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New Outer Membrane-Associated Protease of *Escherichia coli* K-12

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The gene for a new outer membrane-associated protease, designated OmpP, of *Escherichia coli* has been cloned and sequenced. The gene encodes a 315-residue precursor protein possessing a 23-residue signal sequence. Including conservative substitutions and omitting the signal peptides, OmpP is 87% identical to the outer membrane protease OmpT. OmpP possessed the same enzymatic activity as OmpT. Immuno-electron microscopy demonstrated the exposure of the protein at the cell surface. Digestion of intact cells with proteinase K removed 155 N-terminal residues of OmpP, while the C-terminal half remained protected. It is possible that much of this N-terminal part is cell surface exposed and carries the enzymatic activity. Synthesis of OmpP was found to be thermoregulated, as is the expression of *ompT* (i.e., there is a low rate of synthesis at low temperatures) and, in addition, was found to be controlled by the cyclic AMP system.

The T-even-type *Escherichia coli* phage Ox2 (20) uses the outer membrane protein OmpA as a receptor (15, 46). Host range mutants of this phage which had switched receptors in consecutive steps have been described. One of the mutants, Ox2h12, recognized the outer membrane porin OmpC (31). From this mutant, Ox2h12h1.1 was derived, which uses yet another outer membrane protein as a receptor, designated OmpX. This protein was absent in strains selected for resistance to the phage (6). Sequencing of the N terminus of the polypeptide and a computer search in the SwissProt data bank showed that the polypeptide was unknown. We wished to characterize this new outer membrane protein. To this end, we have cloned the corresponding gene, which we propose to be called *ompP*. It is shown that the OmpP protein is closely related to the outer membrane protease OmpT (13, 41). Several properties of the new enzyme are described in this paper.

MATERIALS AND METHODS

Bacterial strains, plasmids, phage, and growth conditions. The bacterial strains used are listed in Table 1. For most cloning and sequencing experiments, plasmids pUC18 or -19 (58) were used. pTK61 (21), a derivative of pBR322 (1), carries a hybrid gene coding for the B subunit of the cholera toxin of *Vibrio cholerae* fused to the β subunit of the immunoglobulin A (IgA) protease of *Neisseria gonorrhoeae* and specifies resistance to ampicillin. In order to have this plasmid together with the cloned *ompP* gene in the same cell, *ompP* was transferred to pACYC184 (2), effecting resistance to chloramphenicol. An *E. coli* gene bank in λgt11 (59) was purchased from Clontech Laboratories, Palo Alto, Calif. The donor for this library was the K-12 strain W1485 (26). Cells were grown in LB medium (30), supplemented, when required, with glucose (0.5%), maltose (0.5%, for cells to be infected with phage λ), isopropylthiogalactoside (IPTG [1 mM]), ampicillin (100 μg/ml), or chloramphenicol (25 μg/ml).

Cloning and sequencing of *ompP* and detection of chromosomal *ompP* and *ompT*. Plaques of λgt11 were generated on strain Y1090 and transferred to nitrocellulose filters soaked in 1 mM IPTG (which was unnecessary [see Results]). The filters were washed with a 3% solution of bovine serum albumin in 20 mM Tris · Cl–140 mM NaCl, pH 7.5. The filters were then incubated for 4 h at room temperature in the same buffer containing antiserum against OmpP (dilution, 5×10^{-4}) and 2% serum albumin and then washed three times with 0.5% Tween 20 in the same buffer. Positive samples were detected by incubation with peroxidase-coupled goat antiserum against rabbit IgG (Dianova, Hamburg [16]). Strain Y1090 was lysogenized with one of these, λgt11-15. The lysogen was thermoinduced, and the phage was precipitated with polyethylene glycol (57) and then centrifuged through a CsCl step gradient (43). The 4.5-kb *Eco*RI insert of this phage was cloned into pUC18, yielding pAK1. The *ompP* gene was present on a 2.2-kb *Bgl*II fragment of this insert (see Results), which was cloned into the *Bam*HI site of pUC18, resulting in pAK1-5. DNA sequencing (44) was performed using plasmids which were purified by CsCl density gradient centrifugation. Sequencing was started with a 17-mer sequencing primer and a 24-mer reverse sequencing primer (New England Biolabs). The sequence from bp 1 to 1020 was obtained from subclones by using restriction sites for *Acc*I (bp 1), *Bgl*II (bp 50), *Hin*dIII (bp 561 and 610), and *Hin*cII (bp 334 and 777; for the positions, see Fig. 4). For further sequencing, new primers annealing within the cloned chromosomal fragment were obtained from Appligene (Heidelberg, Germany [also shown in Fig. 4]). This strategy left a few uncertainties and gaps in the complementary strand upstream from bp 900 and 1400. A site for *Ecl*XI starts at bp 1474 (CGGCCG), and an *Eco*RI site exists in the vector (see Fig. 3). A deletion was obtained by restricting pAK1-5 with the two enzymes, followed by filling-in with the Klenow fragment of DNA polymerase I and religation. The resulting DNA fragment was sequenced by using the reverse sequencing primer, covering the sequence from bp 1450 to 1100. For the generation of two other deletions, pAK1-5 was opened with *Ecl*XI, digested for various time intervals with exonuclease III, and made blunt-ended with nuclease SI. After cleavage with *Eco*RI, the DNA was made blunt ended as above and religated. Two deletions were recovered; one started at bp 1066 and the other started at position 903. Both were sequenced with the reverse primer, providing the sequence from bp 1050 to 650. With these deletions, the sequence was completed for both strands from bp 334 to 1675. Southern blots were probed by using digoxi-

