JOURNAL OF BACTERIOLOGY, Jan. 1980, p. 190-198
0021-9193/80/01-0190/09$02.00/0

Vol. 141, No. 1

Salmonella typhimurium Mutants with Altered Glutamate Dehydrogenase and Glutamate Synthase Activities

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Although glutamate is a key compound in nitrogen metabolism, little is known about the function or regulation of its two biosynthetic enzymes, glutamate dehydrogenase and glutamate synthase. To begin the characterization of glutamate formation in *Salmonella typhimurium*, we isolated mutants having altered glutamate dehydrogenase and glutamate synthase activities. Mutants which failed to grow on media with glucose as the carbon source and less than 1 mM $(NH_4)_2SO_4$ as the nitrogen source (Asm^-) had about one-fourth the normal glutamate synthase activity and one-half the glutamine synthetase activity. The *asm* mutations also prevented growth with alanine, arginine, or proline as nitrogen sources and conferred resistance to methionine sulfoximine. When a mutation (*gdh-51*) causing the loss of glutamate dehydrogenase activity was transferred into a strain with an *asm-102* mutation, the resulting *asm-102 gdh-51* mutant had a partial requirement for glutamate. A strain isolated as a complete glutamate auxotroph had a third mutation, in addition to the *asm-102 gdh-51* lesions, that further decreased the glutamate synthase activities to 1/20 the normal level. Both the *asm-102* and *gdh-51* mutations were located on the *S. typhimurium* linkage map at sites distinct from those found for mutations causing similar phenotypes in *Klebsiella aerogenes* and *Escherichia coli*.

Recent work has helped describe the regulatory mechanisms of repression and attenuation for certain amino acid biosynthetic pathways (27). For most of these well-characterized pathways, the amino acid is used only for protein synthesis. The amino acids glutamate and glutamine, however, differ because they are used as amino and amido donors for other nitrogenous compounds as well as being needed for protein synthesis. Thus, the regulation of the three enzymes that synthesize glutamate and glutamine may have some novel features in addition to control elements common to other biosynthetic pathways. Despite the central importance of the glutamate and glutamine biosynthetic enzymes in ammonia assimilation and nitrogen metabolism, however, there is a paucity of information regarding their regulation. Much of the physiological and genetic work examining the regulation of nitrogen utilization has focused on the glutamine biosynthetic enzyme, glutamine synthetase (EC 6.3.1.2), rather than on the glutamate biosynthetic enzymes. The glutamate enzymes, however, have characteristics that make the study of their regulation particularly interesting.

One unusual aspect of glutamate synthesis is that there are two enzymes that can independently form glutamate. Preliminary work with *Klebsiella aerogenes* and *Escherichia coli* mutants lacking one or both of the glutamate-synthesizing enzymes, glutamate dehydrogenase (EC 1.4.1.4) and glutamate synthase (EC 2.6.1.53), suggests that either enzyme can function as long as sufficient ammonia is available (2, 5, 6). Although mutants lacking glutamate synthase activity grow on media with excess ammonia, they fail to grow on media containing less than 1 mM $(NH_4)_2SO_4$ or with compounds used slowly as nitrogen sources (6). These results suggest that glutamate synthase is required for the assimilation of low concentrations of ammonia and that glutamate dehydrogenase can function for glutamate production only when sufficient ammonia is available. Consistent with this view is the finding that glutamate dehydrogenase activities are low in *K. aerogenes* cells grown with a limiting nitrogen source (6, 17).

Although glutamate synthase may function primarily as a low-ammonia assimilatory enzyme, another unusual aspect of glutamate production is that the highest activities for glutamate synthase are found for cells grown in media with excess ammonia (4, 6). When cells are grown in a highly supplemented medium or in glucose minimal medium with glutamate as a nitrogen source, the glutamate synthase activi-

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ties are lowered 5- to 10-fold (4). Thus, glutamate synthase may be regulated in response to the supply of glutamate. In this respect, glutamate synthase has an interesting position in cell metabolism. It is part of a physiological cycle in which its product, glutamate, is converted to its substrate, glutamine. Considering these characteristics, it is likely that the function and control of glutamate synthase is more complicated than anticipated for an enzyme considered important only for low-ammonia assimilation. Unfortunately, the physiological studies are limited, and no mutants with altered regulation of this enzyme have been characterized.

Because of these unusual features associated with glutamate formation, we wanted to obtain mutants with altered glutamate dehydrogenase and glutamate synthase activities. We were particularly interested in obtaining glutamate dehydrogenase mutants in *Salmonella typhimurium* because, as opposed to *K. aerogenes*, the glutamate dehydrogenase does not decrease in *S. typhimurium* cells grown with a limiting nitrogen source (4, 6). Thus, the function, as well as the regulation, of glutamate dehydrogenase could differ between *K. aerogenes* and *S. typhimurium*.

