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Periplasmic Protein Associated with the Oligopeptide Permeases of *Salmonella typhimurium* and *Escherichia coli*

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A periplasmic protein essential for the function of the oligopeptide transport system of *Salmonella typhimurium* was identified. This protein, encoded by the *oppA* gene, is one of the most abundant proteins in the periplasm and, with an apparent molecular weight of 52,000, is considerably larger than any other known periplasmic transport component. A similarly abundant periplasmic protein forms part of the oligopeptide transport system of *Escherichia coli*.

Peptides serve as an important source of nutrients for the enteric bacteria *Salmonella typhimurium* and *Escherichia coli*. Three genetically distinct transport systems are involved in the uptake of peptides by these bacteria, a specific dipeptide permease and two systems which will handle both dipeptides and oligopeptides containing up to five amino acid residues. These three systems show little specificity for the amino acid residues present in the peptide but have an absolute requirement for an α-peptide bond (14). The best characterized of these three systems is the oligopeptide permease. Four genes are required for the function of this transport system, *oppA*, *oppB*, *oppC*, and *oppD* (8). These genes are organized as a single operon, cotranscribed from a promoter located proximal to the *oppA* gene (Fig. 1). The *opp* locus is near *trp*, at 34 min on the *S. typhimurium* chromosomal map and 27 min on the *E. coli* chromosomal map, within the region of the chromosome inverted between these two species (6, 18). By analogy with other transport systems (2), the multicomponent nature of the oligopeptide permease suggested to us that a periplasmic substrate-binding protein might be required for peptide transport. This possibility was further supported by evidence that peptide transport is sensitive to osmotic shock (3) and is energized directly by ATP (15, 16), properties characteristic of binding protein-dependent transport systems. We show here that the oligopeptide permeases of *S. typhimurium* and *E. coli* do indeed require the function of an abundant periplasmic protein.

All *S. typhimurium* and *E. coli* strains were derivatives of LT2 and K-12, respectively (Table 1). The isolation and mapping of the *opp* mutations used have been described previously (6, 8). Cells were grown at 37°C with aeration in LB (11) or glucose minimal medium (17) containing amino acid supplements (200 μM) as required. Periplasmic shock fluids were prepared by a modification of the method of Nossal and Heppel (13). Cells were grown to saturation in 5 ml of the appropriate medium. A 0.5-ml amount of 0.5 M Tris-chloride (pH 7.8) was added, and the cells were allowed to stand at room temperature for 10 min. The cells were then pelleted, suspended in 0.8 ml of 40% sucrose–30 mM Tris-chloride (pH 7.8)–2 mM EDTA, and shaken at 30°C for 10 min. The cells were then centrifuged in a 1.5-ml microfuge tube, rapidly suspended in 0.5 ml of ice-cold water, and left on ice for 10 min. The cells were again centrifuged, and the supernatant shock fluid was concentrated 10-fold by lyophilization. Samples were stored at −20°C until required. Concentrated shock fluid (30 μl) was prepared for electrophoresis by mixing with 20 μl of 2× Laemmli sample buffer (10) and heating to 98°C for 2 min immediately before loading. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out on 9% gels (acrylamide to bisacrylamide ratio, 19:1), as described by Ames (1). After electrophoresis, gels were fixed in 45% methanol–5% acetic acid, stained with Coomassie blue, and destained in 5% acetic acid–5% methanol. Molecular weight standards used were carbonic anhydrase (27,000), alcohol dehydrogenase (37,000), ovalbumin (43,000), amylase (48,000), and bovine serum albumin (64,000).

To allow identification of any periplasmic proteins associated with the oligopeptide permease, the proteins present in shock fluids isolated from *S. typhimurium* strains harboring defined deletions extending into *opp* (Fig. 1) were compared with the corresponding proteins isolated from their *opp*$^+$ parent (Fig. 2). Three proteins differed between the parental strain

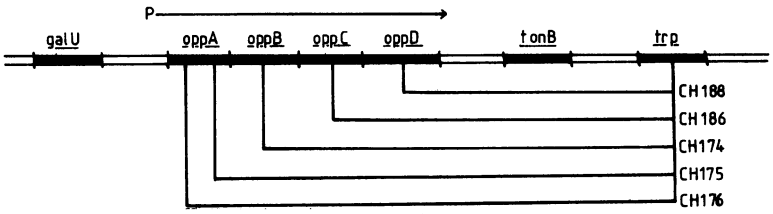

FIG. 1. Genetic map of the *opp* locus. The thick horizontal line represents the chromosome. The order of the *opp* and adjacent genes is shown. The arrow above the chromosome indicates the direction of transcription of *opp* from promoter P. The horizontal lines below the chromosome show the extent of deletions carried by the strains used in this study. All deletions originate from the Tn*10* insertion in *trp* carried by the parent CH56 (Table 1 and references 6 and 8).

