Mol Gen Genet (1992) 233:372–378

MGG

DNA sequence analysis of the *lamB* gene from *Klebsiella pneumoniae*: implications for the topology and the pore functions in maltoporin

Catherine Werts, Alain Charbit, Sophie Bachellier, and Maurice Hofnung

Unité de Programmation Moléculaire et Toxicologie Génétique, CNRS URA 1444 Institut Pasteur, 25 rue du Dr. Roux, F–75015 Paris, France

Received October 18, 1991 / January 16, 1992

Summary. We have determined the sequence of the *lamB* gene from *Klebsiella pneumoniae*. It encodes the precursor to the LamB protein, a 429 amino acid polypeptide with maltoporin function. Comparison with the *Escherichia coli* LamB protein reveals a high degree of homology, with 325 residues strictly identical. The N-terminal third of the protein is the most conserved part of the molecule (1 change in the signal sequence, and 13 changes up to residue 146 of the mature protein). Differences between the two mature proteins are clustered mainly in six regions comprising residues 145–167, 173–187, 197–226, 237–300, 311–329, and 367–387 (*K. pneumoniae* LamB sequence). The most important changes were found in regions predicted by the two-dimensional model of LamB folding to form loops on the cell surface. In vivo maltose and maltodextrin transport properties of *E. coli* K12 and *K. pneumoniae* strains were identical. However, none of the *E. coli* K12 LamB-specific phages was able to plaque onto *K. pneumoniae*. Native *K. pneumoniae* LamB protein forms highly stable trimers. The protein could be purified by affinity chromatography on starch-Sepharose as efficiently as the *E. coli* K12 LamB protein, indicating a conservation of the binding site for dextrins. However, none of the monoclonal antibodies directed against native *E. coli* K12 LamB protein recognized native purified *K. pneumoniae* LamB protein. These data indicate that most of the variability occurs within exposed regions of the protein and provide additional support for the proposed model of LamB folding. The fact that the N-terminal third of the protein is highly conserved is in agreement with the idea that it is part of, or constitutes, the pore domain located within the transmembranous channel and that it is not accessible from the cell surface.

Key words: LamB – *Klebsiella pneumoniae* – Integral membrane protein – Protein topology

Correspondence to: C. Werts

Introduction

In *Escherichia coli* K12, LamB (also called lambda receptor, or maltoporin) is a trimeric integral outer membrane protein. It constitutes pores specifically involved in the diffusion of maltose and maltodextrins across the outer membrane (Charbit et al. 1988 and references therein). LamB also serves as a cell surface receptor for a number of phages including phage lambda (Charbit and Hofnung 1985). In the absence of precise crystallographic data on the three-dimensional structure of the protein, most of our knowledge of LamB organization comes from genetic, immunological, and biochemical approaches. On this basis we have proposed a two-dimensional model of LamB folding in which the protein spans the membrane 16 times (Charbit et al. 1988, 1991; Fig. 3), with loops protruding on either side of the lipid bilayer. This model is compatible with the recently published structure of porin from *Rhodobacter capsulatus*, a trimeric integral membrane protein consisting of three 16-stranded β-sheet barrels (Weiss et al. 1990).

The presence of a maltose-inducible protein homologous to the LamB protein from *E. coli* K12 in the outer membrane of *Klebsiella pneumoniae* has been reported previously (Pick and Wöber 1979). Gene transfers between *E. coli* and *K. pneumoniae* strains showed that LamB proteins from both species could serve as maltodextrin porins. However, only the LamB protein from *E. coli* could confer phage lambda sensitivity, and that also when expressed in *K. pneumoniae* (de Vries et al. 1984; Wehmeier et al. 1989). Moreover, immunological cross-reactivities between these two proteins have been demonstrated (Bloch and Desaymard 1985), suggesting a high degree of homology between the two polypeptide sequences.

Sequences of the *lamB* genes from *E. coli, Shigella sonnei,* and *Salmonella typhimurium* have been previously determined (Clément and Hofnung 1981; Roessner and Ihler 1987; Francoz et al. 1990). Comparison of their deduced amino acid sequences revealed a high degree of homology. *K. pneumoniae* is less closely related to *E. coli*

than *S. sonnei* and *S. typhimurium*, as shown by comparisons of chromosomal DNAs by hybridization methods (Brenner 1978). Therefore, in order to obtain additional information on LamB variability we decided to determine the sequence of the *lamB* gene from *K. pneumoniae*. The deduced peptide sequence was compared to the three other LamB sequences. Some of the biological, biochemical and immunological properties of the LamB protein from *K. pneumoniae* $LamB_{Kp}$ were also studied.

Materials and methods

Bacteria, phages, media, and chemicals. K. pneumoniae strain KAY 2026 : F^- *arg*$^-$ *gua*$^-$ $P1^s$ (Sprenger and Lengeler 1984) was used as a source of *lamB* DNA, as well as for LamB protein purification. *E. coli* strains P4X8 and pop6510 (Charbit et al. 1991) and *S. typhimurium* smooth SL3770 and rough SL3749 strains (Roantree et al. 1977) were used as controls in transport experiments and immunological comparisons.

Phage λ with a wild-type host range and its mutants with extended host range (λh° and λhh*), and phages AC6, AC7, AC28, AC30, AC43, AC50, AC57, AC81, AC95, 21EL, K10, also using LamB from *E. coli* as a specific receptor, were described previously (Braun-Breton and Hofnung 1981; Charbit and Hofnung 1985). Sensitivity to phages was assayed by the spot test procedure on lawns of the different strains plated onto ML plates.