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TABLE 1. Bacterial strains

Organism	Description[a]	*ompP* expression	Source and/or reference
P400	*thi argE proA thr leu rpsL non*/F$^-$	+	P. Reeves (47)
P400ACF	Same as P400, but not expressing *ompA*, *ompC*, *ompF*, or *ompP*[b]	−	6
Y1090	*ΔlacU169 proA*$^+$ *Δlon rpsL supF trpC22*::Tn*10* (pMC9)	−	R. W. Davis (60)
CS1383[c]	*thi supE Δlac-proAB ΔompC168*/F′ *traD36 proAB*$^+$ *lacI*^{Q}*ZΔM15*	+	C. A. Schnaitman
CS1383AC	Same as CS1383, but not expressing *ompA*	+	6
CS1383ACP	Same as CS1383A, but not expressing *ompP*[c]	−	6
UH300	*recA56 proA* or *B ΔlacU169 rpsL relA thi*/F′ *lacI*^{Q}*ZΔM15*	+	28
RW193	*proC leu6 trpE38 entA403 tsx*	−	C. F. Earhart (8, 27)
UT5600	Same as RW193, but *ΔompT*	−	C. F. Earhart (8)

[a] Fermentation markers are not listed.
[b] OmpP was called OmpX in this paper.
[c] Derivative of strain JM101 (58; C. A. Schnaitman, personal communication); for *ΔompC168*, see reference 45.

genin-labeled DNA fragments and anti-digoxigenin serum coupled to alkaline phosphatase (nonradioactive DNA labeling and detection kit; Boehringer, Mannheim, Germany). A probe specific for *ompT* was obtained with chromosomal DNA from strain RW193 and PCR (42) using oligonucleotides (left end primer, 5′-CTTGGAACTCTGAGCGG; right end primer, 5′-TTGCGTTAGGTGTGACG) producing a 0.683-bp fragment from nucleotide positions 571 to 1254 (13). The insert of pAK1-5 served as an *ompP*-specific probe. The "miniset" of the Kohara recombinant lambda phage library (24) was obtained commercially (Gene Mapping Membrane) from Takahara Shuzo Co., Kyoto, Japan. All DNA manipulations were performed as described in detail elsewhere (43).

Determination of the transcription start site of *ompP*. Determination of the transcription start site was performed by primer extension of mRNA. The RNA was isolated from strain UH300 carrying pAK1-5 by the method described in reference 10. The 5′ end of the 30-mer oligonucleotide used as primer was complementary to bp 649 (see Fig. 4). The oligonucleotide was 5′-end labeled by using polynucleotide kinase and [γ-^{32}P]ATP. For primer extension, the protocol of Triezenberg (52) was followed, employing avian myeloblastosis virus reverse transcriptase (Boehringer). For the sequence shown in Fig. 5, the same primer was used; electrophoresis was performed with a 6% polyacrylamide gel containing 7 M urea.

Isolation of OmpP, antiserum, and protein sequencing. OmpP was isolated from cell envelopes of strain CS1383 AC by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (Laemmli type [25]). The part of the gel containing the protein was cut out and electroeluted (sample concentrator model 1750, ISCO), precipitated with acetone (final concentration, 90%), washed two times with 90% acetone, and lyophilized. The protein was suspended in phosphate-buffered saline. A rabbit was injected subcutaneously and intramuscularly with 150 μg of the polypeptide emulsified in complete Freund's adjuvant. A booster with 250 μg of protein was given 5 weeks later. Antiserum (rabbit) against the IgA_β domain of the IgA protease was a gift from T. Klauser. For amino acid sequencing, envelopes of the same strain were subjected to electrophoresis as described above. The proteins were transferred electrophoretically with a dry blot apparatus (Pharmacia-LKB) onto a cellulose-acetate membrane (Millipore). The membrane was stained with Coomassie brilliant blue, and the band representing OmpP was cut out and subjected to automated Edman degradation.

Immuno-electron microscopy. For microscopy with ultrathin, thawed cryosections, cells were fixed with 2% formaldehyde–0.05% glutaraldehyde, embedded in agarose, and soaked in 2.1 M sucrose. Cryosections were obtained by using a Reichert FC-4/Ultracut cryoultramicrotome. For preembedding labeling, cells were fixed onto polylysine-coated Epon blocks. Immunolabeling was performed as described previously (9). Sections or whole cells were incubated with antiserum against OmpP (dilution, 1:20) followed by protein A-13-nm gold particles (48) and then postfixed with 2% glutaraldehyde. Whole cells were treated with 1% osmium tetroxide and 1% aqueous uranyl acetate and embedded in Epon. Thawed cryosections were stained with 2% aqueous uranyl acetate and embedded in methylcellulose containing 0.2% uranyl acetate. For double-label experiments, thawed cryosections were incubated first with antiserum against OmpP and protein A-8-nm gold particles, fixed with 0.1% glutaraldehyde, and then treated with antiserum against OmpA (9) and protein A-13-nm gold particles. Changing the sequence of labeling regarding antibodies and markers did not yield different results. Sections were examined in a Philips 201 electron microscope at 60 kV.

