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Identification and Characterization of *dppA*, an *Escherichia coli* Gene Encoding a Periplasmic Dipeptide Transport Protein

ERIC R. OLSON,[1]* DONNA S. DUNYAK,[1] LYNN M. JURSS,[1]† AND ROGER A. POORMAN[2]

Molecular Biology Research[1] and Biopolymer Research,[2] The Upjohn Company, Kalamazoo, Michigan 49007

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We describe the isolation and analysis of an *Escherichia coli* gene, *dppA*, and its role in dipeptide transport. *dppA* maps near min 79 and encodes a protein (DppA) that has regions of amino acid similarity with a peptide-binding protein from *Salmonella typhimurium* (OppA). Like OppA, DppA is found in the periplasmic space and thus is most likely a dipeptide-binding protein. Insertional inactivation of *dppA* results in the inability of a proline auxotroph to utilize Pro-Gly as a proline source. *dppA*-dependent Pro-Gly utilization does not require any of the three major proline transport systems, demonstrating that DppA is not simply a dipeptidase. An in vivo competition assay was used to show that DppA is probably involved in the transport of dipeptides other than Pro-Gly. Transcription of *dppA* is repressed by the presence of casamino acids, suggesting that the cell alters its dipeptide transport capabilities in response to an environmental signal.

Active transport of small peptides has long been recognized as a strategy for diverse cell types to obtain amino acids (reviewed in reference 25). Although the fundamental importance of this process in organisms with normal amino acid transport and biosynthetic abilities has been difficult to document, the fact that it exists in mammals (25), fungi (31), higher plants (40), and bacteria (33) suggests that the process provides strong selective advantages to these species.

The best understanding of peptide transport comes from investigations with the gram-negative bacteria *Escherichia coli* and *Salmonella typhimurium* (reviewed in reference 33). At a minimum, they both have three genetically separate transport systems. The Opp permease, encoded by *oppABCDF*, can transport peptides of two to five amino acids and is linked to the *trp* operon in both organisms (3, 4, 17, 22). *tppB* of *S. typhimurium*, min 27, and *oppE* of *E. coli*, min 98 (3, 9, 19), have been implicated as being involved in transport primarily of tripeptides. Mutations affecting the transport primarily of dipeptides have been isolated in both *S. typhimurium* and *E. coli*. The loci involved have been named *dpp* for dipeptide permease. A *dpp* allele, *dpp-1*, was mapped in *E. coli* near min 14; however, no subsequent analysis of the role of this locus in peptide transport has been reported (7). A second set of *dpp* mutations have been selected in *E. coli* by their ability to confer resistance to the toxic peptide bacilysin. Fifteen percent of these mutants had reduced levels or were missing a 49-kDa periplasmic protein, called Dpp (24). *dpp* mutations in *S. typhimurium* mapping to min 82 have been identified and shown to cause defects in the uptake of dipeptides as well as some tripeptides (13). The *ami* locus from *Streptococcus penumoniae* has been sequenced and found to contain five genes displaying striking similarity to the *opp* operon of *S. typhimurium* (1). Although not proven, there is evidence that the products of the *ami* operon are involved in the transport of oligopeptides (1).

In gram-negative bacteria, small-molecule transport systems have been divided into several classes. One of these, comprising the osmotic-shock-sensitive transport systems, is responsible for the uptake of a wide range of small molecules, including amino acids, sugars, vitamins, and ions (2). It is this class to which the Opp permease belongs (3, 12, 14, 15, 17). Three main features distinguish this class from other types of transport mechanisms: (i) the requirement for a periplasmic binding protein, (ii) low apparent K_m of transport compared with the other classes, and (iii) inner membrane proteins that, among the members of the class, share similarities in amino acid composition.

The use of chemically modified peptides has led to a detailed picture of the structural features of peptides that allow them to be taken up by *E. coli* (33). These features include (i) a protonated primary terminal amino group (with the exception of peptides beginning with proline), (ii) L-stereospecificity, (iii) an alpha peptide bond, and (iv) at least for dipeptides, a free carboxyl end. Side chain properties are not critical, as evidenced by the fact that peptides comprising different side chains can be transported by the same mechanism. Intriguingly, these properties are also important for transport of di- and tripeptides into mammalian intestinal absorptive cells (25), suggesting that the mammalian peptide permeases share certain structural features with the bacterial proteins.

Although the existence of an *E. coli* dipeptide-specific transport system has been known for several years, the genes and proteins involved have not been well characterized. This report presents the genetic and physical characterization of an *E. coli* gene, *dppA*, and its role in dipeptide transport. In addition, arguments are presented supporting the hypothesis that the *dppA* gene product, DppA, is a periplasmic dipeptide-binding protein.

Following completion of the work described here, we became aware that another group had been characterizing the same gene and protein (23a). Manson has proposed that the gene by called *dppA* and the periplasmic protein be called DppA. The nomenclature used here reflects that proposal. Moreover, the protein identified as Dpp (24) is the same as that described here (23a).

* Corresponding author.
† Present address: Amgen, Inc., Thousand Oaks, CA 91320-01789.

MATERIALS AND METHODS

Strains and media. The *E. coli* strains used are K-12 derivatives and, along with the phage and plasmids, are shown in Table 1. Liquid cultures were grown in either LB, LB+ (LB plus 0.2% glucose), or M9-based medium (28). M9 medium was supplemented with the appropriate sugar at 0.2% and with vitamin-free casamino acids (CAA; Difco, Detroit, Mich.) where noted. Amino acids were added at 50 μg/ml for growth of auxotrophic strains except where noted. Concentrations of antibiotics were: streptomycin sulfate (Sm), 100 μg/ml; kanamycin sulfate (Kan), 50 μg/ml; and ampicillin (Ap), 100 μg/ml. M13-derived clones were grown in YT medium as described, with JM101 as a host (27). Media for λ growth and plating were as described before (5, 10). Phage P1 *vir* was grown and used as described before (28).

E1614 (W3110 *dppA20*::Kan) was made by growing E1532 (see Phage constructions [below] for E1532 derivation) at 30°C in LB and plating for isolated colonies on LB plates at 40°C. Twelve colonies were purified, and eight were Kan^r. One of the Kan^r colonies, E1614, was shown to be sensitive to λ *imm*^{434} infection and to contain the *dppA20*::Kan allele by Southern hybridization of chromosomal DNA and Western immunoblots. E1847 (JM101 *dppA30*::Kan) was isolated in the same manner as E1614 except that the starting strain was E1843 (see Phage constructions for E1843 derivation). The donors and recipients used for P1 transductions to create E1222, E1769, E1772, and E1974 are listed in Table 1. E1222 was selected on LB with streptomycin, E1769 and E1772 were selected on LB with tetracycline, and E1974 was selected on LB with kanamycin containing 250 μM proline. E232 was isolated by growing JM101 in the presence of acridine orange (125 μg/ml) and screening the survivors for loss of the F′ by plating on M9 with and without proline and testing for sensitivity to phage M13. One of the Pro$^-$ M13^r isolates was identified as E232.

Hfr mapping. Donors containing transposon Tn*10* were used essentially as described before (41). Log-phase cultures of both donor and recipient were mixed at a ratio of 5 donor to 1 recipient and mated for 30 min at 37°C with gentle shaking. The mixtures were vigorously vortexed, diluted, and plated onto LB agar containing streptomycin and tetracycline. Transconjugants were purified and assayed for DppA by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

DNA techniques. Restriction digests, agarose gel electrophoresis, large- and small-scale plasmid preparations, transformations, λ DNA isolation, Southern blots, and plaque and colony hybridizations were done by standard techniques (23). Oligonucleotides were supplied by N. Hatzenbuhler (Upjohn Co.) and purified by preparative gel electrophoresis. Hybridizations with the oligonucleotides were done at 50°C in 5× SET (1× SET is 0.15 M NaCl, 0.03 M Tris hydrochloride [Tris HCl, pH 8.0], 1 mM EDTA)–1× Denhardt's solution (0.02% each Ficoll, polyvinylpyrrolidone, and bovine serum albumin)–0.5% SDS and washed at 57°C in 2× SET–0.1% SDS. Dideoxy sequencing was done by the chain termination method (34, 42). Dideoxy sequencing reactions with avian myeloblastosis virus reverse transcriptase, Klenow fragment, and Sequenase (US Biochemicals, Cleveland, Ohio) were used to verify the sequence of ambiguous regions. Both strands of DNA containing the sequence shown in Fig. 3 were sequenced.