Media, chemicals, and growth conditions were as described previously (Charbit et al. 1991).

DNA techniques. The *lamB* gene from *K. pneumoniae* was originally cloned by M. Dahl (personal communication) into a phagemid, λSE6, derived from λ (Elledge and Walker 1985), after partial digestion of chromosomal DNA with *Sau*3A and insertion into the *Bam*HI site of the λSE6 vector. The recombinant phage library was further screened for complementation of an *E. coli* Δ*malE* strain. The complementing SE6/K1 recombinant lambda phage contains a 15 kb *Sau*3A DNA insert (Fig. 1).

Nine oligonucleotides, 20–29 bases in length, were used as primers for DNA sequence analysis. They were synthesized on a Milligen/Bioresearch Cyclone Plus DNA synthesizer (Millipore). The universal M13 reverse primer (rp:17mer −40 from Pharmacia) was also used. Single-stranded phage DNAs were prepared using standard techniques (Sambrook et al. 1989). DNA sequencing was performed using dideoxy chain terminators with a Sequenase Version 2 kit (USB, Cleveland, Ohio). The *lamB* gene sequence was determined on both strands using a step-wise strategy which consisted in moving the priming site for the extension-labelling reactions along the *lamB* DNA in a progressive fashion. Both DNA strands have been sequenced in an overlapping fashion.

Purification of the LamB protein from K. pneumoniae. Purification was conducted in two steps: LamB was solubilized from the outer membrane using the standard procedure described in Gabay and Yasunaka (1980). After concentration by ethanol precipitation, the LamB preparation was resuspended in 2% Triton X100. LamB was then further purified by affinity chromatography on a starch-epoxy activated Sepharose 6B (Pharmacia) column (Francis et al., 1991).

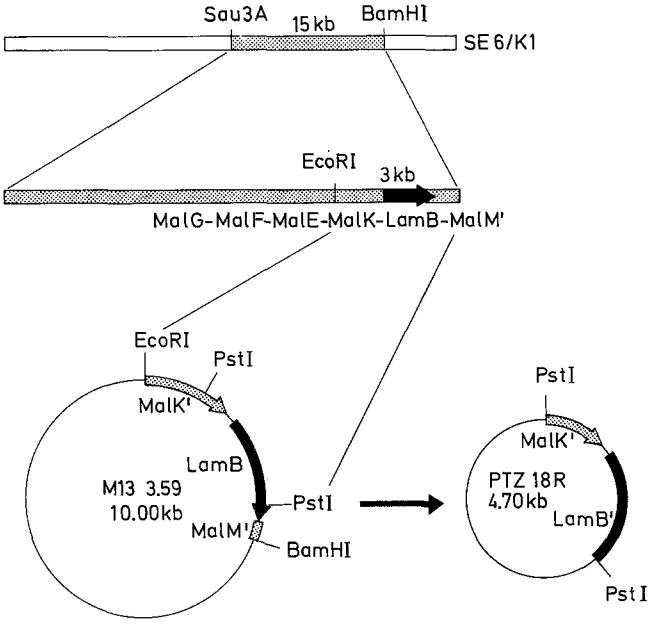


Fig. 1. Recombinant phages and phagemids. A 15 kb DNA insert, comprising almost the entire *malB* region of *Klebsiella pneumoniae*, was subcloned into the λ vector SE6 to yield recombinant phage SE6/K1. The *lamB* gene was further subcloned into the phage vector M13mp18 (Biolabs, New England), after digestion of SE6/K1 DNA with *Eco*RI and *Bam*HI, and subcloning the 3 kb *Eco*RI-*Bam*HI fragment comprising the entire *LamB* gene, into the corresponding sites of M13mp18 phage DNA. Two additional recombinant phagemids were also constructed to facilitate the sequencing. A 1.9 kb *Pst*I fragment carrying the distal part of gene *malK* and most of the *lamB* gene was inserted in both orientations into phagemid vector pTZ18R (Pharmacia LKB, Uppsala)

Transport experiments. Conditions for measuring in vivo maltose and maltodextrin transport by *K. pneumoniae* were those previously described for *E. coli* K12 (Szmelcman and Hofnung 1975). Uptake of [^{14}C] maltose was measured at a low concentration, 4 μM. The rate of maltose uptake was calculated from the slope of the uptake curve obtained for three time points (30 s, 60 s, and 90 s). The permeability of the different strains toward maltopentaose was examined using a competition assay. Uptake of [^{14}C] maltose was measured at a high concentration, 104 μM, in the presence or absence of 240 μM maltopentaose. In wild-type *E. coli* cells, maltopentaose is only able to pass through the LamB pores to the periplasmic space where it competes with the labeled maltose for uptake into the cells (Wandersman et al. 1979). Percentage inhibition was calculated for each strain as 100% minus the fraction of transport activity remaining in the presence of maltopentaose.

Immunodetection experiments. Immunoblotting was performed as described previously (Charbit et al. 1991). Preparations were loaded onto 10% SDS-polyacrylamide gels after heat-denaturation for 5 min at 100° C in sample buffer (Sambrook et al. 1989). After electrophoretic transfer to nitrocellulose filters, the samples were probed with a polyclonal serum raised in rabbits against the denatured LamB protein from *E. coli* K12, which only recognizes denatured monomers.

