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Genetics of the Glutamine Transport System in *Escherichia coli*

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The active transport of glutamine by *Escherichia coli* occurs via a single osmotic shock-sensitive transport system which is known to be dependent upon a periplasmic binding protein specific for glutamine. We obtained a mutant that had elevated levels of glutamine transport and overproduced the glutamine binding protein. From this strain many point mutants and deletion-carrying strains defective in glutamine transport were isolated by a variety of techniques. The genetic locus coding for the glutamine transport system, *glnP*, and the regulatory mutation which causes overproduction of the transport system were both shown to map at 17.7 min on the *E. coli* chromosome, and it was demonstrated that the *glnP* locus contains the structural gene for the glutamine binding protein. Evidence was also obtained that the glutamine transport system, by an unknown mechanism, plays a direct role in the catabolism of glutamate and, hence, of glutamine and proline as well.

The amino acid glutamine is actively accumulated in cells of *Escherichia coli* by a single, very specific, osmotic shock-sensitive transport system (6, 7, 41, 42). This system has a high affinity for glutamine (an apparent K_m of 0.08 μM) as well as a large capacity for its substrate in comparison with many other amino acid transport systems (42). An essential component of the glutamine transport system, the periplasmically located glutamine binding protein, has been characterized by a variety of physical methods (13, 22, 42), and it has been shown that it is possible to obtain mutants which overproduce this protein and also mutants which are defective in this protein (42). This collection of properties makes the glutamine transport system an ideal subject for a genetic study of an osmotic shock-sensitive transport system. In this paper we describe the beginnings of such a study, which we hope will ultimately lead to a complete genetic characterization of glutamine transport and insight into the mechanism of shock-sensitive transport in general.

MATERIALS AND METHODS

Bacterial strains. All strains used in this work were derived from *E. coli* K-12 and are listed in Table 1.

Media. Minimal media contained minimal salts medium E (40) with 0.5% carbon source. To supplement auxotrophs, amino acids were added to 400 μM; vitamin B_1, niacinamide, adenine, uracil, pyridoxal and pyridoxamine were added to 40 μM; and biotin was added to 20 nM. Solid minimal media were made with 1.5% Difco agar; 1.5% Moorehead agar was used to complex media. When glutamine was used in solid media as a sole source of either carbon or nitrogen, it was sterilized by passage through a membrane filter (Millipore Corp.) and added to autoclaved agar plus salts and supplements after the latter had cooled to 50°C to avoid hydrolysis. In media in which glutamine was the sole nitrogen source, nitrogen-free minimal salts were used (10).

For all genetic manipulations with biotin auxotrophs, biotin-free media contained avidin at 2.5×10^{-3} U/ml (9). In transductions involving the *galK* marker, MacConkey galactose agar was used to score phenotypes.

Chlorate-resistant mutants were selected or scored on nutrient broth agar containing 0.2% $KClO_4$ (9). Plates were incubated at 37°C under an atmosphere of H_2, CO_2, and N_2. Lactate-nitrate medium (38), also incubated anaerobically, was often used to complement the scoring of phenotypes on chlorate agar. Phenotypes were also often checked by a qualitative assay for nitrate reductase(2).

Selection of glutamine transport mutants. In selections using toxic glutamine analogs, except for the isolation of strain PSM3, overnight nutrient broth cultures of PSM2 or PSM178 were washed with minimal salts and plated on minimal succinate media containing 30 to 40 μg of either γ-glutamyl-hydrazide or L-methionine-DL-sulfoximine per ml or 0.1% D-glutamine. Strain PSM3 was obtained as one of the colonies occurring within the zone of growth inhibition surrounding crystals of γ-glutamylhydrazide placed in the center of a minimal glucose plate. Glutamine transport mutants were also obtained from PSM2 by *N*-

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TABLE 1. *Bacterial strains*

Strain	Genotype	Parent	Comments
JSH210	F⁻ *thi metC*		
PSM2	F⁻ *thi metC glnPo*	JSH210	
PSM3	F⁻ *thi metC glnPo glnP*	PSM2	Spontaneous, γ-glutamylhydrazide resistant
PSM116	F⁻ *thi metC glnPo glnP*	PSM2	By nitrosoguanidine mutagenesis and tritium suicide selection
JSH1	HfrH *thi*		
JSH1G	HfrH *thi glnPo*	JSH1	By transduction with P1 lysate of PSM2
JSH1Gγ	HfrH *thi glnPo glnP*	FSH1G	Spontaneous, γ-glutamylhydrazide resistant
AT3143	F⁻ *pyrF ilv met his purE proC pdxC xyl lacY str cycA tonA tsx*		A. L. Taylor strain from B. Bachmann
KLF25/KL181	F'125/*thi pyrD his trp recA mtl xyl malA galK str*		Episome F'125, from B. Bachmann
KLF26/KL181	F'126/*thi pyrD his trp recA mtl xyl malA galK str*		Episome F'126, from B. Bachmann
KLF47/KL262	F'147/*thi pyrD tyrA trp thyA recA galK str*		Episome F'147, from B. Bachmann
R871	F⁺ *bioB2 lac*		From A. Campbell (9)
AB468	F⁻ *thi his proA purD mtl xyl galK lacY*		A. L. Taylor strain, from B. Bachmann
JRG97	F⁺ *gal chlE supE*		J. R. Guest strain (38) from B. Bachmann
PSM176	F⁻ *thi metC glnPo bioB2*	PSM3	By transduction with P1 lysate of R871
PSM178	F⁻ *thi metC glnPo galK*	PSM176	By transduction with P1 lysate of AB468
PSM180	F⁻ *thi metC glnPo chlE*	PSM2	Spontaneous, chlorate resistant
PSM219	F⁻ *thi metC* Δ(*glnP-chlE*)	PSM2	
PSM220	F⁻ *thi metC* Δ(*glnP-chlE*)	PSM2	
PSM221	F⁻ *thi metC* Δ(*glnP-chlE*)	PSM2	
PSM222	F⁻ *thi metC* Δ(*gal-glnP*)	PSM2	
PSM223	F⁻ *thi metC* Δ(*nadA-glnP*)	PSM2	
PSM2M	F⁻ *thi glnPo*	PSM2	By transduction with P1 lysate of JSH1
PSM3M	F⁻ *thi glnPo glnP*	PSM3	By transduction with P1 lysate of JSH1

methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis (25) followed by a tritium suicide selection performed by shifting exponentially growing cells from 26 to 42°C and exposing them to 2.7 μM L-[3-^{3}H]glutamine (36.5 Ci/mmol) for 2 h. Cells were then washed once, suspended in minimal salts medium, and stored at 4°C. After a 10^5-fold killing had occurred, survivors were screened for transport ability.

Matings. Episome transfers and Hfr matings were performed as described by Miller (24). Streptomycin resistance was used as the counterselection in Hfr matings.

Bacteriophage P1 transductions. P1kc was used for transductions. Phage lysates were prepared by the plate method (1) and stored at 4°C. Transductions were carried out by a modification (12) of the procedure of Luria et al. (20). For the purposes of mapping, all transductions were scored by streaking of colonies onto nonpermissive and permissive media.

