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Antigenic Polymorphism of the LamB Protein among Members of the Family *Enterobacteriaceae*

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In this study we demonstrate that most members of the family *Enterobacteriaceae* possess a maltose-inducible outer membrane protein homologous to the LamB protein of *Escherichia coli* K-12. These proteins react with polyclonal antibodies raised against the LamB protein of *E. coli* K-12. We compared the antigenic structure of the LamB protein in members of the family *Enterobacteriaceae* with six monoclonal antibodies raised against the LamB protein of *E. coli* K-12. Four of them reacted with epitopes located at the outer face of the membrane, and two reacted with epitopes located at the inner face of the membrane. A great degree of variability was observed for the external epitopes. Even in a single species, such as *E. coli*, an important polymorphism was present. In contrast, the internal epitopes were more conserved.

The *lamB* gene of *Escherichia coli* encodes a maltose-inducible outer membrane protein (LamB protein), which is a component of the maltose and maltodextrin transport system (34). This protein also functions as a receptor for several bacteriophages, including λ and K10 (10, 28, 29). In its native state, the protein is a trimer, composed of three identical subunits (22, 25). Each subunit contains 421 amino acids (11). Attempts to identify the portions of the polypeptide which are exposed at the cell surface have included (i) the sequencing of mutations which impair the interaction of the LamB protein with bacteriophages λ or K10 or both (10, 12, 30), (ii) the preliminary mapping of antigenic determinants recognized by anti-LamB protein monoclonal antibodies (mAbs) which bind to intact cells (16, 17; C. Desaymard, M. Debarbouillé, and M. Shwartz, manuscript in preparation), and (iii) the identification of peptide bonds which are protected by mAbs against proteolysis in vitro (32).

Results obtained by these approaches allowed the identification of three, and perhaps four, small segments of the polypeptide which are probably exposed at the cell surface (T. Gabay, S. Schenkman, C. DeSaymard, and M. Shwartz, in press). These results can be fitted into secondary structure models which predict that the LamB polypeptide may cross the outer membrane up to 17 times (10).

In this study we started a comparison between the LamB protein of *E. coli* K-12 and homologous proteins found in related bacteria. The presence of a protein homologous to the LamB protein has already been reported in *Shigella* sp. (35), *Salmonella typhimurium* (26), and *Klebsiella pneumoniae* (27). We have now extended this observation to other members of the family *Enterobacteriaceae* and have analyzed this family of homologous proteins for the presence of six epitopes, defined by the six mAbs previously characterized in our laboratory (17, 32). The most striking result from this analysis is the finding that the antigenic determinants exposed at the cell surface are very poorly conserved, even in a single species such as *E. coli*. The structural and evolutionary implications of this finding are discussed.

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MATERIALS AND METHODS

Bacterial strains and phages. Strains used in this study are listed in Table 1. MC4100 was our standard Mal$^+$ λ^s strain. Phage K10 was a gift of P. Reeves (18).

Growth conditions. Media were used as described by Miller (23). Induction by maltose was studied by comparing cultures in M63 medium supplemented with 0.4% glycerol or 0.4% glycerol–0.4% maltose, with 0.1% nicotinic acid used for *Shigella* and *Proteus* strains. Maltodextrins were prepared as described by Wandersman et al. (38).

General methods. Bacteriophage λ receptor was extracted with 5 mM EDTA–3% Triton X-100 and assayed by its ability to inactivate λ Vho in vitro, as described by Chapon (9). The outer membrane proteins were separated with Triton X-100 by the method of Schnaitman (33). Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate was performed as described by Anderson et al (1). We used a resolving gel containing 9% polyacrylamide. Transduction of *lamB* genes by phage P1 was performed with strain pop-2502 as the recipient. This *E. coli* K-12 strain carries the *malB101* deletion which covers a part of the *malK* gene and all the *lamB* gene and renders the strain Mal$^-$. The transductants were selected as Mal$^+$.

Antibodies. Polyclonal anti-LamB serum was obtained by injecting Triton X-100-solubilized native LamB protein into a rabbit (17) and was preadsorbed with cells of the *lamB* nonsense mutant pop3205 as described by Schenkman et al. (31). Polyclonal antibodies were purified by DEAE chromatography (21), and mAbs were purified from ascitic fluid with protein A-Sepharose (15).

