Biochimica et Biophysica Acta, 1030 (1990) 165–171
Elsevier

BBAMEM 75063

The apparent permeability coefficient for proton flux through phosphatidylcholine vesicles is dependent on the direction of flux

F. Anderson Norris and Gary L. Powell

Department of Biological Sciences, Clemson University, Clemson, SC (U.S.A.)

(Received 29 January 1990)
(Revised manuscript received 20 August 1990)

Key words: Permeability; Carrier; Carbon dioxide; Carbonic acid; Bicarbonate; Model membrane

A dioleoylphosphatidylcholine unilamellar vesicle model system was used to determine proton permeability. The fluorescence of the pH reporter group, pyranine, trapped within vesicles with a difference in pH across the bilayer, was digitized and analyzed with numerical integration. When H^+ flux was initiated by the acidification of the external buffer (acid jump), the apparent H^+ permeability was found to be a linear function of the reciprocal of the internal H^+ concentration with the slope inversely proportional to the initial size of the H^+ gradient. When flux was initiated by the alkalinization of the external buffer (base jump), the apparent permeability coefficient was constant for each external H^+ concentration. However, the value of the apparent permeability was linearly dependent on the reciprocal of the external H^+. The possibility that carbonates (carbon dioxide, carbonic acid, bicarbonate and carbonate) could be acting as proton carriers was tested by adding millimolar concentrations of bicarbonate to solutions greatly reduced in carbonates. The slopes of the graphs of apparent permeability coefficient vs. reciprocal H^+ were linear functions of added bicarbonate concentration for both acid and base jump conditions. These observations were interpreted in terms of a model suggesting that carbonic acid or carbon dioxide together with bicarbonate was an efficient proton carrier across phospholipid bilayers.

Introduction

Many biological processes depend on pH gradients formed across membranes. The intrinsic permeability of proton/hydroxide to these biomembranes is therefore of interest since it reduces the efficiency of coupling with useful processes. However, the measured permeability of protons * across phospholipid bilayers is five orders of magnitude greater than that for other small univalent cations such as sodium and potassium [1–6]. This dramatic difference indicates that a unique mechanism must he involved to facilitate the movement of protons across bilayers.

One of the earliest mechanisms suggested for the transport of protons through phospholipid bilayers was the proposal that hydrogen bonded chains of water molecules transiently span the bilayer ('water wires'). Suitable rearrangements of the hydrogen bonds would result in the loss of protons at one surface and appearance of protons at the opposite surface [1]. An alternative suggestion postulates the presence of carriers for protons in phospholipid bilayers. For example, a small percent of free fatty acid, could facilitate the transport of protons through the bilayer [7]. Distinction of these mechanisms has proved difficult and many observations have been made that can not easily be explained [8–10]. In the work described below, novel analytical techniques have been developed that reveal characteristics of proton permeability that have not been reported previously.

Materials and Methods

Chemicals. L-α-Dioleoylphosphatidylcholine (DOPC) was a gift from Avanti Polar Lipids, Inc., Alabaster, AL. Pyranine (8-hydroxy-1,3,6-pyrenetrisufonate) (laser grade) was purchased from Eastman Kodak. Valinomycin, nigericin and fatty acid free bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, MO. Hepes (*N*-2-hydroxyethylpiperazine-*N*'-2-

* We will use the term proton for H^+/OH^- flux because of the practical and theoretical difficulties in distinguishing proton flux from a counter flux of hydroxide ions.

Abbreviation: DOPC, L-α-dioleoylphosphatidylcholine.

Correspondence: G.L. Powell. Department of Biological Sciences, Clemson University, Clemson, SC 29634-1903, U.S.A.

ethane sulfonic acid) was obtained from Research Organics, Inc., Cleveland, OH. Potassium sulfate and sodium bicarbonate (reagent grade) were obtained from J.T. Baker Chemical Co., Inc. EDTA from Matheson, Coleman and Bell, Norwood, OH.

