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Identification of the Structural Gene for Glutamine Synthetase in *Klebsiella aerogenes*

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Mutations at two sites of the *Klebsiella aerogenes* chromosome, unlinked by transduction with phages PW52 and P1, result in the lack of enzymatically active glutamine synthetase. A mutation in the *glnB* site leads to a marked decrease in the formation of an apparently normal enzyme. Some of the mutations in the *glnA* site lead to the production of enzymatically inactive material capable of reacting with anti-glutamine synthetase serum. The revertant of a *glnA* mutant was found to produce a glutamine synthetase with less activity and less stability to heat than the enzyme of the wild type. These results locate the structural gene of glutamine synthetase at the *glnA* site. A single mutation in the *glnA* site leads to the production of enzymatically inactive glutamine synthetase antigen, not subject to repression by exogenously added ammonia. This observation suggests that glutamine synthetase is itself involved in the regulation of the synthesis of glutamine synthetase.

We previously reported the isolation of mutants of *Klebsiella aerogenes* that lack glutamine synthetase and of certain of their revertants whose glutamine synthetase is not subject to repression by ammonia (1, 5). The study of these organisms revealed the role of glutamine synthetase as a regulator of the synthesis of enzymes responsible for the formation of glutamate. Thus, an increase in the cellular level of glutamine synthetase activates the synthesis of histidase and inhibits the synthesis of glutamate dehydrogenase.

The mutations leading to the lack of enzymatically active glutamine synthetase were found in two sites of the *Klebsiella* chromosome, *glnA* and *glnB*, unlinked by transduction with phages PW52 and P1 (5, 8). The mutations leading to ammonia-insensitive synthesis of glutamine synthetase, the GlnC$^-$ phenotype, suppress a mutation in *glnB* and map in the *glnA* site (5, 8). We considered it of particular importance to determine whether the structural gene for glutamine synthetase is located at one of the two sites. We therefore searched for inactive material with the antigenic properties of glutamine synthetase in extracts of glutamine-requiring mutants and compared the biochemical and antigenic properties of the glutamine synthetase from revertants with those properties of the enzyme of the prototrophic organism. The results of these experiments, described in this paper, identify *glnA* as the site of the structural gene for glutamine synthetase.

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MATERIALS AND METHODS

Chemicals. 2-Methyl-imidazole and 2,4-dimethyl-imidazole were obtained from Gallard-Schlesinger Chemical Manufacturing Co. They were crystallized from aqueous solution twice and decolorized with Norit. All other chemicals were obtained from commercial sources and used without purification. L-Glutamine (Calbiochem, A grade) was freshly prepared as 0.2 M solution and sterilized by filtration for use in both the γ-glutamyl-transferase assay and as a growth rate-limiting nitrogen source at a concentration of 14 mM (0.2%). Whatman DE-52 (diethylaminoethyl-cellulose) was used.

Cultivation of bacteria and phages. The media used for the cultivation of *K. aerogenes* (and phage PW52) have been described (5).

Strains and mutant selection. The genotype and phenotype designations used in this paper have been defined previously (5).

Strains MK-266 and MK-267 are glutamine auxotrophs isolated by Jean E. Brenchley from strain MK-189 (*asm-200*), a mutant that lacks glutamate synthase (1). Strains MK-266 and MK-267 lack both glutamine synthetase and glutamate synthase. They differ from one another with regard to their ability to produce histidase when grown on glucose under conditions of nitrogen excess or starvation. Strain MK-266 has the Cn^S phenotype of the glutamine auxotrophs described so far (5); it fails to produce histidase even when the nitrogen source is growth rate limiting. Strain MK-267 has the Cn^R phenotype; it produces histidase, even when ammonia is present in excess. In both strains, the mutation leading to the glutamine

requirement was located in the *glnA* site by transduction with phage PW52 (J. E. Brenchley, personal communication), and phage P1 (8). The *asm-200* mutation of strain MK-267 was replaced through transduction with the wild-type allele asm^+ (S. L. Streicher, A. B. DeLeo, and B. Magasanik, unpublished data). The resulting strain, carrying only the *glnA51* mutation, is called MK-922.