Enzyme-linked immunosorbent assays (ELISA) were performed as described previously (Molla et al. 1989). Microtitration plates were coated with LamB proteins purified from a starch-Sepharose column (100 ng of protein per well). The LamB protein from *K. pneumoniae* was tested with six monoclonal antibodies (MAb), directed against cell-surface exposed (E72, E177, E302, and E347), or internal (I141 and I436) determinants of LamB from *E. coli* K12 (Desaymard et al. 1986). MAbs were used at a dilution of 1/2000). Purified LamB proteins from *E. coli* and *S. typhimurium* were used as positive controls.

Results and discussion

We have determined the sequence of the *lamB* gene from *K. pneumoniae* and compared the deduced amino acid sequence of the protein to the sequences of LamB proteins from *E. coli* ($LamB_{Ec}$), *S. sonnei* ($LamB_{Ss}$) and *S. typhimurium* ($LamB_{St}$), which had been previously determined (Clément and Hofnung 1981; Roessner and Ihler 1987; Francoz et al. 1990). We also performed preliminary biochemical, immunological, and functional characterization of the $LamB_{Kp}$ protein. These data will be discussed separately below.

Comparison of four enterobacterial LamB protein sequences

Comparisons of LamB from *S. sonnei* (Roessner and Ihler 1987) with $LamB_{Ec}$ revealed only 7 amino acid substitutions, clustered between positions 381 and 390 in the C-terminal part of the $LamB_{Ss}$ protein. This region was shown to be responsible for the ability of $LamB_{Ss}$ but not $LamB_{Ec}$ to trigger spontaneous ejection of phage lambda DNA in vitro (Roessner and Ihler 1987). In LamB from *S. typhimurium,* more differences were found, providing additional information on the topological organization of the protein (Francoz et al. 1990). The variability here was clustered into five distinct portions of the polypeptide chain. In these variable regions, more than 50% of the residues were changed. Remarkably, they correspond essentially to regions predicted to be extramembranous loops on our 2D model of $LamB_{Ec}$ folding (Charbit et al. 1988). In contrast, the N-terminal third of the $LamB_{St}$ protein appeared to be extremely conserved (only 4% amino acid changes).

Determination of the sequence of the *lamB* gene from *K. pneumoniae* (Fig. 2) confirmed that $LamB_{Kp}$ was more distantly related to $LamB_{Ec}$ than $LamB_{St}$. The *lamB* gene from *K. pneumoniae* encodes a protein of 429 amino acids. There is only one change in the region of the signal sequence of $LamB_{Kp}$ as compared to that of $LamB_{Ec}$ (a met→leu change, at position −2). Thus, it is reasonable to assume that pre-$LamB_{Kp}$ is cleaved during export at the same site to generate a 404 amino acid long mature protein.

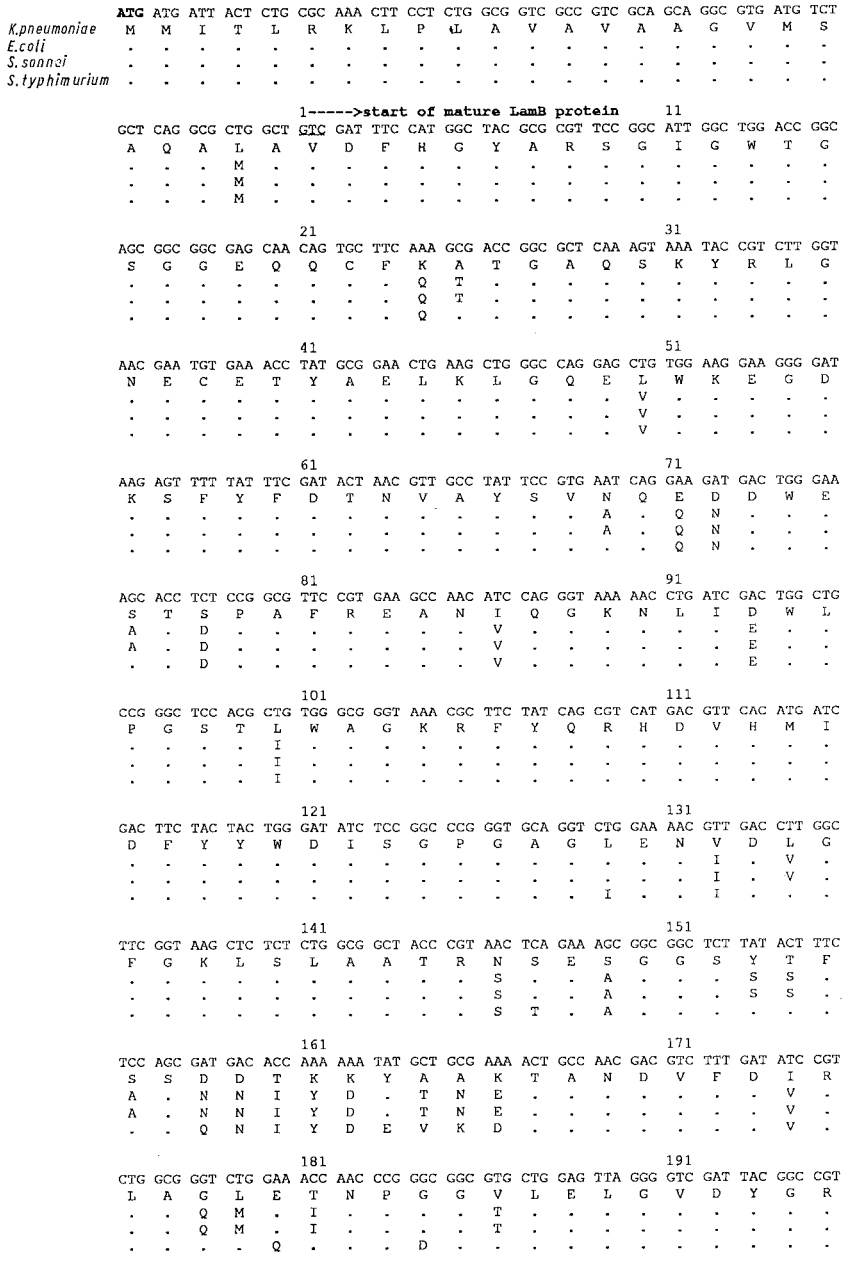
The N-terminal third of $LamB_{Kp}$ is the most conserved part of the protein (9% amino acid changes up to residue 145). Interestingly, it was previously shown that most of the determinants involved in the sugar-binding domain of the *E. coli* maltoporin are clustered towards its N-terminus (Heine et al. 1988; Ferenci and Lee 1989). The sequence of a sucrose-specific porin of *K. pneumoniae* (ScrY) was recently determined (Schmid et al. 1991). This porin was shown to be able to complement maltoporin function but not phage lambda receptor function in a LamB-negative *E. coli* strain. Some regions of homology were found between $ScrY_{Kp}$ and $LamB_{Ec}$ located mainly in the N-terminal third of the two proteins (33.5% identical amino acids between residues 1 and 140 of $LamB_{Ec}$ and residues 95–244 of $ScrY_{Kp}$). We checked whether the sequence of $LamB_{Kp}$ is more closely related to $ScrY_{Kp}$ than $LamB_{Ec}$, but did not find a better homology (32% only).

