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# Bacteriophage λ Receptor Site on the *Escherichia coli* K-12 LamB Protein

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**We have analyzed eight new phage-resistant missense mutations in *lamB*. These mutations identify five new amino acid residues essential for phage λ adsorption. Two mutations at positions 245 and 382 affect residues which were previously identified, but lead to different amino acid changes. Three mutations at residues 163, 164, and 250 enlarge and confirm previously proposed phage receptor sites. Two different mutations at residue 259 and one at 18 alter residues previously suggested as facing the periplasmic face. The mutation at residue 18 implicates for the first time the amino-terminal region of the LamB protein in phage adsorption. The results are discussed in terms of the topology of the LamB protein.**

The LamB protein is an integral outer membrane protein of *Escherichia coli* that functions as a specific pore for the diffusion of maltose and maltodextrins across the outer membrane (reviews in references 9 and 18). LamB also serves as a receptor for phage λh$^+$, its host range derivatives λh° and λhh*, and several other phages (6, 12, 20, 21, 24).

We have undertaken to identify portions of the LamB protein specifically involved in phage adsorption. For this purpose, we have isolated mutations in *lamB* leading to phage resistance that retain at least some of the activities of LamB such as sensitivity to λhh*. We believe that such mutations do not alter severely the overall structure of the protein. By further assuming that the mutated residues belong to or are close to the primary site of interaction with the phage, i.e., the "receptor site," we have devised working models of the folding of the LamB protein in the outer membrane (5) (Fig. 1).

We have already reported the identification of 10 such residues in LamB (5, 7, 22). These residues are clustered in four different regions of the LamB protein and belong to portions that are hydrophilic and mostly predicted as β-turns of the molecule.

In the present study, to identify more residues belonging to the λ receptor site, we selected new resistant mutations by using three different mutagenic treatments and screened the resistant clones with a battery of LamB-specific phages for phenotypes distinct from previous mutations. The analysis of the eight new mutants extends the conclusions of the previous studies and suggests that other parts of the molecule may be exposed at the cell surface.

## MATERIALS AND METHODS

**Strains and bacteriological techniques.** The *lamB* alleles described in this study are carried on phage λσ3*S*am7h°434 that was derived from λσ3h°434 (13). Lysogens of pop725 (F$^-$ *thr leu met lacY malBΔ12*) (2) were used for the preparation of phage, phage DNA, and receptor extracts (prepared as described in reference 3). Spot tests, phage binding and inactivation were analyzed as described previously (2).

**Isolation of mutants.** Phage-resistant mutations were selected in lysogens of λσ3*S*am7h°434, carrying a wild-type copy of *lamB* under the control of the *lacL8UV5* promoter. The *lamB* gene was mutagenized by either passage through a *mutT* mutator strain or by chemical mutagenesis of a lysogen with ethyl methanesulfonate or with a nitrofuran compound R-7000 (7-methoxy-2-nitronaphto-1,2-b-furan) (28). For mutagenesis by the *mutT* strain, a λσ3*S*am7h°434 lysogen of W3807 (*mutT*) (15) was grown overnight at 30°C before selection. Chemical mutagenesis and selection for resistant mutants were performed essentially as described previously (12). Mutagenesis by R-7000 was at a concentration of 2 μg/ml for 30 min at 30°C.

The mutagenized cultures (from either the *mutT* or chemically mutagenized strains) were infected after phenotype expression with virulent λvh$^+$ to select resistant mutants. The resulting colonies were screened by cross-streaking for sensitivity to a mixture of LamB-specific phages: λh°, λhh*, K10, 21EL, AC7, AC30, AC43, AC95, AC6, AC28, AC57, and AC81 (6). Isolates sensitive to the phage cocktail were further screened by a spot test for a pattern of phage sensitivity that was different from previously identified mutations in *lamB*.

**DNA labeling and sequencing.** Phage DNA carrying the mutant *lamB* allele was prepared and labeled with $^{32}$P (7). DNA sequencing was performed by the chemical cleavage reactions as described previously (16).

## RESULTS

**DNA alterations.** After mutagenic treatment, mutants resistant to phage λh$^+$ were selected and screened for sensitivity to a mixture of 12 LamB-specific phages (see Materials and Methods). From about 700 mutants resistant to phage λh$^+$, 8, which yielded a pattern of resistance toward the 12 phages that was different from known LamB mutations (Table 1), were selected for further study. The mutations were mapped against a set of deletions in *lamB* (13), and the corresponding DNA was sequenced. All of the new isolates correspond to new DNA alterations (Table 1), demonstrating the efficiency of screening with the new LamB-specific phages.