Strains MK-710 and MK-933 were isolated as spontaneous revertants on solid minimal medium with low ammonia at 32 C from strains MK-103 (*glnA5*) and MK-922 (*glnA51*), respectively. On solid medium, both revertants require glutamine at 42 C, but grow without glutamine at 32 C, irrespective of the ammonia concentration.

Preparation of cell extracts and protein determinations. Cell extracts for determination of glutamine synthetase activity or antigen were prepared according to procedures described by Prival et al. (5). Protein concentrations were determined by the method of Lowry et al. (4).

Glutamine synthetase assay. Unless otherwise stated, glutamine synthetase activity was assayed in crude extracts and in purified enzyme preparations by reverse γ-glutamyl transferase activity as previously described (5). In this assay, adenylylated and non-adenylylated enzyme are equally active. Glutamine synthetase activity of purified enzyme preparations was also measured by the biosynthetic reaction (7). Glutamine synthetase activity, as measured by either assay, is expressed as 1 μmol of product per min per mg of protein at 37 C.

Purification of glutamine synthetase. Glutamine synthetase (unadenylylated) was purified from extracts of *K. aerogenes* by a modification of the method of R. E. Miller and E. R. Stadtman (personal communication). Unless otherwise stated, all operations were at 2 to 4 C. "Buffer" refers to 10 mM imidazole buffer, pH 7.0. "Low-Mg^{2+} buffer" refers to buffer containing 2.5 mM $MgCl_2$. A typical purification scheme is given in Table 1.

The successful purification of glutamine synthetase from extracts depends on the growth medium and on extensive washing of the cells with 1 M sodium chloride. Cultivation of cells on a growth rate-limiting nitrogen source, i.e., glutamate, histidine, or glutamine, while resulting in increased level of enzyme, also leads to over-production of capsular material, which generally lowered the yield of purified enzyme. Regardless of the culture medium, extensive washing of the cells with 1 M NaCl significantly increased the yield.

TABLE 1. *Purification of glutamine synthetase*

Fraction	Vol (ml)	Protein (mg)	Total activity (U)[a]	Sp act (U[a]/mg)	Yield (%)
Initial extract	35	510	170	0.330	100
Soluble extract (2.5 mM $MgCl_2$)	20	7.2	148	20.6	87
DE-52[b]	6	2.9	57	19.6	33

[a] Amount of enzyme catalyzing synthesis of 1 μmol of γ-glutamyhydroxamate per minute.

[b] Calculations based on a portion of fraction 2 containing 68 U of activity and 3.6 mg of protein.

Strain MK-53 was grown in W medium containing glucose and 1.5 mM ammonium sulfate with vigorous shaking at 32 C to a cell density of 100 Klett units. Cells were harvested by centrifugation at 7,700 × *g* and stored at −18 C until used. The thawed cells were washed three times in 1/10 of their original volume with 1 M sodium chloride, suspended in buffer containing 50 mM $MgCl_2$ (1 g of wet cells per 2 ml of buffer), and disrupted by subjecting 10-ml portions to oscillation in a 60-W M.S.E. ultrasonic disintegrator at 4 C for six 15-s periods with 15-s rest periods. The suspension was centrifuged at 27,000 × *g* for 30 min, and the extract was adjusted to pH 5.85 with 1 M acetic acid. The extract was then treated with streptomycin (1 g/100 ml), and the suspension was stirred for 15 min; the pH was readjusted if necessary, and the extract was centrifuged at 27,000 × *g* for 30 min. The resulting supernatant fluid was warmed to 25 C, and 0.1 M zinc sulfate was added to a final concentration of 0.75 mM. The resulting mixture was allowed to stand for 15 min with only occasional stirring, and was centrifuged at 27,000 × *g* for 30 min. The precipitate obtained, containing the enzyme activity, was washed in one-half the original volume of buffer containing 50 mM $MgCl_2$ and collected again by centrifugation at 27,000 × *g* for 30 min. The precipitate was then suspended in 1/2 of the original volume in low-Mg^{2+} buffer and allowed to stand overnight. The suspension was then centrifuged at 27,000 × *g* for 30 min. Generally, 1/4 to 1/2 of the enzyme activity was soluble at this point. The remaining activity was solubilized by subsequent resuspension in low-Mg^{2+} buffer and repetition of the previous step.

