Hypothesis

Voltage gating is a fundamental feature of porin and toxin β-barrel membrane channels

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Abstract Beta-barrel pores are found in outer membrane porins of Gram-negative bacteria, bacterial toxins and mitochondrial channels. Apart from the β-barrel the three groups show no close sequence or structural homology but these pores exhibit symmetrical voltage gating when reconstituted into planar lipid bilayers. The structures of several of these are known and many site-directed mutants have been examined. As a result it seems evident that the gating is a common characteristic of these unrelated large pores and is not generated by specialised structures in the pore lumen.

Key words: Beta-barrel pore; Voltage gating

1. Introduction

The switching of ion currents through transmembrane channels is called 'gating' [1]. This occurs most clearly in the voltage-dependent sodium, potassium and calcium channels which combine to create the action potential. In each case the channels respond to changes in the transmembrane potential difference by altering their probability of opening. Unfortunately the origin of this fascinating 'voltage gating' is unclear since the three dimensional structures of these proteins are not known. However, a model has been proposed, based upon mutagenesis and structure prediction, in which charged side chains on transmembrane helices provoke conformational changes leading to channel opening and closing [1].

Voltage gating also occurs in a quite separate family of membrane proteins: the porins [2,3]. These large (~1 nm diameter) channels are situated in the outer membranes of Gram-negative bacteria and create a pathway which controls access of solutes to the periplasm [4]. The porins fall into two distinct groups, the non-selective porins which restrict permeability to solutes of $M_r < 600$ Da. and the porins which have precise selectivity for defined solutes [4]. Unusually for membrane proteins, members of both groups have had their structures solved to high resolution by X-ray crystallography. All of the non-selective porins are trimers of 16 stranded β-barrels [5–8] whilst those selective porins solved have 18 stranded barrels [9,10]. All the non-selective porins of known structure, and most of the rest [11], show voltage dependent closing [2,12–24] and are therefore our only high resolution structural key to the way transmembrane voltage interacts with membrane proteins. Here we present a review of the work carried out and put forward the hypothesis that the gating observed in porins and the unrelated β-barrel toxins is essentially the same. Although gating under physiological conditions has been reported [25–27], we make no assumptions as to its physiological relevance and seek merely to understand a distinctive and mysterious feature of these large pores.

2. Beta-barrel pores are specialised structures

The amino acid sequences of most membrane proteins clearly show the long stretches of hydrophobic sequence (20–30 residues) required to create transmembrane α-helices. The porin primary structures do not have this character and resemble water soluble proteins. This paradox was solved by the discovery of the structure of *Rhodobacter capsulatus* porin [5] which showed a complete barrel of β-strands, each neatly hydrogen bonded with its two neighbours (Fig. 1). Thus the secondary structural elements are fixed in a rigid network of hydrogen bonds which cannot be easily broken. This contrasts with the unrestrained association of α-helices which allows helix twisting and translocation to form part of models of gating and transport.

3. What happens when porins close during voltage gating?

The voltage gating of porins is mostly described as two-state gating of individual channels with closing occurring at both positive and negative applied potentials. This results in a bell shaped curve of open probability versus potential (Fig. 2). In several cases a distinct but small 'closed' channel current is observed [13,28] and it is not clear whether uncharged solutes or water can still permeate through the 'closed' state [19]. When the reconstitution conditions are varied, gating can be seen to occur at higher or lower potentials and may be sensitive to the voltage polarity across the membrane (asymmetric voltage gating) [12,24,27] but these are essentially variations on a fundamentally symmetrical behaviour. This symmetry is rarely found in the physiological (and α-helical) voltage-gated channels which respond to polarity changes over a narrow range (Fig. 2). Also the closed state remains closed for periods of seconds or minutes whilst this 'dwell time' for sodium channels is of the order of milliseconds. Finally, porins exhibit much larger single channel conductances than the ion selective channels.

