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A model of maltodextrin transport through the sugar-specific porin, LamB, based on deletion analysis

Phillip E.Klebba[1,2], Maurice Hofnung and Alain Charbit

Unité de Programmation Moleculaire et Toxicologie Génétique CNRS URA 1444, Institut Pasteur, 25 Rue du Dr Roux, 75015, Paris, France and [1]Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 73019, USA
[2]Present address: Unité de Programmation Moleculaire et Toxicologie Génétique CNRS URA 1444, Institut Pasteur, 25 Rue du Dr Roux, 75015, Paris, France

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LamB facilitates the uptake of maltose and maltodextrins across the bacterial outer membrane and acts as a general porin for small molecules. Using directed deletion mutagenesis we removed several regions of the LamB polypeptide and identified a polypeptide loop that both constricts the maltoporin channel and binds maltodextrins. In conjunction with a second sugar binding site that we identified at the rim of the channel, these data clarify, for the first time, the mechanism of transport through a substrate-specific porin. Furthermore, unlike the transverse loops of general porins, which originate from a central location in their primary structure, the loop that regulates LamB permeability originates from a C-terminal site. Thus LamB represents a second distinct class of porins in the bacterial outer membrane that is differently organized and separately evolved from OmpF-type, general porins.

Key words: intracellular transport/LamB/maltodextrins/membrane proteins/porins

Introduction

Outer membrane porins facilitate and regulate the entry of small molecules into Gram-negative bacteria (Nikaido and Vaara, 1985). Four types of porins have been identified (Nikaido, 1994): non-specific, open channels like the general porin OmpF (Weiss *et al.*, 1991; Cowan *et al.*, 1992; Kreusch *et al.*, 1994); substrate-specific, open channels like maltoporin LamB (Charbit *et al.*, 1988); substrate-specific, ligand-gated channels, like the TonB-dependent porin FepA (Rutz *et al.*, 1992); and monomeric channels, like OmpA (Sugiwara and Nikaido, 1991). The fundamental tertiary structure of porins, a barrel of consecutive anti-parallel β-strands, creates a non-specific, water-filled pore through the outer membrane bilayer. This motif occurs in the crystal structures of the general porins of *Rhodobacter*, *Rhodopseudomonas* and *Escherichia coli* (Weiss *et al.*, 1991; Cowan *et al.*, 1992; Kreusch *et al.*, 1994) and it probably exists in other outer membrane proteins that contain aqueous channels (Luckey and Nikaido, 1980a; Killman *et al.*, 1993; Liu *et al.*., 1993). LamB contains significant sequence divergence relative to OmpF (Nikaido and Wu, 1984), but it also contains some structural features comparable with those of general porins (Charbit *et al.*, 1988; Pauptit *et al.*, 1991). LamB facilitates maltose and maltodextrins uptake at low sugar concentrations (Luckey and Nikaido, 1980b; Benz *et al.*, 1986, 1992). Several models of LamB secondary structure have been postulated (Charbit *et al.*, 1988; Shirmer and Cowan, 1993; Jeanteur *et al.*, 1994), but its channel architecture and transport mechanism are unknown.

To elucidate the organization of the LamB channel we deleted surface loops or proposed transmembrane strands of the maltoporin polypeptide (Figure 1) and then measured the transport and recognition functions of the resulting mutant proteins: specific sugar binding and transport, non-specific transport and bacteriophage and antibody adsorption. These experiments identified two features that lead us to propose, for the first time, a mechanism for the action of a substrate-specific porin: first, a maltodextrin binding site at the outer rim of the LamB channel; second, a dominant functional domain near the C-terminus that both regulates the non-specific entry of molecules into the pore and binds maltose and maltodextrins.

Results

Site-directed deletion mutagenesis reveals dual maltose binding sites and a transverse loop within maltoporin