Preparation and characterization of vesicles. Dioleoyphosphatidylcholine was transferred to a small flask and taken to dryness under vacuum or a stream of argon, and then held in a vacuum desiccator overnight. The lipid (about 10 mg phospholipid per ml) was vortexed with 25 mM Hepes, 50 mM potassium sulfate, 1 mM EDTA and 2 mM pyranine at pH 8.0 for an acid jump experiment or pH 7.2 for a base jump experiment. Unilamellar vesicles were prepared from the lipid suspension by the extrusion method [11] using polycarbonate membranes with a pore diameter of 0.1 μm (Nucleopore Corp.) and the extrusion device manufactured by Lipex Biomembranes Inc., Vancouver, BC. Unilamellar vesicles were separated from untrapped pyranine by gel exclusion chromatography on G-25–150 Sephadex (Sigma Chemical Co.). The phosphate concentration of the pyranine loaded vesicles was assayed spectrophotometrically [12]. Vesicles were then diluted to a phosphate concentration of 0.5 mM, and 1 μg/mg lipid of valinomycin was added (0.78 μg/ml) [2,10]. The ratio of valinomycin to phospholipid was kept constant in all experiments; the valinomycin concentration was sufficiently low that small increases in concentration did not change the measured proton permeability. The vesicle suspension was incubated at 4° C overnight before measuring proton fluxes.

Buffer containing limited carbonates was prepared by dissolving the potassium sulfate, acidifing to pH 3 with sulfuric acid and boiling for one hour. Argon (99.99%) was then bubbled through this solution and Hepes (acid form) and EDTA (acid form) added. When cool, the solution was titrated to the appropriate pH with saturated sodium hydroxide which had been centrifuged to remove insoluble carbonates and adjusted to the final volume with boiled water. Centrifuged, saturated sodium hydroxide contains 8.7 mM carbonate [13]. The amount used to prepare the buffer would provide no more than 0.017 mM carbonates in the buffer. The concentration of inorganic carbon was estimated by the difference between determinations of total carbon and organic carbon using the Beckman Model 915B Carbon Analyzer. The values from these differences were smaller than the lower detection limit of 0.02 mM. The vesicles were characterized by freeze-fracture electron microscopy and by quasi-elastic light scattering. The Nicomp model 200 Laser Particle Sizer with a 5 mW Helium-Neon laser at 632.8 nm was used The data obtained using a channel width of 10, a viscosity of 0.9325 cP and index of refraction of 1.333 was consistent with a population of relatively homogeneous vesicles with a mean diameter of 120 nm (Fig. 1).

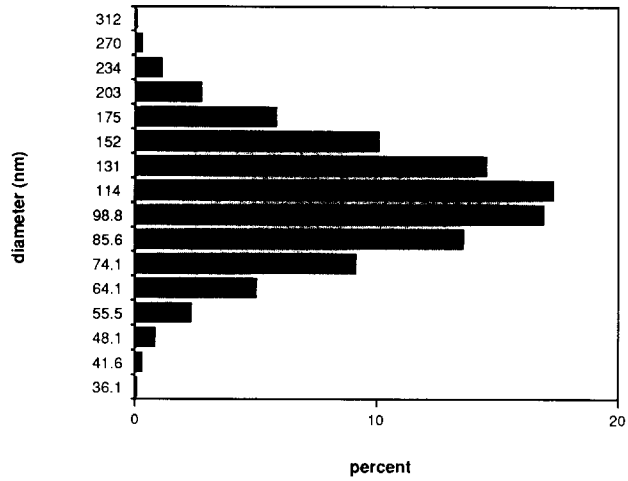

Fig. 1. Distribution and mean diameters of extruded DOPC vesicles. The percent of the total population of vesicles is shown as a function of the diameter (nm) of the vesicles.

Estimation of internal pH. After thermal equilibration, the pH was jumped by adding the desired volume (microliters) of 0.5 M sulfuric acid or KOH, and mixing by inversion. With excitation at 450 nm, the intensity of the emission at 520 nm was observed as a function of time using a Perkin-Elmer 650-40 fluorescence spectrophotometer equipped with a cell holder thermostated at 25° C. The digitized values (approx. 1800 values over 30 min) were saved to a floppy disc (Victor 9000 microcomputer with A/D converter built by the College of Sciences Electronic Shop) for later analysis. The 520 nm emission of pyranine upon excitation at 416 nm (isosbestic point) was measured at the end of each experiment in order to normalize the fluorescence data The ratio of the emission at 520 nm when excited at 450 nm (function of concentration of pyranine anion) and 416 nm (function of total pyranine present) was linearly correlated with pH (glass electrode) between 7 and 8 using nigericin to ensure equilibration of pH across the bilayer.

