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Identification and Characterization of the TolC Protein, an Outer Membrane Protein from *Escherichia coli*

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We used the cloned *tolC* gene to identify, locate, and purify its gene product. Strains carrying pPR13 or pPR42 overproduced a cell envelope protein (molecular weight, 52,000). A protein of the same molecular weight was identified in radioactively labeled minicells carrying pPR13; this protein was absent in pPR11-carrying minicells. This protein was the *tolC* gene product, since pPR11 differed from pPR13 in having a Tn*10* insertion in the *tolC* gene. The protein seen in cell envelopes of whole cells (TolC protein) was found to exist in an aggregated state in the outer membrane; under conditions in which OmpC and OmpF were peptidoglycan associated, TolC protein was not likewise associated. Using these properties, we purified the TolC protein and determined the sequence of twelve amino acids from the amino-terminal end. The location of the TolC protein in the outer membrane was consistent with the proposed function for the *tolC* gene product as a processing protein in the outer membrane.

The outer membrane of *Escherichia coli* K-12 contains several protein species in large amounts and many more of which relatively fewer copies are present. These proteins have been characterized functionally, physically, and genetically to various degrees. They include components of specific transport systems (LamB, BtuB, PhoE, FepA, FhuA, FecA, Tsx), nonspecific pores (OmpC, OmpF), structural proteins (Lpp), enzymes (phospholipase, protease), and conjugal recipient ability (OmpA) (18, 19). Many of these proteins have not been studied in detail, and it is apparent that others are yet to be detected. These minor proteins may have key functions, and a study of these proteins should give insight into the biosynthesis, assembly, structure, and function of the *E. coli* outer membrane (OM).

The *tolC* locus has a major effect on the OM; *tolC* mutants become tolerant to colicin E1, show an altered bacteriophage sensitivity pattern, lack the OmpF protein, and become hypersensitive to detergents, dyes, and certain antibiotics (5, 9, 15, 16).

We recently described the cloning of the *tolC* locus of *E. coli* K-12 into the multicopy plasmids pSF2124 and pBR322 (14). In this paper we identify, characterize, and purify the protein product of the *tolC* gene.

MATERIALS AND METHODS

***E. coli* K-12 strains used.** The bacterial strains used in this work are listed in Table 1. The plasmids used (pSF2124, pPR11, pPR13, pPR42, and pPR29 through pPR37) have been described elsewhere (14, 22). Briefly, plasmid pPR13 is pSF2124 with a 10.5-kilobase (kb) *Eco*RI fragment containing the *tolC* gene cloned into its *Eco*RI site; pPR11 is pPR13 with a transposon Tn*10* insertion inactivating the *tolC* gene; plasmids pPR29 through pPR37 are pPR13 with transposon Tn*5* insertions inactivating the *tolC* gene; pPR42 is pBR322 with an 8.5-kb *Eco*RI-*Pst*I fragment, from the *tolC-Eco*RI fragment of pPR13, inserted between its *Eco*RI and *Pst*I sites and has *tolC*$^+$ function.

Growth media. Bacterial strains were grown in double-strength nutrient broth (Difco Laboratories) with 5 g of sodium chloride per liter unless specified. Nutrient agar was Blood Base Agar (Difco) without any added blood. All incubations were done at 37°C. Plasmid-carrying strains were grown in broth from colonies on nutrient agar containing 16 μg of tetracycline per ml (pPR42) or 25 μg of ampicillin per ml (pSF2124, pPR11, and pPR13).

Chemicals. Tetracycline was Rolitetracycline (Hoechst) and ampicillin was Penbritin (Beecham). [^{35}S]methionine (1,380 Ci/mmol) and [^{3}H]diisopropyl fluorophosphate (6.5 Ci/mmol) were from the Radiochemical Centre. Trypsin was obtained from Hopkin and Williams. Sodium dodecyl sulfate (SDS) used for column chromatography was BDH no. 44244. Sephacryl S-200 was from Pharmacia, Inc.

Transformation. Transformation with plasmid DNA was done as described elsewhere (16).

Minicell methods. Minicells were isolated and labeled with [^{35}S]methionine as described by Achtman et al. (1). Minicells were lysed and fractionated to give soluble and cell envelope-associated protein fractions as previously described (1). Samples for electrophoresis were solubilized by heating at 100°C in sample buffer (12). On a few occasions, minicell preparations were stored at −70°C in 30% glycerol and then thawed and labeled as above.

