Volume 211, number 1, 1–4 FEB 04364 January 1987

Review Letter

Voltage gating in porin channels

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Received 20 October 1986

Data from experiments in which porin channels are reconstituted into planar bilayer membranes are reviewed for their relevance to porin channel gating in vivo. Contradictory evidence concerning voltage gating indicates that the different results may stem from the variety of purification techniques employed. The likelihood of voltage gating as a property of *E. coli* porins in vivo is discussed in relation to the possible magnitude of the membrane potential across tho outer membrane.

Porin; Voltage-dependent channel; Planar bilayer; Membrane reconstitution; Outer membrane; *(E. coli)*

Porins are channel-forming proteins from the outer membrane of Gram-negative bacteria which confer on this protective barrier the properties of a molecular sieve [1,2]. Their role has been studied both in living cells [2] and by the use of purified samples in vitro [1–3]. The incorporation of these proteins into planar lipid bilayers [1,3,4] has provided clear evidence regarding their physicochemical interactions with solvent molecules and membrane components. This work combined with swelling measurements of porin-containing liposomes has allowed the role of porins in the outer membrane to be well characterised, and for a broader understanding of this subject I refer the reader to previous excellent reviews [1–4]. However, the basic question of whether a porin channel can change reversibly from an open (conducting) to a closed (non-conducting) state (i.e. exhibit gating) has not been fully resolved [1,5]. I hope in this short review to gather the main lines of evidence concerning this fundamental dilemma.

The possibility that porin channels can exist in a closed state has been suggested by several authors [6,7] to account for discrepancies between porin and outer membrane permeability properties. One example of this is the outer membrane of *Pseudomonas aeruginosa* which, although it possesses many thousands of non-selective F porins (exclusion limit 6000 Da), is practically impermeable to small hydrophilic antibiotics of around 400 Da. In order to account for this, it has been proposed [6] that very few of these channels are, in fact, open. This, if one can discount permanently disabled channels, indicates some form of gating mechanism.

There are two main gating mechanisms that can be considered. Firstly, ligand-induced gating, as in the nicotinic acetylcholine receptor of vertebrate excitable cells. In this example acetylcholine released from a nerve ending initiates the opening of channels in an adjoining cell causing propagation of an action potential. The binding of acetylcholine alters the conformation of the receptor component of the channel so that an ion pathway is opened across the membrane [8].

Secondly, there is voltage-sensitive gating. In this, a component of, or protein companion to, the channel is sensitive to the sign or size of the transmembrane electrical potential difference (p.d.) and changes in this p.d. cause opening or

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

closing of the channel. As a 100 mV p.d. across a 5 nm wide membrane corresponds to a field strength of 2×10^7 V·m^{-1} it is not surprising that fewer than six strategically placed charges are needed to cause this behaviour [1].

Both gating mechanisms are well known to the electrophysiologist studying animal cells [9], but the normal methods by which they are studied (the intracellular microelectrode and patch-clamp techniques) [9] are, so far, of no use in the present study due to the minute size of the bacterial cells involved. On the other hand, permeation studies using vesicles are excellent tools for studying many aspects of porin behaviour save that of gating [2]. The planar bilayer membrane is the obvious tool to employ in investigating this problem but sadly the results so far are rather equivocal. This is largely due to significant differences in the results obtained from the two most popular reconstitution techniques [1,10].

One method utilises the ability of lipid vesicles in solution to form a surface monolayer [4]. Two such monolayers can then be apposed across a small aperture to form a bilayer containing very little solvent [4]. Either purified porin is pre-incorporated into the vesicle phase [10] or a low concentration of *E. coli* outer membrane vesicles is used instead [11]. In both cases the porins are a constituent part of the membrane from its formation but do not show up immediately as channels or increased conductivity values [10,11]. The channels must first be activated by a p.d. of 100 mV whereupon they close at a rate determined by the magnitude of the p.d. The now 'activated' channels will reopen if the p.d. is reduced to zero and close if it is raised to 100–150 mV [10,11]. Hence, after the initial activation, porins reconstituted by this 'monolayer method' show a form of voltage gating.

Using *E. coli* OmpF porin [10,11] the voltage dependence is always seen whether native outer membrane vesicles, aggregated porin or individual porin trimers are used to cause the channel activity [10,11]. The magnitude of the p.d. needed to close the channels is larger if dextrans are bound to the channel aggregates or if a larger initiation voltage has been applied to bilayers formed with outer membrane vesicles [11]. Finally, it should be remembered that most of these reconstitutions involved little or no detergent in the final product [11] and that the monolayer method is assumed to make 'solvent free' bilayers [4].

With *E. coli* maltoporin [7] (lamB, receptor) the monolayer technique has shown evidence for a two-protein mechanism of voltage gating. This porin facilitates maltose and maltodextrin uptake across the outer membrane into the periplasmic space where attachment to maltose-binding protein (MBP) is instrumental in eventual transcytoplasmic membrane transport. Reconstituted into bilayers, maltoporin showed inconsistent channel activity that was stabilised by the addition of MBP. This two-protein system then showed voltage-dependent closing of the channels [7]. The role and structure of maltoporin are, however, sufficiently different from OmpF porin for there to be no immediate need to postulate a binary system in the previous voltage-dependent result. This method has thus shown at least two forms of voltage dependence in porin channels.

