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Regulation of the Major Proline Permease Gene of *Salmonella typhimurium*

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The structural gene for the major proline permease is located in a tight cluster with genes coding for the proline degradative enzymes, proline oxidase and pyrroline-5-carboxylic acid dehydrogenase. Expression of the permease is regulated in parallel with the two degradative enzymes, and all three functions are subject to catabolite repression. Regulatory mutants (*putC*) have constitutively high levels of all three activities, suggesting that all are regulated by a single mechanism.

Salmonella typhimurium can utilize proline as both a nitrogen and a carbon source. Proline degradation is catalyzed by two enzymes, proline oxidase and pyrroline-5-carboxylic acid (PCA) dehydrogenase (Fig. 1). These enzymes are induced by growth in the presence of proline and are subject to catabolite repression (3, 16).

Dendinger and Brill (3) have described three classes of mutants unable to utilize proline as a carbon or nitrogen source (*put*). One mutant type, designated *putA*, lacks proline oxidase activity. A second mutant type, *putB*, lacks PCA dehydrogenase activity. A third mutant type, *putP*, lacks both activities and was thought to be defective in a regulatory element, such as promoter or activator protein. A class of regulatory mutants, *putC*, was also described in which both enzymatic activities were expressed at a high constitutive level. All loci (*putA,B,C,P*) were shown to be linked to each other by phage P22-mediated transduction (3).

It occurred to us that many attributes of the pleiotropic negative (*putP*) class of mutants could be explained as being due to defects in a permease required directly for transport of proline and indirectly for induction of the degradative enzymes. Permease-defective cells might contain very low levels of degradative enzymes if they were unable to transport the inducer. A precedent for this has been reviewed by Magasanik (12).

This paper presents evidence that the *put* region includes a gene (or genes) that encodes a proline permease. The expression of this permease gene is regulated by the same mechanism that controls production of the proline degradative enzymes. We believe that many of the pleiotropic negative mutants described previously owe their phenotype to loss of permease function. A fine-structure map of the *put* region is presented in the accompanying paper (17).

MATERIALS AND METHODS

Media. Nutrient broth (Difco) was used as complex medium, and the E medium of Vogel and Bonner (20) was used as minimal medium. For a carbon-free minimal medium, a variant of E medium lacking citrate (NCE) was used (1). A medium having neither carbon nor nitrogen source (NCN) contained, per liter: 7 g of K_2HPO_4, 3 g of KH_2PO_4, and 0.119 g of $MgSO_4$. When proline was to be used as sole nitrogen source, succinate was generally added as carbon source at a final concentration of 0.2%. When glucose was used, it was added at a final concentration of 2%. When required, supplements of amino acids and uracil were added to minimal media at a final concentration of 0.1 mM. Proline, when used as either carbon or nitrogen source, was used at a concentration of 0.2%. To solidify any of the above media, Difco agar was used at a concentration of 2%.

Chemicals. L-Azetidine-2-carboxylate (AC) and L-3,4-dehydroproline (DP) were obtained from Calbiochem. Diethyl sulfate was obtained from Eastman Organic Chemicals. Cyclic 3',5'-AMP (cAMP) was obtained from Sigma. ^{14}C- and ^{3}H-labeled proline was purchased from Schwartz/Mann Co. ICR-191 was a gift from H. J. Creech of the Institute for Cancer Research, Fox Chase, Philadelphia.

Bacterial strains. The genotypes of bacterial strains used are presented in Table 1. Strains designated AR were all obtained from Winston Brill; these strains are derived from wild-type *S. typhimurium* strain 1559. All other strains used are derived from *Salmonella* strain LT2 except for mutant *proAB47* and its derivatives (including TR3177, 3275, and 3330), which come from strain LT7. Mutants TR2139 (*putC900*) and TR2140 (*putB609*) were obtained from

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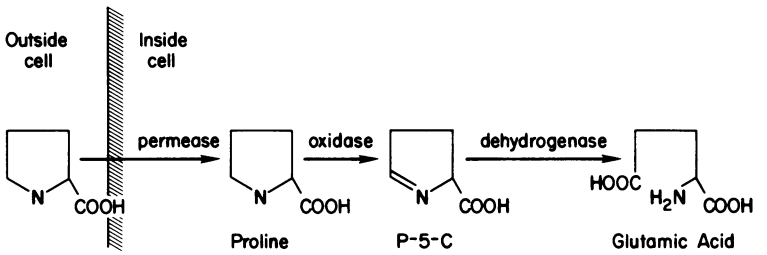