Transport assays. Cells were grown in minimal glucose at 37°C with vigorous shaking. Fresh overnight cultures were diluted, grown for at least four generations, and harvested at a density of 3×10^8 to 4×10^8 cells per ml. Cells were washed three times with carbon-free salts medium (10) and suspended to 1.4×10^9 to 1.5×10^9 cells per ml in the same medium plus 100 μg of chloramphenicol per ml. Active transport was measured by a filter assay (17). Filters were dried and counted in 4 ml of Omnifluor (New England Nuclear Corp.) in toluene. The specific activities and final concentrations of the radioactive substrates used were as follows: L-[*U*-^{14}C]glutamine (243 mCi/mmol), 2.0 μM; L-[*U*-^{14}C]glutamate (263 mCi/mmol), 1.8 μM; L-[*methyl*-^{3}H]methionine (954 mCi/mmol), 1.3 μM; L-[3,4-^{3}H]glutamine (4.0 mCi/mmol), 1.0 mM; and L-[3,4-^{3}H]glutamate (4.0 mCi/mmol), 1.0 mM. For uptake of 1.0 mM glutamine or glutamate, chloramphenicol was omitted from the assay, and transport was measured in minimal salts medium E as described below.

Total uptake and incorporation assays. The total uptake and incorporation by cells of 35 mM

glutamine or glutamate over a period of 4 h was assayed by using cells grown for many generations on minimal glucose at 37°C and harvested at a density of 3.5×10^8 to 5.5×10^8 cells per ml. Cells were washed four times at room temperature with minimal salts containing methionine and vitamin B_1 and were suspended in the same solution to a density of 4.8×10^9 to 6.2×10^9 cells per ml. These suspensions were prewarmed for 5 min in a rapidly shaking water bath at 37°C and were then added to an equal volume of either 70 mM glutamine or 70 mM glutamate (in minimal salts plus methionine and vitamin B_1 at 37°C) and labeled with either L-[3,4-^{3}H]glutamine or L-[3,4-^{3}H]glutamate, and incubation at 37°C with rapid shaking was continued. To measure total uptake, 100-μl samples were removed at each time point, added to 1 ml of minimal salts, and immediately poured through a prewetted nitrocellulose filter and washed with 1 ml of minimal salts. For measurement of incorporation of glutamine or glutamate into macromolecules, 100-μl samples were removed at each time point and added to 0.5 ml of ice-cold 10% trichloroacetic acid. Samples were kept at 0°C for at least 30 min, and precipitates were collected on glass fiber filters and washed with 2 ml of ice-cold 5% trichloroacetic acid followed by 5 ml of 0.1 M HCl. Filters were dried and counted as in transport assays.

Gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by a modification of the procedure of Laemmli (15). Protein bands were stained with Coomassie brilliant blue.

Glutamine binding protein and antisera. Glutamine binding protein from strain PSM2 was purified by the method of Willis and Seegmiller (46). As judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the purified binding protein was essentially homogeneous. A very faint contaminating band of an apparent molecular weight of 31,000 was visible on gels. This impurity was probably the glutamate-aspartate binding protein (43), which is of the same molecular weight and is known to copurify with the glutamine binding protein. The fractionation procedures of Willis et al. (45) were adapted to a miniature scale to partially purify shock fluid from 1-liter cultures of cells to compare various strains. Glutamine binding activity was measured by a nitrocellulose filter binding assay (16).

For the preparation of antisera, two adult female New Zealand albino rabbits were immunized by three footpad inoculations, at weekly intervals, of 1 mg of purified glutamine binding protein in complete Freund adjuvant. Antisera were obtained after 3 and 5 weeks, and immunoglobulins were partially purified by successive ammonium sulfate precipitations (31). These antisera, run against purified glutamine binding protein by Ouchterlony double diffusion, elicited two precipitin bands: a strong, sharp band corresponding to the glutamine binding protein and a weak, trailing band caused by the contaminating antigen mentioned above. Antibodies to the contaminating antigen were completely removed from one stock of serum by combining the serum with partially purified osmotic shock fluid from strain PSM223, a mutant containing a deletion of the glutamine transport genes. After 48 h at 4°C, the resulting precipitate was removed from the mixture by centrifugation, and this "purified" antiserum was used in all subsequent screening of strains.

Rapid screening of transport mutants. For screening large numbers of strains for glutamine binding protein activity and anti-glutamine binding protein cross-reacting material, cell extracts were prepared by chloroform-toluene treatment (14) or by the following technique. Overnight cultures in 5 ml of minimal succinate were harvested by centrifugation and washed once with 10 mM Tris-hydrochloride (pH 8.0). Cell pellets were suspended in 100 μl of 30 mM Tris-hydrochloride (pH 8.0)–20% sucrose, and to this was added 100 μl of 30 mM Tris-hydrochloride (pH 8.0)–20% sucrose–20 mM potassium EDTA–0.2 mg of lysozyme per ml. Suspensions were incubated at 37°C for 1 h and then centrifuged. The supernatants were used in Ouchterlony double-diffusion immunoassays and in binding activity assays.

Enzyme assays. Cell extracts were prepared from cultures of PSM2 and PSM3 with and without exposure of cells to glutamine or glutamate as a sole carbon source. One-liter minimal glucose cultures were grown for many generations at 37°C and were harvested by centrifugation when a density of 4.5×10^8 cells per ml was reached. Cells were then either used for preparation of extracts or washed three times with minimal salts medium and suspended in 100 ml of minimal salts, methionine, vitamin B_1, and either 35 mM glutamine or 35 mM glutamate. These cultures were incubated at 27°C with vigorous shaking for 4 h and were then harvested by centrifugation. All harvested cells were washed twice with 50 mM Tris-hydrochloride (pH 7.4) and suspended in 10 ml of the same. Cells were sonicated by five 15-s bursts on a Branson sonicator (setting 70), with cooling on ice between bursts. Cellular debris was removed by centrifugation at $12,000 \times g$ for 10 min; particles and smaller debris were removed by centrifugation at $50,000 \times g$ for 30 min. The resulting supernatants were quick-frozen in a dry ice-ethanol slurry and kept at −20°C until use.

Glutaminase A (EC 3.5.1.2) was assayed by combining 100 μl of extract with 1 ml of 100 mM sodium acetate (pH 5.0)–30 mM glutamine (26). Reaction mixtures were incubated at 37°C for 50 to 100 min, and reactions were terminated by the addition of 1 ml of ice-cold 10% trichloroacetic acid. After incubation on ice for 15 min, precipitates were removed by centrifugation, and ammonia in the supernatant was determined with Nessler reagent. Reaction mixtures without glutamine and reaction mixtures without cell extract were used to determine background values.

Aspartase (aspartate ammonia-lyase, EC 1.3.1.1) was assayed at 37°C in a reaction mixture of 100 mM potassium L-aspartate–100 mM Tris-hydrochloride (pH 8.5)–2 mM $MgCl_2$. After the addition of 20 μl of cell extract, the rate of appearance of fumarate was monitored at 240 nm (35). Data were converted by using ϵ_{240} (fumarate) = 2,530 mol^{-1} cm^2.

Glutamate-aspartate transaminase (aspartate aminotransferase, EC 2.6.1.1) in cell extracts was determined by a coupled assay exactly as described by Urm et al. (37). Data were converted by using ϵ_{340} (NADH) = 6,220 mol^{-1} cm^2.

Protein determinations. Protein was determined by the method of Lowry et al. (19) with bovine serum

albumin as the standard.

Materials. All radioactive compounds were obtained from New England Nuclear Corp. L-Glutamine, D-glutamine, L-glutamic acid, L-methionine-DL-sulfoximine, avidin, lysozyme (grade I), and Nessler reagent were from Sigma Chemical Co. γ-L-Glutamylhydrazide was from ICN Pharmaceuticals. Malate dehydrogenase, used in the glutamate-aspartate transaminase assay, was obtained from Boehringer Mannheim Corp. Sucrose was Schwarz/Mann special enzyme grade. Nitrocellulose filters used in binding protein assays were from Millipore Corp. Glass fiber filters were from Whatman Ltd. All other materials were reagent grade and were purchased from commercial sources.