The first region showing significant variability (59% changes) corresponds to the fourth predicted external loop of $LamB_{Kp}$ (loop e4 on the 2D model of $LamB_{Kp}$ folding in Fig. 3). The extent of substitutions (from residue 146 to 166) corresponds almost exactly to the proposed size of the loop on the 2D model of $LamB_{Ec}$ folding (Charbit et al. 1988). As in the $LamB_{St}$ protein, the majority of the amino acid changes are located in the distal half of the loop. This suggests that the distal part of the loop may be more readily dispensable to LamB functions than the proximal part. The second and third regions of variability of $LamB_{Kp}$ are comprised within residues 174 to 186 (33% changes), and 198 to 225 (36% changes), respectively. These regions were not predicted as external loops of LamB. Interestingly, we previously showed that amino acid site 183 corresponds to a permissive site of $LamB_{Ec}$ likely to face the periplasm (Charbit et al. 1991).

The fourth variable region (residues 238–299) encompasses the sixth predicted external loop of the protein (loop e6). The alignments of this region of $LamB_{Kp}$ with the corresponding regions of $LamB_{Ec}$ and $LamB_{St}$ suggest that, compared to $LamB_{Ec}$, deletions accompanied by insertions occurred, leading to the net loss of 11 residues in the case of $LamB_{Kp}$ and to the net addition of 10 residues in the case of $LamB_{St}$. It is remarkable that the fourth variable region also comprises residues 267–286 which were predicted to be transmembranous. Compared to $LamB_{Ec}$ there are 12 substitutions in $LamB_{Kp}$, and only 5 in $LamB_{St}$. Such variability may indicate a peculiar position for this segment. One possible explanation is that this peptide sequence is on the surface of LamB but inside the channel, in a portion which is not essential for pore function.

The fifth region of variability, comprised of residues 312–328 (31% changes), was predicted to be extracellular. The sixth variable region (residues 368–386) encom-

Nucleotide sequence of the *lamB* gene from *K.pneumoniae* and alignements of the predicted amino-acid sequences of enterobacterial LamB proteins