Theory

The net flux of protons through a membrane, J, can be expressed in terms of a pH gradient across the membrane:

$$J = B(R/3)\ \mathrm{dpH}/\mathrm{d}t = PG \qquad (1)$$

where: B is the buffer capacity; R is the radius of the vesicle ($R/3$ is the volume to area ratio for a sphere); the gradient, G, is the difference in proton concentration (molar) across the bilayer. If Eqn. 1 is rearranged and integrated, an expression is obtained which can be

evaluated readily over all or any portion of the experimental data set:

$$P = \frac{B(R/3)(\mathrm{pH}_{t2} - \mathrm{pH}_{t1})}{\int_{t_1}^{t_2} G \, dt} \quad (2)$$

where: $\mathrm{pH}_{t2} - \mathrm{pH}_{t1}$ is the change in the internal pH in the time interval between time t_2 and t_1. The integral in Eqn. 2 was numerically evaluated. The internal pH of the vesicles was determined by pyranine fluorescence. The external pH was measured with a pH electrode at the end of each experiment and due to the large excess of external buffer was considered constant following the pH jump. These values were then numerically integrated over subsets of data points, c.f. 100 data points at 1 s per point. Values for P were calculated using eqn. 2 and time intervals of 100 to 180 s (1 data point/s). This technique allows changes that occur in P during the course of the experiment to be detected.

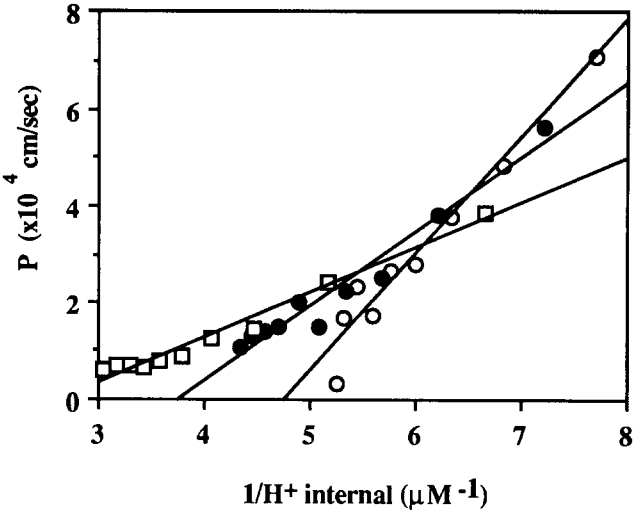

Fig. 3. Permeability coefficients as a function of reciprocal internal hydrogen ion concentration for acid jump conditions. DOPC vesicles were prepared in pH 8.0 buffer; microliters of 0.5 M sulfuric acid were added to obtain final pH values of 7.17 (□), 7.46 (●), and 7.62 (○).

Results

The model system used to study proton flux is shown schematically in Fig. 2. The DOPC vesicle was large and unilamellar with a relatively narrow size distribution (Fig. 1 and Ref. 11). Trapped within was the pH reporter group, pyranine [14], buffer (at pH 8.0 for an acid jump or at pH 7.2 for a base jump), and K_2SO_4 and valinomycin to abolish the electrical potential induced by the proton flux [1]. K_2SO_4 was used because chloride may increase proton movement via an electrically silent mechanism [18,19]. The proton flux was initiated by adding a few microliters of H_2SO_4 (acid jump) or by adding KOH for a base jump and the change in internal pH vs. time observed.

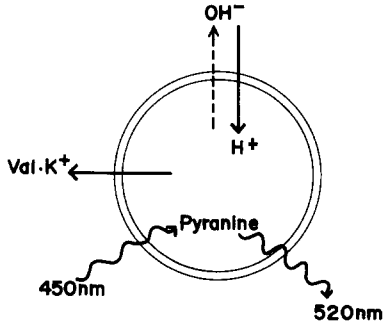

Fig. 2. Schematic diagram of the vesicle system used to measure proton flux. The dioleoyl phosphatidylcholine vesicle is represented by the circles. The influx of protons is represented by the arrow with the H^+ by the arrow head. The dashed line indicates that a flux of protons is indistinguishable from a counter flux of hydroxide ions. The arrow labeled valinomycin with K^+ by the arrow head indicates that the electrical effects of proton entry are compensated for by the counter movement of K^+. Thus the technique measures only the effects of hydrogen ion gradient. In the acid jump experiment shown, the interior was buffered at pH 8.0 and the exterior was adjusted to 7.2 to initiate the proton flux. Sufficient Hepes buffer was present to extend the time required for equilibration into a convenient range for measurement. The interior pH was obtained from the intensity of the fluorescence emission at 520 nm upon excitation of pyranine at 450 nm.

