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Regulation of the Ammonia Assimilatory Enzymes in *Salmonella typhimurium*

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The regulation of glutamate dehydrogenase (EC 1.4.1.4), glutamine synthetase (EC 6.3.1.2), and glutamate synthase (EC 2.6.1.53) was examined for cultures of *Salmonella typhimurium* grown with various nitrogen and amino acid sources. In contrast to the regulatory pattern observed in *Klebsiella aerogenes*, the glutamate dehydrogenase levels of *S. typhimurium* do not decrease when glutamine synthetase is derepressed during growth with limiting ammonia. Thus, it appears that the *S. typhimurium* glutamine synthetase does not regulate the synthesis of glutamate dehydrogenase as reported for *K. aerogenes*. The glutamate dehydrogenase activity does increase, however, during growth of a glutamate auxotroph with glutamate as a limiting amino acid source. The regulation of glutamate synthase levels is complex with the enzyme activity decreasing during growth with glutamate as a nitrogen source, and during growth of auxotrophs with either glutamine or glutamate as limiting amino acids.

The regulation of three major ammonia assimilatory enzymes, glutamate dehydrogenase, glutamine synthetase, and glutamate synthase, has been examined previously in *Klebsiella aerogenes*. In this organism glutamate dehydrogenase functions when the ammonia concentration is greater than 1 mM to synthesize glutamate from α-ketoglutarate and ammonia using reduced nicotinamide adenine dinucleotide phosphate (3). However, cells grown with a limiting nitrogen source have substantially lowered levels of glutamate dehydrogenase, suggesting that this enzyme does not synthesize glutamate during nitrogen limitation (3, 22). Another enzyme, glutamate synthase, provides this function by converting α-ketoglutarate and glutamine to glutamate (11, 22). The characterization of mutants lacking glutamate synthase activity demonstrated that this enzyme is essential for growth of *K. aerogenes* on media containing less than 1 mM ammonia (3). The glutamine used as a substrate for this reaction is produced from glutamate and ammonia by glutamine synthetase. Thus, glutamine synthetase and glutamate synthase constitute a cyclic, low-ammonia assimilatory pathway that can also provide glutamate when ammonia is in excess (2).

In addition to the biosynthetic role of glutamine synthetase, this enzyme functions as a regulator of protein synthesis in *K. aerogenes*. When glutamine synthetase is derepressed during nitrogen-limiting growth, the synthesis of histidase and other nitrogen catabolic enzymes increase, whereas the synthesis of glutamate dehydrogenase decreases (14, 15). Certain mutations in the gene encoding glutamine synthetase (*glnA*) simultaneously cause increased synthesis of histidase and lowered synthesis of glutamate dehydrogenase (3, 5, 20). The role of glutamine synthetase in regulation is substantiated by in vitro studies using *Salmonella typhimurium hut* deoxyribonucleic acid carrying the gene for histidase. These studies show that *hut*-specific messenger ribonucleic acid transcription is stimulated by the addition of purified glutamine synthetase from *K. aerogenes* (25), and a regulatory model with glutamine synthetase as an activator for *hut* transcription and a repressor for glutamate dehydrogenase synthesis has been proposed (9, 10, 25). Recently, the regulatory role of glutamine synthetase has been extended to other genes for enzymes subject to nitrogen catabolite repression, such as nitrogenase (6, 21, 24).

The previous investigations have emphasized the functions of the ammonia assimilatory enzymes in *K. aerogenes*. However, in *S. typhimurium* histidase production is not derepressed when the nitrogen source is growth-rate limiting (15). Whereas *K. aerogenes* overcomes nitrogen catabolite repression of the *hut* genes

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and grows on glucose medium containing histidine as the sole nitrogen source, *S. typhimurium* cultures cannot (12). This difference appears associated with a physiological control signal and not with a difference in *hut* deoxyribonucleic acid since an F′ carrying *hut* deoxyribonucleic acid from *S. typhimurium* is regulated similarly to the *K. aerogenes hut* operon when it is transferred to *K. aerogenes* (15). Two explanations for this difference could be the inability of *S. typhimurium* to derepress the glutamine synthetase necessary to activate *hut* transcription, or the inability of glutamine synthetase to interact with *hut* deoxyribonucleic acid. This paper presents evidence that glutamine synthetase can be derepressed during the nitrogen limitation, but in contrast to *K. aerogenes*, this does not result in decreased levels of glutamate dehydrogenase. However, this enzyme is regulated since the levels increase during the limitation of a glutamate auxotroph for glutamate.

