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Faster-than-anticipated Na^+/Cl^- diffusion across lipid bilayers in vesicles

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ABSTRACT

Maintenance of electrochemical potential gradients across lipid membranes is critical for signal transduction and energy generation in biological systems. However, because ions with widely varying membrane permeabilities all contribute to the electrostatic potential, it can be difficult to measure the influence of diffusion of a single ion type across the bilayer. To understand the electrodiffusion of H^+ across lipid bilayers, we used a pH-sensitive fluorophore to monitor the lumenal pH in vesicles after a stepwise change in the bulk pH. In vesicles containing the ion channel gramicidin, the lumenal pH rapidly approached the external pH. In contrast, the lumen of intact vesicles showed a two stage pH response: an initial rapid change occurred over ~1 min, followed by a much slower change over ~24 h. We provide a quantitative interpretation of these results based on the Goldman–Hodgkin–Katz ion fluxes discharging the electrical capacitance of the bilayer membrane. This interpretation provides an estimate of the permeability of the membranes to Na^+ and Cl^- ions of ~10^{-8} cm/s, which is ~3 orders of magnitude faster than previous reports. We discuss possible mechanisms to account for this considerably higher permeability in vesicle membranes.

1. Introduction

Maintenance of electrochemical potential gradients across lipid membranes is critical for signal transduction and energy generation in biological systems. The lipid bilayer enables formation of these potential gradients by serving as a highly insulating barrier. However, extensive studies have shown ions do slowly diffuse across the lipid bilayer [1]. Using the pH-dependent partitioning and concentration quenching of the fluorescent probe 9-aminoacridine, Deamer and Nichols were able to show that the permeability of lipid membranes is orders of magnitude higher for protons than for other ions [2–5]. Since then, acidity inside vesicles has been measured more directly from the fluorescence of impermeable dyes such as pyranine [6–8] and BCECF [9]. The following picture emerges from these studies. First, the diffusion of protons arising from a pH gradient quickly leads to the formation of an electrical gradient across the membrane. This electrical gradient can be removed by adding an ionophore such as valinomycin. Without such ionophores, the electrical gradient persists and makes additional flow of protons into the lumen energetically unfavorable. Subsequently, this electrical gradient diminishes by diffusion of larger, less permeable ions such as Na^+ and Cl^-. However, previous experiments have not quantitatively measured this Na^+/Cl^- driven change in the context of electrochemical gradients. To quantify its effect, we followed the pH change after a stepwise change in bulk pH over a time course of several hours. We offer a quantitative interpretation of these observations, based on the Goldman–Hodgkin–Katz flux equations and the electrical capacitance of the lipid bilayer membrane. The results suggest that the vesicle membranes could be considerably more permeable for some of the other ions beside protons, compared with what has been deduced from other experiments. We discuss possible mechanisms to account for this faster than expected diffusion.

2. Materials & methods

2.1. Vesicle preparation and measurement of lumenal pH

Vesicles were prepared by extrusion as previously described [10]. Briefly, 50 μL of 50 mg/mL solution of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine in chloroform (POPC, Avanti Polar Lipids, AL, USA) was dried in a glass vial by a stream of nitrogen, and kept in a vacuum for 1 h. The lipids were then rehydrated by vortexing with 1 mL phosphate buffered saline (0.10 M sodium phosphate, 0.15 M sodium chloride, pH 7.2) and 10 μM trisodium 8-hydroxypyrene-1,3,6-trisulfonate (referred to as pyranine; Invitrogen, OR, USA), as a convenient and sensitive probe for pH [6,7]. A concentration of 10 μM pyranine avoids concentration quenching and the inner filter effect. After 15 min, the rehydrated lipid dispersion was extruded eighteen times through two 200 nm pore size track etched polycarbonate membranes, resulting in ~200 nm diameter vesicles, as confirmed using dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments Ltd, UK). Assuming that the pyranine is uniformly distributed in the solution,

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and not preferentially included in or excluded from the liposomes, there will be ~25 pyranine molecules in a vesicle. Based on an area per lipid of ~0.6 nm^2, and assuming that all the lipids end up in vesicle bilayers, we estimate that the vesicles enclose about 8% of the volume of the solution at this point.