Other techniques. Cell envelopes were prepared as described previously (23). All electrophoreses of proteins were performed as described in the previous section, and staining was done with Coomassie brilliant blue. Immunoblots of such electrophoretograms were also obtained as described above, except that nitrocellulose was used (16); proteins were detected as detailed for the plaques of the recombinant λgt11 phages. Molecular weight standard proteins were Rainbow Protein Molecular Weight Markers (Amersham). For digestions with trypsin or proteinase K, cells or envelopes were suspended in phosphate-buffered saline (pH 8.0); incubations were performed at 25°C. Neither higher temperatures nor Tris buffer was used, because cells containing overproduced OmpP tended not to tolerate harsher conditions, e.g., prolonged incubation in Tris buffer, pH 8, at 37°C led to signs of lysis. The action of trypsin was stopped by the addition of soybean trypsin inhibitor (twice the concentration of the enzyme on the basis of weight), and the action of proteinase K was stopped with phenylmethylsulfonyl fluoride (final concentration, 2 mM).

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper appear in the EMBL, GenBank, and DDBJ nucleotide sequence data bases under the accession number X74278.

RESULTS

Cloning and sequencing of *ompP*. The strategy employed to clone the *ompP* gene was to screen a chromosomal gene bank, present on an expression vector, with an antiserum against the corresponding protein. Some characteristics of this polypeptide are shown in Fig. 1A. As reported earlier (OmpP was

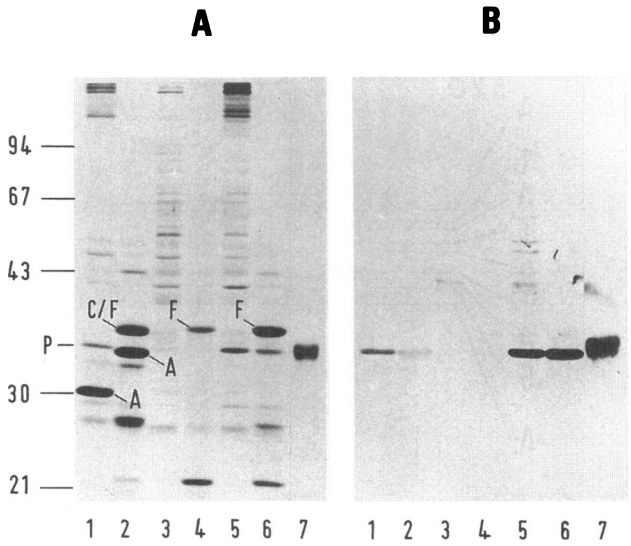

FIG. 1. Identification of OmpP. (A) stained electrophoretogram of cell envelopes; (B) immunoblot of the electrophoretogram using antiserum against OmpP. Lanes 1 and 2, strain P400; lanes 3 and 4, strain CS1383ACP (obtained by selection for resistance to phage Ox2h12h1.1); lanes 5 and 6, strain CS1383AC; lane 7, isolated OmpP (2 μg). Proteins were solubilized at 50°C (lanes 1, 3, and 5) or boiled in sample buffer (lanes 2, 4, and 6). Numbers at the left margin indicate positions of molecular mass markers (in kilodaltons). A, C, F, and P indicate the OmpA, C, F, and P proteins; this gel system does not separate the porins C and F.

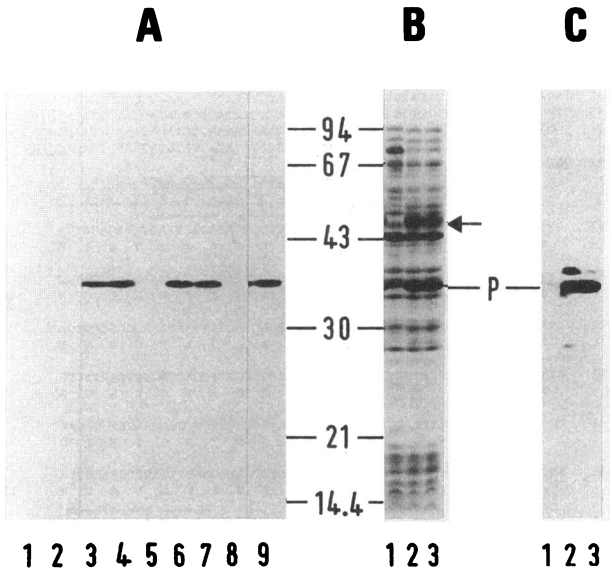

FIG. 2. Expression of the cloned *ompP* gene. (A) Immunoblot; (B) stained electrophoretogram; (C) immunoblot of B. In all cases, whole cells boiled in sample buffer were used. A, lanes 1 through 4, strain Y1090; lanes 5 through 8, strain CS1383ACP. Both were lysogenized with λgt11-15 (lanes 2 through 7) and grown in the presence of glucose (lanes 2 and 5), without additions (lanes 3 and 6), or in the presence of IPTG (lanes 4 and 7). Lanes 1 and 8, strains without prophage; lane 9, strain CS1383AC (expressing the chromosomal *ompP* gene). B and C, *ompP* carried by pAK1 in strain UH300. Cells were grown in the presence of glucose (lanes 1), and cells pregrown in glucose medium were transferred to medium without the sugar and grown for a further 4 (lanes 2) or 2 (lanes 3) h. The band above OmpP in panel C, lane 2 is likely to be the precursor. An arrowhead indicates the 45-kDa protein; P indicates OmpP. Numbers between A and B show positions of molecular mass markers.

previously called OmpX [6]), it was absent from a strain resistant to phage Ox2h12h1.1. Its electrophoretic mobility did not depend on the temperature of solubilization in electrophoresis sample buffer (containing 1% SDS), and, in contrast to the porins OmpC and F (40), it was readily soluble in this buffer at 50°C. It could easily be isolated electrophoretically from strain CS1383AC (Fig. 1A, lane 5). An antiserum was raised against the protein (obtained by electroelution from such gels); the specificity of the serum is shown in Fig. 1B.