Kinetic analysis using Eqn. 2 for an acid jump indicated that the permeability coefficient, P, was not constant but decreased during the course of the experiment (Fig. 3). It was empirically determined that P for acid jump vesicles was, to a good approximation ($R > 0.98$), a linear function of the reciprocal of the internal proton concentration. The slope and intercept of these plots were also found to be dependent on the size of the acid jump (Fig. 3). However, analysis of experiments begun with a base jump yielded a constant P (Fig. 4). For base jumps, the value of P — while constant during the course of the experiment — was also a function of the size of the jump.

The key element common to the acid and the base jump experiments is the side of the vesicle with higher pH, i.e., the proton deficient side. For the base jump experiment the outside is at a lower pH and this pH is constant. For acid jump experiments, the inside is at a lower pH but for this experiment, the pH changes, equilibrating to the external pH. These properties are the hallmark of a carrier mechanism. Experiments were subsequently designed to identify a possible proton carrier.

Addition of 1–4 mol% of oleate to DOPC vesicles had only a small effect on the slope of graphs of P vs. $1/[H]_i$. Oleate was not detectable in the DOPC vesicles using high pressure liquid chromatography with flame ionization detection under conditions where as little as

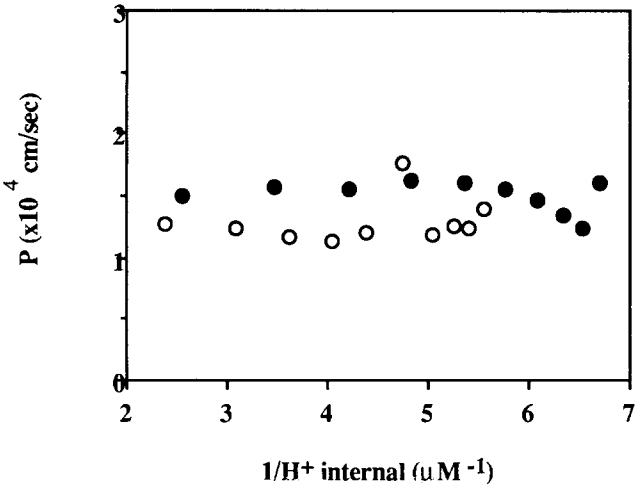

Fig. 4. Permeability coefficients as a function of reciprocol internal hydrogen ion concentration for base jump conditions. DOPC vesicles were prepared in pH 7.2 buffer; microliters of 0.5 M potassium hydroxide were added to obtain final pH values of 7.73 (○), and 7.82 (●).

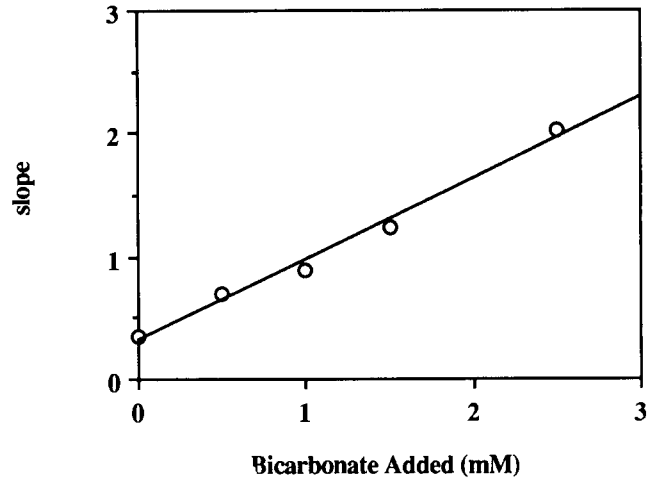

Fig. 6. Linear relationship between bicarbonate added and the slopes from the dependence of permeability coefficients on reciprocol internal hydrogen concentration for acid jumped vesicles. The slopes of the lines shown in Fig. 4 together with that from one additional experiment at a different bicarbonate concentration are graphed as a function of the bicarbonate concentration.

0.1% (weight fraction of the DOPC) free fatty acid would have been readily observable.

Laboratory solutions in equilibrum with air will contain carbon dioxide, the weak acid, carbonic acid, and, depending on the pH, appreciable concentrations of bicarbonate and carbonate. If techniques are used to limit the concentration of these carbonates (see Materials and Methods), and the above experiment conducted using conditions of an acid jump, lowest curve in Fig. 5 was obtained. The permeability coefficient in solutions with limited carbonates had the lowest slope observed for an acid jump experiment. The non-zero slope of the permeability coefficient seen at low bicarbonate concentrations in Fig. 5 could also he a consequence of the presence of the low concentration of carbonates, or of other (presently unidentified) carriers for protons in addition to passive flux.