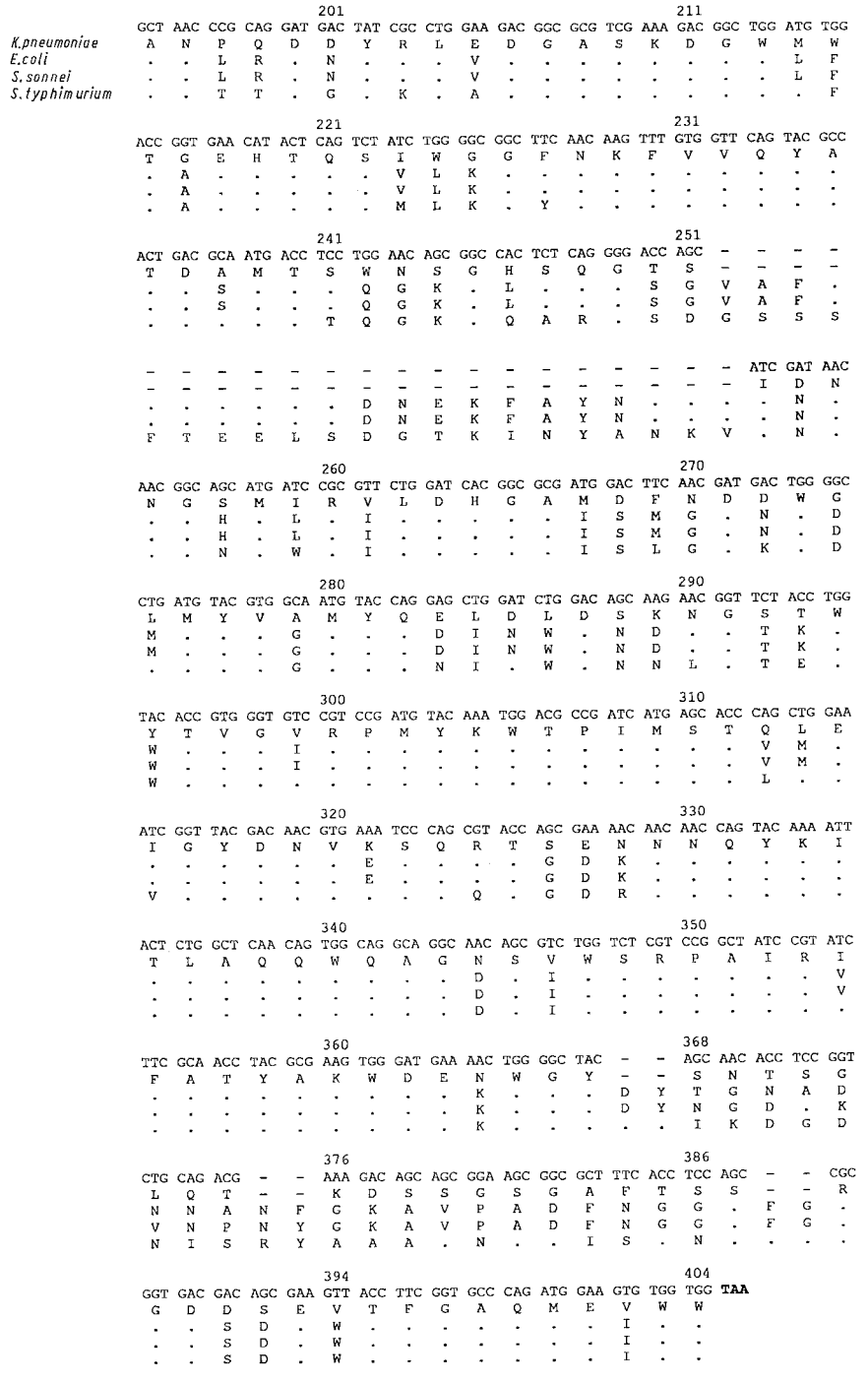


Fig. 2. For continuation, see page 376

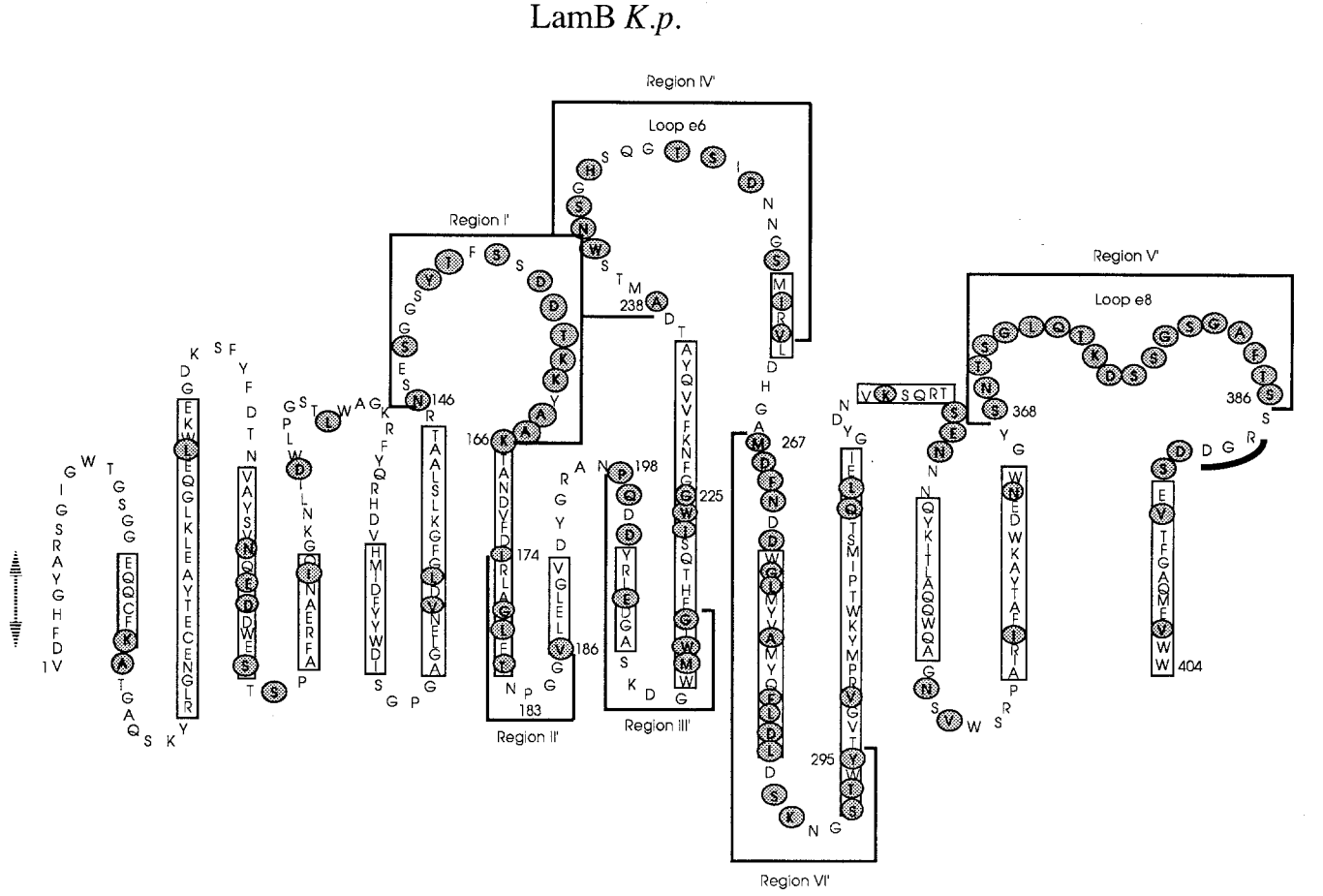


Fig. 3. Two-dimensional *K. pneumoniae* LamB ($LamB_{Kp}$) model. The 404 amino acid $LamB_{Kp}$ mature protein 2–D model is drawn according to the rules presented and discussed in Charbit et al. 1988). The amino acid sequence is shown in the single letter code. The number of transmembranous segments is identical in this model and in the model for *E. coli* LamB ($LamB_{Ec}$). Amino acid changes between $LamB_{Kp}$ and $LamB_{Ec}$ are *circled*. Changes correspond strictly to amino acid substitutions only up to residue 249. From residue 250, *circled* residues are those which differ from the $LamB_{Ec}$ sequence (such as insertions accompanying deletions). The RGD motif, in the last proposed external loop, is *underlined*

passes the eighth predicted external loop of the protein (loop e8). In this region, alignments of the amino acid sequences from the different LamB proteins suggest that, as in loop e6, deletions occurred accompanied by insertions. Compared to $LamB_{Ec}$, loop e8 would be six residues shorter in $LamB_{Kp}$, and four residues shorter in $LamB_{St}$.

Fig. 2. DNA sequence of the *lamB* gene from *K. pneumoniae*. Comparison of the amino acid sequences of the LamB proteins from four enterobacteriae. The upper line shows the nucleotide sequence of the *lamB* gene from *K. pneumoniae*. The deduced amino acid sequence is shown below the nucleotide sequence. The start codon and the corresponding methionine of the precursor form of LamB are indicated in *bold letters*, as well as the translation stop codon. The probable start codon of the mature LamB protein has been *underlined*. Numbers above the nucleotide sequence indicate the numbers of amino acids of the mature protein. The LamB sequences from *K. pneumoniae* KAY 2026 (first line), *Escherichia coli* K12 (second line), *Shigella sonnei* (third line) and *Salmonella typhimurium* LT2 are shown. Identical amino acids are noted as *points*. Gaps for optimal alignment with the LamB *E. coli* sequence are denoted as *dashes*