In general, preparations of glutamine synthetase after precipitation with Zn^{2+} and subsequent solubilization were contaminated with material having high absorbancy at 260 nm (A_{260}). The absorbancy ratio A_{280} to A_{260} was generally 0.6 to 0.8. The contaminating material was separated from glutamine synthetase on DE-52. The enzyme (Table 1, fraction 2; 3.8 mg) was adsorbed on a column of DE-52 (1 by 6 cm) previously equilibrated with low-Mg^{2+} buffer, and eluted with a gradient composed of 20 ml of low-Mg^{2+} buffer containing 0.5 M sodium chloride. Fractions of 0.5 ml were collected. The maximal glutamine synthetase activity was eluted at approximately 0.3 M sodium chloride. Peak fractions were pooled and assayed. The absorbancy ratio A_{280} to A_{260} of the pooled elutants was 1.3 to 1.4. Such preparations (fraction 3) were used as antigen for preparation of anti-glutamine synthetase sera.

Preparations of the enzyme (fraction 2) were subjected to electrophoresis on polyacrylamide gel containing sodium dodecyl sulfate (9). With samples of up to 25 μg of protein, the gels showed one major band, estimated to contain approximately 90% of the protein and several minor bands. Similar gels prepared with marker proteins of known molecular

weights indicated a molecular weight of 50,000 for the major band, the subunit of glutamine synthetase. The specific activity of these preparations was 20 to 25 U per mg of protein.

Immunological methods. Serum with specific anti-glutamine activity was obtained from rabbits injected with purified glutamine synthetase (unadenylylated; fraction 3, Table 2) obtained from strain MK-53. It has been demonstrated by Tronick et al. (10) for *Escherichia coli* that the state of adenylylation of glutamine synthetase has no effect on the antigenicity of the molecule nor could sera be prepared that selectively precipitated adenylylated and unadenylylated enzyme.

Each of two 12-lb (about 5,443 g) New Zealand rabbits received subcutaneous injections of 2 ml of an emulsion containing 1 ml (0.15 mg of protein) of enzyme in low-Mg^{2+} buffer containing 0.3 M sodium chloride, and 1 ml of complete Freund adjuvant (Difco). Three weeks later the animals were bled. Six weeks later, 1 ml (0.2 mg of protein) of a similar enzyme preparation, as previously described, was injected subcutaneously into each animal, and the animals were bled 2 weeks later. Sera of both animals, obtained on the second bleeding, were used without further purification. Sera were stored under sterile conditions at −20 C and anti-glutamine synthetase activity remained relatively constant for at least 12 months.

The anti-glutamine synthetase activity of the sera was determined by the quantitative inhibition method of Hamers and Hamers-Casterman (2) as described by Kaminskas et al. (3). Various volumes of extract (0.05 to 0.10 ml) containing glutamine synthetase were added to a series of tubes containing a fixed amount (0.01 to 0.05 ml) of 10-fold-diluted serum. Total volume was brought to 0.2 ml with low-Mg^{2+} buffer. The tubes were incubated at 37 C for 30 min and at 4 C overnight. At the end of the incubation period, the samples were centrifuged at 7,700 × *g* for 20 min and 0.025 to 0.05 ml was removed for assay. At the equivalence point, 1 ml of the more active serum precipitated 75 ± 5 units of glutamine synthetase activity.