Adding back known concentrations of bicarbonate and using the acid jump provided curves with increasing slope (Fig. 5), i.e., more rapidly changing permeability coefficients over the course of the experiment. If the slopes of these lines are graphed as a function of the concentration of the bicarbonate added, a linear curve with positive slope is obtained (Fig. 6). Extrapolation to zero slope gives a value for bicarbonate concentration

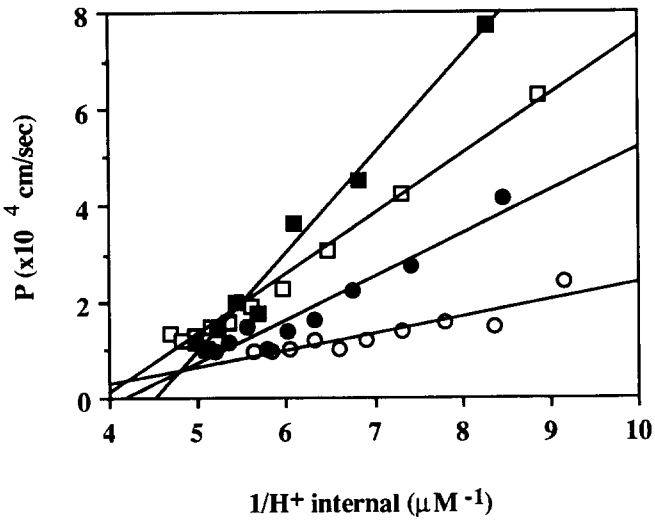

Fig. 5. The effect of bicarbonate on the apparent permeability coefficients as a function of the reciprocal internal hydrogen ion concentration for acid jump conditions. DOPC vesicles were prepared at pH 8.0 and 0.5 M sulfuric acid was added to obtain a pH of 7.4. Sodium bicarbonate was added to a final concentration of 0 mM (○), 1.0 mM (●), 1.5 mM (□), and 2.5 mM (■).

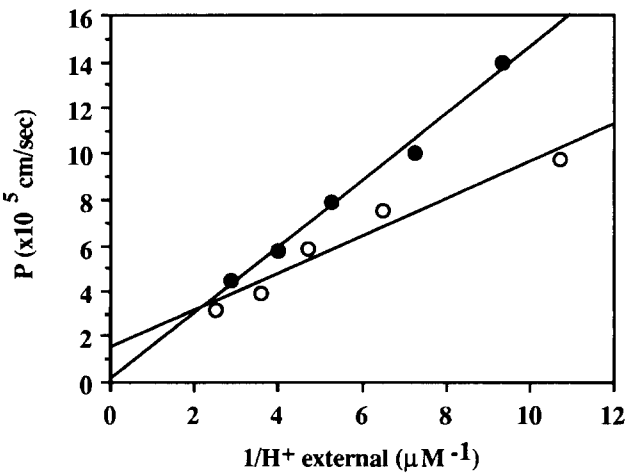

Fig. 7. The effect of bicarbonate on the apparent permeability coefficients as a function of the reciprocol internal hydrogen ion concentration for base jumped conditions. Vesicles were prepared at pH 7.1 and potassium hydroxide added to obtain the hydrogen ion concentrations of 0 mM (○) or 1.6 mM (●).

near 0.5 mM. However, this 0.5 mM concentration is greater than the measured inorganic carbon present in the solutions; moreover the experimentally measured slope was not zero even when carbonates were stringently removed (Fig. 5).

For base jump conditions, the permeability was constant for a given initial external pH (Fig. 4). However, if one graphs the permeability coefficient obtained against the reciprocal of the different external hydrogen ion concentrations, a linear relationship could be obtained (Fig. 7). Repeating this experiment in the presence of added bicarbonate also provided a linear relationship with increased slope (Fig. 7). This behavior is analogous to that observed for the acid jump in the presence of bicarbonate (Fig. 5).

Discussion

The permeability coefficient is the first order rate constant for the flux of permeants across a bilayer per unit concentration gradient:

$$P = J/G \tag{3}$$

This relationship is appropriate for neutral permeants and probably also holds for univalent cations including Na^+ and K^+. When the permeability of protons has been estimated using this relationship, the permeability coefficient was found to be at least eight orders of magnitude larger than for Na^+ and K^+ ions [1–5,7,8,10] which implied that protons do not cross the bilayer by the same mechanism as do other univalent cations.