TABLE 1. Bacterial strains

E. coli strain	Characteristics	Source or reference
AB1133	F⁻ *thr-1 leu-6 proA2 lacY2 supE44 galK2 his-4 rpsL31 xyl-5 mtl-1 argE3 thi-1 ara-14*	A. L. Taylor
P602	AB1133 *tolC203*	5
A586	F⁻ *thr-1 leu-6 tonA21 supE44 thi-1 pro-43 tolC3*	S. E. Luria
DS410	*rpsL*, minicell producer	J. Reeve
P2787	F⁻ *pyrD34 tonA208 ompA* (pPR42)	This work
W1485F⁻	F⁻, prototroph	C. Schnaitman
P2731	W1485F⁻ *tolC210*::Tn*10-48*	This work

Cell envelope preparation (small scale). Cell envelopes were prepared by the small-scale method (10-ml culture) as described elsewhere (15). Essentially, this involved Tris-sucrose-lysozyme-EDTA treatment and then sonication of the spheroplasts and centrifugation (60 min, 15,000 rpm, SM24 rotor [Sorvall]) to pellet the cell envelopes, which were then solubilized in sample buffer. Fewer proteins were seen in cell envelopes prepared in this way than in whole cell envelopes prepared as described below. We believe that these cell envelopes are predominantly OMs, and we found that the TolC protein band was easier to see in these preparations.

One-liter scale preparation of OMs. Large-scale OMs were prepared essentially as described by Schnaitman (21). A 1-liter culture was grown to an optical density at 600 nm (OD_{600}) of 1.0 to 1.2. The cells were collected by centrifugation, washed in 100 ml of 50 mM Tris-hydrochloride (pH 7.5), centrifuged, and suspended in 20 ml of 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.5) with 2 mM $MgCl_2$. The cells were broken in a precooled French pressure cell (Aminco), unbroken cells were removed by centrifugation (7,000 rpm, 10 min, 4°C, SS34 rotor [Sorvall]), and the supernatant was centrifuged at 30,000 rpm for 60 min at 4°C in a Beckman 30 rotor. The resulting whole cell envelope pellet (WM) was suspended in 10 mM HEPES (pH 7.5) containing 2% (vol/vol) Triton X-100 (25 ml), held for 15 min at room temperature, and centrifuged at 20°C and 30,000 rpm for 60 min. The supernatant (Triton X-100-soluble envelope [soluble envelope]) was kept, and the pellet (Triton X 100-insoluble envelope [insoluble envelope]) was reextracted with Triton X-100. The resulting pellet was suspended in water and called the OM pellet. The protein in the supernatant was precipitated with 2.5 volumes of ethanol, collected by centrifugation, washed with 70% (vol/vol) ethanol, and dried under vacuum. The dried pellet was solubilized in sample buffer and termed the cytoplasmic membrane. All membrane fractions were stored at −20°C.

SDS-PAGE. Samples were solubilized, run on 11% linear or 11 to 20% gradient gels, and then stained as previously described (2, 12, 13) for polyacrylamide gel electrophoresis (PAGE). Autoradiography was performed at −70°C with an intensifying screen and Kodak X-Omat S film after the gel was dried onto Whatman no. 1 paper. Gels containing samples labeled with [^{3}H]diisopropyl fluorophosphate were processed for fluorography (4) and then exposed to film. Gels were exposed for 3 to 7 days for [^{35}S]methionine-labeled protein and for 3 to 6 weeks for [^{3}H]diisopropyl fluorophosphate-labeled proteins.

Molecular weight estimation. The following proteins and molecular weight markers were used as calibration standards for estimating the molecular weight of the TolC protein in whole cell envelopes: phosphorylase B (94,000 [94K]), bovine serum albumin (68K), L-glutamic dehydrogenase (53K), DNase I (31K), carbonic anhydrase (29K), α-chymotrypsinogen (25.7K) and RNase A (14K). An 11% linear polyacrylamide gel was used.

Trypsin treatment of membranes. Samples of insoluble envelope (OM) were incubated with and without trypsin (250 μg/ml) in 1.5-ml Eppendorf micro test tubes (200-μl final volume) for 15 or 30 min at 37°C.