RESULTS

Isolation of a strain containing a regulatory mutation involved in the utilization of glutamine. To facilitate both genetic manipulations and the purification of glutamine binding protein, we isolated a derivative of wild-type *E. coli* strain JSH210 containing a regulatory mutation involved in the utilization of glutamine. JSH210 grew very poorly on glutamine as a sole carbon source. When cells of this strain were spread onto minimal glutamine plates, spontaneously arising, fast-growing colonies, which occurred at a frequency of approximately 10^{-7}, could be readily distinguished from the background growth. One such mutant clone, PSM2, was found to have a higher initial rate and steady-state level of glutamine transport than JSH210, and it produced 2.5- to 6-fold higher levels of glutamine binding protein than did the parent strain, depending upon growth conditions. PSM2 was used as the parent strain for all mutants subsequently generated.

Isolation of a mutant defective in the active transport of glutamine. Strain PSM3 was isolated from PSM2 as a spontaneously occurring mutant resistant to the toxic glutamine analog, γ-glutamylhydrazide. As shown in Fig. 1, PSM3 was very defective, compared with PSM2, in the active transport of 2 μM glutamine. PSM3 was also unable to grow on glutamine as a sole source of carbon, and this phenotype was used to isolate revertants. Spontaneously arising revertants of PSM3 able to grow as well as PSM2 on glutamine occurred at a frequency of 10^{-9} to 10^{-8} in fully grown nutrient broth cultures. Three such revertants, isolated independently, were examined and found to have completely regained the ability to transport glutamine. Transport by one of these strains is shown in Fig. 1. The three independent revertants had also regained sensitivity to γ-glutamylhydrazide and were thus indistinguishable from the parent strain, PSM2. This showed that the characteristics of PSM3 were due to a single (revertible)

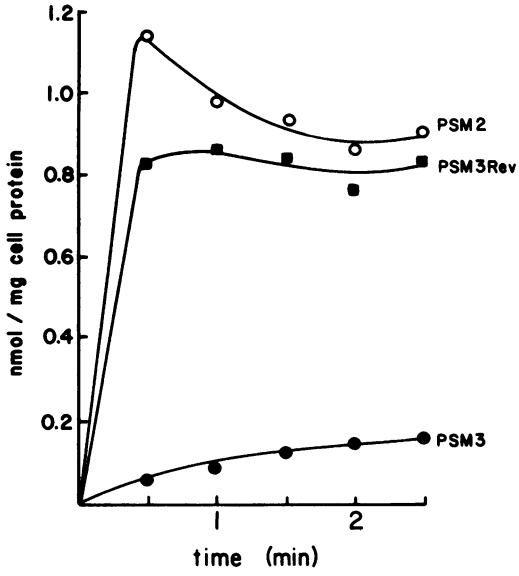

FIG. 1. *Transport of 2 μM glutamine by PSM2, PSM3, and PSM3Rev, a revertant of PSM3.*

point mutation, and that the phenotypes of growth or nongrowth on glutamine and resistance or sensitivity to γ-glutamylhydrazide could be used to map the mutation responsible for the loss of the ability to transport glutamine. The genetic locus defective in PSM3 was designated *glnP*.

Many other glutamine transport mutants were obtained by using four different types of selections. These were (i) resistance to γ-L-glutamylhydrazide, (ii) resistance to L-methionine-DL-sulfoximine, (iii) resistance to D-glutamine, and (iv) long-term survival after incubation with a low concentration (2.7 μM) of [^{3}H]glutamine of very high specific activity. The same strategy was present in all of these selections: at least some of the mutants able to survive exposure to a poisonous analog of glutamine (or glutamine itself) were expected to derive their immunity from an inability to take up the poison. γ-Glutamylhydrazide, which has been shown to compete with glutamine for both transport and binding to the glutamine binding protein (reference 2; data not shown), strongly inhibits 5-amino imidazole ribonucleotide synthetase (33), an enzyme of the purine biosynthetic pathway, and probably inhibits other glutamine-utilizing enzymes. Methionine sulfoximine is a potent inhibitor of glutamine synthetase (23). This compound, which is also an analog of methionine, was used by Betteridge and Ayling (8) to select for methionine transport mutants of *Salmonella typhimurium*. These authors found that methi-

onine sulfoximine-resistant strains were double mutants defective in both methionine transport and glutamine transport. With our strains, selection for resistance to methionine sulfoximine usually yielded mutants defective only in glutamine transport and normal in the transport of methionine. This was most likely due to the necessity of including methionine in all growth media, because the strains we used are methionine auxotrophs. The basis for the toxicity of D-glutamine (which, to inhibit growth, must be used at a concentration 25-fold higher than the other two poisons) is unclear. D-Glutamine is a substrate for glutamine synthetase (23), but another substrate for this crucial enzyme, L-2-pyrrolidone-5-carboxylate, showed no inhibition of the growth of strain PSM2 at any concentration. Kustu et al. (14) have found that D-glutamate is toxic to cells of *S. typhimurium* capable of transporting it. It is possible that the effect of D-glutamine on cell metabolism occurs after its conversion to D-glutamate. The killing action of [^{3}H]glutamine was probably due to its incorporation into macromolecules and subsequent damage to cells resulting both from β-emissions and the decay of carbon-tritium bonds into carbonium ions plus helium atoms.

More than 70 independent glutamine transport mutants were obtained by the methods described above, with and without the use of mutagenesis. We usually found that greater than 90% of the survivors of the four types of selections were glutamine transport defective. The nature of the mutations in survivors which were not impaired in glutamine transport was not pursued. All glutamine transport mutants examined (a total of 52) turned out to be equally resistant to the three toxic glutamine analogs, regardless of the means by which the mutants were selected. All were as defective as PSM3 in the transport of glutamine; all were unable to grow on glutamine as a sole carbon source. Thus, the phenotype of glutamine transport mutants is uniform, irrespective of the means of their isolation.

Mapping of *glnP*. The *glnP* locus was mapped by matings with an Hfr strain, by episome transfers, and finally by phage P1 cotransduction. Strain JSH1, which is an HfrH, was used to construct a strong glutamine grower, JSH1G, by transduction with P1 phage grown on PSM2. A *glnP* derivative of JSH1G was obtained by selection for spontaneous resistance to γ-glutamylhydrazide, and the resulting strain, JSH1Gγ, was shown, like PSM3, to be very defective in the transport of glutamine. This male *glnP*-carrying strain was then mated with female strains containing multiple auxotrophic markers. The linkage of *glnP* to particular markers was established by selecting for the transfer of the wild-type allele of a given marker from JSH1Gγ to the female recipient. Progeny from such a mating were then scored for resistance to γ-glutamylhydrazide. In this manner, a linkage was demonstrated between *glnP* and the markers *purE* and *pdxC*, which are located at 12 and 20 min, respectively, on the *E. coli* linkage map (4). It was then concluded from a three-point cross that the gene order had to be *purE* ... *glnP* ... *pdxC* and that *glnP* is closer to *pdxC* than to *purE*.

The *glnP* gene was further localized by the use of episomes. Strains containing episomes F'125, F'126, and F'147 (18) were mated with PSM3, and colonies capable of growth on glutamine were selected. The matings with F'126 and F'147 gave a high frequency of *glnP*$^+$ progeny. A lower number of *glnP*$^+$ colonies was obtained with F'125, and these were probably due to Hfr formation (18). Taking the results with F'125 as negative, consideration of the chromosome segments carried by F'126 and F'147 (Fig. 2) as well as the results of the three-point cross restricted the location of *glnP* to the region of 16.5 to 20 min on the *E. coli* chromosome.

