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A Role for Residue 151 of LamB in Bacteriophage Lambda Adsorption: Possible Steric Effect of Amino Acid Substitutions

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LamB is the cell surface receptor for bacteriophage λ. LamB missense mutations yielding resistance to λ have been previously grouped in two classes. Class I mutants block growth of λ with wild-type host range (λh$^+$) but support growth of one-step extended-host-range mutants (λh). Class II mutants block λh but support growth of two-step extended host range mutants (λhh*). While Class I mutations occur at 11 different amino acid sites, in five distinct portions of LamB, all the Class II mutations analyzed previously correspond to the same G-to-D change at amino acid 151. We generated by in vitro mutagenesis four different new substitutions at site 151 (to S, V, R, and C). Two of the mutants (G-151→V [G151V] and G151R) were of Class II, while the two others (G151S and G151C) were of Class I, demonstrating that not only the site but also the nature of the substitutions at residue 151 was critical for the phage sensitivity phenotypes. The introduction of a negatively charged, a positively charged, or an aliphatic nonpolar residue at site 151 of LamB prevented both λh$^+$ and λh adsorption, indicating that the block is not due to a charge effect. In contrast to G151D, which was sensitive to all the λhh* phages, G151V and G151R conferred sensitivity to only four of the five λhh* phages. Thus, G151V and G151R represent a new subclass of Class II LamB mutations that is more restrictive with respect to the growth of λhh*. Our results agree with the hypothesis that residue 151 belongs to an accessibility gate controlling the access to the phage tight-binding site and that substitutions at this residue affect the access of the phage to the binding site in relation to the size of the substitute side chain (surface area): the most restrictive changes are G151V and G151R, followed to a lesser extent by G151D and then by G151S and G151C.

LamB of *Escherichia coli* K-12 is an integral outer membrane protein that serves as a cell surface receptor for a number of bacteriophages, including λ (6, 20, 23, 28). Native LamB is a homotrimer (19–21) that forms nonspecific channels through the outer membrane that allow the diffusion of molecules of molecular weights of less than 600 into the cell. In addition, LamB facilitates the diffusion of maltose and maltodextrins across the outer membrane, and hence its other name, maltoporin (12, 27). Mature LamB protein is a 421-amino-acid polypeptide (10). In the absence of high-resolution X-ray structure, two-dimensional models of LamB folding have been proposed from the analysis of its primary sequence and refined by genetic, immunological, and biochemical data (see the references in reference 7). In the current models (5, 7, 26), LamB spans the membrane 16 times, with loops protruding on both sides of the lipid bilayer.

We have previously described class I missense mutations in LamB that prevent phage λh$^+$ adsorption but permit the selection of one-step extended-host-range mutants of λ (λh) (16); they correspond to eleven amino acid sites in mature LamB (residues 18, 148, 151, 152, 163, 164, 245, 247, 249, 250, 259, and 382 [4, 5, 11, 14]). All one-step extended-host-range phages also retain the capacity to use the wild-type LamB receptor and can also utilize any of the class I LamB mutants for infection. Other λh$^+$-resistant missense mutants in *lamB* that confer tight resistance on both λh$^+$ and λh were previously selected after ethyl methane sulfonate mutagenesis in vivo. Six of these mutants, called class II mutants (11), contained a G-to-D change at residue 151 (G151D). Two-step extended-host-range mutants of λ that were selected on G151D, named λhh*, can infect *E. coli* expressing either G151D, any of the class I LamB mutants, or wild-type LamB.

