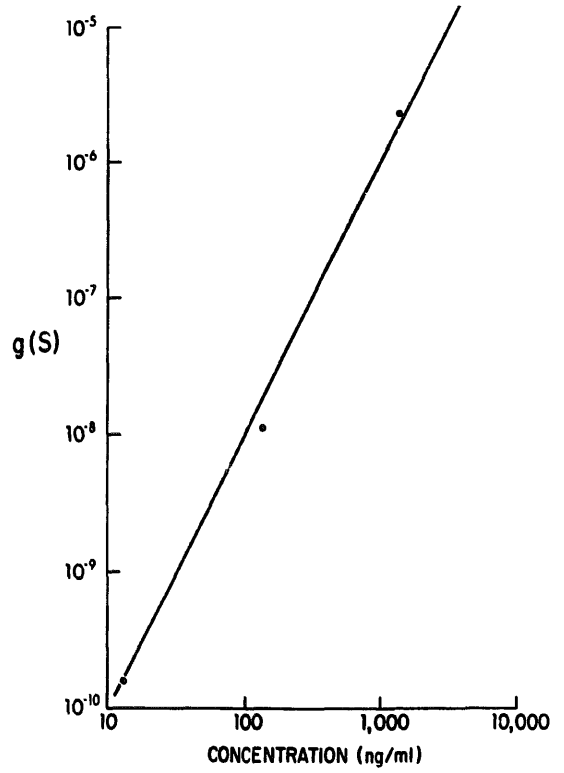

Fig. 3. Double-logarithmic plot of conductance vs. $(C_6F_5)_2Hg$ concentration. The initial conditions were the same as those in Fig. 1, and $(C_6F_5)_2Hg$ was then added to the solutions on both sides of the membrane to the final concentrations indicated on the graph (1 ng/ml = $1.87 \cdot 10^{-9}$ M). The recorded conductances represent measurements taken about 2–3 minutes after each addition of $(C_6F_5)_2Hg$ and were calculated from the ΔI in response to a ΔV of 10 mV. The line is drawn with a slope of 2.0. Solutions were stirred continuously throughout the experiment.

M NaCl on one side of the membrane and 0.05 M NaCl on the other, the reversal potential was only 13.6 mV (an ideally Cl^--permeable membrane would yield a potential of about 42 mV). At pH 9.5 (OH^- concentration $\approx 3 \cdot 10^{-5}$ M), the addition of concentrated NaCl to both sides of the membrane to the final concentration of 0.2 M increased the membrane conductance only by about a factor of 2. Thus, the carrier displays about a 10^4-fold preference for OH^- over Cl^-.

Discussion

We have shown that the addition of $(C_6F_5)_2Hg$ to 100 mM Na_2SO_4 solutions bathing planar lipid bilayers can induce very large membrane conductances. The selectivity data and the linear rise in conductance with $[OH^-]$ imply that the induced permeability is to either H^+ or OH^-; one cannot, on the basis of these data alone, establish which ion is being translocated. The rise in conductance with pH, for example, is also seen with proton carriers, reflecting an increase in the concentration of A^- or HA_2^-, the current-carrying species [1]. However, given the absence of titratable groups, and in light of the well-known ability of Hg to complex with anions and with the ability of $(C_6F_5)_2Hg$ to function as a Cl^- carrier, we conclude that this compound acts as a carrier of OH^- rather than of H^+. The dependence of conductance on the square of the $(C_6F_5)_2Hg$ concentration indicates that the ion-carrying species is a dimer of the form $[(C_6F_5)_2Hg]_2OH^-$. The carrier is about 10^4-fold more selective for OH^- than for Cl^-, as evidenced from our observation that in 0.2 M NaCl at pH 9.5, the OH^- and Cl^- conductances are about equal.