FIG. 1. *Pathway of proline degradation.*

TABLE 1. *Bacterial strains*[a]

Strain	Genotype
AR	Wild-type *S. typhimurium* strain 1559
AR17	*putP23*
AR27	*putA26*
AR65	*putC3*
AR75	*putC3 putP61*
AR111	*putC3 putB86*
SL3684	*galE542 hut*$^+$
TR1976	*putP500*
TR1994	*proAB47 put-638*
TR1995	*proAB47 put-639 proP673*
TR2139	*putC900*
TR2140	*putB609*
TR2228	*pyrD13 hut*$^+$ *galE542*
TR2230	*galE542 hut*$^+$ *hisC3734 hisO1242 pyrC7*
TR2272	*galE542 hut*$^+$ *pyrC7*
TR3177	*argI539 proAB47 amtA1 Δtrp-130*/F'393 *pro*$^+$ *lac*$^+$ *pyrB*$^+$ *argI*$^+$
TR3275	*argI539 proAB47 amtA1 Δtrp-130 cya-961*/F'393 *pro*$^+$ *lac*$^+$ *pyrB*$^+$ *argI*$^+$
TR3294	*pyrC7 Δput-515 leuD798 fol-101 supQ1238* (ProAB$^-$)
TR3298	*pyrC7 Δput-517 leuD798 fol-101 supQ1238* (ProAB$^-$)
TR3301	*pyrC7 Δput-513 leuD798 fol-101 supQ1238* (ProAB$^-$)
TR3330	*argI533 proAB47 amtA1 Δtrp-130 crp-661*/F'393 *pro*$^+$ *lac*$^+$ *pyrB*$^+$ *argI*$^+$
TR3526	*galE542 hut*$^+$ *pyrC7 put-559*
TR3527	*pyrC7 put-600*
TR3528	*galE542 hut*$^+$ *hisC3734 his01242 pyrC7 put-602*
TR3529	*galE542 hut*$^+$ *hisC3734 his01242 pyrC7 put-603*
TR3530	*galE542 hut*$^+$ *hisC3734 his01242 pyrC7 put-604*
TR3531	*galE542 hut*$^+$ *hisC3734 his01242 pyrC7 put-605*

[a] Strains numbered AR are derived from *S. typhimurium* strain 1559 and were isolated by and obtained from Winston Brill. All other strains are derived from strain LT2. TR2139 and TR2140 were also obtained from W. J. Brill.

Winston Brill; both are derived from LT2. Strain SL3684 was obtained from B. A. D. Stocker; this strain was used as donor in order to transmit P1 phage sensitivity to various recipient strains. Strains TR3177, TR3275, and TR3330 were constructed and donated by Christoph Beck.

Transductions. The non-lysogenizing P22 mutant *int-4* (18) was used in P22-mediated transduction. Plates were spread with 1×10^9 phage particles and 2×10^8 recipient cells. Transductional crosses mediated by the coliphage P1 were performed with *Salmonella* strains rendered sensitive to P1 by possession of a *galE* mutation. Transductions were performed by mixing 0.5 ml of log-phase cells (about 5×10^8 cells/ml), 0.5 ml of phage (10^7 to 10^8 plaque-forming units/ml), and 0.5 ml of a solution containing 0.015 M $CaCl_2$ and 0.03 M $MgSO_4$ and incubating for 20 min at 37°C. The cells were centrifuged, washed once in E medium, resuspended in 0.1 ml of E medium, diluted, and plated on selective media.

Assay methods. For assay of proline permease, cells were grown in the desired medium and harvested in either log phase or early stationary phase. Fresh cultures in stationary phase gave results indistinguishable from log-phase cells. Cells were washed twice in NCE medium (no proline present) and resuspended in NCE. These cells either were used immediately for assay or were starved for 90 min at 37°C to deplete proline pools. The assay was performed in NCE medium containing 0.1% glucose, 100 μg of chloramphenicol per ml, and 0.01 mM radioactive proline, labeled with either [^{14}C]proline (50 μCi/μmol) or [^{3}H]proline (3,000 μCi/μmol). A 0.2-ml assay mixture was incubated at either 10°C or room temperature; 0.050-ml samples were removed and filtered onto 0.45-μm membrane filters (Millipore Corp.). The filters were washed with 2.0 ml of NCE medium at room temperature, dried with a heat lamp, and counted in a liquid scintillation counter. Proline oxidase was assayed by the method of Dendinger and Brill (3).