Recombinant phage λgt11 (59, 60), harboring an *E. coli* chromosomal gene bank, was plated on strain CS1383ACP, and the plaques were screened with the antiserum. Several positive plaques were detected. Strain Y1090 (which was found not to possess OmpP) or CS1383ACP was lysogenized with a phage from one of the positive plaques, λgt11-15. Phage λgt11 was designed to yield genes fused to a β-galactosidase gene inducible with IPTG. The lysogens were therefore grown in the presence of either the inducer or glucose or were grown in the absence of either compound. Figure 2A demonstrates that the antiserum detected a protein the size of OmpP. It was absent in glucose-grown cells, but its synthesis was independent of the presence of IPTG. Apparently, the gene existed in the phage genome under the control of its own promoter.

Phage λgt11-15 carried a 4.5-kb *Eco*RI insert which was transferred to pUC18, resulting in pAK1. This plasmid conferred sensitivity toward phage Ox2h12h1.1 to strain CS1383ACP and caused the production of large amounts of OmpP (Fig. 2B and C). Another protein (with an apparent molecular mass of about 45 kDa) was overproduced, suggesting that the gene for this polypeptide is also present on the cloned fragment (see below). Subcloning showed that *ompP* was located on a 2.2-kb *Bgl*II fragment (Fig. 3), which was cloned into pUC18, yielding pAK1-5, and sequenced (Fig. 4). Sequencing of the N terminus of the purified OmpP revealed the sequence Ser-Asp-Phe-Phe (or Trp)-Gly-Pro-Glu-Lys-Ile-Ser-Thr-Glu-Ile-Asn-Leu-Gly-Thr (or Ser)-Leu-Ser-Gly; this demonstrated the presence of a signal peptide. Immediately downstream from *ompP* another open reading frame exists, a candidate for the sequence encoding the 45-kDa protein; this polypeptide also possesses a potential signal sequence (Fig. 4; for the possibility of cleavage at the site by the leader peptidase, see reference 54).

Properties of the *ompP* gene. Transcription of *ompP* starts at bp 520 (Fig. 5); obvious candidates for the −10 and −35 promoter regions are indicated in Fig. 4. Expression of *ompP* is under the control of the cyclic AMP system (see below). A potential site for binding the catabolite activator protein (consensus sequence, 5′-aaaTGTGAtctagaTCACAttt [14]) be-

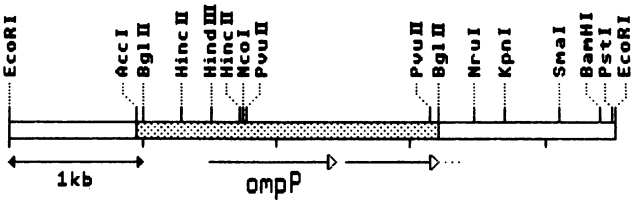

FIG. 3. Structure of the insert in pAK1. The area which has been sequenced (see Fig. 4) is stippled. The arrow following *ompP* represents the open reading frame starting at bp 1592 (Fig. 4). At the point labeled *Hin*dIII, two such sites exist which are only 49 bp apart.

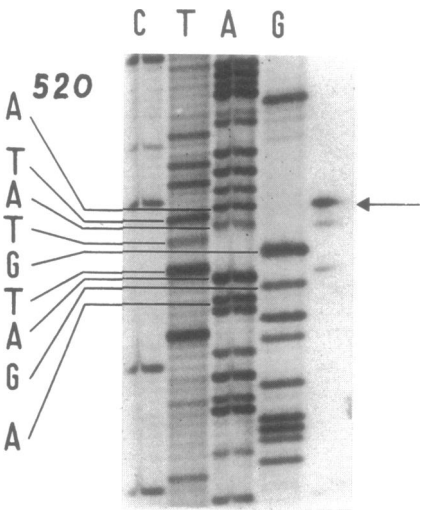

FIG. 5. Transcriptional start site of *ompP*. Right lane, products of primer extension (arrow indicates the full-length product). The sequence ladder was obtained by using the same primer (see Materials and Methods). The position of A at bp 520 is shown in Fig. 4.

FIG. 4. DNA sequence and deduced amino acid sequences. The sequence of the area indicated in Fig. 3 is shown. Promoter sequences are boxed with dots (consensus sequences, 5′-TTGACA and 5′-TATAAT for the −35 and −10 regions, respectively [17]); a −35 region was not discernible for the open reading frame following *ompP*. Shine-Dalgarno sequences are underlined. The potential binding site for the catabolite activator protein, the signal sequence of pre-OmpP, and the signal sequence proposed for the following open reading frame are boxed. The transcription start site is marked with an open triangle; the palindrome possibly forming a stem-and-loop structure downstream from *ompP* is indicated by opposing arrows with open arrow heads. The other arrows indicate the primers used for sequencing.

gins at bp 416: 5′-tatATTGAtataccTCACAaaa. A rather stable stem-and-loop structure (ΔG = −15.9 kcal [66.5 kJ]) in the corresponding mRNA, starting at position 1517, may serve as the terminator.