Immunological techniques. Immunoprecipitation experiments were performed as previously described by Gabay et al. (16) with a few modifications. Proteins were extracted under nondenaturing conditions by a 1-h incubation in 1% octyl polyoxyethylene (a gift from J. P. Rosenbusch)–5 × 10^{-3} M EDTA–10 mM Tris-hydrochloride (pH 7.5). Immune complexes were precipitated with protein A-Sepharose.

Enzyme-linked immunosorbent assay (ELISA) was carried out with an antibody-antigen-antibody system. Disposable microtiter plates (Dynatech; Rungis, France) were first coated with rabbit anti-LamB immunoglobulin G at 5 μg/ml;

TABLE 1. Bacterial strains

Strain or species	Source or reference
Escherichia coli	
K-12 MC4100	8
K-12 MC4100 *lamB102*[a] (pop3205)	20
K-12 MM294	36
K-12 MM294 Δ*malB101*[b] (pop2502)	This study
K-12 (pop2513)[c]	This study
ML30	35
63.71	35
02	Reference strain for O serotype[d]
04	Reference strain for O serotype[d]
06	Reference strain for O serotype[d]
07	Reference strain for O serotype[d]
018	Reference strain for O serotype[d]
028	Reference strain for O serotype[d]
083	Reference strain for O serotype[d]
Shigella sonnei	
482-83	Hospital isolate[d]
484-83	Hospital isolate[d]
488-83	Hospital isolate[d]
491-83	Hospital isolate[d]
493-83	Hospital isolate[d]
495-83	Hospital isolate[d]
496-83	Hospital isolate[d]
499-83	Hospital isolate[d]
185-83[e]	Hospital isolate[d]
Citrobacter freundii CDC 460-61	Reference strain[d]
Enterobacter aerogenes ATCC 13048	Reference strain[d]
Enterobacter cloacae ATCC 13047	Reference strain[d]
Enterobacter hafniae ATCC 13337	Reference strain[d]
Erwinia herbicola NCTC 9381	Reference strain[d]
Erwinia herbicola T	A. Toussaint[f]
Klebsiella pneumoniae K2	Reference strain[d]
Klebsiella pneumoniae ATCC 15050	G. Cornelis; 3
Klebsiella pneumoniae W	G. Wöber; 13
Proteus vulgaris PR1	Reference strain[d]
Salmonella typhimurium LT2	Reference strain[d]
Serratia liquefaciens 866	Reference strain[d]
Serratia rubidaea 864	Reference strain[d]

[a] *lamB102* is a nonsense mutation.
[b] Δ*malB101* is a deletion of the *malB* region, including the entire *lamB* gene (19).
[c] A Mal$^+$ transductant of *pop-2502* obtained by using P1 phage grown on *Shigella sonnei* 495-83
[d] From the collection of the Service des Entérobactéries de l'Institut Pasteur.
[e] *Shigella sonnei* 185-83 is Mal$^-$.
[f] This strain was provided by A. Toussaint because it had the exceptional property of being sensitive to phage λ when grown at 42°C.

bacterial extracts at a final concentration of 0.1% octyl polyoxyethylene were then added to the wells. Additional steps were performed as described previously (14).

RESULTS

Presence of a LamB protein in members of the family *Enterobacteriaceae*. Because the LamB protein is a component of the maltose and maltodextrin transport system, we restricted our analysis to Mal$^+$ bacteria. Although it is not exhaustive, our analysis includes bacteria from most species of the family *Enterobacteriaceae*. A minimum requirement for a protein homologous to the LamB protein of *E. coli* K-12 is a localization in the outer membrane. Additional properties expected for such a protein are (i) inducibility by maltose and (ii) similar molecular weights (47,000). Proteins fulfilling the three requirements were found in all strains tested excepted *Proteus vulgaris* (Fig. 1).