RESULTS

Physiological effects of proline analogs. Under appropriate conditions, the proline analogs AC and DP each strongly inhibited the growth of *S. typhimurium*. The degree of inhibition varied greatly. Growth of cells was only weakly inhibited when glucose was used as the carbon source. The strongest growth inhibition was observed on media containing succinate as sole carbon source. However, all inhibition was reversible by addition of proline or proline-containing peptides to the growth medium. Such observations suggest that AC and DP are proline analogs and that the cell's sensitivity to inhibition is subject to catabolite repression.

Both analogs also inhibited growth of *Escherichia coli* and have been shown to be incorporated into protein (4, 5, 15). Both analogs appeared to enter the cell via the proline permease. This is suggested by the observation that uptake of [^{14}C]proline (0.01 mM) was inhibited by the presence of either analog (1 mM) in the

assay mixture. This point has also been demonstrated in *E. coli* (5).

Mutants resistant to AC. Spontaneous mutation of wild-type *S. typhimurium* to AC resistance is a relatively common event; approximately 1 cell in 10^5 is resistant. Over half of these mutants exhibit two phenotypic characteristics in addition to resistance to the analog: (i) they are unable to utilize proline as a carbon or nitrogen source; and (ii) they excrete proline into the growth medium.

Table 2 presents the phenotype of a typical AC-resistant mutant, TR1976. This should be compared with its wild-type parent (LT2) and with a series of mutants defective in proline utilization, isolated by Brill and co-workers. The following points should be made:

(i) The phenotype of the AC-resistant mutant (TR1976) is quite similar to that of the pleiotropic mutant type (AR17 and AR75) described earlier by Dendinger and Brill (3). Both types are defective in proline utilization and resistant to AC, and both excrete proline. We have designated AC resistance mutations *putP* in the belief that most of the pleiotropic mutations of Dendinger and Brill are identical to the AC-resistant mutants described here. We believe that both sorts of mutations affect the proline permease.

(ii) Mutants defective in only one of the degradative enzymes (*putA* or *putB* mutants) remain sensitive to AC.

(iii) Constitutive mutants (*putC*), which have high levels of degradative enzymes, are especially sensitive to AC.

(iv) The sensitivity of a constitutive mutant is removed by a *putP* mutation (strain AR75) but not by a *putB* mutation (strain AR111).

These results are consistent with the following hypothesis: the AC-resistant mutants and the pleiotropic mutations of Brill and co-workers both affect the proline permease. Both render the cell unable to transport AC (and therefore resistant to inhibition). Due to poor uptake of proline, the cells are unable to induce the proline degradative enzymes and cannot use proline as a nitrogen or carbon source. Proline is excreted in these strains because a permease function is required to maintain internal proline pools. The *putC* regulatory mutants are supersensitive to AC because the proline permease is expressed at a high level in these mutant cells. The following experiments were performed in order to test various aspects of this hypothesis.

Proline transport. Uptake of proline by strains LT2, *putC900*, and *putP500* is presented in Fig. 2. The wild type (LT2) exhibited slow uptake without prior induction, but greatly increased uptake when pregrown in the presence of proline. The *putC* constitutive mutants showed rapid uptake even without prior induction. (We do not understand the drop in the uptake rate by proline-grown *putC900* cells at later times.) The pleiotropic mutant (*putP*) showed no uptake whether or not pregrown in the presence of proline. All the AC-resistant mutants tested were similarly deficient in uptake and will be referred to as *putP* (permease) mutants.