MATERIALS AND METHODS

Strains. The *S. typhimurium* LT2 strain is JL781 obtained from J. L. Ingraham. Strain JL907 is the parent of the glutamine and glutamate auxotrophs and is a *hutR49 galE* transductant of JL781. The glutamine auxotroph, JB667 (*gln-10*), was selected by mutagenesis of JL907 with 10 μg of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine per ml. JB678 (*glt-20*) was selected without mutagenesis for resistance to 10 μg of nalidixic acid per ml as described by Helling and Kukora (7). Although the reason this selection yields auxotrophs is unknown, several glutamate-requiring mutants were isolated easily using this technique. The ability of JB678 to grow with an α-ketoglutarate supplement, but not with citrate, suggests that it lacks isocitrate dehydrogenase activity and is similar to the *Escherichia coli* mutants isolated by Helling and Kukora (7). The *K. aerogenes* strain is MK53 (*hutC515*) from B. Magasanik.

Media. The minimal salts medium has been described (1). Carbon sources are added at 0.4%. For the nitrogen limitation experiments, $(NH_4)_2SO_4$ was omitted from the minimal salt medium. Sodium glutamate was added at 4 mg/ml when used as either a nitrogen or amino acid source. Glutamine was used at 4 mg/ml as a nitrogen source and at 2 mg/ml as an amino acid source. Other supplements are added as described for the individual experiments. The complex medium is Luria broth (1).

Culture conditions and extract preparation. The maintenance of stock cultures and inoculum preparation have been described (1). Cells to be used for extracts were grown at 37 C from an inoculum of 5 to 10 Klett units to a value of 100 ± 5 Klett units, which corresponds to approximately 9×10^8 cells/ml (no. 42 filter for minimal medium, no. 54 filter for Luria broth). The cells were chilled rapidly, centrifuged, washed twice with cold 0.85% NaCl, and stored at 0 to 4 C for 8 to 12 h before sonic treatment. The cells were resuspended (in 1/100 their original volume) in imidazole buffer (10 mM imidazole-hydrochloride, pH 7.15, 10 mM $MnCl_2$, and 0.14 ml of mercaptoethanol per liter) and sonically treated (Bronwill Biosonik III with a needle probe) three times for 15 s with 20-s cooling intervals. After sonication, the extracts were centrifuged for 20 min at 17,000 × *g*, and the supernatant solution was saved and maintained at 0 to 4 C. Enzymes were assayed within 8 h after sonication. Protein determinations were made by the method of Lowry et al. (8) with bovine serum albumin as the standard.

For cultures growth-rate limited for a nitrogen source, ammonia was limited directly using a procedure similar to that of Clark and Marr (4). Cells were inoculated into medium without $(NH_4)_2SO_4$ and an $(NH_4)_2SO_4$ solution was added slowly at a rate allowing linear growth at approximately 20 Klett units per h. Cultures were harvested as described above. The glutamine and glutamate auxotrophs were limited for the respective amino acid using diffusion capsules purchased from Lab-Line Instruments, Inc., Melrose Park, Ill. (13). The growth rates and cell yields can be varied by altering the amino acid concentration within the capsule and the number of dialysis membranes sealing the aperture. In the experiments reported here the quantity of amino acid was adjusted to allow normal growth until approximately 50 Klett units, followed by limited growth to about 100 Klett units (Fig. 1). The culture volume was 60 ml and the capsule with one membrane contained 1 ml of either 12 mg of L-glutamine per ml or 10 mg of sodium glutamate per ml, each dissolved in glucose minimal medium. The capsules were autoclaved separately, and the membranes and solutions were added aseptically before placement in the growth flasks. The use of the capsules provided a growth limitation condition while allowing the continuation of protein synthesis for one doubling, and it has the advantage of avoiding the centrifugation or filtration procedure often necessary to remove amino acids and limit growth.