In addition to the transport of solutes, bacterial outer membrane proteins serve as recognition sites for a variety of noxious agents, including antibodies and bacteriophage. LamB, for example, was named as the cell surface receptor for bacteriophage λ (Thirion and Hofnung, 1972). Point mutations that confer resistance to λ mainly occur in three separate, hydrophilic regions of the protein (Hofnung *et al.*, 1976; Charbit *et al.*, 1988; Francis *et al.*, 1991a; Werts *et al.*, 1994) that have been postulated as cell surface loops (Charbit *et al.*, 1988; Shirmer and Cowan, 1993; Jeanteur *et al.*, 1994). Using site-directed deletion mutagenesis we individually eliminated these three loops [designated P (Δ148–165), C (Δ239–263) and D (Δ376–405) in Figure 1] and in each case the deletions abolished infection by phage λ (Table I). However, LamBΔD maintained the ability to act as receptor for extended host range mutants of λ (Hofnung *et al.*, 1976), demonstrating the dispensability of the D loop for infection by these phages and strongly suggesting that ΔD did not disrupt LamB tertiary and quaternary structure. Furthermore, LamBΔD weakly bound starch, but it neither transported maltose (Figure 2) nor facilitated growth on maltodextrins larger than triose (Table I). Finally, ΔD changed the non-specific permeability of maltoporin: it notably increased the diffusion of large molecules through the outer membrane.

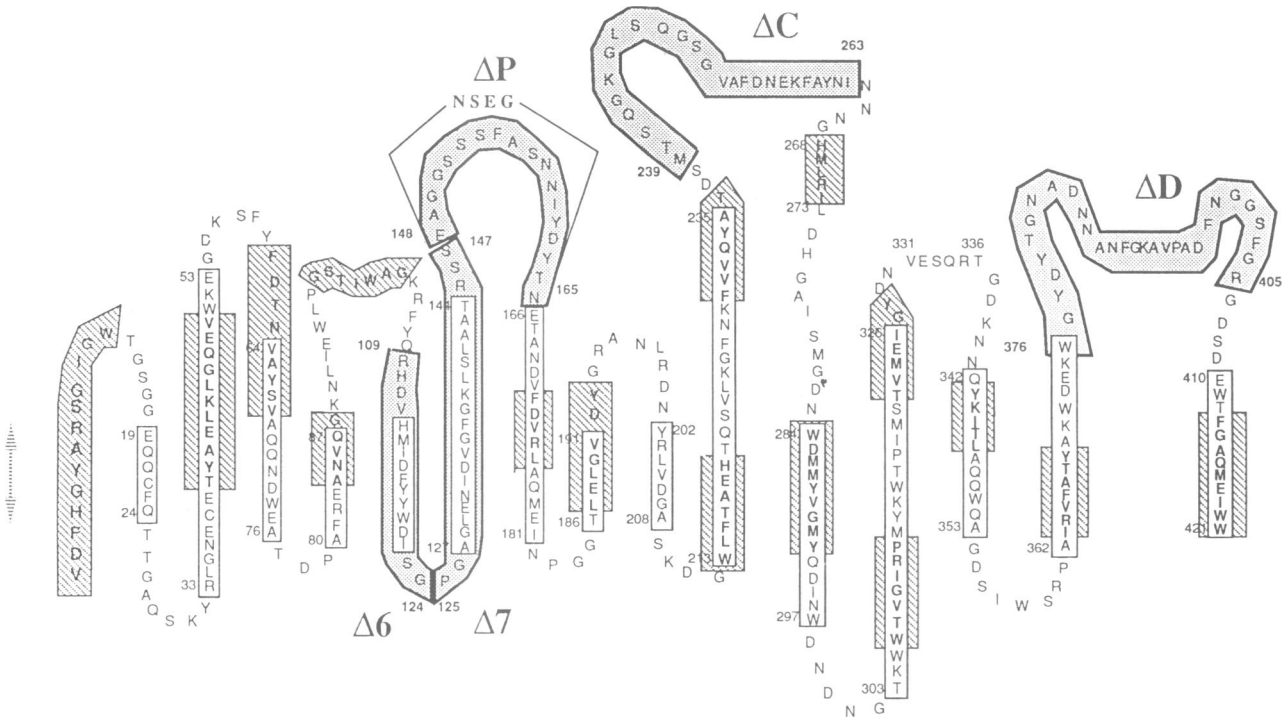

Fig. 1. Location of site-directed deletions within the proposed secondary structure of *E.coli* LamB. Five deletions, named 6 (Δ108–124), 7 (Δ125–148), P (Δ149–165), C (Δ239–263) and D (Δ379–405), displayed here in a model of LamB secondary structure (Charbit *et al.*, 1988), were constructed by mutagenesis of *lamB*. The model of LamB structure shows potential transmembrane strands (rectangles), residues designated as transmembrane strands in another postulated structure (striped; Schirmer and Cowan, 1993) and residues removed by directed deletion mutagenesis (shaded).