Finally, the *glnP* locus was precisely mapped by phage P1 cotransduction with the genes *galK*, *bioB*, and *chlE*. For this purpose, an isogenic set of strains was constructed. The *bioB* and *galK* markers were transferred to a PSM2 background by P1 transduction (Table 1), and the resulting strains, PSM176 and PSM178, were checked for strong growth on glutamine. A *chlE* derivative of PSM2 was obtained by selecting 18 spontaneous chlorate-resistant mutants from PSM2. P1 lysates of these 18 candidates were used to transduce a known *chlE* mutant, JRG97, to *chl*$^+$, the ability to grow anaerobically on lactate-nitrate medium (38). Five of the transductions produced 2 orders of magnitude fewer transductants than the rest, indicating that the chlorate-resistance mutations in the donor strains lay very close to the known *chlE* mutation. One of these five strains, PSM180, was further shown to be a *chlE* mutant on the basis of its linkage to *bioB*. By using strains PMS3, PSM176, PSM178, and PSM180, the linkage map shown in Fig. 2 was generated. The *glnP* marker showed an average P1 cotransduction frequency of 30% with *bioB* and 70% with *chlE*. Linkages were converted to map distances (in minutes) by using the equation derived by Wu and assuming that the P1 transducing fragment has an effective length of 2.0 min (5). By this relation, *glnP* lies at 17.7 min on the revised *E. coli* linkage map (4) between *bioB* and *chlE*: 0.66 min from

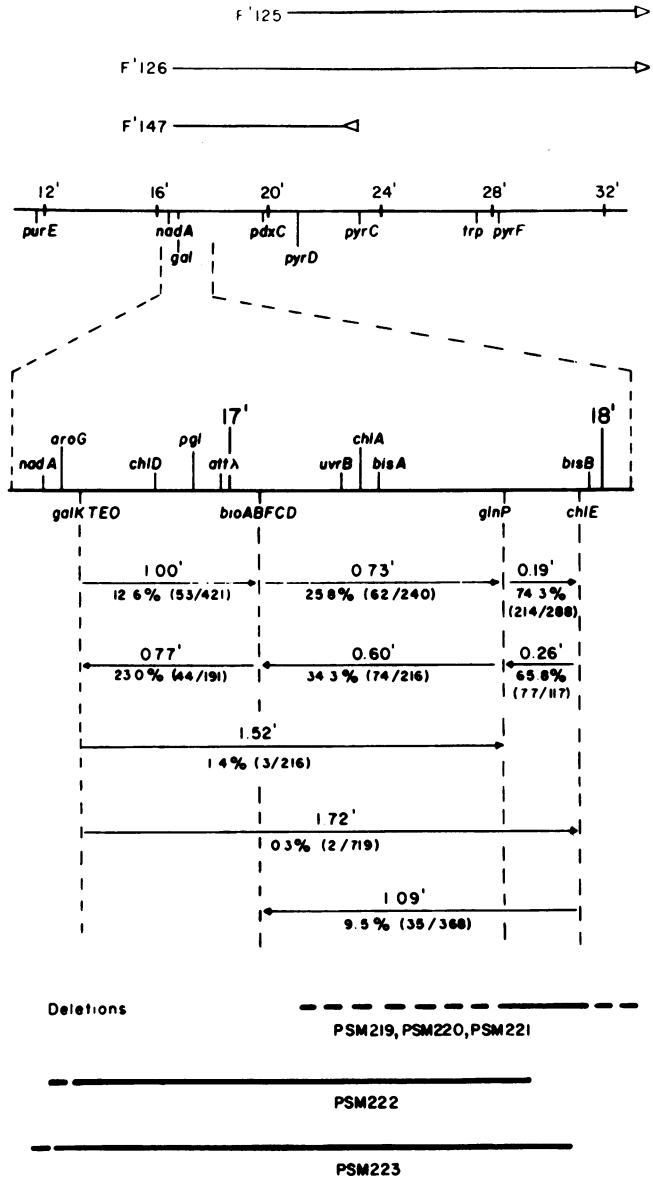

FIG. 2. *Mapping of glnP. A portion of the E. coli linkage map, taken from Bachmann and Low (4), is shown. Above, the region of 12 to 32 min, showing the chromosome segments covered by the episomes F'125, F'126, and F'147 (18). Below, an expansion of the region of 16 to 18 min, showing the P1 linkages between glnP and the markers galK, bioB, and chlE. For each transduction, the arrow points from donor to recipient. Cotransduction percentages (and ratios within parentheses) are given below each arrow. Map distances, in minutes, are given above each arrow. At the bottom are shown strains containing deletions of glnP. Solid lines denote the known extent of deletions in five mutant strains. Dotted lines represent uncertainty in the endpoints of the deletions.*

the former and 0.23 min from the latter.

All of 26 other glutamine transport mutants tested were also found to be linked to *bioB*. This implies that there is only one locus of genes affecting the high affinity glutamine transport system on the *E. coli* chromosome. For the purposes of the present work, the designation *glnP* has been assigned to all mutants defective in glutamine transport, although it is likely that in the future this class will have to be subdivided.

It is shown below that the structural gene for the glutamine binding protein is situated in the *glnP* locus. In addition, in other work in which we demonstrate the reconstitution of binding protein-dependent transport of glutamine by spheroplasts of *E. coli*, evidence is presented which suggests that the gene(s) for at least one other component of the glutamine transport system must be located in the same region (P. S. Masters and J.-S. Hong, Biochemistry, in press). In this laboratory, the glutamine transport genes of *E. coli* have been recently cloned in the vector pBR322 (M. Simon, C. M. Cassidy, P. S. Masters, and J.-S. Hong, unpublished results), and studies with this recombinant plasmid may allow a complete definition of the gene products which constitute the glutamine transport system.

A curiosity in the linkage data shown in Fig. 2 was the low P1 cotransduction frequency of *bio* and *gal* in our strains (13 to 23%). Other investigators have reported this linkage as 33 to 50% (4, 28, 29, 38), placing the two markers almost 0.5 min closer to each other than is implied by our data. If this disparity is not merely due to undefined differences in experimental conditions or anomalies in the interaction of P1 phage with different strains, then it suggests that the strains used in this study contain substantially more DNA in the *gal*... *bio* interval than do the strains used in the other studies cited. The attachment site for the bacteriophages lambda, 434, and 82 is located in this interval, and it has been shown that the cotransduction frequency of *gal* and *bio* drops from 47 to 7% when both donor and recipient strains are lambda lysogens (29). However, all of our strains, which are descendants of a lambda-free Hfr Hayes strain, are sensitive to lambda and are not known to be harboring any cryptic prophage. It is possible that the strains used in this study, which have never been heavily mutagenized, do not contain any extra genetic material, but rather that the strains used in previous studies have accumulated phenotypically silent deletions in the *gal*... *bio* interval through successive contacts with mutagens. Even in the absence of mutagenesis, deletions occur in this region of the *E. coli* chromosome (16 to 18 min) with a relatively high frequency (references 2 and 9 and results discussed below).

Mapping of the mutation in PSM2. Strain PSM176 (*bioB*) was derived in two steps from PSM2 (Table 1), and, like PSM2, it was a strong grower on glutamine as a sole carbon source. When PSM176 was transduced to *bio*$^+$ with P1 phage grown on JSH210, the wild-type parent of PSM2, 29% (18 of 62) of the transductants obtained grew as poorly as JSH210 on minimal glutamine, whereas the remainder grew as well as PSM2 on minimal glutamine (the two extents of growth were very distinct). This showed, then, that the mutation in PSM2, which causes an increased rate of growth on glutamine and elevated levels of glutamine transport and glutamine binding protein, maps at the same position as *glnP*. Therefore, we assume it to be an operator mutation that results in constitutive transcription of the gene(s) for the glutamine transport system. In accordance with the nomenclature suggested by Bachmann and Low (4), the mutation in PSM2 is tentatively designated *glnPo*. A further indication of the linkage of *glnPo* to *glnP* was obtained by transducing the *glnP* mutant PSM3 with P1 phage grown on JSH210 and selecting for transductants able to grow on glutamine. After 2 days of growth at 37°C, a number of glutamine-utilizing transductants were visible, but a far larger population of tiny colonies appeared after 6 days of incubation. The first set of transductants corresponded to cells of PSM3 which had received the wild-type *glnP*$^+$ allele from JSH210, but which had retained the *glnPo* mutation. The second set of transductants were presumably those which had received both the *glnP*$^+$ allele and the wild-type allele corresponding to *glnPo*. Since the tiny transductant colonies appeared in a ratio greater than 15:1 over the large colonies, it can be estimated that the *glnPo* mutation is greater than 93% linked to the *glnP* mutation in strain PSM3.

