Volume 278, number 1, 31–34 FEBS 09296 January 1991

ADONIS 001457939100014P

ompC mutants which allow growth on maltodextrins show increased channel size and greater voltage sensitivity

Jeremy H. Lakey[1], Edward J.A. Lea[2] and Franc Pattus[1]

[1]*European Molecular Biology Laboratory, Meyerhofstrasse 1, Postfach 10.2209, D-6900 Heidelberg, Germany* and [2]*School of Biological Sciences, University of East Anglia, Norwich, Norfolk, NR4 7TJ, UK*

Received 29 October 1990; revised version received 16 November 1990

Misra and Benson [(1988) J. Bacteriol. 170, 3611–3617] showed that point mutations in the *ompC* gene can allow *Escherichia coli* to grow on maltotriose in the absence of LamB. This report shows that these mutants produce OmpC porins with increased single channel conductance compared to the wild type. The mutants showed similar voltage dependence to each other and to PhoE by being totally closed at 200 mV. The wild type from various sources was largely insensitive to voltages below 200 mV and thus 6 point mutations at 3 sites appear to increase the voltage dependence of OmpC channels.

OmpC; Dex$^+$ mutant; Voltage dependence; Single-channel studies; Planar bilayers; Thermal stability

1. INTRODUCTION

The permeability properties of the outer membrane of *Escherichia coli* K-12 are largely determined by the complement of porins they contain. There are two general diffusion porins OmpF and OmpC, the relative level of each being determined by the ambient osmolarity. In addition, there exists a range of selective porins, such as LamB, PhoE and Tsx, which enhance the uptake of certain nutrients. PhoE increases the permeability of the outer membrane to anions in general and phosphate in particular, Tsx provides an additional uptake pathway for nucleotides and LamB allows maltodextrins to pass rapidly into the periplasmic space (for review see [1]). Without LamB cells cannot grow on maltodextrins larger than maltotriose [2] as these are larger than the size exclusion limit of either OmpF or OmpC.

Misra and Benson [3] imposed a strong selection pressure on this system by growing an *E. coli* strain expressing solely OmpC on media with maltodextrins as only carbon source. Spontaneous [3] or mutagen induced [4] colonies which grew under these conditions were termed Dex$^+$ mutants. There was a large proportion which involved mutations of the *ompC* gene itself and these were called *ompC*(Dex) mutants. The mutant proteins showed amino acid substitutions, small deletions and small insertions [4,5]. Point mutations of 3 residues (R37, R74 and D105) can specifically alter pore function by increasing both sensitivity to hydrophilic antibiotics and the rate of [^{14}C]maltose uptake. It was thus concluded that these alterations increased the size of the porin channel.

By reconstituting *E. coli* porins into planar lipid bilayers it is possible to measure the conductance of single channels and thus obtain an estimate of their mean diameter [6,7]. In stable membranes at high transmembrane potentials it has been shown that OmpF [6,8] and PhoE [9] are voltage-dependent and that gating occurs in units which correspond to one third of the trimer conductance. The method can thus resolve the voltage-dependent closing of a single porin monomer within these trimeric proteins. We have recently shown that this voltage-sensitive behaviour is independent of the purification or reconstitution method employed [10]. In this paper we observe the behaviour of *ompC*(Dex) mutants in planar bilayers.

2. METHODS

The *ompC*(Dex) strains were generously supplied by R. Misra and S. Benson. A sample of purified OmpC from plasmid pMY150 expressed in *E. coli* B^E was the kind gift of Dr Lucas Buhler. The strain CSH57B was as used previously [11]. The purification of OmpC porin by differential extraction with octyl-polyoxyethylene or SDS was exactly as described earlier for OmpF [10]. The formation of asolectin bilayers, single channel data collection and analysis were as described previously [10]. A standard buffer of 1 M NaCl, 5 mM $CaCl_2$, 10 mM Tris-HCl, pH 7.4 was used throughout. For temperature-stability measurements samples of protein were incubated at a set temperature for 5 min in Laemmli sample buffer before cooling and SDS-PAGE analysis.

Correspondence address: J.H. Lakey, European Molecular Biology Laboratory, Meyerhofstrasse 1, Postfach 10.2209, D-6900 Heidelberg, Germany

