Eur Biophys J (2004) 33: 280–282
DOI 10.1007/s00249-003-0366-z

BIOPHYSICS LETTER

C. Lindsay Bashford

Ion permeation of pores in model membranes: selectivity, fluctuations and the role of surface charge

Received: 12 August 2003 / Revised: 12 September 2003 / Accepted: 24 September 2003 / Published online: 4 November 2003

Abstract Fluctuation of surface charge on pore walls provides a realistic, additional mechanism for generating fluctuation of ionic current and ionic selectivity in narrow pores.

Keywords Channel switching · Current fluctuations · Ion channels · Ion conductance · Ion flow

Introduction

The ability of biological ion channels to initiate and integrate signals across cellular membranes hinges on a group of interlinked properties. These include: high throughput – individual ion channels have readily measurable ionic conductance; selectivity between differing ions – channels can discriminate between ions of opposite charge and between ions of the same charge; and switching behaviour – channels can fluctuate between states of high ionic conductance and low (or zero) ionic conductance.

The physical chemistry underlying these properties is only recently being informed by detailed structural information of the ion channels in their various conducting states (Jiang et al. 2003; Kuo et al. 2003; Miyazawa et al. 2003). However, there is considerable work, particularly in simulated systems (Capener et al. 2003; Tieleman et al. 2003), that points to key features that might be expected to pertain in bona fide ion channels. In our work (Bashford 1995) we have used artificial systems, both biological – pores induced in phospholipid bilayer membranes by toxins and peptides – and non-biological – plastic materials made porous by tracking and etching – to explore the determinants of ion conductance, selectivity and switching between high (''open'') and low (''closed'') conducting states. In the non-biological systems we have particularly focused on the role of charged groups within the lumen of a pore in determining pore properties.

Presented at the Biophysical Society Meeting on Ion Channels – from structure to disease held in May 2003, Rennes, France

C. L. Bashford (✉)
School of Medicine, Keele University, Keele, Staffordshire, ST5 5BG, UK
E-mail: c.l.bashford@keele.ac.uk
Tel.: +44-1782-583654
Fax: +44-1782-583903

Ion conductance

In our model systems the limiting diameter of the pores – around 2 nm – is larger than that of biological ion channels and, to a first approximation, ion conductance varies as one would expect for an equivalent cylinder of electrolyte. This suggests that diffusion of ions limits pore conductance. For example, Menestrina (1986) calculated a diameter for the pores induced by *Staphylococcus aureus* α-toxin in bilayer membranes of around 2 nm, assuming cylindrical geometry and the value of the single channel conductance. Subsequently, the atomic structure of the α-toxin pore was determined (Song et al. 1996), revealing a central pore which was 10 nm long and with a diameter that varied between 1.4 and 4.6 nm. In plastic membranes made of poly(ethylene terephthalate) (PET), cylindrical pores can be created by tracking and subsequent etching (Spohr 1990). The initial tracks in PET are formed by bombardment with heavy ions; these are then opened up into cylindrical pores by chemical etching with alkaline solutions (Spohr 1990). The density of pores is determined by the intensity of ion bombardment and membranes may contain from 10^0 to 10^9 pores per cm^2. The diameter of the pores depends on the time of etching – longer etching times giving wider pores – and can be estimated by diffusion of electrolytes and non-electrolytes through the porous material (see Rostovtseva et al. 1996).

An additional layer of complexity was revealed when we studied ion conductance of *S. aureus* α -toxin pores under a wider range of conditions (Korchev et al.

1997). Under acidic conditions (pH values around 4), the conductance of single pores is not a linear function of ionic strength. In particular, conductance at low ionic strength is nearly an order of magnitude higher than that predicted from an equivalent cylinder of electrolyte. In track-etched PET membranes this situation is even more pronounced. Under alkaline conditions (pH values around 11), pore conductance is virtually independent of ionic strength over a very wide range (Lev et al. 1993). These observations suggest that conditions within the pore are not well-modelled by the bulk solution bathing the membrane in which the pores are embedded.

Selective ion flow

One criterion for a pore conductance different from the conductance of the bathing electrolyte would be differential flow of ions – either according to size or according to charge. Such selective ion flow can be monitored by studying the voltage dependence of ion current under conditions where the concentrations of electrolyte on either side of the membrane differ. If selective ion flow occurs, the voltage at which there is no net current (the "reversal potential") is not zero. In *S. aureus* α-toxin pores in phospholipid bilayer membranes (Menestrina 1986; Korchev et al. 1997), anions are preferred to cations, particularly at acid pH values. In PET membranes, cations are preferred to anions, particularly at alkaline pH values (Rostovtseva et al. 1996; Korchev et al. 1997). In such large pores there is some evidence for discrimination between ions of the same charge but differing size. In PET pores, for example, small reversal potentials indicate discrimination between K^+ and larger cations such as choline and tetraethylammonium (C.L. Bashford, unpublished observations).