The observed DNA alterations are in agreement with reported mutational specificity of the mutagens. The ethyl methanesulfonate-induced mutation was a transition, and

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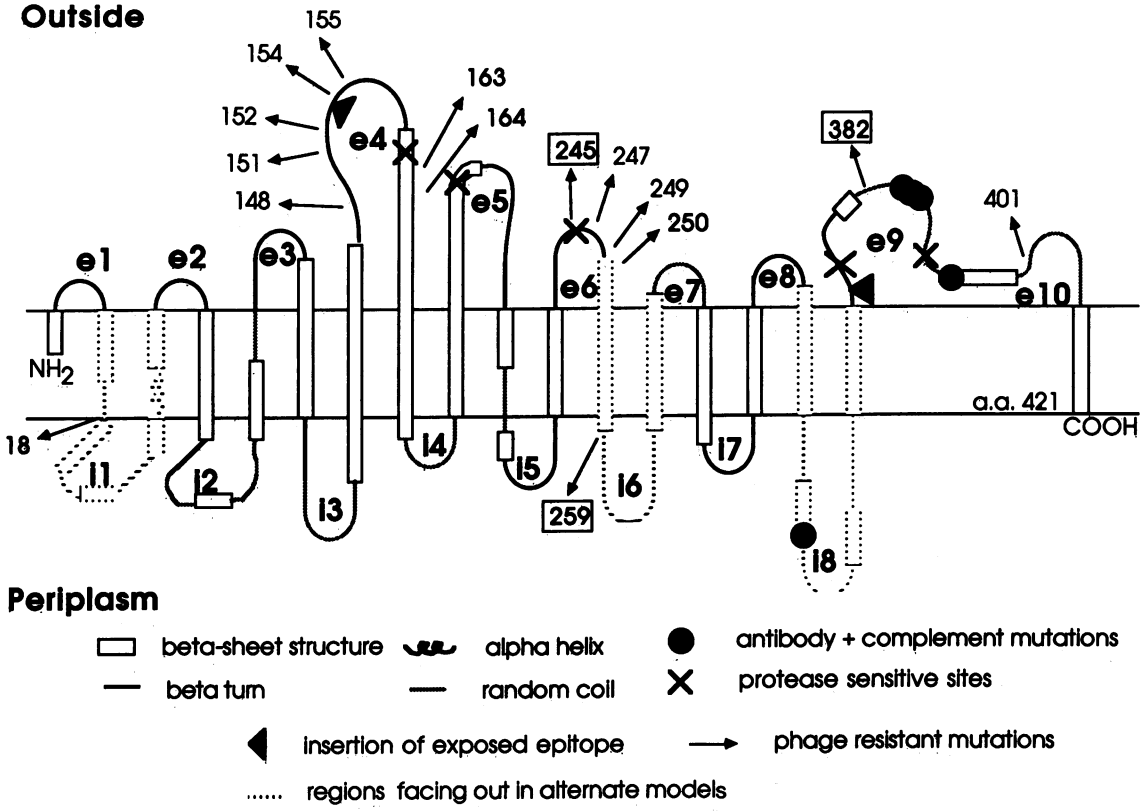


FIG. 1. Proposed model for the folding of LamB in the outer membrane. Loops of the protein, referred to in the text, are identified by a lowercase e or i and a number. Phage-resistant mutations (marked by arrows and residue numbers) have been found in loops i1, e4, e6, i6, e9, and e10. Residues identified in the present study are outlined. Residues identified by two different amino acid changes are boxed. Protease-sensitive sites have been identified in loops e4, e5, e6, and e9. Mutations resistant to killing with monoclonal antibodies plus complement are located in loops i8 and e9. Insertion of a foreign epitope at position 153 (loop e4) or 374 (loop e6) into LamB results in its exposition at the cell surface (4).

the three *mutT* mutations were A-to-C transversions. The nucleotide substitutions due to mutagenesis by the nitrofuran R-7000 are less specific. Of the four sequenced nitrofuran-induced mutations, three were G-to-T transversions and one was a T-to-C transition.

**Mutant receptor activities.** The in vitro receptor activity of seven of the eight mutations was examined in detail (Table 2). The receptor activity of the mutant cell extracts toward phage λh$^+$ was reduced by a factor of at least 100 in comparison with the corresponding wild-type strain. Reversible binding of λh$^+$ (as measured by an in vitro phage protection assay) was also absent in all mutant strains tested. This is in agreement with the idea that the primary site of interaction with this phage was affected.