Enzyme assays. Glutamate dehydrogenase and glutamate synthase activities were measured by following the rate of reduced nicotinamide adenine dinucleotide phosphate oxidation as described (1, 11). The glutamine synthetase assay is the γ-glutamyl transfer reaction described by Stadtman et al. (17). Blanks without adenosine diphosphate and arsenate were included for every assay condition and the values were subtracted from those obtained with the complete reaction mixture. This assay has been used to differentiate the adenylylated and deadenylylated forms of *E. coli* glutamine synthetase (16, 17). In this reaction, both enzyme forms are active at pH 7.15 with Mn^{2+} present. The addition of Mg^{2+} (60 mM) to the reaction mixture inhibits the activity of the adenylylated enzyme, and the adenylylation state ($\bar{n}$) can be determined by comparing the activities measured with and without Mg^{2+} ($\bar{n} = 12 - 12$ [activity

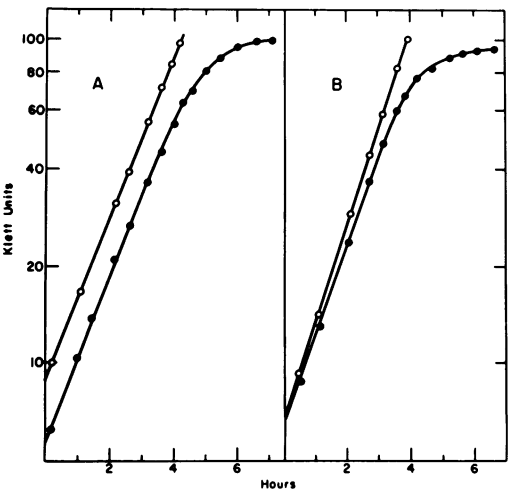

FIG. 1. *Growth of the glutamine and glutamate auxotrophs with the amino acids provided in excess or limiting concentrations. (A) Strain JB667 (gln-10) and (B) strain JB678 (glt-20). Symbols:* O, *excess amino acid (either 2 mg of glutamine per ml or 4 mg of sodium glutamate per ml);* ●, *limiting amino acid (separate diffusion capsules containing either 12 mg of glutamine per ml or 10 mg of sodium glutamate per ml).*

with Mg^{2+}/activity without Mg^{2+}]). As with the *K. aerogenes* enzyme (14), the *S. typhimurium* glutamine synthetase activities sometimes have negative ñ values (completely deadenylylated) for cultures grown in glucose ammonia medium and harvested at 100 Klett units. Thus the specific activities for the two assay conditions are presented rather than an ñ value. The negative values for the deadenylated enzyme reflects a slight activation of activity by Mg^{2+} and may indicate differences in the pH optima and ion specificities of the *K. aerogenes* and *S. typhimurium* enzymes from the *E. coli* enzyme. This assay remains useful for detecting positive ñ values observed with highly adenylylated forms of the enzyme.

All enzyme levels are reported as specific activities and the units are nanomoles of product formed per minute per milligram of protein. All results are the average of at least duplicate assays. Furthermore, because of the variations in enzyme activities between experiments, all values are the average of at least four individual experiments, and a control culture (either JL781 or JL907 in glucose minimal medium) was included for comparison with each experiment. The average specific activities found with these control cultures in over 30 independent experiments are: 218 for glutamate dehydrogenase (range 167 to 300), 132 for glutamate synthase (range 85 to 224), and 203 for the total glutamine synthetase activity (range 151 to 305).

