Rapid Switching of Ion Current in Narrow Pores: Implications for Biological Ion Channels

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Rapid switching of ion current in narrow pores: implications for biological ion channels

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SUMMARY

Ions flowing through purely synthetic filters made of polyethylene terephthalate which have been etched to produce narrow pores show: (i) rapid transitions between a high-conducting and a low-conducting state; (ii) selectivity of ion flow; and (iii) inhibition by divalent cations and protons. These features resemble those displayed by many biological ion channels. We interpret our results in terms of the special properties of ion conductance at an interface that may be observed whenever the contribution of bulk conductance is minimal.

1. INTRODUCTION

The characteristic feature of ion channels in biological membranes is that when an electric potential is applied across the membrane the resultant current fluctuates between a conducting and a non-conducting state (Neher & Sakmann 1976). So far most explanations of this phenomenon have been based on the notion that conducting and non-conducting states represent 'open' and 'closed' configurations of the channel, and that the transition between them results from the physical movement of part of the channel structure: the opening and closing of some kind of 'gate' (Hille 1992). The experiments described below show that current fluctuations typical of those observed with biological channels can be observed with purely synthetic membrane filters that are devoid of any added lipid or protein. Two other features of biological ion channels are ion selectivity and inhibition by divalent cations and protons (Hille 1992); these, too, are displayed when current across synthetic membrane filters is measured. These results have been presented in brief at meetings in May 1992 (Lev *et al.* 1992) and August 1992 (Pasternak *et al.* 1993) and are remarkably similar to observations with patch pipettes in the absence of membranes which were presented at a meeting in September 1992 (Sachs & Feng 1993).

2. RESULTS

The filters used for this work were made of polyethylene terephthalate (PETP) which, after heavy ion bombardment to induce nuclear tracks, were 'etched' with hot alkali; this treatment breaks some of the ester bonds (generating free carboxyl groups), and makes the filters more hydrophilic (Apel *et al.* 1990). Two separate filters with slightly different apparent pore sizes (see figure 1) were used. They are referred to throughout as filter 1 and filter 2.

The selectivity of the filters for cations over anions was deduced from the reversal potential, i.e. the sign and magnitude of the voltage (ψ) at zero current, in the presence of a fivefold gradient of KCl. The experiments illustrated in figure 1*a* show this to be 35 mV for each filter, indicating a selectivity value (t_+) of 0.93 (i.e. a 13.3-fold preference for K^+ over Cl^-). The relative pore sizes of the two filters were estimated by assessing the effect on conductivity of solutions of polyethyleneglycol (PEG) of varying molecular mass (figure 1*b*). It is seen that the pore in filter 2 appears to be somewhat narrower than that in filter 1, compatible with a lower conductance (figure 1*a*).

The dependence of pore conductance on ionic strength is shown in figure 2 as a double-logarithmic plot. Its form may be predicted on the assumption that the filter bears a negative surface charge due to the presence of charged carboxyl groups and polarized water molecules (Lee & McCammon 1984), and that the (high) conductance of the pore (see below) is proportional to the concentration of univalent cations at the surface and hence inside or at the mouths of the pore. The concentration of univalent cations at the surface is given by $[K^+].\exp(-q.V_s/kT)$, where $[K^+]$

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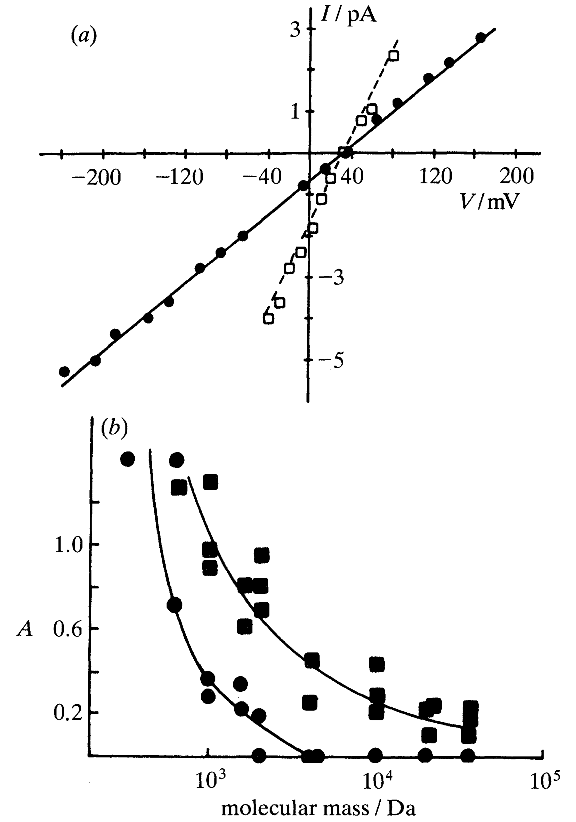