Induction of proline oxidase by a proline-containing peptide. To determine whether *putP* mutants possess a functional regulatory

TABLE 2. *Summary of put mutant types*

Strain	Relevant genotype	Proline utilization[a]	Sensitivity to azetidine carboxylic acid[b]		Proline excretion[c]
			Glucose	Galactose	
LT2	Wild type	+	S	S	−
TR1976	*putP500*	−	R	R	+
TR2140	*putB609*	−+	S	S	−
TR2139	*putC900*	+	S	VS	−
AR	Wild type	+	S	S	−
AR17	*putP23*	−+	R	R	+
AR27	*putA26*	−+	S	S	−
AR65	*putC3*	+	S	VS	−
AR75	*putC3 putP61*	−	R	R	+
AR111	*putC3 putB86*	−	S	S	−

[a] Proline utilization was scored as growth on NCN-proline succinate medium. Symbols: +, Growth on proline as sole nitrogen source; −, no growth; −+, weak but detectable growth.

[b] A filter paper disk containing 5 μmol of AC was placed in the center of a minimal plate containing the indicated sugar as carbon source. A nutrient broth culture of each strain was streaked radially from the disk to the outer edge of the plate. Sensitivity was scored by the distance from the disk at which growth was inhibited. S, VS, and R indicate sensitive, very sensitive, and resistant, respectively.

[c] Proline excretion was scored as the ability to feed the proline auxotroph *proC110* on minimal galactose medium. Symbols: +, Feeding was observed; −, no feeding was observed.

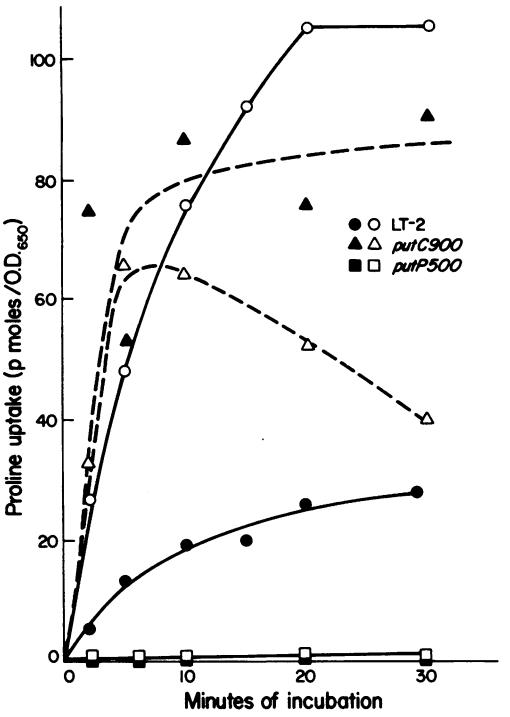

FIG. 2. *Induction of proline transport. Cells were pregrown in NCE plus succinate with* (○, △, □) *or without* (●, ▲, ■) *proline and assayed for transport of proline at 10°C without prior starvation.*

mechanism and an intact oxidase-dehydrogenase gene, the *putP* mutants were tested for growth on the dipeptide leucylproline. This dipeptide can be cleaved by a dipeptidase to yield intracellular proline (13). Since free leucine cannot be used as nitrogen source (7), growth on leucylproline as sole nitrogen source requires a functional proline degradative pathway. Leucylproline enters the cell via the peptide permease (19) and thus is not dependent on the proline-specific uptake system. Most *putP* mutants, whether selected as proline nonutilizers or as AC-resistant mutants, were fully able to grow on leucylproline as sole nitrogen source. Furthermore, leucylproline caused normal induction of proline oxidase levels in *putP* mutants (Fig. 3). The *putP600* strain exhibited poor but detectable induction by proline and normal induction by leucylproline. The *put*$^+$ strain showed induction by both proline and leucylproline. The slow induction of *putP600* may reflect a slight residual permease function in *putP600*.

Parallel induction of proline oxidase and permease. Growth in the presence of proline induced the permease four- to fivefold over the basal level; no further increase was seen following extended growth under inducing conditions. Growth with proline induced oxidase to well over 20 to 30 times its basal level. Thus, it is possible that these two activities are regulated together but not coordinately. At early induction times, however, there was a coordinate increase in the two activities (Fig. 4). Synthesis of these two activities was approximately coordinate for 3 h, after which time the increase in permease activity slowed, and no further increase was seen at sampling times beyond 4 h; oxidase activity continued to increase. The failure of proline transport activity to increase after this time may indicate that there are a limited number of sites in the cell membrane or periplasmic space with which the permease molecules can associate. Thus the increase in their activity is coordinate for only a short time following induction. Gene expression might be coordinate for longer if a direct assay of permease protein were available.

