Monoclonal Antibodies Reveal LamB Antigenic Determinants on Both Faces of the Escherichia coli Outer Membrane

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LamB protein is involved in the transport of maltose across the outer membrane and constitutes the receptor for phage λ. In this study we characterized six previously described anti-LamB monoclonal antibodies (mAbs). Four of these, the E-mAbs, recognized determinants that were exposed at the cell surface, whereas the other two, the I-mAbs, recognized determinants which were not exposed. Competition experiments demonstrated that the domains recognized by these two classes of mAbs were completely distinct. In addition, the E-mAbs prevented LamB from neutralizing phage λ in vitro and protected LamB against proteolytic degradation, whereas the I-mAbs had no such effects. The E-mAbs have been shown previously to constitute two classes; some E-mAbs inhibit maltose transport in vivo, and others do not. Immunoelectron microscopy demonstrated that the I-mAbs also define at least two types of determinants. One of these, which is accessible in membrane fragments from a mutant (lpp) devoid of lipoprotein but not in membrane fragments from an lpp+ strain, probably corresponds to a region of LamB that is involved in the interactions with peptidoglycan. The other determinant, which is fully accessible in LamB-peptidoglycan complexes and in LamB-containing phospholipid vesicles but only slightly accessible in membrane fragments from an lpp mutant, is probably located very close to the inner surface of the outer membrane. LamB also contains at least one additional determinant, which (i) is exposed at the inner surface of the membrane, (ii) is accessible to antibodies in membrane fragments from an lpp+ strain, and (iii) may be involved in the interaction of LamB with the periplasmic maltose-binding protein.

LamB protein is one of the major cellular proteins (approximately 105 copies per cell) when Escherichia coli is grown in the presence of maltose (2, 24). The primary function of this protein is to permit the diffusion of maltose and maltodextrins across the outer membrane (2, 17, 30). In detergent solutions (14, 20) and probably in membranes (19, 23), LamB is a trimer composed of three identical subunits containing 421 amino acids (5). The amino acid sequence of LamB was deduced from the nucleotide sequence of its structural gene (5), whereas circular dichroism studies (14) indicated a predominance of β-sheets in its secondary structure. Like other pore-forming proteins (22, 26), LamB is believed to span the entire outer membrane. At the outer face of the membrane LamB acts as a receptor for phages, such as λ (24) and K10 (25), is recognized by specific antibodies (8), and binds maltose and maltodextrins (6). Evidence that LamB is exposed on the inner face of the outer membrane is more circumstantial. Genetic (11, 31) and biochemical (1, 18) experiments have indicated that this protein interacts with the periplasmic maltose-binding protein, and this feature has been included in models describing the mechanism of maltose transport (11, 28). The interaction with peptidoglycan also suggests that LamB may be exposed on the inner face of the outer membrane. LamB remains associated with the peptidoglycan sacculus when cells are dissolved in sodium dodecyl sulfate at 60°C in the presence of Mg2+ ions (9); it can be eluted from the sacculus by removing the Mg2+ ions or by increasing the salt concentration and can be reassociated with the sacculus under adequate conditions (32). However, reassociation requires the presence of lipoprotein, which is covalently bound to peptidoglycan and which is believed to be at least partially embedded in the outer membrane (3, 22, 32). Therefore, the association of LamB with the sacculus could result from LamB-lipoprotein interactions that occur within the outer membrane and does not necessarily imply that LamB protrudes at the inner face of this membrane.
To examine how LamB is inserted in the membrane, we used monoclonal antibodies (mAbs) directed against the protein, as recently described by Gabay and Schwartz (8). Some of these mAbs were shown previously to recognize determinants that were exposed at the bacterial surface; they inhibited the adsorption of λ and K10, and some also inhibited maltose transport. The other mAbs did not bind to intact cells; one bound to LamB-peptidoglycan complexes, and because of this it was proposed that the antigenic site recognized by this mAb is located within the membrane. In other cases the mAbs which did not bind to the surfaces of intact cells only recognized solubilized LamB that was obtained after elution from the peptidoglycan by extraction with detergent and EDTA; it was assumed that these mAbs recognized sites involved in the LamB-peptidoglycan interaction (8).