Closing of these large diameter pores would therefore appear to require a significant structural change but porins have another feature relevant to their gating. Within the pore is the eyelet region, a narrow passageway created by the invagina-

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tion of a surface loop (loop 3) into the pore lumen. This reduces the channel diameter at a point approximately half way across the bilayer. Furthermore positively charged residues on the barrel wall face carboxylates and backbone carbonyls on loop 3 to create a unique electrostatic field parallel to the plane of the bilayer [29,30]. With structural change in the barrel considered unlikely, the eyelet was proposed as the likely site of voltage gating. The involvement of the eyelet was apparently confirmed when eyelet mutations of OmpC found to increase voltage sensitivity [31]. Two possible origins of voltage gating were considered, firstly that local electrostatic changes in the eyelet subtly altered the channel to stop all conductivity or that large movements of loop 3 occurred which closed the channel lumen [19]. The latter suggestion was supported by simulations using molecular dynamics which showed the possibility of large deformations in *R. capsulatus* loop 3 [32].

These hypotheses provoked site-directed mutagenesis experiments which have been carried out in a number of groups including our own. In one series of papers on PhoE and OmpF, disulphide bonds have been inserted into the loop 3 region to restrict its conformation to that of the solved X-ray structure [19,33,34]. Small differences in gating were observed but in no case did any of the mutations significantly inhibit voltage gating. Thus is quite clear that large deformation of loop 3 is not required for voltage gating.

The other series of mutagenesis papers have modified the charge environment of the eyelet region to try and find the gating charges operating within the transmembrane region. Since the known mutants *increased* voltage gating in OmpC [31], the search was on to discover mutations or chemical modification which abolished the effect. Unfortunately this search has not yet been successful. That is not to say that mutants have not been found which do alter the gating, but abolition of gating has not been achieved. The data are very detailed and the interested reader is referred to the original papers [19,20,31,35–37]. One interesting observation is the discovery that charge mutations have opposite effects on gating

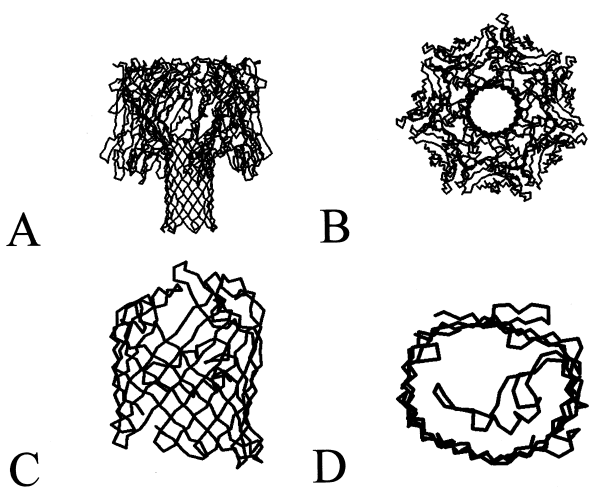

Fig. 1. The structures of two β-barrel pores. Examples of voltage-gated pore proteins. Side (A) and top (B) view of *S. aureus* α-toxin [41]. Note (A) the membrane penetrating β-barrel and large extramembraneous domains, and (B) the clear unobstructed pore lumen. Side (C) and top (D) view of *E. coli* OmpF pore monomer [6]. In (D) note the pore lumen and the internal loop 3 which creates the eyelet.