Table I. Properties of LamB deletion mutants

LamB mutants	λ phage			Maltodextrins										Antibiotic susceptibility							External MAbs		Internal MAbs	
	λ	h	hh*	1	2	3	4	5	6	7	Mc	M*	S	Cs	T	N	E	Ra	B	V	177	302	141	436
pop6510 *lamB*	R	R	R	+	+	−	−	−	−	−	W	3	3	18	14	14	9	R	R	R	−	−	−	−
PAC 1 *lamB*+	S	S	S	+	+	+	+	+	+	+	Re	100	100	18	16	14	11	R	R	R	+	+	+	+
P(Δ149–165)	R	R	R	+	+	+	+	+	+	+	Re	**16**	1	18	16	14	12	R	R	R	+	+	+	+
C(Δ239–263)	R	R	R	+	+	+	+	+	+	+	Re	**11**	1	19	16	14	11	R	R	R	+	+	+	+
PC	R	R	R	+	+	+	+	+	−	−	Re	**65**	**107**	19	16	14	11	R	R	R	+	+	+	+
D(Δ379–405)	R	S	S	+	+	+	−	−	−	−	P	3	**8**	18	16	14	**21**	**12**	**15**	**15**	−	−	+	+
PD	R	R	R	+	−	+	−	−	−	−	P	2	3	18	15	14	**21**	**17**	**18**	**19**	−	−	+	+
CD	R	R	R	+	−	−	−	−	−	−	P	2	2	18	15	14	**21**	**15**	**16**	**16**	−	−	+	+
PCD	R	R	R	+	−	−	−	−	−	−	P	1	3	18	16	14	**21**	**18**	**16**	**16**	−	−	+	+
6(Δ108–124)	R	R	R	+	−	−	−	−	−	−	W	2	3	19	15	14	15	10	R	10	−	−	−	−
7(Δ125–148)	R	R	R	+	−	−	−	−	−	−	W	2	2	19	16	14	15	10	R	R	−	−	−	−
6–7	R	R	R	+	−	−	−	−	−	−	W	2	2	18	nd	14	15	9	R	R	−	−	−	−

Phage sensitivity tests (S, sensitive; R, resistant) were performed with phage λ and its host range mutants. Growth on maltodextrins at 0.05% was tested with glucose, maltose and maltodextrins triose through heptose (labeled 1–7). Bacteria were also streaked on MacConkey agar (Mc: Re, red; P, pink; W, White) containing dextrins (Pfanstiehl; primarily maltotriose) as sole carbon source. [^{14}C]Maltose uptake (M*; Charbit *et al.*, 1988) and starch binding (S; Francis *et al.*, 1991b) were measured and are expressed as a percentage of wild-type activity. Susceptibility to antibiotics is expressed as the diameter in millimetres of the zone of growth inhibition. The amounts of the compounds tested and their molecular masses were as follows: cycloserine (Cs, 100 μg, 320 Da), tetracycline (T, 30 IU, 444 Da), neomycin (N, 30 IU, 614 Da), erythromycin (E, 15 IU, 734 Da), rifamycin (Ra, 30 μg, 823 Da), bacitracin (B, 10 IU, 1421 Da) and vancomycin (V, 30 μg, 3.3 kDa). At high concentrations (0.6%), sugars as large as maltohexose supported growth of bacteria expressing ΔD-containing LamB proteins (data not shown), confirming the increase in non-specific permeability conferred by these mutant porins (Misra and Benson, 1988). The tertiary structure of the mutant proteins was assessed by recognition of cell surface (external) and periplasmic (internal) epitopes by monoclonal antibodies (Charbit *et al.*, 1991). All monoclonal antibodies to external surface determinants of LamB bind epitopes that lie in the D loop, therefore, mutants carrying ΔD are not recognized by these antibodies.

These data imply that the D loop restricts non-specific entry into the LamB channel and also binds maltodextrins within the pore, thereby facilitating their specific transport.

