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Comparison of *Escherichia coli* K-12 Outer Membrane Protease OmpT and *Salmonella typhimurium* E Protein

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The predicted amino acid sequence of OmpT, an *Escherichia coli* outer membrane protease, was found to be highly homologous to that predicted for the *pgtE* gene product of *Salmonella typhimurium*. In this paper, it is shown that *pgtE* codes for a protein functionally homologous to OmpT as judged by its ability to proteolyze T7 RNA polymerase and to localize in the outer membrane of *E. coli*.

Escherichia coli possesses over one dozen proteolytic enzymes (13, 14, 17). Some of these proteases have known roles in protein export (1, 19) or protein turnover (5, 13); however, the biological functions for many remain to be elucidated. Recently, it has been shown that bacteriophage T7 RNA polymerase is very sensitive to proteolysis in cell extracts (2, 18). Subsequent genetic studies demonstrated that the product of the *ompT* gene, which encodes an outer membrane-associated protease, was the major source of this proteolytic activity (7).

The nucleotide sequence of *ompT* and the N-terminal sequence of the mature OmpT protein have been determined (8). These data indicate that OmpT is synthesized as a 317-amino-acid proprotein. During export to the outer membrane, a 20-amino-acid signal sequence is removed.

The affinity of OmpT for a benzamidine matrix (9, 15), together with its inhibitor profile and specificity for cleaving after lysine or arginine residues (7; 18), suggests functional homology with other microbial and pancreatic serine proteases. A highly conserved feature between these two classes of proteases is conservation of sequence surrounding the catalytic histidine and serine residues (Thr-Ala-Gly-His-Cys and Gly-Asp-Ser-Gly-Gly) (3). None of the 3 histidine residues or the 25 serine residues in OmpT is flanked by a sequence that has strong homology with catalytic residues in other serine proteases. Searching current sequence data banks also failed to demonstrate any convincing regions of homology with any other known proteases. However, this search did reveal extensive homology between OmpT and one other protein, the E protein specified by the *pgtE* gene of *Salmonella typhimurium* (20).

The predicted amino acid sequences of the entire OmpT and *pgtE* open reading frames were aligned to exhibit the best homology possible and are shown in Fig. 1. Of the 278 amino acid residues that overlap, 135 (48.2%) are identical and another 97 (34.9%) represent conservative amino acid changes between the two proteins.

Although the *pgtE* gene is part of an operon whose gene products are involved in phosphoglycerate uptake, it is not essential for phosphoglycerate transport and no enzymatic or structural role has been previously ascribed to the protein (20). The high degree of homology of the *pgtE* gene product with OmpT suggested that it also may be an outer membrane protease. However, isogenic *Salmonella* strains with or without *pgtE* are not available; thus, direct assay of *Salmonella* cells for the presence or absence of a *pgtE*-encoded protease activity was not possible. In order to test the idea that the E protein is an outer membrane protease, we introduced a plasmid carrying the *pgtE* gene into an *ompT* deletion strain of *E. coli*, BL21. The plasmid we used, pJH556, carries a 2.05-kilobase-pair *S. typhimurium* *Eco*RI-*Hin*dIII chromosomal fragment known to code for the E protein (10, 20). As a positive control, BL21 was independently transformed with a plasmid, pML19, encoding functional OmpT (4, 6).

Whole cells were tested for their ability to cleave T7 RNA polymerase as described previously (7). Briefly, washed cells were mixed with the polymerase and, after a short incubation period, the cells were pelleted by centrifugation. The supernatants were then analyzed by electrophoresis on sodium dodecyl sulfate–polyacrylamide gels to determine whether the T7 RNA polymerase had been cleaved.

Transformants were grown overnight at 37°C as standing cultures in 2.5 ml of a complex medium containing 1% tryptone, 0.5% yeast extract, 0.5% NaCl, and 0.4% glucose, to which was added ampicillin at a final concentration of 40 μg/ml (2). Duplicate 1-ml portions of each culture were centrifuged for 2 min in a Fischer microcentrifuge, and the cell pellets were washed in 1 ml of 10 mM Tris hydrochloride (pH 8.0), recentrifuged, and then suspended in 100 μl of 50 mM Tris hydrochloride (pH 8.1)–20 mM NaCl. The samples were adjusted if needed to have equal A_{600}s. In addition, we tested whole-cell lysates in case the E protein could not localize in the outer membrane. One sample from each culture was mixed with 10 μl of lysozyme (1 mg/ml) in 100 mM trisodium EDTA. After 30 min on ice, the samples were frozen and thawed three times to complete lysis.

BL21 whole-cell lysates and intact BL21 cells did not cleave added T7 RNA polymerase (Fig. 2); however, BL21 cells transformed with either pJH556 or pML19 could cleave T7 RNA polymerase. Interestingly, intact BL21(pJH556) cells could cleave T7 RNA polymerase. These observations suggest that the *pgtE* gene product is analogous to the OmpT protease in both catalytic activity and ability to localize in the cell outer membrane.