The tubes were centrifuged (4°C, 15,000 rpm, 60 min, SS34 rotor) with plastic adaptors used for the micro test tubes. The resulting membrane pellets were then solubilized (12), and samples were run on an acrylamide gel.

Peptidoglycan association of the TolC protein. Samples of insoluble envelope (OM) were added to sample buffer (200-μl final volume) in Eppendorf micro test tubes. The tubes were heated at 37 or 56°C for 30 min and then centrifuged as for the trypsin treatment method. The supernatant was removed to a fresh tube, and the pellet was suspended in 200 μl of sample buffer. Both were then heated at 100°C before PAGE was performed.

Purification of the TolC protein. TolC protein was purified from strain P2787. This strain was grown in broth (21 liters; OD_{600}, 1.2), and OMs were prepared with Triton X-100. The OMs were suspended in 25 ml of water to which was added 25 ml of double concentrated Rosenbusch extraction buffer (20) (final concentration was 2% SDS, 10 mM Tris-hydrochloride [pH 7.3], 0.7 M β-mercaptoethanol, 10% glycerol), heated at 37°C for 40 min, and centrifuged (20,000 rpm, 50 min, Beckman 35 rotor). The supernatant was concentrated to 20 ml in an Amicon ultrafiltration cell (PM30 membrane). A 10-ml amount of this sample was applied to a 140 by 4-cm Sephacryl S-200 column with a flow rate of 20 ml/h in column buffer (10 mM Tris-hydrochloride [pH 7.4], 0.2% [wt/vol] SDS, 5 mM EDTA, 0.02% azide). Fractions (3.5 to 4.0 ml) were collected. The eluate was monitored by measurement of the OD_{280}. Samples in the peak fractions were electrophoresed on gels; fractions enriched in TolC protein were pooled and concentrated, as described above, to 2.0 ml. Glycerol and β-mercaptoethanol were added to 10% and 0.7 M, respectively, and the sample was heated at 100°C for 3 min before being reapplied to the same column and eluted as above. Peak fractions were analyzed on gels, and those containing only TolC protein (four fractions) were pooled and dialyzed twice against 2 liters of 0.2% (wt/vol) SDS. The protein was precipitated with 9 volumes of acetone, washed twice with 90% (vol/vol) acetone, twice with 100% acetone, and once with diethyl ether, and dried under vacuum.

Amino acid analysis. An amino acid analysis of a 1-mg sample of TolC protein was performed by M. Calder of the Department of Biochemistry, The University of Adelaide.

Amino-terminal sequence of the TolC protein. The sequence of the amino-terminal amino acids of the TolC protein was determined with an automated Beckman sequenator. Samples ranging from 1 to 5 mg of TolC protein were sequenced by P. Martin, Botany Department, The University of Adelaide.

RESULTS

Overproduction of a cell envelope protein due to the cloned *tolC* gene. Analysis of the cell fractions of *tolC* mutants did not at first reveal the absence of any protein band which might be the *tolC* gene product (15). It presumably is normally only a minor protein and hence was not detected by PAGE. We used the cloned *tolC* gene to identify the gene product.

The plasmids pSF2124, pPR13 (*tolC*$^+$), and pPR11 (*tolC*::Tn*10*) were transformed into strains AB1133 and P602 (*tolC*). Cell envelopes were prepared by the small-scale method and analyzed by PAGE. The presence of pPR13 in AB1133 resulted in the hyperexpression of a cell envelope protein that was not consistently detected with either pPR11 or pSF2124 (Fig. 1). Although this protein was only recognized as the TolC protein after the gene had been cloned, we subsequently observed that under some conditions the protein can be seen in AB1133 and other strains, but not in P602 or several other *tolC* mutants (unpublished data). The TolC protein was thus present at levels detectable by PAGE but was at first missed due to the proximity of other bands in the gels. Figure 1 also shows strain A586 carrying plasmid pPR42, which has the *tolC* gene cloned in pBR322 (14). This strain produced the same cell envelope protein, but in even greater amounts (also see below). We believe that this was due to a gene dosage effect, as pBR322 has a higher copy number than does pSF2124, from which pPR13 was derived.

We also isolated Tn*5* insertions in *tolC* in pPR13. When strain C600 was transformed with these plasmids (pPR29 through pPR37), the cell envelope protein detected above was not produced (data not shown). The data suggested that this cell envelope protein was the *tolC* gene product (called the TolC protein).