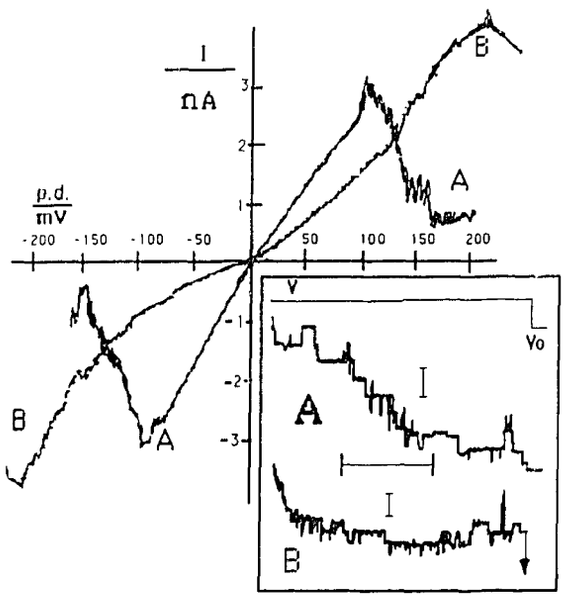

Fig. 1. OmpC porins in asolectin bilayers. Current–voltage curves for A, R37C and B, wild-type. Inset shows current vs time traces for porins in response to applied voltage. Channels close in response to applied voltage. R37C at 120 mV (A) shows almost complete closure whilst in wild-type OmpC at 200 mV (B) only 20% of channels are closed and the baseline at V_0 cannot be shown. Methods as in [9].

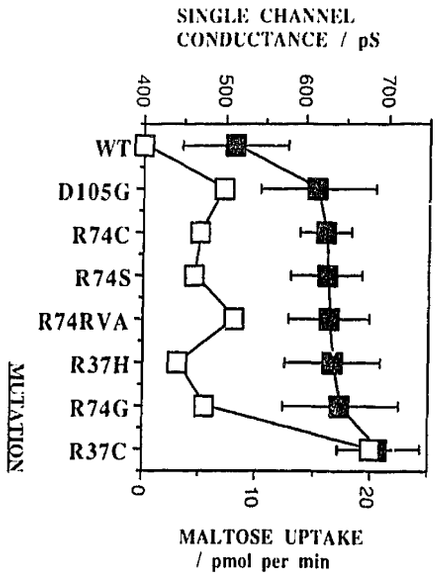

Fig. 2. Mean single channel conductance of OmpC wild-type (WT) and mutants (■); error bars are ±1 standard deviation. Maltose-uptake rates for cells with the indicated mutation (□) are taken from [3].

3. RESULTS

OmpC trimers were incorporated into planar bilayers by injecting detergent solubilised protein into the bathing solution. The appearance of channels was shown by the stepwise increases in current when the bilayers were voltage clamped at 50 mV. When the potential across the bilayer was raised to 100–250 mV the closure of individual monomers provided current steps which were used to measure the single channel conductance (Fig. 1). Relaxing the applied potential reopened the channels allowing the procedure to be repeated and thus the data shown refer to the monomer conductance (Table I, Fig. 2). For all the mutants a voltage of 200 mV completely closed the channels whereas the wild-type RAM105 showed only a small proportion of closure even at 250 mV. This was unexpected as the homologous proteins OmpF and PhoE show largely reproducible voltage sensitivity below 200 mV [10].

The 'wild-type' was also prepared from a second sample of RAM105 and from RAM473 which carries the same gene expressed on a low copy number plasmid. RAM105 was also purified using both the SDS–salt extraction procedure of [12] and the maltoporin purification procedure of [13] which involves a 65°C incubation. In each case no voltage gating under 200 mV was observed. Wild-type OmpC purified by L. Buhler [14] showed voltage gating above

Table I

Strain	Mutation	G (channel conductance)/pS	ΔG pS	n_{obs}	Δ^{a} Å	Voltage[b] sensitivity	Thermal[c] stability
RAM105	wild-type	512 ± 66	0	191	0.0	–	+ + +
RAM272	R37 to H	633 ± 59	119	310	0.96	+	+ + ÷
RAM276	R37 to C	690 ± 51	178	197	2.34	+	+ +
RAM120	R74 to S	626 ± 44	114	191	2.44	+	+ + +
RAM124	R74 to G	643 ± 70	131	136	3.72	+	+ +
RAM270	R74 to C	623 ± 31	121	145	2.34	+	+ + +
RAM280	D105 to G	614 ± 70	102	257	1.99	+	+ +
RAM121	R74 to RVA	629 ± 50	117	217	.??.	+	+

[a] α-carbon to side chain centroid distance from [21]
[b] Voltage sensitivity is defined as (+) when the majority of channels are closed at 200 mV
[c] Thermal sensitivity is defined as (+ + +) when small amounts of monomers occur at 75°C with none at 56°C, (+ +) when mostly trimeric at 75°C and small amounts of monomer at 56°C, (+) when no trimer remains at 75°C with small amounts of monomer at 56°C (see Fig. 3)