Protein techniques. SDS-PAGE for analyzing cell extracts was done as described, with DATD (*N*,*N*′-diallyltartardiamide) as the cross-linker, and stained with Coomassie brilliant blue (21, 30). Cell fractionation was done by osmotic shock essentially as described by Nossal and Heppel (32). Overnight cultures were grown in the indicated media and diluted to an OD_{550} of 1. Then, 1 ml was centrifuged, washed in 1 ml of TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), centrifuged, suspended in 100 μl of room-temperature 20% sucrose–30 mM EDTA, pH 8.0, and held for 10 min. The cells were centrifuged, suspended in 100 μl of ice-cold 0.5 mM $MgCl_2$ held on ice for 5 min, and then centrifuged for 5 min in a microfuge. The supernatant was saved, and the pellet was suspended in 100 μl of TE. For total cellular extract preparation, overnight cultures were centrifuged, suspended in lysis buffer (21), and boiled for 5 min before loading.

Western blots were done with nitrocellulose as described before (37). Procedures for subsequent treatment and processing of filters were as described by the supplier of the immunologic reagents (Bio-Rad Laboratories, Richmond, Calif.). After reaction with primary antibody, filters were reacted with protein A-horse radish peroxidase (HRP) conjugate and developed according to the manufacturer's recommendations. DppA was purified from 400 ml of JM101 grown to saturation in M9–glucose–0.02% CAA. Cells were centrifuged, sonicated, and centrifuged; the supernatant was lyophilized, suspended in 0.1 M ammonium bicarbonate, pH 7.8, and centrifuged. The pellet was discarded, and the supernatant was applied to a column (1.5 by 97.5 cm) of Bio-Gel P-6 (Bio-Rad) developed in 0.1 M ammonium bicarbonate at a flow rate of 4 ml/h. The effluent fractions were monitored for the presence of proteins and peptides by UV absorbance at 230 nm. The fraction eluting at the void volume of the column was diluted fivefold with water, applied to a column (0.9 by 10 cm) of Whatman DE-52 equilibrated with 0.02 M ammonium bicarbonate, and developed with a linear gradient to 0.2 M ammonium bicarbonate. The effluent fractions were monitored as above. An aliquot of the peak eluting at a buffer concentration of approximately 0.05 M yielded a single band of 50 kDa on SDS PAGE. Automated Edman degradation was carried out on a 1-nmol sample with an Applied Biosystems Inc. (Foster City, Calif.) model 470A gas-phase sequencer equipped with an on-line phenylthiohydantoin analytical system provided by the manufacturer.

Antibody was prepared by injecting six CD-1 female mice (8 weeks old) subcutaneously with purified DppA in complete Freund's adjuvant. A 0.15-ml amount containing 40 μg of protein was used for the initial injection and for a booster 3 weeks later. Six weeks later, the mice were injected with cells from an S180 ascites tumor in the peritoneal cavity. Ascites fluid was harvested from each mouse 8 to 10 days later. For Western analysis, antibody was diluted 1:500.

RNA isolation and Northern hybridizations. RNA was isolated from cells subcultured 1:100 in the indicated medium from overnight cultures as follows. When the culture reached mid-log phase, 5 ml of cells was added to an equal volume of semifrozen 100 mM sodium azide–400 μg of chloramphenicol per ml and mixed until thawed. The cells were pelleted and suspended in 0.180 ml of buffer (100 mM Tris-HCl [pH 7.3], 100 mM KCl, 5 mM $MgCl_2$), and 0.02 ml of lysozyme solution (3 mg of lysozyme per ml of buffer) was added. The cell suspensions were held on dry ice until frozen, 1.0 ml of RNazol (CINNA/BIOTECX, Friendswood, Tex.), was added, and the tubes were gently mixed until the suspensions thawed and the cells were completely lysed. Then 0.120 ml of chloroform was added, and the lysates were vigorously shaken for 15 s, held on ice for 15 min, and

TABLE 1. Strains, phages, and plasmids

Strain, phage, or plasmid	Relevant characteristics	Source, reference, or derivation
E. coli K-12		
JM101	*supE thi* Δ(*lac-proAB*) F′ *traD36 proA*$^+$ *proB*$^+$ *lacI*^{q} ZΔM15	ATCC[a] (26)
WE110	F$^-$ IN(*rrnD-rrnE*)	F. Neidhardt (University of Michigan) (16)
AB1157	F$^-$ *gal thr xyl mtl leu his pro*	18
S1228	F$^-$ *trp lac thr metF ilv lys his xyl rpsL tsx zjh-428*::Tn*10*	6
BW5659	HfrKL98, *xyl-7 lacY1 mglP1 zdh-57*::Tn*10*	41
BW6156	HfrP4X, *lacY1 mglP1 relA1 spoT1*	41
BW6159	HfrKL14, *ilv-691*::Tn*10 thi-1 relA1 spoT1*	41
BW6160	Hfr Broda 8, *zdh-57*::Tn*10 metB1 relA1 spoT1*λ^r	41
BW6163	HfrKL16, *zed-977*::Tn*10 thi-1 relA1 spoT1*	41
BW6164	HfrRa-2, *thr-43*::Tn*10 mal-28 sfa-4 supE42*	41
BW6165	HfrP801, *argE*::Tn*10 lacY1* or *lacY40 xyl-7 mtl-2 ara-41* λ *ind*	41
BW6166	HfrJ4, *zhf-721*::Tn*10 thi-1 malB16*::*F*1 (λ) *supE44*	41
BW6169	HfrAB313, *argA81*::Tn*10 thi-1 leuB6 gal-6 lacY1* or *lacZ4 supE44*	41
BW6175	HfrPK3, *argE*::Tn*10 thr-1 leuB6 thi-1 lacY1 azi-15 tonA21 supE44*	41
BW7261	Hfr Cavalli, Δ(*argF-lac*)*205 leu-63*::Tn*10 relA1 tonA22 pit-10 spoT1 ompF627*	41
BW7620	HfrKL99, *zed-977*::Tn*10 thi-1 relA1 lac-42*	41
BW7623	Hfr Broda 7, *purE*::Tn*10 relA1*?	41
WG208	F$^-$ *trp lacZ rpsL thi* Δ*putPA101 proP219 proU205 pro-81*::Tn*10*	11
WPS18	C600, *hsdR rpsL150 proC*::Tn*10* Δ*lacU169* λ Δ*B*am N^+ *imm*λ *c*Its857 ΔH1	36
K37	F$^-$ *galK* Sm^r	D. Friedman (University of Michigan)
C600	F$^-$ *thi thr leu supE tonA lacY*	D. Friedman (University of Michigan)
K124	K37 (λ)	D. Friedman (University of Michigan)
E232	JM101 cured of F′ by acridine orange	This work
E1222	E232, Sm^r	This work; P1(K37) × E232[b]
E1532	W3110, Kan^r lysogen of λ1532-3	This work
E1614	W3110, *dppA20*::Kan, from E1532 prophage curing	This work
E1769	W3110, *proC*::Tn*10* Δ*lacU169*	This work; P1(WPS18) × W3110
E1772	E1614, *proC*::Tn*10* Δ*lacU169*	This work; P1(WPS18) × E1614
E1843	JM101, Kan^r lysogen of λ1843-3	This work
E1847	JM101, *dppA30*::Kan, from E1843 prophage curing	This work
E1974	WG208, *dppA20*::Kan	This work; P1(E1614) × WG208
Plasmids		
pSRlac	pBR322 derivative with *lac* promoter used for expressing *dppA*, Ap^r	S. Rockenbach (The Upjohn Co.)
pBS	Ap^r	Stratagene Inc., La Jolla, Calif.
pfppl-2	Ap^r, *dppA* expression plasmid (detailed map available upon request)	This work
pBSEB-2	Ap^r, pBS with *Eco*RI-*Bam*HI *dppA* fragment from λ10	This work
pUC18	Ap^r	27
pUC3-13	Ap^r, pUC18 containing *Bam*HI *dppA* fragment from λ9F6	This work
pUC3-13K	Ap^r Kan^r, pUC3-13 with Kan^r cassette from pUC4K in *Eco*RI site of *dppA*	This work
pUC4K	Ap^r Kan^r	Pharmacia Inc.
pCWB-1	Ap^r, pUC18 with *Bam*HI *dppA* fragment from λ10	This work
pCWBK-2	Ap^r Kan^r, pCWB-1 with Kan^r cassette from pUC4K in *Eco*RI site of *dppA*	This work
pACDF	Tet^r, pACYC184 with 9-kb *Eco*RI fragment from λ22 containing 3′ end of *dppA*	This work
Phages		
λ9F6, λ3H5, λ1D1, λ9H8, λ25B125, λ9G3, λ4D9	W3110 chromosomal fragments from min 79 region	20
λEMBL4		8
λ10, λ22	JM101 chromosomal *Sau*3A fragments from *dppA* region in λEMBL4	This work
λ2-2	λ3H5 with *imm*^{434} *c*Its	This work; λ3H5 × G501
G501	λ *imm*^{434} *c*Its *N*am7,53	D. Friedman (University of Michigan)
λ1532-3	λ2-2 containing *dppA20*::Kan	This work; λ2-2 × pUC3-13K
λ22i4	λ22 *imm*^{434} *c*Its	This work; λ22 × G501
λ1843-3	λ22i4 containing *dppA30*::Kan	This work; λ22i4 × pCWBK-2
mp18, mp19		27