A computer search of the EMBL gene bank revealed that pre-OmpP is 70% identical with the precursor of the *E. coli* outer membrane protease OmpT (13), previously also called protease VII (51) or protein a or 3b (41). When conservative substitutions are included and signal peptides are omitted, the degree of identity becomes 87%. The nucleotide sequences flanking the *ompP* and *ompT* genes are entirely different (Fig. 4 and reference 13), indicating that the chromosomal locations of the two genes are different. This was substantiated by probing chromosomal DNA restriction digests with *ompP* and *ompT*-specific fragments (Fig. 6). The results obtained with the *ompP* probe agreed with the restriction map shown in Fig. 3; it also turned out that both strains RW193 (*ompT*$^+$) and the *ompT* deletion strain UT5600 lacked *ompP*. The patterns generated with the *ompT* probe prove that the two genes occupy different chromosomal locations, although the sizes of the fragments do not agree with published data (e.g., *ompT* should be present on a 2.035-kb *Eco*RI-*Pst*I fragment [13], while this fragment from the three different strains used had a size of 3 kb). This is most likely due to strain differences. Attempts to locate *ompP* in a recombinant λ phage library, representing most of the *E. coli* chromosome (miniset of Kohara et al. [24]), failed. By using *ompT*- and *ompP*-specific probes (Fig. 6), *ompT* was detected at the correct position. The absence of a hybridization signal with the *ompP*-specific probe may have been due to the absence of the gene from the strain that was used in construction of the library. Alternatively, *ompP* may be located within the few gaps existing in this library.

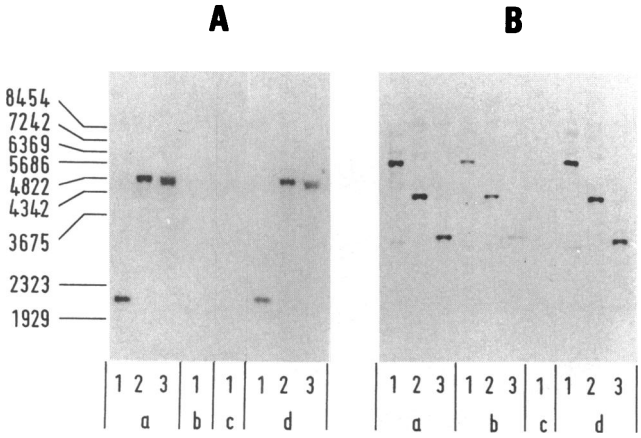

FIG. 6. Restriction analyses. Chromosomal DNA from strains UH300 (a), RW193 (b), UT5600 (c), and CS1383 AC (d) was digested with *Bgl*II (lanes 1), *Eco*RI (lanes 2), or *Eco*RI plus *Pst*I (lanes 3), subjected to agarose gel electrophoresis, and probed with an *ompP*-specific (A) or an *ompT*-specific (B) DNA fragment. Numbers at left margin indicate positions of fragments of phage λ DNA digested with *Bst*EII.

Location and properties of OmpP. As we expected, OmpP is an outer membrane protein. In sucrose density gradients (34), it migrated together with this membrane (not shown), and immuno-electron microscopy demonstrated the cell surface exposure of the polypeptide (Fig. 7). This exposure was substantiated by a double-label experiment using antisera against OmpP and OmpA (Fig. 8); the serum specific for the outer membrane protein OmpA is known to react with the protein almost exclusively at the inner side of the membrane (for an example, see reference 9).

OmpT cleaves predominantly between two basic residues (11) and cannot attack chromogenic protease substrates. This inability was also exhibited by OmpP. The *ompT* deletion strain UT5600, with or without pAK1-5, was grown for 5 h, causing overproduction of OmpP. Neither culture could cleave *N*-benzyloxycarbonyl-lysine-*p*-nitrophenylester or *N*-benzyloxycarbonyl-glycine-glycine-arginine-β-naphthylester. A fusion protein has been constructed which acts as a substrate for OmpT. It consists of the signal peptide followed by the B subunit (CtxB [12 kDa]) of the cholera toxin of *V. cholerae*, which in turn was fused to the β domain (45 kDa) of the IgA protease of *N. gonorrhoeae* (21). The IgA_β domain causes translocation of the B subunit across the outer membrane, and OmpT releases the whole subunit and a short part of the IgA_β domain into the medium. The protease rapidly hydrolyzes two nearby Arg-Arg bonds, creating a membrane-bound 37-kDa fragment; at another site further downstream an Arg-Ala sequence is hydrolyzed more slowly and leads to a 32-kDa fragment (22), the true membrane moiety of the IgA_β domain. Figure 9 shows that OmpT and OmpP appear to possess the same specificity towards the hybrid protein, i.e., fragments of identical sizes were generated. This could be proven by an Edman degradation of the 37-kDa fragment generated from the hybrid protein by OmpP (Fig. 9, lane 5). The sequence was Arg-Ala-Ile-?-?-Glu-Pro. The N-terminal sequence of this fragment obtained by digestion with OmpT was reported to be Arg-Ala-Ile-Ser-Ser-Glu (22). Since this sequence occurs only once in the hybrid protein (38), the specificities of OmpT and OmpP towards this substrate are identical.

Information concerning the membrane topology of a protein can often be obtained by treating a membrane with proteases.

