JOURNAL OF BACTERIOLOGY, Nov. 1983, p. 481–486
0021-9193/83/110481-06$02.00/0
Copyright © 1983, American Society for Microbiology
Vol. 156, No. 2

Two Proline Porters in *Escherichia coli* K-12

MARY E. STALMACH, SUZANNE GROTHE, AND JANET M. WOOD*

Guelph-Waterloo Centre for Graduate Work in Chemistry, University of Guelph, Guelph, Ontario, Canada N1G2W1

Received 20 May 1983/Accepted 1 August 1983

Escherichia coli mutants defective at *putP* and *putA* lack proline transport via proline porter I and proline dehydrogenase activity, respectively. They retain a proline uptake system (proline porter II) that is induced during tryptophan-limited growth and are sensitive to the toxic L-proline analog, 3,4-dehydroproline. 3,4-Dehydroproline-resistant mutants derived from a *putP putA* mutant lack proline porter II. Auxotrophic derivatives derived from *putP*$^+$ or *putP* bacteria can grow if provided with proline at low concentration (25 μM); those derived from the 3,4-dehydroproline-resistant mutants require high proline for growth (2.5 mM). We conclude that *E. coli*, like *Salmonella typhimurium*, possesses a second proline porter that is inactivated by mutations at the *proP* locus.

Escherichia coli K-12 actively accumulates L-proline. In this paper, we provide evidence that L-proline uptake in that organism is catalyzed by two independent uptake systems or porters. Active uptake via proline porter I (PP-I) is required if proline is to be utilized as the sole carbon or nitrogen source by *E. coli* or *Salmonella typhimurium*, and PP-I is inactivated by mutations at the *putP* locus in either organism (13, 14, 18, 19). Both genetic and biochemical data have revealed a second proline porter (PP-II) in *S. typhimurium* (1, 8). Proline auxotrophic strains of that organism defective at *putP* can grow if provided with L-proline at a low concentration (16 μM). Further mutations at the *proP* locus elevate the proline requirement 500-fold (8). The mutations at *proP* inactivate a second, proline-specific active uptake system that is induced during amino acid-limited growth (1). We call that system PP-II.

The proline analogs L-azetidine-2-carboxylate (AC) and 3,4-dehydro-D,L-proline (DHP) are toxic and can be used to select bacteria with altered metabolic and permeability characteristics. *E. coli* strains defective at *putP* show increased resistance to AC and DHP (18). Those defective at *putP* and the adjacent gene, *putA*, are resistant to AC but not DHP (18). Proline dehydrogenase, the *putA* gene product, is thought to detoxify DHP but not AC (18).

Beginning with a *putPA* deletion strain of *E. coli*, we have selected DHP-resistant mutants. We provide evidence here that those mutants are defective for a proline porter (PP-II) that is induced during tryptophan-limited growth. Genetic experiments suggest that PP-II is independent of the previously described proline uptake system, PP-I.

MATERIALS AND METHODS

Materials. All reagents used were from the sources described previously, and culture media were prepared as described before (20). Defined media were based on the morpholinopropanesulfonic acid medium described by Neidhardt et al. (12). Solid media contained D-glucose (2 mg/ml) and NH_4Cl (9.5 mM) as carbon and nitrogen sources, respectively; liquid media contained D-fructose (2 mg/ml) and NH_4Cl (9.5 mM) as carbon and nitrogen sources, respectively, unless otherwise stated.

Strains. The bacterial strains prepared and used during this study, all derivatives of *E. coli* K-12, are listed in Table 1. All genetic manipulations were performed as previously described (10, 18). Strains WG139, WG140, WG141, WG148, WG174, and WG175 are P1 transductants selected on LB medium (10) containing kanamycin sulfate (75 μg/ml). The proline auxotrophy of WG139, WG140, and WG141 was confirmed by streaking on defined media (see below), the *put* defects of WG148 and WG175 were confirmed by streaking on triphenyltetrazolium chloride-containing indicator medium and by the radial streak test (20), and the *proP* lesion of WG174 was confirmed by the radial streak test ([20] see below). Strain WG143 was selected on defined medium containing D-lactose (2 mg/ml) as the carbon source, and its L-proline auxotrophy was confirmed by streaking on defined media (see below). Strain WG145 is a uracil auxotrophic Tn*10* insertion mutant of CSH4 isolated as described by Davis et al. (5). Strains WG146, WG147, and WG95 are P1 transductants selected on LB medium (10) containing tetracycline hydrochloride (25 μg/ml). Their uracil auxotrophy was confirmed by streaking on defined media and their ability or inability to utilize L-proline was confirmed by streaking on triphenyltetrazolium chloride indicator medium (20). Strains WG146 and WG147 are transductants of RM2 from WG145. Strain WG146 is 1 of 44 *put*$^-$ transductants, and strain WG147 is 1 of 3 *put*$^+$ transductants, arising from that cross. Strain WG138 is a uracil