[a] ATCC, American Type Culture Collection.
[b] P1-mediated transductions are shown as P1 (donor) × recipient.

centrifuged for 15 min to separate the layers. The aqueous phase was transferred to a fresh tube, and an equal volume of isopropanol was added to precipitate the RNA. After incubation at −20°C for 45 min, the samples were centrifuged for 15 min at 10,000 rpm in a Microfuge and the resulting pellet was washed twice with 0.8 ml of 75% ethanol. The pellets were briefly dried and suspended in 0.05 ml of diethyl pyrocarbonate-treated water. Then 4.5 μg of each RNA sample was suspended in loading dye (23), heated at 90°C for 5 min, and electrophoresed through a 1.3% formaldehyde–agarose gel (23). RNA size standards from BRL-Gibco (Gaithersburg, Md.) were treated identically. The gel was electrophoresed overnight at 20 mA and transferred to Nytran (Schleicher & Schuell) with an LKB (Bromma, Sweden) vacuum blotter, and the RNA was fixed onto the filter by UV cross-linking. A [^{32}P]-rCTP-labeled single-stranded antisense RNA probe was synthesized by using T7 polymerase as described by the supplier (Stratagene Inc., La Jolla, Calif.). The template for this synthesis was pBSEB-2 (see below). The filter was prehybridized for 30 min and hybridized overnight in 5× SSC–5× Denhardt's solution–0.1% SDS–100 μg of salmon sperm DNA per ml–50% formamide at 65°C (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate). The filter was washed twice in 2× SSC–0.1% SDS at 65°C and once in 0.1× SSC–0.1% SDS for 15 min at the same temperature. The RNA markers and rRNA were visualized by staining the filter, after autoradiographs were exposed, with methylene blue, as described before (29).

Isolation of λ10 and λ22. Chromosomal DNA was isolated from E1222 as described before (35) and partially digested with *Sau*3A to yield fragments of 10 to 20 kbp. The resulting fragments were ligated with *Bam*HI-digested λEMBL4 arms (Promega, Madison, Wisc.), packaged in vitro with Packagene Lambda DNA Packaging System (Promega) and plated on JM101, and the resulting plaques were screened with nick-translated 1.5-kbp *Bam*HI fragment from pUC3-13 containing part of *dppA*. Two of the hybridizing plaques were characterized further (λ10 and λ22) and shown by restriction enzyme mapping and Southern hybridizations to contain *dppA* and flanking DNA.

Plasmid constructions. pUC3-13 and pCWB-1 were constructed by ligating the 1,465-bp *Bam*HI fragments from λ9F6 and λ10, respectively, into the *Bam*HI site of pUC18. pUC3-13K and pCWBK-2 were constructed by ligating an *Eco*RI fragment from pUC4K (Pharmacia, Inc., Piscataway, N.J.) containing the gene for Kan^r into the *Eco*RI site internal to *dppA* in pUC3-13 and pCWB-1, respectively.

pfppl-2 was constructed as follows. Plasmid pSRlac contains the pBR322 *ori* and *bla* genes and a resynthesized version of the *lac* promoter (used to replace the *Eco*RI-*Bam*HI fragment of pBR322) situated so that transcription from *lacp* proceeds clockwise with respect to the standard pBR322 map. The promoter is followed by restriction sites for *Cla*I, *Hin*dIII, *Kpn*I, *Sma*I, and *Bam*HI. Oligonucleotides containing the start of *dppA* (5′-CGATAATGCGTA TTTCCTTGAAAAAGTCAGGGATGCTGA and 5′-AGCT TCAGCATCCCTGACTTTTTCAAGGAAATACGCATTAT) were annealed and ligated to *Cla*I-*Hin*dIII-digested pSRlac to create pfls-3. A *Hin*dIII-*Pst*I fragment from λ10 (*Pst*I site made blunt-ended with Klenow and dNTPs), containing all but the very beginning of *dppA* plus 1.1 kbp downstream of *dppA*, was ligated into *Hin*dIII-*Sma*I-digested pfls-3 to generate pfppl-2.

pACDF was constructed by ligating a 9-kbp *Eco*RI fragment from λ22 containing the 3′ end of *dppA* into the *Eco*RI site of pACYC184.

pBSEB was constructed by ligating the *Eco*RI-*Bam*HI fragment internal to *dppA* from λ22 into *Eco*RI-*Bam*HI-digested pBS.

Phage constructions. λ2-2 was made by infecting C600 with λ3H5 and G501, each at a multiplicity of infection of 5, and selecting for *imm*^{434} *N*$^+$ recombinants on a lawn of K124. One of the resulting recombinants, λ2-2, was shown by restriction enzyme mapping and hybridization to contain the *dppA* region as in λ3H5. λ22i4 was made similarly to λ2-2 except that the cross was between λ22 and G501. λ1532-3 was made by growing λ2-2 in JM101(pUC3-13K) and using the resulting lysate to lysogenize W3110. Lysogens were selected on LB-kanamycin plates at 30°C. One of these lysogens, E1532, was resistant to λ *imm*^{434} but not λ at 30°C. Phage was isolated from the resulting lysogen by growing the cells at 40°C and plating the resulting supernatant on a lawn of W3110. The resulting plaques were hybridized to a nick-translated DNA fragment from pUC4K containimg the gene for Kan^r, and one of those that hybridized, λ1532-3, was shown by restriction enzyme analysis to contain the Kan^r cassette in the *Eco*RI site of *dppA*. The same steps were used to make λ1843-3 except that λ22i4 was grown in JM101(pCWBK-2) and the resulting phage were used to lysogenize JM101. One of these lysogens was called E1843. Phage were isolated from E1843 by heat induction, and an isolate shown to contain the Kan^r insertion was identified and called λ1843-3.

Dipeptide utilization. Cells were grown overnight in M9-glucose containing required amino acids, pelleted, washed in 1× M9 salts, pelleted, and suspended in 1× M9 salts (28). The cultures were then diluted 1:100 in M9-glucose containing the appropriate peptide at 50 μM unless otherwise noted. The cultures were shaken overnight at 37°C, and the density was read at 550 nm. Peptides were purchased from Bachem Bioscience Inc., Philadelphia, Pa.

Nucleotide sequence accession number. The sequence shown in Fig. 3 has GenBank accession number M35045.

RESULTS

Characterization of DppA levels and cellular location. In efforts to expand the repertoire of elements (genetic and physiological regulatory elements, signal sequences, etc.) that would be useful in expressing heterologous genes in *E. coli*, we investigated the following observation. When the *E. coli* strain JM101 was grown in minimal medium, a 50-kDa protein accumulated in the periplasm to extremely high levels (about 10% of total cell protein and 90% of periplasmic protein, as determined from Coomassie-stained gels). If this high expression was due to a single copy of a gene, then the elements controlling its expression and secretion would be of interest. For this reason, we wished to characterize and ultimately identify the gene encoding this protein, understand the basis for its high expression, and determine the suitability of any of the genetic elements for use in expression vectors.