To render the liposomes porous to ions and bring down the transmembrane potential, in some experiments 0.19 μg of the ion channel gramicidin A (Enzo Life Sciences, NY, USA) was added to the lipid mixture prior to drying. Assuming that all the gramicidin is incorporated in the lipid bilayer, the 2.5 mg of 760 g/mol POPC and 0.19 μg of 1882 g/mol gramicidin A result in a molar ratio of 1:33,000 gramicidin:POPC molecules within each liposome, or approximately 3 gramicidin monomers per liposome.

Vesicles containing pyranine were separated from external aqueous pyranine by size exclusion chromatography using a Sepharose CL-6B matrix on a separation column 10 mm wide and 6 cm tall, loaded with 100 μL of the vesicle suspension, and eluted with buffer. Fractions of 1 mL were collected, and analyzed using a spectrofluorometer (FluoroMax-4, Horiba Jobin-Yvon) to select the vesicle fractions. The elution volume of the vesicles (~5 mL) was well-separated from the elution volume of free pyranine (beyond 9 mL). Fluorescence from the pooled vesicle fractions was collected using a 2 mL, 10 mm path length cuvette which was magnetically stirred continuously in a thermostated holder kept at 25 °C. An excitation spectrum was recorded every 30 s, with detector wavelength set to 510 nm and slit widths for excitation and collection of 5 and 10 nm, respectively. The pH was changed stepwise by adding microliter volumes of 1 N hydrochloric acid and sodium hydroxide.

The pH in the lumen of the vesicles was determined from the fluorescence excitation spectra of the pyranine. The excitation spectra as a function of pH, $S(\lambda,\mathrm{pH})$, consist of two independent contributions, due to pyranine and due to its deprotonated conjugate base: $S(\lambda,\mathrm{pH}) = S_{\mathrm{acid}}(\lambda)\cdot c_{\mathrm{acid}}(\mathrm{pH}) + S_{\mathrm{base}}(\lambda)\cdot c_{\mathrm{base}}(\mathrm{pH})$. The component spectra were determined from excitation spectra of free pyranine measured in phosphate buffers at various known pH (Fig. S1, Supplementary material). The pH dependence of the percentage of base in these spectra fits the Henderson–Hasselbalch equation, as expected, and we find $\mathrm{pK_a} \sim 7.33$, close to the 7.2 obtained by Clement & Gould [11–13]. The pH in the vesicle lumen is thus determined ratiometrically, making it insensitive from variations in excitation intensity, dye loading, or vesicle concentration.

2.2. Modeling the luminal pH using the Goldman–Hodgkin–Katz (GHK) flux equation

To model the pH time response, we estimate the ion fluxes using the Goldman–Hodgkin–Katz (GHK) flux equation for the current density Φ_S across a lipid membrane due to ions of type S:

$$\Phi_S = Pz_S F\frac{z_S F\,V}{RT}\frac{[S]_{\mathrm{in}} - [S]_{\mathrm{out}}e^{\frac{-zFV}{RT}}}{1-e^{\frac{-zFV}{RT}}}, \tag{1}$$