Catabolite repression of proline permease. We had expected that the proline permease might be subject to catabolite repression, because wild-type cells are less sensitive to the proline analog AC when growing in the presence of glucose. It has also been observed that growth of cells is inhibited by addition of glucose or even the poorer carbon sources, glycerol and galactose, to succinate medium containing pro-

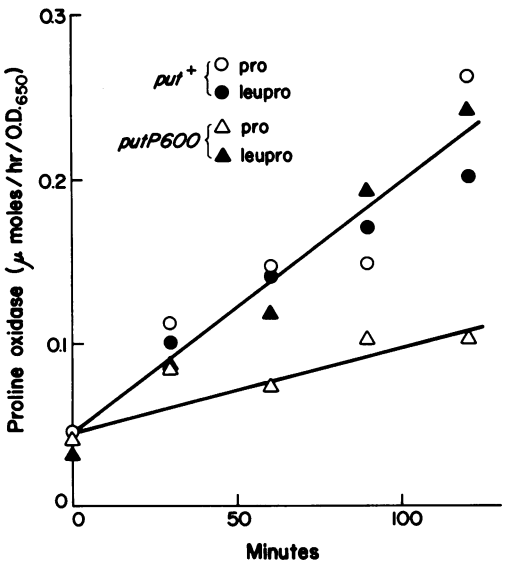

FIG. 3. *Induction of oxidase by leucylproline. Strains pyrC7 (put*$^+$*) and TR3527 (pyrC7 putP600) were pregrown in NCE + succinate + uracil, and the cultures were divided into two portions. One part received 2 mM leucylproline, and the other part received 2 mM proline. Samples were removed at 30-min intervals and assayed for proline oxidase. Uninduced oxidase levels remained at or near a level of 0.03 μmol/h per unit of optical density at 650 nm.*

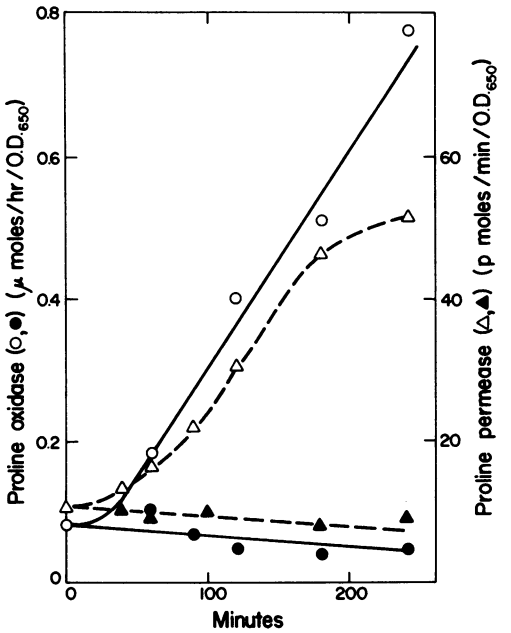

FIG. 4. *Induction of permease and oxidase. A culture of LT2 was grown in NCE + succinate to mid-log phase and divided. One culture received 0.2% proline. Samples were removed and assayed for permease and oxidase activities with* (○, △) *and without* (●, ▲) *added proline. Addition of proline caused the doubling time to change from 300 min to 136 min.*

line as sole nitrogen source; apparently these carbon sources interfere with utilization of proline. Brill and co-workers (3, 16) have shown that glucose can repress synthesis of proline oxidase and dehydrogenase. The effect of growth in the presence of glucose upon permease levels is presented in Table 3.

Growth of strain LT2 on succinate as sole carbon source resulted in five- to eightfold-higher permease levels than for cells grown in glucose; this was true for both induced and uninduced levels. Similarly, the constitutive permease levels found in strain *putC900* were partially repressed by growth in the presence of glucose. The regulatory mutant *putC900* showed some repression of permease by proline (Table 3). This has been observed repeatedly but is not understood. For comparison, proline oxidase levels are also presented in Table 3. These data confirm the earlier results of Brill and co-workers (3, 16).