Materials. All chemicals are reagent grade and commercially available. Amino acids were from Sigma Chemical Co. (St. Louis, Mo.). Vitamin-free Casamino Acids was from Difco Laboratories, Detroit, Mich.

RESULTS

Effect of nitrogen limitations on enzyme levels. To compare the enzyme levels for *S. typhimurium* and *K. aerogenes*, strains JL781 and MK53, respectively, were grown in glucose minimal medium containing either excess (15 mM) or limiting ammonia. For the limitation conditions, the $(NH_4)_2SO_4$ was omitted from the medium and then added slowly to give a constant cell increase of 20 Klett units per h. Table 1 shows the results of enzyme assays for these cultures. Although the glutamine synthetase for the ammonia-limited *K. aerogenes* (MK53) culture is not as elevated in these experiments as in others, it is clear that the increased glutamine synthetase activity is coincident with a decrease level of glutamate dehydrogenase. However, the glutamate dehydrogenase activities in *S. typhimurium* are not reduced in the limited cultures even though the glutamine synthetase levels are substantially elevated.

The effect of supplying one of the products of these reactions, glutamate, as the nitrogen

TABLE 1. *Enzyme levels for cultures grown with excess and limiting ammonia*

Strain	Growth condition	Enzyme activities[a]			
		GltD	GltS	GlnS	
				$-Mg^{2+}$	$+Mg^{2+}$
JL781	Excess NH_3	255	116	231	255
JL781	Limiting NH_3	244	156	989	809
MK53	Excess NH_3	356	194	153	174
MK53	Limiting NH_3	87	164	630	670

[a] Abbreviations for the enzymes are: GltD, glutamate dehydrogenase; GltS, glutamate synthase; GlnS, glutamine synthetase. Glutamine synthetase were assayed in the absence and presence of Mg^{2+} to indicate the adenylylation state as described.

source was examined. The growth rate of cells in glucose medium containing glutamate as the sole nitrogen source was reduced to about 2.5 h per generation as opposed to about 1 h with ammonia as the nitrogen source. The results in Table 2 show that the glutamine synthetase levels in *S. typhimurium* are elevated, but again the glutamate dehydrogenase levels are not lowered. Similar experiments with *K. aerogenes* demonstrated that the glutamine synthetase increased from 208 to 1,401 units and the glutamate dehydrogenase decreased from 376 to 5 units for cultures grown with ammonia versus glutamate, respectively. As opposed to the limiting ammonia growth condition where the glutamate synthase activity did not vary significantly from that found with excess ammonia (Table 1), the glutamate synthase level decreased when glutamate was the nitrogen source.

Because the nonrepression of glutamate dehydrogenase is not due to the inability of *S. typhimurium* cells to derepress the glutamine synthetase, the adenylylation properties of the *S. typhimurium* and *K. aerogenes* glutamine synthetases were compared. Cells were grown with excess ammonia and harvested 3 h after the beginning of stationary phase (about 350 Klett units). Table 3 presents the glutamine synthetase activities and the calculated adenylylation states ($\bar{n}$) for extracts from *S. typhimurium* and *K. aerogenes* cultures. Both cultures formed highly adenylylated enzymes. Since these enzymes appear similar in their regulation and modification properties, the difference in glutamate dehydrogenase control could result from either a more subtle difference in glutamine synthetase, or a different control mechanism for glutamate dehydrogenase synthesis.

Effects of glutamate and glutamine as amino acid sources. Most investigations of these enzymes have emphasized their control by the availability of nitrogen. However, in addition to their role in ammonia assimilation, these enzymes produce glutamate and glutamine as amino acids for protein synthesis, and it is possible their levels would respond to excess or limiting supplies of these amino acids. To examine this possibility, cells were grown in glucose medium containing either glutamate or glutamine supplements (Table 4). As opposed to growth with glutamate as a nitrogen source, glutamate in addition to ammonia did not repress the glutamate synthase levels. The simultaneous addition of glutamate and glutamine reduced the glutamine synthetase level to about 50% of the glucose ammonia control. Although *S. typhimurium* is not readily permeable to glutamate, the concentration used in these experiments (4 mg/ml) allows a normal

TABLE 3. *Adenylylation states of glutamine synthetase from stationary-phase cultures*

Strain	Glutamine synthetase activity		$\bar{n}$ values[a]
	$-Mg^{2+}$	$+Mg^{2+}$	
JL781	131	20	10
MK53	144	48	8

[a] See text for definition of $\bar{n}$ value.

