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Peptide transport and chemotaxis in *Escherichia coli* and *Salmonella typhimurium*: characterization of the dipeptide permease (Dpp) and the dipeptide-binding protein

W. N. Abouhamad,[1] M. Manson, [1] M. M. Gibson[2] and C. F. Higgins[2*]

[1]*Department of Biology, Texas A & M University, College Station, Texas 77843, USA.*

[2]*Imperial Cancer Research Fund, University of Oxford, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK.*

Summary

The dipeptide permease (Dpp) is one of three genetically distinct peptide-transport systems in enteric bacteria. Dpp also plays a role in chemotaxis towards peptides. We have devised three selections for *dpp* mutations based on resistance to toxic peptides (bacilysin, valine-containing peptides, and bialaphos). All *dpp* mutations mapped to a single chromosomal locus between 77 and 78 min in *Salmonella typhimurium* and at 79.2 min in *Escherichia coli*. Expression of *dpp* was constitutive in both species but the absolute level of expression varied widely between strains. At least in part this difference in expression levels is determined by *cis*-acting sequences. The *dpp* locus of *E. coli* was cloned. The first gene in the operon, *dppA*, encodes a periplasmic dipeptide-binding protein (DBP) required for dipeptide transport and chemotaxis. Downstream of *dppA* are other genes required for transport but not for chemotaxis. The dipeptide-binding protein was found to share 26.5% sequence identity with the periplasmic oligopeptide-binding protein OppA.

Introduction

Peptides can serve as important sources of nutrients for most species of bacteria, fungi, plants and animals (Matthews, 1975; Payne, 1980; Higgins and Payne, 1980). Small peptides also serve many specific biological functions as hormones, toxins or antibiotics, and synthetic peptides are increasingly being used in the design of novel antibiotics and chemotherapeutic agents (Higgins, 1987). Peptides are particularly suited to such uses because of the unique properties of peptide uptake systems, which exhibit broad substrate specificity with little regard for the nature of the amino acids which comprise the peptide. Thus, normally impermeant toxic moieties can be attached to a peptide backbone and thereby gain access to cytoplasmic targets (Ames *et al.*, 1973; Fickel and Gilvarg, 1973).

The enteric bacteria *Escherichia coli* and *Salmonella typhimurium* possess three distinct peptide-transport systems with overlapping substrate specificities (Gibson *et al.*, 1984; Higgins and Gibson, 1986). The oligopeptide permease (Opp) transports essentially any peptide containing up to five amino acid residues, more or less irrespective of its amino acid composition (Payne and Gilvarg, 1968). Opp is a periplasmic binding protein-dependent transport system encoded by an operon of five genes which have been cloned and sequenced (Hiles and Higgins, 1986; Hiles *et al.*, 1987). In addition to taking up peptides as a source of nutrients, Opp is also essential for the recycling of cell-wall peptides (Goodell and Higgins, 1987). Very similar oligopeptide transport systems are found in the Gram-positive species *Streptoccocus pneumoniae* (Alloing *et al.*, 1990) and *Bacillus subtilis* (Perego *et al.*, 1991). In *B. subtilis*, uptake of peptides via Opp plays an important role in signalling the onset of sporulation (Perego *et al.*, 1991). The tripeptide permease (Tpp) has a more restricted substrate specificity, having highest affinity for hydrophobic tripeptides although it will handle dipeptides to some extent (Gibson *et al.*, 1984). Expression of *tpp* is induced by anaerobic growth and is positively regulated by the gene products of *ompR* and *envZ* (Jamieson and Higgins, 1984; Gibson *et al.*, 1987). The physiological role of Tpp is not understood.

The third peptide-uptake system is the dipeptide permease (Dpp). Like Opp, Dpp is a periplasmic binding protein-dependent transport system with greatest affinity for dipeptides (Manson *et al.*, 1986). In addition, the dipeptide-binding protein (DBP) is required for peptide chemotaxis (Manson *et al.*, 1986). Peptide taxis also involves the Tap chemotactic signal transducer, with which DBP is assumed to interact (Manson *et al.*, 1986). Dipeptides are the only attractant for which Tap is known to be required.

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To investigate the dual role of the dipeptide transport system in transport and chemotaxis, we have undertaken a genetic characterization of *dpp*. The isolation of *dpp* mutants has proved to be difficult in the past because dipeptides can also enter the cell through Opp and Tpp. In this paper we describe several methods for isolating and analysing *dpp* mutations in both *S. typhimurium* and *E. coli*. All *dpp* mutations map to a single locus on the chromosomes of *E. coli* and *S. typhimurium*. The *E. coli dpp* locus has been cloned, and the gene encoding DBP has been sequenced; the locus contains at least one additional gene. Cells lacking Opp, Tpp and Dpp show no residual peptide uptake, demonstrating that these three transport systems provide the only normal routes for peptide uptake and utilization.

Results

Isolation of dpp *mutations in* S. typhimurium

Dipeptides can enter *S. typhimurium* via three distinct transport systems with overlapping substrate specificities: these are Opp, Tpp and Dpp. In order to select *dpp* mutations it was necessary to use cells deficient in both Opp and Tpp. We have devised three different selections, using toxic peptides, to isolate mutants deficient in dipeptide transport and chemotaxis.

Bacilysin. Bacilysin is a toxic dipeptide produced by certain species of *Bacillus*. After uptake into the cell, bacilysin is cleaved by peptidases to yield alanine and anticapsin which inhibits glucosamine synthesis and results in cell lysis (Kenig and Abraham, 1976; Kenig *et al.*, 1976). Preliminary experiments showed that *opp tpp* strains remain sensitive to bacilysin and that resistant mutants arise spontaneously at a frequency of about 10^{-5}, suggesting that a single genetic lesion could give resistance. Since resistant mutants did not arise in an *opp*$^+$ *tpp*$^+$ strain, and since many peptidases can cleave dipeptides (Miller, 1975), it seemed probable that these mutants were defective in uptake of the toxic dipeptide. We therefore selected bacilysin-resistant mutants from a collection of random Tn*5* kanamycin-resistant (Kan^R) insertions in strain CH356 (*opp tpp*). Ten independently isolated, resistant mutants were purified and studied further.

To show that each derivative contained just a single transposon insertion and that this insertion was responsible for conferring bacilysin resistance, P22 lysates prepared on each strain were used to transduce strain CH356 (*opp tpp*) to Kan^R. The transductants were then screened for bacilysin resistance. Of 20 Kan^R transductants screened from each cross, more than 90% simultaneously became bacilysin resistant. The remaining bacilysin-sensitive transductants presumably resulted from transposition of Tn*5* during transduction; transposition of this element is known to occur at about this frequency upon introduction into a virgin background. One of the Tn*5* insertions in CH356 conferring bacilysin resistance (designated *dpp-101*:Tn*5*; strain CH725) was studied further. Each of the other insertions was later shown to be at the same chromosomal locus.

To demonstrate that the Tn*5* insertion in strain CH725 resulted in a deficiency in peptide transport, the Tn*5* was transduced into various amino acid auxotrophs, selecting for Kan^R, and the ability to utilize peptides as the sole source of a required amino acid was tested. Table 1 shows that, when introduced into an *opp tpp* strain, the putative *dpp*::Tn*5* insertion completely abolished the ability of amino acid auxotrophs to utilize a number of peptides. Because cells contain a battery of peptidases that hydrolyse dipeptides (Miller, 1975), the inability to utilize peptides is unlikely to arise from a peptidase mutation. Furthermore, the ability to utilize these peptides was restored when a wild-type *opp* locus was introduced (data not shown), showing that resistance is the result of a transport defect. To confirm this conclusion, peptide uptake was measured by the fluorescamine procedure (Nisbet and Payne, 1979; Payne and Bell, 1979; Higgins and Gibson, 1986). Figure 1 shows that the Tn*5* insertion in strain CH725 abolishes uptake of glycyl-L-isoleucine. Thus the gene containing the insertion can be unambiguously assigned to the *dpp* locus. Furthermore, strain CH725 failed to transport any dipeptide tested (Ala-Gly, Val-Val, Gly-Met, Asp-Ala, Lys-Lys, Ala-Glu, Lys-Ala,

Table 1. Utilization of dipeptides as sources of required amino acids for *S. typhimurium* auxotrophic mutants.