The LamB protein of *E. coli* K-12 interacts with phage λ in vitro. Under adequate conditions (presence of chloroform in the reaction mixture or use of a host range mutant of λ, λ Vho) it inactivates the phage (28). We extended the findings of Schwartz and Le Minor (35) that extracts from strains other than *E. coli* or *Shigella* generally fail to inactivate λ Vho in vitro (data not shown). Therefore, although these other bacteria possess a protein which seems to be homologous to the LamB protein, this protein fails to react with λ in vitro. These bacteria are all resistant to λ, but results of previous studies have indicated that even *E. coli* and *Shigella* strains which possess a λ receptor active in vitro fail either to fix or to support growth of phage λ (35). The only exception was a strain of *Erwinia herbicola* (also called *Enterobacter agglomerans*) which was sensitive to phage λ, at least when grown at 42°C (D. Expert and A. Toussaint, personal communication). This strain (*Erwinia herbicola* T in our nomenclature) contains a protein which inactivates λ Vho in vitro. Moreover, it is also sensitive to phage K10.

Immunological cross-reactivity between the LamB proteins from various *Enterobacteriaceae*. The immunological cross-reactivity between the different LamB proteins was studied by using both polyclonal antibodies and mAbs raised against the LamB protein of *E. coli* K-12 (17, 31). Of the six mAbs used, four (mAbs 72, 177, 302, and 347) were specific for epitopes exposed at the outer face of the outer membrane, whereas two (mAbs 141 and 436) reacted with epitopes which are only accessible in cell extracts (17, 31). The ELISA and the immunoprecipitation assays were used as appropriate, both involving detergent extracts of the bacterial cells. ELISA, which is more convenient, was used systematically. However, it was not very sensitive because it failed to detect cross-reactivity at levels less than 10%. Therefore, immunoprecipitation was used in many instances to verify the results obtained by ELISA and to test for low-level cross-reactivity.

With all strains tested, the maltose-inducible protein of the outer membrane could be immunoprecipitated with anti-LamB polyclonal antibodies (Fig. 2), thus confirming that this protein can be considered homologous to the LamB protein of *E. coli* K-12. However, the degree of cross-reactivity was not the same in all cases; it was high when other *E. coli* strains were used and much lower with many of the more distantly related bacteria. In the latter cases, the protein was present in large amounts (Fig. 1), but it could only be detected by immunoprecipitation and not by ELISA. These differences in the degree of cross-reactivity were generally reflected in the number of conserved epitopes, as detected when mAbs were used (Table 2). However, some additional conclusions can be drawn from the results shown in Table 2. The most striking result with the *E. coli* strains is the variability in the presence of the four cell surface epitopes and the complete conservation of the two internal epitopes. With *Shigella* strains the pattern resembles that found with *E. coli*, except that some *Shigella* spp. lost one or two of the internal epitopes. When the *lamB* gene was transduced from some of these *E. coli* and *Shigella* strains (*E. coli* 018 and 075, and *Shigella sonnei* 482-83 and 495-83) into *E. coli* K-12, the pattern of antibody recognition was the