To initiate this study on the relative functions and regulation of these two enzymes in *S. typhimurium*, mutants with low glutamate synthase activity were first isolated. These mutants were unable to assimilate low concentrations of ammonia and thus have an Asm$^-$ phenotype. A glutamate auxotroph isolated from an Asm$^-$ mutant had an absolute requirement for glutamate caused by the loss of glutamate dehydrogenase plus an additional mutation that further reduced the glutamate synthase activity. Conjugation and transduction studies showed that the mutation causing low glutamate synthase activity (*asm-102*) and the one eliminating glutamate dehydrogenase activity (*gdh-51*) lie in regions of the chromosome that differ from those of mutations abolishing the corresponding enzyme activities in *K. aerogenes* (1, 12) and *E. coli* (20).

MATERIALS AND METHODS

Bacterial strains. The properties of the *S. typhimurium* strains used in this study, all derivatives of LT-2, are summarized in Table 1.

TABLE 1. *Genotype and origin of bacterial strains*

Strain	Genotype	Source (reference)[a]
JL907	*galE hutR49*	
JB664	*galE hutR49 asm-101*	NTG mutagenesis of JL907
JB665	*galE hutR49 asm-102*	NTG mutagenesis of JL907
JB801	*galE hutR49 asm-102 gdh-51*	NTG mutagenesis of JB665
JB810	*galE hutR49 asm-102 glt-759*	NTG mutagenesis of JB665
JB1075	*galE hutR49 asm-102 zcd-2*::Tn*10*	Transduction (see text)
JB1173	*galE hutR49 gdh-51*	Transduction of JB801 with JL907 as donor
JB1174	*galE hutR49 gdh-51*	Transduction of JB801 with JL907 as donor
JB1175	*galE hutR49 asm-102*	Transduction of JB801 with JL907 as donor
JB1176	*galE hutR49 asm-102*	Transduction of JB801 with JL907 as donor
JB1177	*galE hutR49 asm-102 gdh-51 zcd-2*::Tn*10*	Transduction of JB801 with JB1075 as donor
JB1178	*galE hutR49 asm-102 gdh-51 zcd-2*::Tn*10*	Transduction of JB665 with JB1177 as donor
JB1179	*galE hutR49 asm-102 gdh-51 zcd-2*::Tn*10*	Transduction of JB665 with JB1177 as donor
JB1180	*galE hutR49 asm-102 gdh-51 zcd-2*::Tn*10*	Transduction of JB665 with JB1177 as donor
SA486	HfrK3 *serA13 rfa-3058*	K. E. Sanderson (24)
SA534	HfrK4 *serA13 rfa-3058*	K. E. Sanderson (24)
SA536	HfrK6 *serA13 rfa-3058*	K. E. Sanderson (24)
SA654	HfrK9 *thrA9*	K. E. Sanderson (24)
SA955	HfrK20 *serA13 rfa-3058*	K. E. Sanderson (24)
SA965	HfrK17 *leuBCD39 ara-7*	K. E. Sanderson (24)
JB1102	*argG*	Laboratory collection
JB1120	*put-11*	Laboratory collection
JB1182	*purB12*	J. Gots
JB1105	HfrK4 *serA13 rfa-3058 zcd-2*::Tn*10*	Transduction of SA534 with JB1075 as donor
JB1113	HfrK6 *serA13 rfa-3058 zcd-2*::Tn*10*	Transduction of SA536 with JB1075 as donor
JL67	*trp-2 metA22 tyr-40 str xyl-1*	J. Ingraham
JL68	*trp-2 metA22 his-1009 strA201*	J. Ingraham
JL72	*trp-4 metG365 thr-97 leu-197 pro-24 pyrC-197 purB210*	J. Ingraham
JL436	*pyrC7 cod-101 metC30 strA*	J. Ingraham
JL615	*pyrD135*	J. Ingraham
SK75	*nit-9 galE1797*	S. Kustu (7)

[a] NTG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

Chemicals. L-Methionine-DL-sulfoximine (MS) and tetracycline were obtained from Sigma Chemical Co., St. Louis, Mo. All other reagents are commercially available.

Media. The Luria broth (LB) and glucose minimal medium, which contains excess ammonia [15 mM $(NH_4)_2SO_4$], were described previously (3). For ammonia-limiting conditions, either 0.5 mM $(NH_4)_2SO_4$ or an organic nitrogen source present at a concentration of 0.4% was substituted for the excess ammonia in glucose minimal medium. The minimal medium designations give the carbon source followed by the nitrogen source. For example, glucose-ammonia refers to a medium containing glucose as the carbon source and $(NH_4)_2SO_4$ as the nitrogen source, and glucose-arginine refers to a medium with glucose as the carbon source and arginine as the nitrogen source. MS sensitivity was scored on glucose-ammonia medium plus MS (1 mM). The inability to grow on glucose-arginine medium was used to score the Asm$^-$ phenotype. Tetracycline (Tet) sensitivity was scored on LB-tetracycline (50 μg/ml) or glucose-ammonia-tetracycline (50 μg/ml) medium. L-Glutamate was added as a supplement at 4 mg/ml; other amino acid supplements were added at 20 μg/ml and vitamin supplements at 2 μg/ml, unless indicated otherwise.