Recently, we selected and analyzed a series of different one-step and two-step extended-host-range mutants of λ (30). Three different class I LamB missense mutants (at residues 247, 245, and 148 in the LamB protein) were used to select eleven independent one-step host range mutants (λh phages). In each case, a single amino acid change was responsible for the alteration in host range. The changes affected only three residues in the distal part of J, corresponding to V1077A, to T1040M, and to L1127P. The class II LamB mutant G151D was used to select two-step extended-host-range mutants (λhh* phages) from these three λh phages. The λhh* mutants that resulted corresponded either to double or to triple point mutations located at the distal end of the J protein, in the same region of J that produced the λh phenotype. The nature of the mutations in J did not depend on the LamB class I mutants used to select them: each of the λh mutants could utilize any of the class I mutants that we used to generate them. Moreover, all the two-step host range phages could grow on the class II LamB mutant used for their selection as well as on all the class I mutants and on wild-type LamB. Therefore, we concluded that the mutated residues in J and in LamB were probably not involved in allele-specific protein-protein interactions. Rather, all class I LamB mutants create a block in phage λ adsorption that can be relieved in one selection step by different mutations

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TABLE 1. Characteristics of the mutated residues at site 151

Residue	Class of mutations[a]	MW[b]	Hydrophobicity index[c]	Accessible surface area[d]	Side chain
Wild type		57	0.16	75	—H
G151S	I	87	−0.26	115	—CH_2OH
G151C	I	103	−0.04	135	—CH_2—SH
G151R	II	156	−1.76	225	—$(CH_2)_3$—NH—C(=NH)—NH_2
G151V	II	99	0.54	155	—CH(CH_3)—CH_3
G151D[e]	II	115	−0.72	150	—CH_2—COOH

[a] According to in vivo sensitivity to λh⁺, λh, and λhh*.
[b] According to reference 8: molecular weight of amino acid minus that of water.
[c] According to the Eisenberg normalized scale (22).
[d] According to reference 8 (in Ångstroms).
[e] LamB 113 class II mutation in pop1091.

in the J protein of the phage (λh mutants). In contrast, the class II mutation can be compensated for only in two selection steps by different mutations in the same region of the J protein of the phage (λhh* mutants) (2).

Since G151D was the only substitution in LamB preventing both λh⁺ and λh adsorption (class II), we decided to study the effects of four new amino acid changes at site 151, generated by directed mutagenesis. Therefore, we chose four residues (S, C, V, and R) with different biochemical characteristics (Table 1). The data are discussed in terms of a possible mechanism of phage adsorption.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. *E. coli* K-12 strain C600 (F⁻ *thr leu tonA lacY1 supE*) carries a wild-type *lamB* gene on its chromosome. Strain pop6510 (*thr leu thi metA lacY dex-5 tonA supE recA srl*::Tn*10*) (1) was our standard λ-resistant strain. Strain pop1091, the class II mutant strain previously described (11), carries the chromosomal *lamB113* allele in the background of Hfr G6 (Hfr *metA trpE galE galY rpoB*).

Transformations with pAC1 recombinant plasmids and transfections with M13mp18 recombinant phages were performed by standard procedures (15, 25). JM 501 (30) was used as a recipient for plasmid constructions and for infections with M13mp18 recombinant phages. Strain pop6510 was the recipient for all the pAC1-derived plasmids.

The virulent λ derivative of wild-type λ phage (λh⁺) and its mutants of extended host range (λh and λhh*) were previously described (λ*b2virho*, and λ*b2virhoh16* in reference 2). We recently reported that phage λh (from the laboratory collection) contained two distinct mutations at the distal end of its *J* gene (S1011G and T1040A [30]). For this work, we selected four new λhh* derivatives from λh, on the Class II LamB mutant G151D. Sequence analysis revealed that the four new λhh* were identical (they were called λhh* 1011) and contained three distinct mutations at the distal end of the J protein (T1040A, S1011G, and D996E; Fig. 1). In this case, as in the case of all the other two-step host range λhh* phages that we have previously analyzed (30), λhh* 1011 contained the two mutations that were present in the parental λh phage (T1040A and S1011G) and had acquired an additional mutation upstream, in the same region (D996E). Phage λhh* (from the laboratory collection) was also analyzed in this work and we found that it contained three distinct mutations all located at the distal end of its gene *J* (Q1078R, V1077A, and S1011G). However, the fact that λhh* contained only one of the two mutations present in λh (S1011G), from which it was supposed to derive, tends to suggest that it might have derived from a λh phage which carried only one mutation (the conserved S1011G change).