We have also examined the role of cAMP in induction of proline permease and oxidase activities. Enzyme levels were measured in mutant strains lacking adenyl cyclase (*cya*) and in strains lacking the cAMP binding protein (*crp*). For these experiments, mutants were grown on adenosine as a carbon source, because the required adenosine degradative enzymes are not subject to catabolite repression (8). The results are presented in Table 4. There was substantial induction of oxidase by proline in the wild-type strain (TR3177) as well as a small induction of permease activity. However, both the *crp* and the *cya* strains, TR3330 and TR3275, showed approximately fivefold reduction in the induced levels of oxidase and permease. The addition of cAMP to proline-induced cultures caused the oxidase level in a *cya* strain to reach wild-type levels. As expected, added cAMP showed no effect on the *crp* strain.

Although the proline utilization pathway was subject to control by catabolite repression, there was still substantial expression of the *put* genes

TABLE 3. *Effect of glucose upon enzyme levels in wild type and putC900*[a]

Strain	Carbon source	Proline	Permease[b]	Oxidase[c]
LT2	Glucose	−	13	0.001
		+	36	0.09
	Succinate	−	97	0.03
		+	160	1.10
TR2139 (*putC900*)	Glucose	−	23	0.04
		+	14	0.01
	Succinate	−	104	0.44
		+	89	0.81

[a] Cells were grown in NCE medium with the carbon sources and proline supplementation indicated and assayed as described in Materials and Methods.

[b] Specific activity is expressed as picomoles per minute per unit of optical density at 650 nm. Assay was performed at 10°C on unstarved cells.

[c] Specific activity is expressed as micromoles per hour per unit of optical density at 650 nm.

TABLE 4. *Effect of cAMP upon enzyme levels*[a]

Strain	Additions to the growth medium			Permease[b]	Oxidase
	cAMP	Proline	Glucose		
TR3177	−	−	−	167	0.2
(*cya*$^+$ *crp*$^+$)	−	+	−	382	1.3
	+	+	−	NA	2.8
	−	+	+	180	0.4
TR3330	−	−	−	16	0.01
(*cya*$^+$ *crp*)	−	+	−	41	0.34
	+	+		NA	0.35
	−	+	+	53	0.26
TR3275 (*cya*	−	−	−	27	0.02
crp$^+$)	−	+	−	64	0.34
	+	+	−	NA	1.8
	−	+	+	62	0.28

[a] Cells were grown in NCE with 0.2% adenosine as the carbon source in all cases except those where glucose (2%) was used. Proline was added at 0.2%, and cAMP was added at 1 mM.

[b] Assays on starved cells at 25°C. NA, Not assayed.

in the absence of cAMP (Table 4). Both *cya* and *crp* mutants grew slowly (doubling time, 4 to 6 h) on proline as sole nitrogen source (using glucose as carbon source). Wild-type cells grew at approximately the same rate when using proline as sole nitrogen source in the presence of glucose. In addition, the assay of oxidase and permease in cells grown with glucose and proline (Table 4) shows that glucose represses enzyme levels in wild type to levels approaching those seen for *crp* and *cya* mutants; these assays are done in the presence of NH_3. Thus, the glucose effect appears to be mediated by the cAMP, which is required for maximal *put* expression.

Nature of the proline transport system. The proline uptake system is an active-transport process, since energy is required. Permease activity was eliminated by 10 mM azide. Transport was stimulated fourfold by addition of glucose to cells. In cells that had been starved by incubation for 90 min in medium without a carbon source, proline transport was completely dependent on addition of glucose to the assay mixture.

Over 100 proline permease mutants were selected as AC-resistant clones from cultures mutagenized with ICR-191. All of these mutations mapped at the *put* locus. This suggests that if the proline permease involves several functions, all the genes critical to AC transport (at the low concentration used) must map in this *put* region.

Assay of permease levels in mutant strains suggests that a single permease is responsible for uptake of proline under conditions of our assay (0.01 mM proline). Table 5 presents permease levels for a series of spontaneous *putP* mutants. All mutations except the last were revertible and are presumed to be point mutations. Several of the point mutations caused loss of over 99% of the proline permease activity. The residual activity was almost as low as that seen with a deletion mutant (Table 5, last line). These residual levels did not increase following growth in the presence of proline and may have been due to entry of proline by diffusion. Therefore, we conclude that the *putP* gene product is part of the major proline uptake system. The assay data presented would not preclude the existence of low-capacity permeases or permeases with lower affinity for proline (K_m > 0.01 mM). However, data presented make it unlikely that additional permeases exist that are physiologically significant for proline utilization.