Mutagenesis. Cultures were mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine at 10 μg/ml for 20 min at 37°C. After growth in a permissive medium, the cells were washed, diluted, and suspended in a restrictive medium as described in the text for each selection. After two doublings, penicillin G (20,000 U/ml) was added, and the culture was allowed to grow for three doublings. The survivors were grown in permissive media to stationary phase and then plated on appropriate media.

Growth of cells and preparation of extracts. We used the procedures described earlier (10), with the exception of the buffer used for sonication. It contained 0.1 M *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES; pH 7.1 at 25°C), 10 mM $MnCl_2$ and 14 mM mercaptoethanol.

Enzyme assays. The glutamate synthase and glutamate dehydrogenase assays measured the rate of oxidation of NADPH at 37°C by using modifications of the procedures of Meers et al. (17). The glutamate synthase assay mixture contained: 50 mM TES, pH 7.2 at 25°C; 5 mM α-ketoglutarate, pH 7.2 at 25°C; 0.25 mM NADPH; and 20 mM L-glutamine. The glutamate dehydrogenase assay mixture contained: 50 mM Tris, pH 8.1 at 25°C; 5 mM α-ketoglutarate, pH 8.1 at 25°C; 0.25 mM NADPH; and 40 mM NH_4Cl.

Glutamine synthetase activity was determined by the γ-glutamyl transferase assay of Stadtman et al. (25) with the modification that 50 mM TES buffer, pH 7.3 at 25°C, replaced the mixed imidazole buffer.

Proteins were determined by the method of Lowry et al. (15), using bovine serum albumin as the standard. Specific activities for all enzymes are reported as nanomoles of product formed per minute per milligram of protein. The results are averages of at least three separate experiments with duplicate or triplicate assays for each experiment.

Transductions and conjugations. We used either KB1 (*int-1*) (16), P22 HT105/*int* (21) or P1 *kc-vir* (9) phage for transductions. When galactose-negative, P1-sensitive strains were used with KB1 or P22, the LB was supplemented to contain 0.2% D-glucose and 0.3% D-galactose. Transductions with P1 followed the procedures of Enomoto and Stocker (9), and P1-sensitive derivatives were selected by the method of Ornellas and Stocker (18).

The donors and recipients for conjugation were grown overnight, without shaking, in LB medium. Donor cultures were then diluted 1:20 in fresh LB and allowed to grow for 2 h. The recipient cultures (0.1 ml) were spread onto selective media, and 0.075 ml of the donor culture was added. The concentration of donor was adjusted by dilution to yield several hundred exconjugants per plate. Donor and recipient cells alone were plated as controls.

Selection of Tn*10* insertion strains and preparation of a transducing lysate. The vehicle used for introducing TN*10*, P22 Tc10 *c2*ts29 12$^-$ am*N*11 13$^-$ am*H*101 *int3 sieA*44 *mnt*ts *ant*$^-$, was maintained as a lysogen (13), and the transducing lysate was prepared by S. Rosenfeld using the following procedure. Strains with Tn*10* inserted into the chromosome at random were obtained by infecting a wild-type strain of *S. typhimurium*, JL781, with this phage. Samples of the infected culture were spread on green indicator plates (8) containing 50 μg of tetracycline per ml and incubated at 40°C to select for tetracyline resistant (Tet^r) cells. Approximately 5,000 Tet^r colonies, each arising by an independent insertion, were scraped from the plates and pooled. A generalized transducing lysate of phage P22 was prepared from this mixed culture by standard methods and used to look for cotransduction of Tet^r with specific mutations (14).

RESULTS

Isolation of Asm$^-$ mutants. One objective of the study of glutamate synthesis in *S. typhimurium* was to obtain glutamate auxotrophs lacking glutamate synthase and glutamate dehydrogenase activities. However, it seemed likely that the loss of both enzyme activities would be necessary before a strain required glutamate and that an auxotroph would have to be obtained by selecting for the sequential loss of each activity. Assuming that the loss of glutamate synthase activity in *S. typhimurium* would cause an Asm$^-$ phenotype similar to that found for *K. aerogenes* and *E. coli*, we first selected mutants unable to grow on low concentrations of ammonia or certain amino acids as nitrogen sources. These mutants were isolated by penicillin counterselection in glucose-alanine medium. Colonies able to grow on glucose-ammonia medium, but unable to grow with limiting ammonia [0.5 mM $(NH_4)_2SO_4$] or on glucose-alanine medium were isolated as possible Asm$^-$ mutants.