Figure 1. Reversal potential and pore size of PETP nuclear filters. The filters, which were approximately 5 μm thick, contained approximately 1 pore per cm². (*a*) Reversal potential was determined in a fivefold gradient of KCl for filter 1 (open squares) and filter 2 (filled circles); the modal current of the high conducting state (obtained from amplitude histograms; see figure 3*b*) is plotted as a function of applied potential. Selectivity (t_+) was calculated from the reversal potential (ψ) where t_+ is defined as

$$\frac{1}{2}\left[1+\psi\Big/\left(\frac{RT}{F}\ln\frac{[K^+]\mathrm{trans}}{[K^+]\mathrm{cis}}\right)\right].$$

(*b*) Pore size was estimated by use of an 'exclusion' technique (Krasilnikov *et al.* 1988) in which the conductivity of the pore in the presence or absence of 20% (by volume) polyethyleneglycol (PEG) of differing molecular mass is compared. The conductances (calculated from the modal current of the highest conducting state at a potential of 0.2 V) of filter 1 (filled squares) and filter 2 (filled circles) in the presence of 20% PEG relative to that in the absence of PEG are expressed as the ratio (A) of that parameter with the same parameter but measured in the absence of the filter:

$$A=\frac{(G^{\mathrm{filter}}/G^{\mathrm{filter+PEG}})-1}{(G^{\mathrm{solvent}}/G^{\mathrm{solvent+PEG}})-1}.$$

The approximate 'cut-off' size of PEG sufficient to yield values for A of 0.5 was found at molecular masses of ≈ 3000 Da (filter 1) and ≈ 1000 Da (filter 2); based on experimental and theoretical data given by Krasilnikov *et al.* (1992), these correspond to radii of about 1.4 nm (filter 1) and 1 nm (filter 2). Values of $A > 1$ may arise from an effect of polyethylene glycol on ion activity (Bezrukov & Vodyanoy 1993). Method: filters were clamped between two small chambers (volume 0.3 ml each) which contained KCl (0.1–3 M), buffered with 0.005 M HEPES pH 7.4, respectively. Current was measured at room temperature with Ag/AgCl electrodes monitored with a virtual grounded operational amplifier with a feed-back resistor of $10^9\ \Omega$.

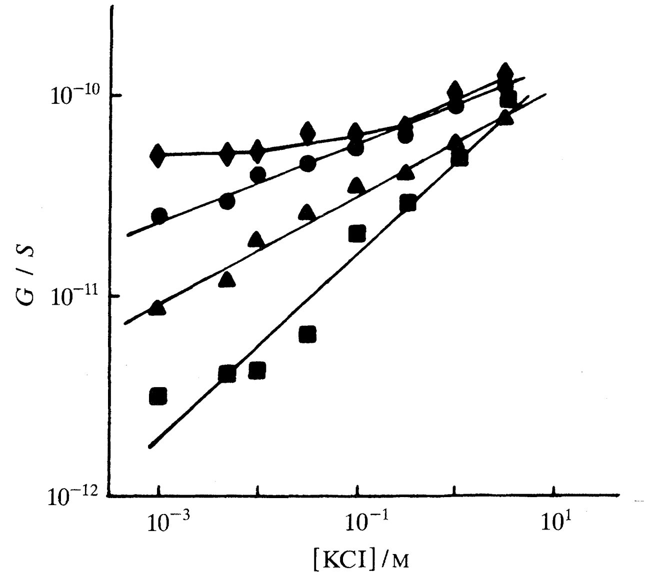

Figure 2. Ionic strength dependence of the conductance of a PETP nuclear filter. Identical solutions containing KCl at the concentration indicated with 10^{-4} M to 3×10^{-3} M EDTA and 0.005 M HEPES at pH 11 (filled diamonds), 8.3 (filled circles), 7.5 (filled triangles) or 6.5 (filled squares) bathed each face of filter 2. Conductance (G) of the highest conducting state at an applied potential of 0.8 V is shown. Similar data were obtained at an applied potential of 0.2 V, except that at pH 6.5 the high conducting state was not observed. The lines through the points were drawn by eye.