In this paper we further characterize the sites recognized by the different classes of mAbs and provide more direct evidence that LamB is accessible at the periplasmic face of the outer membrane.

MATERIALS AND METHODS

Bacterial strains and medium. Strain pop130 (ompR his) was used for LamB purification (9). The following other strains were also used in this work: pop3 (araD139 ΔlacU169 rpsL relA thi; strain MC4100 of Casadaban [4]); pop3205, a derivative of pop3 carrying the nonsense mutation lamB102 (12); JE5506 (pps his proA argE thi lac xyl mtl tsx); and JE5505, an lpp mutant of strain JE5506 (29). These bacteria were grown in M63 medium (24) supplemented with 0.4% maltose and the necessary amino acids and vitamins. Purification of LamB. LamB-peptidoglycan complexes were obtained as described by Gabay and Yasunaka (9). These complexes were dissociated in 10 mM Tris-hydrochloride (pH 7.4) containing 5 mM EDTA and 2% octylpolyoxyethylene (octyl-POE) a gift from J. P. Rosembusch (26). After 1 h of centrifugation at 100,000 × g, the supernatant was adjusted to 20 mM MgCl2, phenylmethylsulfonyl fluoride-treated DNase I (0.01 mg/ml; Sigma Chemical Co.) was added, and the mixture was incubated for 1 h at 37°C. During this step, DNA hydrolysis led to a reduction in the viscosity of the preparation, and the addition of Mg2+ ions induced precipitation of the LamB protein. After additional centrifugation at 100,000 × g for 1 h, the resulting pellet was suspended in 10 mM Tris-hydrochloride (pH 7.4)-5 mM EDTA-2% octyl-POE and incubated for 1 h at 37°C. The insoluble material was removed by centrifugation at 100,000 × g for 1 h, and the supernatant was loaded onto a column containing mAb302 coupled (13) to Sepharose 4B. LamB was eluted by washing the column with 0.1 M glycine hydrochloride buffer (pH 2.8) containing 1% octyl-POE. The protein solution was brought to pH 7 with 1 M Tris-hydrochloride buffer (pH 8.3) and dialyzed against 10 mM Tris-hydrochloride buffer (pH 7.4) containing 1% octyl-POE. In a standard preparation, 2 liters of culture and a 2-ml Sepharose 4B column containing 7 mg of immobilized mAb yielded approximately 1 mg of LamB. The concentration of purified LamB was determined spectrophotometrically by using 24.5 as the extinction coefficient of a 1% solution at 280 nm (20).

Radioactively labeled LamB was prepared as described above, after the bacteria were grown in a low-sulfur medium containing 0.1 mCi of 35S (25 to 40 Ci/ml; Amersham France) per ml (8). More than 98% of the radioactivity present in the final preparation could be precipitated by adding any of the mAbs directed against LamB and Staphylococcus aureus cells. The specific activity of the purified protein was generally between 5 and 20 nCi/μmol of LamB monomer.

Preparation of antibodies and Fab fragments. A conventional anti-LamB serum (polyclonal antibodies) had been obtained previously by injecting Triton X-100-solubilized LamB into a rabbit (9). This serum was preadsorbed (27) in two different ways. Preadsorption with strain pop3205 cells (lamB102) yielded a preparation of polyclonal antibodies (total serum [Ig]) which, when tested by immunofluorescence (8), reacted with the surfaces of lamB+ cells, but not with the surfaces of cells of strain pop3205, a mutant devoid of LamB protein. Preadsorption with strain pop3 cells (lamB+) yielded a preparation of antibodies (adsorbed serum [aIg]) which failed to react either with lamB+ cells or with lamB mutants. When assayed for its ability to immunoprecipitate purified LamB, the aIg had a titer which was approximately one-tenth the titer of the Igs. For electron microscopy, aIg was further adsorbed with vesicles from strain pop3205, because this strain contained contaminating antibodies which reacted with components of the E. coli membranes which were inaccessible in whole cells and unrelated to LamB. After this additional adsorption step the serum reacted with membrane fragments from lamB+ cells, but not with fragments from strain pop3205.