The pattern of accumulation of this 50-kDa protein, which for the reasons described in the introduction is called DppA, is shown in Fig. 1. The laboratory K-12 strain JM101 accumulated DppA to a high level when grown in minimal glucose medium; the level was substantially reduced when the medium was supplemented with CAA (Fig. 1A, lanes 2 and 3). Strain W3110, another common laboratory strain,

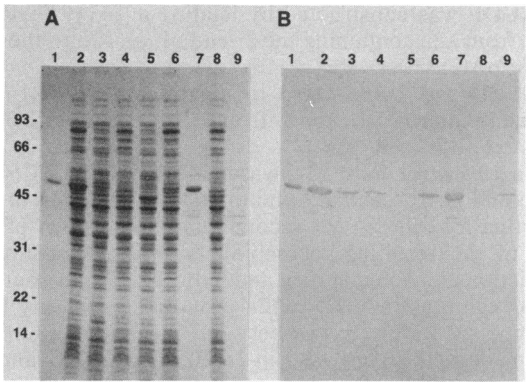

FIG. 1. Expression of DppA in JM101 and W3110. (A) Coomassie-stained 14% polyacrylamide–SDS gel. Migration and sizes (in kilodaltons) of protein standards are indicated on the left. Samples in each lane are as follows: 1, purified DppA; 2, JM101 grown in M9-glucose; 3, JM101 grown in M9–glucose–2% CAA; 4, W3110 grown in M9-glucose; 5, W3110 grown in M9–glucose–2% CAA; 6, pellet from osmotically shocked JM101 grown in M9-glucose; 7, supernatant from osmotically shocked JM101 grown in M9-glucose; 8, pellet from osmotically shocked W3110 grown in M9-glucose; 9, supernatant from osmotically shocked W3110 grown in M9-glucose. (B) Western blot of duplicate gel shown in panel A reacted with anti-DppA antibody and protein A-horseradish peroxidase conjugate.

was found not to accumulate substantial amounts of DppA (Fig. 1A, lanes 4 and 5). To better quantitate the expression, DppA was purified from JM101 grown in minimal medium and used to raise polyclonal antibody. Western hybridizations with this antibody against JM101- and W3110-derived proteins revealed that W3110 also produced DppA, and, as with JM101, the level was reduced when cells were grown in the presence of CAA (Fig. 1B, lanes 2 to 5). The nature of the compound in CAA responsible for the regulation was not identified; however, addition of the 20 common L-amino acids, either singly, as a complete mixture, or in various combinations, to M9-glucose did not significantly reduce DppA synthesis in JM101 (data not shown). Likewise, the addition of several different vitamins, nucleotides, and alternative carbon sources had minimal effects on the level of DppA (data not shown). Osmotic shock treatment of overnight cultures of JM101 and W3110 resulted in the release of DppA into the supernatant, suggesting that it is localized in the periplasmic space (Fig. 1A and B, lanes 6 to 9).

Genetic and physical mapping of the gene encoding DppA. Since the function of DppA was unknown, there was no way to genetically map its structural gene, *dppA*, directly. However, through Hfr-mediated crosses, it was found that when the region between min 70 and 90 was crossed from strains that made low levels of DppA (as determined from Coomassie-stained gels) into an F^- derivative of JM101 (E1222), expression of DppA from some of the resulting transconjugants was low, like that observed in the donor Hfr strain. Although these crosses only resulted in mapping an element that is responsible for the difference in expression between these strains, it was hoped that this element would be near the structural gene itself.

The crosses were carried out between Hfr strains shown in Table 1 (BW strains) and E1222. The BW strains had the following important characteristics (40); (i) a Tn*10* inserted near the origin of transfer, (ii) low DppA level when grown in minimal medium (like W3110), and (iii) sensitivity to streptomycin (for counterselection). The recipient in these crosses was E1222, an F^- Sm^r derivative of JM101, and transconjugants were selected on LB plates with streptomycin and tetracycline. Recombinants were grown in minimal medium and screened for their DppA level by SDS-PAGE. Only with BW6159, BW6169, and BW6166 as donors were low-level DppA-containing recombinants recovered; frequencies were 48, 8, and 50%, respectively. From the locations of the origins and Tn*10* insertions in these Hfr donors, the region responsible for the difference in expression was localized between min 70 and 90.

To more precisely map this region, phage P1-mediated recombinations between JM101 and strains with selectable markers in this region of the chromosome were carried out. Specifically, when AB1157 (*xyl mtl*, low DppA level) was transduced to xyl^+ by using P1 grown in JM101 (xyl^+ mtl^+, high DppA level), 17% of the transductants were high DppA expressors, demonstrating that the element responsible for the expression difference was less than 2 min from *xyl* (min 80). No linkage was observed between this locus and *mtl* (min 81), suggesting that it lies counterclockwise to *xyl*. This was confirmed by a three-factor cross between S1228 (*xyl zhj-428*::Tn*10*, low DppA level), in which the Tn*10* is between *xyl* and *mtl*, and JM101. P1 grown on S1228 was used to transduce JM101 to Tet^r, and the resulting recombinants were scored for *xyl* and DppA expression. The frequency of Tet^r *xyl* recombinants was higher than of the Tet^r, low-DppA-expressing class.

The mapping described above suggested that the gene encoding DppA (*dppA*) might be near min 80. The following experiment was carried out to test this hypothesis and physically locate *dppA*. First, the amino-terminal sequence of purified DppA was determined, and degenerate oligonucleotides based on that sequence were prepared (Fig. 2A). All four oligonucleotides were used as hybridization probes against DNA from λ clones that contained DNA fragments from the min 78 to 80 region of the W3110 chromosome (Fig. 2B) (18). Figure 2C shows that two of these phages, λ9F6 and λ1D1, hybridized strongly to the probes, suggesting that *dppA* (at least the 5′ end) was contained in these clones. Restriction enzyme maps of λ9F6 and λ1D1 were constructed, and the region of hybridization was narrowed to a 0.5-kbp *Bam*HI-*Eco*RI fragment. This fragment was cloned into the vectors M13mp18 and M13mp19, and the DNA sequence was determined. An open reading frame (ORF) was identified 231 bp from the *Bam*HI site that matched the amino acid sequence of DppA in all but the first position; DppA began with Asp-Thr, and the ORF contained codons for Lys-Thr. To obtain the analogous sequence from E1222, a *Sau*3A partial library of chromosomal DNA was made in the vector λEMBL4, and clones were identified that hybridized to the 1.5-kbp *Bam*HI fragment from λ9F6 that contained the putative *dppA* 5′ end. DNA fragments from one of these clones, λ10, were used to generate subclones in the vector pBS to use as sequencing templates and for generating RNA probes.

DNA sequence of *dppA*. The DNA sequence of 1,950 bp from the λ10-derived subclones revealed an ORF (nucleotides 231 to 1751) that corresponded to the size and amino-terminal sequence of DppA (Fig. 3). The ORF, beginning with Lys-231, appears to encode a processed form of the protein, since there is an adjoining upstream sequence that resembles a typical bacterial signal sequence (38, 39). Assignment of the beginning of this putative signal sequence is complicated by the fact that there are three in-frame Met codons that could potentially serve to signal translation