is the bulk concentration of potassium ions, V_s is the voltage at the surface of the filter due to its negative charge, q is the proton charge, k is Boltzmann's constant and T the temperature. For a given pH and [K⁺], the surface voltage is obtained by combining (Israelachvili 1992) the Grahame equation which gives the surface charge for a given charge density and ionic strength with the expression for the degree of dissociation of the surface acid sites, which depends upon the proton concentration at the surface, $[H^+].\exp(-q.V_s/kT)$. The variation of conductivity shown in figure 2 then largely mirrors changes in the surface charge and hence of potassium ion concentration inside or at the mouths of the pore. The change of slope over the range of pH values tested suggests that the relevant groups have an effective pK around neutrality. Current fluctuations are maximal around neutrality, with smaller, fewer fluctuations above or below it (Pasternak *et al.* 1993). Such an effect has recently been observed with *S. aureus* α-toxin-induced pores across lipid bilayers also (Bezrukov & Kasianowicz 1992).

Typical records of current are shown in figure 3*a*. Discrete changes between high-conducting and low-conducting states are seen. Analysis of the two predominant states (figure 3*b*) shows the higher one to be 11 pA and the lower one 2.5 pA (filter 1; for filter 2 the values are 3.3 pA and 1.2 pA). The lower state is unlikely to represent a 'leak' current, because: (i) it changes on the addition of Ca^{2+} (see below); (ii) it disappears altogether at low pH; and (iii) it is not seen if the filter is moved so that there is no pore at all between the two chambers. The difference in current between the higher and lower conductance states