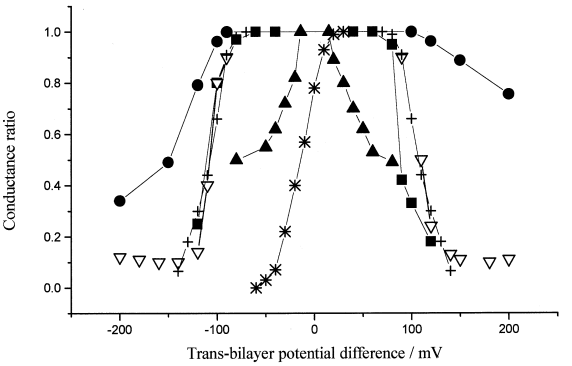

Fig. 2. The voltage dependence of five unrelated β-barrel pores. The graph shows the ratio of conductance (R) at each voltage compared to the conductance measured at a low voltage that does not induce gating. All data from planar lipid bilayer experiments replotted from original references. ●, α-toxin in diphytanoyl-phosphatidylcholine bilayer, 100 mM KCl, 5 mM HEPES, pH 7.4 [44]; ■, aerolysin in soybean lecithin bilayer, 1 M NaCl, 10 mM HEPES, 1 mM EDTA, pH 7.4 [38]; ▲, *Paramecium* mitochondrial porin in 0.5 M KCl pH 7.2 [45]; ▽, *E. coli* PhoE in 1 M NaCl, 10 mM $MgCl_2$, 10 mM Tris-HCl pH 7.4; soybean lecithin bilayer [28]; +, *Neisseria gonorrhoaeae* porin in phosphatidylethanolamine, phosphatidylcholine, cholesterol bilayer, 0.1 M NaCl, 10 mM HEPES pH 7.4 [13]. *, Voltage dependence of squid giant axon sodium channels for comparison [1].

when they are made in highly homologous cation or anion selective porins [20] and this was recently extended in a patch clamp study of intact outer membrane fragments [27]. Under these conditions with the asymmetric outer membrane still intact porins exhibit an asymmetry not present in their purified state in bilayer lipid membranes (BLM). Curiously this induced asymmetry of anion selective PhoE is opposite to that of homologous but cation selective OmpF and the authors have shown that one lysine residue in PhoE may be the key to this behaviour [27].

4. Clues from other voltage gated pores

In 1990 it was noted [38] that the pore-forming toxin aerolysin formed porin-like voltage gated pores in BLM (Fig. 2). This toxin is first synthesised in a water soluble form before forming a heptameric pore structure in the membrane of the target cell [39]. Since the toxin was known to be rich in β-structure the authors suggested that it may form a similar structure to porin and this would be the origin of the gating in both systems. Since then the structures of aerolysin (in water soluble and membrane states [40]) and the membrane inserted form of the α-haemolysin from *Staphylococcus aureus* (which has clear structural but no sequence similarity to aerolysin) have been solved [41]. This revealed a 14 stranded β-barrel made by using a single β-hairpin loop from each of the seven subunits (Fig. 1). On the side of membrane insertion there is a large water soluble domain whilst on the other side of the membrane the channel is unlikely to protrude significantly beyond the membrane surface. This highly asymmetric structural pattern is considered to be conserved widely in toxins of this form [39,42]. The important feature for this hypothesis is that there is no loop 3 equivalent and no eyelet. The pore is a smooth open cylinder with no significant change in radius along its length.

The α-haemolysin voltage gating is similar to porins [43,44]

(Fig. 2) but like aerolysin its gating is also sensitive to divalent cations. The origin of the divalent cation effect has been extensively studied and does not appear to be a simple voltage dependent channel block by an ion forced into the channel by a strong transmembrane potential difference.

The final group of symmetrically gated β-barrel pores are the VDAC family or 'mitochondrial porins' [45]. Whether these are in fact related to Gram-negative porins via the endosymbiont theory for the origin of mitochondria is debatable [46] but their large pore size and symmetrical voltage gating is well established. It is very likely that they are dimeric β-barrels although the existence of some α-helical element is possible. Their fundamental voltage gating occurs at very low voltages and is also affected by a wide variety of soluble proteins and molecules [45]. Furthermore it is possible that voltage gating in VDAC has a physiological role for permeability control of the mitochondrial outer membrane [47]. Since evidence exists for a large structural change in VDAC [48] and significant closed channel permeability [45], which are both at variance with the current data for bacterial porins, the analogy is unclear between the two groups [48]. Nevertheless the type of gating and the pore structure suggest an underlying similarity of mechanism (also proposed by [23]).