TABLE 4. *Enzyme levels for S. typhimurium cultures grown with glutamate of glutamine as amino acid supplements*

Growth condition	Enzyme activities[a]			
	GltD	GltS	GlnS	
			$-Mg^{2+}$	$+Mg^{2+}$
Minimal	217	149	158	209
Minimal + glutamate	184	138	134	170
Minimal + glutamine	235	144	105	140
Minimal + glutamate +glutamine	195	117	79	98

[a] See Table 1 for abbreviations of enzymes.

TABLE 2. *Enzyme levels for cultures grown with glutamate as nitrogen source*

Strain	Nitrogen source	Enzyme activities[a]			
		GltD	GltS	GlnS	
				$-Mg^{2+}$	$+Mg^{2+}$
JL781	Ammonia	222	125	219	248
JL781	Glutamate	210	47	1,383	1,275
MK53	Ammonia	376	284	208	251
MK53	Glutamate	5	21	1,401	1,234

[a] See Table 1 for abbreviations of enzymes.

growth rate of glutamate auxotrophs and thus should provide sufficient glutamate in these experiments.

Cells grown in Luria broth had low levels of all three enzymes (Table 5), and a similar effect is observed for cultures grown in glucose ammonia medium plus 0.4% Casamino Acids (this amount of Casamino Acids does not contain sufficient glutamine to support the growth of a glutamine auxotroph). This reduction is similar to that reported by Woolfolk et al. (26) for *E. coli* cells grown in media containing peptone-yeast extract. To determine whether a few or several compounds are important in reducing the enzyme levels, cells were grown with various supplements and the enzyme levels were measured. Aspartate alone, or the combination of aspartate, serine, proline, and arginine, lowers the glutamate dehydrogenase and glutamate synthase levels (Table 5). Products which either inhibit glutamine synthetase activity, or are precursors of inhibitors, were added to the medium to determine if they might also be involved in repression of glutamine synthetase. Although there may be a small reduction in glutamine synthetase activity (Table 5; minimal plus combination), this effect may be caused by the compounds sparing glutamine and allowing the glutamine pools to increase. Whether the addition of Casamino Acids also causes the lower enzyme levels because of sparing glutamate and glutamine from the biosynthesis of other compounds or because of direct effects by some of the compounds is not known. Since the reduction caused by Luria broth and Casamino Acids is greater than that observed with glutamate and glutamine additions alone, it may be that more than glutamate and glutamine are involved. These effects are being investigated further.

TABLE 5. *Effects of supplements to the growth media on S. typhimurium enzyme levels*

Growth media	Enzyme activities[a]		
	GltD	GltS	GlnS
Minimal	204	151	228
Luria broth	17	20	7
Minimal + Casamino Acids[b]	44	26	79
Minimal + Asp + Ser + Pro + Arg[c]	86	44	189
Minimal + Asp[d]	110	68	248
Minimal + combination[e]	236	61	145

[a] Abbreviations for enzymes are given in Table 1. The values for glutamine synthetase are for assays without Mg^{2+}.

[b] Vitamin-free Casamino Acids added at 0.4%.

[c] Amino acids each added at 250 μg/ml.

[d] Sodium aspartate added at 1 mg/ml.

[e] Supplements were alanine (200 μg/ml), glycine (200 μg/ml), histidine (200 μg/ml), tryptophan (200 μg/ml), cytosine (50 μg/ml), uracil (50 μg/ml), and glucosamine (50 μg/ml).