The P and C loops together comprise a maltodextrin binding site, as evidenced by the starch binding of LamBΔD and the inability of LamB proteins with single deletions of either P or C to bind starch. Although ΔP and ΔC reduced maltose uptake rates ~10-fold (Figure

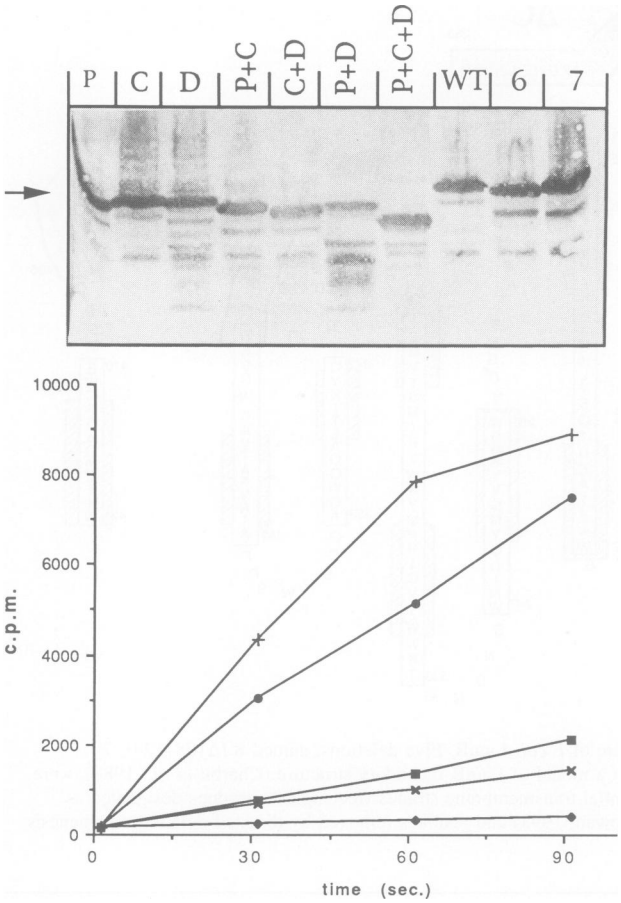

Fig. 2. [^{14}C]Maltose uptake by LamB and LamB deletion mutants. Bacteria were cultured in M63 minimal media and washed. Top: whole cell lysates were assayed for the presence of LamB proteins by Western immunoblot (Werts *et al.*, 1993) with polyclonal anti-LamB sera. The arrow indicates the position of the denatured LamB monomer. See text for explanation of the various deletion mutants. Δ6–7 is not shown, but equivalent expression was observed in other experiments. Bottom: the initial rates of [^{14}C]maltose transport (Charbit *et al.*, 1988) were also measured: ◆, LamB$^-$; +, LamB$^+$; ■, ΔP; ×, ΔC; ●, ΔPC. Maltose uptake by other lamB mutants was equivalent to the LamB$^-$ strain pop6510 (Table I). The data were averaged from two separate experiments.

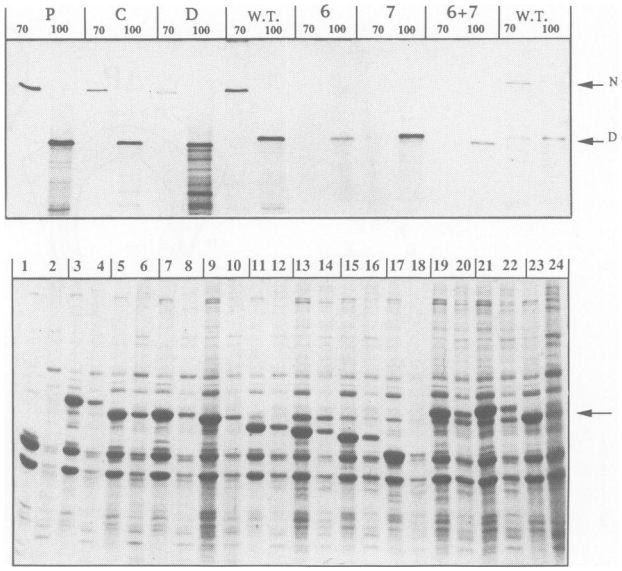

Fig. 3. Expression, localization in the cell envelope and trimer formation of LamB deletion mutants. Pop6510/pAC1 with *lamB* deletion alleles was grown as in Figure 2. Top: LamB proteins were immunoprecipitated and analysed by SDS–PAGE (Werts *et al.*, 1993) to evaluate their quaternary structure at 70 or 100°C. The contents of the lanes are indicated. Note the presence of native LamB trimers (N) at 70°C for ΔP, ΔC and ΔD, but not Δ6, Δ7 and Δ6–7. Trimers were also observed for ΔPC, ΔPD, ΔCD and ΔPCD (data not shown), but the removal of multiple surface loops reduced the efficiency of the immunoprecipitation, even though polyclonal antisera were utilized. Bottom: outer membranes (odd lanes) and inner membranes (even lanes) were prepared (Klebba *et al.*, 1990) and analysed by SDS–PAGE, stained with Coomassie blue. Lanes 1 and 2, LamB$^-$; 3 and 4, LamB$^+$; 5 and 6, ΔP; 7 and 8, ΔC; 9 and 10, ΔD; 11 and 12, ΔPC; 13 and 14, ΔPD; 15 and 16, ΔCD; 17 and 18, ΔPCD; 19 and 20, Δ6; 21 and 22, Δ7; 23 and 24, Δ6–7.