Growth characteristics of Asm$^-$ strains. Two Asm$^-$ mutants, JB664 (*asm-101*) and JB665 (*asm-102*), obtained from independent selections were characterized. Although they grew in glu-

cose-ammonia medium, their growth rates were slower than that of the parent, JL907, (84 and 78 min for JB664 and JB665, respectively, compared with 55 min for JL907). The addition of glutamine restored the wild-type rate. Although the Asm$^-$ mutants cannot use some nitrogen sources, specifically alanine, arginine, and proline, they grow on other compounds such as asparagine, aspartate, glutamate, glutamine, serine, adenosine, and cytidine that are readily used as nitrogen sources by *S. typhimurium*. Because the difference in growth between Asm$^+$ and the Asm$^-$ mutants was greatest on glucose-arginine medium, this was used to score the Asm phenotype in subsequent experiments.

Mutants isolated previously in this laboratory for their resistance to the analog MS also had an Asm$^-$ phenotype (26). Therefore, we examined the growth of JB664 and JB665 with this analog to determine if they had simultaneously become MS resistant (MS^r). The parent strain JL907 is completely inhibited by 1 mM MS and partially inhibited by 0.2 mM; strains JB664 and JB665 are resistant to 1 mM MS. Therefore, the mutants selected for an Asm$^-$ phenotype are also MS^r.

Activities of the ammonia assimilatory enzymes in Asm$^-$ strains. We measured the three ammonia assimilatory enzymes to determine whether the new Asm$^-$ MS^r mutants had altered activities (Table 2). Both JB664 and JB665 had greatly reduced glutamate synthase activity (approximately 20 to 40% of the level in JL907). In addition, both strains had reduced glutamine synthetase activity (approximately 50% of the level in JL907). The glutamate dehydrogenase activity was within the normal range associated with the parent, JL907.

These mutants had pleiotropic properties since they were Asm$^-$ MS^r and had reduced activities for both glutamate synthase and glutamine synthetase. To determine whether these properties resulted from a single lesion, Asm$^+$ transductants of both JB664 and JB665 were constructed and analyzed. These Asm$^+$ transductants were similar to the original parent JL907 in that they were sensitive to MS and had normal glutamine synthetase and glutamate synthase activities (data not shown). Thus, the altered sensitivity to MS and the reduced levels of both glutamate synthase and glutamine synthetase are all due to either a single mutation or closely linked mutations.

In *S. typhimurium*, MS inhibits both glutamine synthetase and glutamate synthase activities in vitro (26). Because both glutamate synthase and glutamine synthetase activities are lowered in the Asm$^-$ strains, we wanted to determine whether either enzyme activity had become resistant to methionine sulfoximine. Glutamine synthetase assays demonstrated that the activity was equally sensitive to MS with extracts from both Asm$^+$ and Asm$^-$ cells (Fig. 1). Thus, the mutation conferring growth resistance to MS appears not to have altered glutamine synthetase directly. This result is consistent with genetic experiments showing that the mutation causing MS resistance in JB664 and in JB665 was not linked by KB1-mediated transduction (<1%) to *glnA*, the structural gene for glutamine synthetase. Because of the low level of glutamate synthase in the mutants, we were unable to determine whether the MS sensitivity of this enzyme activity was altered.

Isolation and characterization of a mutant lacking glutamate dehydrogenase activity. Although strains JB664 and JB665 retained some glutamate synthase activity, they had a clear Asm$^-$ phenotype on glucose-arginine

TABLE 2. *Activities of the ammonia assimilatory enzymes in Asm$^-$ mutants*

Strain	Sp act[a]		
	GltS	GS	GDH
JL907 (Asm$^+$)	214	84	422
JB664 (Asm$^-$)	88	44	414
JB665 (Asm$^-$)	44	41	554

[a] GltS, Glutamate synthase; GS, glutamine synthetase; GDH, glutamate dehydrogenase. All enzyme activities are specific activities in which one unit represents the synthesis of one nanomole of product per minute per milligram of protein.

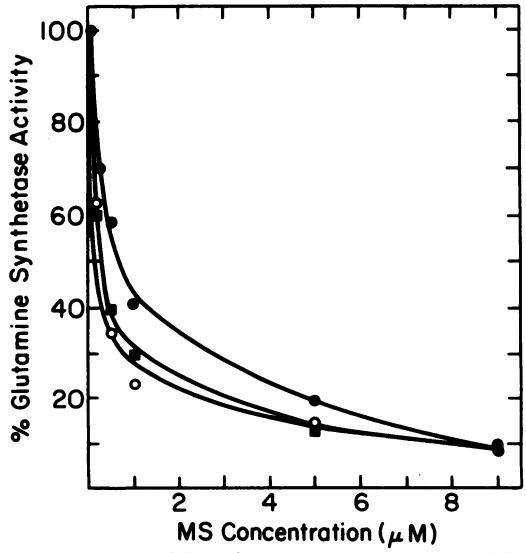

FIG. 1. *Inhibition of glutamine synthetase activity by MS. Crude extracts were assayed as described in the text. Symbols: ■, JL907; ○, JB664; ●, JB665.*