(CH56) and the *opp* deletions derived from it. One of these proteins (labeled *oppA*) was present in periplasmic shock fluids from the parent (CH56) and from strains deleted for *oppB*, *oppC*, and *oppD*, (CH188, CH186, and CH174) but was consistently absent from strains harboring deletions extending into *oppA* (CH175 and CH176).

To confirm that this protein was indeed associated with the *oppA* gene, we examined the shock fluids of strains harboring point mutations in *opp*. Strains with point mutations, polar or nonpolar, in *oppB*, *oppC*, or *oppD* had no effect on the presumed *oppA* protein (data not shown). However, mutations in *oppA* did affect the presumed *oppA* protein in one of several ways (Fig. 3). All strains carrying polar mutations in *oppA*, for example, CH164, completely lacked the *oppA* protein. Many nonpolar mutations, on the other hand, had no obvious effect on the *oppA* protein (e.g., CH161). However, a number of nonpolar *oppA* mutations which still retained partial transport activity (strains CH34, CH138, CH163 [6]) also retained the *oppA* protein, but its mobility on polyacrylamide gels was altered. In the case of strains CH163 and CH138, the apparent molecular weight of the protein was increased by 2,000 to 3,000, whereas in the case of CH34, the apparent molecular weight was decreased by about 1,000. Such large changes in apparent molecular weight on sodium dodecyl sulfate gels caused by a single amino acid change have been noted previously (12). The altered mobility of the 52,000-dalton protein in strains carrying point mutations within the *oppA* gene confirms that *opp* is indeed the structural gene for this periplasmic protein.

In addition to the 52,000-dalton *oppA* protein, certain other proteins present in shock fluids were found to vary among strains. One of these proteins, labeled c in Fig. 3, was not strain specific and was shown to be an outer membrane protein which sometimes contaminated

TABLE 1. Bacterial strains

Strain	Genotype[a]	Origin or reference
S. typhimurium		
CH56	*pro-594 trp-1012*::Tn*10*	6, 8
CH188	*pro-594 Δopp (trp-tonB-oppD)300*	6, 8
CH186	*pro-594 Δopp (trp-tonB-oppCD)298*	6, 8
CH174	*pro-594 Δopp (trp-tonB-oppBCD)286*	6, 8
CH175	*pro-594 Δopp (trp-tonB-oppABCD)287*	6, 8
CH176	*pro-594 Δopp (trp-tonB-oppABCD)288*	6, 8
CH2	*pro-594*	G. F.-L. Ames
CH161	*pro-594 oppA273*	6, 8
CH163	*pro-594 oppA275*	6, 8
CH164	*pro-594 oppA276*	6, 8
CH138	*pro662*::Tn*10 oppA266*	6, 8
CH34	*oppA240*	6, 8
E. coli		
HB101	*hsdS20* (r_B^- m_B^-) *recA13 ara-14 proA2 lacY1 galK2 rpsL20* (Sm^r) *xyl-5 mtl-1 supE44*	D. M. J. Lilley
CH212	*hsdS20* (r_B^- m_B^-) *recA13 ara-14 proA2 lacY1 galK2 rpsL20* (Sm^r) *xyl-5 mtl-1 supE44 oppA462*	This study

[a] See Fig. 1 for genetic loci and extents of deletions.