TABLE 2. *Relationship of glutamine synthetase activity to level of antigen*

Expt	Strain[a]	Pertinent genotype	Pertinent phenotype[b]	Ammonia (30 mM)	Relative glutamine synthetase	
					Antigen[c]	Activity
1	MK-53	*gln*+	Gln+Cn+	−	100	100
				+	23	27
2	MK-93	*glnB3*	Gln−Cn^s	−	7	7
3	MK-104	*glnA6*	*Gln*−Cn^S	−	0	0
4	MK-266	*glnA50*	Gln−Cn^S	−	0	0
5	MK-103	*glnA5*	Gln−Cn^S	−	5	0
				+	7	0
6	MK-710	*glnA5, sup-1*	Gln^TS Cn^S	−	52	12
				+	12	3
7	MK-9021	*glnA10*	Gln−Cn^R	−	0	0
				+	0	0
8	MK-267	*glnA51*	Gln−Cn^R	−	95	0
				+	90	0
9	MK-933	*glnA51, glnA53*	Gln^TS Cn+	−[d]	125	123
				+[d]	ND[e]	62

[a] All strains were grown on glucose and glutamine at 32 C.

[b] The phenotype designations have been defined in Table 1 of reference 5, and in the text of this paper.

[c] The same serum was used for all experiments. For experiments 1 to 8, activity and antigen levels were normalized to those measured in an extract of strain MK-53 grown on glucose and glutamine (specific activity, 1.45 U per mg of protein; serum titer, 79 U/ml). For experiments 3 to 6, the levels of activity and antigen were also normalized to those measured in an extract of strain MK-53 grown on glucose and 30 mM ammonia (specific activity, 0.44 U per mg of protein; serum titer, 70 U/ml). For experiment 9, levels of activity and antigen were normalized to those measured in an extract of strain MK-53 grown on glucose and glutamine (specific activity, 1.40 U per mg of protein; serum titer, 72 U).

[d] L-Histidine (0.2%) was also present in the growth medium.

[e] ND, Not determined.

The same procedure was used in testing extracts for the presence of antigen. To the tube containing an appropriate amount of 10-fold diluted serum, a fixed amount of extract was added. The tubes were incubated overnight at 4 C, an then various amounts of titered extracts of strain MK-53 were added. The final volume of each sample was 0.2 ml. Samples were again incubated overnight at 4 C. Samples were centrifuged, and the supernatant fluids were assayed for glutamine synthetase activity. The presence of antigen in sample extracts was revealed by the difference in the amount of enzyme precipitated by the serum. The level of antigen in extracts having glutamine synthetase activity was determined as described for the prototrophic strain, MK-53. For each experiment, the serum used was titered in duplicate against an extract of strain MK-53, grown on either histidine or glutamine as a nitrogen source, and all data were normalized to this value. In general, the titer of a serum varied 10% in a series of experiments conducted over a 1-year period. In addition, the level of antigen for any given strain was confirmed either on several separate occasions or by using several fixed levels of extract.

Heat denaturation studies. The purified glutamine synthetase (unadenylylated) from both strains MK-53 and MK-710, in a volume of 0.1 ml at a protein concentration of 0.5 mg/ml, was heated in a water bath for the prescribed time and/or temperature and quickly chilled in an ice-water bath. Volumes (0.005 and 0.020 ml) of each sample were then assayed.

RESULTS

Relationship of glutamine synthetase activity with the level of antigen. Analyses of glutamine synthetase activity in cell extracts of the prototrophic strain, MK-53, grown in media with or without an excess of ammonia, are presented in experiment 1, Table 2. There was an approximate fourfold increase in glutamine synthetase activity when the cells were grown on a growth rate-limiting nitrogen source, e.g., glutamine, rather than on excess ammonia in addition to glutamine. Our results show that the increase in glutamine synthetase activity corresponded to a similar increase in the level of antigen. Thus, the increased activity of the enzyme in relation to the decreasing ammonia concentration of the growth medium was a consequence of increased production of the enzymatically active protein.

Cell extracts of six glutamine auxotrophs, representing mutations at both the *glnA* and *glnB* loci, were assayed for material capable of reacting with anti-glutamine synthetase serum.

Strain MK-93, a *glnB3* mutant that gives rise at high frequency to revertants that lie within the *glnA* locus, had a level of antigen equivalent to the low level of glutamine synthetase activity present in the extract (experiment 2, Table 2).