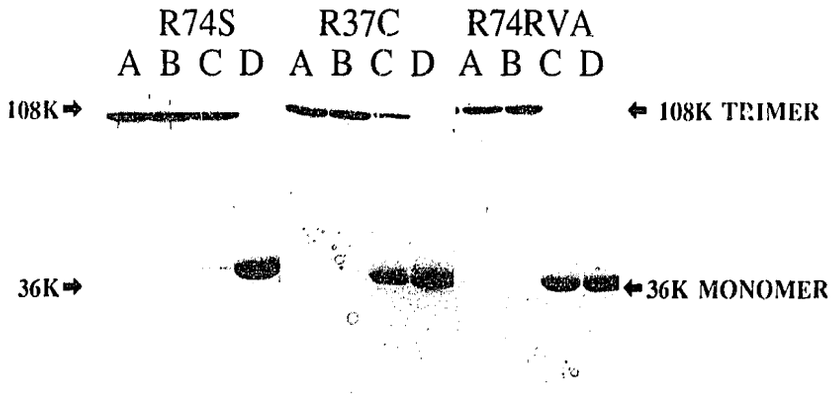

Fig. 3. Three classes of thermal stability. After 5 min at the indicated temperature the proteins were analysed on SDS-PAGE [4]. A = 37°C, B = 56°C, C = 75°C and D = 95°C. For comparison with other mutants see Table I. R74S has wild-type (+ + +), R37C intermediate (+ +) and R74RVA (+) the most unstable behaviour.

180 mV as previously shown [14]. Finally another wild-type strain CSH57B producing only OmpC was purified [11] and showed no closing tendency at all up to 250 mV.

The closing events seen at high potentials with RAM105 gave a value for the wild type conductance which agreed with [14] whilst the *ompC*(Dex) mutants all had significantly higher values (Table I, Fig. 1). R37C was significantly larger (*t*-test statistic <0.001) and D105G significantly smaller ($t < 0.01$) than the other mutants. The remaining mutants were more or less similar. There was no effect on the conductance of any mutant when maltotriose was added to the medium at concentrations which reduce the conductance of maltoporin and it is unlikely that the mutants possess a maltodextrin binding site [15].

The stability of the proteins was investigated by heat denaturation with the appearance of monomers used as a measure of the trimer stability in each mutant (Fig. 3 and Table I). R37C, R74G and D105G were slightly more liable to break down into monomers whilst R74RVA (the only insert mutant) was singularly unstable.

4. DISCUSSION

Na^+, the main charge carrier in these experiments is much smaller than maltodextrins and may not provide a clear idea of how these molecules traverse the channel. Nevertheless R37C had both the highest conductance and the highest growth rates on maltose (Fig. 2) [2,3]. An R to C mutation at R37 had a greater effect than the same change at R74, whilst the conductance at these two points increased as side chains became smaller. The conduction of maltodextrins is probably an all or nothing event depending on whether a certain threshold pore size has been exceeded, hence the relative increase in maltose permeability is expected to be much greater than the Na^+ permeability changes measured here. The bulk of side chains decreases in the order R > H > C > S > G which is the order in which the conductance increases when such substitutions occur at R37 or 74 (Table I). This infers that these side chains constitute part of the lumen of the channel. The aligned sequences of 7 *E. coli* porins (Fig. 4) shows that the mutations occur among residues completely conserved within this group. The mutations produced in

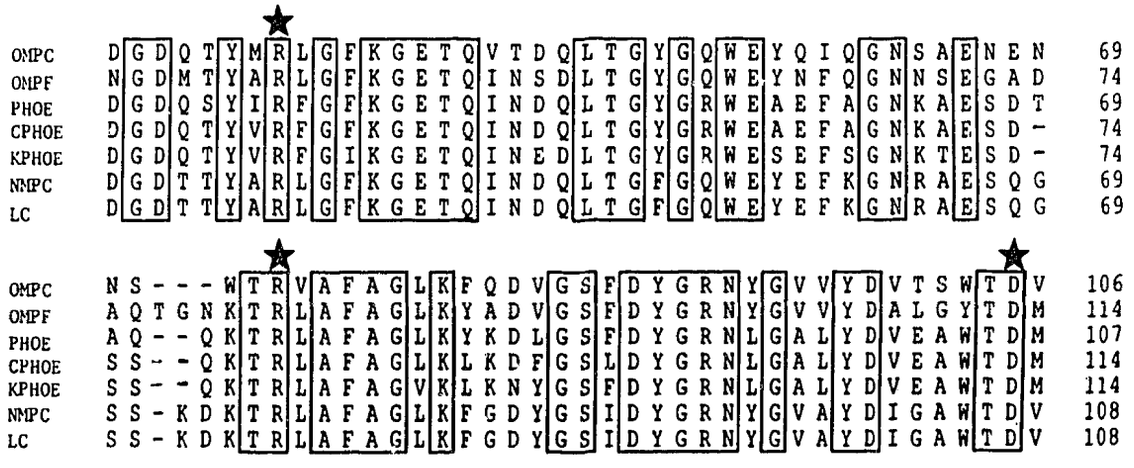

Fig. 4. Partial sequence alignment of *E. coli* and related porins. Fully conserved residues are boxed. ★ = positions of mutations investigated in this study. CPhoE is PhoE porin from *Enterobacter cloacae* and KPhoE is from *Klebsiella pneumoniae* data [19]. Other data are taken from Swissprot Version 15 and [20] for NmpC. Alignment was made using UWGCG programmes.