The monoclonal anti-LamB antibodies (8) were purified from ascitic fluids by protein A-Sepharose 4B chromatography (13), and the total immunoglobulin concentration was determined (16) by using bovine serum albumin as a standard. To prepare Fab fragments, the purified immunoglobulin G preparations were digested with papain (Worthington Diagnostics) (13). The reduction was stopped by dialyzing the mixture against 5 mM sodium phosphate buffer (pH 8.0). The insoluble Fc fragments were eliminated by centrifugation, and the Fab fragments were purified by Whatman DE-52 chromatography (13) followed by a single passage through a protein A-Sepharose 4B column.

As a control we used an mAb (IDA 7) directed against an antigen which is totally unrelated to LamB (a gift from G. Buttin).

Immunoprecipitation and competition experiments. For all immunoprecipitation experiments 35S-labeled LamB was diluted in 0.6 ml of 50 mM Tris-hydrochloride (pH 8.0)-0.5 M NaCl-0.5 mM EDTA-2% Triton X-100 (8) (Triton buffer) containing the desired amount of immunoglobulin. After 4 h at 37°C, the immunocomplexes were precipitated by adding 0.05 ml of a 10% suspension of formalized S. aureus cells. The precipitate was collected by centrifugation, washed with Triton buffer, and added to 7 ml of Bioflour (New England Nuclear Corp.). The radioactivity of this preparation was determined with an Intertechnique scintillation counter.
For competition experiments [³⁵S]-labeled LamB was incubated first for 4 h at 37°C in Triton buffer with the Fab fragment and then for an additional 4 h with the intact immunoglobulin. The Fab fragment was used in large excess (approximately 100 to 1,000 times the amount of antibody necessary to precipitate 50% of the [³⁵S]-labeled LamB). The intact immunoglobulin was used in a limiting amount at a concentration that was independently determined to precipitate about 30% of the [³⁵S]-labeled LamB in the absence of competing Fab fragment.

For proteolysis experiments the preparations were incubated in 50 mM Tris-hydrochloride (pH 8.0) containing 1% octyl-POE at 37°C. [³⁵S]-labeled LamB was preincubated for 4 h with the antibodies, and subtilisin (protease type VII; Sigma) was then added at a molar ratio of 0.5 with respect to the concentration of LamB monomer. The reaction was stopped by adding phenylmethylene sulfonyl fluoride to a final concentration of 1 mg/ml and incubating the mixture for 30 min at room temperature. The samples were then dissolved in sodium dodecyl sulfate sample buffer (0.0625 M Tris-chloride [pH 6.8], 2% sodium dodecyl sulfate buffer), boiled for 3 min, and electrophoresed in 19.3% polyacrylamide gels containing 0.1% sodium dodecyl sulfate and 6 M urea (15). The radioactive bands were revealed by fluorography after the gel was treated with Enhance (New England Nuclear Corp.).

**Membrane preparation and electron microscopy.**

E. coli membrane fragments were obtained from maltose-grown cells. The procedure used was essentially that of Osborn et al. (21) and involved treatment of cells with lysozyme and EDTA to produce spheroplasts, sonication to fragment spheroplasts, and ultracentrifugation. No attempt was made to separate outer and inner membranes. Each preparation contained a mixture of large and small fragments. Since only the former could be labeled with anti-LamB antibodies (see below), they were believed to correspond to outer membrane fragments.

LamB-containing reconstituted vesicles were prepared by the dialysis method (26). Briefly, 1 ml of LamB dissolved in 1 ml of a solution containing 3% octyl-POE, 0.1 M NaCl, 1 mM MgCl₂, 0.02% NaN₃, and 10 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) (pH 7.4) was transferred to a tube that was internally coated with a dried film of soybean phosphatidylycholine (type III; Sigma). The resulting suspension was vigorously mixed with a Vortex mixer for 5 min and dialyzed first for 16 h and then twice for 4 h against 300 ml of the same buffer without detergent.