All the other one-step and two-step host range mutants of λ were described in reference 30.

Sensitivity to phages was assayed by the spot test procedure on lawns of the different strains plated on Luria-Bertani agar.

Maltose transport experiments. Conditions for measuring in vivo maltose transport were previously described (27). ^{14}C-maltose uptake was measured at a low maltose concentration (4 μM), at which LamB is specifically required for maltose entry, at three time points (30, 60, and 90 s). For each strain, results in counts per minute were plotted against time. Maltose transport is expressed as percent wild-type activity. It corresponds to the ratio between the slope of the mutant and the slope of the wild-type LamB strain. For the four plasmid-encoded LamB mutants, the wild-type LamB strain was AC1 [pop6510(pAC1)]. The growth conditions were as in the study reported in reference 29.

Isolation and DNA sequence analysis of a λh-resistant and λhh*-sensitive LamB mutant. A culture from strain C600 was irradiated with UV. The mutagenized culture (1.35×10^8 PFU) was then grown in 5 ml of 63 B1 minimal medium containing threonine, leucine (0.02% final), dextrins (0.4% final), and phage λh (10^9 PFU). At an optical density at 600 nm of 0.3, an aliquot of the culture was spread onto tryptone plates with phage λh (2×10^9 PFU of phage per plate). Colonies were replica plated onto MacConkey dextrin solid medium, and the dextrin-positive (Dex⁺) colonies were reisolated. The phage sensitivity phenotype of the selected clones was checked by cross-streaking and by spot test against λh and λhh*. One Dex⁺, λh-resistant, and λhh*-sensitive strain was studied.

We focused our sequence analysis on the region of *lamB* corresponding to residues 121 to 280 of the mature LamB protein, because this region is known to contain most of the residues affecting phage lambda adsorption (for a review, see reference 5). The chromosomal DNA portion of the mutant encoding residues 121 to 280 was amplified by asymmetric PCR as described previously (25). We used two primers: Left, 5′ TCT GGT CCT GGT GCC GGT CTG G 3′; and Right, 5′ CAT CAT GTC CCA GTT GTC GCC C 3′. The single-stranded amplified DNA fragments were sequenced by PCR according to the procedure described in reference 25. We used primer Left as a sequencing primer. It was labelled at its 5′ end with T4 polynucleotide kinase.

Oligonucleotide-directed mutagenesis of amino acid site 151 of LamB. (i) Subcloning of *lamB* into M13mp18. Gene *lamB* was first subcloned into the pTZ18R vector. A 1.2-kb DNA fragment comprising the *lamB* gene was obtained from plasmid pHSF1 (9) by double digestion with restriction enzymes *Eco*RI and *Stu*I (in this plasmid, the *Eco*RI site is located a few bases upstream of the ATG of the signal sequence of LamB, and the *Stu*I site occurs 60 bp downstream from the last TGG codon of the *lamB* gene). The 1.2-kb fragment was subcloned into the *Eco*RI and *Sma*I sites of plasmid pTZ18R. On this plasmid (constructed by Eliette Touati, Institut Pasteur, and called pET1), *Bam*HI sites flank the *lamB* gene. Plasmid pET1 was then cut with *Bam*HI, and the 1.2-kb *Bam*HI-*Bam*HI fragment containing *lamB* was subcloned into the *Bam*HI site of the replicative form of M13mp18 DNA according to stan-