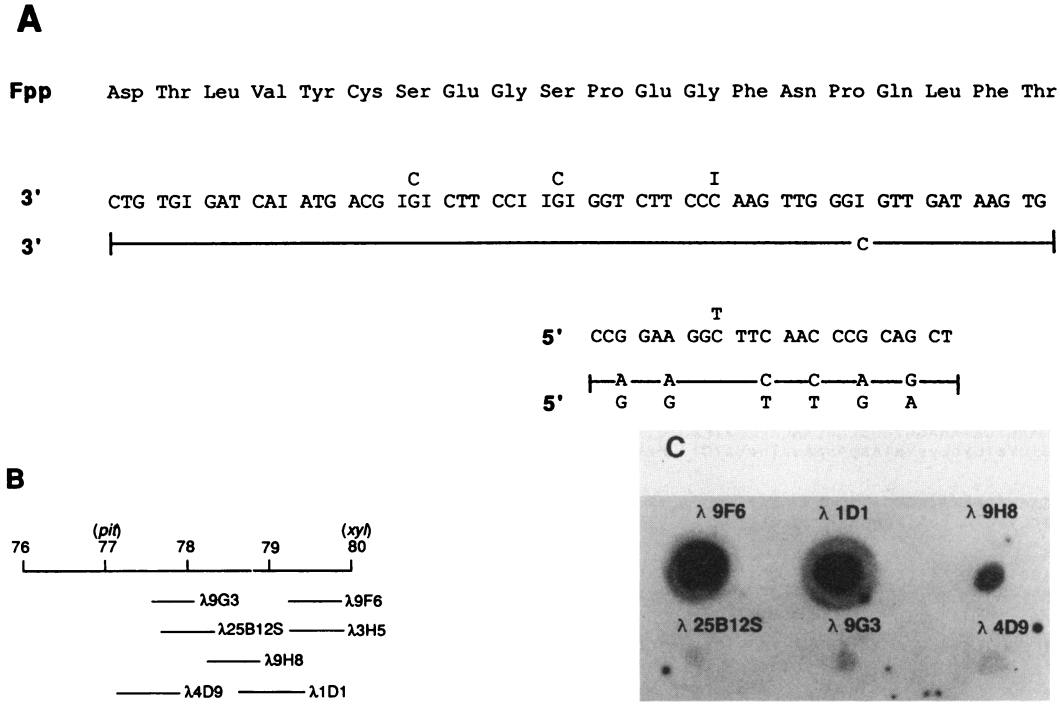

FIG. 2. Localization of DppA coding region. (A) Amino terminus of DppA and the four oligonucleotides used as probes. Nucleotides listed above or below the sequence were included at that position during synthesis at a ratio of 1:1 with the other nucleotide. The solid line represents positions where the oligonucleotide was identical to the one above it. (B) Genetic map of the min 79 region of *E.coli* W3110 with respect to the location of DNA fragments in recombinant phages derived from that region (20). (C) Hybridization of ^{32}P-labeled probes shown in panel A to DNA isolated from phages shown in panel B.

initiation (Met-204, Met-174, and Met-146). From theoretical considerations, a signal sequence beginning with Met-204 would be too short to function effectively. Translation initiating at Met-146 would result in a leader peptide of 28 amino acids, having three positive charged residues within the first 7 and a core rich in hydrophobic residues, and would end with a sequence fitting the −3, −1 rule for signal peptide cleavage sites.

Expression of *dppA* from a plasmid. To prove that the ORF identified above encoded DppA, an expression plasmid (pfppl-2) was constructed containing a DNA fragment extending from nucleotide 146 to a *Pst*I site approximately 1 kb downstream from the end of the ORF (see Materials and Methods). The fragment was placed downstream from the *lac* promoter on a pBR322-based replicon (pSRlac) and introduced into JM101. A protein was observed in these cells that, for the following reasons, we believe to be DppA: (i) it was the same size as purified DppA (Fig. 4A and B, lane 1), (ii) synthesis was induced upon addition of IPTG (isopropylthiogalactopyranoside) (Fig. 4A and B, lanes 4 and 5), (iii) it was localized to the periplasm (Fig. 4A and B, lanes 6 through 9), and (iv) it reacted with polyclonal antibody made against purified DppA (Fig. 4B).

DppA is similar to the peptide transport protein OppA. Comparison of *dppA* with DNA sequences in Gen-Bank and of the deduced DppA amino acid sequence with proteins in the NBRF protein data base revealed that DppA has weak similarity to the oligopeptide-binding protein OppA from *S. typhimurium* (Fig. 5) (13). In fact, with these parameters (Fig. 5 legend), it was more similar to OppA than any other protein or ORF in the data base. Of the 234 amino acids marked as being identical or conservative substitutions, 128 were identical. Especially noteworthy are the regions from residues 76 to 83 of DppA, in which 7 residues are identical, and from 300 to 323, in which 13 are identical.

***dppA* is required for dipeptide utilization.** Two questions were asked concerning the function of DppA. One, is it an essential *E. coli* protein? Two, because of the similarities in size, amino acid sequence, and cellular location to OppA, is it involved in peptide transport? More specifically, since the genes and proteins specific for dipeptide transport have not been clearly identified, could DppA be a periplasmic binding protein for this transport pathway? To address these issues, a DNA fragment (Kan) encoding kanamycin resistance was inserted into *dppA* in both the JM101 and W3110 chromosomes (see Materials and Methods for details). Briefly, the procedure involved integrating a λ derivative that carried *dppA20*::Kan into *dppA* in the chromosome, resulting in two copies of *dppA*, one wild type and one with the Kan^r insertion. The resulting strain was cured of the prophage, resulting in only one of the *dppA* alleles remaining in the chromosome. If *dppA* were essential, only cured cells that retained the *dppA*$^+$ allele would survive. Alternatively, if *dppA* was dispensable under these conditions, cured cells containing the *dppA20*::Kan allele would also survive. For both JM101 and W3110 derivatives, both Kan^r and Kan^s cured cells were isolated, showing that *dppA* is not an essential gene. The presence of the Kan^r insertion in these derivatives was confirmed by obtaining the expected banding pattern on Southern blots (data not shown) and demonstrating that the strains did not produce DppA (Fig. 4C). The resulting alleles in the W3110 and JM101 backgrounds were called *dppA20*::Kan and *dppA30*:Kan, respectively.

To determine whether *dppA* was involved in peptide

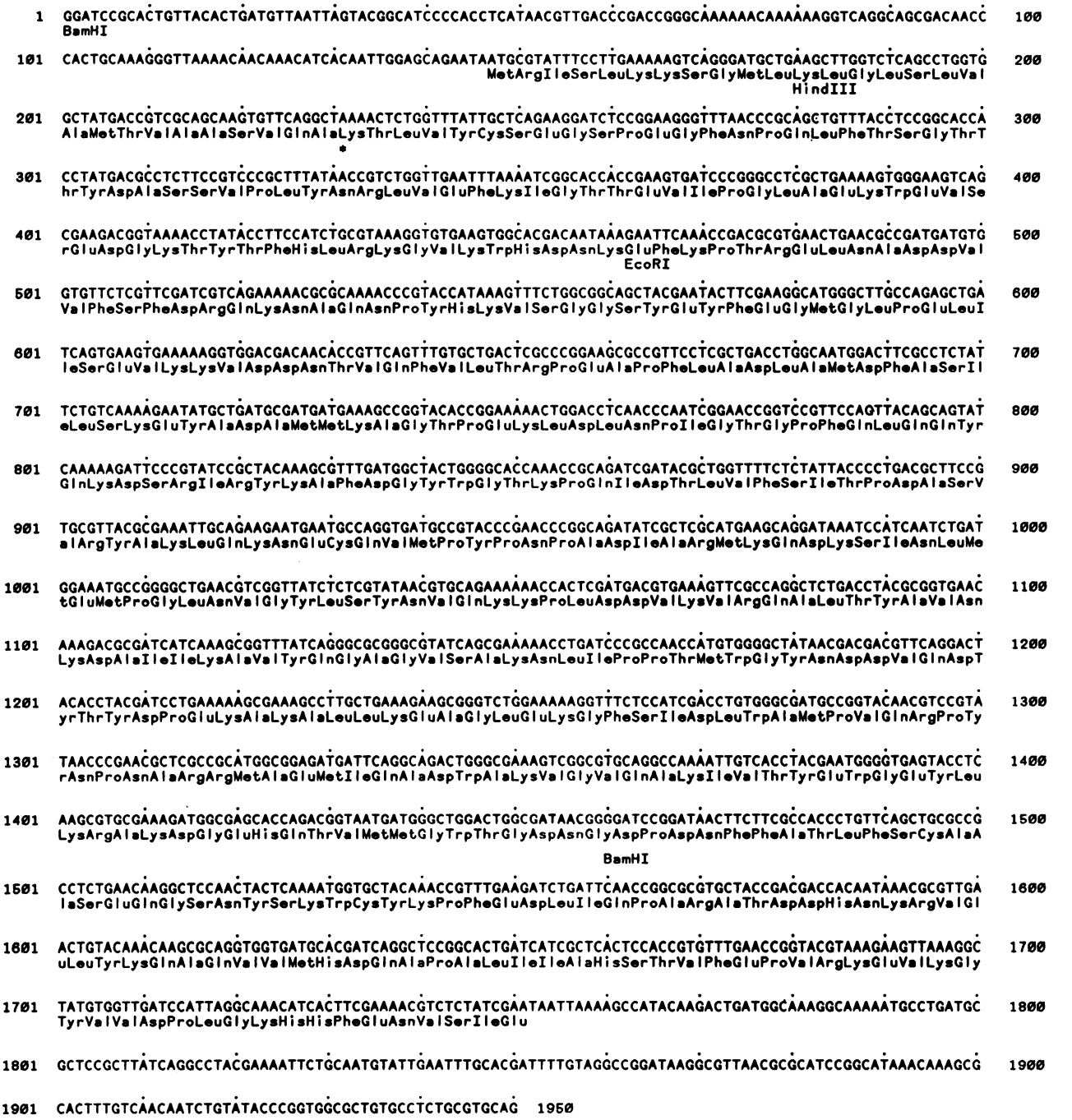

FIG. 3. DNA sequence of *dppA*. Sequence derived from subclones of fragments from λ10. The ORF corresponding to *dppA* is indicated. The signal peptide cleavage site is indicated (*).