	Growth Zone (cm)					
Strains	Leu–Val	Leu–Gly	Pro–Val	Pro–Gly	Gly–His	His–Ala
CH749 (*pro leu dpp*$^+$)	2.5	2.5	2.5	2.5	ND	ND
CH1830 (*pro leu dpp*$^-$)	0	0	0	0	ND	ND
CH751 (*his leu dpp*$^+$)	2.5	2.5	ND	ND	2.5	2.5
CH1831 (*his leu dpp*$^-$)	0	0	ND	ND	0	0

Cells (2×10^8) were spread onto VBCG-agar plates. A filter disc, impregnated with the indicated peptides as the sole source of one of the required amino acids, was placed in the centre of the plate. (The other required amino acid was provided in the agar.) The zones of growth around the discs were measured after 24 h incubation at 37°C. ND = not determined. All strains were *opp tpp*.

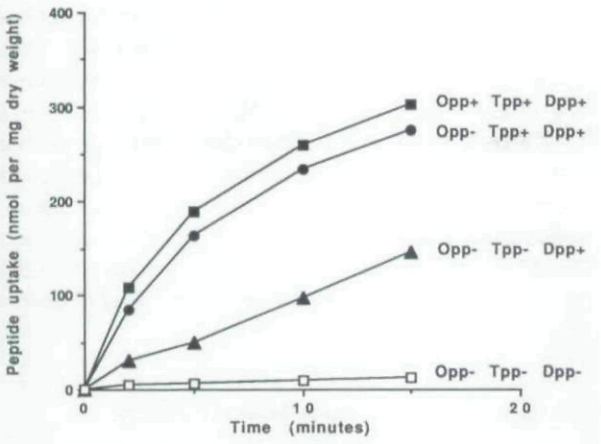

Fig. 1. Uptake of glycyl-L-leucine by various peptide transport-deficient mutants of *S. typhimurium*. Glycyl-L-isoleucine was at 100 μM, a saturating concentration, and uptake was measured using the fluorescamine procecure as described previously (Higgins and Gibson, 1986). No detectable uptake was seen with strain CH725 over extended periods; an uptake rate as low as 0.2 nmol min^{-1} would have been detected. ■, LT2 (*opp*$^+$ *tpp*$^+$ *dpp*$^+$ parent); ●, CH44 (*opp*$^-$); ▲, CH356 (*opp*$^-$ *tpp*$^-$); ❑, CH725 (*opp*$^-$ *tpp*$^-$ *dpp*$^-$).

Lys-Leu, Met-Met, Tyr-Tyr, Ala-Tyr, Arg-Asn, Ala-Asp), although amino acid transport was normal (data not shown). Thus, Opp, Tpp and Dpp appear to provide the only general routes for peptide transport into *S. typhimurium*.

The role of Opp and Tpp in bacilysin uptake was also examined. An *opp*$^+$ *tpp dpp* strain was found to be bacilysin resistant, while an *opp tpp*$^+$ *dpp* strain remained sensitive. Thus, bacilysin enters the cell via Tpp and Dpp but not to a significant extent via Opp.

Valine-containing peptides. *E. coli* K-12 strains are sensitive to valine because of a deficiency in acetohydroxy acid synthetase (AHAS II), which is encoded by *ilvG*. Valine inhibits the other two AHAS isoenzymes (AHAS I and III), resulting in a requirement for isoleucine or methionine and hence inhibits growth on minimal medium unless isoleucine or methionine are supplied (Berg and Shaw, 1980). In contrast, *S. typhimurium* has a functional *ilvG* gene, although it becomes valine-sensitive if an *ilvG* mutation is introduced (Primerano and Burns, 1982). We reasoned that valine peptides would be toxic to an *ilvG* mutant of *S. typhimurium* and could be used to select peptide transport-deficient mutants. Preliminary experiments showed that growth of *S. typhimurium* strain CH407 (*opp tpp ilvG*) was inhibited by divaline or glycyl-valine on minimal-glucose agar and that this sensitivity was overcome by the addition of isoleucine. Mutants resistant to 4 μM divaline arose at a frequency of about 10^{-6}. However, when purified and screened, all were resistant to valine as well as to divaline. Thus, resistance could not be due to a simple peptide transport defect but was presumably due to an altered sensitivity of one of the AHAS isoenzymes to valine. It seemed likely that this failure to obtain *dpp* mutants might be due to the release of free valine from cells following the uptake and intracellular cleavage of divaline. Any *dpp* mutants would still take up and be killed by this released valine. (It is well known that amino acid cleavage products can exit the cell following peptide uptake; Payne and Bell, 1977; 1979.)

In an attempt to overcome this problem we added excess leucine (0.4 μM) to the selection medium to inhibit, competitively, the uptake of valine since valine and leucine enter the cell by means of a common transport system (Quay *et al.*, 1977). Twenty spontaneous divaline-resistant mutants were selected in the presence of leucine, and eight of these remained sensitive to valine. These eight mutants (e.g. CH450) were also resistant to bacilysin and failed to take up any of the dipeptides tested (data not shown). Finally, as described below, each of these valine-resistant mutations mapped to the same chromosomal location as all other *dpp* mutations.

Bialaphos resistance. Bialaphos is a peptide antibiotic produced by *Streptomyces hygroscopicus* (Murakami *et al.*, 1986). It was found that *opp* mutants and *opp tpp* double mutants were partially resistant to bialaphos, but that an *opp*$^+$ *tpp*$^-$ strain was still fully sensitive (Table 2). Complete resistance was conferred by the introduction of a *dpp* mutation. Thus bialaphos must enter the cell through both Opp and Dpp, but not via Tpp. At very high concentrations (10 mg ml^{-1}) *opp tpp dpp* strains were

Table 2. Bialophos sensitivity of peptide transport mutants of *S. typhimurium*.

Strains	Functional transport systems			Zone of killing (cm)
A.				
LT2	Opp	Tpp	Dpp	1.6
CH44	—	Tpp	Dpp	0.3
CH356	—	—	Dpp	0.3
CH725	—	—	—	0.0
B.				
CH1432	—	Tpp	Dpp	1.0
CH1279	Opp	Tpp	Dpp	2.2
CH1104	—	—	Dpp	1.0
CH1105	—	Tpp	—	0.0
CH1110	—	—	—	0.0
CH1417	Opp	—	Dpp	2.2
CH1421	Opp	—	—	2.2

The zone of inhibition around a filter disc containing 10 μl of a 1 mg ml^{-1} solution of bialaphos was measured after 24 h incubation. Strains in set A are isogenic derivatives of strain LT2. Set B are isogenic derivatives of strain LT2 Mot$^+$, a separately maintained stock that, unlike our standard laboratory stock of LT2, retained good motility.

killed by bialaphos, implying that there is another means of entry (possibly non-specific permeation of the cell membrane). Interestingly, a motile derivative (used for chemotactic studies) of our standard LT2 strain (CH1279) showed greater sensitivity to bialaphos than did LT2 (Table 2), suggesting that the dipeptide permease is more active in this strain. Furthermore, the levels of periplasmic dipeptide binding protein were also higher in the motile strain (data not shown). This difference is the result of a mutation at the *dpp* locus of the motile strain that causes increased transcription of *dpp* (see below).

We exploited bialaphos resistance (Bap^R) to select *dpp–lacZ* fusions in *S. typhimurium*. A collection of random Mu*d*1-8 insertions were constructed in strain CH356 (see the *Experimental procedures*), and cells plated on minimal-glucose agar containing 1mg ml^{-1} bialaphos and X-gal. Two independent, Lac^+ Bap^R derivatives were purified and characterized (designated CH1191 and CH1194). The Mu*d*1-8 insertion from each was transduced back into strain CH356, selecting for ampicillin resistance (Amp^R): all Amp^R transductants were Bap^R, demonstrating that a single Mu *d*1-8 insertion was responsible for the resistant phenotype. The insertion also conferred bacilysin resistance and loss of the ability to take up a number of peptides (data not shown). Finally, the Mu*d*1-8 insertions were 95% cotransducible with the *dpp*-101::Tn*5* insertion of strain CH725. Thus it is clear that these two mutants carry Mu*d*1-8-mediated *dpp-lacZ* fusions.