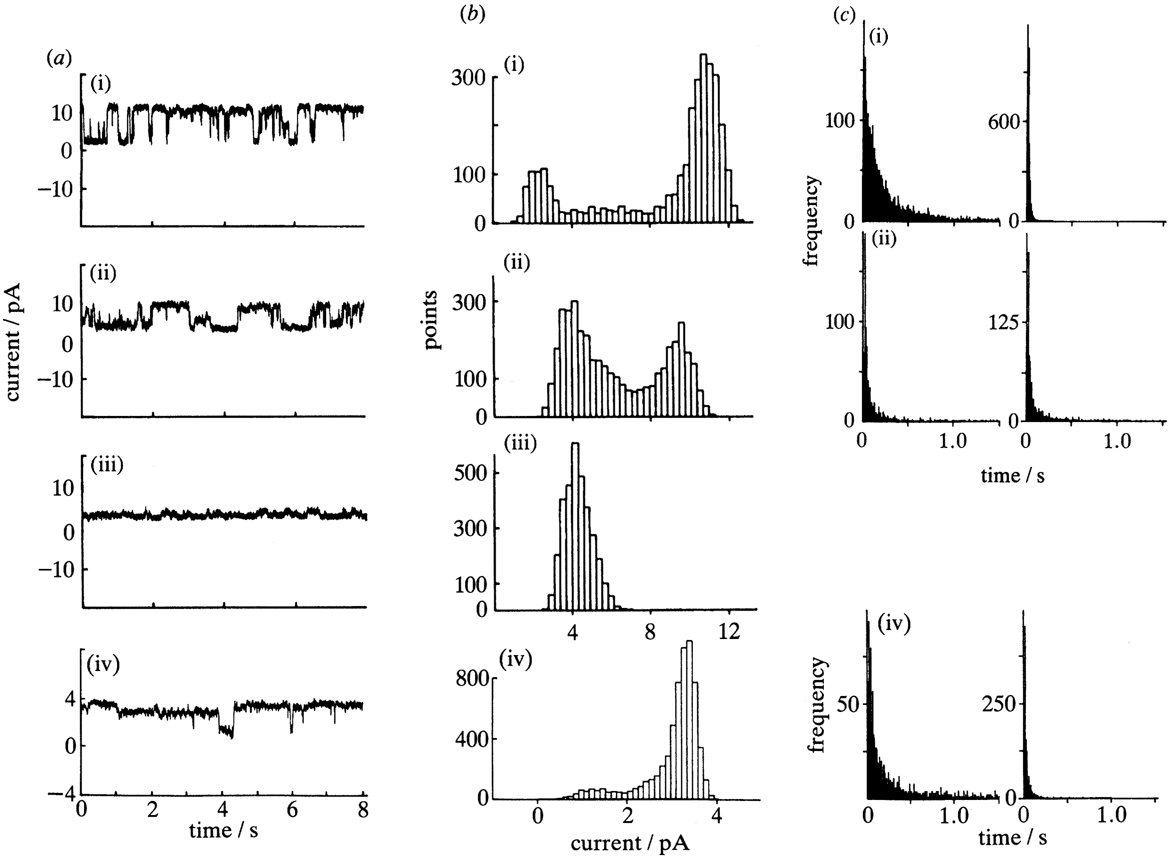

Figure 3. Current fluctuations across PETP nuclear filters. Filter 1 was bathed in 0.1 M KCl, 0.005 M HEPES pH 7.4 (i) without or (ii) with 3×10^{-4} M or (iii) with 3×10^{-3} M $CaCl_2$; (iv) filter 2 was bathed in a similar medium without $CaCl_2$ at pH 8.0; currents at 0.2 V were recorded on videotape by using a Biologic PCM Instrumentation Recorder and subsequently analysed by using Cambridge Electronic Design Patch and Voltage-Clamp software. (*a*) representative traces; (*b*) amplitude histograms of currents filtered at 200 Hz (filter 1) or 150 Hz (filter 2); (*c*) time distribution histograms for the high and low conducting states, respectively. The time constants for each state obtained by fitting a single exponential to the distribution were: (i) high, $\tau = 250$ ms; low, $\tau = 20$ ms; (ii) high, $\tau = 80$ ms; low, $\tau = 72$ ms; (iv) high, $\tau = 230$ ms; low, $\tau = 30$ ms. For filter 1 mean values of the time constant for the high conducting state (τ_H) of 210 ± 59 ms and of the low conducting state (τ_L) of 23 ± 8.7 ms ($n = 8$) were found. Table 1 shows the amplitude of the difference between the current of the high and low conducting states as a function of applied voltage or of the ionic concentration of the bathing medium.

Table 1. *Difference in current between the high and low conducting states of filter 2 at different potentials and ionic strengths*

(For experimental details, see legend to figure 3; n.d. is not determined.)

applied potential/V	current/pA 0.1 M KCl	 0.2 M KCl
0.2	2.7	4.0
0.4	5.5	7.0
0.6	8.8	n.d.

varies with voltage and with ionic strength (table 1). The absolute value of the higher conductance state depends on the pre-treatment of the filter before use (e.g. exposure to ethanol, lipids, other chemicals, etc. (Pasternak *et al.* 1993)). However, the difference in amplitude between the higher conductance state and the lower one is remarkably constant. Other factors, such as pH (figure 2) or the presence of divalent cations (see below), do affect both conductance states. On the addition of 0.3 mM Ca^{2+}, for example, the higher conducting state is decreased to 9.5 pA, whereas the lower conducting state is increased to 3.5 pA for filter 1 (figure 3*b*). Such intermediate amplitudes are rare in the absence of Ca^{2+}; they are detected in recordings of > 1 min. Addition of 3 mM Ca^{2+} abolishes the high-conducting state completely; high conductance is restored by the addition of EDTA (not shown). Similar effects are observed by addition of Zn^{2+} or Mg^{2+}, or by reduction of pH.