transport, an in vivo assay was used based on the observation that amino acid auxotrophies could be phenotypically corrected by transporting into the cytoplasm a peptide containing the required amino acid. Derivatives of JM101 and W3110 that were *pro* and either *dppA*$^+$ or *dppA*::Kan were tested for growth in M9-glucose containing either proline or various proline-containing peptides. The results from one of these experiments (with the W3110 derivatives E1769 and E1772) are shown in Fig. 6 (a similar result was obtained with JM101 derivatives; data not shown). The role of *dppA* in tripeptide transport was not thoroughly investigated (primarily due to the presence of the other peptide transport systems); however, cells carrying the *dppA20*::Kan allele were checked for their ability to utilize the tripeptide Pro-Gly-Gly as a proline source. In this experiment, E1769 and E1772 grew to an OD_{550} of 0.48 and 0.50, respectively, in M9-glucose supplemented with the tripeptide at a final concentration of 100 μM, indicating that this system is distinct from one or more of the systems involved in the uptake of this tripeptide. This result also

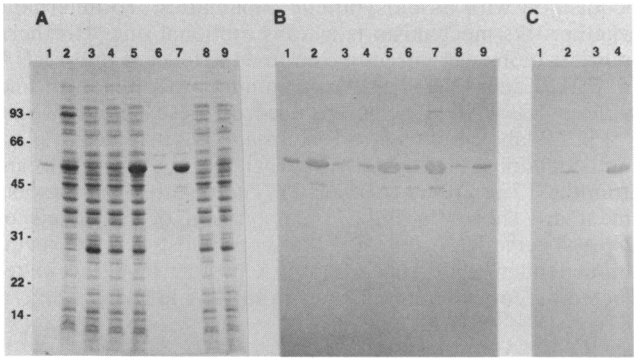

FIG. 4. DppA expression from pfppl-2 and loss of expression in W3110 and JM101 carrying *dppA*::Kan. (A) Coomassie-stained 14% polyacrylamide–SDS gel. All cultures were grown in M9-glucose with or without 1 mM IPTG as noted. Lanes: 1, purified DppA; 2, JM101; 3, E1838 plus IPTG; 4, E1859; 5, E1859 plus IPTG; 6, E1859 osmotic shock supernatant; 7, E1859 plus IPTG osmotic shock supernatant; 8, E1859 osmotic shock pellet; 9, E1859 plus IPTG osmotic shock pellet. (B) Western blot of duplicate gel shown in panel A reacted with anti-DppA antibody and protein A-horseradish peroxidase. (C) Western blot treated as in panel B with extracts isolated from E1614 (lane 1), W3110 (lane 2), E1847 (lane 3), and JM101 (lane 4).

suggests that the mutation does not confer on the cell an inability to use peptides in general or proline-containing peptides specifically.

Since the *dppA20*::Kan mutation is an insertion, it is possible that *dppA* was not required for dipeptide utilization, but rather the Kan^r insertion was polar on the expression of a downstream gene encoding a function that is both necessary and sufficient for Pro-Gly utilization. To test this, two plasmids, the *dppA* expression plasmid pfppl-2 (see above) and pACDF (a pACYC184 derivative containing a 9-kbp DNA fragment extending from the *Eco*RI site in *dppA* to an *Eco*RI site downstream, see Materials and Methods), were introduced into the *pro dppA20*::Kan and *dppA30*::Kan derivatives, and growth on Pro-Gly was determined. Only when both plasmids were present were the insertion mutants complemented for growth on Pro-Gly (data not shown). This result shows that *dppA* is necessary but not sufficient for dipeptide utilization. Although it is not known exactly what sequence downstream from *dppA* is required in addition to *dppA* to complement the Kan^r insertion mutation, this result was not unexpected. The organization of genes encoding periplasmic transport systems generally consists of the periplasmic binding protein gene first, followed by genes encod-

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DppA  ....MRISLKKSGMLK..LGLSLVAMTVAASVQ...AKTLVYCSEGSPEG 41
OppA  MSNITKKSLIAAGILTALIAATPTAADVPAGVQLADKQTLVRNNGSEVQS 50

  42  FNPQLFTSGTTYDASSVPLYNRLVEFKIGTTEVIPGLAEKWEVSEDGKTY 91
  51  LDPHKIEGVPESNVSRDLFEGLLI..SDVEGHPSPGVAEKWE.NKDFKVW 97

  92  TFHLRKGVKWHDNKEFKPTRELNADDVVFSFDRQKNAQNPYHKVSGGSYE 141
  98  TFHLRENAKWSDGT......PVTAHDFVYSWQRLADPNTASPYASYLQYG 141

 142  YFEGMG......LPELISEVKKVDDNTVQFVLTRPEAPFLADLAMDFASI 185
 142  HIANIDDIIAGKKPATDLGVKALDDHTFEVTLSEPVPYFYKLLVHPSVSP 191

 186  LSKEYADAMMKAGTPEKLDLNPIGTGPFQLQQYQKDSRIRYKAFDGYWGT 235
 192  VPK...SAVEKFGDKWTQPANIVTNGAYKLKNWVVNERIVLERNPQYWDN 238

 236  KPQIDTLVFSITPDASVRYAKLQKNECQVMPYPNPAD..IARMKQDKSIN 283
 239  AKTVINQVTYLPISSEVTDVNRYRSGEIDMTYNNMPIELFQKLKKEIPNE 288

 284  LMEMPGLNVGYLSYNVQKKPLDDVKVRQALTYAVNKDAIIKAVYQGAGVS 333
 289  VRVDPYLCTYYYEINNQKAPFNDVRVRTALKLALDRDIIVNKVKNQGDLP 338

 334  AKNLIPPTMWGYNDDVQDY.....TYDPEKAKALLKEAGLEKGFSIDLWA 378
 339  AYSYTPPYTDGAKLVEPEWFKWSQQKRNEEAKKLLAEAGFTADKPLTFDL 388

 379  MPVQRPYNPNARRMAEMIQADWAK.VGVQAKIVTYEWGEYLKRAKDGEHQ 427
 389  LYNTSDLH...KKLAIAVASIWKKNLGVNVNLENQEWKTFLDTRHQGTFD 435

 428  TVMMGWTGDNGDPDNFFATLFSCAASEQGSNYSKWCYKPFEDLIQPARAT 477
 436  VARAGWCADYNEPTSFLNTML....SDSSNNTAHYKSPAFDKLIADTLKV 481

 478  DDHNKRVELYKQAQVVMHDQAPALIIAHSTVFEPVRKEVKGYVVDPLGKH 527
 482  ADDTQRSELYAKAEQQLDKDSAIVPV....YYYVNARLVKPWVGGYTGKD 527

 528  HFENVSIE.......  535
 528  PLDNIYVKNLYIIKH  542
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FIG. 5. Comparison of DppA and OppA. *E. coli* DppA and OppA from *S. typhimurium* (15) were compared by using the GAP program from the University of Wisconsin Genetics Computer Sequence Analysis software package. The gap weight was set at 5 and the length weight was set at 0.3. Lines join identical and related amino acids.