2), the resulting mutant proteins still allowed enough maltodextrin uptake (maltotriose to maltoheptose) to support growth (Table I), through the action of the second, stronger maltodextrin binding region in D. Neither the P nor the C loop affected non-specific permeability through maltoporin as did the D loop (Table I). LamB proteins with either ΔP or ΔC conferred non-specific permeability that was indistinguishable from that of wild-type LamB.

Combined deletions confirm the prominence of the D loop in maltoporin function

As was observed for ΔD, both ΔP and ΔC were well tolerated within the LamB tertiary and quaternary structure, either singly or in combinations. The P, C and D deletions and the multiple deletions PC, PD, CD and PCD all formed trimeric porins that localized to the outer membrane at expression levels that were indistinguishable from wild-type LamB (Figure 3). Their native structures were properly recognized by polyclonal antisera during immunoprecipitations (Figure 3) and their epitopes were correctly localized by monoclonal antibodies to the appropriate surfaces of the outer membrane (Table I). The combined deletions reiterated the findings that resulted from their individual analysis: deletions involving only P and C did not alter non-specific permeability through LamB, but any genetic constructions including ΔD opened the maltoporin channel to the passage of large molecules, up to 3.3 kDa (vancomycin in Table I). The enhanced permeability of such mutant LamB proteins was confirmed by the non-specific diffusion of large maltodextrins through them. This phenomenon is distinct from the specific facilitation by LamB of maltose and maltodextrin transport at low concentrations. At high concentrations (0.6%) maltotetraose, maltopentaose and maltohexaose supported the growth of bacteria carrying D deletions in LamB (data not shown), as has been observed previously for L3 loop deletions of OmpF (Misra and Benson, 1988). One last finding with the combined mutations illuminated the pivotal role of the D loop in regulating permeability through the LamB pore: strains expressing the PC deletion, which leaves the D loop intact, showed wild-type susceptibility to antibiotics, starch binding, maltose uptake (65%) and growth on maltodextrins. Thus even in the absence of the two major surface loops, P and C, maltoporin functioned normally, but subsequent deletion of the D loop (PCD in Table I) eliminated all specific functions and transformed LamB into a large, non-specific channel. These data illustrate the unforeseen resilience of LamB to multiple deletions of surface polypeptides. Either singly

or in combinations, we removed three disparate loops that together contain over 70 amino acids without disrupting the native quaternary structure of the maltoporin trimer, but with markedly different effects on the function of the maltoporin channel.

LamB channel organization differs from that of general porins

A prominent feature of general, OmpF-type porins is a polypeptide loop (L3, the third loop in the primary structure) that extends across their respective channels, diminishing the effective size of the openings (Weiss *et al.*, 1991; Cowan *et al.*, 1992; Kreusch *et al.*, 1994). This approximately 30 residue sequence, termed the L3, transverse or eyelet loop, is a conserved feature among related porins within the OmpF family and may be a hallmark structural determinant of these proteins. One model of LamB secondary structure (Schirmer and Cowan, 1993) suggests that maltoporin contains an eyelet loop at an analogous, central location in the primary structure. Other concepts of LamB folding designate the same region as a pair of transmembrane strands (Figure 1). To test these alternatives we constructed deletions 6 (Δ108–124), 7 (Δ125–147) and 6–7 (Δ108–147). Deletions in the OmpF L3 loop greatly increased the permeability of its channel (Misra and Benson, 1988), without compromising the structure of the OmpF trimer (Klebba *et al.*, 1990). However, neither Δ6, Δ7 nor Δ6–7 created comparable phenotypes in LamB. These mutations did not affect LamB expression (Figures 2 and 3), but they destroyed the native structure of maltoporin. From λ phage sensitivity tests, immunochemical analyses and maltodextrin utilization and uptake studies, the resulting mutant LamB proteins did not assemble into trimers, did not fold properly in the outer membrane bilayer (from immunochemical characterization) and did not facilitate growth on, binding of or uptake of maltodextrins (Figures 2 and 3 and Table I). In general, Δ6, Δ7 and Δ6–7 did not resemble deletions in the OmpF transverse loop. Rather, their properties support the idea that Δ6 and Δ7 remove transmembrane strands of the LamB barrel: similar results have been observed for deletions that eliminate single strands of the PhoE porin (Bauer *et al.*, 1989) or an odd number of strands of the FepA porin (Rutz *et al.*, 1992). The slight increase in permeability observed in strains expressing such deletions is difficult to interpret in the light of the inability of the mutant proteins to trimerize and their improper folding. It may reflect some retention of pore formation or unspecific membrane perturbation by the resulting monomeric proteins, which at least fractionate with the outer membrane during sedimentation through sucrose gradients (Figure 3).