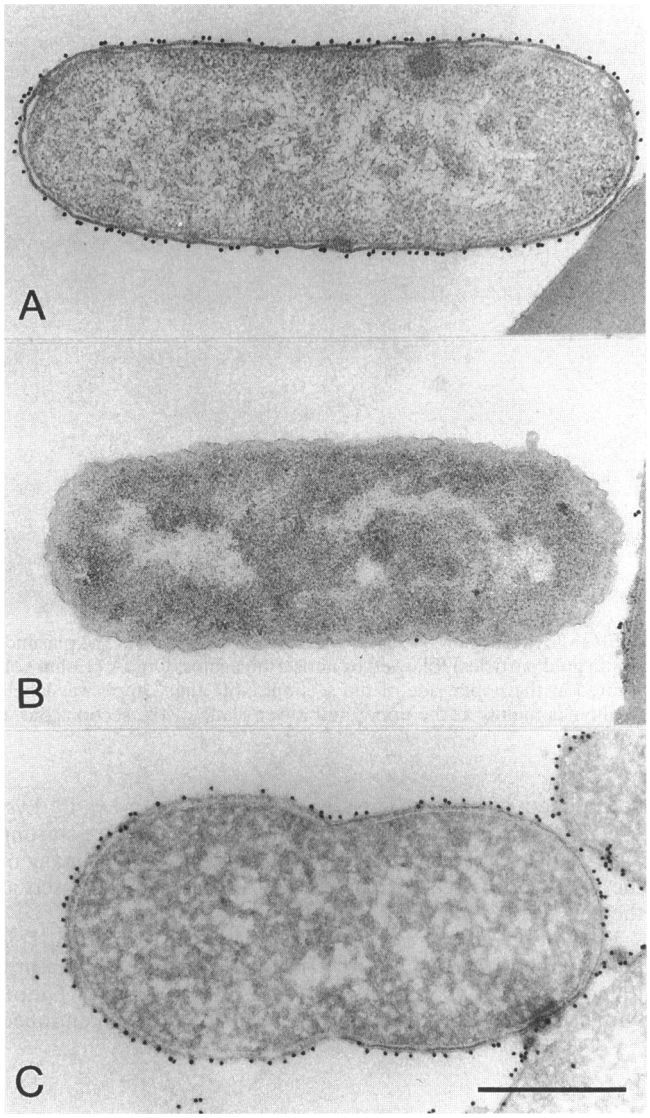

FIG. 7. Immuno-electron micrographs. Whole cells (A and B) or ultrathin thawed cryosections (C) were subjected to immunogold labeling with antiserum against OmpP. (A and C) Strain UH300 carrying pAK1-5; (B) strain UH300 without the plasmid. The concentration of chromosomally encoded OmpP is too low to be easily detectable under the experimental conditions used. Bar, 0.5 μm.

Therefore, envelopes from cells expressing the chromosomal *ompP* gene were treated with trypsin or proteinase K. The former enzyme had no effect on OmpP, while the latter caused its complete disappearance; fragments that would have been stainable or immunoreactive were not detectable. The kinetics of degradation of OmpP was determined with a reduced concentration of proteinase K (20 μg/ml instead of 500 μg/ml). Figure 10A shows that an intermediate with an apparent molecular mass of 16 kDa existed. Intact cells were subjected to the action of the two proteases. As with membranes, trypsin had no effect on OmpP. Proteinase K generated a stable 16-kDa fragment (Fig. 10B) which is likely to be identical with the intermediate just mentioned. When the protein was overproduced, part of it was degraded in the absence of added protease to yield an approximately 18-kDa fragment (Fig. 10B). The N-terminal sequence of the 16-kDa fragment was

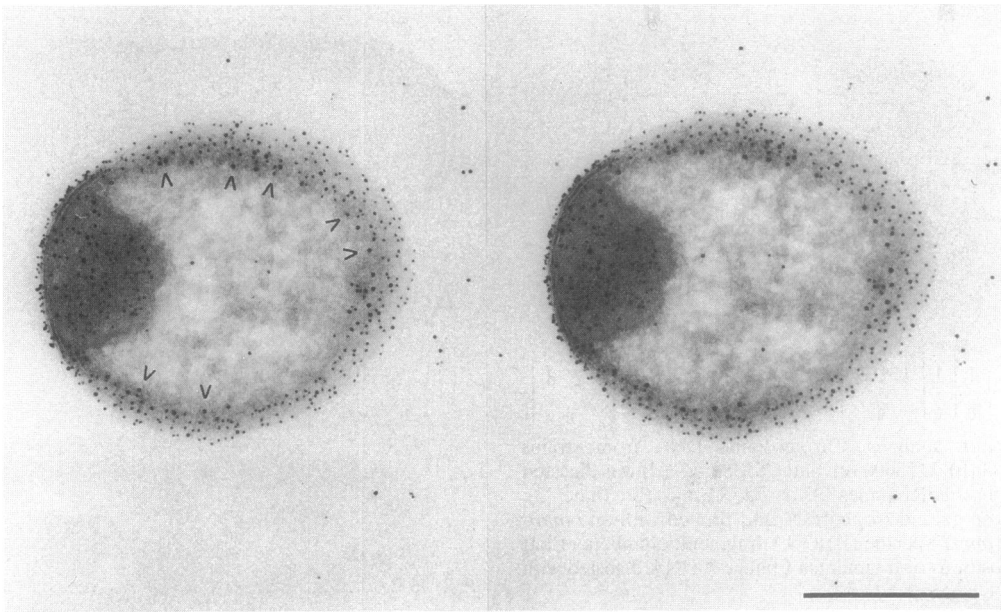

FIG. 8. Stereoview. A semithick thawed cryosection of the plasmid pAK1-5-harboring strain UH300 was labeled using antiserum against OmpP (8-nm gold particles) followed by antiserum against OmpA (13-nm gold particles). The arrowheads point to regularly arranged 13-nm gold particles located at the upper side of the section. Note that OmpP was labeled over the whole outer surface of the cell, while OmpA was accessible to antibodies mainly at the upper and lower plane of the section. Bar, 0.5 μm.

determined to be Glu-Thr-Gly-Ala-Leu-Pro-Asp-Lys-Ile-Lys-Val-Ile-Gly-Tyr-Lys. This corresponds to the sequence starting at residue 156 of OmpP (Fig. 4). This indicated that many of the mature protein's 155 N-terminal residues are exposed at the cell surface (however, see Discussion).