In the last predicted external region of LamB, between residues 368 and 392, there are only four residues conserved with $LamB_{Ec}$ (SRGD). A tetrapeptide RGDS, present in the extracellular protein fibronectin, was previously shown to be responsible for the ability of fibronectin to bind mammalian cells. In this motif, the RGD residues are critical for the interaction. RGDS has been found in several other proteins, including $LamB_{Ec}$ (Pierschbacher and Ruoslahti 1984). It is tempting to speculate that this RGD motif, which is conserved in the four LamB sequences available, may have a functional role in the bacterial infection process allowing, for example, efficient adhesion of bacteria to cells expressing fibronectin receptor on their surface. This point is presently under investigation in our laboratory (D. O'Callaghan, in preparation).

Finally, the size and extent of changes found in the different LamB proteins are significantly more important than those found in OmpA (Cole and Maldener 1986) and PhoE (Van Der Ley et al. 1987) proteins. This could be related to the existence in LamB of loops larger than these in the two other proteins. There are two main

hypotheses to explain the high degree of local variability that may lead to different topological interpretations:

1. Selective pressure. Polymorphism represents a safety mechanism by which the various enterobacterial species can avoid cross-sensitivity to noxious external agents. It is thus clustered in exposed at the cell surface.

2. Structural constraints. Variable regions correspond to regions of the proteins the role of which is not tightly sequence-dependent. Such regions would, thus, allow passive accumulation of mutations without affecting the localization, folding, and functions of the protein. In agreement with this second hypothesis, we previously showed that "permissive" regions can be identified in LamB, which allow the insertion of foreign peptide sequences without affecting most of the biological properties of the protein (Charbit et al. 1986, 1991). These regions generally correspond to loops on the surface of the protein which protrude at *either* side of the outer membrane. Therefore, we favor this interpretation of the variability for the topological organization of the LamB protein.