Effect of glutamate and glutamine limitations. Glutamine and glutamate auxotrophs were grown in media containing the required amino acid in excess or in growth-rate limiting amounts as described above. Representative growth curves for these mutants are presented in Fig. 1 and the results of enzyme assays are shown in Table 6. Strain JB667 (*gln-10*) lacks most of the glutamine synthetase activity, as expected for a glutamine auxotroph. Although the glutamate dehydrogenase and glutamate synthase activities are slightly lower than control cultures containing glutamine, this reduction is not seen with all glutamine auxotrophs and it may not be the result of a mutation in glutamine synthetase (Brenchley, unpublished data). The glutamate synthase levels are further reduced, however, when the strain is limited for glutamine. The glutamate auxotroph (JB678) has enzyme activities comparable to the control when grown with 4 mg of sodium

TABLE 6. *Enzyme levels for glutamine and glutamate auxotrophs grown with excess or limiting supplements*

Strain	Growth condition	Enzyme activities[a]			
		GltD	GltS	GlnS	
				$-Mg^{2+}$	$+Mg^{2+}$
JL907	Minimal	218	169	176	141
JB667 (*gln-10*)	Excess glutamine	182	101	4	5
JB667 (*gln-10*)	Limiting glutamine	129	53	6	5
JB678 (*glt-20*)	Excess glutamate	209	213	164	203
JB678 (*glt-20*)	Limiting glutamate	520	61	119	138

[a] Abbreviations for the enzymes are given in Table 1.

glutamate per ml, but the glutamate dehydrogenase increases and the glutamate synthase decreases when glutamate is limited (Table 6). Cultures more severely limited for glutamate had higher glutamate dehydrogenase activities so 520 units may not represent the maximum derepression level.

The glutamate synthase activity decreases with a variety of growth conditions, including glutamate and glutamine limitations. Because this enzyme from *K. aerogenes* is unstable in the absence of glutamine (1), one possibility for the low levels might be a reduction in the glutamine pool needed for enzyme stability. To examine this, strains JB667 (*gln-10*) and JB678 (*glt-20*) were limited for the required amino acid, and at 102 Klett units, the cultures divided and one flask with strain JB667 was supplemented with 2 mg of glutamine per ml and one flask of strain JB678 was supplemented with 4 mg of glutamate per ml. After 10 min, the two supplemented cultures resumed growth and the turbidity had increased to 108 Klett units whereas the growth of the two limited cultures remained at 102 Klett units. All cultures were harvested and the enzyme levels were determined. There is no significant difference in enzyme levels between the limited and supplemented cultures (Table 7), indicating that the lowered glutamate synthase level was not directly caused by lowered pools causing enzyme instability during sonication.

TABLE 7. *Effects of glutamine or glutamate additions to limited cultures*

Strain	Growth condition[a]	Enzyme activities[b]		
		GltD	GltS	GlnS
JL907	Minimal	179	126	158
JB667	Limiting glutamine	121	69	6
JB667	Limited + 10 min excess glutamine	130	53	5
JB678	Limiting glutamate	440	82	174
JB678	Limited + 10 min excess glutamate	396	68	177

[a] Strains JB667 and JB678 were grown with capsules containing 1 ml of 12 mg of glutamine per ml and 10 mg of sodium glutamate per ml, respectively. At 102 Klett units each culture was divided and 2 mg of glutamine per ml added to one flask of the JB667 culture and 4 mg of sodium glutamate per ml added to one JB678 culture. After 10 min of incubation, the supplemented cultures were harvested at 108 Klett units.

[b] Abbreviations for the enzymes are presented in Table 1. The glutamine synthetase activity is measured without Mg^{2+}.

DISCUSSION

The results in this paper demonstrate that the levels of glutamate dehydrogenase in *S. typhimurium* do not decrease when glutamine synthetase levels increase. Thus, in contrast to *K. aerogenes*, *S. typhimurium* neither derepresses histidase synthesis nor represses glutamate dehydrogenase synthesis during nitrogen-limiting growth. This difference in control is not caused by the inability of the *S. typhimurium* glutamine synthetase to be derepressed since it can be elevated to the levels found in *K. aerogenes* without affecting the *S. typhimurium* glutamate dehydrogenase levels (Tables 1 and 2).