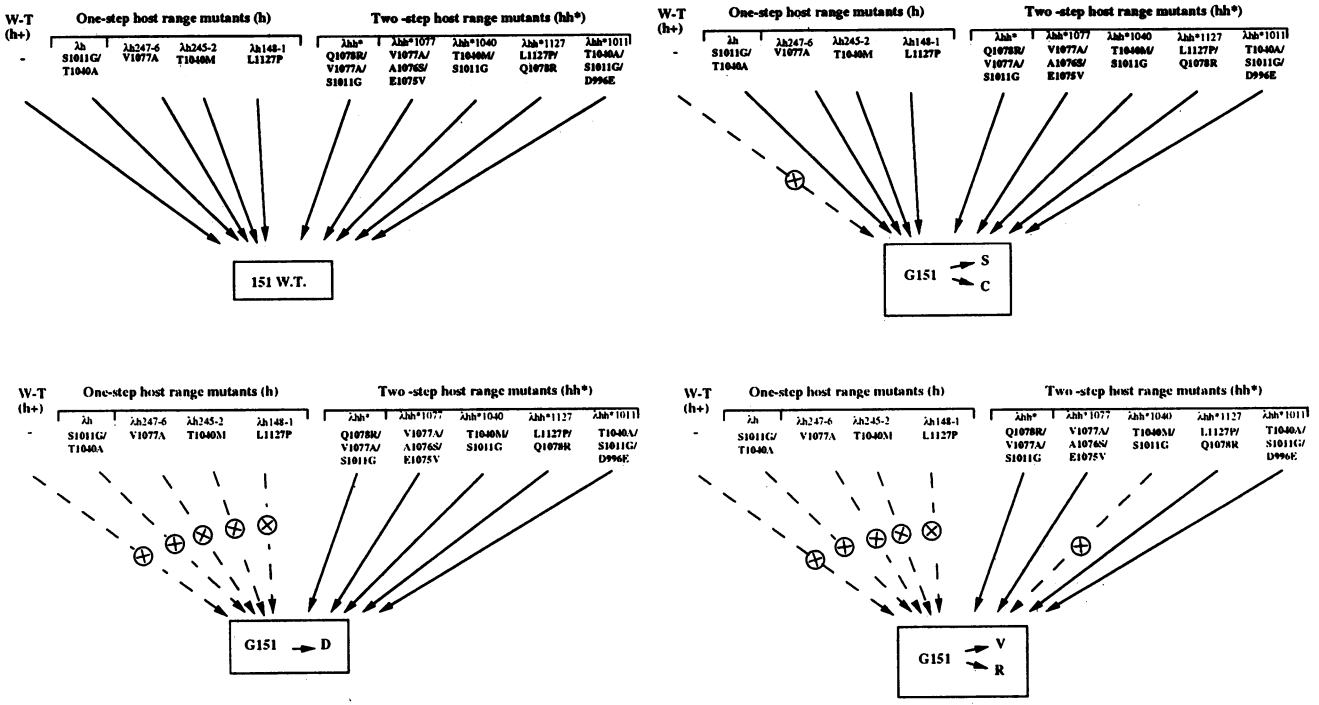

FIG. 1. Summary of the in vivo properties of the mutant phages on the LamB mutants at amino acid site 151. Plain arrows indicate sensitivity to infection by the phage. Dotted arrows with circled X indicate tight resistance to the phage (below 10^{-5}). The general effect of both class I and class II mutations is to decrease the binding between λ and its receptor, and the mutations in λh and λhh* compensate by at least partially restoring this binding. In this respect, it is particularly striking to compare the sequence of the two-step host range phage λhh* 1127 with the sequence of its parental one-step host range phage, λh148-1. λhh* 1127 contains one additional mutation (Q1078R) that is sufficient to restore wild-type in vivo binding (EOP = 1) on the two class II LamB mutants G151V and G151R (Table 2). λhh* 1040 (T1040M and S1011G), deriving from λh245-2 (T1040M), was the only two-step host range phage unable to adsorb to G151R and G151V. In this case, the additional S1011G mutation in λhh* 1040 was only sufficient to compensate for the class II G151D change. It is also worth noting that the two mutations in λhh* 1040 fell at the same two sites as did the mutations in the one-step phage λh (T1040A and S1011G). However, in λhh* 1040, T changes to M at site 1040, while in λh, T becomes A. Therefore the T1040M change permits the adsorption of λhh* 1040 (and not of λh) to LamB G151D, and both the G151R and G151V mutations in LamB eliminate this binding.

dard procedures (15). One M13mp18 recombinant phage carrying an insert corresponding to the complementary strand of *lamB* gene was kept.