For the data shown above, the average proton permeability coefficients over the course of the experiment were of the same order of magnitude as those reported using earlier techniques [1–5,7,8,10]. However, procedures we have developed clearly demonstrate that the apparent permeability coefficient was not constant but changed as a function of pH during the course of the experiment, c.f. correlations between the permeability coefficient and $1/[H^+]$ are apparent from the graphical treatment shown above (Figs. 3–5 and 7).

For the two variations of the proton permeability experiments, the base jump and the acid jump, markedly different results were obtained. When the vesicles were prepared in pH 7.2 and the exterior pH altered with KOH to initiate the measurement (base jump), the permeability coefficient was constant during the course of an experiment (Fig. 4). Conversely, when vesicles prepared in pH 8.0 buffer and the external pH was altered by the addition of H_2SO_4 (acid jump), the permeability decreased linearly with the reciprocal of the interior proton concentration ($1/[H]_i$) (Fig. 3). This complex behavior was unexpected for a simple diffusion mechanism and suggested the participation of carriers for protons.

Carbonic acid (or carbon dioxide) was suggested as a carrier of protons and addition of millimolar concentrations of sodium bicarbonate had marked effects on the observed permeabilities. For the acid jump, the slope of the line for the graph of the permeability coefficient vs. the reciprocal of internal hydrogen ion concentration was directly proportional to the amount of bicarbonate added to the vesicle preparation (Fig. 5). Furthermore, for the base jump case, the slope of the line for the permeability coefficient vs. the reciprocal of the external hydrogen ion concentration was shown to increase after addition of bicarbonate (Fig. 7). These results strongly suggest that the carbonates were important participants in the reported measurements shown above (and probably in measurements made earlier by others). By carbonates we mean carbonic acid, carbon dioxide and bicarbonate; carbonate ($pK_2 = 10.36$) is assumed to be present in negligible concentrations in the pH ranges studied. The differences between the acid jump and the base jump measurements also imply that the rate determining step of the carrier-mediated transport occurs on the side of the bilayer which has the higher pH.

The results obtained in the presence of bicarbonate can be described by the following hypothesis. Protons can he transported across bilayers as carbonic acid ($pK_1 = 6.37$). Alternatively (and indistinguishably using these techniques), carbon dioxide can diffuse across the bilayer forming its hydrate, carbonic acid. We will use the term 'carbonic acid' to refer to either carbon dioxide or its hydrate in the following discussion. Flux of carbonic acid is assumed to be much faster than the second process and also faster than the flux of bicarbonate ion. When the pH is jumped in the presence of the carbonates (predominantly CO_2, H_2CO_3 and HCO_3^- in this pH range), a gradient of carbonic acid across the vesicle bilayer is also created. But because the flux of carbonic acid is fast, the carbonic acid concentration equilibrates across the bilayer relatively quickly. In the case of the acid jump (Fig. 8A), the influx of carbonic acid also increases the bicarbonate concentration within the vesicle (interior alkaline). Because of the presence of buffer within the vesicle, and the slow flux rate of bicarbonate, the bicarbonate concentration within the vesicle soon greatly exceeds the external bicarbonate concentration. Once the carbonic acid concentration has equilibrated across the bilayer, for additional protons to enter the vesicle as carbonic acid, bicarbonate must leak from the vesicle. In the acid jump, after the short time required for the initial equilibration of carbonic acid, the apparent proton flux is actually the sum of the passive proton flux and the proton flux resulting from the flux of bicarbonate. This bicarbonate flux is proportional to the concentration of internal bicarbonate and thus a function of the internal pH. Hence, the apparent measured proton permeability will change during the course of the experiment as a