The relative efficacy of divalent cations and protons at reducing current is shown in figure 4*a*, which shows data obtained in 0.1 M KCl, and figure 4*b*, which shows data obtained in 3 M KCl. It is seen that in both cases $H^+ > Zn^{2+} > Ca^{2+} \geqslant Mg^{2+}$ with 50% inhibition at molar concentrations of approximately $10^{-7.7}$, $10^{-4.5}$, $10^{-3.4}$ and $10^{-3.0}$ (0.1 M KCl) and $10^{-7.5}$, $10^{-2.8}$, $10^{-1.7}$ and $10^{-1.7}$ (3 M KCl), respectively, for filter 1, and $10^{-8.4}$, $10^{-6.0}$, $10^{-4.2}$ and $10^{-4.2}$ (0.1 M KCl) for filter 2 (not shown). Some part of this behaviour is explicable on the basis that the conductivity of the high-

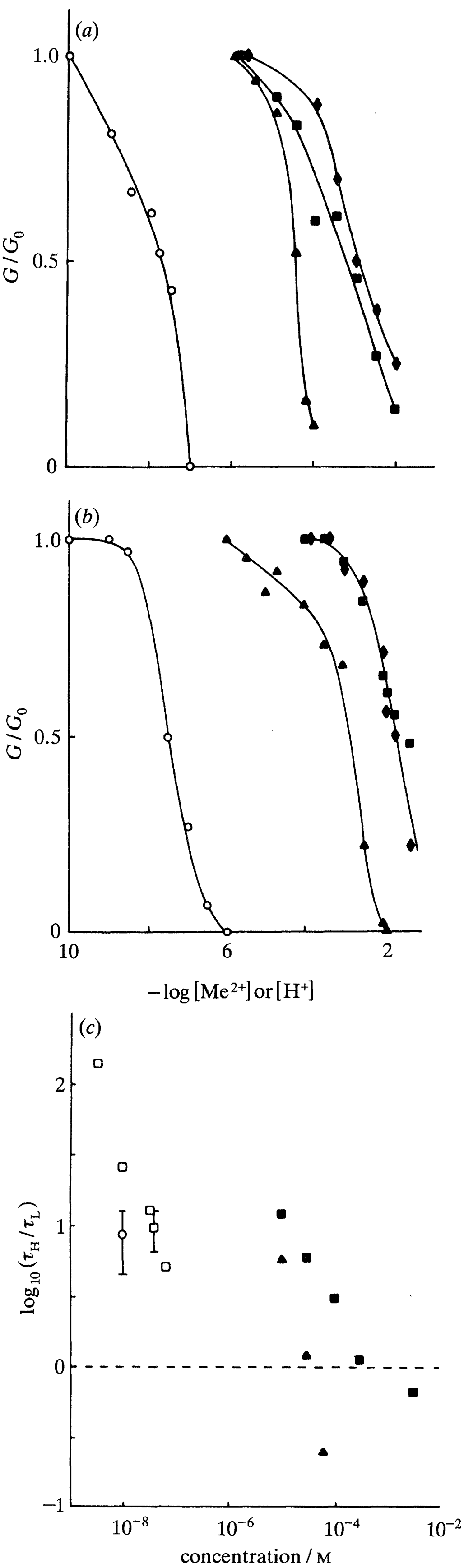

Figure 4. Effect of protons and divalent cations on the conductance of PETP nuclear filters. Filter 1 was bathed in (*a*) 0.1 M KCl and (*b*) 3 M KCl, with 0.005 M HEPES, initial pH 7.4. H^+/OH^- (open circles) or divalent cations (at pH 7.4: Zn^{2+} (filled triangles), Ca^{2+} (filled squares), Mg^{2+} (filled diamonds)) were added to give the final concentrations indicated. The time-averaged conductance (G) at 0.2 V is expressed relative to the maximum observed conductance (G_0) in each titration. (*c*) The logarithm of the ratio of the time constants for the high (τ_H) and low conducting states (τ_L) obtained at 0.2 V in 0.1 M KCl, 0.005 M HEPES at the pH specified (filter 1, open squares; filter 2, open circles) or in a similar medium at pH 7.4 (filter 1) containing the concentration of $CaCl_2$ (filled squares) or $ZnSO_4$ (filled triangles) indicated; error bars represent the standard deviation found for filter 1 ($n = 8$) at pH 7.4 and for filter 2 ($n = 4$) at pH 8.0.

conductance state (figure 2) reflects the concentration of univalent cation at the filter surface. However, inhibition also arises from the promotion of the low-conductance state (figures 3 and 4) by divalent cations and protons. The latter still occurs in 3 M KCl, which suggests that it arises from processes other than the simple screening of surface charge. The data points in figure 4*a*, *b* are derived from time-averaged conductances. Kinetic analysis (as in figure 3*c*) of fluctuations observed in the presence of divalent cations and protons is shown in figure 4*c*. Both sets of data show that filter 2 is more sensitive to inhibition by divalent cations and protons, which may be related to a smaller apparent pore size (see figure 1*b*).