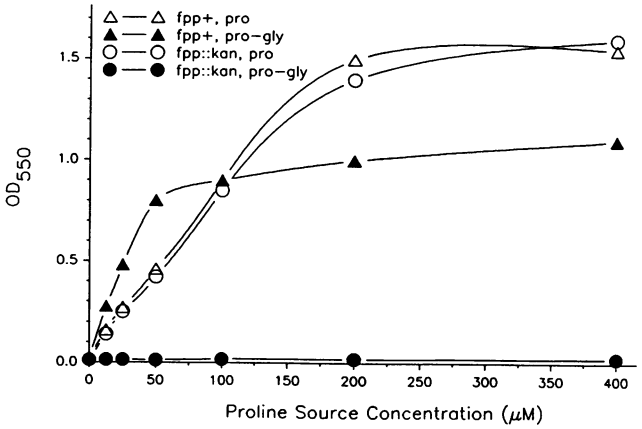

FIG. 6. Growth of *dppA*$^+$ and *dppA20*::Kan strains in Pro-Gly. Cells were grown overnight in M9-glucose-proline, centrifuged, washed, resuspended in 1× M9 salts, and diluted 1:100 in M9-glucose supplemented with either proline (open symbols) or Pro-Gly (solid symbols) at the indicated concentration. Symbols: △, ▲, E1769; ○, ●, E1772. The cultures were grown at 37°C for 18 h, and the OD_{550} was measured.

ing inner membrane components that are also required for transport.

***dppA*-dependent dipeptide utilization is not dependent on the major proline transport systems.** *dppA*-dependent utilization of Pro-Gly could take place by one of the following mechanisms: (i) active transport of the dipeptide across the inner membrane, followed by cleavage in the cytoplasm, or (ii) DppA cleavage of Pro-Gly in the periplasm, followed by transport of free proline into the cytoplasm. The second possibility was ruled out by showing that a proline auxotroph defective for the three major proline transport systems (strain WG208) could still utilize Pro-Gly. The OD_{550} of WG208 grown in M9-glucose with either 50 μM proline or 50 μM Pro-Gly was 0.03 and 0.90, respectively. That this growth was *dppA* dependent was demonstrated by introducing the *dppA20*::Kan allele into WG208 and showing that the resulting strain, E1974, could no longer use Pro-Gly (OD_{550} in M9 with 50 μM proline was 0.03). These results show that *dppA*-dependent Pro-Gly utilization does not require the normal proline transport systems, and therefore, DppA is part of a transport system for dipeptides rather than merely a Pro-Gly dipeptidase.

The ability of the DppA system to recognize a dipeptide other than Pro-Gly was tested in a competition assay. This assay was based on the assumption that if another peptide could be recognized by DppA, that peptide would lower the growth of a proline auxotroph in limiting concentrations of Pro-Gly. E1769 was grown overnight in M9 supplemented with either proline, Pro-Gly, or a combination of proline plus His-Glu or Pro-Gly plus His-Glu. Proline and the dipeptides were all used at 100 μM. The resulting growth was measured by the OD_{550}. The OD_{550} of overnight cultures was as follows: no proline source, 0.06; proline, 1.6; Pro-Gly, 0.42; proline plus His-Glu, 1.6; Pro-Gly plus His-Glu, 0.07. Thus, the presence of His-Glu blocked the ability of the cells to use Pro-Gly, indicating that the pathway involving DppA also recognizes other dipeptides.

***dppA* expression is regulated at the level of mRNA.** Identifying the mechanism responsible for the differential expression of *dppA* (as shown in Fig. 1) would allow a better understanding of how the cell regulates its external nutrient acquisition with its biosynthetic capabilities. To determine whether this mechanism is a transcriptional one, Northern hybridizations were used to examine the level of *dppA* RNA in W3110 and JM101 grown in minimal medium with and without 2% CAA. The probe used in these studies was a [^{32}P]CTP-labeled antisense RNA made in vitro from a plasmid containing a fragment internal to *dppA* downstream from the T7 promoter (pBSEB-2). The results of this experiment are shown in Fig. 7. Comparison of the levels of *dppA*-specific RNA revealed that both the strain- and medium-dependent differences in DppA protein levels were reflected by concomitant changes in mRNA levels.

DISCUSSION

Reported here is the identification and characterization of a gene, *dppA*, that encodes a periplasmic protein that appears to be a structural component of a dipeptide permease. The major results described in this work include (i) genetic and physical mapping of *dppA*, (ii) DNA sequence of *dppA*, (iii) examination of *dppA* involvement in dipeptide transport, (iv) identification of conserved amino acid residues between DppA and the periplasmic oligonucleotide-binding protein OppA, (v) DppA cellular location, and (vi) transcriptional regulation of *dppA* expression.

The data presented argue that DppA is part of a general dipeptide transport system. Most compelling is the observation that disruption of the chromosomal copy of *dppA* by a Kan^r insertion eliminates the ability of Pro-Gly to phenotypically correct proline auxotrophy (Fig. 6). The following alternative explanations for this result were addressed experimentally. (i) *dppA* is involved in some other aspect of proline utilization. This was ruled out by showing that growth in minimal medium containing proline was identical with isogenic *dppA*$^+$ and *dppA20*::Kan strains (Fig. 6). (ii) DppA cleaved Pro-Gly in the periplasm, releasing free proline. This hypothesis does not seem likely, since Pro-Gly utilization was not dependent on any of the three major proline transport systems. (iii) DppA is not involved in

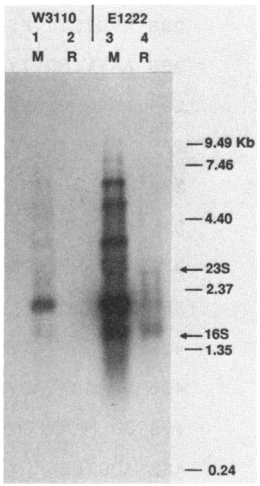

FIG. 7. Northern hybridization of RNA isolated from W3110 and JM101. RNA was isolated, electrophoresed, blotted, and hybridized to a *dppA* antisense probe as described in Materials and Methods. The sizes and migration of RNA standards are shown on the right along with the migration of 23S and 16S rRNAs. M, M9-glucose; R, M9–glucose–2% CAA.

Pro-Gly utilization, but rather the Kan insertion in *dppA* is polar on the expression of a downstream gene(s) that is both necessary and sufficient for transport. The finding that expression of *dppA* (from pfppl-2), as well as downstream genes (in pACDF), was required for complementation of the *dppA20*::Kan mutation ruled out this possibility.

Osmotic-shockable transport systems of gram-negative bacteria are responsible for the uptake of many types of small molecules, including amino acids, sugars, vitamins, ions, and oligopeptides (2). The properties of DppA and its role in dipeptide transport lead to the suggestion that DppA is part of an osmotic-shockable transport system. More specifically, DppA appears to be the periplasmic binding protein for this permease. Although direct biochemical evidence has not been obtained (i.e., we have not attempted in vitro binding of dipeptides by DppA), comparison of DppA and OppA supports this hypothesis. Since both proteins are localized to the periplasm, are similar in size, and have several conserved amino acids, we think it is likely that they function similarly in peptide transport. One obvious possibility is that the conserved amino acids constitute part of a dipeptide recognition domain, possibly involved in recognizing one of the structural features common to dipeptides (i.e., amino or carboxyl terminus or the peptide bond).

Another feature common to osmotic-shockable transport systems is the genetic organization of the structural genes (2). Typically, the first gene in the operon encodes the periplasmic binding protein, followed by genes encoding at least two inner membrane components. If *dppA* encodes a periplasmic binding protein, it would not be surprising to discover genes downstream that are also required for dipeptide transport. The finding that pACDF (containing a 9-kbp fragment beginning in *dppA* and extending downstream with respect to *dppA*) was required, in addition to pfppl-2, to complement the *dppA20*::Kan mutation suggests that such genes reside in the same operon as *dppA*. However, the most abundant *dppA*-hybridizing RNA species (Fig. 7) appears to be too small to encode a multigene operon characteristic of this type of transport system. Either this gene is not part of an operon, or else the higher-molecular-weight RNAs present in the induced culture (although less abundant) represent more of the operon. We think the latter possibility is more likely. If this is correct, then the most abundant band would result from some sort of RNA processing or transcription termination event. Since the promoter for *dppA* has not been identified and the 3′ and 5′ ends of the RNA species shown in Fig. 7 have not been localized, the events that lead to these RNA species are unknown.

The ability to genetically map *dppA* was based on the difference in expression levels between JM101 and most other K-12 strains (including the BW series of Hfr strains). When the min 79 region of the chromosome was crossed from these strains into the JM101 derivative E1222, expression of *dppA* decreased. Conversely, when this region was crossed from JM101 into other strains (specifically W3110 and S1228), *dppA* expression increased to resemble that of JM101. Although we do not know what accounts for the difference in expression, steady-state levels of *dppA* mRNA (Fig. 7) suggest that the two strains differ in a transcriptional element (i.e., promoter, enhancer, repressor, or activator) involved in *dppA* expression.