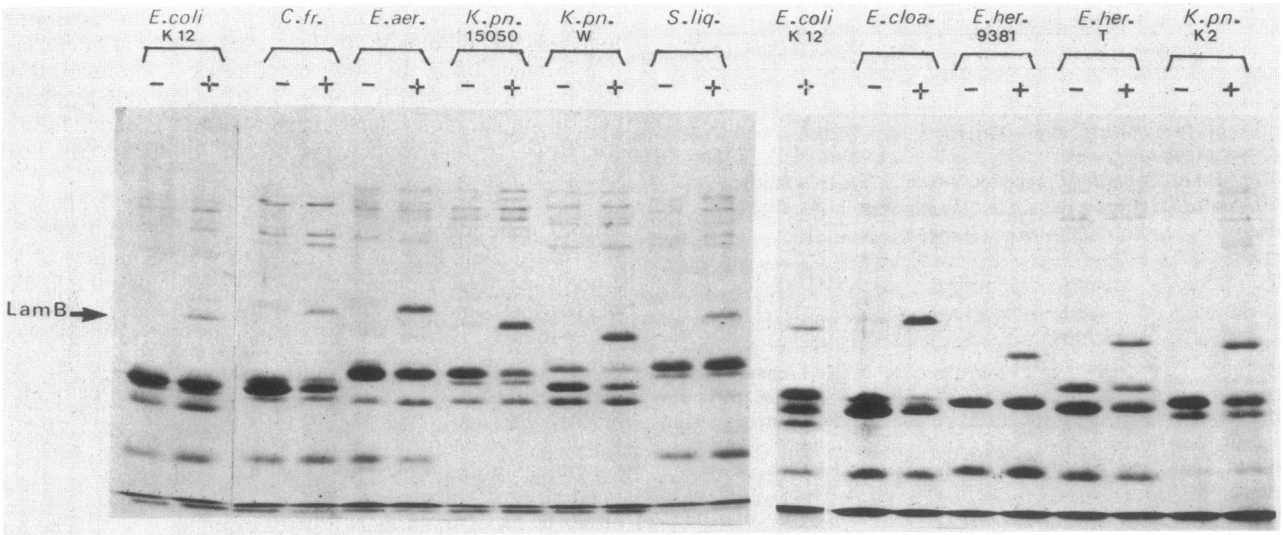

FIG. 1. Outer membrane proteins of members of the family *Enterobacteriaceae*. The proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the gel was stained with Commassie brilliant blue. The samples were obtained from cultures in minimal medium induced (+) or not induced (−) by maltose. The arrow indicates the LamB protein of *E. coli* K-12. Abbreviations *C.fr.*, *Citrobacter freundii* CDC 460-61; *E.aer.*, *Enterobacter aerogenes* ATCC 13048; *K.pn.* 15050, *Klebsiella pneumoniae* ATCC 15050; *K.pn.*W, *K. pneumoniae* W; *S.liq.*, *Serratia liquefaciens* 866; *E.cl.*, *Enterobacter cloacae* ATCC 13047; *E.her.* 9381, *Erwinia herbicola* NCTC 9381; *E.her.*T, *Erwinia herbicola* T; *K.pn.* K2, *K. pneumoniae* K2.

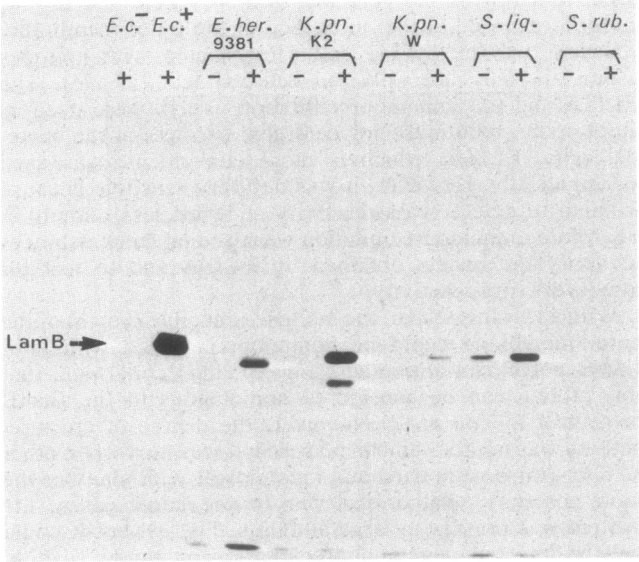

FIG. 2. Immunological cross-reactivity of the LamB proteins of some members of the family *Enterobacteriaceae* with polyclonal antibodies. Proteins were extracted from cells grown in minimal medium and induced (+) or not induced (−) by maltose. They were immunoprecipitated with the polyclonal serum raised against the LamB protein of *E. coli* K-12. Abbreviations: *Ec*⁻: *E. coli* K-12 *pop-3205* (*lamB*⁻); *Ec*⁺, *E. coli* K-12 *pop-3* (*lamB*⁺); *E.her.* 9381, *Erwinia herbicola NCTC 9381; K.pn.* K2, *K. pneumoniae* K2, *K.pn.* W, *K. pneumoniae* W; *S.liq.*, *Serratia liquefaciens* 866; *S.rub.*, *Serratia rubidaea* 864. Similar results (data not shown) were obtained with the LamB proteins from all strains shown in Fig. 1. (The seemingly lower degree of LamB protein inducibility in this experiment as compared in Fig. 1 is probably due to the fact that cross-reactive antibodies are limiting.)

same in the transductants as it was in the donor strain, indicating that the observed reactions are indeed LamB specific (Fig. 3). With more distantly related species, the exposed determinants were very rarely conserved (two in the strain of *Enterobacter hafniae*, and one in a strain of *Klebsiella pneumoniae*). A somewhat better conservation of the internal determinants can be seen (Fig. 4), especially of the one recognized by mAb 436 (Table 2).