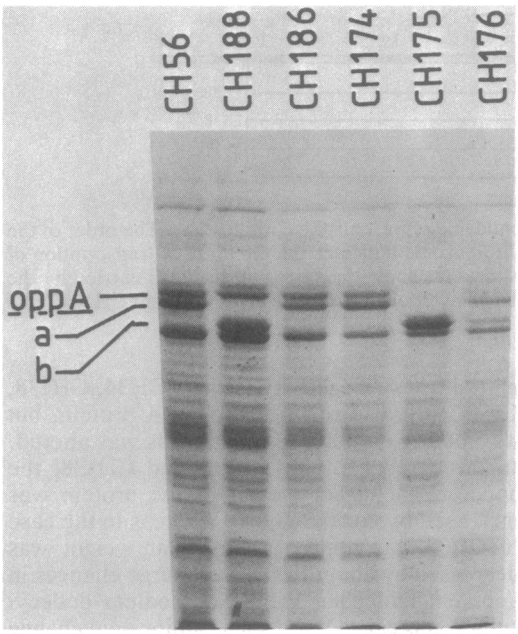

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of periplasmic proteins from strains carrying *trp-tonB-opp* deletions. Strain CH56 is the parent from which all the other strains carrying *opp* deletions were derived (Table 1). Protein bands a and b are the two flagellar antigens H1 and H2 (see text).

shock fluid preparations (data not shown). The other two proteins (labeled a and b in Fig. 2 and 3) are of molecular weights 47,000 and 42,000, respectively, and were identified as the two antigenic variants of flagellin (19), being absent in flagellin-deficient mutants (data not shown). The relative amounts of these two proteins varied from strain to strain but showed no obvious relationship to the genotypes of the strains. Single colonies purified from any of the strains used produced only one of the two proteins, but after growth for a number of generations, an intermediate amount of each protein was produced. This is due to the high frequency of antigenic phase variation (19).

The oligopeptide permeases of *E. coli* and *S. typhimurium* are very similar, and indeed, components from the two species are interchangeable (8). To determine whether or not the *E.coli* system also involved a similar periplasmic protein, we isolated a number of spontaneous *opp* mutations in strain HB101 by resistance to the toxic peptide triornithine (6). One of these mutations, *opp-462*, was mapped to the *oppA* gene by complementation (8). Figure 4 shows a comparison of the periplasmic proteins from this *E. coli oppA* mutant with its *opp*$^+$ parent. Clearly, a single protein is absent from the *opp* strain. This protein, like the *oppA* protein of *S. typhimurium*, has a molecular weight of 52,000. Thus, both species have very similar *oppA* proteins. The abundance of the *oppA* protein in *E. coli* is even more apparent than in *S. typhimurium*. Even under conditions in which other periplasmic proteins are specifically induced (e.g., the maltose-, arabinose-, or galactose-binding proteins), these proteins do not attain the level of the *oppA* protein in the periplasm (data not shown). Interestingly, regardless of the medium in which cells were grown, the amount of *oppA* protein present in shock fluids showed no significant variation (Fig. 5). Although the overall protein composition of the periplasm varied considerably, the amount of *oppA* protein was unaffected by the carbon or nitrogen source or the presence or absence of peptides and/or amino acids (data not shown).

Thus, we have identified a periplasmic protein of molecular weight 52,000 as a component of the oligopeptide permease of *S. typhimurium*. This protein is absent from strains deleted for part or all of the *oppA* gene but is unaffected by deletions of the *oppB*, *oppC*, or *oppD* genes. The altered electrophoretic mobility of this protein in strains harboring certain point mutations in the *oppA* gene confirms that *oppA* is indeed the structural gene for this protein rather than coding for a positive regulatory signal. A peri-

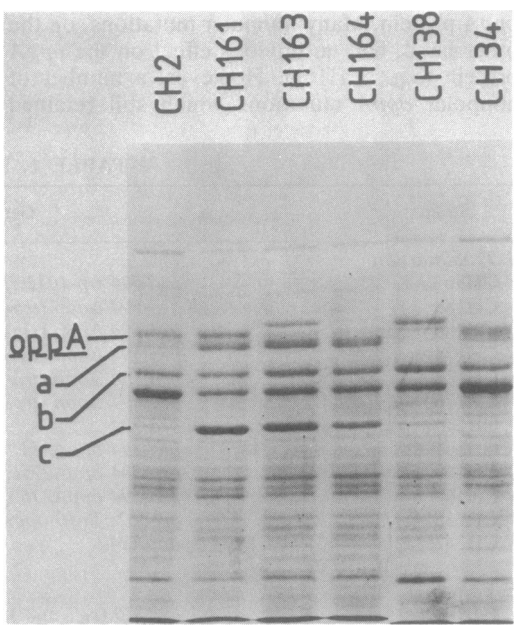

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of periplasmic proteins from strains with point mutations in *oppA*. CH2 is the *opp*$^+$ parent from which the other strains were derived. The position of the *oppA* protein is indicated. The identities of bands a, b, and c are discussed in the text.