Generation of deletions. Strains carrying deletions of the *glnP* locus and arising spontaneously in fully grown nutrient broth cultures of PSM2 were isolated in two ways. By the first method, the simultaneous acquisition of resistance to chlorate and γ-glutamylhydrazide was selected. Three deletion strains independently selected in this manner were PSM219, PSM220, and PSM221. All three of these strains were as defective in glutamine transport as PSM3, produced no cross-reacting material with antibodies raised against purified glutamine binding protein, and did not have any measurable frequency of spontaneous reversion to the ability to utilize glutamine as a sole carbon source. In addition, these strains were devoid of nitrate reductase activity, and phage P1 grown on these strains could not transduce a *chlE* mutant (PSM180) to *chlE*$^+$. This indicated that the deletions in PSM219, PSM220, and PSM221 extend at least from *chlE* into *glnP* and terminate somewhere before the biotin operon (Fig. 2).

Strains containing much larger deletions were obtained by selecting for the simultaneous resistance to γ-glutamylhydrazide and the inability to utilize galactose as a sole carbon source.

Such mutants were isolated on MacConkey agar containing 0.5% galactose and 90 to 120 μg of γ-glutamylhydrazide per ml. The deletion-carrying strains generated in this way were PSM222 and PSM223. PSM222 was defective in glutamine transport, required biotin for growth on minimal medium, and could not use glutamine or galactose as a sole source of carbon. This strain had no measurable spontaneous reversion rate to *glnP*$^+$, *gal*$^+$, or *bio*$^+$. However, P1 phage grown on PSM222 were capable of transducing PSM180 to *chlE*$^+$ at a high frequency. Thus, the deletion in this strain has one terminus between *chlE* and *glnP* and extends through the galactose operon (Fig. 2).

The deletion in strain PSM223 turned out to be even more extensive than that in PSM222. PSM223 could not transport glutamine and was unable to use either glutamine or galactose as a sole carbon source. In addition, it was found to require both niacinamide and biotin for growth on minimal medium, indicating that the left terminus of the deletion in this strain lies in the *sucAB* . . . *nadA* interval. PSM223 was unable to revert to biotin or niacinamide prototrophy, nor was it able to revert to the ability to grow on galactose or to the ability to transport glutamine. P1 phage grown on PSM223 were capable of transducing PSM180 to *chlE*$^+$ only at a very low, but not zero, frequency (10^2-fold lower than the frequency of *chlE*$^+$ transductants obtained with a P1 lysate of the deletion strain PSM222 and 10^3-fold lower than the frequency of *chlE*$^+$ transductants obtained with a P1 lysate of a wild-type strain). This indicates that the right terminus of the deletion in this strain comes very close to, but does not overlap, the *chlE* mutation in PSM180. Thus, the deletion harbored by PSM223 extends for at least 1.4 min of the *E. coli* chromosome (Fig. 2), entirely eliminating the glutamine transport genes.

The structural gene for the glutamine binding protein is situated in the *glnP* locus. Crude lysozyme-EDTA supernatants from a collection of *glnP* point mutants were screened to see whether there existed among them a strain devoid of glutamine binding activity but containing material that cross-reacted with antibodies raised against purified wild-type glutamine binding protein. It was found that one of the strains in the collection, PSM116, had these characteristics.

Osmotic shock fluid was prepared and concentrated by a carboxymethyl cellulose batch step (43) from PSM116 (*glnPo*, *glnP*) and also from JSH210 (wild type), PSM2 (*glnPo*), and PSM223 (*glnP* deletion). The specific activity of glutamine binding in such partially purified shock fluid was 1.33 nmol/mg of protein for JSH210 and almost 2.5-fold higher (3.19 nmol/mg of protein) for PSM2. In contrast, shock fluid from both PSM116 and PSM223 had less than 4% of the glutamine binding activity of PSM2 (0.057 and 0.097 nmol/mg of protein, respectively). At least in the case of PSM223, this residual glutamine binding activity was probably attributable to the glutamate-aspartate binding protein, which also has a low affinity for glutamine (43). Equal amounts of the shock fluid samples were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis with purified glutamine binding protein as a reference sample (Fig. 3), and the gel patterns clearly showed (i) an elevated intensity of the glutamine binding protein band of PSM2 in comparison with that of JSH210; (ii) a band in the shock fluid of PSM116 which had intensity and molecular weight identical to those of the glutamine binding protein band of PSM2; and (iii) the complete absence of a glutamine binding protein band in shock fluid from the *glnP* deletion strain, PSM223. Because this latter result was somewhat obscured by a faint band of slightly higher molecular weight than the glutamine binding protein, the carboxymethyl cellulose-concentrated shock fluid samples were further purified by passage through DEAE-cellulose, which binds many of the periplasmic proteins, but not the glutamine binding protein (45). The electrophoresis patterns of these DEAE-cellulose-purified samples are shown on the same gel (Fig. 3). In addition (Fig. 4), the carboxymethyl cellulose-concentrated shock fluid samples from PSM2 and PSM116 produced equally intense precipitin bands with antibodies raised against purified glutamine binding protein. The corresponding band elicited with JSH210 shock fluid was considerably less intense.

The mutation in PSM116 was shown to map in the same place as the mutation in PSM3, and transport of 2 μM glutamine by PSM116 was as defective as that of PSM3 (Fig. 1). PSM116 was essentially unable to utilize glutamine as a sole carbon source in comparison with PSM2, although it grew slightly better on this substrate than did PSM3. Like all *glnP* strains, PSM116 was found to be resistant to γ-glutamylhydrazide, methionine sulfoximine, and D-glutamine. Spontaneously arising revertants of PSM116 capable of growing as well as PSM2 on glutamine occurred at a frequency of 10^{-9} in fully grown nutrient broth cultures. Two such revertants, indepently isolated, were shown to have completely regained the ability to transport glutamine. These results prove that the *glnP* mutant PSM116 has a (revertible) point mutation in the