Regulation of dpp *expression in* S. typhimurium

To ascertain whether expression of *dpp* is regulated, we examined *lacZ* expression in strains carrying *dpp–lacZ* fusions grown in different media. Expression of *lacZ* from both fusions was unaffected by carbon source (glycerol or glucose), or by nitrogen excess or limitation (data not shown). The addition of peptides or casamino acids to minimal medium also had no effect on expression. Furthermore, expression was unaffected by the introduction of *opp* or *tpp* mutations. Finally, because Tpp is anaerobically induced (Jamieson and Higgins, 1984), we also examined the effect of anaerobic growth on *dpp–lacZ* expression: again, no significant effect was observed. Thus, *dpp* expression appears to be constitutive in *S. typhimurium*.

As described above, a motile derivative of *S. typhimurium* strain LT2 (CH1279) appeared to have increased Dpp activity relative to our standard LT2 isolate. To determine whether this was the result of increased *dpp* transcription, the *dpp*::Mu *d*1-8 insertion from strain CH1194 was transduced with phage P22 into CH1279 selecting for Amp^R. Of 50 transductants, 45 were dark red on MacConkey-lactose plates, while 5 were pink. Two red and two pink colonies were purified, grown in MMA glucose media, and the cultures assayed for β-galactosidase activity. Cells from red colonies expressed 520 units of β-galactosidase (Miller units; Miller, 1972) while cells from the pink colonies expressed only 53 units. Marker rescue experiments showed that in each transductant the insertion remained in the *dpp* locus. Thus it appears that the increased level of *dpp* expression CH1279 is caused by a genetic lesion closely linked to *dpp*. It is not known whether a high or low level of expression is 'normal'.

Map location of the S. typhimurium dpp *locus*

The *dpp*-101::Tn*5* insertion from strain CH725 was transduced into the Hfr strains SA486, SA534 and SA722 (Sanderson and Roth, 1983), selecting for Kan^R. It was shown by marker rescue that the Tn*5* insertion had not transposed in the transductant chosen for each strain. These Kan^R donors were mated for 40 min with the streptomycin-resistant (Str^R) strain CH330 and the number of Kan^R Str^R transconjugants determined. Only strain SA722 transferred *dpp*-101::Tn*5* with high efficiency, indicating a map location close to the origin of transfer at 83 min. To determine the location more accurately, interrupted matings with this Hfr derivative were performed, selecting for Kan^R transconjugants. Extrapolation of the data (not shown) suggested a map location at 77–80 min.

The ability to cotransduce *dpp*-101::Tn*5* with markers in this region of the chromosome was also tested. Using a phage P22 lysate grown on strain CH725, no Tet^S transductants were found out of 100 Kan^R transductants with recipient strains containing Tn*10* insertions in *spoT*, *cysE*, *pyrE* or *gltC*. However, we obtained 5% linkage with *xyl*. As no cotransduction with *mtl* was found, *dpp* must be located counterclockwise of *xyl*. Thus in *S. typhimurium* *dpp* is located between 77 and 78 min on the chromosome. A similar map location was found for the *dpp* locus of *E. coli* (see below). Finally, all *dpp*::Tn*5*, *dpp*::Mu *d1-8* and *dpp* point mutations, regardless of the selection employed to isolate them, were found to map at 77–78 min.

*Mini-Tn*10 *insertions at the* E. coli dpp *locus*

To characterize the *E. coli dpp* locus, six independently isolated Bap^R mutants were isolated in strains MM500 and MC4100 using the Tn*10Δ16Δ17* transposon (Tet^R, mTn*10*; Way *et al.*, 1984 — see the *Experimental procedures*). MM500 and MC4100 are opp^- (Manson *et al.* 1986) and hence Bap^R requires just a single mutation in *dpp*. It should be noted that MM500 and MC4100 appeared equally sensitive to bialaphos, although MC4100 has substantially reduced levels of DBP and of

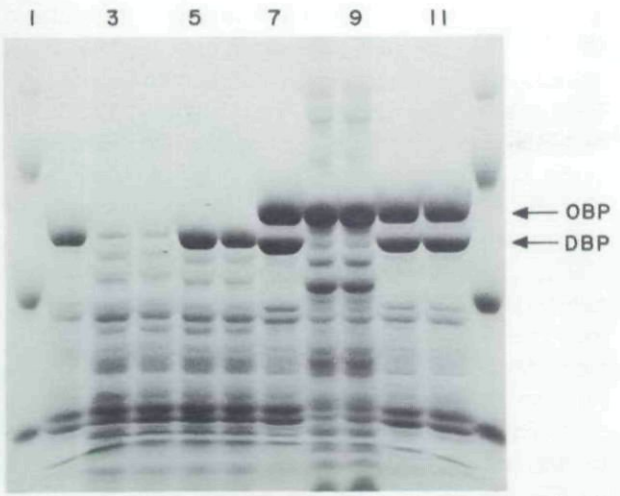

Fig. 2. SDS–PAGE analysis of periplasmic proteins from *dpp*::mTn10 insertion mutants of *E. coli*. Strains were grown overnight at 37°C in MMA-glycerol medium supplemented with 0.2% casamino acids, and periplasmic proteins were isolated from the cells by osmotic shock. Samples of crude shock fluid were adjusted to contain equal amounts of total protein before loading the gel. Lanes 1 and 12, molecular mass standards; lane 2, MM500 (*dpp*$^+$ *opp*$^-$); lane 3, MM1105, lane 4, MM1114; lane 5, MM1107; lane 6, MM1108; lane 7, MM564 (*dpp*$^+$ *opp*$^+$); lanes 8–11, strain MM564 containing the same dpp::mTn*10* insertions in the same order as the strains in lanes 3–6. To avoid cluttering, only odd lanes are numbered. The positions of the oligopeptide-binding protein (OBP) and the dipeptide-binding protein (DBP) are indicated by arrows. Molecular mass markers are (from the bottom) 31 kDa, 45 kDa, 66.2 kDa and 97.4 kDa.

glycyl-glycine transport activity relative to MM500 (Manson *et al.*, 1986).

To show that the Bap^R mutations were indeed in *dpp*, Dpp activity was assessed by growth on dipeptides as sole source of a required amino acid. L-prolyl-L-leucine (Pro–Leu) is a good attractant for *E. coli* (Manson *et al.*, 1986) and is therefore presumed to be a good substrate for Dpp. Phage P1$_{vir}$ lysates grown on four strains with independent, putative *dpp*::mTn*10* insertions were used to transduce a *proC*::Tn*5* (Kan^R) derivative of strain MM500 to Tet^R. Pro–Leu was supplied to these transductants as sole source of the required amino acids. While the parental strain grew on minimal-glucose agar supplemented with 10 μg ml^{-1} Pro–Leu, none of the 100 transductants from each of the crosses could utilize this peptide.

Production of DBP in dpp *mutants*

We previously reported (Manson *et al.*, 1986) that the *E. coli dpp* locus codes for a 49 kDa periplasmic protein, which was proposed to be a dipeptide-binding protein (DBP). This protein has now been purified, and fluorescence quenching has been used to show that it specifically binds dipeptides containing L-amino acids or glycine (Blank, 1987). A re-estimated relative mobility of 55 kDa obtained by sodium dodecyl sulphate/polyacrylamide gel electrophoresis (SDS–PAGE) and comparison with the 58 kDa oligopeptide-binding protein (Hiles and Higgins, 1986; Hiles *et al.*, 1987) and other markers of known molecular weight corresponds well with the value predicted from the nucleotide sequence of the structural gene for DBP (see below). Of 24 spontaneous *E. coli dpp* mutations isolated (presumed point mutations) by bacilysin resistance, 20 still produced DBP (Manson *et al.*, 1986). This result suggested that the *dpp* locus consists of more than one gene. However, these could have been missense mutations in the DBP gene that generated inactive, but normal-sized, polypeptides. To resolve this point, we examined periplasmic proteins from 12 independently isolated *E. coli* mutants with *dpp*::mTn*10* insertions. Four of the insertions generated in strain MM500 and four generated in strain MC4100 still produced normal amounts of DBP, demonstrating that the *dpp* locus consists of at least one gene in addition to that which codes for DBP (Fig. 2). DBP and the oligopeptide-binding protein (OppA) were the most abundant periplasmic proteins in cells grown under these conditions. Other experiments (data not shown) demonstrated that these two proteins are equally abundant in cells grown in minimal-glycerol medium, minimal-glucose medium, either of these media supplemented with 0.2% casamino acids and/or 20 μM L-prolyl-L-leucine, or in cells grown in rich medium (L-broth). Thus, as in *S. typhimurium*, in *E. coli* we have no evidence that *opp* or *dpp* expression are regulated by the composition of the growth medium.