Of the *glnA* mutant strains tested, only two, MK-103 (*glnA5*) and MK-267 (*asm-200, glnA51*), had detectable levels of glutamine synthetase antigen. Strain MK-103 (*glnA5*) had a low level of the antigen, the synthesis of which did not vary in relation to the ammonia concentration of the medium (experiment 5, Table 2). In contrast, high levels of antigen corresponding to the levels found in the protrophic strain when grown without ammonia were found in extracts of cells of strain MK-267 (*asm-200, glnA51*) regardless of whether the strain had been grown on a growth rate-limiting nitrogen source or on excess ammonia plus glutamine (experiment 8, Table 2). Similar results were obtained for the isogenic strain, MK-922 (*glnA51*).

The level of glutamine synthetase activity of strain MK-710, a temperature-sensitive glutamine auxotroph obtained as a revertant from strain MK-103, is shown in experiment 6, Table 2. Although there was an increase of glutamine synthetase activity when the strain was grown on the growth rate-limiting nitrogen source, glutamine, as compared with excess ammonia plus glutamine, the levels of activity were only 1/10 those of strain MK-53 grown under similar conditions. Determination of the antigen levels in these extracts confirmed the increased levels of antigen in response to decreasing levels of ammonia and also indicated that the ratio of antigen to enzymic activity is approximately 4 to 1. This result suggests that the glutamine synthetase of strain MK-710 has only approximately 25% of the catalytic activity of the normal enzyme.

A similar analysis of strain MK-933, a temperature-sensitive revertant of the glutamine auxotroph MK-922, is shown in experiment 9, Table 2. The results indicate that the reversion has restored the normal catalytic activity of the glutamine synthetase.

Growth characteristics of strains MK-710 and MK-933. The temperature-sensitive glutamine auxotrophs, MK-710 and MK-933, were isolated from their parental strains as spontaneous revertants to glutamine independence at 32 C. On solid media, they are strict glutamine auxotrophs at 42 C. The temperature-sensitive revertants had growth rates at 32 C comparable to those of the wild type on glucose minimal media containing 30 mM or 3 mM ammonia or glutamine. Slower growth rates were observed for strain MK-710 in the presence of citrate as a carbon source or when either histidine or proline were present as growth rate-limiting nitrogen sources at 32 C.

Although strain MK-710 is phenotypically a strict glutamine auxotroph on solid media at 42 C, prompt arrest of growth in liquid media at the restrictive temperature could not be demonstrated. Temperature shift experiments with cells of strains MK-53 and MK-710 grown in the presence of excess ammonia are shown in Fig. 1. When a growing culture of strain MK-710 was shifted from 32 to 42 C at a cell density of 10 Klett units, there was no immediate arrest in growth, but there was a gradual decrease in growth rate. Similar experiments with strain MK-933 also required very low levels of inoculum as well as a growth rate-limiting nitrogen source, i.e., histidine, to demonstrate temperature sensitivity in liquid culture at 42 C.

Biochemical comparison of glutamine synthetase of strains MK-53 and MK-710. The comparison of glutamine synthetase activity and antigen in extracts of strain MK-710 suggests that the revertant enzyme should have approximately 25% of the catalytic activity of the normal enzyme. Purified enzyme preparations of both MK-53 and MK-710 (fraction 2, Table 1) were judged by sodium dodecyl sulfate polyacrylamide gel electrophoresis to be of comparable purity. Analyses of these preparations indicated that the normal enzyme had specific activity of 26 U per mg of protein as measured by the reverse γ-glutamyl transferase assay and of 27 U per mg of protein as measured by the biosynthetic assay. The mutant enzyme had 15% of the activity of the normal enzyme measured by the reverse γ-glutamyl transferase assay and 13% of the activity measured by the biosynthetic assay. The K_m for adenosine 5'-triphosphate and glutamine of both enzyme preparations in the biosynthetic reaction showed no discernible differences (A. B. DeLeo and B. Magasanik, unpublished data). The K_m for ammonia was not determined owing to interference from exogenous ammonia present in the enzyme and reagent preparations. We compared the heat stability of the glutamine synthetase of strain MK-53 and of strain MK-710 (Fig. 2). It can be seen that there was a sharp and rapid inactivation of the revertant enzyme above 55 C, as compared with the wild-type enzyme. The time course of inactivation of both enzymes at 57 C is also illustrated in Fig. 2 and shows complete inactivation of the revertant enzyme within 3 min, as compared with only an approximated 30% loss of wild-type enzyme activity.