The carrier action of $(C_6F_5)_2Hg$ must involve the shuttling back and forth within the membrane of both the neutral species and its charged complex with OH^-, analogous to the mechanism of action of the H^+ carriers [1]. It cannot simply result from the partitioning of the complex between the aqueous solution and the membrane and its subsequent diffusion through the membrane *. The currents are much too large for such a mechanism. For example, in the experiment in Fig. 1A, even if all of the $(C_6F_5)_2Hg$ were in the $[(C_6F_5)_2Hg]_2OH^-$ form, its concentration (c) in solution would be only approx. 1 μM. Given an unstirred layer thickness (δ) on one side of the membrane of about 50 μm [10], and assuming a free diffusion coefficient (D) of the complex in water of about $5 \cdot 10^{-6}$ cm/s, then even if, in the face of a large applied voltage, the concentration of the complex at the membrane/solution interface were near zero, the maximum flux (Φ) of the complex through the 10^{-2} cm^2 area (A) membrane (which, in the steady state, must equal its

* Some transport can occur via this pathway and can lead, under certain circumstances, to non-Nernstian potentials in response to a ΔpH across the membrane (see Refs. 1 and 9 for discussions of this point). This is probably the reason for the deviations from ideality seen in Table I.

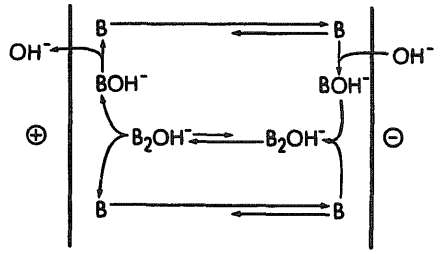

Fig. 4. Diagram illustrating the shuttling back and forth carrier mechanism of $(C_6F_5)_2Hg$ (denoted as B) and $[(C_6F_5)_2Hg]_2OH^-$ (denoted as B_2OH^-) within the membrane. The circled positive and negative signs indicate that a voltage is being applied across the membrane. This diagram is a plagerization of Fig. 9 in a paper by Cohen et al. [15], which pertains to a proton carrier.

flux through the unstirred layer) would be approx. 1 nA. That is,

$$\Phi = DAc/\delta = (5\cdot10^{-6}\,cm^2/s)(10^{-2}\,cm^2)(10^{-9}\,mol/cm^3)$$

$$/(5\cdot10^{-3}\,cm) = 10^{-14}\,mol/s \approx 1\,nA.$$

Yet we can see in the figure that even for only a 20 mV stimulus, there is a steady-state current of 35 nA. To achieve this current level, the carrier must shuttle back and forth within the membrane, picking up an OH^- at one interface and discharging it at the other (Fig. 4). (See Refs. 1 and 9 for an elaboration, with respect to proton carriers, of the analysis in this paragraph.)

To our knowledge, this is the first report of a molecule functioning as an electrogenic OH^- carrier across a lipid bilayer membrane, with the possible exception of a paper by Young and Feldberg [4], in which they suggest that the photogenerated magnesium octaethylporphyrin cation may act in this capacity. There are several barene mercury derivatives that have been shown to function as anion carriers in lipid bilayers [11], but their capacity to carry hydroxide ion has not been tested. Conceivably, they too could act as electrogenic OH^- carriers.

It should be appreciated that the distinction between H^+ and OH^- carrier-induced transport across lipid bilayers depends on information other than that arising from permeability and conductance measurements. Thus, both the Nernstian potentials arising from a ΔpH across the membrane and the increase in membrane conductance with pH are equally consistent with H^+ or OH^- transport. (In the case of the weak acid carriers, the increase in conductance with pH is a consequence of the increase in A^- or HA_2^- concentration, which are the current-carrying species.) The known weak acid properties of molecules such as 2,4-dinitrophenol (DNP) and *m*-chlorocarbonylcyanidephenylhydrazone (CCCP) are what make their identification as proton transporters so obvious. Similarly, the additional information that $(C_6F_5)_2Hg$ can function as a Cl^- carrier, plus the exclusion of any reasonable interaction of this molecule with H^+, makes its identification as an OH^- carrier compelling. At present, there are no comparable auxilliary data for the so-called proton pumps (e.g., mitochondrial ATPase and bacteriorhodopsin), and the presumed proton efflux generated by these systems can be equally well characterized as a hydroxide influx. In fact, given that halorhodopsin is a Cl^- pump [12] and that it is structurally similar to bacteriorhodopsin [13], one might guess, by analogy, that the latter is actually an OH^- pump. Similarly, Nicholls and Locke [14] suggest that since the so-called proton conductance pathway in brown fat mitochondria also conducts Cl^-, that pathway may actually conduct OH^-.

Acknowledgements

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