LOCATION OF A PHAGE BINDING REGION ON AN OUTER MEMBRANE PROTEIN

M. ROA and J. M. CLÉMENT+

Unité de Génétique Moléculaire (LA 270 CNRS) and +Unité de Programmation moléculaire et Toxicologie génétique (LA 271 CNRS and U163 INSERM), Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France

Received 19 September 1980

1. Introduction

Although several of the proteins present in the outer membrane of Gram negative bacteria are known to constitute phage receptors [1,2], in no instance has the exact site recognized by these phages been identified. The identification of these sites should provide information not only on the nature of phage–receptor interactions, but also on the structure–function relationships in outer membrane proteins, which have biological roles in addition to being phage receptors [2,3]. Here we report on the identification of part of a phage binding site on the *lamB* protein of *Escherichia coli.*

The product of gene *lamB* is a 50 000 M_r maltose-inducible protein which facilitates the diffusion of maltose and maltodextrins across the outer membrane [4–6]. In addition it serves as a receptor for phages λ, K10 and TP1 [7–9]. As a first attempt to locate the different functional sites on this polypeptide we have studied the binding site for phage K10. Mutations specifically affecting this site should render the cells unable to adsorb K10 without altering the other functions of the *lamB* protein. Such mutations were obtained and genetically mapped in [8]. The exact location of some of these mutations has now been determined by using DNA-sequencing techniques.

2. Materials and methods

2.1. *Transfer of the mutations onto a λ transducing phage*

The structure of phage λp*malB lac* 863 (constructed by J. M. C., unpublished) is shown in fig.1. Deletion *malB*Δ500 which corresponds to the 745 basepair *SalI–SalI* fragment [10] was transferred onto this phage by homogenization. The resulting phage was used to lysogenize a strain carrying *malB*Δ12 which encompasses the whole *lamB* gene [11]. The K10 resistant mutations were transduced into this lysogen by using P1 stocks grown on strains pop. 1433, 1434, 1435, 1437 and 1442 [8], the selection being for growth on maltodextrins, essentially as in [12]. The prophages present in these transductants were verified to carry the K10-resistant mutations.

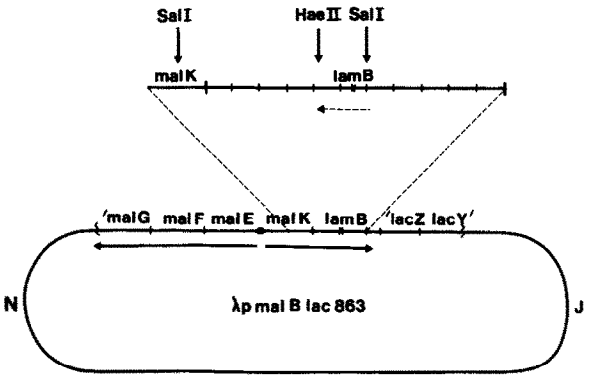

Fig.1. Genetic location of the K10-resistant–λ-sensitive mutations. The mutations (x) all map in the same deletion interval of *lamB* [8,11]. This interval is limited on one side by a *SalI* site since the deletion ending at that point was constructed in vitro using the *SalI* enzyme [10]. The positions of the other *SalI* site and of the *Hae*II site were also determined in [10]. The mutations were recombined onto λp*malBlac*863. The direction of sequencing is shown by the interrupted arrow. Primes, as in *'lacZ* or *lacY'* indicate that the genetic region referred to is deleted on the side the prime is written. The arrows indicate the directions of transcription of the *mal* operons.

2.2. *DNA sequencing*

The phages carrying the mutations were thermo-

induced, and purified on CsCl gradients. Their DNA was phenol extracted and hydrolyzed with the *Sal*I enzyme (Boehringer). The 745 basepair fragment was purified in each case by electrophoresis on a 5% polyacrylamide gel and 5′-labeled using [γ-^{32}P]-ATP (Amersham, >3000 Ci/mmol) and T4 polynucleotide kinase (a gift of M. Katinka). It was then treated with the *Hae*II enzyme (New England Biolabs), and the smaller *Hae*II–*Sal*I fragment (~250 basepairs) was purified and sequenced using the technique in [13].