where P_S is the membrane permeability of ion type S, z_S is its valence ($+1$ or -1), $F = eN_A$ is the Faraday constant, $R = kN_A$ is the molar gas constant, T is the temperature, and $[S]_{\mathrm{in}}$ and $[S]_{\mathrm{out}}$ are the ion concentrations inside and outside the vesicle. The current density Φ is positive for outward flow, and V is the electrostatic potential difference across the vesicle membrane. For small V, the denominator cancels the zFV/RT prefactor and $\Phi \cong PzF([S]_{\mathrm{in}} - [S]_{\mathrm{out}})$, so excess positive ions inside indeed give rise to a positive outward current; for large $|V|$, the current density is $\Phi \cong PzF(zFV/RT)[S]$, where $[S] = [S]_{\mathrm{in}}$ when $zV > 0$ and $[S] = [S]_{\mathrm{out}}$ when $zV < 0$, i.e., the polarity of V selects the relevant ion concentration (inside or outside). To estimate the GHK fluxes, we have varied permeabilities P of Na^+ and Cl^-, but kept their ratio fixed at $P_{Na^+} : P_{Cl^-} = 10^{-12}$ cm/s : $7\cdot 10^{-12}$ cm/s [14].

The GHK fluxes lead to changes in the electric charge Q_S of a vesicle due to ions of type S:

$$\frac{d}{dt}Q_S = -A\Phi_S \tag{2}$$

where A is the surface area of a vesicle. The charges result in a potential across the bilayer,

$$V = \frac{Q}{C} = \frac{\sum_S Q_S}{C} = \frac{Q_{Na^+} + Q_{Cl^-} + Q_{H^+}}{C}, \tag{3}$$

where $C = cA$ is the electrical capacitance of a vesicle, mainly due to the areal capacitance of its lipid bilayer membrane, $c \sim 1$ μF/cm^2 [15].

The GHK ion fluxes also change the ion concentrations inside the vesicles. For unbuffered ions like Na^+ and Cl^-,

$$[S]_{\mathrm{in}} = [S]_{\mathrm{in},t=0} + \frac{Q_S}{z_S F\mathcal{V}}, \tag{4}$$

where $\mathcal{V}$ is the volume of a vesicle. We consider the outside concentrations to remain essentially unaffected, since the volume of fluid outside the vesicles is so much larger. In contrast with the unbuffered Na^+ and Cl^-, for buffered $[H^+]$ the buffer capacity B needs to be taken into account [16]:

$$\frac{d\mathrm{pH}}{dt} = \frac{1}{B}\frac{A}{\mathcal{V}}\frac{\Phi_{H^+}}{z_{H^+}F}, \quad \text{with} \quad B = \frac{\partial n_{\mathrm{acid}}}{\partial \mathrm{pH}} = \frac{\mathcal{P}K[H^+]}{(K+[H^+])^2}\ln(10), \tag{5}$$

where $\mathcal{P}$ is the total concentration of buffer, $\mathcal{P} = [H_2PO_4^-] + \left[HPO_4^{2-}\right] = 100$ mM, K is its equilibrium constant $K_a = [H^+][HPO_4^{2-}]/[H_2PO_4^-]$, and $\mathrm{p}K_a = -\log_{10}(K_a/[\mathrm{mol/L}]) = 7.20$ for dihydrogen phosphate. This ignores contributions due to the dissociation of hydrogen phosphate or phosphoric acid and due to the dissociation of water, since these only occur at pHs more than 5 units removed from the pH in our experiment.

The expressions for dQ/dt and $d\mathrm{pH}/dt$, together with those for the GHK fluxes and the electrostatic potential V, form a closed system of ordinary differential equations that we solved numerically using Matlab's ode45 solver, with initial conditions $[Na^+] = [Cl^-] = 150$ mM, $Q_{H^+} = 0$, and $\mathrm{pH} = 7.1$.

The model can be simplified if we consider that the bilayer is thought to be much more permeable to H^+ than to the other ions [1,4,17,18]. Thus, one would expect the H^+ flux Φ_{H^+} to quickly subside. According to the GHK equation, the potential then follows directly from the pH gradient across the bilayer alone. The change in electrical charge dQ_{H^+}/dt due to H^+ flow is related to the change in pH via the buffer capacity B; the pH relates to voltage via the GHK equation as just explained, and the voltage is related to the net charge Q, leading to