TABLE 1. *E. coli* K-12 strains[a]

Strain	Genotype	Source or Derivation
CBK130	F⁻ *thyA36 proC*::Tn*5*	Claire Berg, University of Connecticut
CSH4	F⁻ *trp lacZ rpsL thi*	Cold Spring Harbor Laboratory (10)
JT31	CSH4 *putA1*::Tn*5*	This laboratory (18, 20)
JT34	CSH4 *putP3*::Tn*5*	This laboratory (18, 20)
RM2	CSH4 Δ*(putPA)101*	Rolf Menzel, University of Utah (18, 20)
WG95	CSH4 *pyr-76*::Tn*10 proP219*	P1 transductant of WG170 from WG146
WG138	CSH4 *proP219*	P1 transductant of WG95 from CSH4
WG139	RM2 *lac*⁺ *proC*::Tn*5*	P1 transductant of RM2 from CBK130
WG140	CSH4 *proC*::Tn*5*	P1 transductant of CSH4 from CBK130
WG141	WG170 *lac*⁺ *proC*::Tn*5*	P1 transductant of WG170 from CBK130
WG143	WG174 *lac*⁺ *proC*::Tn*5*	P1 transductant of WG174 from CBK130
WG145	CSH4 *pyr-76*::Tn*10*	See Materials and Methods
WG146	CSH4 *pyr-76*::Tn*10*	See Materials and Methods
WG147	RM2 *pyr-76*::Tn*10*	See Materials and Methods
WG148	CSH4 *putA1*::Tn*5 proP219*	P1 transductant of WG170 from JT31
WG170	RM2 *proP219*	See Results
WG171	RM2 *proP220*::Tn*5*	See Results
WG174	RM2 *proP220*::Tn*5*	See Results
WG175	WG170 *putP3*::Tn*5*	P1 transductant of WG170 from JT34

[a] The genetic nomenclature is that of Bachmann and Low (2) with the exception of *proP*, which is defined in the text. The deletion in strain RM2 was previously named, incorrectly, as Δ*(putPA)100* (18, 20).

prototrophic transductant of WG95 selected on defined medium lacking uracil.

Transport assays. Bacteria were cultured for transport experiments as previously described (17, 20) with the following modification for amino acid-limited cultures. Bacteria were cultured in LB medium (10) and subcultured in defined medium containing excess tryptophan (250 μM) as before (17, 20). When this subculture had reached an optical density (600 nm) of 1, 15 ml of culture was harvested by centrifugation, and the bacteria were resuspended in 50 ml of fresh medium containing only 24 μM L-proline. The second subculture was grown to an optical density of 1 and harvested for transport measurements as described previously (18, 20). Transport was measured by a filtration assay based on that of Berger and Heppel (3). The reaction mixture (0.5 or 1.0 ml) contained morpholinopropanesulfonic acid medium supplemented with chloramphenicol (0.5 mg/ml), D-glucose (0.2 mg/ml), bacteria (0.1 to 0.5 mg of protein per ml), and (unless otherwise stated) L-[^{14}C]proline (214 μM), L-[^{14}C]glutamine (20 μM), or [^{14}C]glycine (40 μM). The bacteria were incubated in the reaction mixture for 5 min at room temperature before initiation of the reaction with radioactive substrate. Samples (100 or 200 μl) were withdrawn at the indicated times and applied to 25-mm diameter, 0.45-μm pore size cellulose acetate filters (Gelman Sciences, Inc.) under a vacuum of 30 mm of Hg on a Hoefer filtration apparatus. Each filter was washed immediately with 10 ml of a solution containing 0.01 M Tris-hydrochloride, 0.15 M NaCl, and 0.5 mM $MgCl_2$ (pH 7.3), dried, and counted as described previously (3). The phenotypes of all cultures were thoroughly tested before harvesting to confirm the absence of contaminants or revertants.