Dependence of dipeptide chemotaxis on DBP

Our earlier work (Manson *et al.*, 1986) indicated that DBP is required for dipeptide taxis. However, since we did not know the nature of the *dpp* mutations in those strains, we could not determine whether dipeptide transport activity was necessary for taxis. This question was answered using *dpp*::mTn*10* mutants that lacked significant Dpp activity but retained normal amounts of DBP. In capillary assays of chemotaxis (Fig. 3) peak accumulation of cells occurred at 1 mM Pro–Leu (26 000 cells) and Pro–Gly (40 000 cells) with strain MM500. These values are 13 and 20% of the peak accumulations seen with 1 mM L-aspartate. Strain MM1105, which contains a *dppA*::Tn*10* insertion that eliminates DBP, gave no response to either Pro–Leu or Pro–Gly. In contrast, peak accumulations were at 100 μM Pro-Leu (11 000 and 8000 cells) for strains MM1107 and MM1108, which contain *dpp*::mTn*10* insertions but make normal amounts of DBP. Thus, apart from the gene encoding DBP, genes in the *dpp* locus which are required for peptide transport are not required

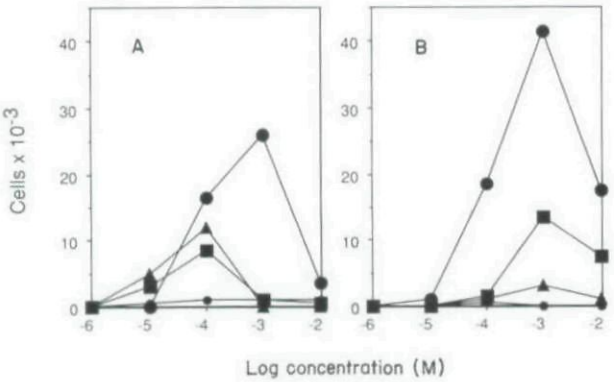

Fig. 3. Capillary assays of dipeptide chemotaxis. The results shown are the mean values of the number of cells accumulating in duplicate capillaries containing: (A) L-prolyl-L-leucine, and (B) L-prolyl-glycine at the indicated concentrations. A background value of the mean number of cells accumulating in capillaries containing buffer only was subtracted from the values shown. ●, MM500; ●, MM1105 (dppA473::mTn*10*); ▲, MM1107 (*dpp*-474::mTn*10*); ■, MM1108 (*dpp*-475::mTn*10*).

for peptide chemotaxis. Thus, dipeptide transport and chemotaxis are independent processes, as has been seen with other binding protein-dependent systems. However, unexpectedly, *dpp*::mTn*10* insertions that did not alter DBP levels in the periplasm still reduced the chemotactic response. This is probably due to the accumulation of peptides, generated by proteolytic activity, in the periplasm of mutants blocked in peptide transport. These peptides would then interfere with chemotaxis. The reintroduction by transduction of a functional *opp* locus into these strains did not substantially enhance their responses to dipeptides (data not shown), suggesting that Dpp is better than Opp at clearing endogenously produced dipeptides from the periplasm.

Map location of the E. coli dpp *locus*

The *dppA473*::mTn*10* insertion from strain MM1105 (a derivative of strain MM500) was transduced with phage $P1_{vir}$ into 17 Hfr strains (obtained from the *E. coli* Genetic Stock Center, Yale University). These transductants were used as conjugation donors into the recipient, MM500 (Str^R), which has multiple auxotrophies and catabolic defects. Tet^R Str^R transconjugants were selected in uninterrupted matings and screened for donor markers. With most Hfr donors, more than 95% of the transconjugants became *xyl*$^+$. However, with a few of the Hfr donors all the transconjugants remained *xyl*. Since all the Hfr donors were originally *xyl*$^+$, the *xyl* mutation must have been introduced by cotransduction together with the *dppA473*::mTn*10*. Furthermore, 21 out of 50 *dppA-473*::mTn*10* transductants of Hfr strain PK3 (Kahn, 1968) had lost the Hfr origin. Thus the *dppA473*::Tn*10* insertion must be closely linked to *xyl* and the origin of transfer in HFr PK3. To determine the relative order of these three markers, Tet^R transductants of PK3 that remained Hfr were mated with MM500. This mating yielded *xyl*$^+$ transconjugants at high frequency and Tet^R transconjugants at a very low frequency. Thus the Hfr origin (PO131) of PK3 must lie between the *dpp* locus and *xyl* and transfer *xyl* early and *dpp* late.

$P1_{vir}$ cotransduction linkages were determined between the *xyl* and *mtl* loci and *dpp*. Lysates grown on five independently isolated *dpp*::mTn*10* insertions in strain MM500 (*xyl mtl*) were transduced into MC4100 (*xyl*$^+$ *mtl*$^+$). Out of about 50 Tet^R transductants from each cross, cotransduction frequencies with *xyl* ranged from 34 to 56% (118/269 total colonies, or 44% overall). Cotransduction frequencies with *mtl* ranged from 0 to 14% (18/269 total colonies, or 7% overall). Similarly, $P1_{vir}$ lysates grown on six independent *dpp*:::Tn*10* insertions in MC4100 were used to transduce strain MM500 to Tet^R. Cotransduction frequencies between *dpp* and *xyl* ranged from 46 to 65% (220/397 total colonies, or 55% overall) and from 10 to 19% for *dpp* and *mtl* (48/397 total transductants, or 12% overall). Since the majority of transductants from all crosses that received the donor *mtl* marker also received the donor *xyl* marker, *dpp* cannot lie between *xyl* and *mtl*. These results indicate that all 11 independently isolated *dpp*:::Tn*10* insertions examined are closely linked to each other and lie counterclockwise from *xyl*. Based on a mean cotransduction frequency of 50% between *dpp* and *xyl*, *dpp* is located 0.5 min from *xyl* at 79.2 min on the *E. coli* chromosome. Thus the location of *dpp* with respect to adjacent genes is the same in *E. coli* as it is in *S. typhimurium*.

Cloning and restriction analysis of the E. coli dpp *locus*

The *dpp* locus was cloned on an 11.4 kb fragment from a *Sau*3A partial digest of the MM500 chromosome inserted into λSE6. The rationale for the selection was based on that devised for the *opp* operon (Hiles *et al.*, 1987), selecting for complementation of a *proC*::Tn*5* derivative of strain MM1114 (*opp dppA*::mTn*10*) for growth on Pro–Leu as sole source of the required proline. The cloned DNA fragment contained three *Bam*HI fragments, a 1.5 kb fragment flanked by 4.7 and 5.2 kb fragments. By comparing the restriction map of this DNA fragment with the restriction map of the *E. coli* chromosome (Kohara *et al.*, 1987) in the 79–80 min interval, we were able to position the *dpp* locus at 79.2 min, consistent with the genetic mapping. The *Bam*HI sites linking the insert to the vector are not present in the chromosome and were generated by joining the *Sau*3A ends of the insert to the *Bam*HI ends of the vector arms.