$$\frac{d}{dt}Q_{H^+} = -z_{H^+}F\mathcal{V}B\frac{z_{H^+}F}{RT\ln(10)}\frac{1}{C}\frac{dQ}{dt} \equiv -\zeta\frac{dQ}{dt}, \tag{6}$$

where we defined a dimensionless number ζ that gauges whether the electrical charge of one pH unit of buffer capacity $B\mathcal{V}$ leads to an appreciable potential compared with kT. In our case, $\zeta \gg 1$. Substituting the expression for Q above and solving for dQ_{H^+}/dt, we find that

$$C\frac{dV}{dt} = \frac{1}{1+\zeta}\frac{d}{dt}(Q_{Na^+} + Q_{Cl^-}). \tag{7}$$

This removes dQ_{H^+}/dt from the equations, and expressing $d\mathrm{pH}/dt$ via dV/dt, it eliminates one degree of freedom. Solving this reduced set of equations yielded identical results as solving the full set when P_{H^+} was large, confirming the accuracy of the numerical method.

3. Results and discussion

3.1. The time-dependent pH response of the vesicle lumen suggests a higher than expected Na^+ and Cl^- flux across lipid bilayers

Fig. 1 outlines our experiment. Pyranine encapsulated inside the vesicles with or without gramicidin reveals the pH of the lumen. The vesicles are prepared in phosphate buffer at pH 7.1, and HCl is added to step the pH down. The pH inside the vesicles is then determined from fluorescence excitation spectra of the pyranine within. The measurements of pH changing over time are shown in Fig. 2. When gramicidin is present in the vesicles, a change of lumen pH quickly ensues, and the change is almost complete in less than a minute. These observations are in agreement with other literature reports [19]. These observations indicate that gramicidin makes the bilayer permeable to other ions, which lowers the electrical gradient arising from diffusion of protons arising from the pH gradient. Gramicidin's lowering of electrical gradient then allows the pH in the lumen to rapidly reach equilibrium with the external solution (Fig. 1B). In contrast, in the vesicles without gramicidin, the pH does not change much initially, and takes many hours to decrease. In fact it can be argued that the true contrast is even more pronounced than shown in Fig. 2, since the gramicidin concentration in the 'with gramicidin' vesicles is not high: part of the 'with gramicidin' vesicle population actually does not contain gramicidin. If we assume that all the supplied gramicidin is incorporated in vesicle membranes, then there would be $n \sim 3$ gramicidin monomers in a single vesicle. Presumably the incorporation of gramicidin follows a Poisson distribution, so the probability of encountering a vesicle with less than two gramicidins would be $(1+n)\exp(-n)$. For $n \sim 3$, about 20% of the vesicles would not have any gramicidin dimers. The 'with gramicidin' curve in Fig. 2 thus would contain a 20% contribution of 'without gramicidin' result. As a consequence, the 'with gramicidin' pH curve, essentially an intensity ratio, would be expected to follow the 'without gramicidin' time course. This is indeed the case: Fig. 2 shows a copy of the 'without gramicidin' curve, offset vertically, in yellow; the yellow and red curves in the plot do indeed overlap.

In these experiments, gramicidin was added preceding the extrusion of the vesicles. We have also tried adding gramicidin during the experiment. To this end, the gramicidin was dissolved in ethanol and 10 μL of this solution was added to the 2 mL cuvette. This did collapse the pH at once. However in control experiments, it appears that adding ethanol without gramicidin has a similar effect. This suggests that even a small amount of ethanol can be disruptive, at least for our POPC lipid bilayers, and contrasts with the experience of Clement and Gould using liposomes composed of asolectin [19].

Without gramicidin, the initial change in pH is less than 0.1 pH unit (Fig. 2). This observation is consistent with a much higher permeability of H^+ than that of the other ions Na^+ and Cl^-, leading to formation of an electrical gradient across the vesicle membrane due to the separation of H^+ from its counter ions remaining outside. This electrostatic potential prevents further influx of H^+ and the pH change in the vesicle lumen halts. The actual charging time is shorter than the time between spectra acquired by our spectrometer (30 s); the mixing time in our magnetically stirred 2 mL cuvette is probably considerably shorter than 30 s.