Transcription of *dppA* is repressed by the presence of CAA (Fig. 7). Thus, the cell alters its ability to transport dipeptides as its nutritional environment changes. This is similar to what is observed with amino acid transport systems. Although each pathway is slightly different, in general, high nitrogen availability represses the expression of genes encoding these systems. In the case of cells grown in minimal medium, it appears that the cell senses a need for amino acids and responds, in part, by inducing transcription of *dppA* (and thus dipeptide transport capabilities). The components of this signaling system and the mechanism for controlling *dppA* transcription have not been identified.

As described in the introduction, a 49-kDa periplasmic protein in *E. coli* has been implicated as being involved in the ability of the cell to utilize and chemotaxis toward dipeptides (24). This protein, called Dpp, appears to be the same as the protein reported here (23a). Manson et al. (24) also reported that *S. typhimurium* made Dpp. Consistent with this is our observation that extracts from *S. typhimurium* LT2 have a protein of about 50 kDa that reacts with antibody to DppA (unpublished data).

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REFERENCES

1. **Alloing, G., M.-C. Trombe, and J.-P. Claverys.** 1990. The *ami* locus of the gram-positive bacterium *Streptococcus pneumoniae* is similar to binding protein-dependent transport operons of gram-negative bacteria. Mol. Microbiol. **4**:633–644.
2. **Ames, G. G.-L.** 1986. Bacterial periplasmic transport systems: structure, mechanism, and evolution. Annu. Rev. Biochem. **55**:397–425.
3. **Andrews, J. C., and S. A. Short.** 1985. Genetic analysis of *Escherichia coli* oligopeptide transport mutants. J. Bacteriol. **161**:484–492.
4. **Andrews, J. C., and S. A. Short.** 1986. *opp-lac* fusions and transcriptional regulation of the *Escherichia coli trp*-linked oligopeptide permease. J. Bacteriol. **165**:434–442.
5. **Baron, L. S., E. Penido, I. R. Ryman, and S. Falkow.** 1970. Behavior of coliphage λ in hybrids between *Escherichia coli* and *Salmonella*. J. Bacteriol. **102**:221–233.
6. **Campillo-Campbell, A. D., and A. Campbell.** 1982. Molybdenum cofactor requirement for biotin sulfoxide reduction in *Escherichia coli*. J. Bacteriol. **149**:469–478.
7. **De Felice, M., J. Guardiola, A. Lamberti, and M. Iaccarino.** 1973. *Escherichia coli* K-12 mutants altered in the transport systems for oligo- and dipeptides. J. Bacteriol. **116**:751–756.
8. **Frischauf, A., H. Lehrach, A. Poustka, and N. Murray.** 1983. Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. **170**:827–842.
9. **Gibson, M. M., M. Price, and C. F. Higgins.** 1984. Genetic characterization and molecular cloning of the tripeptide permease (*tpp*) genes of *Salmonella typhimurium*. J. Bacteriol. **160**:122–130.
10. **Gottesman, M. E., and M. B. Yarmolinsky.** 1968. Integration-negative mutants of bacteriophage λ. J. Mol. Biol. **31**:487–505.
11. **Grothe, S., R. L. Krogsrud, D. J. McClellan, J. L. Milner, and J. M. Wood.** 1986. Proline transport and osmotic stress response in *Escherichia coli* K-12. J. Bacteriol. **166**:253–259.
12. **Guyer, C. A., D. G. Morgan, N. Osheroff, and J. V. Staros.** 1985. Purification and characterization of a periplasmic oligopeptide binding protein from *Escherichia coli*. J. Biol. Chem. **260**: 10812–10818.
13. **Higgins, C. F., and M. M. Gibson.** 1986. Peptide transport in bacteria. Methods Enzymol. **125**:365–377.
14. **Higgins, C. F., and M. M. Hardie.** 1983. Periplasmic protein

associated with the oligopeptide permeases of *Salmonella typhimurium* and *Escherichia coli*. J. Bacteriol. **155**:1434–1438.
15. **Hiles, I. D., M. P. Gallagher, D. J. Jamieson, and C. F. Higgins.** 1987. Molecular characterization of the oligopeptide permease of *Salmonella typhimurium*. J. Mol. Biol. **195**:125–142.
16. **Hill, C. W., and B. W. Harnish.** 1981. Inversions between ribosomal RNA genes of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **78**:7069–7072.
17. **Hogarth, B. G., and C. F. Higgins.** 1983. Genetic organization of the oligopeptide permease (*opp*) locus of *Salmonella typhimurium* and *Escherichia coli*. J. Bacteriol. **153**:1548–1551.
18. **Howard-Flanders, P., R. P. Boyce, and L. Theriot.** 1966. Three loci in *Escherichia coli* K12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics **53**:1119–1139.
19. **Jamieson, D. J., and C. F. Higgins.** 1984. Anaerobic and leucine-dependent expression of a peptide transport gene in *Salmonella typhimurium*. J. Bacteriol. **160**:131–136.
20. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50**:495–508.
21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
22. **Lenny, A. B., and P. Margolin.** 1980. Locations of the *opp* and *supX* genes of *Salmonella typhimurium* and *Escherichia coli*. J. Bacteriol. **143**:747–752.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23a. **Manson, M. D. (Texas A&M).** Personal communication.
24. **Manson, M. D., V. Blank, G. Brade, and C. F. Higgins.** 1986. Peptide chemotaxis in *E. coli* involves the Tap signal transducer and the dipeptide permease. Nature (London) **321**:253–256.
25. **Matthews, D. M.** 1987. Mechanisms of peptide transport. Beitr. Infusionther. Klin. Ernaehr. **17**:6–53.
26. **Messing, J.** 1979. A multipurpose cloning system based on the single-stranded DNA bacteriophage M13. Recombinant DNA Tech. Bull. **2**:43–48.
27. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–78.
28. **Miller, J. H.** 1982. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Monroy, A. F.** 1988. Staining immobilized RNA ladder. BRL Focus **10**:1.
30. **Morse, L. S., L. Pereira, B. Roizman, and P. A. Schaffer.** 1978. Anatomy of herpes simplex virus DNA. X. Mapping of viral genes by analysis of polypeptides and functions specified by HSV-1 × HSV-2 recombinants. J. Virol. **26**:389–410.
31. **Naider, F., and J. M. Becker.** 1988. Peptide transport in *Candida albicans*: implications for the development of antifungal agents. Curr. Top. Med. Mycol. **2**:170–198.
32. **Nossal, N. G., and L. A. Heppel.** 1966. The release of enzymes by osmotic shock from *Escherichia coli* in exponential phase. J. Biol. Chem. **241**:3055–3062.
33. **Payne, J. W., and C. Gilvarg.** 1978. Transport of peptides in bacteria, p. 325–383. *In* B. P. Rosen (ed.), Bacterial transport. Marcel Dekker, New York.
34. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
35. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
36. **Sisk, W. P., J. G. Chirikjian, J. Lautenberger, C. Jorcyk, T. S. Papas, M. L. Berman, R. Zagursky, and D. L. Court.** 1986. A plasmid vector cloning and expression of gene segments: expression of an HTLV-I envelope gene segment. Gene **48**:183–193.
37. **Towbin, H., T. Staehelin, and J. Gordon.** 1989. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
38. **von Heijne, G.** 1983. Patterns of amino acids near signal-sequence cleavage sites. Eur. J. Biochem. **133**:17–21.
39. **von Heijne, G.** 1985. Signal sequences: the limits of variation. J. Mol. Biol. **184**:99–105.
40. **Walker-Smith, D. J., and J. W. Payne.** 1984. Characteristics of the peptide-transport system in the scutellum of germinating barley embryos. Planta **162**:166–173.
41. **Wanner, B. L.** 1986. Novel regulatory mutants of the phosphate regulon in *Escherichia coli* K-12. J. Mol. Biol. **191**:39–58.
42. **Zagursky, R. J., K. Baumeister, N. Lomax, and M. L. Berman.** 1985. Rapid and easy sequencing of large linear double-stranded DNA and supercoiled plasmid DNA. Gene Anal. Techniques **2**:89–94.