5. What is the physical basis of porin voltage gating?

We have shown in this paper the generality of symmetrical voltage gating in all large β-barrel pores. In view of their lack of other homology we believe that this indicates that voltage gating is a general property of β-barrel pore structure rather than any particular sub-structure. The alternative is to assume that the similarities arise by chance. Clearly the gating can be inhibited since OmpC [31,49], LamB [50] and anthrax protective antigen [51,52] do not show clear voltage gating. Gating can also be modified by interactions with other molecules (toxins and porins and VDAC), hydrostatic pressure [53] and reconstitution method [12,54] but the general feature is of two state open and closed channels at either polarity [23]. As all the channels are β-barrels, is this gating a feature of such barrels (Fig. 2)?. The β-structure results in the minimum separation between the hydrophobic membrane core and the channel lumen since only one layer of amino acids is used to define the pore. The only large water filled pores known to be formed by groups of α-helices arise from oligomeric barrels of short peptides and these show asymmetric rectification and/or gating [1,55]. Since they are modelled as parallel bundles they will have a clear asymmetry from NH_2 to COOH terminus [55]. Anti-parallel β-barrels are, on the other hand extremely symmetrical within the membrane. The extra-membranous regions of porins and especially pore forming toxins are very asymmetric but this clearly does not affect the voltage sensitivity of the pore. Hence the origin of the gating is most likely situated within the transmembrane β-barrel. The β-structure does appear to allow for a symmetrical behaviour but its role in provoking closing is less clear.

In general the pores offer a 1–2 nm diameter pathway and ions can travel either through the lumen or by interaction with the charged or dipolar groups at the periphery. Voltage *independent* gating can be observed in very large pores in synthetic membranes where conformational change leading to closure of the channel is unlikely. This behaviour has been ascribed to fluctuations in the peripheral ion conductance pathways of the channel [56]. This may provide the basis for a general electrostatic model to explain the closure of large channels without conformational change. Channels which are large in comparison with ionic radii can be modelled using electrostatics and bulk dielectric constant values. This approach shows that even in such large pores significant energy barriers are presented to ions (due to image forces etc.) unless appropriate dipoles are present [57]. Furthermore description of the ion conduction pathway through a synthetic α-helical channel using a Poisson-Nernst-Planck approach shows that variations in charge and charge shielding have large effects on ion flux and similar studies are under way in porin [55]. One important feature of this simulation is that even impermeant ions can radically alter the charge potential over the whole of a 30 Å channel into which they cannot enter. The mechanism of gating may thus depend upon a voltage (rather than solute) dependent breakdown of the delicate ion conducting pathway. The voltage and polarity at which the breakdown occurs is further modified by lipid surface charge [24], cations [38,44], eyelet residues [20,27,31,35,37], membrane thickness [12,24] etc.

In conclusion, the current knowledge of porin and pore-forming toxin structures indicates an common but unknown mechanism of voltage gating. It is still possible that conformational changes occur but the underlying symmetry indicates a switching within the pore lumen which is dependent purely on features common to all the large β-barrel pores. We further suggest that where the gating does have a physiological role it has not evolved independently in each case but has modified an existing idiosyncrasy.

Note added in proof: A potassium channel structure has recently been published by Doyle et al., Science 280 (1998) 69–77.