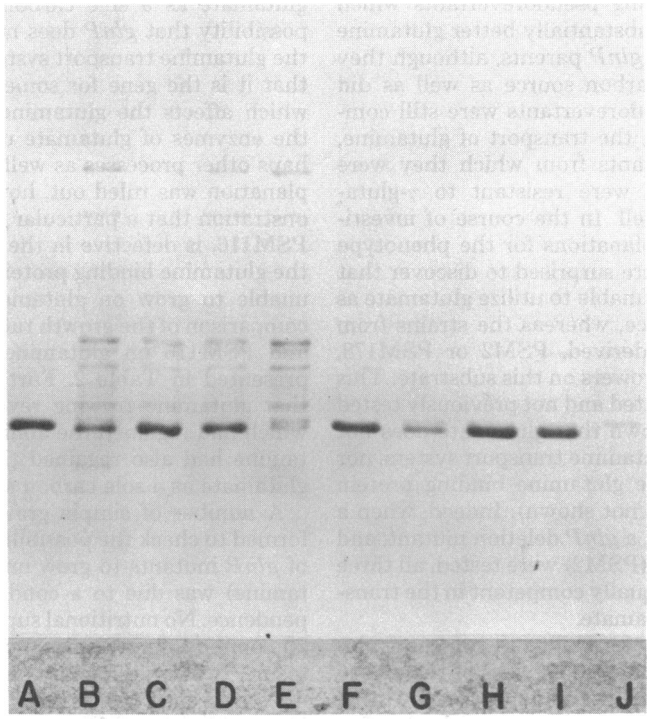

FIG. 3. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified glutamine binding protein and osmotic shock fluid from strains JSH210 (wild type), PSM2 (glnPo), PSM116 (glnPo, glnP), and PSM223 (glnP deletion). Samples of 2 μg of purified glutamine binding protein or 5 μg of shock fluid proteins were run on sodium dodecyl sulfate gel containing 10% polyacrylamide. Tracks: A and F, glutamine binding protein; B through E, carboxymethyl cellulose-concentrated samples of (B) JSH2, (C) PSM, (D) PSM116, and (E) PSM 223; G through J, DEAE-cellulose-passaged samples of (G) JSH210, (H) PSM2, (I) PSM116, and (J) PSM223.*

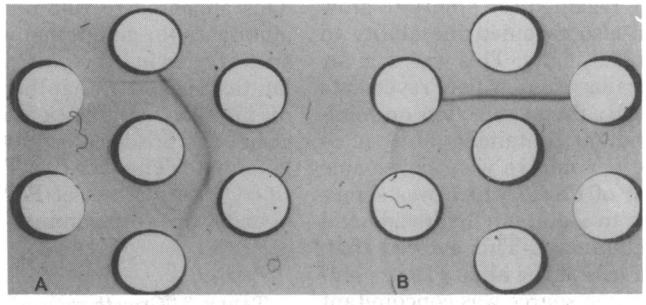

FIG. 4. *Ouchterlony double diffusion of carboxymethyl cellulose-concentrated osmotic shock fluid samples and antiserum which was raised against glutamine binding protein and purified as described in the text. Antiserum (8 μl) was placed in each center well and allowed to diffuse for 2.5 h at room temperature before the addition of shock fluid samples to surrounding wells. (A) Wells: 1.5 μg of shock fluid protein—JSH210 at 12 o'clock, PSM2 at 2 o'clock, PSM116 at 4 o'clock, and PSM223 at 6 o'clock. (B) Wells: 15 μg of shock fluid protein—JSH210 at 12 o'clock and PSM223 at 6 o'clock.*

glutamine binding protein which produces no attenutation of the amount of synthesis of the binding protein or any detectable alteration in the molecular weight or immunological cross-reactivity of the protein, but abolishes its ability to bind glutamine. Therefore, the structural gene for the glutamine binding protein must be contained within the *glnP* locus.

Inability of *glnP* strains to grow on glutamate. When certain *glnP* mutants (particularly deletions) were plated onto minimal glutamine medium, we often obtained a class of

spontaneously arising pseudorevertants which were found to be substantially better glutamine growers than their *glnP* parents, although they did not use this carbon source as well as did PSM2. These pseudorevertants were still completely defective in the transport of glutamine, like the *glnP* mutants from which they were derived, and they were resistant to γ-glutamylhydrazide as well. In the course of investigating possible explanations for the phenotype of this class, we were surprised to discover that *glnP* mutants was unable to utilize glutamate as a sole carbon source, whereas the strains from which they were derived, PSM2 or PSM178, were very strong growers on this substrate. This fact was unanticipated and not previously tested because it was known that glutamate does not compete for the glutamine transport system, nor does it bind to the glutamine binding protein (reference 42; data not shown). Indeed, when a *glnP* point mutant, a *glnP* deletion mutant, and their parent strain (PSM2) were tested, all three were found to be equally competent in the transport of 1.8 μM glutamate.

That the loss of the ability to transport glutamine always resulted in the loss of the ability to grow on glutamate was demonstrated by three criteria. (i) All *glnP* mutants tested (the five deletion strains discussed above and 53 independent point mutants), regardless of the method of their isolation, were unable to utilize glutamate as a sole carbon source. (ii) The three independent revertants of the *glnP* strain PSM3 mentioned above, which had been isolated on the basis of having regained the ability to grow on glutamine, had also regained the ability to grow very well on glutamate. This was true for all other *glnP* strains from which revertants were selected. (iii) By direct selection on minimal glutamate medium containing 30 μg of γ-glutamylhydrazide per ml, we were never able to obtain a mutant of PSM2 which was simultaneously resistant to γ-glutamylhydrazide and able to grow on glutamate. This showed that, without exception, loss of the ability to use glutamate as a sole carbon source was concomitant with the acquisition of an impairment in the glutamine transport system. It was also interesting to note that although the wild-type strain JSH210 grew reasonably well on glutamate, PSM2 (which differs from JSH210 only in the *glnPo* mutation) grew significantly better on this carbon source.

Examination of possible reasons for the inability of *glnP* mutants to grow on glutamate. The finding that single point mutations in the *glnP* locus rendered strains both unable to transport glutamine and unable to grow on glutamate as a sole carbon source raised the possibility that *glnP* does not directly code for the glutamine transport system itself, but rather that it is the gene for some regulatory protein which affects the glutamine transport system, the enzymes of glutamate utilization, and perhaps other processes as well. This potential explanation was ruled out, however, by the demonstration that a particular *glnP* point mutant, PSM116, is defective in the structural gene for the glutamine binding protein and is also totally unable to grow on glutamate. A quantitative comparison of the growth rates of PSM2, PSM3, and PSM116 on glutamine and glutamate is presented in Table 2. Furthermore, we found that glutamine-growing revertants of PSM116 which had regained the ability to transport glutamine had also regained the ability to utilize glutamate as a sole carbon source.

A number of simple growth tests were performed to check the possibility that the inability of *glnP* mutants to grow on glutamate (or glutamine) was due to a conditional nutrient dependence. No nutritional supplement tested (the 20 common amino acids, adenine, guanine, cytosine, thymine, uracil, *p*-aminobenzoate, *p*-hydroxybenzoate, pantothenate, biotin, niacinamide, pyridoxal, and folate) was able to restore the ability of PSM3, or of two other *glnP* point mutants or two *glnP* deletions, to use either glutamate or glutamine as a sole carbon source. Also, addition of either 1 or 35 mM glutamate or glutamine had no effect on the ability of *glnP* mutants to grow on minimal succinate medium. This appeared to rule out the possibility that glutamate (or glutamine) was somehow toxic to strains carrying the *glnP* mutation. In particular, this suggested that there was no impairment of nitrogen assimilation in *glnP* mutants in either the presence or absence of glutamine or glutamate. The fact that PSM2M and PSM3M (*metC*$^+$ derivatives of PSM2 and PSM3) grew equally well on minimal glucose with 1 mM

TABLE 2. *Growth rates of PSM2 (glnPo), PSM3 (glnPo, glnP), and PSM116 (glnPo, glnP) on minimal glutamine and glutamate media*[a]

Strain	Doubling time (h) on:	
	Glutamate	Glutamine
PSM2	2.3	2.9
PSM3	>30	>30
PSM116	>30	5.2

[a] Media were as described in the text with glutamine or glutamate at 35 mM (0.5%). Cultures were grown at 37°C with rapid swirling, and growth rates were monitored by following optical density at 660 nm.

glutamine as the sole nitrogen source also supports this conclusion.