In PET pores, selectivity is a function of etching time and hence pore diameter (Rostovtseva et al. 1996). The selectivity implied by electrophysiological measurements of single or small numbers of pores has been confirmed by monitoring diffusion through many pores using conventional techniques (Rostovtseva et al. 1996). Selectivity is greater in narrow pores, which in this context means pores with diameters that are within an order of magnitude of the Debye length, usually less than 10 nm. Ion selectivity is a function of both ionic strength (lower selectivity at higher ionic strength) and pH (lower cation selectivity at low pH). Diffusion of water and non-electrolytes through the same pores is independent of ionic strength and pH and scales with pore diameter (Rostovtseva et al. 1996). Recently, Tieleman et al. (2003) have modelled similar behaviour of selectivity with ionic strength in alamethicin K18 channels. The simplest explanation of these results is that charged residues within the pore or near its mouth contribute to selective flow and that screening and ionization determine the magnitude of the effect.

Switching behaviour

Biological ion channels and the pores in our artificial systems can switch between states which differ in conductance by an order of magnitude or more. Time records of such behaviour exhibit "random telegraph signalling" and it is the organization and regulation of such switching by native ion channels that determines cell and membrane behaviour. We have made detailed studies of the high- and low-conducting states in both *S. aureus* α-toxin pores and pores in PET membranes. In the former case we found (Korchev et al. 1995) that the high- and low-conducting states for ions (nearly ten-fold difference) do not always correlate with those in permeability to non-electrolytes (less than two-fold difference). It is thus not clear what "closed state" means for a pore that can permit glycerol and water but relatively little electrolyte to pass through it. We find a similar situation in track-etched PET membranes (Rostovtseva et al. 1996). In both systems we found that switching of ion current had a similar time-dependence to switching of reversal potential (Korchev et al. 1997). This situation resembles that found in channels formed by the aquaporin family (Stroud et al. 2003), where structural studies suggest that it is possible to organize pores permeable to non-electrolytes but not electrolytes by having long hydrophobic segments separating hydrophilic portions of the pore (Fu et al. 2000; Sui et al. 2001). In PET, un-ionized carboxyl groups could provide relatively hydrophobic segments of the pore wall such that water but not ions could permeate.

The correlation of switching of ion current with changes in ionic selectivity suggests that changes in surface charge may underpin both phenomena. In the low-conducting state the neutral lining of the pore has little impact on ion flow through the membrane and single channel conductance is that expected from pore length and diameter. In very narrow channels the hydrophobicity of the un-ionized pore wall may also act as a gate (Beckstein et al. 2001) in the manner proposed for the acetylcholine receptor pore (Miyazawa et al. 2003). In the high-conducting state, surface charges attract counterions and reject ions of the same charge. Now, in an electric field, cations are much more likely to flow through because there are so many more of them there. In effect, cation concentration inside the pore approaches that found in 1 M electrolyte and that is the major contribution to ion conductance. Throughput will be high because the energetic cost of acquiring a counterion at one end of the pore, bumping all the other ions along the surface charges and ejecting a similar ion at the other end of the pore, is small. This mechanism has similarities to the single filing of ions through the selectivity filter in potassium channels (Doyle et al. 1998; Morais-Cabral et al. 2001; Zhou et al. 2001; Biggin and Sansom 2002).

Track-etched, PET membranes offer a unique system to validate the hypothesis that switching of surface

charge underpins switching of pore conductance and pore selectivity because they are sufficiently thick to permit longitudinal sectioning pores by confocal microscopy using indicators that are sensitive to changes in surface charge. We used the fluorescent cationic dye 3,3'-diethyloxacarbocyanine iodide (diO-C_2-(3)) for such experiments (Bashford et al. 2002) and found that the time dependence of staining of the pores shows fluctuations of fluorescence intensity that occur on the same time scale as do fluctuations of ionic current.

What mechanism(s) can there be for the apparently simultaneous switching of neighbouring ionizable groups on the pore wall? The simplest explanation is that there is a cooperative interaction between neighbouring groups. A Monte Carlo computer simulation of the state of charge of a hexagonal array of acid residues located in a plane surface provides a significant insight (Korchev et al. 1997). The ionizable sites (carboxyl groups) are assumed to lie in a hexagonal array on the wall of the pore. When any site ionizes, an additional negative surface charge is created which attracts protons away from neighbouring, un-ionized sites. Thus there is a positive feedback mechanism in place: ionization at one site effectively increases the pH at neighbouring sites and thus promotes their ionization. To construct the model we assumed that when a given acid site ionizes, the effective pH at the six neighbouring sites increases by 0.5 of a pH unit and that at each site in isolation the value of (pK−pH) is 1.5. The time evolution of the ionization state of all sites under these conditions generates two important effects. The first is that the dominant states of the system are those in which most sites are charged or in which few sites are charged. The second effect of the coupling is that the number of switches of the assembly between its dominant states in a given time is very small in comparison with the average switching rate of an isolated acid site (Korchev et al. 1997). This helps to explain why a very rapid process, ionization, can drive a relatively slow one, change of conductance state, in a cooperative network of chemical groups.