medium, indicating that the low glutamate synthase and glutamine synthetase activities prevented normal assimilation of this growth-rate-limiting nitrogen source. We next wanted to determine whether a glutamate auxotroph lacking glutamate dehydrogenase could be obtained from an Asm$^-$ mutant. Strain JB665 was mutagenized, and a penicillin counterselection was used to obtain glutamate auxotrophs. These auxotrophs were scored for their ability to use α-ketoglutarate or citrate instead of a glutamate supplement to distinguish mutants having lost glutamate dehydrogenase from those having lost a tricarboxylic acid cycle enzyme. The growth of two mutants, JB801 and JB810, was examined in detail. Both strains grew when supplemented with glutamate, but strain JB810 also grew with α-ketoglutarate or citrate as a supplement, indicating that its glutamate requirement was caused by an alteration in the tricarboxylic acid cycle. Strain JB801 grew only when glutamate was added. Enzyme assays showed that JB801 (*gdh-51*) lacked glutamate dehydrogenase activity, whereas JB810, as suggested by its ability to use citrate or α-ketoglutarate, had this enzyme activity.

The Asm$^-$ mutants grew without exogenous glutamate, indicating that glutamate dehydrogenase activity alone was sufficient to give a Glt$^+$ phenotype. However, it was not known whether the glutamate synthase activity alone is also sufficient for glutamate production, since the glutamate auxotroph carries both the *gdh-51* and *asm-102* mutations. To construct a strain carrying only the *gdh-51* mutation, Glt$^+$ transductants of JB801 (*asm-102 gdh-51*) were selected by using P1 phage prepared on the wild-type strain. Two phenotypic classes, Asm$^-$ and Asm$^+$, were obtained. Several of each type were purified and the enzymes assayed (Table 3). Glt$^+$ transductants with the Asm$^-$ phenotype had regained glutamate dehydrogenase, but not glutamate synthase, activity and resembled the parent, JB665. Those with the Asm$^+$ phenotype still lacked glutamate dehydrogenase activity but had glutamate synthase activity. These results show that the presence of either glutamate synthase activity alone or glutamate dehydrogenase activity alone allows for the synthesis of enough glutamate to give a prototrophic phenotype. In addition, since none of the transductants regained both activities, the results show that the *asm-102* and *gdh-51* mutations are not closely linked.

TABLE 3. *Enzyme activities in Glt$^+$ transductants of strain JB801*

Strain[a]	Sp act[b]	
	GltS	GDH
JL907	203	377
JB665 (*asm-102*)	39	506
JB801 (*asm-102 gdh-51*)	12	2
JB1175 (*asm-102*)	18	309
JB1176 (*asm-102*)	9	196
JB1173 (*gdh-51*)	136	0
JB1174 (*gdh-51*)	126	3

[a] All cultures were grown in glucose-ammonia medium supplemented with 0.4% glutamate.

[b] GltS, Glutamate synthase; GDH, glutamate dehydrogenase.

We also scored these transductants for MS sensitivity. As expected, Asm$^-$ transductants were still MS^r, whereas the Asm$^+$ transductants were no longer as resistant to MS as the parent strain JB665. However, this latter class grew slowly in the presence of 1 mM MS, appearing slightly more resistant than the wild type, JL907. Furthermore, when the glutamate synthase activity of these Asm$^+$ transductants was compared with that of JL907 (Table 3), the levels were slightly lower than expected. We had earlier noted that this enzyme activity in JB801 was consistently lower than the already reduced level of its Asm$^-$ parent, JB665. The two Asm$^-$ transductants were like JB801 in that the level of glutamate synthase activity was even lower than that in JB665.

One simple explanation for these results is that JB801 carries a third, unlinked mutation in addition to the *asm-102* and *gdh-51* mutations. This mutation could cause further reduction in glutamate synthase activity and would account for the glutamate synthase levels in JB801 and the Asm$^+$ transductants being lower than those in strains JB665 and JL907, respectively. One method of testing this would be to reconstruct a double mutant (*asm-102 gdh-51*) by transducing *gdh-51* back into JB665 (*asm-102*) and to compare its enzyme levels with those in JB801. If there is a third mutation in JB801 causing the lower glutamate synthase activity, then these reconstructed strains should have glutamate synthase levels comparable to the level in JB665 rather than the lower level found for JB801. To reconstruct an *asm-102 gdh-51* strain, a method had to be found to select for the transfer of *gdh-51* into the original JB665 strain. This presented a difficulty because the map position for *gdh-51* was unknown (see below) and the loss of glutamate dehydrogenase alone has no detectable phenotype. To circumvent this problem, a strain was constructed in which the Tn*10* transposon was inserted near the *gdh-51* mutation.