(ii) Site-directed mutagenesis of residue 151. Two different 33-base oligonucleotides, encompassing residues 146 to 156 of mature LamB, were used. The sequence of the first oligonucleotide was 5′ TCC TCT GAA GCT GGT XYZ TCT TCC TCT TTC GCC 3′ (where X is A, C, T, or G; Y is A, T, or G; and Z is T or G). The second oligonucleotide was 5′ TCC TCT GAA GCT GGT XGT TCT TCC TCT TTC GCC 3′ (where X is C or T). In this oligonucleotide, the composition of codon XGT allows the formation of codon CGT determining an arginine and of codon TGT determining a cysteine. We followed the Kunkel method for the in vitro mutagenesis of residue 151 of LamB (17). Single-stranded DNA and DNA replicative forms were prepared from the recombinant plaques and sequenced by the standard techniques (15, 25). We used the primer 5′ GGC TCC ACC ATC TGG GCA GGT 3′, corresponding to the coding strand of *lamB* upstream of the mutagenized codon (residues 97 to 103 of mature LamB). After DNA sequence analysis of a series of recombinant clones, we selected four different substitutions: two with the first mutagenic oligonucleotide, GGT to AGT and GGT to GTT, corresponding respectively to G151S and G151V substitutions, and two with the second mutagenic oligonucleotide, GGT to CGT and GGT to TGT, corresponding to G151R and G151C substitutions, respectively.

The four mutations were subcloned into the expression vector pAC1 (1). Double-stranded 440-bp *Cla*I-*Nco*I fragments from the M13-*lamB* recombinant phage DNAs (encoding the mutagenized residue) were subcloned into the 5.3-kb *Cla*I-*Nco*I fragment of pAC1 and transformed into the LamB negative strain pop6510.

RESULTS

Generation of specific mutations at LamB residue 151. In vitro mutagenesis was performed on the *lamB* gene cloned into the M13mp18 vector. We obtained four different missense mutations, which changed Gly-151 of LamB to S, V, R, or C (codons AGT, GTT, CGT, and TGT, respectively). Then, the four corresponding *lamB* mutant genes were placed under the control of the inducible *ptac* promoter (see Materials and Methods) and expressed in the background of pop6510 (Fig. 2).

Maltose and maltodextrin utilization. The ability of the LamB 151 mutants to utilize maltose and maltodextrins was first tested on 63 B1 minimal medium plates containing limiting concentrations of maltodextrins (0.05% final) with an increasing number of glucose units, from glucose (glu1) to maltoheptaose (glu7). The four mutant strains grew in maltodextrins with glucose unit levels up to that of maltopentaose as well as the wild-type LamB strain (AC1). They also formed red colonies on MacConkey dextrin medium, confirming that they

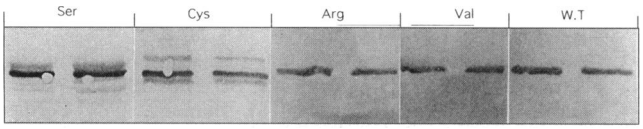

FIG. 2. Immunodetection of the LamB mutants. LamB extracts were prepared as described previously (3). The amounts of LamB protein present in the different extracts were compared by Western blot (immunoblot) analysis. The samples were heat denatured and loaded onto sodium dodecyl sulfate–10% polyacrylamide gels. After electrophoretical transfer to nitrocellulose, the LamB proteins were revealed by use of anti-I 12 anti-LamB antibody (31) at a final dilution of 1/20,000. In each case, two different amounts of extract were analyzed (corresponding to 7.5×10^6 bacteria in the left well and 1.125×10^7 bacteria in the right well). The amounts of LamB detected in the four mutants were comparable to that of the wild-type LamB (in AC1). For the chromosomally encoded *lamB* mutant G151D previously described (pop1091), the amount of LamB detected was approximately twofold lower (not shown).

were able to utilize dextrins as a carbon source. However, they showed a somewhat reduced growth capacity on maltohexaose and maltopentaose. Then, we measured the kinetics of in vivo maltose uptake in the different LamB mutants at a low maltose concentration (4 μM). The two class I mutants G151C and G151S showed a twofold reduction of maltose uptake (50% of the wild-type level), like the previously studied class II mutant G151D (5), while the two class II mutants G151V and G151R showed a slightly stronger reduction in maltose uptake (with 36% of the wild-type activity for both of them). This confirmed previous observations showing that although the phage λ-binding domain and the maltose and maltodextrins-binding domains are distinct, they overlap on the surface of LamB (5).