We conclude that fluctuation of surface charge on pore walls provides a realistic, additional mechanism for generating fluctuation of ionic current and ionic selectivity in narrow pores.

References

Bashford CL (1995) Membrane pores: from biology to track-etched membranes. Biosci Rep 15:553–565

Bashford CL, Alder GM, Pasternak CA (2002) Fluctuation of surface charge in membrane pores. Biophys J 82:2032–2040

Beckstein O, Biggin PC, Sansom MSP (2001) A hydrophobic gating mechanism for nanopores. J Phys Chem B 105:12902–12905

Biggin PC, Sansom MSP (2002) Open-state models of a potassium channel. Biophys J 83:1867–1876

Capener CE, Proks P, Ashcroft FM, Sansom, MSP (2003) Filter flexibility in a mammalian K channel: models and simulations of Kir6.2 mutants. Biophys J 84:2345–2356

Doyle DA, Cabral JM, Pfuetzner RA, Kuo AL, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science 280:69–77

Fu DX, Libson A, Miercke LJW, Weitzman C, Nollert P, Krucinski J, Stroud RM (2000) Structure of a glycerol-conducting channel and the basis for its selectivity. Science 290:481–486

Jiang YX, Lee A, Chen JY, Ruta V, Cadene M, Chait BT, MacKinnon R (2003) X-ray structure of a voltage-dependent K^+ channel. Nature 423:33–41

Korchev YE, Bashford CL, Alder GM, Kasianowicz JJ, Pasternak CA (1995) Low conductance states of a single ion channel are not 'closed'. J Membr Biol 147:233–239

Korchev YE, Bashford CL, Alder GM, Apel PY, Edmonds DT, Lev AA, Nandi K, Zima AV, Pasternak CA (1997) A novel explanation for fluctuations of ion current through narrow pores. FASEB J 11:600–608

Kuo AL, Gulbis JM, Antcliff JF, Rahman T, Lowe ED, Zimmer J, Cuthbertson J, Ashcroft FM, Ezaki T, Doyle DA (2003) Crystal structure of the potassium channel KirBac1.1 in the closed state. Science 300:1922–1926

Lev AA, Korchev YE, Rostovtseva TK, Bashford CL, Edmonds DT, Pasternak CA (1993) Rapid switching of ion current in narrow pores: implications for biological ion channels. Proc R Soc London B 252:187–192

Menestrina G (1986) Ionic channels formed by *Staphylococcus aureua* alpha-toxin: voltage-dependent inhibition by divalent and trivalent cations. J Membr Biol 90:177–190

Miyazawa A, Fujiyoshi Y, Unwin N (2003) Structure and gating mechanism of the acetylcholine receptor pore. Nature 423:949–955

Morais-Cabral JH, Zhou YF, MacKinnon RM (2001) Energetic optimisation of ion conduction rate by the K^+ selectivity filter. Nature 414:37–42

Rostovtseva TK, Bashford CL, Alder GM, Hill GN, McGiffert C, Apel PY, Lowe G, Pasternak CA (1996) Diffusion through narrow pores: movement of ions, water and non-electrolytes through track-etched PETP membranes. J Membr Biol 151:29–43

Song LZ, Hobaugh MR, Shustak C, Cheley S, Bayley H, Gouaux JE (1996) Structure of staphylococcal alpha-hemolysin, a heptameric transmembrane pore. Science 274:1859–1866

Spohr R (1990) In: Bethge K (ed) Ion tracks and microtechnology. Vieweg, Braunschweig, Germany, pp XX–XX

Stroud RM, Nollert P, Miercke L (2003) The glycerol facilitator G1pF, its aquaporin family of channels, and their selectivity. Adv Protein Chem 63:291–316

Sui HX, Han BG, Lee JK, Walian P, Jap BK (2001) Structural basis of water-specific transport through the AQP1 water channel. Nature 414:872–878

Tieleman DP, Borisenko V, Sansom MSP, Woolley GA (2003) Understanding pH-dependent selectivity of alamethicin K18 channels by computer simulation. Biophys J 84:1464–1469

Zhou YF, Morais-Cabral JH, Kaufman A, MacKinnon RM (2001) Chemistry of ion coordination and hydration revealed by a K^+ channel-Fab complex at 2.0 angstrom resolution. Nature 414:43–48