Both types of vesicles were diluted 1:10 in phosphate-buffered saline (PBS) and adsorbed onto glow-discharged Formvar-coated carbon grids for 1 min at room temperature. The grids were rinsed extensively with PBS containing 0.5% bovine serum albumin (M buffer) and then incubated for 1 h in a solution of antibody diluted in M buffer. The grids were then washed once more with M buffer and floated for 1 h at room temperature over a solution of a protein A-gold complex (10). The grids were washed again with M buffer and then with PBS, floated for 30 s over double-distilled water, and stained with a 2% sodium phosphotungstate solution at pH 7.0. Micrographs were obtained with a Siemens model 101 electron microscope.

**RESULTS**

Affinity of the mAbs for LamB. Gabay and Schwartz distinguished four classes of mAbs directed against LamB. The mAbs which recognize determinants exposed at the cell surface (mAbs 302, 177, 347, and 72) are referred to collectively as E-mAbs. The other mAbs, which are believed to recognize internal determinants, are referred to as I-mAbs. An estimate of the affinity of the mAbs for LamB protein was obtained by using radioactive LamB protein and precipitating the immune complexes with *S. aureus* cells. With the four mAbs tested we obtained linear Scatchard plots from which apparent *Kₐ* values were calculated by using 141,000 as the molecular weight of the LamB trimer. The apparent *Kₐ* values were 0.27, 0.24, 1.1, and 1.4 nM for mAbs 302, 347, 436, and 141, respectively (Fig. 1). With such high affinities the interactions between LamB and the mAbs could be considered essentially irreversible. This greatly facilitated the design of the competition experiments described below.

**Competition between Fab fragments and antibodies.** The possible overlapping of the binding
sites for the different mAbs were determined in competition studies. We used the fact that all of the available anti-LamB mAbs are recognized by protein A (8). Fab fragments, which are not recognized by protein A, were used as competitors in immunoprecipitation experiments with intact antibodies and \textit{S. aureus} cells. The results obtained with the Fab fragments from one E-mAb (E-mAb 302) and one I-mAb (I-mAb 436) are shown in Fig. 2. An excess of E-mAb 302 Fab inhibited the immunoprecipitation observed in the presence of the E-mAbs, but had no effect on the immunoprecipitation induced by the I-mAbs. Conversely, the I-mAb 436 Fab fragment inhibited the immunoprecipitation observed in the presence of mAbs 436 and 141, but had little or no effect in the presence of the E-mAbs. This result indicates that the domain recognized by I-mAbs 141 and 436 is completely distinct from the domain recognized by the E-mAbs.

Additional information was obtained by performing competition experiments involving two preparations of polyclonal anti-LamB antiserum. One preparation (tS) had been preadsorbed with cells devoid of LamB protein and therefore probably contained antibodies that recognized all domains of LamB. The other preparation (aS) had been preadsorbed with lamB\(^+\) cells and was thereby depleted of the antibodies which recognized determinants located at the cell surface. The Fab fragment from mAb 302 failed to compete with aS. This was predictable since mAb 302 recognized an external determinant, whereas aS was specific for internal determinants. However the mAb 302 Fab fragment was also very inefficient in competing with tS. This indicates that LamB has E determinants which do not overlap with the determinant recognized by mAb 302 and are therefore different from the determinants recognized by the four available E-mAbs. Similarly, the incomplete competition between mAb 436 and aS indicates that LamB has I determinants which do not overlap with the determinant recognized by mAb 436.

**Effect of the mAbs on the interaction between LamB and phage \(\lambda\).** The E-mAbs were shown previously to prevent the adsorption of phage \(\lambda\) in vivo. Therefore, it was not surprising to find that mAbs 302 and 347 prevented LamB from inactivating \(\lambda\)H\(\phi\) in vitro (Fig. 3). In contrast, the two I-mAbs failed to prevent LamB from inactivating \(\lambda\)H\(\phi\) in vitro; this result was less predictable. Indeed, although these two mAbs had no effect on \(\lambda\) adsorption in vivo, this was an obvious consequence of their inability to react with the cell surface. In vitro, where they could interact with LamB, they could have altered the conformation of this protein in such a way that it would have lost its ability to inactivate the phage.