DISCUSSION

Results of this study demonstrate that most Mal⁺ members of the family *Enterobacteriaceae* possess a maltose-inducible outer membrane protein which is recognized by antibodies raised against the LamB protein of *E. coli* K-12. The only exception was *P. vulgaris* which, of all the strains tested, had the least homology with *E. coli* by DNA-DNA hybridization (6). This result is reminiscent of that found with other outer membrane proteins (2, 24).

Immunological cross-reactivity between homologous proteins, as studied with polyclonal antibodies, has provided a means for the taxonomic classification of bacteria, with the results being generally consistent with those obtained by other techniques (7, 37). A similar classification of the different species could also be made on the basis of reactivity with our anti-LamB mAbs. However, the analysis of several strains within one species exhibited an unexpectedly large variation with overlap between species, for example between *E. coli* and *S. sonnei*. Hence, the *E. coli* strains tested had from two to six of the epitopes recognized by the six available mAbs, whereas *S. sonnei* had from none to five. With this type of variation, it is not surprising to find that a strain of *Enterobacter hafniae* has more epitopes in common with *E. coli* K-12 than do several *Shigella* strains, or even another strain of *E. coli*.

Of the six antigenic sites tested, the four which are exposed at the cell surface are the most variable. They are the only ones to vary among *E. coli* strains, and they are almost systematically absent in strains other than *E. coli* and *S. sonnei*. This suggests that the variability may be, in great

TABLE 2. Immunological cross-reactivity of the LamB proteins of some members of the family *Enterobacteriaceae* with mAbs

Strains	Binding of mAbs at the following sites:					
	Exposed				Hidden	
	302	177	72	347	436	141
Escherichia coli						
K-12[a]	+	+	+	+	+	+
07	+	+	+	+	+	+
010	+	+	+	+	+	+
028	+	+	+	+	+	+
ML30	+	+	+	+	+	+
018[a]	−	+	+	+	+	+
02[a]	−	+	+	−	+	+
04	−	+	+	−	+	+
06	−	+	+	−	+	+
075[a]	−	+	+	−	+	+
083	−	−	−	−	+	+
Shigella sonnei						
482.83[a]	−	+	+	+	+	+
493.83	−	+	+	+	+	+
495.83[a]	−	+	+	+	+	+
491.83[a]	−	+	+	+	+	+
185.83	−	−	+	+	+	+
496.83	−	+	+	+	−	+
484.83	−	−	−	−	−	−
488.83	−	−	−	−	−	−
499.83	−	−	−	−	−	−
Citrobacter freundii CDC 460-61[a]	−	−	−	−	+	−
Enterobacter aerogenes ATCC 13048[a]	−	−	−	−	+	+
Enterobacter cloacae ATCC 13047[a]	−	−	−	−	+	−
Enterobacter hafniae ATCC 13337[a]	−	−	+	+	+	−
Erwinia herbicola						
T[a]	−	−	−	−	+	+
NCTC 9381[a]	−	−	−	−	+	−
Klebsiella pneumoniae						
K2[a]	−	−	−	+	+	−
ATCC 15050[a]	−	−	−	−	+	−
W[a]	−	−	−	−	−	−
Serratia liquefaciens 866[a]	−	−	−	−	−	−
Serratia rubidaea 864[a]	−	−	−	−	−	−

[a] Reactivity of the mAbs with the LamB protein was studied both by ELISA and by immunoprecipitation. Immunoprecipitation was a more sensitive method so that some results that were negative in ELISA were slightly positive in immunoprecipitation. The results shown are those from the immunoprecipitation. Reactivity of all other was determined by ELISA.

part, the consequence of a selective pressure against the maintenance of exposed portions of the polypeptide, which may act as receptors for toxic agents. In this respect it is interesting to compare the antigenic variability of the LamB protein with its variability in terms of phage binding ability. It turns out that the LamB protein exhibits different degrees of conservation among species, depending on the parameters that are considered. The largest variability was observed for the epitopes located at the surface of the bacteria. There is no correlation between the loss of antigenic determinants and the loss of phage binding ability. For instance, some strains of *S. sonnei* and *Erwinia herbicola* still bind both phages λ and K10, but they have lost the four exposed epitopes. This may result from a selective pressure exerted by toxic agents which bind to this part of the molecule, like phages other than λ and K-10 or antibodies.