Protein assay. The protein content of cell suspensions was determined by the method of Lowry et al. (6), with bovine serum albumin as a standard.

RESULTS

DHP-resistant mutants. Strain RM2 [Δ*(putPA)101*] is much more resistant to proline analog AC than to DHP (18). We propose that the DHP sensitivity of that strain depends on DHP uptake via a proline porter analogous to PP-II of *S. typhimurium* (1, 8). If so, DHP-resistant mutants of RM2 may lack that porter.

Strain RM2 was grown for approximately 16 h at 37°C in LB medium, and the bacteria were harvested by centrifugation and resuspended in an equal volume of saline (0.85% [wt/vol] NaCl). Spontaneous DHP-resistant mutants were selected by spreading 10^6 to 10^7 bacteria on defined medium containing DHP (50 μg/ml). They arose at a frequency of 4×10^{-5} after 2 days of incubation at 37°C. Tn*5* insertion mutants were isolated by spreading 10^6 bacteria suspended in saline from a Tn*5* insertion mutant stock of RM2 (18) on defined medium containing DHP (25 μg/ml). They arose at a frequency of 10^{-4} after 2 days of incubation at 37°C. The mutants were purified and tested for DHP resistance by radial streak (19). All of the mutants tested (29 spontaneous and 9 Tn*5* insertions) shared similar analog resistance phenotypes to each other and to 30 spontaneous mutants selected on medium

containing 25 μg of DHP per ml and 10 Tn*5* insertion mutants selected on medium containing 50 μg of DHP per ml, implying that the mutation selected was the same at both DHP concentrations. Strains WG170 (containing allele *pro-219*) and WG171 (allele *pro-220*::Tn*5*) were retained for further analysis.

To confirm that the DHP resistance of strain WG171 was attributable to a single insertion mutation, that insertion was moved by P1 transduction from WG171 back into strain RM2, selecting for kanamycin resistance. Five such transductions were performed, yielding cotransduction frequencies of DHP and kanamycin resistance of 41 of 49, 38 of 38, 13 of 48, 36 of 50, and 27 of 49. DHP-resistant strain WG174 was retained for further analysis. In a subsequent series of experiments, kanamycin resistance was moved by transduction from WG174 into strain CSH4, then back into strain RM2, again yielding DHP-resistant tranductants. These data show that the Tn*5* insertion and DHP resistance are linked. The variable frequency of cotransduction of kanamycin and DHP resistance may result from Tn*5* transposition during some of these transductions (15, 18).

Proline requirement. Proline auxotrophic strains of *S. typhimurium* that are defective at both *putP* and *proP* require a high concentration of L-proline for growth (8 mM), whereas *putP proP*$^+$ strains can grow in low proline medium (16 μM) (8). To determine whether our *E. coli* mutants would share those phenotypes, we have introduced proline auxotrophy into strains CSH4, RM2, WG170, and WG174 (see above). Glycyl-L-proline (0.1 mg/ml) was added as the proline source to all media on which auxotrophic derivatives of strains WG170 and WG174 were grown. No growth was observed if glycyl-L-proline or high levels of proline were omitted (see below).

To assess their proline requirement, the proline auxotrophs were asked to form isolated colonies on defined media at a series of L-proline concentrations. Bacteria from fresh LB (10) overnight cultures were harvested by centrifugation, resuspended, and diluted in saline. Dilution samples containing approximately 50 bacteria were spread on the test plates which were incubated for 36 h at 37°C. Strains WG140 (*proC*::Tn*5*) and WG139 [Δ(*putPA*)*101* *proC*::Tn*5*] formed colonies as large as 1 mm in diameter on medium containing 25 μM L-proline, whereas strains WG141 [Δ(*putPA*)*101* *pro-219* *proC*::Tn*5*] and WG143 [Δ(*putPA*)*101* *pro-220*::Tn*5* *proC*::Tn*5*] did not grow. Medium containing 2.5 mM L-proline supported strong growth of all four strains. Since these phenotypes correspond with those of the *proP* mutants of *S. typhimurium*, we hereafter refer to the mutations in strains WG170 and WG174 as *proP219* and *proP220*::Tn*5*, respectively.