Discussion

Deletion of the D loop of LamB transformed maltoporin into a non-specific channel. This finding defines the relationship of LamB to general porins like OmpF. Although the LamB and OmpF sequences are only distantly related (Nikaido and Wu, 1984), they create pores that are generally similar: the D loop of LamB restricts the channel of maltoporin, as the L3 loop restricts the channel of general porins. Yet the evolutionary distance between LamB and general porins appears in their specific channel architectures: the D loop of LamB differs substantially in sequence from L3 of OmpF, originates from a different relative position in the primary structure and contains elements that are exposed at the cell surface, since they are recognized by bacteriophage and antibodies. The L3 loop of OmpF, on the other hand, which is stabilized by electrostatic interactions within the channel (Cowan *et al.*, 1992), is not accessible at the cell surface to antibodies that recognize it (Klebba *et al.*, 1990). Also unlike general porins, the LamB D loop adds binding specificity for maltodextrins, which accounts for most of maltoporin's specialized transport properties. So, although LamB and OmpF-type porins may have descended from a common, β-barrel-containing ancestral porin, they constitute two separate manifestations of a single mechanistic theme: bacteria regulate permeability through outer membrane porins by the evolution of peptide loops that restrict channel permeability and, as exemplified by maltoporin, provide specificity for substrates.

Our results suggest an explanation for the maltodextrin-specific diffusion that LamB facilitates. Starch adsorption by both LamBΔD and LamBΔPC (Table I) showed the presence of two independent dextrin binding sites within maltoporin. Sugars adsorb first to a binding site at the rim of the channel, formed by the P and C surface loops (Figure 4). This initial binding by the P and C loops agrees with their known role as a selective filter for maltodextrins (Dargent *et al.*, 1988). Localization of sugars at this site within the channel vestibule favours their subsequent interaction with a second, stronger binding region in the D loop at the constriction of the pore. The establishment of this latter binding within the channel probably orients linear maltodextrins and facilitates their passage into the periplasm by diffusion. Proper orientation of linear maltodextrins (larger than triose) may be critical to their passage through the pore, because the Stokes'

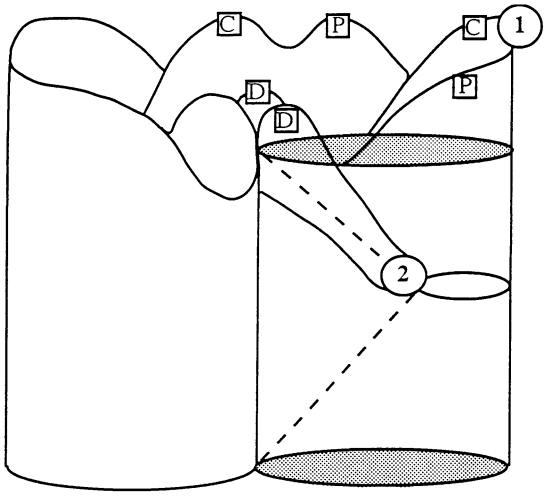

Fig. 4. Deduced model of the maltoporin channel and its transport mechanism. The presence of two substrate binding domains, one situated at the rim of the channel (1) and the other at the constriction of the pore (2), promotes in at least two steps the flux of sugars into and through maltoporin, across the outer membrane.

radius of such sugars exceeds the estimated dimensions of the maltoporin channel (Luckey and Nikaido, 1980a). Our results do not exclude other sites within the LamB channel, distinct from the D loop, from maltodextrin binding that facilitates transport. Finally, further argument for this mechanism could come from *in vitro* confirmation of the phenotypes of the mutant LamB proteins we have generated, by analysis of sugar binding to the purified proteins and their reconstitution in liposomes and lipid bilayers.