TABLE 5. *Permease activities in point mutants resistant to AC*

Strain	Permease activity[a]
Wild type	430
TR3526 (*putP599*)	35.3
TR3527 (*putP600*)	8.3
TR3528 (*putP602*)	7.3
TR3529 (*putP603*)	4.7
TR3530 (*putP604*)	4.7
TR3531 (*putP605*)	3.5
TR1976 (*putP500*) (deletion)	2.0

[a] Cells were grown in NCE + succinate + proline. Permease units presented are picomoles per minute per unit of optical density at 650 nm.

Strains were constructed that carry a deletion mutation for the proline biosynthetic genes (*proA* and *proB*) as well as a permease deletion mutation. To supply their need for proline in protein synthesis, these strains depend upon uptake of proline by diffusion or by secondary uptake systems. The growth rates of these strains depend upon the exogenous proline concentration. The behavior of three deletion mutant strains is shown in Fig. 5. All grew very poorly even at proline concentration approaching 2 mM. This is quite a sensitive assay for proline uptake, since very little proline is required to satisfy the requirement of protein synthesis. Mutant *put-513* showed one-half of maximal growth rate at a proline concentration of roughly 0.5 mM. Even at high proline concentrations, a normal doubling time was not achieved. While the deletion mutants could take up sufficient proline to supply the needs for

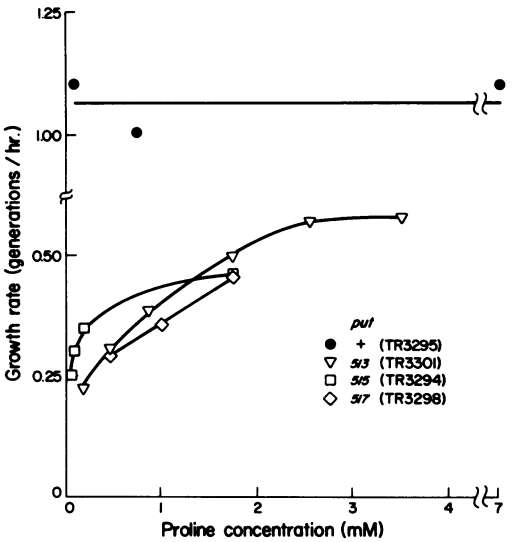

FIG. 5. *Proline-dependent growth rates of permease mutants. The growth rates of strains containing proAB deletions and permease mutations at various proline concentrations were measured in strains TR3294 (Δput-515), TR3298 (Δput-517), and TR3301 (Δput-513). Cells were grown in nutrient broth and washed twice before being inoculated into E + glucose medium. Growth rates were determined from the exponential portion of the growth curves as determined by absorbance at 650 nm.*

proline synthesis at a reduced growth rate, none of the three deletion mutants could grow on proline as carbon or nitrogen source even at 17 mM exogenous proline.

As far as we know, the only mutation in these strains which affects proline uptake is the *putP* deletion. However, recently Rolf Menzel (unpublished data) has studied other strains with the same growth properties as those presented in Fig. 5; Menzel can demonstrate that a second mutation, perhaps affecting a minor proline permease, is needed in addition to the *putP* mutation in order to drastically reduce transport. Further genetic analysis of the deletion strains *put-513*, *-515*, and *-517* will be needed to determine whether these strains carry unknown mutations in other uptake systems. An additional point must be kept in mind in interpreting these data: *putP* mutants excrete proline. If the *putP* function is essential to maintenance of an internal proline pool, then a low-capacity uptake system might function to no avail in strains carrying a *putP* mutation.

DISCUSSION

This paper presents evidence that the major, if not the only, proline permease in *Salmonella* is closely associated with the degradative system for proline. (i) The permease maps near the structural genes for the proline degradative enzymes. (ii) Like the degradative enzymes, the permease is induced by proline and is subject to catabolite repression. (iii) Regulatory mutants constitutive for the proline degradative enzymes also possess constitutively high levels of the proline permease.