The *dpp*::Tn*10* insertions were located relative to the

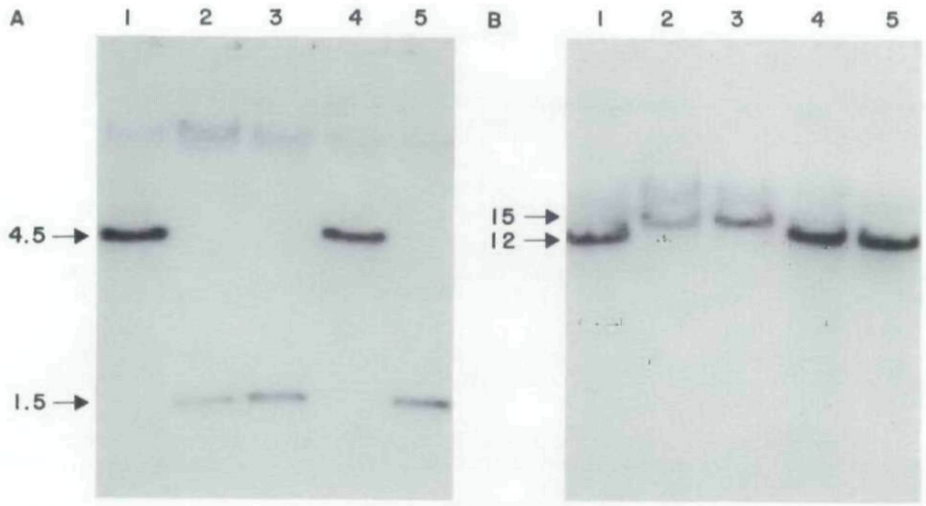

Fig. 4. Southern blots of chromosomal DNA from *dpp*::mTn*10* insertion strains. The probes were: (A) the 1.5 kb *Bam*HI fragment, and (B) the 5.6 kb *Bam*HI fragments from *dpp*. The strains are: lane 1, MM1105; lane 2, MM1107; lane 3, MM1108; lane 4, MM1114; and lane 5, MM500. The first four strains contain *dpp* insertions, MM500 is the *dpp*$^+$ parent. The sizes (in kilobases) of the primary DNA bands are indicated. The slower running, fainter bands seen in all lanes are incompletely digested DNA.

restriction map by Southern blot analysis of *Bam*HI-digested chromosomal DNA from strains containing different insertions (Fig. 4). Two insertions that eliminate DBP (in strains MM1105 and MM1114) were shown to be within the 1.5 kb fragment while two insertions that leave normal amounts of DBP (strains MM1107 and MM1108) were located within the 5.2 kb fragment. Thus, the *dpp* operon appears to begin on the 1.5 kb fragment and to extend into the 5.2 kb fragment.

DNA sequence of the gene encoding E. coli *DBP*

The DNA sequence of the gene that encodes DBP (designated *dppA*) and the amino acid sequence predicted from it are shown in Fig. 5. An *N*-terminal amino acid analysis of DBP isolated from the periplasm gave the sequence KTLVY(C)SEGSPEGFNPQLFT(T)G(T)Y(D)A(A)A, in which residues that were determined with a lower level of confidence are in parentheses (P. Dunten, personal communication). Except for the T at residue 21 and the two A residues at the end of the sequence (all three of which are S residues based on the DNA sequence) this sequence corresponds to the amino acid sequence predicted from nucleotides 231 to 317 of our DNA sequence. This confirms, unambiguously, that the *dppA* open reading frame encodes DBP and predicts the AUG codon at residues 146–148 as the probable translation start site. It also shows that, as expected, DBP is synthesized with signal peptide which is removed post-translationally. The predicted signal peptide contains 28 amino acids and has four Arg or Lys residues towards the *N*-terminus, followed by a run of 13 uncharged, largely non-polar residues. The residues at positions –3 to –1 constitute an acceptable leader peptidase cleavage site (von Heijne, 1983).

The calculated molecular mass for the predicted 507 residues of mature DBP is 57 406 Da. This value is in reasonable agreement with the estimated molecular mass from sodium dodecyl sulphate/polyacrylamide gel electrophoresis (SDS–PAGE) of 55 kDa. A comparison of the predicted amino acid sequences of DBP and the oligopeptide-binding protein (OppA; Hiles *et al.*, 1987) shows 26.5% identity for the mature proteins and 26.8% for the preproteins. The identities are distributed rather uniformly over the entire length of the two proteins and no regions of exceptionally strong homology are obvious. (For a more detailed comparison of these two proteins, consult Olson *et al.*, 1991.)