After the H^+-driven initial charging of the vesicles, the pH change is determined by the flux of other small ions, Na^+ and Cl^-. Our data provide an opportunity to quantitatively model these fluxes. The initial concentrations of these ions are accurately known and serve as input to the numerical model. Likewise, the initial and final pHs are set to match the pH in the experiment, and the extra chloride outside due to added acid is accounted for. This leaves the permeability of Na^+ and Cl^- as the only remaining parameter that we have adjusted. The results of this numerical model are plotted in Fig. 2, together with the observed pH time course. The figure shows that the model captures the qualitative features of the pH change over time well. However, the permeabilities that we find in this way are higher than literature permeabilities P, measured using radioactive tracers, of 10^{-12} cm/s for Na^+, and $7 \cdot 10^{-12}$ cm/s for Cl^- [14] by a factor 1500, i.e., up to 10^{-8} cm/s. Similar results are obtained by then adding NaOH. Our result is further confirmed by an order-of-magnitude estimate of the initial rate of change of the pH in our model:

$$\frac{d\mathrm{pH}}{dt} \cong \frac{AP}{B\mathcal{V}}[\mathrm{NaCl}]. \tag{8}$$

This estimate contains fewer parameters than the full GHK model (Eqs. (1)–(5)), and all the parameters in Eq. (8) (vesicle diameter $d = \mathcal{V}/(6A)$, buffer capacity B, and concentration of salt) are known with good accuracy. Again, the initial slope of the graph in Fig. 2 can only be accounted for when the permeability P of the membrane is increased by a factor of ~1500. This analysis indicates that the higher than expected permeability is observed throughout the entire experiment and is not a result of a change in the vesicles long after the start of the experiment.

Changing the permeability amounts to rescaling the time coordinate, since only the permeability has the dimension of time. The high

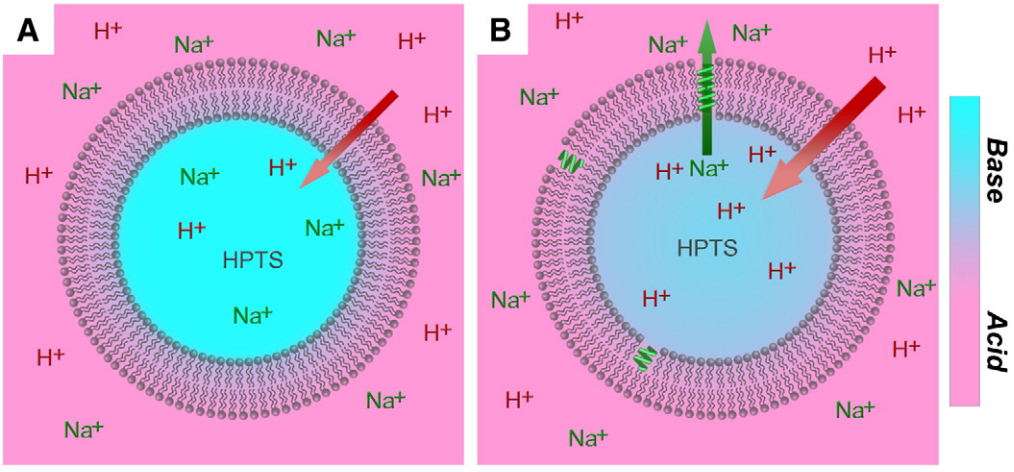

Fig. 1. Illustration of ion fluxes across a vesicle wall after adding HCl outside, for vesicles extruded without (A) and with (B) gramicidin. The gramicidin monomers are symbolized as green coils. The pH gradient is represented by the color transition from pink to blue. Pyranine dye senses the pH in the vesicle lumen. In (A), the influx of H^+ has charged the vesicle, preventing further entry of protons. In (B), the gramicidin dimers make the bilayer permeable to other ions, thus averting the electrical charging, so the pH inside and outside can equilibrate more quickly.