Proline uptake activity. Mutations at the *proP* locus of *S. typhimurium* specifically eliminate a proline uptake activity that is induced during amino acid-limited growth (1). Since our strains are tryptophan auxotrophs, we have examined the effect of tryptophan limitation on their proline uptake activity. The growth rate of strain RM2 was tryptophan limited when 24 μM tryptophan was provided to batch cultures (M. Stalmach, M.S. thesis, University of Guelph, Ontario, Canada). Strain RM2 reverted to tryptophan prototrophy at a frequency of 2×10^{-8} when spread on defined medium lacking tryptophan. Routine analysis of batch cultures of strains RM2, WG170, and WG174 after tryptophan-limited growth failed to reveal any prototrophic revertants, however.

Proline uptake was measured by using 214 μM L-proline, since our previous data showed a high K_m for PP-II in *S. typhimurium* (1). Tryptophan limitation caused a threefold increase in L-proline uptake by strain RM2 [Δ(*putPA*)*101*; Table 2]. No further increase in activity was observed if subsequent cycles of growth in low tryptophan medium were completed (data not shown). Proline uptake by strains WG170 [Δ(*putPA*)*101* *proP219*] and WG174 [Δ(*putPA*)*101* *proP220*::Tn*5*] was similar to that of RM2 when all were grown with excess L-tryptophan, but the uptake activity of the mutant strains was not increased by tryptophan limitation (Table 2). The glutamine and glycine uptake activities of the three strains were similar, and they were not altered by growth in low tryptophan medium (Table 2). These data are consistent with the prediction that mutations *proP219* and *proP220*::Tn*5* specifically eliminate an inducible proline uptake activity.

The time course of proline uptake by strain RM2 has been examined further (Fig. 1). Uptake was measured at three proline concentrations with bacteria from tryptophan-limited cultures. In each case, uptake was a linear function of time from 1 to 5 min, and additional experiments in which uptake was also measured at 15, 30, and 45 s failed to reveal an additional kinetic component (data not shown). Strikingly, each line extrapolates to a high level of uptake at 0 min: 4.2, 8.8, and 19 nmol/mg of protein for uptake at 39, 214, and 914 μM L-proline, respectively. Assuming a cell volume of 0.73 μl of cell protein per mg (16), equilibration of the radiolabeled proline across the cell membrane would yield approximately 0.03, 0.16, and 0.67 nmol of proline per mg of cell protein, respectively, at these three proline concentrations. These data imply that the linear phase of proline uptake is preceded by a high level of proline binding to

TABLE 2. Amino acid uptake by strains RM2, WG170, and WG174[a]

Strain	Tryptophan[b]	Transport substrate[c]		
		Glycine	L-Glutamine	L-Proline
RM2	Excess	1.4	1.2	3.8
	Limiting	1.8	1.2	9.6
WG170	Excess	1.4	1.4	3.7
	Limiting	1.6	1.2	3.9
WG174	Excess	1.2	0.9	2.8
	Limiting	1.1	1.0	2.6

[a] Uptake activities are cited as nanomoles of substrate accumulated per milligram of cell protein in 30 s.

[b] Uptake was measured by using cultures provided with L-tryptophan either in excess (250 μM) or at a growth-limiting concentration (24 μM) (see text).

[c] Substrate concentrations were glycine, 40 μM; L-glutamine, 20 μM, and L-proline, 214 μM.

cells or filters, or a rapid phase of concentrative proline uptake. The initial phase cannot be attributed to the binding of L-proline to the assay filters, since the quantity of substrate retained in the absence of bacteria was negligible. A similar pattern of uptake was observed for bacteria from tryptophan-sufficient cultures of strain RM2. Although we have not demonstrated that proline is accumulated in a soluble and unmodified form, active accumulation is probable since proline dehydrogenase is absent and chloramphenicol is included in the transport assay mixture (cf. reference 1).

PP-I and PP-II are independent. We have begun to assess the relationship between PP-I and PP-II by constructing bacterial strains that possess both, either, or neither uptake activity. The influence of *putA* lesions on the properties of these strains has also been examined. Sensitivity of bacteria to growth inhibition by the proline analogs AC and DHP reflects the rates of uptake and catabolism of the analogs, as well as the intracellular concentration of their competitor, L-proline. Since uptake and metabolism of the analogs is frequently mediated by enzymes for which L-proline is the native substrate, analog sensitivity can be used as an indicator of proline uptake and catabolic activity. Analog sensitivity is determined by the radial streak test (see Materials and Methods) employing tryptophan-sufficient growth medium containing D-glucose as the carbon source. It is therefore expected to reflect the levels of PP-I, PP-II, and proline dehydrogenase activity present under conditions of catabolite repression and amino acid sufficiency.