Discussion

As Gram-negative bacteria lack extracellular peptidases, utilization of peptides requires the function of specific transport systems. Two peptide transport systems, the oligopeptide permease (Opp) and tripeptide permease (Tpp) of *S. typhimurium*, have been characterized in some detail (Gibson *et al.*, 1984; Hiles *et al.*, 1987). In this paper we describe a genetic characterization of a third system, the dipeptide permease (Dpp) of *E. coli* and *S. typhimurium*. Dpp is of special interest, since it plays a key role in peptide-chemotaxis (Manson *et al.*, 1986). We devised three methods, using toxic peptides, for selecting *dpp* mutations in *S. typhimurium* and *E. coli*. That these mutations inactivate a peptide transport system is shown by several findings: they confer resistance to several peptide antibiotics that have different modes of action, they

```
Bam HI  .         .         .         .        50        .         .         .         .       100
GGATCCGCACTGTTACACTGATGTTAATTAGTACGGCATCCCCACCTCATAACGTTGACCCGACCGGGCAAAAAACAAAAAAGGTCAGGCAGCGACAACC

101     .         .         .         .       150        .         .      Hind III   .       200
CACTGCAAAGGGTTAAAACAACAAACATCACAATTGGAGCAGAATAATGCGTATTTCCTTGAAAAAGTCAGGGATGCTGAAGCTTGGTCTCAGCCTGGTG
                                                MetArgIleSerLeuLysLysSerGlyMetLeuLysLeuGlyLeuSerLeuVal

201     .         .         .         .       250        .         .         .         .       300
GCTATGACCGTCGCAGCAAGTGTTCAGGCTAAAACTCTGGTTTATTGCTCAGAAGGATCTCCGGAAGGGTTTAACCCGCAGCTGTTTACCTCCGGCACCA
AlaMetThrValAlaAlaSerValGlnAlaLysThrLeuValTyrCysSerGluGlySerProGluGlyPheAsnProGlnLeuPheThrSerGlyThrT

301     .         .         .         .       350        .         .         .         .       400
CCTATGACGCCTCTTCCGTCCCGCTTTATAACCGTCTGGTTGAATTTAAAATCGGCACCACCGAAGTGATCCCGGGCCTCGCTGAAAAGTGGGAAGTCAG
hrTyrAspAlaSerSerValProLeuTyrAsnArgLeuValGluPheLysIleGlyThrThrGluValIleProGlyLeuAlaGluLysTrpGluValSe

401     .         .         .         .       450     . EcoRI     .         .         .       500
CGAAGACGGTAAAACCTATACCTTCCATCTGCGTAAAGGTGTGAAGTGGCACGACAATAAAGAATTCAAACCGACGCGTGAACTGAACGCCGATGATGTG
rGluAspGlyLysThrTyrThrPheHisLeuArgLysGlyValLysTrpHisAspAsnLysGluPheLysProThrArgGluLeuAsnAlaAspAspVal

501     .         .         .         .       550        .         .         .         .       600
GTGTTCTCGTTCGATCGTCAGAAAAACGCGCAAAACCCGTACCATAAAGTTTCTGGCGGCAGCTACGAATACTTCGAAGGCATGGGCTTGCCAGAGCTGA
ValPheSerPheAspArgGlnLysAsnAlaGlnAsnProTyrHisLysValSerGlyGlySerTyrGluTyrPheGluGlyMetGlyLeuProGluLeuI

601     .         .         .         .       650        .         .         .         .       700
TCAGTGAAGTGAAAAAGGTGGACGACAACACCGTTCAGTTTGTGCTGACTCGCCCGGAAGCGCCGTTCCTCGCTGACCTGGCAATGGACTTCGCCTCTAT
leSerGluValLysLysValAspAspAsnThrValGlnPheValLeuThrArgProGluAlaProPheLeuAlaAspLeuAlaMetAspPheAlaSerIl

701     .         .         .         .       750        .         .         .         .       800
TCTGTCAAAAGAATATGCTGATGCGATGATGAAAGCCGGTACACCGGAAAAACTGGACCTCAACCCAATCGGAACCGGTCCGTTCCAGTTACAGCAGTAT
eLeuSerLysGluTyrAlaAspAlaMetMetLysAlaGlyThrProGluLysLeuAspLeuAsnProIleGlyThrGlyProPheGlnLeuGlnGlnTyr

801     .         .         .         .       850        .         .         .         .       900
CAAAAAGATTCCCGTATCCGCTACAAAGCGTTTGATGGCTACTGGGGCACCAAACCGCAGATCGATACGCTGGTTTTCTCTATTACCCCTGACGCTTCCG
GlnLysAspSerArgIleArgTyrLysAlaPheAspGlyTyrTrpGlyThrLysProGlnIleAspThrLeuValPheSerIleThrProAspAlaSerV

901     .         .         .         .       950       EcoRV      .         .         .      1000
TGCGTTACGCGAAATTGCAGAAGAATGAATGCCAGGTGATGCCGTACCCGAACCCGGCAGATATCGCTCGCATGAAGCAGGATAAATCCATCAATCTGAT
alArgTyrAlaLysLeuGlnLysAsnGluCysGlnValMetProTyrProAsnProAlaAspIleAlaArgMetLysGlnAspLysSerIleAsnLeuMe

1001    .         .         .         .      1050        .         .         .         .      1100
GGAAATGCCGGGGCTGAACGTCGGTTATCTCTCGTATAACGTGCAGAAAAAACCACTCGATGACGTGAAAGTTCGCCAGGCTCTGACCTACGCGGTGAAC
tGluMetProGlyLeuAsnValGlyTyrLeuSerTyrAsnValGlnLysLysProLeuAspAspValLysValArgGlnAlaLeuThrTyrAlaValAsn

1101    .         .         .         .      1150        .         .         .         .      1200
AAAGACGCGATCATCAAAGCGGTTTATCAGGGCGCGGGCGTATCAGCGAAAAACCTGATCCCGCCAACCATGTGGGGCTATAACGACGACGTTCAGGACT
LysAspAlaIleIleLysAlaValTyrGlnGlyAlaGlyValSerAlaLysAsnLeuIleProProThrMetTrpGlyTyrAsnAspAspValGlnAspT

1201    .         .         .         .      1250        .         .         .         .      1300
ACACCTACGATCCTGAAAAAGCGAAAGCCTTGCTGAAAGAAGCGGGTCTGGAAAAAGGTTTCTCCATCGACCTGTGGGCGATGCCGGTACAACGTCCGTA
yrThrTyrAspProGluLysAlaLysAlaLeuLeuLysGluAlaGlyLeuGluLysGlyPheSerIleAspLeuTrpAlaMetProValGlnArgProTy

1301    .         .         .         .      1350        .         .         .         .      1400
TAACCCGAACGCTCGCCGCATGGCGGAGATGATTCAGGCAGACTGGGCGAAAGTCGGCGTGCAGGCCAAAATTGTCACCTACGAATGGGGTGAGTACCTC
rAsnProAsnAlaArgArgMetAlaGluMetIleGlnAlaAspTrpAlaLysValGlyValGlnAlaLysIleValThrTyrGluTrpGlyGluTyrLeu

1401    .         .         .         .      1450      BamHI       .         .         .      1500
AAGCGTGCGAAAGATGGCGAGCACCAGACGGTAATGATGGGCTGGACTGGCGATAACGGGGATCCGGATAACTTCTTCGCCACCCTGTTCAGCTGCGCCG
LysArgAlaLysAspGlyGluHisGlnThrValMetMetGlyTrpThrGlyAspAsnGlyAspProAspAsnPhePheAlaThrLeuPheSerCysAlaA

1501    .         .         .         .      1550        .         .         .         .      1600
CCTCTGAACAAGGCTCCAACTACTCAAAATGGTGCTACAAACCGTTTGAAGATCTGATTCAACCGGCGCGTGCTACCGACGACCACAATAAACGCGTTGA
laSerGluGlnGlySerAsnTyrSerLysTrpCysTyrLysProPheGluAspLeuIleGlnProAlaArgAlaThrAspAspHisAsnLysArgValGl

1601    .         .         .         .      1650        .         .         .         .      1700
ACTGTACAAACAAGCGCAGGTGGTGATGCACGATCAGGCTCCGGCACTGATCATCGCTCACTCCACCGTGTTTGAACCGGTACGTAAAGAAGTTAAAGGC
uLeuTyrLysGlnAlaGlnValValMetHisAspGlnAlaProAlaLeuIleIleAlaHisSerThrValPheGluProValArgLysGluValLysGly

1701    .         .         .         .      1750        .         .         .         .      1800
TATGTGGTTGATCCATTAGGCAAACATCACTTCGAAAACGTCTCTATCGAATAATTAAAAGCCATACAAGACTGATGGCAAAGGCAAAAATGCCTGATGC
TyrValValAspProLeuGlyLysHisHisPheGluAsnValSerIleGluEnd
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Fig. 5. DNA sequence of the *dppA* gene and predicted amino acid sequence of DBP. The open reading frame contains 535 codons. The predicted Shine-Dalgarno sequence is underlined, and restriction sites used in the sequence analysis are indicated; promoter elements have not been characterized. The site of cleavage by leader peptidase is shown by the vertical arrow. The signal peptide contains 28 residues and the mature protein contains 507 residues. These sequence data will appear in the EMBL/GenBank/DDBJ Nucleotide Sequence Data Libraries under the accession number X58051 (*E. coli dppA*).

abolish the ability of dipeptides to serve as sole source of a required amino acid, and they abolish any detectable peptide transport. Over 50 mutations in *S. typhimurium* and *E. coli*, isolated using a variety of selections, mapped to a single chromosomal site counterclockwise from *mtl* and *xyl* at 77–78 min and 79.2 min in the two species, respectively. Thus, we believe that a single locus encodes all components of the dipeptide permease. This location differs from the map position of about 14 min reported previously for *E. coli* (Bachmann, 1990; De Felice *et al.*, 1973). We cannot explain the discrepancy. Finally, we were unable to detect uptake of any peptide in an *opp*$^-$ *tpp*$^-$ *dpp*$^-$ strain, demonstrating that these are the only general peptide transport systems in these two species.

Mutants in *dpp* are defective in the uptake of a wide variety of different peptides, implying an ability to recognize general features of dipeptides unrelated to their side chains. Similarly, in Dpp-dependent chemotaxis assays, cells respond to a variety of dipeptides (Manson *et al.*, 1986). This broad specificity with regard to amino acid composition is similar to that of other peptide-transport systems and is consistent with competition data (Payne, 1980). However, in both chemotaxis (Manson *et al.*, 1986) and transport studies there is a relatively tight specificity for peptide length. At first sight, Dpp appears to be functionally redundant since Opp can also transport dipeptides efficiently. However, each transport system has at least one unique function. Opp recycles cell-wall peptides (Goodell and Higgins, 1987), which contain D-amino acids and are not substrates for Dpp, whereas Dpp but not Opp mediates peptide chemotaxis. Clearly, a peptide chemoreceptor must avoid recognizing peptides generated by peptidoglycan turnover, or the cell would continually jam its peptide sensor. Thus it seems that the primary role of Dpp is to detect and transport peptides from the extracellular environment, whereas the principal role of Opp is in peptidoglycan metabolism.