Surprisingly, although the mutant receptors retained the capacity to adsorb phage λh° in vivo (Table 1), the in vitro activity toward phage λh° was greatly reduced in some strains. In particular, the *lamB603* and *lamB602* mutants showed no detectable activity in vitro toward λh°; however, in vivo, the kinetics of phage inactivation were identical to those of the wild type (data not shown). On sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels, the amount

TABLE 1. Characteristics of the mutant LamB alleles[a]

| Allele | Codon and amino acid substitution | Phage resistance | | | | | | | | | | | | Mutagen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | λh | λh° | λhh* | K10 | 21EL | AC30 | AC43 | AC50 | AC6 | AC28 | AC57 | AC81 | |
| *lamB601* | GGT (Gly-18)→GTT(Val) | R | S | S | R | R | R | R | I | R | I | R | R | R-7000 |
| *lamB602* | TAT (Tyr-163)→GAT(Asp) | R | S | S | R | R | I | R | I | S | I | R | R | *mutT* |
| *lamB603* | ACC (Thr-164)→CCC(Pro) | I | S | S | R | S | S | S | S | S | S | S | S | *mutT* |
| *lamB604* | GGG (Gly-245)→GTG(Val) | R | S | S | R | R | R | I | S | S | I | R | I | R-7000 |
| *lamB605* | TCT (Ser-250)→TTT(Phe) | R | S | S | I | R | R | R | I | R | R | R | R | Ethyl methanesulfonate |
| *lamB606* | TTT (Phe-259)→TCT (Ser) | R | S | S | R | I | I | I | S | I | I | I | R | R-7000 |
| *lamB607* | TTT (Phe-259)→GTT(Val) | R | S | S | R | I | I | I | S | R | I | I | R | *mutT* |
| *lamB608* | GGT (Gly-382)→GTT(Val) | I | S | S | S | R | R | R | I | R | I | R | R | R-7000 |

[a] Efficiencies of plating were measured, compared with those of the wild type, and scored as follows: S, 1 to 0.1; I, 0.1 to $10^{-4}$; R, below $10^{-4}$. Phages 21EL and AC30 through AC81 were described previously (6). Strains tested were λσ3S7h°434 lysogens of pop725 carrying the *lamB* mutant alleles: pop6337 (*lamB601*), pop6449 (*lamB602*), pop6447 (*lamB603*), pop6445 (*lamB604*), pop6336 (*lamB605*), pop6444 (*lamB606*), pop6448 (*lamB607*), and pop6446 (*lamB608*).

of LamB protein detectable was not diminished. It appears that for some mutant receptors, extraction leads to a loss of activity. This phenomenon has to be borne in mind when comparing in vivo and in vitro activities of the mutant proteins in other functions such as transport.

**Location of the mutated amino acids.** Of the eight missense mutations, five are located in regions where mutations had been previously found and that were predicted as outward-facing loops in the model of Fig. 1: *lamB602* and *lamB603* at amino acid positions 163 and 164 (in loop e4), *lamB604* and *lamB605* at positions 245 and 250 (in loop e6), and *lamB608* at position 382 (in loop e9). Three mutations occur in regions that were previously predicted as turns facing the periplasm. Mutations *lamB607* (*mutT* induced) and *lamB606* (nitrofuran induced) both change phenylalanine 259 (in loop i6) to valine and serine, respectively. The mutation *lamB601* (nitrofuran induced) changes glutamine 18 (in loop i1) to alanine. Significantly, this is the first phage-resistant missense mutation identified in the N-terminus region of LamB.

## DISCUSSION

Fifteen amino acid residues are now identified on the LamB polypeptide as essential for λ or phage K10 adsorption. The limited number of residues and the fact that several have been found repeatedly (5) justify the definition of the receptor site as the set of these residues.

As expected from the selection procedure, the eight newly identified λh$^+$-resistant mutations have not lost all LamB functions. All retain the capacity to adsorb phages λh° and λhh* in vivo. The mutants strains also transport maltose and maltodextrins significantly better than do strains with *lamB* nonsense mutations (data not shown). These limited functional defects are compatible with our hypothesis that the overall structure of the LamB protein is conserved in these mutants and that the mutations affect essentially the receptor site for λh$^+$.

Using the assumption that the phage-resistant mutations are located on the outer surface of the protein, we proposed a model of the folding of LamB in the membrane (Fig. 1 (5). Secondary structure predictions, the high percentage of β-sheet structure in LamB (17), and the hydropathy profile were also used in designing the model.

Since our model is based on a number of assumptions, it is important to examine independent experimental evidence. Three other lines of evidence are available.

First, protease sensitivity studies (23) have shown that the protease-sensitive sites of native solubilized LamB are located in four regions predicted as exposed loops on our model (Fig. 1). These sites are protected against proteolytic digestion by monoclonal antibodies that recognize cell surface determinants of LamB.