In vivo and in vitro properties of K. pneumoniae *LamB protein*

We first performed a functional, biochemical, and immunological characterization of the $LamB_{Kp}$ protein. In liquid culture, *K. pneumoniae* can utilize maltose as efficiently as *E. coli* K12 (comparable growth rates). As previously observed (Wehmeier et al. 1989), no difference was found between the in vivo maltose transport efficiencies of *E. coli* K12 and *K. pneumoniae* KAY 2026). We assayed maltodextrin transport by measuring inhibition of maltose transport by maltopentaose (see Materials and methods). In both *E. coli* K12, and *K. pneumoniae,* inhibition effects recorded were strong (87% and 92%, respectively), indicating that the two strains allowed the diffusion of maltopentaose with comparable efficiencies. Thus, the differences between the two protein sequences have no effect on the pore function of LamB. We also assayed the ability of extracted $LamB_{Kp}$ to bind to a starch-Sepharose column. In our assay, $LamB_{Kp}$, and also $LamB_{St}$, were able to bind to a starch-Sepharose column as efficiently as $LamB_{Ec}$. This result indicates that the starch-binding domain is probably highly conserved.

We then tested the ability of $LamB_{Kp}$ to serve as a phage receptor. It had been previously shown that $LamB_{Kp}$ cannot serve as a phage receptor for wild-type λ phage (de Vries et al. 1984). Therefore, we tested the sensitivity of *K. pneumoniae* to two host-range mutants of λ : λh° (a one-step derivative) and λhh* (a two-step derivative), as well as to 11 different $LamB_{Ec}$-specific phages (Charbit and Hofnung 1985). None of the 13 phages produced plaques on *K. pneumoniae*.

Stability of the $LamB_{Kp}$ trimers was assayed by incubating $LamB_{Kp}$ extracts at four temperatures (37° C, 50° C, 70° C and 100° C) in a buffer containing 2% SDS. In these conditions, like $LamB_{Ec}$, and $LamB_{St}$ trimers, $LamB_{Kp}$ trimers were still fully stable at 70° C. At 100° C, the three types of trimers dissociated and single bands corresponding to the monomeric form of the protein were detected (data not shown). This suggests that the differences in the three sequences probably occur in regions which are not involved in subunit association. One way to address this point would consist in expressing simultaneously at controllable levels LamB from two different origins in the same strain, and testing for stability and functionality of the heterotrimers (work in progress).

$LamB_{Ec}$ and $LamB_{Kp}$ appeared to be immunologically cross-reactive. In Western blotting experiments, denatured $LamB_{Kp}$ protein extract (in 2% Triton X100) could be detected by a polyclonal anti-$LamB_{Ec}$ serum. Native purified $LamB_{Kp}$ was further tested in ELISA for its ability to react with six monoclonal antibodies (MAb) directed against native $LamB_{Ec}$ (four MAbs directed against external determinants, E-MAb, and two MAbs against internal determinants, I-MAb; Desaymard et al. 1986). None of them was able to react with native purified $LamB_{Kp}$. In contrast, it was shown previously that with native $LamB_{St}$, although the protein was not recognized by the E-MAb, it was recognized by the I-MAb. Thus, immunologically, *S. typhimurium* is also more closely related to *E. coli* than *K. pneumoniae*.

Taken together, these data indicate that, although the main changes between the different LamB proteins occurred in loops exposed on the cell surface, changes also occurred in periplasmic loops of the protein and in one case in a putative transmembranous segment. These observations are in agreement with our hypothesis explaining variability as modifications in dispensable parts of the protein and should be complemented by further biochemical and immunological characterization, as well as crystallographic analysis, of the LamB protein from *K. pneumoniae*.

Acknowledgments. We thank M. Dahl for providing cosmid SE6/K1. We are grateful to E. Gilson for helpful discussions. This work was supported by grants from the Association pour le Développement de la Recherche sur le Cancer, the Ligue Nationale contre le Cancer, the Fondation pour la Recherche Médicale and the North Atlantic Treaty Organization (NATO).

References

Bloch M-A, Desaymard C (1985) Antigenic polymorphism of the LamB protein among members of the Enterobacterial. J Bacteriol 163:106–110

Braun G, Cole S (1984) DNA sequence analysis of the *Serratia marcescens ompA* gene: Implications for the organization of an enterobacterial outer membrane protein. Mol Gen Genet 195:321–328

Braun-Breton C, Hofnung M (1981) *In vivo* and *in vitro* functional alterations of the bacteriophage lambda receptor in *lamB* missense mutatns of *E. coli* K12. J Bacteriol 148:845–852

Brenner DJ (1978) Characterisation and clinical identification of enterobacteriaceae by DNA hybridization. Progress in clinical pathology. In: Grune and Stratton (eds) vol 7. New York, pp 71–117

Charbit A, Hofnung M (1985) Isolation of different bacteriophages using the LamB protein for adsorption on *E. coli* K12. J Virol 53:667–671

Charbit A, Boulain J-C, Ryter A, Hofnung M (1986) Probing the topology of a bacterial membrane protein by genetic insertion of a foreign epitope: expression at the cell surface. EMBO J 5:3029–3037

Charbit A, Clément JM, Hofnung M (1984) Further sequence analysis of the phage lambda receptor site. Possible implications for the organization of the LamB protein in *E. coli* K12. J Mol Biol 175:395–401

Charbit A, Gehring K, Nikaido H, Ferenci T, Hofnung M (1988) Maltose transport and starch binding in phage-resistant point mutants of maltoporin: functional and topological implications. J Mol Biol 201:487–496

Charbit A, Ronco J, Michel V, Werts C, Hofnung M (1991) Permissive sites and the topology of an outer-membrane protein with a reporter epitope. J Bacteriol 173:262–275

Clément JM, Hofnung M (1981) Gene sequence of the lambda receptor, an outer membrane protein of *E. coli* K12. Cell 27:507–514