Phage receptor activity in vivo. We tested the sensitivity of the four mutants to λh$^+$ and to its extended one-step and two-step extended-host-range derivatives, λh and λhh*, two phages from the laboratory collection (2). Two of the LamB mutants (G151S and G151C) conferred resistance to λh$^+$ but were still fully sensitive to λh and λhh* (with an efficiency of plating [EOP] of 1 with the two host range mutants), while the two other LamB mutants (G151R and G151V) conferred tight resistance to λh$^+$ and λh and were still fully sensitive only to λhh*. According to the previous classification based on in vivo sensitivity to these phages, the two G151S and G151C changes thus correspond to class I LamB mutants, while the two G151R and G151V changes correspond to class II LamB mutants.

We next tested the sensitivity of the four LamB mutants to other different one-step and two-step host range mutants of λ (30) (Fig. 1). In agreement with the previous classification, G151S and G151C mutants were fully sensitive (EOP = 1) to the three one-step host range mutants tested (λh148-1, λh247-6, and λh245-2), while G151R and G151V mutants were resistant to these three phages. But conversely, when the four LamB mutants were tested with the new λhh* two-step host range phages, a new sensitivity pattern emerged. While G151S and G151C mutants were fully sensitive to all the λhh* phages (EOP = 1), G151R and G151V mutants were resistant to λhh* 1040 and strongly impaired in λhh* 1077 infection (EOP = 10^{-3}). Therefore, G151R and G151V represent a new subclass in class II mutants with a phage sensitivity pattern different from that of the previously studied LamB G151D.

As recalled previously, all the two-step host range phages were selected on the class II G151D mutant. It is noteworthy that four of these five two-step host range phages showed a reduced EOP on G151D (10^{-2}).

Isolation and DNA sequence analysis of a λh-resistant and λhh*-sensitive LamB mutant. We next wanted to see if class II point mutations could be found at other sites in LamB. Since selection for λh$^+$ yields mostly class I mutants (i.e., sensitive to λh), we selected λh-resistant mutants directly. *E. coli* K-12 strain C600 was mutagenized in vivo with UV light. To avoid deletion and frameshift mutations in *lamB*, we selected λh-resistant mutants that still expressed a functional LamB protein, i.e., one that conferred the ability to use dextrins as a carbon source (Dex$^+$; see Materials and Methods). The λh-resistant, Dex$^+$ mutants were then tested against λhh*, the two-step host range mutant of λ. One Dex$^+$, λh-resistant, and λhh*-sensitive mutant was isolated by this procedure. The DNA sequence analysis of this mutant showed that it corresponded to a GGT-to-GAT transition at site 151, changing G-151 to D, exactly like the class II mutant G151D previously described (11).

TABLE 2. In vivo properties of the mutated and wild-type LamB proteins

Phage[a]	Relative efficiency of plating[b] of:						
	New LamB mutants[c]				LamB (WT)[d]	Other LamB mutants[e]	*lamB* 113[f]
	Class I		Class II				
	G151S	G151C	G151V	G151R			
λh$^+$	0	0	0	0	1	0	0
λh	1	1	0	0	1	1	0
λhh*	1	1	1	1	1	1	1
λh 148-1	1	1	0	0	1	1	0
λh 247-6	1	1	0	0	1	1	0
λh 245-2	1	1	0	0	1	1	0
λhh* 1011	1	1	1	1	1	1	0.01
λhh* 1127	1	1	1	1	1	1	0.01
λhh* 1040	1	1	0	0	1	1	0.01
λhh* 1077	1	1	0.001	0.001	1	1	0.01

[a] The virulent λ derivative of the wild-type λ phage, λ*b2vir* (abbreviated as λh$^+$), and its one-step (λh) and two-step (λhh*) extended-host-range mutants were previously described (2). Sensitivity to phages was assayed by spot tests on lawns of the different strains plated on Luria-Bertani agar. The titers of the different phage stocks were of about 10^{10} PFU/ml on the wild-type LamB$^+$ strain.