Regulation of expression of *ompP*. Synthesis of OmpT is thermoregulated (29), i.e., the protein is produced in greatly reduced quantities during growth at 32°C or below. OmpP synthesis exhibited exactly the same property. As mentioned above, OmpP (and the 45-kDa protein shown in Fig. 2) was absent in cells grown in the presence of glucose. The addition of cyclic AMP (1 mM) to glucose-containing medium relieved the glucose effect almost completely (not shown). This restoration of expression was also observed for the 45-kDa protein.

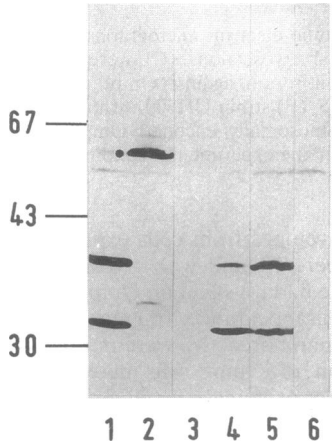

FIG. 9. Enzymatic activities of OmpT and OmpP. An immunoblot of an electrophoretogram of whole cells boiled in sample buffer is shown; antiserum against the IgA_β domain of IgA protease was used. Lanes: 1, strain RW193 (*ompT*$^+$ *ompP*) carrying pTK61 (expressing the CtxB-IgA_β hybrid [dot in lane 2]); 2, strain UT5600 (*ompP ompT*) carrying the same plasmid; 3, strain RW193; 4, strain RW193 carrying both pTK61 and *ompP* on pACYC184; 5, strain UT5600 harboring pTK61 and pACYC184; 6, strain UT5600.

DISCUSSION

As expected for an outer membrane protein, there are no lipophilic stretches within the mature part of OmpP which would serve as membrane anchors. Analogously to other proteins of this membrane (4, 32, 53, 55, 56), the protease may exist in its membrane in the form of antiparallel β-sheets. Yet, it represents an unusual outer membrane protein in that, in contrast to other such proteins (3, 33), bilateral action of proteinase K on the membrane did not produce any stable protected fragment. Only one intermediate of this degradation, with a molecular mass of ~16 kDa, could be identified. A fragment of the same size was obtained when intact cells were treated with the protease, and it was shown to consist of the C-terminal half of OmpP; the intermediate and this fragment are probably identical. The data presently available do not allow us to propose any detailed model for the membrane topology of OmpP. One possibility would be that much, if not all, of the N-terminal half of the protein is exposed at the cell surface. The disappearance of the C-terminal moiety of OmpP upon bilateral proteolytic digestion of the membrane would be understandable if the protease cleaved at turns of the polypeptide chain at the inner side of the membrane, thus creating single transmembrane strands. Since it is known that even lipophilic α-helices can be lost from a membrane upon bilateral proteolytic attack (7, 19), such loss is also conceivable for β-strands. Alternatively, the hypothetical single strands may simply lack antigenic epitopes and remain undetected in the immunoblots. The same mechanisms could also be responsible for the disappearance of the N-terminal part after the action of

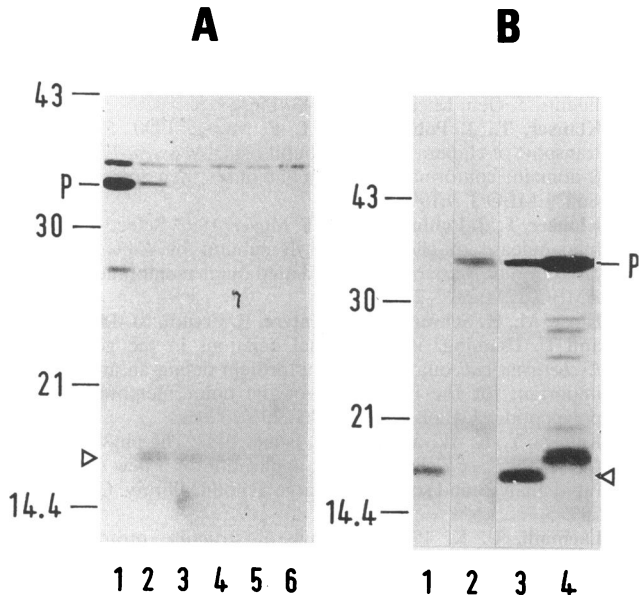

FIG. 10. Cleavage of OmpP with proteinase K. Immunoblots (with anti-OmpP serum) of electrophoretograms are shown. (A) Treatment of cell envelopes with proteinase K (20 μg/ml). Lanes: 1, no protease; 2 through 6, incubation with protease for 2, 4, 6, 8, and 10 min, respectively. (B) Intact cells incubated without (lanes 2 and 4) or with (lanes 1 and 3) proteinase K (200 μg/ml) for 1 h. Strain CS1383AC with no plasmid (lanes 1 and 2) or carrying pAK1-5 (lanes 3 and 4) was used. P, OmpP; arrowhead, 16-kDa fragment. Note the approximately 18-kDa fragment arising from overproduction of OmpP in the absence of the protease (lane 4).