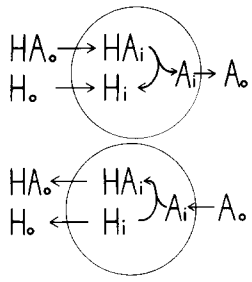

Fig. 8. Mechanism of proton flux catalyzed by carbonic acid and bicarbonate. (A, top) The process envisioned for an acid jump at steady state is shown schematically. The circle represents the vesicle bilayer and carbonic acid, HA_o and protons, H_o, are shown entering the vesicle from the outside (subscript 'o') in response to conditions of the H^+ gradient, excess outside. The influx of HA is fast relative to the passive influx of H^+ and faster than the efflux of bicarbonate, A_i, available from dissociation of HA_i within the vesicle as indicated by the arrows. Thus the internal concentration of bicarbonate, A_i, exceeds the external concentration, A_o, but equilibration is retarded by the relatively low permeability of bicarbonate. The measured rate of proton influx is assumed to come primarily from movement of HA into the vesicle rather than passive H^+ flux and this HA movement is linked to the rate limiting loss of bicarbonate, A_i. The linkage comes from the equilibration between HA_i, A_i, and H_i as shown as the entering HA further increases the A_i concentration within the vesicle. In this experiment, the apparent permeability coefficient will be proportional to the internal pH which controls the relative concentrations of HA_i and rate limiting A_i. (B, bottom) The process envisioned for a base jump at steady state is shown where the efflux of protons is again limited by the flux of bicarbonate, A. The measured proton flux is the flux for HA across the bilayer rather than through passive H flux (lower arrow). In this case, HA_i at steady state is available only from the influx of A, bicarbonate, which then is rate limiting. The apparent permeability coefficient will be proportional to the external pH which controls the concentration of A_o, the rate limiting bicarbonate concentration. Because carbonic acid is formed by hydration of carbon dioxide, the rapid transport of carbonic acid, HA shown above could be accomplished with similar facility by the diffusion of CO_2 and subsequent hydration.

function of the internal pH with the permeability coefficient decreasing as the internal pH decreases. Moreover, the internal bicarbonate concentration changes as a function of the size of the jump, which sets the internal bicarbonate concentration.

These same processes, during a base jump, yield different results (Figs. 4 and 8B). The efflux of carbonic acid is fast, quickly depleting the carbonates within the vesicle (the interior pH is more acidic) until the carbonic acid concentration approaches the exterior carbonic acid concentration. The carbonic acid gradient decreases and the bicarbonate gradient increases until the carbonic acid efflux is limited by the bicarbonate influx. Because the external pH is constant during the experiment, and the external bicarbonate concentration is constant, the rate of bicarbonate influx will be constant as long as the flux is directly proportional to the bicarbonate gradient. The apparent proton flux will again be the rate of bicarbonate influx (plus the passive proton flux). This results in an apparent permeability coefficient that is a constant for a given external pH (Fig. 4). Because the internal concentration of bicarbonate during the steady state condition is that remaining after the initial loss of carbonic acid, the internal bicarbonate concentration is a function of the size of the jump. The apparent proton permeability coefficient will then be a function of the size of the jump, i.e., the external pH for that experiment. Clearly for weak acids $[A^-] = [HA]\ K/[H^+]$ which may be the basis for the observed correlation between the permeability coefficient and $1/[H^+]$.

The above hypothesis makes several assumptions which have not been tested: the relative permeabilities of carbonic acid, carbon dioxide, and of bicarbonate through bilayers, to our knowledge, have not been described. The assumptions of rapid carbonic acid or carbon dioxide permeability and slower bicarbonate permeability seem plausible. Addition of a weak acid like acetic acid to our system quickly equilibrates protons across these vesicles and gives permeability as a function of $1/[H^+]$ for acid jump conditions. If the flux of protons measured for an acid jump were truly limited by the bicarbonate flux in the presence of 3.2 mM added bicarbonate (Fig. 5), a permeability for bicarbonate of $1 \cdot 10^{-7}$ cm/s would limit the observed proton flux to the observed values. This permeability about three orders of magnitude greater than the value of $5.5 \cdot 10^{-11}$ cm/s for for chloride [15,18,19]. We do not know the concentrations of bicarbonate, CO_2 and carbonic acid within vesicles even when we have deliberately added bicarbonate, nor can we currently assess the changes in these concentrations within vesicles during the experiment. The most convincing way to test the above hypothesis would be direct kinetic measurement of concentrations of bicarbonate, CO_2 and/or carbonic acid within vesicles but such methodology is not currently available.

Even though the bicarbonate/CO_2 carbonic acid system provides the fastest pathway for proton flux we have observed, these results do not rule out the existence of other mechanisms and/or carriers which may be significant for proton flux [16]. Systematic measurements of permeability coefficients as a function of bicarbonate gave a value near $1 \cdot 10^{-4}$ cm/s for the lowest bicarbonate concentration achieved in our current experiments (Fig. 5). Accordingly, we suggest that the passive proton permeability is no larger than the above value and may be within an order of magnitude of the lowest average permeability coefficient reported here. Certainly additional experiments using additional special precautions to eliminate carbonates or other weak acids from the system would continue to be of interest.