The biphasic nature of the heat denaturation curves was repeatedly observed in both crude extract and purified preparations of glutamine synthetase. The presence of 10 mM Mn^{2+} rather

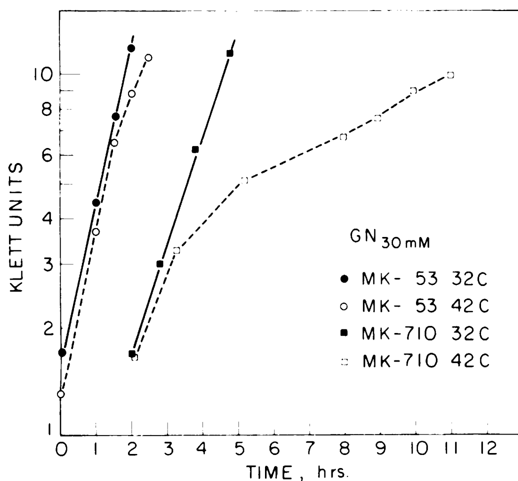

FIG. 1. *Growth of strains MK-53 (Gln+) and MK-710 (GlnTS) at 32 and 42 C on glucose and 30 mM ammonia. The cells used as innoculum had been grown in the same medium at 32 C.*

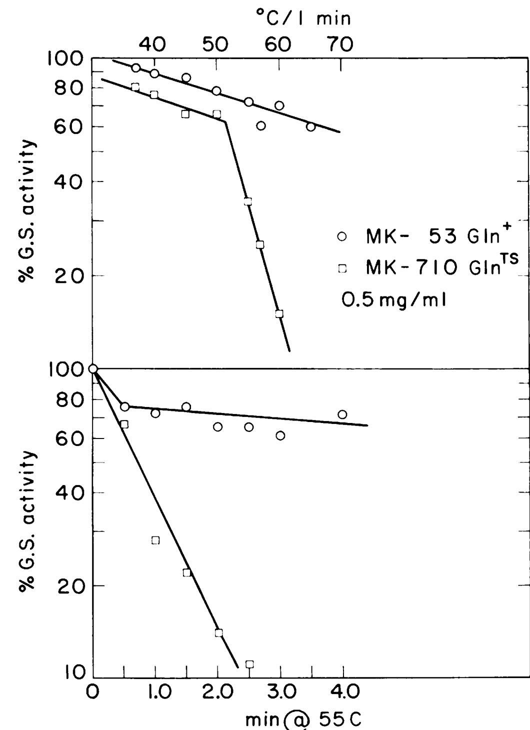

FIG. 2. *Purified glutamine synthetase of strain MK-53 (Gln+) and of strain MK-710 (GlnTS) was assayed after 1 min of exposure to the indicated temperatures (upper panel) or at different time intervals at 55 C (lower panel).*

than 2.5 mM Mg^{2+} increased the heat stability of both wild-type and mutant enzymes, but the biphasic curve was still observed. Whether these observations reflect differential stability of cationic binding sites in the protein was not investigated further.

Similar studies of MK-933 failed because the enzyme activity was unstable to purification, and heat lability studies with extracts did not show an altered thermosensitivity.

DISCUSSION

The glutamine synthetase of *Escherichia coli* is a dodecamer composed of identical subunits whose molecular weight is 50,000 (6). The enzyme from the related enteric organism *K. aerogenes* appears to have a very similar structure. We would therefore expect a single gene to determine the primary structure of the enzyme molecule. Nevertheless, we have found that mutations at two sites of the chromosome of *K. aerogenes*, *glnA* and *glnB*, result in the failure to produce enzymatically active glutamine synthetase at a level sufficient to support the growth of the mutant in media free of glutamine (5).