3. DISCUSSION

It has long been known that the conductivity of aqueous solutions near a solid interface may be anomalous (Smoluchowski 1903); surface viscosity (Granick 1991) also shows deviations from bulk behaviour. More recently, anomalies in conductivity along a surface (Lundstrom 1977) or through capillaries (Bezrukov *et al.* 1989) have been reported. The type of conductance observed by us should not be confused with the so-called proton conductance (Pratts *et al.* 1986) at a phospholipid–water interface, as protons decrease conductance through PETP filters (figure 4). The type of conductance described here, which we term 'surface conductance', should be displayed whenever narrow pores, i.e. pores having a high internal surface area to volume ratio, are induced across a lipid bilayer or biological membrane, and this is precisely what is observed. Current fluctuations, interpreted as 'open–closed' transitions, have been described for antibiotics, porins, bacterial and animal toxins (Stein & Lieb 1986), detergents at submicellar concentration (Schlieper & de Robertis 1977) and even phospholipids on their own (Yoshikawa *et al.* 1988; Woodbury 1989), in particular at the transition temperature (Antonov *et al.* 1980). Ion selectivity (Stein & Lieb 1986; Schlieper & de Robertis 1977) is also seen provided the pore is narrow enough (Korchev *et al.* 1992), and the same is true of inhibition by divalent cations and protons (Pasternak *et al.* 1992) with efficacy often $H^+ > Zn^{2+} > Ca^{2+} > Mg^{2+}$ (Bashford *et al.* 1986).

What we have demonstrated here is that current-switching behaviour typical of that observed for a patch-clamped single ion channel may be reproduced by a voltage-clamped narrow water-filled pore in the absence of both lipid and protein. By using other PETP filters with many and larger pores that have been treated with lipid we have demonstrated similar rapid switching of ion current and have shown that the flow of rubidium ions, chloride ions, uncharged molecules and water are similarly inhibited by addition of protons and divalent cations (Pasternak *et al.* 1993); experiments with patch pipettes applied to PETP filters show that the pores showing the lowest conductance have the highest selectivity (A. A. Lev, Y. E. Korchev, T. K. Rostovtseva, C. L. Bashford, D. T. Edmonds & C. A. Pasternak, unpublished results).

Ion conduction in narrow pores will differ from conduction in bulk solution when the ratio of cross-sectional area (πr^2) to circumference ($2\pi r$), namely $r/2$, is low. We find that this occurs at values of r of approximately 2–3 nm, i.e. a distance equal to approximately five hydrated ions away from the surface. There are several reasons for the effect. For example, ion concentrations within the pore will vary from their values in the bulk solution if the wall of the pore is charged due to ionized groups or to an ordered water layer (Lee & McCammon 1984). Direct ion binding at the pore wall will alter this surface charge. Protons and divalent ions are particularly effective, as indicated by their effect on ionic diffusion in zeolite pores (Barrer & Hinds 1953) and on the forces observed between closely apposed surfaces in water (Israelachvili 1992). In very narrow pores the water structure may remain essentially static while the ion moves, as has been shown for some narrow zeolite pores (Barrer & Rees 1960). Calculations predict (Edmonds 1980) that the centres of the planar pentagon and puckered hexagon rings that preserve tetrahedral bonding in water arrays do provide low-energy binding sites for particular ions. Lattice sums (Edmonds 1984) and molecular orbital calculations (MacKay *et al.* 1984) also predict that within narrow pores water may be electrically ordered. The abrupt switching we have observed could be caused by changes in surface charge at the pore wall, or by changes in water structure.

The anomalous properties of ion current described by us, and by Sachs & Feng (1993), point to a form of ion conductivity that occurs close to a water–solid (or water–lipid) interface. We believe that such properties are relevant to an understanding of ion flow through biological ion channels. Channel proteins provide, in addition, voltage-sensitive (see, for example, Papazian *et al.* 1991) and ligand-sensitive (Hille 1992) gating mechanisms, selectivity between divalent cations (see, for example, Yool & Schwarz 1991) and the phenomenon of inactivation (see, for example, Stuhmer *et al.* 1989).

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