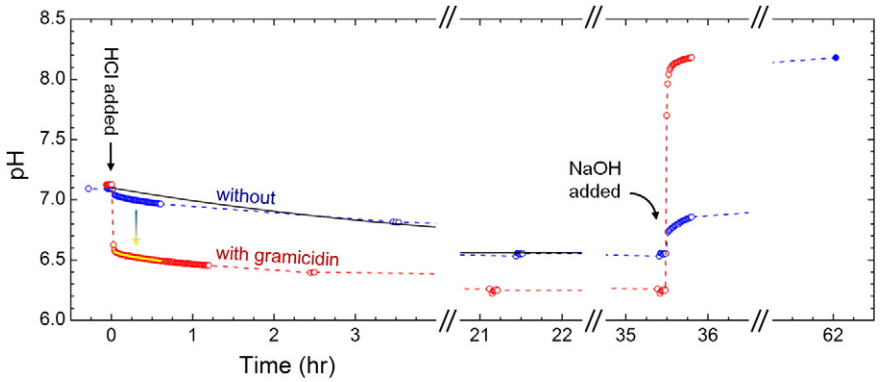

Fig. 2. Lumenal pH as a function of time in vesicles with (red symbols) and without (blue symbols) gramicidin; the dashed curves are a guide to the eye. The pH is deduced from pyranine in the vesicle lumen. First HCl and later NaOH are added. The 'with gramicidin' vesicle population (red curve) contains some vesicles without gramicidin, hence the red trace and blue trace follow a similar time evolution, i.e., the blue trace can be made to overlap the red by vertical displacement (shown in yellow). The solid curve shows a numerical model of the GHK ion fluxes electrically charging the vesicle membrane capacitance, taking into account buffer capacity, for permeabilities $P_{Na^+} = 1500 \cdot 10^{-12}$ cm/s and $P_{Cl^-} = 1500 \cdot 7 \cdot 10^{-12}$ cm/s.

permeability of ~10^{-8} cm/s that we find makes the pH equilibrate in the course of a day or so; with the conventional low permeabilities, the pH would change on much longer time scales, staying essentially unaltered over our observation period.

3.2. A high Na^+/Cl^- permeability is the most likely cause of the observed pH response

The low permeability of lipid bilayer membranes on the order of 10^{-11} cm/s has been well documented [14]. However, the quantitative modeling of the short and long time pH changes of membranes (Fig. 2) indicates that the actual permeabilities in our experiment are much higher. One potential reason for the deduced high permeability is that there are additional ion fluxes across the bilayer that we did not take into account. Possible unaccounted fluxes include leakage of the pyranine itself across the bilayer or rupture of the vesicles. To test for these possibilities, we added a base one day after the initial acid-driven pH step. As shown in Fig. 2, the gramicidin containing vesicles immediately show a large change in pH. In contrast, the vesicles without gramicidin take a long time to arrive at the final pH. If free pyranine was present in the bulk solution of the vesicles without gramicidin, we should observe an instantaneous change in pH induced by adding NaOH. The observations in Fig. 2 rule this possibility out, supporting a previous report that estimated the leakage rate of pyranine to be less that 1% per day.[6] Additionally, the absence of a fast change in observed pH in the vesicles without gramicidin after adding a base shows that the vesicles do not rupture. Thus, the high permeability cannot be explained by unaccounted-for fluxes.