Both pPR13 and pPR42 restored OmpF protein production in strains P602 and A586, respectively (Fig. 1), as would be expected if they provide *tolC*$^+$ function (14, 15). *recA* derivatives of strains AB1133 and P602 harboring the various plasmids also expressed the TolC protein. Strain AB1133 carrying pPR13 or pPR42 showed the normal medium-dependent fluctuations in the levels of OmpC and OmpF proteins (6, 7)

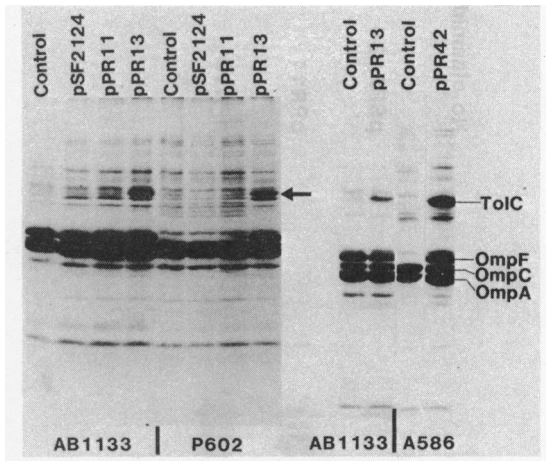

FIG. 1. Cell envelopes of whole cells carrying pPR11, pPR13, or pPR42. Cell envelopes, prepared by the small-scale method, from strains AB1133 and P602 carrying pSF2124, pPR11, pPR13, or no plasmid (control), as well as strain A586 with and without pPR42, were electrophoresed on a linear 11% polyacrylamide gel.

(data not shown), showing that excess *tolC* gene product had no effect on this regulation.

Identification of the *tolC* gene product in minicells. Plasmids pPR13 (*tolC*$^+$), pPR11 (*tolC*::Tn*10*), and pSF2124 were transformed into the minicell-producing strain DS410. Minicells were isolated from these strains, labeled with [^{35}S]methionine, solubilized in sample buffer, and run on an 11% SDS-polyacrylamide gel. Radioactive plasmid-encoded proteins were detected by autoradiography. The results (Fig. 2) showed that pPR13 encoded a protein which was absent from pPR11. This protein was assumed to be the *tolC* gene product, as pPR11 differed from pPR13 by having a Tn*10* insertion in the *tolC* gene. The protein seen in the stained gels described in the previous section comigrated with the radioactively labeled *tolC* gene product, and hence was the *tolC* gene product.

Neither plasmid produced one of the proteins made by pSF2124; this protein ran slightly slower than the TolC protein and was probably colicin E1, which was not expected in minicells harboring pPR11 or pPR13, both of which have cloned DNA inserted in the colicin E1 gene (22). In some minicell preparations derived from DS410(pPR13), the presumed TolC protein band migrated as a doublet, with the lower band being less intense than the upper band; we do not have an explanation for this observation.

We attempted to locate the TolC protein produced in minicells by fractionating radioactively labeled DS410(pPR13) minicells into soluble and insoluble (cell envelope-associated) protein frac-

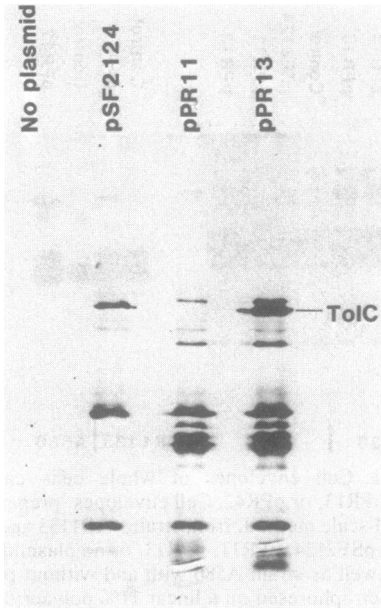

FIG. 2. Identification of the *tolC* gene product with minicells. Minicells prepared from strain DS410 and derivatives carrying pSF2124, pPR11, or pPR13 were labeled with [^{35}S]methionine. This figure is a typical autoradiograph of a linear 11% polyacrylamide gel run of the labeled samples.

tions. Some labeled proteins were clearly identified as either soluble or cell envelope associated (data not shown). The stained gel also showed that fractionation had occurred (data not shown). The TolC protein was partially (about 25%) solubilized under these conditions, but densitometer scanning of the autoradiogram clearly showed an enrichment of the TolC protein in the cell envelope fraction.