All of the data presented above are consistent with the interpretation that *glnP* mutants are, by an unknown mechanism, directly impaired in the utilization of glutamate (and, hence, of glutamine) as a sole source of carbon. Compatible with this was the finding that *glnP* mutants were unable to grow on proline as a sole source of carbon, whereas the parent strains PSM2 and PSM178 grew well on this substrate. Proline is catabolized via conversion to pyrroline-5-carboxylate and then to glutamate (27). There was, however, a set of anomalous observations deriving from various growth experiments. It was possible, at a very high frequency, to select pseudorevertants of PSM3 (and other *glnP* mutants) which were able to grow on proline as a sole carbon source, but which were still nongrowers on glutamine or glutamate. We also obtained pseudorevertants of PSM223 and other *glnP* deletion strains, as mentioned above, which had recovered an intermediate capacity to utilize either glutamine or glutamate as a sole carbon source. Glutamine-utilizing pseudorevertants were still unable to grow on glutamate; glutamate-utilizing pseudorevertants were still unable to grow on glutamine. These pseudoreversion characteristics had to be due to second-site mutations, because they arose in strains in which the *glnP* locus had been completely removed by deletion, and because these strains remained defective in glutamine transport and were still resistant to γ-glutamylhydrazide. The existence of these classes of pseudorevertants with mutually exclusive growth capabilities seems to undermine the notion that *glnP* exerts a direct effect on glutamate utilization. However, little can be inferred from these secondary lesions since they are only partially compensatory and may point to alternate routes of catabolism of glutamine, glutamate, and proline.

All of the transport data which have been presented thus far in this work were obtained by using substrate present at external concentrations in the range of 1 to 2 μM. However, all growth experiments examined the ability of cells to use carbon sources available in media at a concentration of 0.5%; for glutamate and glutamine, this was equal to 35 mM. To check the possibility that transport of substrates in this concentration range differed drastically from the transport properties which had been noted at a 10^3-fold lower concentration, we assayed the uptake of 1 mM glutamine and 1 mM glutamate by PSM2, PSM3, and PSM116 in the same salts buffer (minimal salts medium E) in which cells were always grown. As shown in Fig. 5A, PSM3 and PSM116 remained grossly defective in the transport of glutamine at this external concentration, possibly enough so to account for their inability to grow on this substrate. However, transport of 1 mM glutamate was identical in the *glnP* mutants and their parent strain (Fig. 5B) and, hence, cannot account for the inability of *glnP* mutants to use this substrate as a sole carbon source. To pursue this further, we looked at the total uptake (transport and incorporation via metabolism) over 4 h of glutamine and glutamate by cells of PSM2, PSM3, and PSM116 which had been harvested during exponential growth on minimal glucose and shifted, after extensive washing, to minimal media containing either 35 mM glutamine or 35 mM glutamate. The results of these experiments are presented in Fig. 6. In parallel experiments it was shown that virtually all glutamine or glutamate taken up was incorporated in trichloroacetic acid-pre-

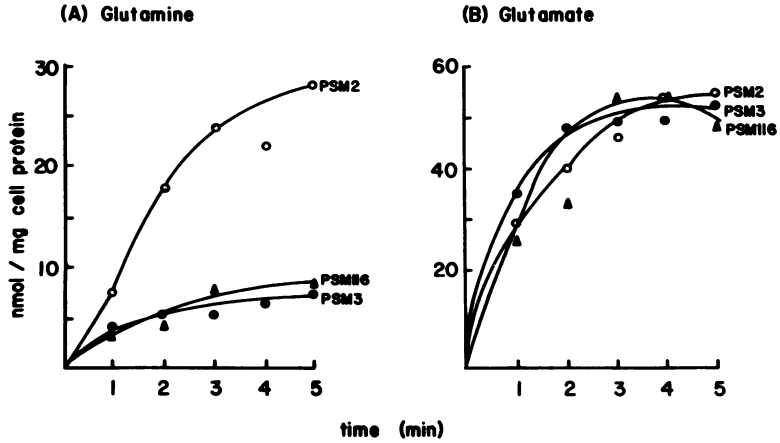

FIG. 5. *Transport by PSM2, PSM3, and PSM116 of 1 mM glutamine (A) and 1 mM glutamate (B).*

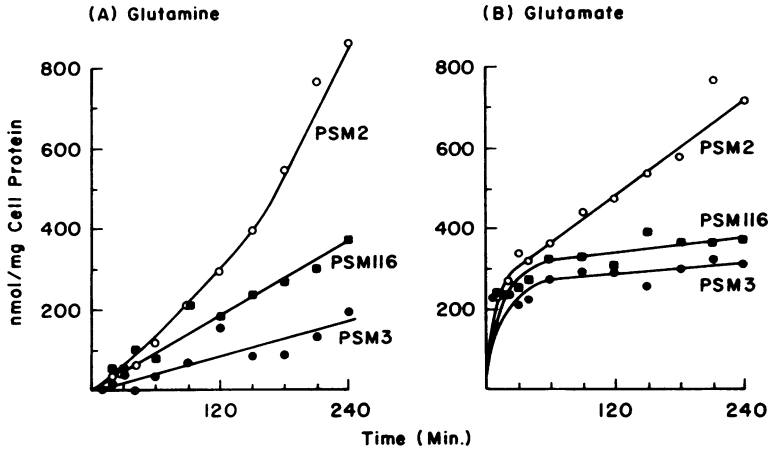

FIG. 6. *Total uptake by PSM2, PSM3, and PSM116 of 35 mM glutamine (A) and 35 mM glutamate (B).*

cipitable material. These data, which correlate with the observed growth rates on glutamine and glutamate (Table 2), are consistent with the interpretation that at least for glutamate, the inability of *glnP* mutants to grow on this substrate is due to an impairment in glutamate catabolism.

The principal pathway of glutamate utilization in *E. coli* is shown in Fig. 7 (21). Glutamate is metabolized via the Krebs cycle after its conversion to α-ketoglutarate. Its cosubstrate in the transamination reaction, oxaloacetate, then must be regenerated through the deamination of aspartate to fumarate. Mutants defective in either the glutamate-aspartate transaminase or aspartase lose the ability to grow on glutamate (21). Further, it has been shown that the NADP-dependent glutamate dehydrogenase, which can also catalyze the conversion of glutamate to α-ketoglutarate, plays no role in the degradation of glutamate (39). The fact that all *glnP* mutants tested grew as well as PSM2 or PSM178 on either α-ketoglutarate or succinate indicated that the lesion in these strains does not affect the Krebs cycle, and this suggested that we should examine the activities of glutamate-aspartate transaminase and aspartase. The levels of these two enzymes were assayed in extracts prepared from exponentially growing cells of PSM2 and PSM3 both before and after cells were incubated for 4 h in minimal media containing either 35 mM glutamine or 35 mM glutamate. We also assayed glutaminase A activity because the results of Willis et al. (44) suggested that this enzyme is induced by growth in the presence of glutamine. As shown in Table 3, the specific activities of transaminase and glutaminase A remained essentially constant in both strains under all conditions of growth. The levels

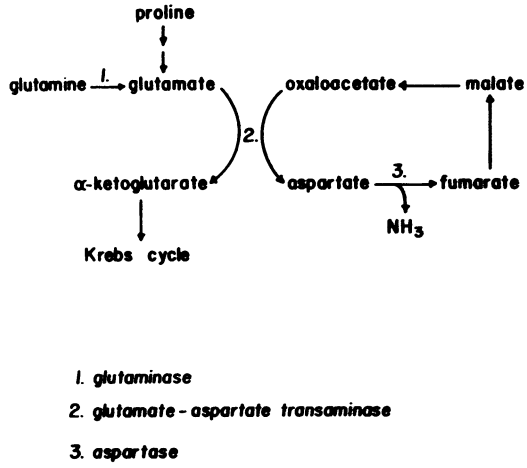

FIG. 7. *The principal pathway of the catabolism of glutamate in E. coli, adapted from Marcus and Halpern (21).*

TABLE 3. *Levels of aspartase, glutamate-aspartate transaminase, and glutaminase A in PSM2 and PSM3 under various growth conditions*[a]

Strain	Incubated with	Sp act (μmol/min per mg of protein)		
		Aspartase	Transaminase	Glutaminase A
PSM2		0.055	0.559	0.015
PSM3		0.056	0.717	0.013
PSM2	Glutamine	0.245	0.520	0.014
PSM3	Glutamine	0.203	0.489	0.003
PSM2	Glutamate	0.254	0.486	0.013
PSM3	Glutamate	0.208	0.640	0.013

[a] Growth conditions, preparation of extracts, and assays are described in the text.

of aspartase were significantly induced by exposure of cells to either glutamine or glutamate, and the same degree of induction was seen in both the *glnP* mutant and its parent strain. Thus, there is no obvious defect in the enzymes involved in the initial steps of glutamate catabolism in extracts prepared from cells containing a *glnP* mutation.