LamB proteins lacking one of the P or C loops, but retaining the other, did not bind starch. These results may be explained by the idea that the P and C loops function together to form a dextrin binding site and extend beyond the D loop on the cell surface, which is consistent with their function in λ phage adsorption (see Werts *et al.*, 1994, and references therein). According to this view, single deletions of either ΔP or ΔC disrupt both the initial dextrin binding site and the conformation of the remaining isolated loop, such that it sterically hinders access of maltodextrins to the second binding site in D. In LamBΔPC, on the other hand, the elimination of both superior loops allows direct access of starch to the D loop.

Materials and methods

Bacterial strains and plasmids

All phenotypic assays were performed on the LamB$^-$ strain pop6510, containing derivatives of the pBR322 plasmid pAC1 (Werts *et al.*, 1994) with various *lamB* alleles, which are described below.

Directed deletion mutagenesis

lamB was mutagenized on bacteriophage M13 (Kunkel, 1989). Single-stranded oligonucleotide primers containing the mutations were synthesized and incorporated into *lamB*. The mutations were transferred to *lamB* on the pBR322 derivative pAC1 (Charbit *et al.*, 1988) by restriction endonuclease excision from the replicative form of M13*lamB* and insertion into similarly restricted pAC1. Combinations of the individual mutations were also generated (Table I). The nucleotide sequences of each of the M13 constructions and their pAC1 subclones were determined (Sequenase; US Biochemical Corp., Cleveland, OH). The boundaries of the deletions were engineered into known surface domains of LamB containing strongly predicted β-turns (Wilmot and Thornton, 1989). In all cases except that of ΔP, the codons GCATGC, which introduce a *Sph*I site into *lamB* and when translated the residues Ala–Cys into LamB, were incorporated at the site of the deletion. In ΔP, a known β-turn (NSEG, residues 69–72 of OmpF) was inserted at the site of the deletion.

Specific functions of LamB: bacteriophage adsorption and sugar binding and transport

Phage sensitivity tests and host range mutants of λ have been previously described (Werts *et al.*, 1994). For maltose transport assays, the *lamB* strain pop6510 (Charbit *et al.*, 1988), either without plasmid or carrying *lamB*$^+$ or *lamB* deletion alleles on pAC1 (Charbit *et al.*, 1988) was utilized. The initial rates of [^{14}C]maltose transport (Charbit *et al.*, 1988) were measured relative to wild-type LamB, expressed from pAC1. Growth on maltodextrins was measured on M63 minimal plates (Misra and Benson, 1988) containing 0.05% (L) sugars; glucose, maltose and maltodextrins triose through heptose (labeled 1–7) were tested. Bacteria were also assayed for maltodextrin utilization on MacConkey agar (Mc: Re, red; P, pink; W, white) containing dextrins (Pfanstiehl; primarily triose) as sole carbon source.

Non-specific permeability through LamB: antibiotic sensitivity and maltodextrin diffusion

To determine non-specific permeability through LamB, bacteria expressing the mutations were tested for sensitivity to antibiotics that are too large to penetrate through OmpF-type pores (Misra and Benson, 1988; Rutz *et al.*, 1992). Susceptibility is expressed as the diameter in millimetres of the zone of growth inhibition. As a further measure of non-specific permeability, the diffusion of large maltodextrins (maltotetraose through maltoheptaose) was measured at high concentrations (0.6%; Misra and Benson, 1988).

Immunological characterization of LamB proteins

Immunoprecipitation of LamB proteins and subsequent analysis by SDS–PAGE was performed (Charbit *et al.*, 1991) to evaluate their quaternary structure at 70 or 100°C. The tertiary structure of the mutant proteins was also assessed by recognition of cell surface (external) and periplasmic (internal) epitopes by monoclonal antibodies (26) in enzyme-linked immunosorbent assays (ELISA). All monoclonal antibodies to external surface determinants of LamB bind epitopes that lie in the D loop. Therefore, mutants carrying ΔD are not recognized by these antibodies.