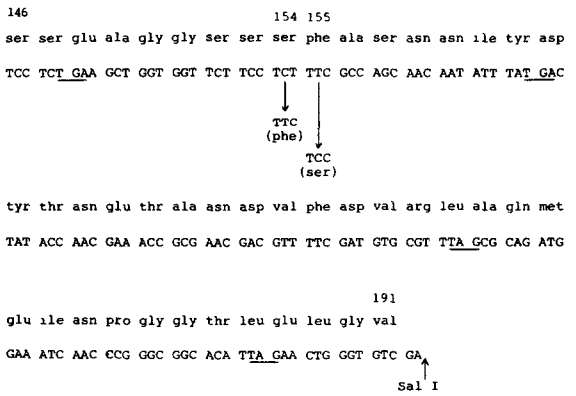

Fig.2. The wild-type nucleotide sequence of the *Sal*I end of the *Hae*II–*Sal*I fragment is given, together with the corresponding amino acid sequence. Only the DNA strand with the same polarity as the message is shown. The reading frame can be deduced from the sequence shown since there are nonsense codons (underlined) in the two other frames. The *Sal*I site is known from [16] to be located in a region corresponding to amino acids 180–200 in the mature protein. The exact numbering of residues, as shown here, was deduced from the sequence of the whole gene (J. M. C. and Maurice Hofnung, in preparation). The codon and amino acid changes resulting from the mutations are indicated under the sequence. The nucleotide change in codon 154 was found in 4 mutants (*lamB* N33, N35, N37 and N42) and that in codon 155 in one mutant (*lamB* N34).

3. Results and discussion

Most of the K10-resistant *lamB* mutants are also resistant to λ and TP1 and, in addition, are unable to grow on maltodextrins ([8], M. R. unpublished). However, after mutagenizing with ethylmethane sulfonate (EMS) we isolated K10-resistant, but λ-sensitive strains which grew like the wild-type on maltodextrins, and whose *lamB* protein interacted normally with λ in vitro [8]. These mutants represented only 1–2% of all K10-resistant *lamB* mutants obtained after EMS mutagenesis. The mutations present in 6 of these strains have been mapped and found to lie in the same region of the *lamB* gene [8]. This region is located in a 745 basepair *Sal*I–*Sal*I DNA fragment (fig.1). Five of the mutations (*lamB* N33, N34, N35, N37 and N42) were recombined onto a λ-transducing phage carrying gene *lamB*, and the *Sal*I–*Sal*I fragment was purified from the DNA of each of the resulting phages. A 250 basepair *Hae*II–*Sal*I subfragment carrying the mutation was then sequenced as in [13]. The wild-type sequence was determined in the same way, and the results were in agreement with data obtained by sequencing both strands using the techniques of Maat and Smith, and of Sanger et al. (J. M. C. and Maurice Hofnung, in preparation).

In 4 of the mutants, the only change in the *Hae*II–*Sal*I fragment was found to be a GC–AT transition at the same location. The deduced amino acid change is from serine to phenylalanine at position 154 in the mature polypeptide chain. The fifth mutation corresponds to an AT–GC transition in the next codon, leading to a change of phenylalanine into a serine (fig.2). The nature of the base changes is in agreement with observations regarding the mechanism of EMS-induced mutagenesis [14]. The results in fig.2 clearly demonstrate that the amino acids at positions 154 and 155 play a crucial role in the interaction of the *lamB* protein with phage K10. They probably constitute part of the binding site for this phage. However, it is also possible that instead, K10 binds to a site located in the vicinity of Ser 154 and Phe 155, and whose conformation is altered by the above amino acid changes. According to the model developed in [15] the change of Phe 155 to Ser would be expected to alter the secondary structure of the polypeptide from residue 159–161 (fig.3). It seems likely that the portion of the *lamB* polypeptide shown in fig.2 bears part of the binding site for phage K10, and is therefore most probably located at the outer surface of the cell. This region may be the only one which specifically interacts with K10. Indeed, K10-resistant–λ-sensitive mutations, such as those described here, are very rare [8], and it is striking that the 5 studied are located in the same very small region of the *lamB* gene. However it is possible that alterations located elsewhere but leading to the same phenotype could be uncovered by using other