Previously Dendinger and Brill (3) described a class of pleiotropic mutants (*putP*) that lack both proline oxidase and PCA dehydrogenase activities. Many of these mutants are indistinguishable from the analog-resistant permease mutants described here. Apparently loss of permease prevents proline from entering the cell and inducing the degradative pathway. The failure to induce results in the pleiotropic negative phenotype described earlier. We have used the *putP* designation for mutants defective in proline permease. The proline permease has been studied extensively in *E. coli* (9–11, 14). Many of these studies have been done using cells that are not induced for maximum permease expression. However, induction of permease levels by proline was noted by Morikawa et al. (14). In comparing data on proline uptake, growth conditions should be carefully controlled in light of the data presented here.

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LITERATURE CITED

1. **Berkowitz, D., J. Hushon, H. Whitfield, J. R. Roth, and B. N. Ames.** 1968. Procedure for identifying nonsense mutations. J. Bacteriol. **96**:215–220.
2. **Condamine, H.** 1971. Sur la régulation de la production de proline chez E. coli K12. Ann. Inst. Pasteur (Paris) **120**:126–143.
3. **Dendinger, S., and W. J. Brill.** 1970. Regulation of proline degradation in *Salmonella typhimurium*. J. Bacteriol. **103**:144–152.
4. **Fowden, L., S. Neale, and H. Tristram.** 1963. Effect of 3,4-dehydro-DL-proline on growth and protein synthesis. Nature (London) **199**:35–38.
5. **Fowden, L., and M. H. Richmond.** 1963. Replacement of proline by azetidine-2-carboxylic acid during biosynthesis of protein. Biochim. Biophys. Acta **71**:459–461.
7. **Gutnick, D., J. M. Calvo, P. Klopotowski, and B. N. Ames.** 1969. Compounds which serve as the sole source of carbon or nitrogen for *Salmonella*. J. Bacteriol. **100**:215–219.
8. **Hammer-Jespersen, K., A. Munch-Petersen, P. Nygaard, and M. Schwartz.** 1971. Induction of enzymes involved in the catabolism of deoxyribonucleosides and ribonucleosides in *Escherichia coli* K12. Eur. J. Biochem. **19**:533–538.
9. **Hirata, H., K. Altendorf, and M. H. Franklin.** 1974. Energy coupling in membrane vesicles of *Escherichia coli*. I. Accumulation of metabolites in response to an electrical potential. J. Biol. Chem. **249**:2939–2945.
10. **Kasahara, M., and Y. Anraku.** 1974. Transport of sugars and amino acids in bacteria. XI. Mechanism of energy coupling reaction for the concentrative uptake of proline by *Escherichia coli* membrane vesicles. J. Biochem. **76**:977–983.
11. **Kobayashi, H., E. Kin, and Y. Anraku.** 1974. Transport of sugars and amino acids in bacteria. X. Sources of energy and energy coupling reactions of the active transport systems for isoleucine and proline in *E. coli*. J. Biochem. **76**:251–261.
12. **Magasanik, B.** 1970. Glucose effects: inducer exclusion and repression, p. 189–219. *In* J. R. Beckwith and D. Zipser (ed.) The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Miller, C. G., and K. Mackinnon.** 1974. Peptidase mutants of *Salmonella typhimurium*. J. Bacteriol. **120**:355–363.
14. **Morikawa, A., H. Suzuki, and Y. Anraku.** 1974. Transport of sugars and amino acids in bacteria. VIII. Properties and regulation of the active transport reaction of proline in *Escherichia coli*. J. Biochem. **75**:229–241.
15. **Morris, H., and M. Schlesinger.** 1972. Effects of proline analogues on the formation of alkaline phosphatase in *Escherichia coli*. J. Bacteriol. **111**:203–210.
16. **Newell, S. L., and W. J. Brill.** 1972. Mutants of *Salmonella typhimurium* that are insensitive to catabolite repression of proline degradation. J. Bacteriol. **111**:375–382.
17. **Ratzkin, B., and J. R. Roth.** 1978. Cluster of genes controlling proline degradation in *Salmonella typhimurium*. J. Bacteriol. **133**:744–754.
18. **Smith, A. O., and M. Levine.** 1967. A phage P22 gene controlling integration of prophage. Virology **31**:207–216.
19. **Sussman, A. J., and C. Gilvarg.** 1971. Peptide transport and metabolism in bacteria. Annu. Rev. Biochem. **40**:397–408.
20. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.