Cole ST, Maldener M (1986) Identification of a region of the enterobacterial OmpA protein used as a bacteriocin receptor. FEMS Microbiol Lett 33:133–136

Dahl MK, Francoz E, Saurin W, Boos W, Manson MD, Hofnung M (1989) Comparison of sequences from the *malB* regions from *Salmonella typhimurium* and *Enterobacter aerogenes* with *E. coli*. A potential new regulatory site in the inter-operonic region. Mol Gen Genet 218:199–207

Dargent B, Charbit A, Hofnung M, Pattus F (1988) Effect of point mutations on the *in vivo* pore properties of maltoporin, a protein of *E. coli* outer membrane. J Mol Biol 201:497–506

Desaymard C, Debarbouillé M, Jolit M, Schwartz M (1986) Mutations affecting antigenic determinants on an outer membrane protein of *Escherichia coli*. EMBO J 5:1383–1388

Elledge SJ, Walker GC (1985) Phasmid vectors for identification of genes by complementation of *Escherichia coli* mutants. J Bacteriol 162:777–783

Ferenci T, Lee KS (1982) Directed evolution of the lambda receptor of *Escherichia coli* through affinity chromatography selection. J Mol Biol 160:431–444

Ferenci T, Lee KS (1989) Channel architecture in maltoporin: dominance studies with *lamB* mutations influencing maltodextrin binding provide evidence for independent selectivity filters in each subunit. J Bacteriol 171:855–861

Francis G, Brennan L, Ferenci T (1991) Affinity-chromatographic purification of sixteen cysteine-substituted maltoporin variants: thiol reactivity and cross-linking in an outer membrane protein of *Escherichia coli*. Biochem Biophys Acta 1067:89–96

Francoz E, Molla A, Dassa E, Saurin W, Hofnung M (1990) The maltoporin of *Salmonella typhimurium:* sequence and folding model. Res Microbiol 141:1039–1059

Gabay J, Yasunaka K (1980) Interaction of the LamB protein with the peptidoglycan layer in *E. coli* K12. Eur J Biochem 265:896–901

Heine HG, Francis G, Lee KS, Ferenci T (1988) Genetic analysis of sequences in maltoporin that contribute to binding domains and pore structure. J Bacteriol 170:1730–1738

Molla A, Charbit A, Leguern A, Ryter A, Hofnung M (1989) Antibodies against synthetic peptides and the topology of LamB, an outer-membrane protein from *Escherichia coli* K12. Biochemistry 28:8234–8241

Nikaido H, Vaara M (1985) Molecular basis of bacterial outer membrane permeability. Microbiol Rev 49:1–32

Pick KH, Wöber G (1979) Maltodextrin pore proteins in the outer-membrane of *Escherichia coli* and *Klebsiella pneumoniae:* immunological comparisons. FEMS Microbiol Lett 5:119–122

Pierschbacher MD, Ruoslahti E (1984) Variants of the cell recognition site of fibronectin that retain attachment-promoting activity. Proc Natl Acad Sci USA 81:5985–5988

Roantree RJ, Kuo TT, MacPhee DG (1977) The effect of defined lipopolysaccharide core defects upon antibiotic resistances of *Salmonella typhimurium*. J Gen Microbiol 103:223–234

Roessner CA, Ihler GM (1987) Sequence of amino-acids in LamB responsible for spontaneous ejection of bacteriophage lambda DNA. J Mol Biol 195:963–966

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: A laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Schmid K, Ebner RT, Jahreis K, Lengeler JW, Titgemeyer F (1991) A sugar-specific porin, ScrY, is involved in sucrose uptake in enteric bacteria. Mol Microbiol 5:941–950

Schülein K, Benz R (1990) LamB (maltoporin) of *Salmonella typhimurium:* isolation, purification and comparison of sugar binding with LamB of *Escherichia coli*. Mol Microbiol 4:625–632

Sprenger GA, Lengeler JW (1984) L-sorbose metabolism in *Klebsiella pneumoniae* and sor+ derivatives of *Escherichia coli* K12 and chemotaxis toward sorbose. J Bacteriol 157:39–45

Szmelcman S, Hofnung M (1975) Maltose transport in *Escherichia coli* K12; involvment of the bacteriophage lambda receptor. J Bacteriol 124:12–118

Van Der Ley P, Bekkers A, Van Meersbergen J, Tommassen J (1987) A comparative study of the *phoE* genes of three enterobacterial species. Implications for structure function relationships in a pore-forming protein of the outer membrane. Eur J Biochem 164:469–475

de Vries GE, Raymond CK, Ludwig RA (1984) Extension of bacteriophage lambda host-range: selection, cloning and characterization of a constitutive lambda receptor gene. Proc Natl Acad Sci USA 81:6080–6084

Wandersman C, Schwartz M, Ferenci T (1979) *Escherichia coli* mutants impaired in maltodextrin transport. J Bacteriol 140:1–13

Wehmeier U, Sprenger GA, Lengeler JW (1989) The use of λplac-*Mu* hybrid phages in *Klebsiella pneumoniae* and the isolation of stable Hfr strains. Mol Gen Genet 215:529–536

Weiss MS, Waeker T, Weckesser J, Welte W, Schulz GE (1990) The three-dimensional structure of porin from *Rhodobacter capsulatus* at 3Å resolution. FEBS Lett 267:268–272

Communicated by J. Lengeler