Second, monoclonal antibodies raised against cell surface-exposed regions of the protein were shown to recognize residues located within the last 70 C-terminal amino acids of LamB (11). Recently, mutations preventing complement killing in the presence of monoclonal antibody binding have been sequenced, and residues 333, 386, 387, 389, and 394 have been identified (8). No phage resistance mutations or protease-sensitive sites have been detected in the region of residue 333.

Third, we have been able to insert foreign epitopes into several positions in LamB without perturbing appreciably its folding and export. When the epitopes were inserted after residues 153 and 374, they were exposed at the cell surface. This confirmed the location of loops e4 and e9 on the cell surface (Fig. 1, 4).

In conclusion, the exposure of the distal part of the protein (corresponding to loop e9) outside the cell is clearly demonstrated by the four different approaches: phage resistance mutations, protease accessibility, monoclonal antibody-binding studies, and foreign epitope insertion. For loop e4, three independent approaches agree: phage resistance mutations, protease accessibility, and foreign epitope insertion. For loop e6 two independent approaches suggest its placement at the cell surface: phage resistance mutations and protease accessibility. We consider that in all of these cases the evidence for exposure of the corresponding regions to the outside is compelling.

For four regions of the protein (i1, e5, i6, i8), a single approach indicates their possible placement at the surface. We consider here that the evidence is less compelling, especially since at this stage we cannot exclude the possibility that some of the mutations have long-range effects within a subunit or affect subunit-subunit interactions. In particular, residues 18 and 259, identified in the present study, and residue 333, identified by monoclonal antibody studies, are situated in loops (i1, i5, i8) that are proposed to face the periplasm in the initial model. These loops could be pulled out to the cell exterior. We have therefore drawn the corresponding parts of the model as dotted lines, indicating one possibility for an alternate model. In fact several alternate models are possible at this stage: for example, loop e8 could be pulled in, whereas loop i8 could be pulled out. More data, including transport and dextrin-binding data on these mutants as well as the study of other LamB mutants (A. Charbit, T. Ferenci, K. Gehring, H. Nikaido, and M. Hofnung, manuscript in preparation), should help to choose between various possible models.

Recent models for the folding of other outer membrane proteins bear a striking resemblance to our early model (5). For the OmpF protein, biophysical studies have unequivocally established the existence of multiple anti-parallel β-sheets traversing the membrane (14), leading to the suggestion that OmpF crosses the outer membrane as 16 β-sheets (19). Genetic and biophysical studies of OmpA have also engendered models rich in transmembrane β-sheets (10, 27).

TABLE 2. Phage binding properties of the mutant receptors in vitro

| Allele | Inactivation[a] | | λh$^+$ protection[b] | | | | |
|---|---|---|---|---|---|---|---|
| | λh$^+$ | λh° | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ |
| Wild type | 100 | 100 | 60 | 58 | 21 | 6 | 2 |
| *lamB601* | 0.8 | 5 | <5 | <2 | <2 | <2 | <2 |
| *lamB602* | 0.5 | <0.05 | 5 | <2 | <2 | <2 | <2 |
| *lamB603* | <0.5 | <0.05 | 5 | <2 | <2 | <2 | <2 |
| *lamB604* | <0.5 | 0.05 | <2 | <2 | <2 | <2 | <2 |
| *lamB606* | <0.5 | 0.3 | <2 | <2 | <2 | <2 | <2 |
| *lamB607* | 0.6 | 4 | <5 | <2 | <2 | <2 | <2 |
| *lamB608* | 0.5 | 9 | <5 | <2 | <2 | <2 | <2 |

[a] Relative activities of the Triton X-100 extracts in inactivating phage λh$^+$ and λh°, expressed as a percentage of the wild type. Inactivation was measured by incubating dilutions of the receptor extracts with the phage in the presence (for λh$^+$) or absence (for λh°) of 20% ethanol for 30 min at 37°C. Remaining viable phages were then counted by plate titering (2). Activities are calculated from the dilution yielding 50% inactivation. For the wild-type extract, this was between $1 \times 10^{-4}$ and $4 \times 10^{-4}$ for λh$^+$ and between $8 \times 10^{-5}$ and $6 \times 10^{-5}$ for λh°.

[b] Reversible binding of phage λh$^+$ by the Triton X-100 extracts was measured by a phage protection assay (2). The column headings indicate the dilutions of the receptor extracts. Values in the table represent percentage of phages protected by the mutant extracts from inactivation by a second extract that does not require ethanol for inactivating phage λh$^+$.

The PhoE protein presents extensive homology with OmpF and can thus be expected to have a similar structure. Genetic studies of PhoE have, indeed, identified a tendency for adjacent surface-exposed regions to be separated by approximately 40 amino acids, as is the case for LamB and OmpA (25, 26). These features appear to be common motifs in the structure of outer membrane proteins.

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