It should be noted that the major OM proteins (OmpA and OmpC plus OmpF) were also partly (5 to 10%) solubilized in this experiment.

TolC protein is an OM protein. WM from strain AB1133 carrying pPR13 or pPR42 was fractionated by Triton X-100 extraction as described above. Fig. 3 shows the results of fractionating strain AB1133(pPR42). The TolC protein was clearly associated with the insoluble envelope (OM). The TolC protein in the WM was estimated to have a molecular weight of 52K; but in the example shown (Fig. 3), the TolC protein in the OM (form B) migrated faster than the TolC protein in the WM (form A). The amount of form B (TolC*, Fig. 3) varied between OM preparations: some had only form A (see Fig. 6), whereas others had both forms.

On a few occasions some TolC protein appeared to be present in the soluble fraction, but under the same conditions a similar proportion of the major OM proteins were also present. OMs from strain AB1133(pPR42) had more TolC protein than did those from strain AB1133(pPR13), a result consistent with the levels of TolC protein seen in the WM and in cell envelopes as described above.

Characterization of the TolC protein. The major OM proteins of *E. coli* have been characterized by several methods, including sensitivity to trypsin treatment (8), solubility in SDS (20), and solubilization temperature (17). We used these treatments to characterize the TolC protein and compare it with other OM proteins.

Figure 4 shows the effect of treating the OMs from strains AB1133 and AB1133(pPR13) with trypsin. The TolC protein was digested by trypsin. It is not known why the particular preparation used had a lower than usual amount of TolC protein. Samples of AB1133 and AB1133(pPR13) OMs were heated at 37 or 56°C in sample buffer (equivalent to a Rosenbusch extraction [20]). Both procedures solubilized the TolC protein from the OM, with the 56°C treatment being fully effective, although some (about 80%) solubilization did occur at 37°C. Samples of WM were solubilized at 37 or 100°C before being loaded on a polyacrylamide gel. The TolC

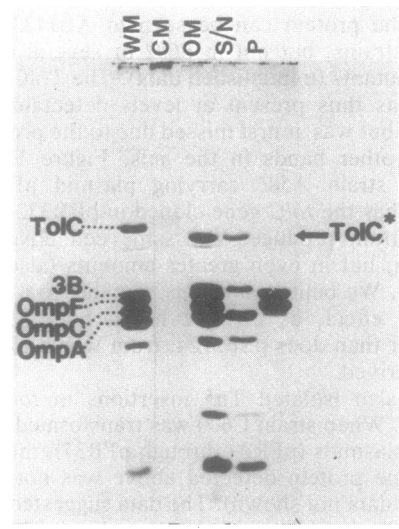

FIG. 3. Localization of the TolC protein. WM from strain AB1133(pPR42) were fractionated into OM and cytoplasmic membrane (CM) components by extraction with Triton X-100. Samples were run on an 11% polyacrylamide gel. The position of the TolC protein is indicated, as is the TolC* (form B) protein, which migrated faster. The two other tracks are samples of the supernatant (S/N) and pellet (P) derived from a Rosenbusch extraction (see the text) and are included to show that the modified protein is not peptidoglycan associated (see also Fig. 6).

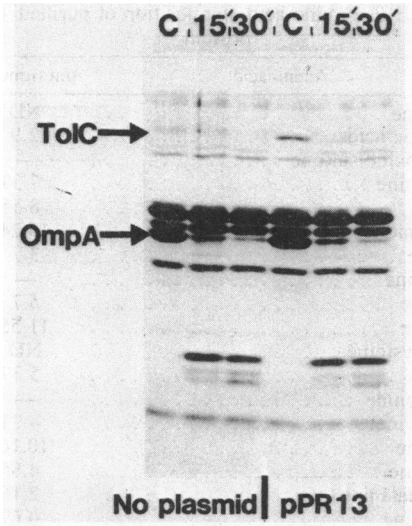

FIG. 4. Trypsin digestion of the TolC protein. OMs prepared from strains AB1133 and AB1133(pPR13) were treated with trypsin as described in the text for 15 or 30 min or not treated (C). Samples were run on an 11% polyacrylamide gel. The positions of the TolC and OmpA proteins are indicated.