**Proteolytic digestion of LamB: effect of the mAbs.** Like the other pore-forming proteins of \textit{E. coli}, LamB is extremely resistant to proteolysis (22). However, we found that by using high concentrations of subtilisin (ratio of enzyme to LamB, 1:2) and long incubation times (48 h at 37\(^\circ\)C) it was possible to obtain essentially complete inactivation of LamB, as assayed by \(\lambda\)H\(\phi\) neutralization, and conversion of LamB to smaller peptides. The major hydrolysis product was a 22,000-dalton peptide, but 14,000- and 34,000-dalton peptides were also detected. When incubated under the same conditions but in the absence of subtilisin, LamB remained fully active.

To study the effect of the mAbs on this proteolytic reaction, we used a somewhat shorter incubation time (24 h). Under these conditions approximately 80\% of LamB was converted to smaller peptides (Fig. 4). Preincubation of LamB with I-mAb 436 or 141 had no effect on this reaction. On the other hand, the E-mAbs protected LamB against proteolytic degradation. Results identical to those shown in Fig. 4 were obtained when we used 100-fold-higher concentrations of mAbs or Fab fragments. When the gel shown in Fig. 4 was stained with Coomassie blue, we found that practically all of the immunoglobulin G had been converted.

![Figure 2. Fab competition experiments. \(^{35}\text{S}\)-labeled LamB (0.1 \(\mu\)g/ml) was preincubated for 4 h at 37\(^\circ\)C with or without 50 \(\mu\)g of mAb 302 Fab (A) or mAb 436 Fab (B) per ml. Antibodies (as shown on the abscissa) were then added, and, after an additional 4 h of incubation at 37\(^\circ\)C, immunoprecipitation was obtained by adding \textit{S. aureus} cells. The percentages correspond to the relative amounts of immunoprecipitation in the presence and absence of Fab. tS and aS represent the total anti-LamB serum and the serum preadsorbed with lamB\(^+\) cells, respectively. The values correspond to the means of three determinations.](http://jb.asm.org/content/155/5/1385/F2.large.jpg)
before reaction with the One id immunoelectron shows, successful due attempts artificial in mAbs in the force the membrane, were determinants was LamB located at the sites described below domain recognized by E-mAbs is composed from the sites of OmpA, a bacterial membrane protein which has a protease-sensitive site at the inner face of the membrane (22), could be cleaved by trypsin (data not shown). Second, the aS serum which did not recognize external determinants of LamB did interact with these fragments (Fig. 6b).

Although the results described above indicated that the periplasmic face of the membrane was accessible in the outer membrane fragments and even that some LamB determinants were exposed on this face, no labeling was obtained with mAbs 436 and 141 (Fig. 6c and d). This result was somewhat surprising since the determinants recognized by these mAbs had been to 22,000- to 26,000-dalton peptides similar to the Fab and Fc fragments (data not shown).

All of the experiments described above reinforce the notion that the E-mAbs recognize a domain of the LamB protein which is different from the domain recognized by the I-mAbs. The domain recognized by the E-mAbs is clearly located at the cell surface. The experiments described below provided information regarding the locations of the sites recognized by the I-mAbs.

Locations of the antigenic determinants when LamB is inserted in a membrane. The E-mAB determinants were known to be accessible when LamB was inserted in the bacterial outer membrane, but very little was known about the accessibility of the sites recognized by the I-mAbs or even about the existence of these sites in the absence of detergent. A first set of experiments was performed after LamB was inserted in artificial phosphatidylcholine vesicles. Attempts to evaluate the accessibility of the antigenic sites by immunoprecipitation were unsuccessful due to the retention of the vesicles by the immunoadsorbent. Instead, we developed a rapid immunoelectron microscopy technique in which the vesicles were preadsorbed to the grids before reaction with the antibodies. As Fig. 5 shows, two kinds of vesicles were identified. One population was composed of small clear vesicles which were not labeled, and the other population was composed of large isolated vesicles, which were labeled by all of the anti-LamB mAbs, including I-mAbs 436 and 141, but not by the IDA 7 control immunoglobulin.