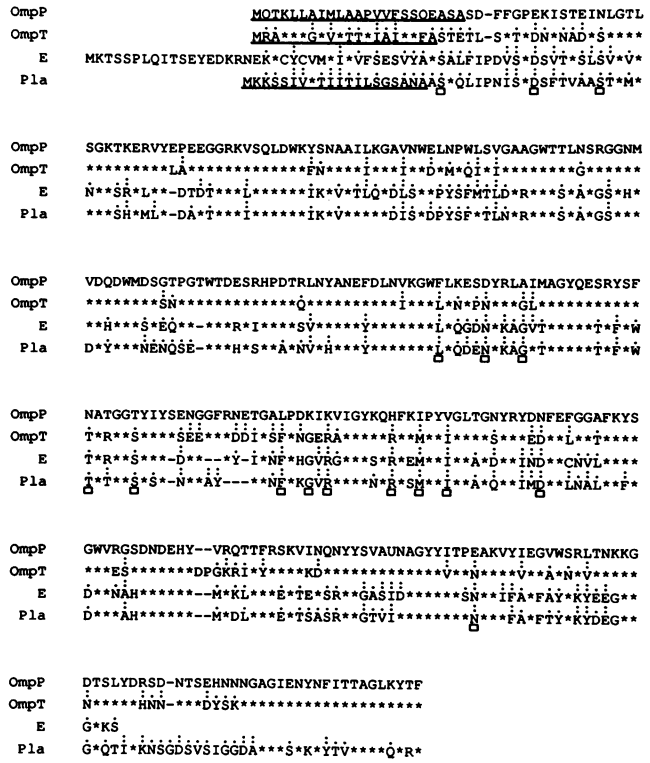

FIG. 11. Alignment of OmpP with outer membrane proteases. The alignment was performed by using the Smith and Waterman algorithm (49) with the Dayhoff matrix (subprogram "best fit" from the programs of the Genetics Computer Group of the University of Wisconsin [5]). E, the *S. typhimurium* protein; Pla, the *Y. pestis* protein. The known signal sequences are underlined. *, identical residues; :, conservative substitutions; ·, substitutions not drastically altering the predicted secondary structure; −, gaps. The boxes indicate identities between OmpT, E, and Pla that do not extend to OmpP.

proteinase K on intact cells. Hence, this part need not be exposed entirely at the cell surface. Since only one intermediate was detected when the kinetics of digestion of cell envelopes with the protease was monitored, we favor the possibility that most of the N-terminal moiety of OmpP is indeed cell surface exposed. OmpT is a trypsinlike protease with narrow specificity (18). OmpP, as expected from the high degree of identity with OmpT, was shown to possess the same specificity. Such an activity is likely to demand a tightly folded structure, and this also tends to argue for surface exposure of much of the N-terminal part of OmpP. Such a structure would be consistent with the complete resistance of outer membrane-associated OmpP toward trypsin. (If so, it will be interesting to find out how a large fraction of the protease is translocated across the outer membrane, as this may not be a spontaneous process. Help may be required, be it by other polypeptides [39] or by self-help, as in the case of an IgA protease [38].) Other membrane topologies are possible, and there is some indication that the OmpT protein is present in the membrane as an oligomer (51). OmpP could then, of course, also exist in oligomeric form in the membrane, and the anchoring could be achieved by a participation of all monomers.

Aside from being thermoregulated, *ompP* is under the control of the cyclic AMP system. This regulation may offer an explanation for the strange fact that the two very closely related proteases OmpP and OmpT can coexist in *E. coli* K-12 strains. We found that OmpT synthesis is not subject to catabolite repression (18a). Both OmpP and OmpT are not required for growth under usual laboratory conditions. Whatever the physiological function of OmpP may be, however, conditions causing catabolite repression of OmpP synthesis but requiring the presence of such an enzyme may have existed in the wild. The solution to this problem could have been gene duplication, generating a protease gene that was not subject to catabolite repression which then evolved to the present-day OmpT. A duplication event is consistent with the different chromosomal locations of *ompT* and *ompP*.

Other cell envelope-associated proteases have been described which, however, are not identical to OmpT or OmpP. Protease IV is located at the cytoplasmic membrane, and protease V was found to be associated with both envelope membranes (35, 36). The exact location of the about 43-kDa protease VI has not been determined (37). It is clearly different from OmpT; e.g., the activity of OmpT is not inhibited by phenylmethylsulfonyl fluoride (18), while that of protease VI is (38). A protease of *Salmonella typhimurium*, protein E (61; later called PrtA [50]) exhibited an enzymatic activity similar to that of OmpT when expressed in *E. coli* (12). The Pla protein from *Yersinia pestis* represents an outer membrane plasminogen activator/coagulase (50). It has been noted that OmpT, E (PrtA), and Pla share a high degree of identity (50). Obviously, OmpP also belongs to this family, although it is less related to Pla and E than to OmpT (Fig. 11). It is interesting that the *pla* gene is preceded, starting at position 129 (50), by the sequence ttaTGTGAgcaaagTCACAtaa, an excellent candidate for recognition by the catabolite activator protein; it is not known whether it is active.

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