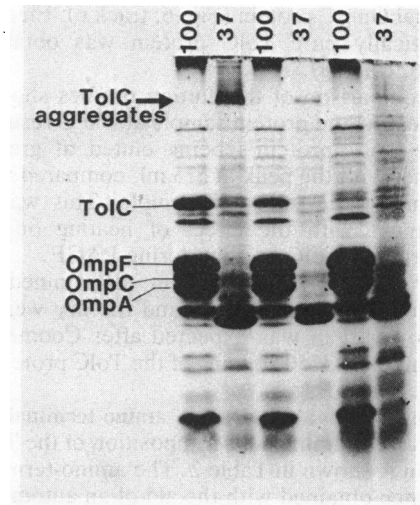

FIG. 5. Effect of solubilization temperature on the TolC protein. WM prepared from (right to left, two tracks each) strains AB1133, AB1133(pPR13), and AB1133(pPR42) were solubilized in sample buffer at 37°C for 30 min or 100°C for 3 min (indicated at top of figure). Samples were then run on an 11% polyacrylamide gel. The position of the TolC, OmpF, OmpC, and OmpA proteins (100°C conditions) is shown, as is the position of aggregated TolC protein.

protein did not run at its usual position when WM were solubilized at 37°C; a slower-migrating protein band at approximately the 150K position appeared as a major band in the WM from AB1133(pPR13), and even more was seen with AB1133(pPR42) (Fig. 5). *tolC* mutants have only a minor band at this position, and it was concluded that the major band was a form of TolC protein, probably an aggregate. The minor band in the control was presumably a different protein of 150K. This change in apparent molecular weight after heating at 100°C was confirmed by the molecular sieve chromotography used in the purification of the TolC protein (see below).

Purification of the TolC protein. Based on some of its properties, described above, the TolC protein was purified as described above.

The insoluble envelope fraction (OM) was prepared from strain P2787, which carries pPR42 and lacks the OmpA protein (Fig. 6). TolC protein was solubilized from these membranes at 37°C to separate it, in an aggregated form, from the porin proteins which are peptidoglycan associated (Fig. 6). The sample was applied to a Sephacryl S-200 column to separate the aggregated TolC protein from proteins of lower molecular weight. Fractions enriched for TolC protein were pooled and heated at 100°C to fully solubilize the TolC protein. These pooled, heated fractions were then applied to the same column to separate the TolC protein from contaminating higher-molecular-weight proteins and

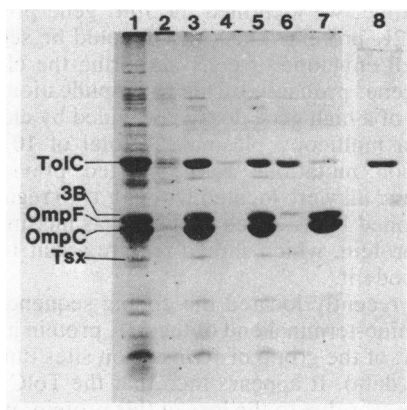

FIG. 6. Purification of the TolC protein: preparation of membrane sample. Strain P2787 was fractionated as described in the text to prepare a sample enriched for the TolC protein. Tracks: 1, Wm; 2 and 4, soluble material from the first and second extractions, respectively; 3 and 5, insoluble material from the first and second extractions respectively; 6 and 7, supernatant and pellet, respectively, from solubilizing the insoluble material from the second extraction in extraction buffer at 37°C; 8, purified TolC protein derived from the second sephacryl S-200 column run. Solubilized samples (100°C) were run on an 11% polyacrylamide gel. Note that some TolC protein is present in tracks 4 and 7.

residual OmpC protein (Fig. 6, track 6). Electrophoretically pure TolC protein was obtained (Fig. 6, track 8).

A comparison of the elution profiles showed that heating the protein sample at 100°C resulted in the TolC protein's being eluted at greater volume, with the peak at 575 ml, compared with 535 ml for the unheated sample. This was in agreement with the effect of heating on the migration of TolC protein during PAGE.

The yield of TolC protein, determined by measurement of the OD_{280} and the dry weight, was lower than was expected after Coomassie brilliant blue G250 staining of the TolC protein in polyacrylamide gels.