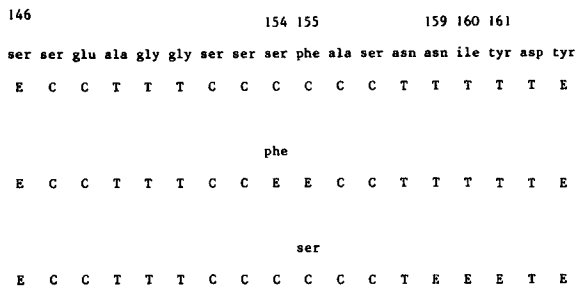

Fig.3. Amino acid sequences and predicted secondary structures in the relevant regions of wild-type and mutant *lamB* proteins. The predictions were made according to the directional method in [15] which takes into account the nature of eight amino acids on each side of any given residue. The symbols E, T and C stand for extended or β sheet, reverse turn, and random coil structures, respectively. The fourth possible conformation, α helix, was not found in that small region.

mutagens [14]. Additional regions of the polypeptide probably interact with phage K10, but in a less specific way, being also part of the binding site for phage λ and/or TP1. Unfortunately, the mutations leading specifically to an alteration in those regions would be pleiotropic (K10-resistant and λ-resistant, for instance) and may be difficult to distinguish from those affecting the conformation of the protein in a less localized fashion.

Acknowledgements

We thank Olivier Raibaud for very useful discussions, Jean Garnier for communication of his program and help with the computer, Maurice Hofnung for unpublished results, and Maxime Schwartz for help in preparing the manuscript. M. R. thanks Michael Katinka, Pascale Cossart and Michel Débarbouillé for introducing her to DNA sequencing techniques. This work was supported by grant 77 7 1294 from the Délégation à la Recherche Scientifique et Technique to Maxime Schwartz and ATP 4248 from the Centre National de la Recherche Scientifique to Maurice Hofnung.

References

[1] Schwartz, M. (1980) in: Virus Receptors (Randall, L. and Philipson, L. eds) vol. 7, Receptors and Recognition, ser. B, Chapman and Hall, London, in press.
[2] Braun, V. and Hantke, K. (1977) in: Microbial Interactions (Reissig, J. L. ed) vol. 3, Receptors and Recognition, ser. B, pp. 101–137, Chapman and Hall, London.
[3] Konisky, J. (1979) in: Bacterial Outer Membrane: Biogenesis and Functions (Inouye, M. ed) pp. 319–359, Wiley, New York.
[4] Szmelcman, S. and Hofnung, M. (1975) J. Bacteriol. 124, 112–118.
[5] Szmelcman, S., Schwartz, M., Silhavy, T. J. and Boos, W. (1976) Eur. J. Biochem. 65, 13–19.
[6] Wandersman, C., Schwartz, M. and Ferenci, T. (1979) J. Bacteriol. 140, 1–13.
[7] Randall-Hazelbauer, L. L. and Schwartz, M. (1973) J. Bacteriol. 116, 1436–1446.
[8] Roa, M. (1979) J. Bacteriol 140, 680–686.
[9] Wandersman, C. and Schwartz, M. (1978) Proc. Natl. Acad. Sci. USA 75, 5636–5639.
[10] Raibaud, O., Clément, J. M. and Hofnung, M. (1979) Mol. Gen. Genet. 174, 261–267.
[11] Raibaud, O., Roa, M., Braun-Breton, C. and Schwartz, M. (1979) Mol. Gen. Genet. 174, 241–248.
[12] Emr, S. D. and Silhavy, T. J. (1980) J. Mol. Biol. 141, 63–90.
[13] Maxam, A. M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560–564.
[14] Coulondre, C. and Miller, J. M. (1977) J. Mol. Biol. 117, 577–606.
[15] Garnier, J., Osguthorpe, D. J. and Robson, B. (1978) J. Mol. Biol. 120, 97–120.
[16] Hedgpeth, J., Clément, J. M., Marchal, C., Perrin, D. and Hofnung, M. (1980) Proc. Natl. Acad. Sci. USA 77, 2621–2625.