[b] The value for the wild type is taken arbitrarily as 1.

[c] The initial classification of the LamB mutants was according to the in vivo growth of phages λh$^+$, λh, and λhh* (2).

[d] WT, wild type.

[e] All class I: E148K, G245R, S247L, and G382D.

[f] Class II: G151D.

DISCUSSION

We generated four different amino acid substitutions at site 151 (S, V, R, and C). When tested against λh$^+$, λh, and λhh*, two mutants fell into class II (G151R and G151V) while the two others fell into class I (G151S and G151C), demonstrating that the nature of the substitutions at residue 151 was critical for the different phage sensitivity phenotypes. The two class I mutants were, as expected, fully sensitive to all the other different one-step and two-step host range mutants of λ tested (Table 2). The two class II mutants were resistant to all the one-step host range phages, but while they remained fully sensitive to two of the two-step host range phages, they were partially or fully resistant to the two others. These results show that G151R and G151V correspond to a subclass of class II mutations different from G151D. The fact that the introduction of a negatively charged (D), a positively charged (R), or an

aliphatic nonpolar (V) residue blocks both λh^+ and λh phage adsorption demonstrates that the block is not due to a charge effect.

Our previous results indicated that the mutated residues in the LamB mutants and in the J proteins of the extended-host-range mutants of λ were probably not involved in allele-specific protein-protein interactions (30). LamB mutants were interpreted as provoking changes in a region of the phage receptor called the accessibility gate, which blocks λ adsorption by preventing access of the phage tail fiber to its tight binding site. This block would be relieved by different types of amino acid changes in the distal part of the *J* genes of the extended-host-range mutants of λ, restoring the ability of the mutated tail fiber to have access to the binding site. The data presented here can also be interpreted in terms of a modification of the accessibility gate, by holding that residue 151 belongs to the accessibility gate and that the different substitutions affect to various extents the accessibility to the phage tight binding site. The class I changes (G151S and G151C) would cause the same type of modifications as all the other class I mutations in LamB. The class II mutations would restrict the access to the binding site more than class I mutations and among them G151V and G151R changes would be more restrictive than G151D changes. The fact that the accessible surface areas (8) of the two class I LamB 151 mutants are smaller than that of G151D, which in turn is smaller than G151V and G151R (G151V and G151R > G151D > G151S and G151C; see Table 1) fits with the idea that the restriction is due to steric hindrance.

In addition to the mutations at site 151, ten different mutations have now been identified in the surface-exposed region of LamB encompassing residues 146 to 164 (S146C, E148K, S152F, S152C, S153C, S154F, S154C, F155S, Y163D, and T164P [4, 11, 13, 24]). Among these mutations, only three completely block λh^+ adsorption (E148K, S152F, and Y163D), while all the others do not affect (or affect only partially) λ adsorption. It is striking that these mutations as well as the mutations at site 151 correspond to amino acid sites located at each extremity of this surface loop. Moreover, we selected by in vivo UV mutagenesis a λh-resistant and λhh*-sensitive LamB mutant. DNA sequence analysis of this mutant showed that it corresponded to a GGT-to-GAT transition at site 151, changing G-151 to D, exactly like the class II mutant G151D previously described (11), confirming the unique role of amino acid site 151 in λ adsorption (h^+ and h). Thus, it is tempting to propose that, among the residues that participate in the formation of the accessibility gate in the loop formed by residues 146 to 164, residue 151 is the most protruding or the closest to the phage tight binding site.

Further biochemical and biophysical studies on the J protein and the determination of the three-dimensional structure of LamB will be required to understand fully some of the observations presented here.

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