Parent strain CSH4 (*putP*$^+$ *putA*$^+$ *proP*$^+$) is defined as having intermediate sensitivity to both AC and DHP. The *putP* defect dramatically reduces sensitivity to both compounds by preventing their uptake via PP-I (strain JT34; Table 3 [18]). The *proP219* defect, alone or in combination with the *putP* lesion, has no effect on analog sensitivity (strains WG138 and WG175; Table 3). This suggests that PP-II activity is negligible in strain CSH4 under the growth conditions employed. Clearly, mutation *proP219* does not inactivate PP-I.

A slightly different pattern is observed among bacteria lacking proline dehydrogenase (*putA*). The *putA* defect alone increases sensitivity to AC and DHP by leading to constitutively high PP-I activity and, in the case of DHP, preventing detoxification by proline dehydrogenase (strain JT31; Table 3 [18]). The *putA* strain lacking PP-I (*putP*) but not PP-II (*proP*$^+$) is fully resistant to AC but only partially resistant to DHP (strain RM2; Table 3 [18]). This implies that DHP is taken up by PP-II in this strain which cannot detoxify it. The *putA* strain retaining PP-I (*putP*$^+$) but lacking PP-II (*proP*) shows partial resistance to both AC and DHP (strain WG148; Table 3). These data suggest that PP-II activity is significant in strains JT31 and RM2 under these conditions. Finally, strain WG170, which lacks the dehydrogenase and both proline porters, is strongly resistant to both proline analogs.

Since allele *proP219* has no detectable phenotype in strain WG138, we wished to demonstrate indirectly that the *proP* defect is still present in that strain. Strain WG138 (*put*$^+$ *proP219*) was transduced to tetracycline resistance with phage P1 grown on strain WG147 [*pyr-76*::Tn*10* Δ(*putPA*)*101*], and the transductants were screened for their ability to utilize L-proline. All

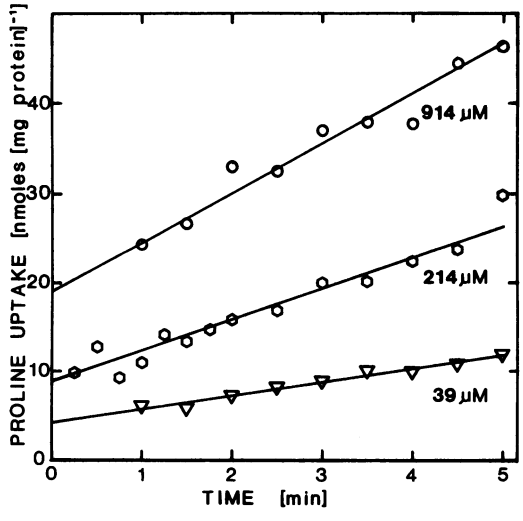

FIG. 1. Time course of proline uptake by strain RM2. Proline uptake was measured with tryptophan-limited cultures of strain RM2 as described in the text. Proline was provided at 914, 214, and 39 μM as indicated.

TABLE 3. Phenotypes attributable to *putP*, *putA*, and *proP* defects

Strain	Genotype			Sensitivity to[a]:		Proline utilization[b]
	putP	*putA*	*proP*	AC	DHP	
CSH4	+	+	+	4	3	+
JT34	−	+	+	2	1	−
WG138	+	+	−	4	3	+
WG175	−	+	−	2	1	−
JT31	+	−	+	5	5	−
RM2	−	−	+	2	3	−
WG148	+	−	−	3	4	−
WG170	−	−	−	2	2	−

[a] Proline analog sensitivity was measured by the radial streak text (20). Analog sensitivities are designated by arbitrary units, 1 indicating least sensitivity and 5 greatest sensitivity to growth inhibition by either compound. The designation 1 represents no growth inhibition by up to 1 mg of AC or DHP.

[b] Proline utilization was scored with triphenyltetrazolium chloride indicator medium (12, 20).

transductants (13 of 14 tested) that were unable to utilize proline had also regained the DHP resistance characteristic of strain WG170 [Δ(*putPA*)*101 proP219*].