Construction of strains with Tn*10* linked to *gdh-51*. Strains with Tn*10* cotransducible

with *gdh-51* were obtained by using a generalized transducing phage lysate prepared on a mixed population of cells with random Tn*10* insertions (see Materials and Methods) to transduce JB801 to Glt$^+$. Glt$^+$ transductants were then replica-plated onto LB-tetracycline plates. Since a Glt$^+$ Tet^r phenotype would result from simultaneous transfer of either the *asm-102*$^+$ allele and the transposon, or of the *gdh-51*$^+$ allele and the transposon, the Asm phenotype was scored to distinguish these two types. All seven Glt$^+$ Tet^r transductants retained the Asm$^-$ phenotype, indicating that each resulted from transfer of the *gdh-51*$^+$ allele.

To verify that the double phenotype was a result of a single transductional event in the original cross, these strains were examined for transductional linkage of the insertion to *gdh-51*$^+$. All seven strains showed linkage by KB1 phage, with frequencies of cotransduction ranging from 10 to 95%. These strains carry the wild-type *gdh-51* allele; the backcrosses provided us with corresponding strains carrying the mutant allele. The strain with the greatest linkage (95% cotransduction) between *gdh-51* and Tn*10*, JB1075 (*asm-102 gdh-51*$^+$ *zcd-2*::Tn*10*), and its corresponding derivative, JB1177 (*asm-102 gdh-51 zcd-2*::Tn*10*), were used in subsequent experiments.

Reconstruction of a double mutant carrying the *asm-102* and *gdh-51* mutations. The *gdh-51* mutation was transduced into JB665 (*asm-102*) by using phage grown on JB1177 and selecting for Tet^r. As expected from cotransduction data, a high percentage of these transductants, 82%, required glutamate due to transfer of the *gdh-51* allele. Three of the Glt$^-$ transductants were purified and assayed for glutamate dehydrogenase and glutamate synthase activity. If JB801 indeed carried a third mutation which further reduced the already low glutamate synthase level of its parent strain, then these transductants should have a level comparable to that of JB665, not of JB801. That was the case (Table 4). The level in the three reconstructed strains, JB1178, JB1179, and JB1180, was less than that of the wild type but significantly higher than that of JB801.

Consistent with the difference in glutamate synthase levels between JB801 and the reconstructed strains is the fact that the latter were glutamate bradytrophs whereas JB801 was a strict auxotroph (Table 5). Another growth property which further distinguished JB801 and the reconstructed strains was the ability of glutamine to satisfy the glutamate requirement. JB801 was unable to use glutamine, whereas the reconstructed strains grew as well with glutamine as with glutamate (Table 5). Thus, the difference in growth properties and alteration in glutamate synthase activities suggests that strain JB801 carried a third mutation affecting its ammonia assimilatory properties. This indicates the existence of at least one additional site involved in ammonia assimilation and may be valuable in the further analysis of the regulation of this process.

Mapping of the *gdh-51* and *asm-102* mutations. In *K. aerogenes* (1, 5, 6) and in *E. coli* (20), strains lacking glutamate synthase and glutamate dehydrogenase activities have been isolated and their mutations have been mapped. However, the precise function of these loci has not yet been determined. In both organisms, mutations abolishing glutamate synthase activity are linked by P1 to *argG* (12, 20), and those resulting in lack of glutamate dehydrogenase activity are linked to *trp* (1, 20). P1-mediated transductions were carried out (Table 6) to determine whether the mutations in the *S. typhimurium* mutants have similar loci. Strain JB801 (*asm-102 gdh-51*) was used as a recipient, and Glt$^+$ recombinants were selected and scored for the Asm phenotype to distinguish between the *asm*$^+$ and *gdh*$^+$ Glt$^+$ transductants. The appropriate class was then scored for coinheritance of the unselected marker. Because of the possibility that there could be a selection pressure against *asm* as an unselected marker, we looked for

TABLE 4. *Enzyme activities in the reconstructed glutamate auxotrophs*[a]

Strain[a]	Sp act	
	GltS	GDH
JL907	203	377
JB665	39	506
JB801	12	2
JB1178	64	3
JB1179	78	0
JB1180	75	0

[a] See footnotes to Table 3.

TABLE 5. *Differences in growth properties between JB801 and reconstructed glutamate auxotrophs*

Strain	Doubling time (min) with the following media[a]		
	No addition	+ Glutamate	+ Glutamine
JB801	ND	86	ND
JB1178	170	98	108
JB1179	154	106	108

[a] The basic medium was glucose-ammonia medium. Glutamate was present at a concentration of 0.4%; glutamine at 0.04%. ND, No doubling.

coinheritance of *asm* and *argG*, using both *argG*$^+$ (cross 1) and *asm*$^+$ (cross 2) as the selected markers. *asm-102* is not linked to *argG* (Table 6). Neither is *gdh-51* linked to *trp*. Thus, these mutations are at different locations from those described for *K. aerogenes* and *E. coli*.