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References

[1] Aidley, D.J. and Stanfield, P.R. (1996) Ion Channels – Molecules in Action, Cambridge University Press, Cambridge.
[2] Schindler, H. and Rosenbusch, J.P. (1978) Proc. Natl. Acad. Sci. USA 75, 3751–3755.
[3] Lakey, J.H. (1987) FEBS Lett. 211, 1–4.
[4] Benz, R. and Bauer, K. (1988) Eur. J. Biochem. 176, 1–19.
[5] Weiss, M.S., Wacker, T., Weckesser, J., Welte, W. and Schulz, G.E. (1990) FEBS Lett. 267, 268–272.
[6] Cowan, S.W., Schirmer, T., Rummel, G., Steiert, M., Ghosh, R., Paupit, R.A., Jansonius, J.N. and Rosenbush, J.P. (1992) Nature 358, 727–733.
[7] Kreusch, A. and Schulz, G.E. (1994) J. Mol. Biol. 243, 891–905.
[8] Hirsch, A., Breed, J., Saxena, K., Richter, O., Ludwig, B., Diederichs, K. and Welte, W. (1997) FEBS Lett. 404, 208–210.
[9] Schirmer, T., Keller, T.A., Wang, Y.F. and Rosenbusch, J.P. (1995) Science 267, 512–514.
[10] Forst, D., Welte, W., Wacker, T. and Diederichs, K. (1998) Nature Struct. Biol. 5, 37–46.
[11] Jeanteur, D., Lakey, J.H. and Pattus, F. (1991) Mol. Microbiol. 5, 2153–2164.
[12] Lakey, J.H. and Pattus, F. (1989) Eur. J. Biochem. 186, 303–308.
[13] Young, J.D.-E., Blake, M., Mauro, A. and Cohn, Z. (1983) Proc. Natl. Acad. Sci. USA 80, 3831–3835.
[14] Besnard, M., Martinac, B. and Ghazi, A. (1997) J. Biol. Chem. 272, 992–995.
[15] Brunen, M. and Engelhardt, H. (1993) Eur. J. Biochem. 212, 129–135.
[16] Vachon, V., Laprade, R. and Coulton, J. (1986) Biochim. Biophys. Acta 861, 74–82.