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References

Bauer,K., Struyve,M. Bosch,D. Benz,R. and Tommassen,J. (1989) *J. Biol. Chem.*, **264**, 16393–16398.
Benz,R., Schmid,A., Nakae,T. and Vosscheperkeuter,G.H. (1986) *J. Bacteriol.*, **165**, 978–986.
Benz,R., Francis,G., Nakae,T. and Ferenci,T. (1992) *Biochim. Biophys. Acta*, **1104**, 299–307.
Charbit,A., Gehring,K., Nikaido,H., Ferenci,T. and Hofnung,M. (1988) *J. Mol. Biol.*, **201**, 487–496.
Charbit,A., Ronco,J., Michel,V., Werts,C. and Hofnung,M. (1991) *J. Bacteriol.*, **173**, 262–275.
Cowan,S.K., Schirmer,T., Rummel,G., Steiert,M., Gosh,R., Pauptit,R.A., Jansonius,J.N. and Rosenbusch,J.P. (1992) *Nature*, **358**, 727–733.
Dargent,B., Charbit,A., Hofnung,M. and Pattus,F. (1988) *J. Mol. Biol.*, **201**, 497–506.
Francis,G., Brennan,L., Stretton,S. and Ferenci,T. (1991a) *Mol. Microbiol.*, **5**, 2293–2301.
Francis,G., Brennan,L. and Ferenci,T. (1991b) *Biochim. Biophys. Acta*, **1067**, 89–96.
Hofnung,M., Jezierska,A. and Braun-Breton,C. (1976) *Mol. Gen. Genet.*, **145**, 207–213.
Jeanteur,D., Lakey,J.H. and Pattus,F. (1994) In Guysen,J.R. and Hackenbeck,R. (eds), *Bacterial Cell Wall*. Elsevier Science, New York, NY, pp. 363–380.
Killman,H., Benz,R. and Braun,V. (1993) *EMBO J.*, **12**, 3007–3016.
Klebba,P.E., Benson,S.A., Bala,S., Abdullah,T., Reid,J., Singh,S.P. and Nikaido,H. (1990) *J. Biol. Chem.*, **265**, 6800–6810.
Kreusch,A., Neubuser,A., Schlitz,E., Weckesser,J. and Schulz,G.E. (1994) *Protein Sci.*, **3**, 58–63.
Kunkel,T.A. (1989) *Proc. Natl Acad. Sci. USA*, **82**, 488–492.
Liu,J., Rutz,J.M., Feix,J. and Klebba,P.E. (1993) *Proc Natl Acad. Sci. USA*, **90**, 10653–10657.
Luckey,M. and Nikaido,H. (1980a) *Proc. Natl Acad. Sci. USA*, **77**, 167–171.
Luckey,M. and Nikaido,H. (1980b) *Biochem. Biophys. Res. Commun.*, **93**, 166–171.
Misra,R. and Benson,S.A. (1988) *J. Bacteriol.*, **170**, 3611–3617.
Nikaido,H. (1994) *J. Biol Chem.*, **269**, 3905–3908.
Nikaido,H and Vaara,M. (1985) *Microbiol. Rev.*, **49**, 1–32.
Nikaido,H. and Wu,H. (1984) *Proc. Natl Acad. Sci. USA*, **81**, 1048–1052.
Pauptit,R.A., Schirmer,T., Jansonius,J.N., Rosenbusch,J.P., Parker,M.W., Tsernoglou,D., Weiss,M.S. and Schulz,G.E. (1991) *J. Struct. Biol.*, **107**, 136–145.
Rutz,J.M., Liu,J., Lyons,J.A., Goranson,J., Armstrong,S.K., McIntosh,M.A., Feix,J.B. and Klebba,P.E. (1992) *Science*, **258**, 471–475.
Schirmer,T. and Cowan,S.W. (1993) *Protein Sci.*, **2**, 1361–1363.
Sugiwara,E., and Nikaido,H. (1991) *J. Biol. Chem.*, **267**, 2507–2511.
Thirion,J.-P. and Hofnung,M. (1972) *Genetics*, **71**, 207–216.

Weiss,M.S., Abele,U., Weckesser,J. Welte,W., Schiltz,E. and Schulz,G.E. (1991) *Science*, **254**, 1627–1630.
Werts,C. O'Callaghan,D., Hofnung,M. and Charbit,A. (1993) *J. Gen. Microbiol.*, **139**, 881–887.
Werts,C., Michel,V., Charbit,A. and Hofnung,M. (1994) *J. Bacteriol.*, **176**, 941–947.
Wilmot,C. and Thornton,J. (1989) *J. Mol. Biol.*, **203**, 221–232.

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