The antigenic polymorphism of the LamB protein may provide a new handle to study the folding of the protein in the membrane. By sequencing the *lamB* genes of *E. coli* and *S. sonnei* strains which differ by the presence of one or several epitopes, it may be possible to identify the amino acid changes responsible for the difference. This, combined with the analysis of *E. coli* K-12 mutants which have lost one or several epitopes (Desaymard et al., in preparation), may lead to a precise localization of the polypeptide segments exposed at the cell surface. These segments might represent ideal locations to insert foreign antigens if desired, as suggested by others (4), to use *E. coli* cells for the preparation of live vaccines.

The OmpA protein is another outer membrane protein present in most members of the family *Enterobacteriaceae* (2). Its structural gene has been sequenced in representative strains of five enterobacterial species (5). Small variable regions have been uncovered which are believed to correspond to exposed portions of the polypeptide. The authors have suggested, as we do again here for LamB, that the variability in such regions is the consequence of a selective pressure resulting from the presence of toxic agents, using the OmpA protein as their receptor. In view of the findings of

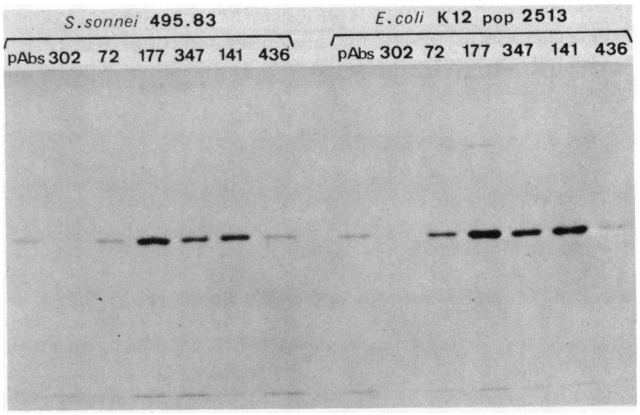

FIG. 3. Cross-reaction between the LamB proteins of *Shigella sonnei* 495-83 and *E. coli* K-12. The proteins were extracted from maltose-induced cultures of *S. sonnei* 495-83 and pop-2513 (a strain constructed by transduction of the *lamB* gene from *S. sonnei* 495-83 into *E. coli* K-12). Then they were immunoprecipitated with polyclonal antiserum (pAbs) or mAbs 302, 177, 72, 347, 141, and 436.

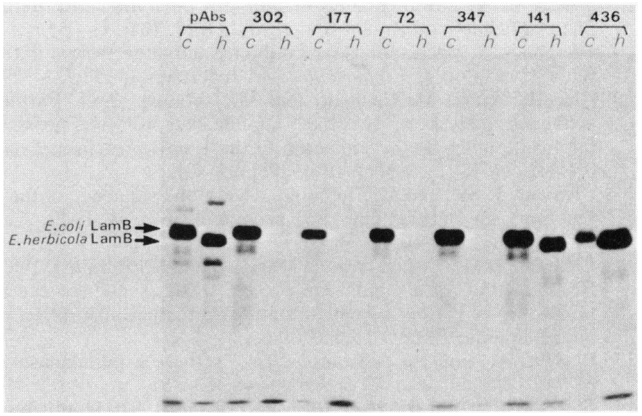

FIG. 4. Cross-reaction between the LamB proteins of *Erwinia herbicola* T and *E. coli* K-12. The proteins were extracted from cultures in minimal medium induced with maltose and immunoprecipitated with polyclonal antiserum (pAbs) or mAbs 302, 177, 72, 347, 141, and 436. Abbreviations: c, *E. coli* K-12 MC4100; h, *Erwinia herbicola* T.

the present study one wonders whether similar variable regions of OmpA might not also have been uncovered with several strains of the same species, rather than with strains from different species.

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