Because Tyler et al. (25) reported that the stimulation of *hut* transcription in vitro is dependent on the deadenylylated form of glutamine synthetase, one explanation for the inability of the *S. typhimurium* glutamine synthetase to function in regulation might be the presence of adenylylated rather than deadenylylated enzyme during nitrogen limitation. However, the adenylylation and deadenylylation properties of the glutamine synthetase from *S. typhimurium* and *K. aerogenes* appear similar. Cells grown with excess ammonia and harvested at 100 Klett units are deadenylylated (Table 1) whereas cells in stationary phase have adenylylated enzyme (Table 3). These results agree with the report by Tronick et al. (23) that glutamine synthetases from gram-negative bacteria are structurally and antigenically similar. Despite this general biochemical relatedness, it is likely that subtle differences exist which account for the nonregulatory property of the *S. typhimurium* glutamine synthetase. Another possibility for the absence of repression of glutamate dehydrogenase is that it is essential during ammonia-limited growth whereas the *K. aerogenes* activity is dispensible (2). Although the ammonia assimilatory enzymes in *S. typhimurium* have not been thoroughly investigated, mutants of *S. typhimurium* lacking glutamate synthase activity have phenotypes similar to *gltS* mutants of *K. aerogenes* (19; Brenchley, unpublished data), suggesting that the enzymes have the same functions.

A second observation reported here is that the glutamate dehydrogenase activity increases during glutamate limitation of an auxotroph, indicating that this enzyme is regulated by the availability of glutamate as an amino

acid source. The addition of excess glutamate and/or glutamine to the medium, however, did not substantially decrease the enzyme levels below those obtained for cells grown in minimal medium. This is analogous to the regulation of the histidine biosynthetic pathway where the addition of histidine to the medium causes only slight repression, but mutants limited for histidine can derepress the enzyme levels severalfold. Our studies do not determine whether glutamate itself or derivatives serve as the regulatory signals. Because glutamate is an amino donor during the synthesis of several diverse compounds, the regulation of glutamate dehydrogenase could involve a form of cumulative repression similar to that found for serine transhydroxymethylase (18), and the dramatic decrease for cultures grown in Luria broth or Casamino Acids would be consistent with a cumulative repression control. However, since there may be different control mechanisms for cells growing in minimal media versus rich media, more direct experiments are necessary to define the control signal(s). The observation that a mutant with several altered transfer ribonucleic acid species has reduced glutamate dehydrogenase levels (Brenchley, unpublished data) may be relevant to the derepression of this enzyme during limitation of glutamate as an amino acid source.

The regulation of glutamate synthase is harder to interpret. Several growth conditions result in decreased glutamate synthase levels: growth with glutamate as a nitrogen source, limitation of glutamine as an amino acid, limitation of glutamate as an amino acid, and addition of various amino acids. These results could be consistent with glutamate synthase having the primary function of converting glutamine to glutamate during nitrogen limitation. Such an enzyme might be repressed during growth with glutamate as a nitrogen source because the cell is provided with the product of the reaction. (However, the addition of glutamate to glucose ammonia medium did not decrease glutamate synthase activity, even though theoretically it should be unnecessary for glutamate production.) Also, during growth of an auxotroph with limiting glutamine, the cell could synthesize adequate glutamate using glutamate dehydrogenase and would not use glutamate synthase to further deplete the limited glutamine supply. The limitation of the glutamate auxotroph probably simultaneously lowers glutamate, glutamine, and α-ketoglutarate (because the strain requires α-ketoglutarate) supplies. Such a growth condition might make the glutamate synthase reaction unprofitable and account for the lowered enzyme levels. Although these results can be explained, the mechanism is not clear, and it may be that several separate controls are involved.

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