The other major method [1,7,12] involves the solubilisation of the purified porins by detergent and their subsequent incorporation into a preformed 'black film' (i.e. a planar bilayer that contains a solvent such as decane). The porins are initially added to the aqueous phase and then partition into the non-polar membrane phase [12] where they cause stepwise increases in the membrane conductivity [1,6,12] consistent with the opening of transmembrane pores. However, the sign or size of this p.d., whilst controlling ion flow rate (current) and direction (polarity), has no effect on the opening or closing of these channels in black films [1,6,12].

The two clear and consistent features of the black film technique are the presence of solvent and detergent, most of the other details having been changed by the many groups who have used this technique. They have varied in the way that the porins are released from the peptidoglycan [1,12] and in the use of different detergents for the solubilisation step [1,3,6,12]. The black films themselves have been formed from most of the major lipid groups [1,12], whilst the porins have originated from many of the commonly studied enteric bacteria [1,3,6,12]. Porin activity has even been demonstrated in soluble periplasmic proteins released from *E. coli* after osmotic shock [1]. The complete lack of porin voltage dependence in black film studies is thus not due to any single one of

these treatments and so the residual solvent and detergent have been regarded as the most likely causes of the differences observed between the two sets of results [7].

Each method has provided useful data on other aspects of these pores and it would certainly be unwise to discount the results of either. What is inescapable, though, is that given identical starting materials the differences must be due to technique.

Fortunately, clarification is afforded by results gained from a third, less well known, technique [13]. This procedure involves the fusion of porin-containing vesicles with a preformed bilayer, thus incorporating features of both previous methods. When using *E. coli* OmpF porin purified similarly to that used in the monolayer technique, it was observed that no initiation voltage was required, vesicle fusion being marked by immediate channel activity. These channels could then be closed by membrane p.d.s of similar size to those necessary in the monolayer reconstitution. However, such gating was also shown after solubilising these porins in detergent (rather than in vesicles) and incorporating them in either solvent containing or solventless films by simple addition to the aqueous phase [13].

This set of results indicates that neither detergent nor solvent per se can be construed as inhibiting an innate voltage gating, whilst the initial activation by voltage appears not to be a constituent feature of these proteins [5,13]. The porins used in this series of experiments would appear to share only one similarity with those which consistently showed voltage dependence in the monolayer method and that is in the purification procedure [10,13]. Hence, purification is a third way (i.e. in addition to detergent and solvent) which may account for the observed differences in voltage sensitivity.

Other evidence that the black film method is capable of reconstituting voltage-dependent channels comes from the accepted voltage gating of mitochondrial porin [1]. This channel, which closes at a p.d. of 35 mV, has been successfully reconstituted by the black film method. Also of interest here is the discovery that 3–5 lysine residues provide the charges for the closing action [1]. It is thus possible that subtle changes affecting as few as three amino acids could be responsible for the observed variation of voltage dependence in the 340-residue OmpF porin. Unfortunately the purification of mitochondrial porin is too dissimilar to that of OmpF to be helpful in this context.

It is clearly not yet possible to decide which of the *E. coli* porin purification methods provides channels most like those occurring in the living bacterium. However, in examining the case for in vivo voltage-gated channels in *E. coli*, one should determine whether the necessary closing voltage of 130 mV can at some stage occur across the outer membrane. The most straightforward approach is to assume that the outer membrane p.d. is a Donnan equilibrium potential as suggested by Stock et al. [14]. It then appears that the required potential would occur when the external salt concentration approaches zero. This indicates a short term protective mechanism rather than accounting for the constant high proportion of closed channels suggested for *P. aeruginosa*. It should be borne in mind that any model of the outer membrane is complicated by the extreme variability of its porin content. For example, it is well known that the anion-selective porins of *E. coli* (PhoE) and *P. aeruginosa* (protein P) are expressed only if external phosphate levels are growth-limiting [5,6]. This complicates not only the ion selectivity of the membrane but also its proposed voltage dependence for it has recently been shown that PhoE has a smaller closing p.d. than OmpF [5]. In addition, when attempting to decide whether the required voltages will occur, a membrane model must include a reasonable estimate for outer membrane electrical resistance [1]. As this is largely a function of total porin conductivity we have returned by a circular argument to our initial problem, namely, how many porins are open?

In conclusion, a careful reappraisal of the purification of *E. coli* OmpF is necessary to complement the current work on its structure [15]. This may enable the voltage-gating process to be clarified and its role in the function of the outer membrane to be more clearly understood.

ACKNOWLEDGEMENTS

The author wishes to thank Drs P.C. Croghan and E.J.A. Lea for their advice and criticism. J.H.L. is an SERC/NATO Postdoctoral Fellow.

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