[17] Dahan, D., Vachon, V., Laprade, R. and Coulton, J.W. (1994) Biochim. Biophys. Acta 1189, 204–211.
[18] Lea, E., Bishop, N.D. and Lakey, J.H. (1993) Biophys. J. 64, 94.
[19] Phale, P.S., Schirmer, T., Prilipov, A., Lou, K.L., Hardmeyer, A. and Rosenbusch, J.P. (1997) Proc. Natl. Acad. Sci. USA 94, 6741–6745.
[20] Van Gelder, P., Saint, N., Phale, P., Eppens, E.F., Prilipov, A., Van Boxtel, R., Rosenbusch, J.P. and Tommassen, J. (1997) J. Mol. Biol. 269, 468–472.
[21] Kleivdal, H., Benz, R. and Jensen, H.B. (1995) Eur. J. Biochem. 233, 310–316.
[22] Trias, J. and Benz, R. (1993) J. Biol. Chem. 268, 6234–6240.
[23] Berrier, C., Besnard, M. and Ghazi, A. (1997) J. Membr. Biol. 156, 105–115.
[24] Wiese, A., Schroder, G., Brandenburg, K., Hirsch, A., Welte, W. and Seydel, U. (1994) Biochim. Biophys. Acta 1190, 231–242.
[25] Rudel, T., Schmid, A., Benz, R., Kolb, H.A., Lang, F. and Meyer, T.F. (1996) Cell 85, 391–402.
[26] Dela Vega, A.L. and Delcour, A.H. (1995) EMBO J. 14, 6058–6065.
[27] Samartzidou, H. and Delcour, A.H. (1998) EMBO J. 17, 93–100.
[28] Dargent, B., Hofmann, W., Pattus, F. and Rosenbusch, J.P. (1986) EMBO J. 5, 773–778.
[29] Karshikoff, A., Spassov, V., Cowan, S.W., Ladenstein, R. and Schirmer, T. (1994) J. Mol. Biol. 240, 372–384.
[30] Weiss, M.S., Abele, U., Weckesser, J., Welte, W., Schiltz, E. and Schulz, G.E. (1991) Science 254, 1627–1630.
[31] Lakey, J.H., Lea, E.J.A. and Pattus, F. (1991) FEBS Lett. 278, 31–34.
[32] Soares, C.M., Bjorksten, J. and Tapia, O. (1995) Mol. Simul. 15, 35–46.
[33] Bainbridge, G., Mobasheri, H., Armstrong, G.A., Lea, E.J.A. and Lakey, J.H. (1998) J. Mol. Biol. 275, 171–176.
[34] Eppens, E.F., Saint, N., Van Gelder, P., van Boxtel, R. and Tommassen, J. (1997) FEBS Lett. 415, 317–320.
[35] Gokce, I., Bainbridge, G. and Lakey, J.H. (1997) FEBS Lett. 411, 201–205.
[36] Saint, N., Prilipov, A., Hardmeyer, A., Lou, K.L., Schirmer, T. and Rosenbusch, J.P. (1996) Biochem. Biophys. Res. Commun. 223, 118–122.
[37] Saint, N., Lou, K.L., Widmer, C., Luckey, M., Schirmer, T. and Rosenbusch, J.P. (1996) J. Biol. Chem. 271, 20676–20680.
[38] Wilmsen, H.-U., Pattus, F. and Buckley, J.T. (1990) J. Membr. Biol. 115, 71–81.
[39] Wilmsen, H.U., Leonard, K.R., Tichelaar, W., Buckley, J.T. and Pattus, F. (1992) EMBO J. 11, 2457–2463.
[40] Parker, M.W., Buckley, J.T., Postma, J., Tucker, A.D., Leonard, K., Pattus, F. and Tsernoglou, D. (1994) Nature 367, 292–295.
[41] Song, L., Hobaugh, M.R., Shustak, C., Cheley, S., Bayley, H. and Gouax, J.E. (1996) Science 274, 1859–1866.
[42] Goaux, J.E., Hobaugh, M. and Song, L. (1997) Protein Sci. 6, 2631–2635.
[43] Bashford, C.L., Alder, G.M., Fulford, L.G., Korchev, Y.E., Kovacs, E., MacKinnon, A., Pederzolli, C. and Pasternak, C.A. (1996) J. Membr. Biol. 150, 37–45.
[44] Korchev, Y.E., Alder, G.M., Bakramov, A., Bashford, C.L., Joomun, B.S., Sviderskaya, E.V., Usherwood, P.N.R. and Pasternak, C.A. (1995) J. Membr. Biol. 143, 143–151.
[45] Benz, R. (1994) Biochim. Biophys. Acta 1197, 167–196.
[46] Harold, F.M. (1986) The Vital Force, W.H. Freeman, New York.
[47] Hodge, T. and Colombini, M. (1997) J. Membr. Biol. 157, 217–279.
[48] Peng, S., Blachly-Dyson, E., Forte, M. and Colombini, M. (1992) Biophys. J. 62, 123–135.
[49] Buehler, L.K., Kusumoto, S., Zhang, H. and Rosenbusch, J.P. (1991) J. Biol. Chem. 266, 24446–24450.
[50] Dargent, B., Rosenbusch, J. and Pattus, F. (1987) FEBS Lett. 220, 136–142.
[51] Petosa, C., Collier, R.J., Klimpel, K.R., Leppla, S.H. and Liddington, R.C. (1997) Nature 385, 833–838.
[52] Blaustein, R.O., Lea, E.J.A. and Finkelstein, A. (1990) J. Gen. Physiol. 96, 921–942.
[53] Ledain, A.C., Hase, C.C., Tommassen, J. and Martinac, B. (1996) EMBO J. 15, 3254–3528.
[54] Berrier, C., Coulombe, A., Houssin, C. and Ghazi, A. (1992) FEBS Lett. 306, 2–3.
[55] Chen, D., Lear, J. and Eisenberg, R. (1997) Biophys. J. 72, 97–116.
[56] Korchev, Y.E. et al. (1997) FASEB J. 11, 600–608.
[57] Hoyles, M., Kuyucak, S. and Chung, S.-H. (1996) Biophys. J. 70, 1628–1642.