In addition, two *S. typhimurium* mutants requiring glutamate have been reported. One mutation presumably affects glutamate dehydrogenase and is cotransducible with *metB* (19). The other (*glt-3*) was mapped at approximately 20 min (22) but has not been characterized. We found that neither the *asm-102* nor *gdh-51* mutation was transducible with *metB* and the *zcd-2*::Tn*10* near *gdh-51* was unlinked (0/202) by KB1 transduction with *glt-3* in a mutant obtained from J. Calvo.

Because the *gdh-51* mutation by itself confers no detectable phenotype, it is difficult to map by conventional methods. We therefore used the linked Tn*10* insertion (95% cotransduction) as a scorable marker. Hfr strains carrying the *zcd-2*::Tn*10* insertion were constructed by transduction, using a P1 lysate propagated on JB1075, selecting for Tet^r. The transposon in these Hfr strains, JB1113 and JB1105, was shown to be cotransducible with *gdh-51* by appropriate backcrosses. The results of two conjugations between the Hfr strains carrying the *gdh-51*-linked transposon and multiply marked recipients placed *gdh-51* in the region between *pro* and *trp* (7 and 34 min, respectively, on the *Salmonella* linkage map [23]) and close to *pyrC* (22 min) (Table 7).

By using P1-mediated transductions we tested for linkage of Tet^r with markers in the *pyrC* region (20 to 25 min on the *S. typhimurium* linkage map). We found no cotransduction (0/125) of Tet^r with *pyrD* (20 min), *put* (21 min), *pyrC* (22 min), or *purB* (25 min). However, Tet^r is 26% cotransducible with *nit*. The *nit* locus was mapped by Kustu and co-workers at approximately 21 min (7); it is unlinked by P22-mediated transductions with the same markers that we tested for linkage with *gdh-51*.

The approximate location of *asm-102* was determined by mating various Hfr strains with JB801 (*asm-102 gdh-51*), selecting for Glt$^+$ recombinants, and scoring these for the Asm phenotype. This mutation was located between the points of origin of Hfr strains SA486 and SA536, and thus lies in the interval of 61 to 76 min on the *S. typhimurium* linkage map. Linkage with several markers was examined by using P1 transduction. We found no (0/150) cotransduction of *asm-102* with *serA* (63 min), *metK* (64 min), *metC* (66 min), *argG* (68 min), *cod* (69 min), *aroE* (71 min), *cysG* (73 min), or *glpD* (75 min).

DISCUSSION

The characterization of mutants with altered glutamate dehydrogenase and glutamate synthase activities demonstrates that the presence of either of these activities allows growth of *S. typhimurium* in media containing excess ammonia. Although the glutamate dehydrogenase activity is not repressed in *S. typhimurium* grown with a limiting nitrogen source (4), this enzyme activity is not essential during this growth condition when glutamate synthase activity is present. In fact, the loss of glutamate dehydrogenase appears to have no consequence for growth with either excess or limiting ammonia conditions even though its activity is normally high (unpublished data). It appears that glutamate synthase could be solely responsible for glutamate production in *S. typhimurium*; however, glutamate dehydrogenase may have a function during other growth conditions and its

TABLE 6. *Linkage analysis of asm-102 with argG and gdh-51 with trp*

Cross	Donor	Genotype	Recipient	Genotype	Selected marker	Unselected marker	% Cotransduction
1	JB665	*asm-102*	JB1102	*argG*	*argG*$^+$	*asm*	0 (0/235)
2	JB1102	*argG*	JB801	*asm-102 gdh-51*	*asm*$^+$	*argG*	0 (0/140)
3	JL68	*trp*	JB801	*asm-102 gdh-51*	*gdh*$^+$	*trp*	0 (0/109)

TABLE 7. *Conjugations using Hfr strains carrying the gdh-51-linked Tn10 insertion*

Cross[a]	Donor	Recipient	No. with selected marker	No. with unselected marker	% Coinheritance
1	JB1113	JL69	*metA*$^+$ (219)	Tet^r (21)	10
				trp$^+$ (13)	6
2	JB1105	JL72	*pro*$^+$ (169)	*pyrC*$^+$ (135)	80
				Tet^r (131)	78
				trp$^+$ (31)	18

[a] Indicated gene order: (cross 1) *metA-gdh-trp*; (cross 2) *pro-(pyrC-gdh)-trp*.

regulation may be more involved than expected from physiological studies.