Amino acid analysis and amino-terminal sequence. The amino acid composition of the TolC protein is shown in Table 2. The amino-terminal sequence obtained with the aid of an automated Beckman sequenator was NH_2-Glu-Asn-Leu-Met-Gln-Val-Tyr-Gln-Gln-Ala-(Ala)-Leu- . . . -COOH. Methionine was not detected in the amino acid analysis; however, this probably reflects a lack of sensitivity in the method. Methionine was known to be present, as [^{35}S]methionine was used to label the TolC protein produced in minicells (see above).

TABLE 2. Amino acid composition of purified TolC protein

Amino acid	Amt (μmol%)[a]
Cysteine	ND
Aspartic acid	12.9
Methionine sulfone	—
Threonine	7.39
Serine	8.55
Glutamic acid	13.24
Proline	3.7
Citrulline	—
Glycine	5.7
Alanine	11.55
Half-cysteine	ND
Valine	5.39
Methionine	—
Isoleucine	4.23
Leucine	10.16
Tyrosine	4.85
Phenylalanine	2.16
Ornithine ?	0.69
Lysine	3.7
Histidine	0.54
Arginine	5.23
Tryptophan	ND

[a] ND, Not determined; —, none found.

DISCUSSION

Using minicells carrying plasmids with either the wild-type *tolC* gene or a transposon insertion derivative, we identified the *tolC* gene product as a 52K protein. This protein could be seen in the cell envelope of cells harboring the cloned *tolC* gene, probably owing to amplification as a result of a high gene dosage provided by cloning onto a multicopy plasmid. A total of 10 *tolC* insertion mutations were isolated previously (14), and all were located within a 1-kb region of the cloned DNA. Each of these was missing the 52K protein, which should require about 1.5 kb to encode it.

We recently located the coding sequence for the amino-terminal end of the 52K protein just to the left of the group of 10 insertion sites (unpublished data). It appears then that the TolC phenotype was due to the loss of this protein, which we therefore identified as the TolC protein. We are aware of the possibility that the TolC phenotype may be due to a protein encoded to the right of the region encoding the 52K protein, with the insertion mutations having a polar effect on it, but consider it highly unlikely, as it would require that all 10 insertions occur in the upstream gene and none in the *tolC* gene itself.

Several other proteins were encoded by pPR13 apart from the TolC protein, and one of these was associated with the cell envelope (data not shown). These proteins were not studied further.

The TolC protein was localized in the OM by showing that it was associated with the insoluble cell wall material. Some anomalies were found, however, in some fractionations; the insoluble envelope contained a protein with the mobility of the TolC protein seen in cell envelopes (form A) and a second protein that electrophoresed slightly faster (form B). Triton X-100 may interact with the TolC protein, resulting in form B being present.

From the data presented, we believe that the TolC protein is present in the OM as an aggregated protein which is accessible to trypsin and that although it may be loosely peptidoglycan associated, it does not behave like OmpC and OmpF proteins in this regard.

The location of the TolC protein in the OM was compatible with our hypothesis (18) that the *tolC* gene product was required at a post-transcriptional step in the expression of the OmpF protein in the OM.

The hypersensitivity to detergents, dyes, and certain antibiotics can also be attributed to altered functioning of the OM; however, the effect on colicin E1 sensitivity requires some reinterpretation of existing data. Bhattacharyya et al. (3) showed that membrane vesicles from the wild type and receptor mutants are sensitive to colicin E1, as measured by inhibition of proline uptake, whereas vesicles from a *tolC* mutant are insensitive to colicin E1 action. They concluded

that the *tolC* defect which affects colicin E1 action is located in the cytoplasmic membrane, which is also the site of colicin E1 action. We interpret the data as showing that the *tolC* protein must be present in the membrane vesicles so that colicin E1 can be directed to its target, perhaps by being processed into an active form. This interpretation allowed the TolC protein to be located in the OM. Since Kaback vesicles contain a high percentage of OM material (10), this role for the TolC protein in colicin E1 action can be accommodated. The data obtained with synthetic membrane vesicles (11) does not reflect the in vivo complexity of colicin E1 action, as shown by the *tolC* requirement for colicin E1 action on *E. coli* membrane vesicles.

We suggest that the TolC protein may be involved in a processing step(s) that several membrane proteins have in common, thus explaining the pleiotropic effects observed in *tolC* mutants. We are continuing our studies to determine the role of the TolC protein in OmpF incorporation into the cell envelope.

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