In our hands, using *lacZ* fusions and by examining the amount of DBP in the periplasm, expression of *dpp* appears to be constitutive and is not induced or repressed by peptides or affected by the carbon or nitrogen source, in either *S. typhimurium* and *E. coli*. This result differs from that reported by Olson *et al.* (1991), who report that casamino acids, but not a combination of all 20 amino acids, repress *dpp* expression significantly. The reason for this difference is not known. The constitutive expression of *dpp* is similar to that observed for Opp (Hiles *et al.*, 1987). However, in both *S. typhimurium* and *E. coli*, different strains express *dpp* at different levels. At least in *S. typhimurium*, the level of expression is determined by mutations linked to the *dpp* locus. The source of the selective pressure that has led to these differences in absolute levels of expression is unknown.

The periplasmic protein encoded by the *dpp* locus has been purified and shown to bind dipeptides with high affinity (Blank, 1987). Its substrate affinities are consistent with its role as the recognition component of the peptide chemoreceptor (Manson *et al.*, 1986). We therefore suggest that this protein be designated a dipeptide-binding protein (DBP). DBP, at 57 kDa, is by far the largest soluble periplasmic chemoreceptor known in *E. coli*, with maltose-binding protein, at 40 kDa (Duplay *et al.*, 1984), being the next largest. DBP is also the only known soluble periplasmic chemoreceptor in *E. coli* that does not have a sugar as a substrate. DBP binds substrate and then interacts with the Tap chemoreceptor (Manson *et al.*, 1986). We therefore propose that the Tap acronym signify 'taxis towards peptides' instead of the original designation as 'taxis-associated protein' (Boyd *et al.*, 1981).

Like other binding protein-dependent transport systems, Dpp presumably requires the function of several membrane-associated proteins in addition to the periplasmic component (Higgins *et al.*, 1990a,b). For other systems the membrane-associated components are normally encoded within the same operon as the periplasmic binding protein. Our data show that the *dpp* locus contains at least one gene, besides that encoding DBP (designated *dppA*), which is required for transport but not for chemotaxis. The gene 3' to *dppA* is homologous to *oppB*, the second gene in the *opp* operon. Thus, the *dpp* locus may be organized like the *opp* operon where the gene encoding the periplasmic binding protein, *oppA*, is the first of five genes in a polycistronic operon (Hiles *et al.*, 1987). Interestingly, the dipeptide-binding protein DBP is homologous to OppA, the periplasmic oligopeptide-binding protein. *In vivo* and *in vitro* data suggest that, unlike other periplasmic binding proteins, OppA may have two distinct substrate-binding sites (Guyer *et al.*, 1986; Goodell and Higgins, 1987). However, OppA is not involved in chemotaxis. The OppA protein of *S. typhimurium* has been crystallized (Tolley *et al.*, 1988) and a structural and functional comparison of OppA and DBP will be extremely instructive in identifying determinants of peptide-binding specificity, and sites of interaction with other transport and chemotactic proteins.

Experimental procedures

Bacterial strains and growth conditions

The genotypes and sources of all strains used in this study are shown in Table 3. All *S. typhimurium* strains were derivatives of strain LT2, and all *E. coli* strains were derivatives of strain K-12. Cells were grown aerobically with vigorous shaking at 37°C, unless otherwise stated. Minimal medium A (MMA), M9 minimal medium (Miller, 1972), or Vogel-Bonner citrate minimal medium (VBC; Vogel and Bonner, 1956), contained 0.2%

Table 3. Bacterial strains used.

Strain	Genotype	Source/Reference
E. coli		
BRZ39	MC4100 *glpR, phoS, proC*::Tn*5*	P. Brzoska
MC4100	F⁻ *araD139,* Δ(*argF–lac*)*U169, ptsF, deoC1, thi, relA, flbB, rpsL150*	Casadaban (1976)
MM500	F⁻ *thr*-$_{Am}$, *leuB6, his-4, metF159*$_{Am}$, *thi-1, ara-14, lacY1, mtl-1, xyl-5, rpsL136, tonA31, tsx78, opp-466*	Identical to strain RP437 (Parkinson and Houts, 1982)
MM564	MM500 *opp*⁺	This study (spontaneous *opp*⁺ revertant of MM500)
MM1105	MM500 *dppA473*::mTn*10*	This study
MM1107	MM500 *dpp-474*::mTn*10*	This study
MM1108	MM500 *dpp-475*::mTn*10*	This study
MM1114	MM500 *dppA476*::mTn*10*	This study
RM41	Q358 F⁻ (r⁻ m⁺) *supE* $\phi 80^R$	Karn *et al.* (1980)
RM42	Q359 P2 lysogen	Karn *et al.* (1980)
S. typhimurium		
CH44	Δ*oppBC250*	Higgins *et al.* (1983)
CH330	*str-1216*	Gibson *et al.* (1984)
CH349	Δ*oppBC250, leu-1151*::Tn*10, tppB9*::Tn*5*	Gibson *et al.* (1984)
CH356	Δ*oppBC250, tppB16*::Tn*10*	Gibson *et al.* (1984)
CH384	Δ*oppBC250,* Δ*leu-3051*	Jamieson and Higgins (1984)
CH407	Δ*oppBC250, ilvG*::Tn*10, tppB9*::Tn5	Recipient CH44; phage 22 donors CH349 and TT63
CH450	Δ*oppBC250, ilvG*::Tn*10, tppB9*::Tn5, *dpp-152*	Valine-peptide resistant derivative of CH356
CH685	Δ*oppBC250,* Δ*leu-3051, tppB16*::Tn*10*	Recipient CH384; phage P22 donor CH356
CH725	Δ*oppBC250, tppB16*::Tn*10, dpp-101*::Tn*5*	This study; Bac^R of CH356
CH748	Δ*oppBC250,* Δ*leu-3051,* Δ*tppB93*	Tet^S deviate of CH685
CH749	Δ*oppBC250,* Δ*leu-3051,* Δ*tppB93, pro-662*::Tn*10*	Recipient CH748; phage P22 donor TT184
CH751	Δ*oppBC250,* Δ*leu-3051,* Δ*tppB93, hisF8539*::Tn*10*	Recipient CH748; phage P22 donor TT29
CH1104	*Mot*⁺, *oppA324, tppB16*::Tn*10*	Goodell and Higgins (1987)
CH1105	*Mot*⁺, *oppA324, dpp-101*::Tn*5*	Goodell and Higgins (1987)
CH1110	*Mot*⁺, *oppA324, tppB16*::Tn*10, dpp-101*::Tn*5*	Goodell and Higgins (1987)
CH1191	Δ*oppBC250, tppB16*::Tn*10, dpp-119*::Mu dl-8	This study; parent CH356
CH1194	Δ*oppBC250, tppB16*::Tn*10, dpp-120*::Mu dl-8	This study; parent CH356
CH1279	LT2 Mot⁺	Goodell and Higgins (1987)
CH1417	Mot⁺, *tppB16*::Tn*10*	Goodell and Higgins (1987)
CH1421	Mot⁺, *tppB16*::Tn*10, dpp-101*::Tn*5*	Goodell and Higgins (1987)
CH1432	Mot⁺, *oppA324*	Goodell and Higgins (1987)
CH1830	Δ*oppBC250,* Δ*leu-3051,* Δ*tppB93, pro-662*::Tn*10, dpp-101*::Tn*5*	Recipient CH749; phage P22 donor CH725
CH1831	Δ*oppBC250,* Δ*leu-3051,* Δ*tppB93, hisF8539*::Tn*10, dpp-101*Tn*5*	Recipient CH751; phage P22 donor CH725
SA486	*serA13, rfa-3058*, HFrK3	K.E. Sanderson
SA534	*serA13, rfa-3058*, HFrK4	K.E. Sanderson
SA722	*serA15, pur-268*, HFrK10	K.E. Sanderson
TT29	*hisF8539*::Tn*10*	J. Roth
TT63	*ilvG*::Tn*10*	Primerano and Burns (1982)
TT184	*pro-662*::Tn*10*	J. Roth
TT3416	*pyrB655*/F'$_{ts}$114, *lac*⁺, *zzf-771*::Tn*5*	J. Roth
TT7610	*zeb-609*::Tn*10, supD10*	Hughes and Roth (1984)
TT7674	*pncA212*::Mu dl-8	Hughes and Roth (1984)

D-glucose as carbon source and 1 μg ml^{-1} thiamine. When carbon sources were varied, appropriate sugars were added at 0.4% or 0.5% (w/v). Carbon- and nitrogen-deficient medium (N^-C^-) has been described (Gutnick *et al.*, 1969). For excess nitrogen, 20 mM NH_4Cl and 0.4% glucose were added to this medium; for nitrogen-limiting (derepressing) conditions this medium was supplemented with 3 mM L-glutamine, 0.2 mM arginine, 0.2 mM uracil, 0.2 mM hypoxanthine and 0.4% glucose (Kustu *et al.*, 1979). Amino acids were added to minimal media at 20 μg ml^{-1}, as required. LB and double-strength yeast-tryptone (DYT) nutrient media were prepared according to Miller (1972). Tetracycline was added to media for *E. coli* at 5 μg ml^{-1} and for *S. typhimurium* at 10 μg ml^{-1}. Ampicillin (Amp), kanamycin (Kan) and streptomycin (Str) were added at 25 μg ml^{-1}, 15 μg ml^{-1} and 100 μg ml^{-1}, respectively. Anaerobic growth was in gas jars using Gas-Paks (Oxoid).

Genetic manipulation

Transductions in *S. typhimurium* were carried out with a high-frequency derivative of phage P22-*int4* (Roth, 1970). When *galE* strains were used, cells were grown in LB containing 0.2% glucose and 0.2% galactose to ensure efficient synthesis of phage receptors. Transductions in *E. coli* were done with $P1_{vir}$ according to Silhavy *et al.* (1982). Hfr matings were carried out as described for S. *typhimurium* (Gibson *et al.*, 1984) and *E. coli* (Miller, 1972), using streptomycin to select against the donor. Tetracycline-sensitive excisions of Tn*10* insertions were obtained using fusaric acid plates (Bochner *et al.*, 1980).