One more option is to call the applicability of the GHK flux equations into question. The GHK flux equation is derived assuming that the lipid bilayer is a homogeneous dielectric, that the electric field is constant across the interior, and that the ions do not interact. This may not be accurate. In experiments, it has been observed that proton conductance is surprisingly constant as pH is varied [4,17]. Then again, the magnitude of the H^+ flux is not of primary importance as an ingredient in the simple model presented, since the proton permeability is so large compared with the permeability of other ions. The main consequence of the GHK proton flux is to link the potential difference to the pH difference across the membrane via the Nernst equation, and this has solid thermodynamic underpinnings. In support of our model, GHK flux equations do accurately describe sodium fluxes, suggesting that a higher than expected permeability is responsible; the flux equations still apply, but with a higher permeability coefficient. A likewise higher than expected permeability for K^+ and DOPC has been observed by Kuyper et al., but this was attributed to strain and higher tension in the vesicles adsorbed to glass.[20] In contrast, our vesicles were freely suspended. The vesicle extrusion method of preparation is well-established, and we have successfully used similar vesicle preparations to form high-impedance free-standing lipid membranes before [10]. We would be surprised to find that this state-of-the-art preparation method is deficient and if true, we think this would be an interesting finding in itself. The anticipated small permeability of large ions such as Na^+ is usually attributed to the high energy barrier of dehydration required to enter into the hydrophobic core of the lipid bilayer. However, there are indications that the ionic distribution near the water–lipid interface is much more subtle, with preferential binding of Na^+ to the lipid head groups, while Cl^- does not directly interact with the lipids [21]. Moreover, in an interesting recent paper Vorobyov et al. suggested that ion permeation might be determined by the energetics of membrane deformation, rather than hydration [22].

It would be desirable to determine the nature of the unexpectedly large ion flux, and to verify the accuracy of our model. Interestingly, the model predicts a slight charging of the vesicles due to the difference in permeability between Na^+ and Cl^- [23]. Adding acid also adds Cl^- and gives rise to a slow upward drift of the pH on a longer time scale than that of the measurements presented here. It also indicates that the eventual equilibrium pH inside does not quite coincide with the pH outside. Experimental observation of these predicted effects would provide a strong confirmation of the accuracy of the model.

4. Conclusions

To study the ion diffusion across a lipid bilayer, we followed the change in pH inside vesicles after a stepwise decrease in the pH outside, using pyranine. The observations are consistent with a rapid formation of an electrical gradient, arising from the inflow of H^+. Based on the Goldman–Hodgkin–Katz flux of the ions, the buffering capacity of the pH buffer inside the vesicles, and the vesicle membrane capacitance, we estimate the membrane permeability to be ~10^{-8} cm/s, about a thousand times faster than expected. We are able to rule out leakage of the indicator dye and rupture of the vesicles as contributors to this high permeability, and our observations are not consistent with systematic errors in the Goldman–Hodgkin–Katz model. Thus our results strongly suggest that the relatively fast equilibration of the pH is attributable to the permeability of the sodium and chloride ions. Thus it is likely that lipid bilayer membranes of cleanly prepared vesicles are more permeable than expected, with charge relaxing on a time scale of hours rather than weeks.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2014.05.010.