To learn more about the localization of the *pgtE* gene product in *E. coli*, we separated the whole-cell extracts into cytoplasmic and detergent-solubilized inner and outer membrane fractions (12). As expected, nearly all of the OmpT activity in the BL21(pML19) extract was recovered in the

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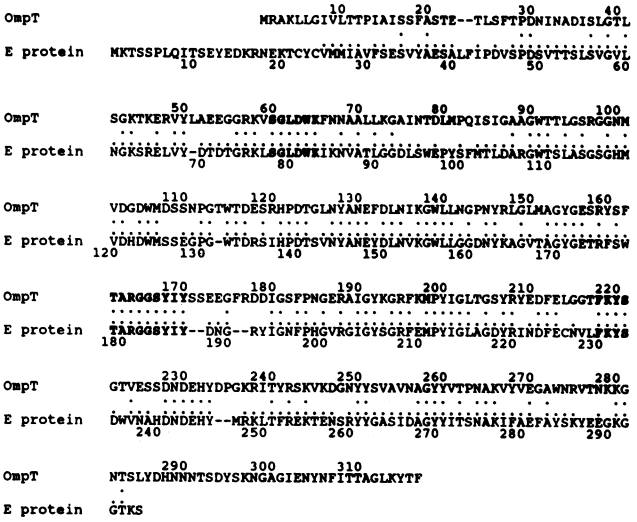

FIG. 1. Comparison of the predicted amino acid sequences of OmpT and the E protein. Double dots between the sequences indicate amino acid residues that are identical in the two proteins; single dots indicate conservative substitutions. Regions in boldface type signify the positions of identical amino acid sequences containing serine residues.

outer membrane fraction. However, somewhat different results were obtained with BL21(pJH556) extracts. While significant amounts of OmpT-like protease activity were present in the BL21(JH556) outer membrane fraction, considerable amounts of activity were also seen in the other fractions (data not shown). These results may indicate that the translocation rates across the *E. coli* membranes of the *ompT* and *pgtE* gene products are different.

It is generally accepted that most proteins destined to be exported from the cytoplasm are synthesized as precursors with signal sequences. Such signals usually have one or more positively charged amino acids near their amino terminus, a hydrophobic middle segment, and a conserved P_{-3}, P_{-1} amino acid sequence found immediately preceding the signal peptidase processing site (11). In *E. coli*, most cleavable signal sequences are about 15 to 20 residues long.

Although the N-terminal signal sequence of OmpT is fairly typical for an *E. coli* outer membrane protein, the N-terminal portion of the E protein is somewhat atypical for a protein that can associate with the *E. coli* outer membrane. However, as the alignment in Fig. 1 shows, residues 25 to 40 of the E protein are homologous to the middle and C-terminal parts of the OmpT signal sequence. Whether this region serves as a processing site in either *E. coli* or *S. typhimurium* remains to be established.

As shown here, the *pgtE* gene codes for a protease with extensive sequence homology to OmpT, a serine protease located in the outer membrane of *E. coli*. Presumably, E protein also has a catalytically important serine residue. Of the 25 serine residues in the mature OmpT protein, only 3 are located in regions in which the two proteins are extremely homologous (indicated by boldface type in Fig. 1). These amino acids are being used as starting points for site-directed mutagenesis experiments aimed at identifying the catalytically active serine residue.

Since this paper was submitted, Sugimura and Nishihara (16) have independently noted the high degree of homology between the *E. coli* OmpT protein and the E protein from *S. typhimurium*.

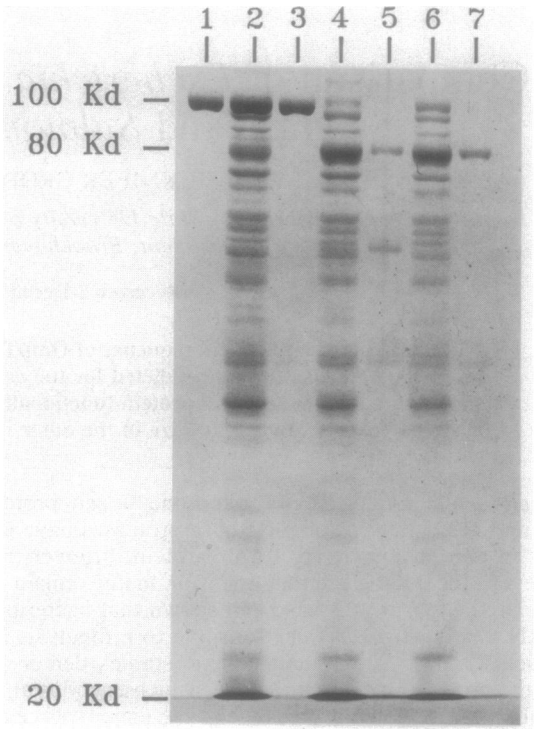

FIG. 2. E protein can cleave T7 RNA polymerase. Purified T7 RNA polymerase was incubated as described previously (7) with whole cells (lanes 3, 5, and 7) or with cell extracts (lanes 2, 4, and 6). Lanes: 1, purified T7 RNA polymerase incubated in buffer alone; 2 and 3, BL21, an *ompT* deletion strain; 4 and 5, BL21 transformed with the plasmid pML19, which carries *ompT*; 6 and 7, BL21 transformed with plasmid pJH556, which carries *pgtE* (encoding the E protein). The positions of T7 RNA polymerase (100 kilodaltons [Kd]) and its most prominent cleavage products (80 and 20 Kd) are indicated.