OmpF by a similar study [16] occurred at these sites and so the formation of larger channels normally involves the replacement of residues which are normally highly conserved and thus subject to strong selection pressure in nature. These mutations increase the penetration of certain antibiotics [2] into the cells and thus the mutated residues may form an important filter in porin proteins. OmpC has a smaller conductance and higher cation selectivity than OmpF [10,14,17] but it appears that the residues responsible for this extra restriction cannot confer an *ompC*(Dex) phenotype by simple substitution.

Some of the mutations reduce trimer stability with the clearest effect being with the insertion mutant, R74RVA. The voltage dependencies of the mutants were indistinguishable from each other within the reproducibilty of the respective *I/V* curves. The wild-type RAM105 was, on the other hand, significantly less voltage-dependent than the mutants or OmpF or PhoE [9]. This effect was investigated carefully by the use of other sources of OmpC but in each case this porin was only voltage-dependent above 200 mV. This is slightly higher than that reported by [14] in which channels begin to close at around 180 mV but even this is markedly different from the mutants. Rocque and McGroarty [4] have reported a change in structure, conductance and gating in an in depth study of the OmpC(Dex) deletion mutant RAM122. The mutations tried so far always affect both conductance and gating and thus the 'size filter' of these channels may also be involved in their voltage dependence. The other possibility is that these residues are generally structurally important and their alteration simply results in a phenotype that is more sensitive to the powerful forces imposed by the transmembrane electric field. It is to be hoped that the imminent resolution of the 3D structure of these proteins will provide some answers [18].

Acknowledgements: The gift of strains by R. Misra and S. Benson is gratefully acknowledged, as is the technical support given by N. Didat in the numerous purifications. J.H.L. and E.J.A.L. thank the European Molecular Biology Organisation (EMBO) and European Molecular Biology Laboratory (EMBL) for fellowships.

REFERENCES

[1] Benz, R. and Bauer, K. (1988) Eur. J. Biochem. 176, 1–19.
[2] Wandersman, C., Schwartz, M. and Ferenci, T. (1979) J. Bacteriol. 140, 1–13.
[3] Misra, R. and Benson, S.A. (1988) J. Bacteriol. 170, 528–533.
[4] Misra, R. and Benson, S.A. (1988) J. Bacteriol. 170, 3611–3617.
[5] Rocque, W.J. and McGroarty, E.J. (1990) Biochemistry 29, 5344–5351.
[6] Schindler, H. and Rosenbusch, J.P. (1978) Proc. Natl. Acad. Sci. USA 75, 3751–3755.
[7] Benz, R., Janko, K., Boos, W. and Läuger, P. (1978) Biochim. Biophys. Acta 511, 305–319.
[8] Schindler, H. and Rosenbusch, J.P. (1981) Proc. Natl. Acad. Sci. USA 78, 2302–2306.
[9] Dargent, B., Hofman, W., Pattus, F. and Rosenbusch, J.P. (1986) EMBO J. 5, 773–778.
[10] Lakey, J.H. and Pattus, F. (1989) Eur. J. Biochem. 186, 303–308.
[11] Lakey, J.H., Watts, J.P. and Lea, E.J.A. (1985) Biochim. Biophys. Acta 817, 208–216.
[12] Nikaido, H. (1983) Methods Enzymol. 97, 85–100.
[13] Neuhaus, J.-M. (1982) Ann. Microbiol. (Inst. Pasteur) 133A, 27–32.
[14] Buhler, L., Ph.D. Thesis, University of Basel.
[15] Dargent, B., Rosenbusch, J.P. and Pattus, F. (1987) FEBS Lett. 220, 136–142.
[16] Benson, S.A., Occi, J.L.L. and Sampson, B.A. (1988) J. Mol. Biol. 203, 961–970.
[17] Benz, R., Schmidt, R. and Hancock, R.E.W. (1985) J. Bacteriol. 162, 722–727.
[18] Weiss, M.S., Wacker, T., Weckesser, J., Welte, W. and Schultz, G.E. (1990) FEBS Lett. 267, 268–272.
[19] Van der Ley, P., Bekkers, A., Van Meersbergen, J. and Tommassen, J. (1987) Eur. J. Biochem. 164, 469–475.
[20] Blasband, A.J., Marcotte, W.R. and Schnaitman, C.A. (1986) J. Biol. Chem. 261, 12723–12732.
[21] Levitt, M. (1976) J. Mol. Biol. 104, 59–107.