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References

[1] S. Paula, A.G. Volkov, A.N. van Hoek, T.H. Haines, D.W. Deamer, Permeation of protons, potassium ions, and small polar molecules through phospholipid bilayers as a function of membrane thickness, Biophys. J. 70 (1996) 339–348.
[2] J. Wylie Nichols, M.W. Hill, A.D. Bangham, D.W. Deamer, Measurement of net proton-hydroxyl permeability of large unilamellar liposomes with the fluorescent pH probe, 9-aminoacridine, Biochim. Biophys. Acta Biomembr. 596 (3) (1980) 393–403.
[3] D.W. Deamer, J.W. Nichols, Proton–hydroxide permeability of liposomes, Proc. Natl. Acad. Sci. 80 (1) (1983) 165–168.
[4] D.W. Deamer, Proton permeation of lipid bilayers, J. Bioenerg. Biomembr. 19 (5) (1987) 457–479.
[5] J. Gutknecht, Proton conductance through phospholipid bilayers: water wires or weak acids? J. Bioenerg. Biomembr. 19 (5) (1987) 427–442.
[6] K. Kano, J.H. Fendler, Pyranine as a sensitive pH probe for liposome interiors and surfaces. pH gradients across phospholipid vesicles, Biochim. Biophys. Acta Biomembr. 509 (2) (1978) 289–299.
[7] N.A. Dencher, P.A. Burghaus, S. Grzesiek, Determination of the net proton–hydroxide permeability across vesicular lipid bilayers and membrane proteins by optical probes, Methods Enzymol. 127 (1986) 746–760.
[8] M. Rossignol, P. Thomas, C. Grignon, Proton permeability of liposomes from natural phospholipid mixtures, Biochim. Biophys. Acta Biomembr. 684 (2) (1982) 195–199.
[9] M. Horie, H. Yanagisawa, M. Sugawara, Fluorometric immunoassay based on pH-sensitive dye-encapsulating liposomes and gramicidin channels, Anal. Biochem. 369 (2) (Oct. 2007) 192–201.
[10] C.E. Korman, M. Megens, C.M. Ajo-Franklin, D.A. Horsley, Nanopore-spanning lipid bilayers on silicon nitride membranes that seal and selectively transport ions, Langmuir 29 (14) (Apr. 2013) 4421–4425.
[11] N.R. Clement, J.M. Gould, Pyranine (8-hydroxy-1, 3, 6-pyrenetrisulfonate) as a probe of internal aqueous hydrogen ion concentration in phospholipid vesicles, Biochemistry (Mosc) 20 (6) (1981) 1534–1538.
[12] Y. Avnir, Y. Barenholz, pH determination by pyranine: medium-related artifacts and their correction, Anal. Biochem. 347 (1) (Dec. 2005) 34–41.
[13] Y. Avnir, Y. Barenholz, Erratum to "pH determination by pyranine: medium-related artifacts and their correction", Anal. Biochem. 351 (2006) 317.
[14] G. Cevc, Phospholipids Handbook, Marcel Dekker, New York, 1993.
[15] R.B. Gennis, Biomembranes: Molecular Structure and Function, Springer-Verlag, New York, 1989. (etc.).
[16] E.D. Urbanski, M.R. Schock, Understanding, deriving, and computing buffer capacity, J. Chem. Educ. 77 (2000) 1640–1644.
[17] D.W. Deamer, J.W. Nichols, Proton flux mechanisms in model and biological membranes, J. Membr. Biol. 107 (2) (1989) 91–103.
[18] J.W. Nichols, R.F. Abercrombie, A view of hydrogen/hydroxide flux across lipid membranes, J. Membr. Biol. 237 (1) (Sep. 2010) 21–30.
[19] N.R. Clement, J.M. Gould, Kinetics for the development of gramicidin-induced ion permeability in unilamellar phospholipid vesicles, Biochemistry (Mosc) 20 (6) (1981) 1544–1548.
[20] C.L. Kuyper, J.S. Kuo, S.A. Mutch, D.T. Chiu, Proton permeation into single vesicles occurs via a sequential two-step mechanism and is heterogeneous, J. Am. Chem. Soc. 128 (10) (Mar. 2006) 3233–3240.
[21] R. Vácha, S.W.I. Siu, M. Petrov, R.A. Böckmann, J. Barucha-Kraszewska, P. Jurkiewicz, M. Hof, M.L. Berkowitz, P. Jungwirth, Effects of alkali cations and halide anions on the DOPC lipid membrane, J. Phys. Chem. A 113 (26) (Jul. 2009) 7235–7243.
[22] I. Vorobyov, T.E. Olson, J.H. Kim, R.E. Koeppe, O.S. Andersen, T.W. Allen, Ion-induced defect permeation of lipid membranes, Biophys. J. 106 (3) (Feb. 2014) 586–597.
[23] E.G. Sedgwick, P.D. Bragg, Differential movement of ions in artificial phospholipid vesicles, FEBS Lett. 272 (1) (1990) 81–84.