In addition to examining the functions of these two glutamate biosynthetic enzymes in *S. typhimurium*, we have identified mutations affecting glutamate synthase and glutamate dehydrogenase that have locations different from those described for *K. aerogenes* or *E. coli* (1, 12, 20). The *asm-102* mutation is not linked to *argG* as are the *asm* and *gltB* mutations affecting glutamate synthase activities in *K. aerogenes* (12) and *E. coli* (20), respectively. Nor is the *gdh-51* mutation linked to *trp* as are the *K. aerogenes* (1) and *E. coli* (20) mutations causing the loss of glutamate dehydrogenase activity. Although the *trp* genes lie in a region which is inverted between *S. typhimurium* and *E. coli*, if *gdh-51* has the same relative position as these mutations, it should show linkage to *trp* by P1-mediated transduction. The *gdh-51* mutation is also genetically distinct from the mutation described by Ortega and Aguilar (19) that results in a loss of glutamate dehydrogenase activity in their *S. typhimurium* glutamate auxotroph. Since they did not measure glutamate synthase, it is not known whether the glutamate auxotrophy results from the simultaneous loss of both glutamate dehydrogenase and glutamate synthase activities or whether some other aspect of glutamate metabolism is affected. Our results indicate that the loss of glutamate dehydrogenase alone would not yield a glutamate auxotroph.

The linkage of *gdh-51* with the *nit-9* mutation characterized by S. Kustu and co-workers is intriguing (7). Mutants carrying *nit* mutations fail to use a variety of nitrogen sources and are phenotypically Asm^- even though glutamate synthase is present. The defect in the *nit* mutants is unknown. The fact that *nit* and *gdh-51* both affect nitrogen utilization may only be coincidental, especially since the cotransduction of 26% by P1 suggests they are separated by several genes. However, it is possible that other genes affecting nitrogen utilization lie in this region and that there is a functional relationship between *nit* and *gdh*.

Another segment of this work has been the characterization of mutants with low glutamate synthase activities. The activity remaining in strain JB665 is too low to allow growth on glucose-arginine medium, but the activity is sufficient for strains JB1178, JB1179, and JB1180 also lacking glutamate dehydrogenase activity to grow slowly on glucose-ammonia medium. Superficially, it appears that glutamate synthase functions as a low ammonia assimilatory enzyme and that its loss causes an Asm^- phenotype. However, the inability of the Asm^- strains to grow on glucose-arginine is a paradox. Wild-type cells grown on glucose-arginine medium have low glutamate synthase activities comparable to those found for JB664 and JB665. If wild-type cells normally have low glutamate synthase activities while growing on glucose-arginine, then why are mutants with a similar low activity unable to grow? One possible explanation lies in the observation that in addition to low glutamate synthase, the Asm^- strains have reduced glutamine synthetase activities (Table 2). We have shown that the mutations in the Asm^- strains are unlinked to the structural gene for glutamine synthetase (*glnA*) and that the MS sensitivity of glutamine synthetase in the mutants is unaltered. Thus, the reduction is not due to an altered glutamine synthetase protein but to lower levels of this enzyme. Either the low glutamine synthetase levels, or a reduction in some other component needed for nitrogen control may actually cause the Asm^- phenotype on glucose-arginine medium. Consistent with this is the prevalence of revertants of *K. aerogenes*, *E. coli*, and *S. typhimurium* Asm^- mutants that have high glutamine synthetase activities caused by mutations near the *glnA* gene (6, 11, 20). Thus, glutamate synthase may indeed be needed during growth with some nitrogen sources; however, the inability of strains lacking glutamate synthase to use certain nitrogen sources is not sufficient evidence that glutamate synthase itself is essential during this growth condition. In fact, a mutant of *S. typhimurium* lacking glutamate synthase, but having normal glutamine synthetase and nitrogen control, might be expected to grow on glucose-arginine medium.

It is not known how the mutations in JB664 and JB665 cause the reduction in both glutamate synthase and glutamine synthetase activities. One possibility is that the loss of glutamate synthase activity increases the glutamine pools and this causes the glutamine synthetase levels to be repressed. If this were true, then any mutant without glutamate synthase activity would, as a consequence, have low glutamine synthetase activity. The observation that the glutamate synthase mutants of *K. aerogenes* (6) and *S. typhimurium* (and possibly *E. coli*) (20) have slightly lower glutamine synthetase activities is consistent with this. However, this does not explain the inability of these mutants to increase the glutamine synthetase activities normally on glucose-arginine medium. Other possibilities are that glutamate synthase itself affects the regulation of either glutamine synthetase or a nitrogen control factor or that these *asm* mutations affect a common regulator needed for both glutamate synthase and glutamine synthetase production. If the latter possibility is true, then the *asm* mutations described here not only

define a gene affecting glutamate synthase activity but also identify a new factor necessary for glutamine synthetase regulation. The further characterization of these mutants should provide insight into the regulation of the interesting pathways for glutamate production as well as the overall control of ammonia assimilation.

ACKNOWLEDGMENTS

We thank J. Calvo and S. Kustu for making their strains available and S. Rosenfeld for providing the generalized transducing phage lysate of Tn*10*-containing cells and for helpful discussions.

This investigation was supported by Public Health Service grant GM25251 from the National Institute of General Medical Sciences and by grant PCM20882 from the National Science Foundation. J.E.B. is a recipient of Research Career Development Award (GM00449) from the National Institute of General Medical Sciences.

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