Uptake specificity. Proline analogs AC and DHP both inhibit proline uptake by strain RM2 (Fig. 2). This observation is consistent with the view that PP-II mediates their uptake by that strain, although accumulation of AC is apparently insufficient to cause growth inhibition under the conditions of the radial streak test. These data do not, however, reveal whether there are additional uptake routes shared by L-proline, AC, and DHP. By the same criterion, both agents also inhibit proline uptake via PP-II in *S. typhimurium* (1).

DISCUSSION

E. coli K-12 strain RM2 lacks the previously characterized proline uptake system, PP-I (17, 18). We show here that it retains a second proline uptake system induced during tryptophan-limited growth (Table 2), and that uptake is inhibited by the proline analogs AC and DHP (Fig. 2). The time course of proline uptake is unusual (Fig. 1). Our data reveal a slow, linear phase of proline uptake subsequent to 15 s and imply that a rapid, concentrative uptake phase is completed before that time. A more detailed examination of those uptake characteristics is underway.

Dehydroproline-resistant mutants derived from strain RM2 seem to lack the inducible uptake component (Table 2). We are currently examining in more detail the effects of those mutations on uptake kinetics. Auxotrophic derivatives of RM2 and its parent, strain CSH4 (*putP*$^+$*A*$^+$), can grow if provided with L-proline at a low concentration (25 μM). Auxotrophs derived from the DHP-resistant mutant strains, WG170 and WG174, require a high proline concentration for growth (2.5 mM).

These data suggest that *E. coli* possesses a proline uptake system analogous to PP-II of *S. typhimurium* (1, 8). We conclude that we have devised a direct selection for *E. coli* mutants defective in PP-II and denote those mutations *proP*. The *proP* mutations do not inactivate PP-I (Table 3), reinforcing the view that PP-I and PP-II are independent uptake systems.

A number of genetic loci have been shown to influence proline uptake by *E. coli* and *S. typhimurium*. The *proP* mutations of *S. typhimurium* are linked to *melA* and *melB* at 92 min on the chromosome map (8). They are complemented by *E. coli* F'117 (94 to 98 min). Motojima et al. have isolated *E. coli* mutants defective for proline uptake and designated them *proT* (11). The *proT* mutations are said to elevate the proline requirement of auxotrophic bacteria and to be linked to *unc* at 82 min on the *E. coli* chromosome map (11). Csonka has employed selection with DHP to isolate *proP* mutants of *S. typhimurium* (4). Beginning with a *putP proP* parent strain, he has isolated further mutants, designated *proU*, which are resistant to proline analog AC in media of high osmolarity (4). These mutations are at 59 min on the chromosome map and are said to eliminate a third proline uptake system. No active uptake by the *putP proP*

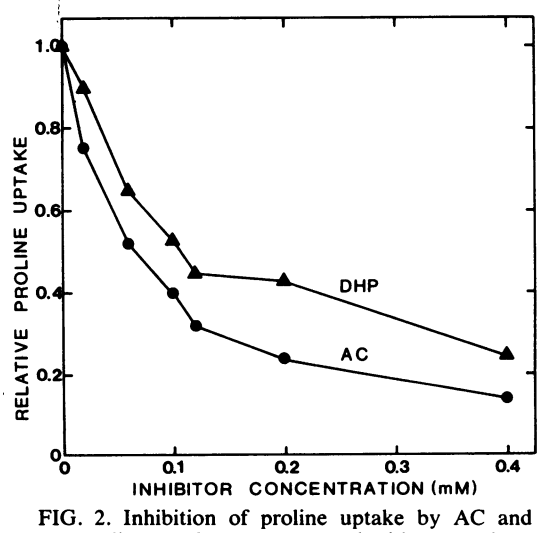

FIG. 2. Inhibition of proline uptake by AC and DHP. Proline uptake was measured with tryptophan-limited cultures of strain RM2 as described in the text. Uptake is cited as a fraction of the uninhibited 2-min uptake level at 25 μM L-Proline, which was 9.7 nmol of proline per mg of protein.

proU$^+$ parent strains has been demonstrated, however.