plasmic protein of similar molecular weight is encoded by the *oppA* gene of *E. coli*. Although no direct evidence has yet been obtained, by analogy with other well-characterized systems (2, 5, 7), it seems probable that the *oppA* protein functions as the initial substrate-binding component during transport. Evidence that mutations in *oppA* can alter the specificity of peptide transport (6) supports this view.

Two properties of the *oppA* protein are noteworthy: its relatively high molecular weight and its abundance. Approximately a dozen periplasmic substrate-binding proteins have been identified to date. All these proteins have molecular weights of between 26,000 (histidine-binding protein [4]) and 38,500 (maltose-binding protein [9]). The *oppA* protein is thus considerably larger than all previously identified periplasmic binding proteins and indeed is one of the largest proteins in the periplasm. In *S. typhimurium* and

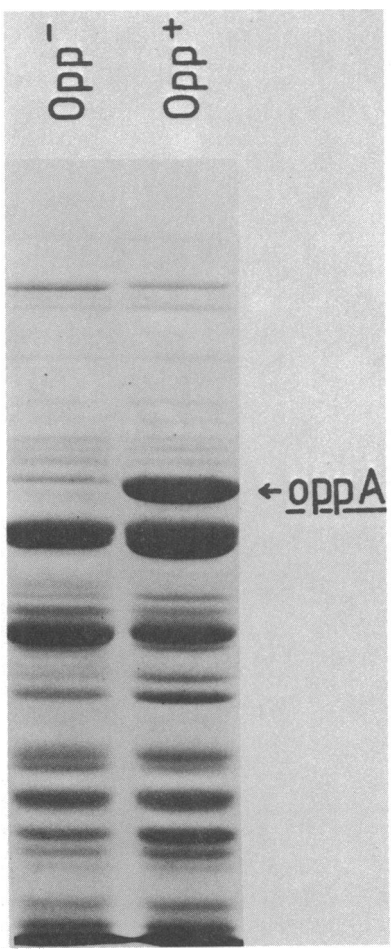

FIG. 4. Periplasmic proteins from *E. coli*. The *opp*$^+$ strain is HB101, and its *oppA* derivative is CH212 (Table 1).

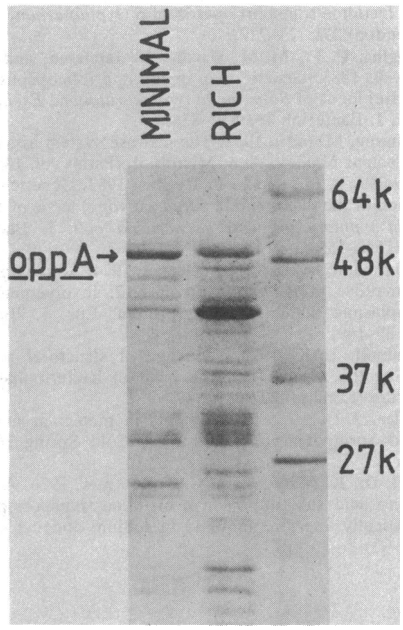

FIG. 5. Periplasmic proteins from *S. typhimurium* LT2 grown in rich (LB) or minimal glucose medium. The *oppA* protein is shown. Molecular weight standards are described in the text.

E. coli the *oppA* protein is one of the most abundant periplasmic proteins. The high levels of this protein in the periplasm are consistent with evidence that the peptide transport system has a relatively high V_{max} compared with most periplasmic transport systems (J. W. Payne, personal communication). The *oppA* protein is also constitutively expressed regardless of the medium in which the cells are grown. These features of the peptide transport system, its high capacity and constitutive expression, are presumably an adaption to the high and relatively constant amounts of peptide found in the mammalian gut, the organism's natural environment.

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