DISCUSSION

The active transport of glutamine by *E. coli* is due almost exclusively to a high-affinity, osmotic shock-sensitive active transport system which is specific for glutamine and no other naturally occurring amino acid (6, 7, 41, 42). The periplasmic glutamine binding protein, an indispensable component of this transport system, has been well studied (13, 22, 42); the existence of other components essential to glutamine transport has not as yet been demonstrated. As shown in this paper, it is possible, by a variety of selections, to obtain strains possessing single-point mutations that render them almost completely unable to transport glutamine. At least one of these mutations, the one in strain PSM116, is located within the gene coding for the glutamine binding protein. In every glutamine transport mutant that we have obtained, including strains with deletions that encompass the glutamine transport genes, there is a residual amount of glutamine transport not due to the high-affinity system. Weiner and Heppel (42) also noted this secondary transport in a γ-glutamylhydrazide-resistant strain they isolated and found that it could be completely suppressed by a 10-fold excess of glutamate. It is thus probably attributable to the shock-sensitive glutamate-aspartate transport system, the binding protein for which also has a low affinity for glutamine (43). This minor contribution to glutamine transport is insignificant in transport studies involving glutamine at micromolar external concentrations (Fig. 1), but it may be of importance when glutamine is presented to cells at millimolar concentrations (Fig. 5A), as it is in growth studies. Indeed, this second mode of glutamine transport is apparently sufficient to sustain the growth on glutamine of pseudorevertants derived from strains which have lost the high-affinity glutamine transport system through deletion.

Mutations in the glutamine transport system reside in a single locus, *glnP*, which maps at 17.7 min on the revised *E. coli* linkage map (4), between the *bio* and *chlE* genes. It is interesting to note that a methionine sulfoximine-resistant glutamine transport mutant isolated in *S. typhimurium* by Betteridge and Ayling (8) was shown, by conjugation, to map approximately in the vicinity of the *galOEK* gene cluster in that organism. It is possible that this mutant, also designated *glnP*, will prove to map between *bio* and *chlE* since there is a very great degree of homology between the *E. coli* and *S. typhimurium* chromosomes in that immediate region (30).

In the work presented here we have shown that the presence of another mutation, *glnPo*, which is closely linked to *glnP*, elevates the levels of the glutamine binding protein and of glutamine transport. In this respect, *glnPo* is analogous to the *dhuA* mutation in *S. typhimurium* (3) which induces or derepresses the high-affinity histidine transport operon and maps immediately proximal to the gene for the histidine binding protein. Kustu et al. (14) have described a class of *S. typhimurium* nitrogen regulatory mutants, *glnR*, which have elevated synthesis of the histidine, glutamine, lysine-arginine-ornithine, and glutamate-aspartate periplasmic binding proteins. The *glnR* gene is distinct from, but closely linked to, *glnA*, the structural gene for glutamine synthetase. That the *glnPo* mutation is different from *glnR* is evidenced by two observations: (i) *glnPo* is distant from *glnA* which, in *E. coli*, maps at 86 min (4); and (ii) a comparison of the proteins in osmotic shock fluid from strains JSH210 (wild type) and PSM2 (*glnPo*) shows an apparent increase of only the glutamine binding protein as a result of the *glnPo* mutation (Fig. 3). This suggests that *glnPo* affects only the glutamine transport operon, whereas *glnR* influences a number of separate operons. It is possible that the wild-type product of the *E. coli* gene homologous to *glnR* interacts with the *glnPo* region of the chromosome.

Another effect of the *glnPo* mutation is to significantly increase the rate at which cells are able to grow on either glutamine or glutamate as a sole source of carbon. In the case of glutamine, it could be argued that this is due solely to the heightened transport of this substrate. For glutamate, however, this explanation is untenable since the glutamine transport system plays no role in glutamate uptake. Related to this, and equally perplexing, is the observation that *glnP* mutants are unable to grow on glutamate (or glutamine) as a sole carbon source. Again, for glutamine, it is possible, but not likely, that a 3-fold difference in the rate of transport of this substrate (Fig. 5) can account for a greater than 10-fold difference in the growth rates of PSM2 and PSM3 on minimal glutamine (Table 2). For glutamate, however, there are no transport differences between PSM2 and PSM3. It is

conceivable that differences in glutamate uptake are manifested only after cells are exposed to this substrate for a long period of time, but we do not favor this hypothesis because of the multiplicity of transport systems which enable *E. coli* cells to accumulate glutamate (32).

The present evidence implies that the glutamine transport system directly interacts with some cell component which is involved in the catabolism of glutamate. The most convincing piece of data in this regard is that a particular *glnP* mutant, PSM116, which has been clearly shown to be a point mutant in the structural gene for the glutamine binding protein, has the identical characteristics of all other *glnP* mutants. Most notably, it is unable to grow on glutamate. There is precedent for certain periplasmic binding proteins serving multiple functions. The binding proteins for galactose, maltose, and ribose, in addition to their transport role, act as the primary receptors in the chemotaxis of cells toward these three carbohydrates (11). It is not unreasonable that the glutamine binding protein, or the entire intact transport system, could be required for the modulation of the degradation of glutamate (and, hence, glutamine), although the advantage afforded cells by such a regulatory process is not evident. An alternative explanation for the observed data is that some component essential to glutamate utilization is coded for by a gene distal to the glutamine transport genes and that every *glnP* mutation we have isolated exerts a polar effect on that gene. This hypothesis is not easy to reconcile with the known binding protein point mutant, PSM116. If the mutation in this strain is polar, then it would have to be a nonsense mutation. The fact that the defective glutamine binding protein produced by PSM116 has a molecular weight indistinguishable by sodium dodecyl sulfate-polyacrylamide gel electrophoresis from that of wild-type binding protein argues against this.

If *glnP* mutants are, indeed, impaired in the catabolism of glutamine and glutamate, the nature of this lesion is not obvious. The two enzymes requisite for the utilization of glutamate, aspartase and glutamate-aspartate transaminase, are present in the same amounts in cell extracts from PSM2 and PSM3, both before and after a 4-h shift to minimal glutamine or minimal glutamate growth medium. In particular, aspartase levels are induced more than threefold in both strains in the presence of glutamine or glutamate. Induction of aspartase by growth on glutamate has also been observed by others (21, 34). It is possible that *glnP*-mediated differences in glutamate catabolism are too subtle to be detected in sonicated cell extracts. The intact transport system may be directly responsible for the observed growth properties of cells. It has been asserted that aspartase associates with membranes of *E. coli* (36). Conceivably, this association could involve the glutamine transport system and could regulate the activity of this enzyme.

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