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LITERATURE CITED

1. **Benson, S. A., M. N. Hall, and T. J. Silhavy.** 1985. Genetic analysis of protein export in *Escherichia coli* K12. Annu. Rev. Biochem. **54**:101–134.
2. **Davanloo, P., A. H. Rosenberg, J. J. Dunn, and F. W. Studier.** 1984. Cloning and expression of the gene for bacteriophage T7 RNA polymerase. Proc. Natl. Acad. Sci. USA **81**:2035–2039.
3. **Delbaere, L. T. J., W. L. B. Hutcheon, M. N. G. James, and W. E. Thiessen.** 1975. Tertiary structural differences between microbial serine proteases and pancreatic serine enzymes. Nature (London) **257**:758–763.
4. **Gayda, R. C., and A. Markovitz.** 1978. Cloned DNA fragment specifying major outer membrane protein *a* in *Escherichia coli* K-12. J. Bacteriol. **136**:369–380.
5. **Goff, S. A., and A. L. Goldberg.** 1987. An increased content of protease La, the *lon* gene product, increases protein degradation and blocks growth in *Escherichia coli*. J. Biol. Chem. **262**:4508–4515.
6. **Gordon, G., R. C. Gayda, and A. Markovitz.** 1984. Sequence of the regulatory region of *ompT*, the gene specifying major outer membrane protein *a*(3b) of *Escherichia coli* K-12: implications for regulation and processing. Mol. Gen. Genet. **193**:414–421.
7. **Grodberg, J., and J. J. Dunn.** 1988. *ompT* encodes the *Esche-*

richia coli outer membrane protease that cleaves T7 RNA polymerase during purification. J. Bacteriol. **170**:1245–1253.
8. **Grodberg, J., M. D. Lundrigan, D. L. Toledo, W. F. Mangel, and J. J. Dunn.** 1988. Complete nucleotide sequence and deduced amino acid sequence of the *ompT* gene of *Escherichia coli* K-12. Nucleic Acids Res. **16**:1209.
9. **Hollifield, W. C., Jr., E. H. Fiss, and J. B. Nielands.** 1978. Modification of ferric enterobactin receptor protein from the outer membrane of *Escherichia coli*. Biochem. Biophys. Res. Commun. **83**:739–746.
10. **Masui, Y., T. Mizuno, and M. Inouye.** 1984. Novel high-level expression cloning vehicles: 10^4-fold amplification of *Escherichia coli* minor protein. Bio/Technology **2**:81–85.
11. **Oliver, D. B.** 1985. Protein secretion in *Escherichia coli*. Annu. Rev. Microbiol. **39**:615–648.
12. **Oliver, D. B., and J. Beckwith.** 1982. Regulation of a membrane component required for protein secretion in *Escherichia coli*. Cell **30**:311–319.
13. **Pacaud, M.** 1982. Identification and localization of two membrane-bound esterases from *Escherichia coli*. J. Bacteriol. **149**: 6–14.
14. **Pacaud, M., and C. Richaud.** 1975. Protease II from *Escherichia coli*. Purification and characterization. J. Biol. Chem. **250**: 7771–7779.
15. **Scott, N. W., and C. R. Harwood.** 1980. Studies on the influence of the cyclic AMP system on major outer membrane proteins of *Escherichia coli* K12. FEMS Microbiol. Lett. **9**:95–98.
16. **Sugimura, K., and T. Nishihara.** 1988. Purification, characterization, and primary structure of *Escherichia coli* protease VII with specificity for paired basic residues: identity of protease VII and OmpT. J. Bacteriol. **170**:5625–5632.
17. **Swamy, K. H. S., and A. L. Goldberg.** 1982. Subcellular distribution of various proteases in *Escherichia coli*. J. Bacteriol. **149**:1027–1033.
18. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82**:1074–1078.
19. **Wolfe, P. B., C. Zwizinski, and W. Wickner.** 1983. Purification and characterization of leader peptidase from *Escherichia coli*. Methods Enzymol. **97**:40–46.
20. **Yu, G.-Q., and J.-S. Hong.** 1986. Identification and nucleotide sequence of the activator gene of the externally induced phosphoglycerate transport system of *Salmonella typhimurium*. Gene **45**:51–57.