We have been unable to show linkage of *proP219* or *proP220*::Tn*5* with *melA* or *melB* in *E. coli* (M. Stalmach, M.S. thesis, University of Guelph), and those loci are not linked to *unc* or to the adjacent locus, *bgl* (J. M. Wood, unpublished data). The phenotypes attributable to these mutations are directly analogous to those of the *proP* mutants of *S. typhimurium*, however. The relationship between *proT* and *proP* remains obscure.

Genetic and biochemical data suggest that the *putA* gene product is a negative effector controlling its own expression and that of *putP* (7, 9, 18). The high proline uptake activity of *putA* strains has therefore been attributed to PP-I. Our data suggest that part of the high proline uptake activity observed in *putP*$^+$ *putA* strains of *E. coli* is attributable to PP-II (Table 3). (That system is not active under comparable conditions in *putA*$^+$ strains [Table 3].) The increase in PP-II activity may reflect either a further regulatory function involving the *putA* product or a physiological effect due to the absence of proline oxidative activity.

ACKNOWLEDGMENTS

We are grateful to Claire Berg for strain CBK130 and to C. W. Forsberg, A. Mellors, and K. F. Gregory for their comments on the data.

This research was supported by the Natural Sciences and Engineering Research Council of Canada.

LITERATURE CITED

1. **Anderson, R. R., R. Menzel, and J. M. Wood.** 1980. Biochemistry and regulation of a second L-proline transport system in *Salmonella typhimurium*. J. Bacteriol. **141**:1071–1076.
2. **Bachmann, B. J., and K. B. Low.** 1980. Linkage map of *Escherichia coli* K-12, edition 6. Microbiol. Rev. **44**:1–56.
3. **Berger, E. A., and L. A. Heppel.** 1974. Different mechanisms of energy coupling for the shock-sensitive and shock-resistant amino acid permeases of *Escherichia coli*. J. Biol. Chem. **249**:7747–7755.
4. **Csonka, L. N.** 1982. A third L-proline permease in *Salmonella typhimurium* which functions in media of elevated osmotic strength. J. Bacteriol. **151**:1433–1443.
5. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
6. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
7. **Maloy, S. R., and J. R. Roth.** 1983. Regulation of proline utilization in *Salmonella typhimurium*: characterization of *put*::Mu *d*(Ap,*lac*) operon fusions. J. Bacteriol. **154**:561–568.
8. **Menzel, R., and J. Roth.** 1980. Identification and mapping of a second proline permease in *Salmonella typhimurium*. J. Bacteriol. **141**:1064–1070.
9. **Menzel, R., and J. Roth.** 1981. Regulation of the genes for proline utilization in *Salmonella typhimurium*: autogenous repression by the *putA* gene product. J. Mol. Biol. **148**:21–44.
10. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
11. **Motojima, K., I. Yamato, and Y. Anraku.** 1978. Proline transport carrier-defective mutants of *Escherichia coli* K-12: properties and mapping. J. Bacteriol. **136**:5–9.
12. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119**:736–747.
13. **Ratzkin, B., M. Grabnar, and J. Roth.** 1978. Regulation of the major proline permease gene of *Salmonella typhimurium*. J. Bacteriol. **133**:737–743.
14. **Ratzkin, B., and J. Roth.** 1978. Cluster of genes controlling proline degradation in *Salmonella typhimurium*. J. Bacteriol. **133**:744–754.
15. **Shaw, K. J., C. M. Berg, and T. J. Sobol.** 1980. *Salmonella typhimurium* mutants defective in acetohydroxy acid synthases I and II. J. Bacteriol. **141**:1258–1263.
16. **Winkler, H. H., and T. H. Wilson.** 1966. The role of energy coupling in the transport of β-galactosides by *Escherichia coli*. J. Biol. Chem. **241**:2200–2211.
17. **Wood, J. M.** 1975. Leucine transport in *Escherichia coli*: the resolution of multiple transport systems and their coupling to metabolic energy. J. Biol. Chem. **250**:4477–4485.
18. **Wood, J. M.** 1981. Genetics of L-proline utilization in *Escherichia coli*. J. Bacteriol. **146**:895–901.
19. **Wood, J. M., and D. Zadworny.** 1979. Characterization of an inducible porter required for L-proline catabolism by *Escherichia coli* K12. Can. J. Biochem. **57**:1191–1199.
20. **Wood, J. M., and D. Zadworny.** 1980. Amplification of the *put* genes and identification of the *put* gene products in *Escherichia coli* K12. Can. J. Biochem. **58**:787–796.