Susceptibility to toxic peptides

Sensitivity to toxic peptides was determined on minimal-

glucose plates by radial streaking away from a filter disc impregnated with sufficient peptide to give about a 1-cm zone of killing with a sensitive strain and no inhibition with a resistant strain. Bacilysin (the gift of E. Abraham) and bialaphos (obtained from C. Thompson) were added at 0.5 mg and 0.01 mg per disc, respectively. Other peptides were obtained from Sigma Chemical Co. and were used at 200 nmol per disc.

To select resistant mutants, valine-containing peptides were present in minimal-glucose agar plates at 4 μM and bacilysin was present at 100 μg ml^{-1}. Bialaphos-resistant mutants were selected on minimal-glucose agar from the zone of inhibition around a filter disc impregnated with 100 μg of the peptide. Resistant colonies growing after 48 h incubation at 37°C were purified for further characterization. Mucoid colonies that often appeared with *E. coli* were discarded.

Isolation of transposon insertions in S. typhimurium

The phage vector Mu *d*1-8 can be used to isolate *lacZ* operon fusions in *S. typhimurium* (Hughes and Roth, 1984). This phage confers resistance to ampicillin (Amp^R) and contains an amber mutation in the transposase gene so that it can only transpose in a strain containing an amber suppressor. A collection of 10 000 random insertions was obtained by transducing the *supD* host strain TT7610 to Amp^R with a P22 lysate of strain TT7674 (*pncA212*::Mu *d*1-8). The collection was pooled, and a phage P22 lysate was grown on the pooled cells and used to transduce an appropriate $supD^+$ strain to Amp^R. Insertions in *dpp* were identified as blue colonies on minimal-glucose agar plates containing 20 μg ml^{-1} 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal).

A collection of random Tn*5* insertions was obtained by transduction of the appropriate host strain to Kan^R using a phage P22 lysate grown on strain TT3416, as described previously (Cairney *et al.*, 1984). Strain TT3416 contains a Tn*5* insertion on an F factor and, upon introduction into a recipient cell, stable Kan^R transductants can arise only by transposition from the F factor onto the chromosome. A collection of at least 10 000 independent insertions was pooled and washed twice with VBC medium before further selections were carried out.

*Isolation of mini-Tn*10 *insertions in* E. coli

Mini-Tn*10* insertions were constructed using phage λ809 as the donor of Tet^R transposon Tn*10*Δ*16*Δ*17* (Way *et al.*, 1984). The transposase function of this element was supplied *in trans* from the Amp^R plasmid pNK474 (Way *et al.*, 1984). Strains MM500 and MC4100 were transformed with pNK474, and plasmid-containing cells were grown to a OD_{578nm} of 0.2 (*c.* 10^8 cells ml^{-1}) in LB containing 25 μg ml^{-1} ampicillin and 0.2% maltose. Cells were concentrated 10 times by centrifugation and resuspension in LB, and phage λ809 was added at a multiplicity of infection of 0.3. The cells were incubated for another 30 min at room temperature to allow absorption of the phage and then 90 min at 37°C to allow for transposition and phenotypic expression of tetracycline resistance. Aliquots of 0.2 ml were plated onto DYT plates containing 5 μg ml^{-1} tetracycline and 1.25 mM sodium pyrophosphate. At least 10 000 Tet^R colonies for each strain were pooled by resuspension in 5 ml of VBC medium, washed twice with 5 ml of VBC medium, and resuspended in the same volume of VBC medium. These cells were diluted 10-fold in VBC medium, and bialaphos-resistant (Bap^R) mutants were isolated as described above.

Eight purified isolates from each selection plate were pooled for preparation of $P1_{vir}$ lysates. These lysates were used to transduce strain MM500 or MC4100 (whichever was the source of the isolates) to Tet^R. Twenty-eight transductants from each lysate were tested for Bap^R by radial streaking away from a 6 mm filter disc impregnated with 100 mg of bialaphos. Bap^R isolates grew up to the edge of the filter and were found with six out of six lysates from strain MM500 and six out of seven from strain MC4100. To ensure independent transposition events, only one Bap^R isolate per lysate was chosen for further analysis.

Analysis of periplasmic proteins

Periplasmic proteins were released from cells by the cold osmotic shock procedure of Neu and Heppel (1965). Crude shock fluids were analysed by electrophoresis on an 11% polyacrylamide slab-gel in the presence of SDS according to Laemmli (1970). Samples were boiled for 2 min before loading onto the gel. Proteins were visualized with Coomassie Brilliant Blue.

Cloning of the E. coli dpp *locus*

Chromosomal DNA from *E. coli* strain MM500 (dpp^+ opp^-) was isolated, and a partial digest of this DNA was prepared with *Sau*3A and then electrophoresed on a 0.5% agarose gel. DNA fragments of 10–20 kb were eluted from the gel and ligated into the *Bam*HI sites of λSE6 (Elledge and Walker, 1985) DNA to replace the stuffer fragment. The ligated mixture was packaged *in vitro* and used to infect strain RM42, a P2 lysogen which will allow only the red^- gam^- (Spi^-) phage (with an insert) to form plaques. Phages from these plaques were pooled and propagated on the non-lysogenic strain RM41 to yield a high-titre lysate.

This lysate was used to infect cells of strain MM1114 *proC*::Tn*5* (λGT4*lac5*) in 10 mM $MgCl_2$. This strain carries a mTn*10* insertion in the *dpp* locus, is opp^-, and requires L-proline and L-leucine. (The lysogenized λGT4*lac5* provides $cI857^{ts}$ repressor, which maintains the $cI^-\lambda$ phage DNA derived from λSE6 as a stable plasmid at 30°C). The infected cells were plated on M9 minimal-glycerol agar containing 20 μg ml^{-1} L-threonine, L-histidine and L-methionine, 1 μg ml^{-1} thiamine, and 10 μg ml^{-1} L-prolyl-L-$leucine^{-1}$ (Pro–Leu agar) and incubated at 30°C. A number of colonies that appeared after 48 h were purified on the same agar and then used to produce lysates in LB liquid medium after induction at 42°C. Dilutions of these lysates were spotted onto Pro–Leu agar plates spread with about 5×10^8 washed cells of strain MM1114 *proC*::Tn*5* (λGT4*lac5*), and the plates incubated at 30°C. Lysates giving rise to large numbers of colonies after 48 h could contain λSE6 with a dpp^+ insert. Such lysates were diluted and plated out on strain RM42. Single plaques were purified, and high-titre lysates were prepared from the purified plaques. The phage in one of these lysates was designated λ*dpp*. Digestion of λ*dpp* with *Bam*HI generated five fragments in the following order: left λarm, chromosomal fragments of 4.7, 1.5, and 5.2 kb, and right λarm. Each internal fragment was cloned into the *Bam*HI site of plasmid pBR322 and subjected to restriction analysis.

Southern blot analysis of chromosomal DNA

Chromosomal DNA was prepared from *E. coli* strains MM500, MM1105, MM1107, MM1108 and MM1114. Total chromosomal DNA was digested with *Bam*HI, and the fragments were separated by electrophoresis in polyacrylamide gels. The DNA was transferred from the gel to nitrocellulose sheets. DNA labelled with [^{32}P]-dCTP by nick-translation was prepared for the three cloned *Bam*HI fragments and used to probe the DNA transferred to nitrocellulose.

DNA sequence analysis

The 1.5 kb *Bam*HI fragment and subfragments of it, and the 5.2 kb *Bam*HI fragment, were cloned into the multilinker sites of phages M13mp18 and M13mp19 for DNA sequence analysis. Sequencing was done using the dideoxynucleotide chain termination method (Sanger *et al.*, 1977). All sequences were obtained on both DNA strands. A universal *lacZ* primer or custom-synthesized primers corresponding to *dpp* sequences were used. Products were labelled with [α-^{32}P]-dATP. Analysis of sequences was carried out using the Sequence Analysis Software Package of the Genetic Computer Group, Version 6.0, which is based on the algorithm of Needleman and Wunsch (1970).

Notes added in proof

During the preparation of this manuscript, we learned that Eric Olson and his collaborators had cloned, mapped and sequenced an *E. coli* gene that they initially called *fpp* (fifty-kilodalton periplasmic protein), and which proved to be *dppA*. We have co-ordinated with Olson to establish a uniform nomenclature for the genes and gene products of the *dpp* operon. Their studies are to be published elsewhere (Olson *et al.*, 1991).

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