The same type of experiment was then performed with natural membrane fragments that were obtained after sonication of bacterial spheroplasts. These fragments were labeled by E-mAb 302 (Fig. 6a), showing that the external face of the outer membrane was exposed in these fragments. However, two lines of evidence indicated that these membranes were also accessible from the periplasmic face. First, OmpA, an outer membrane protein which has a protease-sensitive site at the inner face of the membrane (22), could be cleaved by trypsin (data not shown). Second, the aS serum which did not recognize external determinants of LamB did interact with these fragments (Fig. 6b).

Although the results described above indicated that the periplasmic face of the membrane was accessible in the outer membrane fragments and even that some LamB determinants were exposed on this face, no labeling was obtained with mAbs 436 and 141 (Fig. 6c and d). This result was somewhat surprising since the determinants recognized by these mAbs had been...
shown to be accessible in artificial vesicles (Fig. 5).

One major difference between the artificial membrane and the *E. coli* outer membrane is the presence in the latter of lipoprotein, which anchors the outer membrane to the peptidoglycan sacculus (22). To test whether the lack of reactivity of mAbs 436 and 141 with the outer
FIG. 6. Immunoelectron microscopy of wild-type (lpp⁰) membrane fragments. Vesicles prepared as described in the text from strain pop3 were diluted 10-fold in PBS, adsorbed onto grids, and incubated with 8 µg of mAb 302 per ml (a), a 1/10 dilution of antisera (b), 5 µg of mAb 141 per ml (c), or 10 µg of mAb 436 per ml (d). The grids were washed, treated with gold-labeled protein A, washed, and negatively stained. Results identical to those shown were obtained by using vesicles from strain JE5506, which is the lpp⁰ parent of strain JE5505.
membrane fragments might be related to the presence of lipoprotein, we prepared vesicles from a mutant devoid of this protein (strain JE5505), as well as from its lpp\(^+\) parent (strain JE5506). The results obtained with strain JE5506 were the same as those obtained with strain pop3 (data not shown), but they differed from those obtained with strain JE5505. I-mAb 436 yielded strong labeling of the vesicles from this lpp mutant (Fig. 7c). Since mAb 436 failed to give a positive immunofluorescence response with intact bacteria, this labeling of JE5505 membranes most probably corresponded to an interaction with the inner face of the outer membrane. The other I-mAb, mAb 141, reacted very poorly with the membrane fragments from the lpp mutant (Fig. 7d).

**DISCUSSION**

The properties of the six mAbs which we studied are summarized in Table 1. These properties allow us to define the following two distinct, nonoverlapping domains in the LamB protein: the E domain, which is exposed at the cell surface, and the I domain, which is not.

The E domain was previously shown to contain at least two antigenic sites, one defined by mAbs 347 and 72, which inhibit maltose transport in intact bacteria, and the other defined by mAbs 302 and 177, which do not (8). In this paper we show that these two determinants must overlap or at least be very close to one another. Indeed, the Fab fragment from mAb 302 inhibited the binding of the three other E-mAbs. In addition, the four E-mAbs had identical effects on the \(\lambda\) phage-neutralizing activity of LamB in vitro and on LamB proteolysis. The simplest interpretation of these results is that the sites recognized by the four E-mAbs share a region which (i) is exposed at the bacterial surface in vivo, (ii) is involved in the interaction of LamB with phage \(\lambda\), and (iii) contains the primary target for proteolytic attack of LamB in vitro.

The I domain contains at least three antigenic sites. Two of these sites are defined by mAbs 141 and 436. The third, which may consist of several antigenic sites, is defined by antibodies which are present in aS, a polyclonal anti-LamB antiserum which has been preadsorbed with intact lam\(B^+\) bacteria. The sites recognized by mAbs 141 and 436 overlap or are close to one another since Fab fragments from mAb 436 inhibit the binding of mAb 141. However, these two sites are clearly distinct from those recognized by the E-mAbs since (i) the Fab fragments from mAb 436 failed to inhibit the binding of E-mAbs, (ii) the Fab fragments from E-mAb 302 failed to inhibit the binding of mAbs 141 and 436, and (iii) mAbs 141 and 436 have no effect on the sensitivity of LamB to proteolysis or on its ability to neutralize phage \(\lambda\).