Bicarbonate and carbonic acid are not only ubiquitous in laboratory solutions but are significant components in living organisms. The observations given here demonstrate that carbonic acid and/or carbon dioxide are also facile proton carriers. Proton gradients

are particularly important across the inner mitochondrial membrane for the synthesis of ATP [20]. Decarboxylation of di- and tricarboxylic acids in the Krebs cycle and the β-oxidation of fatty acids within the matrix of the mitochondria also provide carbon dioxide [21]. Either the kinetics of proton gradient formation and its utilization must be rapid compared with the flux rates observed in the presence of carbonates or mechanisms must be present which are designed to limit the concentration of carbonates at the surface of the inner mitochondrial membrane where millimolar concentrations of this carrier could dissipate the proton gradient. One simple mechanism which comes to mind is simply the presence of acidic phospholipids which confer a negative charge to the inner mitochondrial membrane [22]. A negative surface charge would further decrease the movement of a negatively charged carrier across this barrier. Additional mechanisms may include bicarbonate transport systems, carbon dioxide fixing reactions and efficient removal of carbonic acid and carbon dioxide from the local environment.

Acknowledgements

We want to thank Dr. Thomas Madden, The Canadian Liposome Co., Ltd. Vancouver, BC for providing a size distribution for these vesicles using quasi elastic light scattering and Dr. Sek Wen Hui, Roswell Park Memorial Institute, Buffalo, NY and his laboratory for preparing freeze-etch electron micrographs of samples of our vesicle preparations for size and homogeneity studies. Dr. Walter Shaw, Avanti Polar Lipids, Inc., Alabaster, AL kindly provided analyses of the fatty acid content of our DOPC vesicles and Dr. John J. Hains, Biological Sciences, Clemson University analyzed our buffers for total and organic carbon content. Dr. Dorota A. Abramovitch provided valuable comments during the progress of this work. We are indebted to Prof. D. Deamer, University of California at Davis, for his comments on an earlier version of this manuscript and for suggesting to us that carbonates may be important carriers for protons. This research was supported by the US Public Health Service (HL 38190).

References

1 Nichols, J.W. and Deamer, D.W. (1980) Proc. Natl. Acad. Sci. USA 78, 4324–4328.
2 Rosignol, M., Thomas, P. and Grignon, C. (1982) Biochim. Biophys. Acta 684, 194–199.
3 Elamrani, K. and Blume, A. (1983) Biochim. Biophys. Acta 727, 22–30.
4 Nichols, J.W. and Deamer, D.W. (1983) Proc. Natl. Acad. Sci. USA 80, 165–168.
5 Gutknecht, J. (1984) J. Membr. Biol. 82, 105–112.
6 Perkins, W.R. and Cafiso, D.S. (1986) Biochemistry 25, 2270–2276.
7 Gutknecht, J. (1987) Biochim. Biophys. Acta 898, 97–108.
8 Gutknecht, J. (1987) J. Bioenerg. Biomembr. 19, 427–442.
9 Perkins, W.R. and Cafiso, D.S. (1987) J. Bioenerg. Biomembr. 19, 443–455.
10 Deamer, D.W. (1987) J. Bioenerg. Biomembr. 19, 457–479.
11 Mayer, L.D., Hope, M.J., and Cullis, P.R. (1986) Biochim. Biophys. Acta 858, 161–168.
12 Lowry, R.R. and Tinsley, I.J. (1974) Lipids 9, 491–492.
13 Allen, N. and Low, G.W. (1933) Ind. Eng. Chem. Anal. Edn. 5, 192–195.
14 Kano, K. and Fendler, J.H. (1978) Biochim. Biophys. Acta 509, 289–299.
15 Jain, M.K. and Wagner, R.C. (1980) Introduction to Biological Membranes, p. 134, J. Wiley and Sons, New York.
16 Nage, J.F. (1987) J. Bioenerg. Biomembr. 19, 413–426.
17 Haydon, D.A. (1970) in Membranes and Ion Transport (Bittar, E.E., ed.), pp. 64–92, Wiley-Interscience, New York.
18 Toyoshima, Y. and Thompson, T.E. (1975) Biochemistry 14, 1518–1524.
19 Toyoshima, Y. and Thompson, T.E. (1975) Biochemistry 14, 1525–1531.
20 Hatefi, Y. (1985) Annu. Rev. Biochem. 54, 1015–1069.
21 Rawn, J.D. (1989) Biochemistry, p. 341, Neil Patterson, Carolina Biological Supply Co., Burlington, NC.
22 Daum, G. (1985) Biochim. Biophys. Acta 822, 1–42.