On the basis of the experiments described in this paper, we conclude that the structural gene for glutamine synthetase is located at the *glnA*, and not the *glnB* site. This conclusion is based on the observations that some *glnA* mutants produce a protein with the antigenic but without the enzymatic properties of glutamine synthetase, and that a revertant of a *glnA* mutant produces a heat-sensitive glutamine synthetase with reduced enzymatic activity. On the other hand, the *glnB* mutant produces an apparently normal enzyme, but in a greatly reduced quantity. We base this conclusion on the fact that the ratio of glutamine synthetase enzyme to antigen is the same in extracts prepared from wild-type cells and in the extract prepared from cells of the *glnB* mutant.

The temperature sensitivity of the revertants of strains MK-103 and MK-267 appears to result from increased lability of the subunit of glutamine synthetase. When revertant cells in liquid culture are shifted from the permissive temperature of 32 C to the restrictive temperature of 42 C, no immediate arrest of growth, but rather a gradual decline in the growth rate is observed. In the case of mutation *glnA51*, the mutation responsible for the reversion appear to be in the *glnA* site (unpublished data). When measured in a crude cell extract, the enzyme of the revertant strain did not differ in heat stability from the enzyme of the wild type. In the case of mutation *glnA5*, the mutation responsible for the recovery of enzyme activity is not located in the *glnA* site; it is a suppressor mutation that causes a decrease in the activity of histidase and β-galactosidase when present alone, or together with the *glnA5* mutation (A. B. DeLeo, K. Struhl, and B. Magasanik, unpublished data). The revertant enzyme is as stable as the enzyme of the wild strain at the growth restrictive temperature of 42 C, but is considerably more labile than the enzyme of wild strain at temperatures above 55 C. Whether this property is due to the original *glnA5* mutation or to the suppressor mutation that has partly restored the enzymatic activity is presently not known.

We have previously shown that glutamine synthetase is an activator of the synthesis of histidase and of other enzymes responsible for the formation of glutamate (5, 11). Several of the observations described in this paper bear on this property of glutamine synthetase. In the presence of glucose, histidase is formed rapidly only by cells whose growth rate is limited by the nitrogen source. Under these conditions, the cells of the wild type contain an increased level of glutamine synthetase activity. We have now shown that these cells contain a correspondingly increased amount of antigenically active glutamine synthetase molecules. The *glnA* mutants we have previously investigated (strains MK-103 and MK-104) have the Cn^s phenotype; during growth on glucose histidase is not produced even during starvation for a source of nitrogen. We find that extracts of these mutants contain no, or very little material antigenically related to glutamine synthetase, whether grown on a growth rate-limiting nitrogen source or with an excess of ammonia. Similarly, the *glnB* mutant produces very little material of this sort and has the Cn^s phenotype. On the other hand, one of the *glnA* mutants newly isolated by Jean Brenchley (MK-267) has the Cn^R phenotype; it produces histidase at a high level, whether grown on a limiting nitrogen source or with an excess of ammonia. We have now shown that cells of this mutant strain, whether grown with or without an excess of ammonia, contain glutamine synthetase antigen, but not enzymatic activity, at a level corresponding to the level of enzyme in cells of the wild strain grown on a limiting nitrogen source (see Table 2). We assume that this inactive glutamine synthetase is responsible for the activation of histidase formation. It is of considerable interest that a single mutation can apparently result in both the loss of glutamine synthetase activity and the loss of regulation of glutamine synthetase

formation by ammonia. This finding suggests that glutamine synthetase is itself involved in the regulation of glutamine synthetase formation.

One of the *glnA* mutants MK-9021 has the Cn^R phenotype, but produces neither active enzyme, nor antigen. It is possible that the mutation has affected the site at which adenylylation occurs; according to a recent report by Tronick et al. (10), this site is the major antigenic determinant of the enzyme molecule. The mutant may then produce a molecule without the enzymatic or antigenic properties of glutamine synthetase, but with the ability to activate the formation of histidase.

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