The sites recognized by the two I-mAbs are both exposed when LamB is inserted into artificial vesicles, but not when LamB is present in outer membrane fragments obtained from wild-type strains. Despite these common properties, the two sites are nevertheless clearly distinct. First, the site recognized by mAb 141 is exposed in LamB-peptidoglycan complexes, whereas that recognized by mAb 436 is not (8). Second, the site recognized by mAb 436 is fully exposed in outer membrane fragments isolated from an lpp mutant, whereas the site recognized by mAb 141 is not. These results indicate that the site recognized by mAb 436 is located on the periplasmic face of the membrane and is presumably involved in the interaction of LamB with peptidoglycan. More specifically, this site may correspond to a region of LamB that interacts with lipoprotein, which is itself covalently bound to peptidoglycan. Indeed, the inaccessible of this site in outer membrane fragments from lpp\(^+\) bacteria is probably due to the presence of the lipoprotein itself and not to the presence of large peptidoglycan fragments which might have resisted the lysozyme treatment used to prepare

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**TABLE 1. Main characteristics of the anti-LamB antibodies**

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<tr>
<th>Antibody</th>
<th>Domain*</th>
<th>Binding to LamB-peptidoglycan complexes(^a)</th>
<th>Effect on LamB neutralizing activity</th>
<th>Protection of LamB against proteolysis</th>
<th>Binding to artificial vesicles</th>
<th>Binding to wild-type E. coli vesicles</th>
<th>Binding to lpp mutant vesicles</th>
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* Domains E and I are defined based on immunofluorescence labeling of intact bacteria (8) and from Fab competition experiments.

\(^{a}\) From reference 8.

\(^{c}\) NT, Not tested.
FIG. 7. Immunoelectron microscopy of membrane fragment from \textit{lpp} strain JE5505. Membrane fragments prepared as described in the text were diluted 10-fold in PBS, adsorbed onto grids and incubated with 8 \( \mu \)g of mAb 302 per ml (a), 16 \( \mu \)g of control mAb per ml (b), 5 \( \mu \)g of mAb 141 per ml (c), or 10 \( \mu \)g of mAb 436 per ml (d). The grids were washed, treated with gold-labeled protein A, washed, and negatively stained.
the vesicles. If such large fragments had been present (21), they would have been expected to prevent trypsin from attacking the carboxyl end of the OmpA protein (22) and to prevent αS from interacting with the membrane fragments. This was not the case.

The site recognized by mAb 141 is clearly not involved in the interaction of LamB with peptidoglycan since it is accessible in LamB-peptidoglycan complexes. Gabay and Schwartz (8) have suggested that this site could be buried within the membrane. However, since (i) this site is fully accessible when LamB is inserted in phospholipid vesicles and (ii) it overlaps the site of mAb 436, which is fully accessible in the same membrane fragments it seems that the mAb 141 site is not deeply embedded in the lipid bilayer; rather, it may be mostly located on the periplasmic face of the outer membrane.

The existence of at least one additional antigenic site in the I domain is suggested by the fact that unlike mAbs 141 and 436, the LamB-specific serum which has been preadsorbed with lamB" cells, can react with outer membrane vesicles from a wild-type strain. The fact that αS reacts with outer membrane vesicles of lpp" bacteria indicates that a region of LamB must be accessible at the periplasmic face of the outer membrane, even in the presence of lipoprotein. Part or all of this region could be the region which interacts with the periplasmic maltose-binding protein (1).

Figure 8 shows our present view of the location of the antigenic sites studied in this work with respect to the outer membrane and to functional sites on the LamB protein. This analysis is now being pursued in two directions. One involves the mapping of the antigenic sites on the LamB polypeptide. In this respect the site recognized by E-mAbs 177 and 302 has been shown to be located in the vicinity of the COOH-terminal end of the polypeptide (7). The other direction is the characterization of additional antigenic determinants. The results of competition experiments performed with Fab fragments and complete sera, both preadsorbed with whole cells and not preadsorbed (Fig. 2), indicate that there are determinants which do not overlap those described here.

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FIG. 8. Model showing antigenic sites in LamB protein. Lpo, Lipoprotein.

LITERATURE CITED


