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Regulation of Enzyme Formation in *Klebsiella aerogenes* by Episomal Glutamine Synthetase of *Escherichia coli*

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We studied the physiology of cells of *Klebsiella aerogenes* containing the structural gene for glutamine synthetase (*glnA*) of *Escherichia coli* on an episome. The *E. coli* glutamine synthetase functioned in cells of *K. aerogenes* in a manner similar to that of the *K. aerogenes* enzyme: it allowed the level of histidase to increase and that of glutamate dehydrogenase to decrease during nitrogen-limited growth. The phenotype of mutations in the *glnA* site was restored to normal by the introduction of the episomal *glnA*$^+$ gene. These results are consistent with the hypothesis that glutamine synthetase regulates the function of its own structural gene.

In previous papers we have presented evidence that the structural gene for glutamine synthetase in *Klebsiella aerogenes* and in *Escherichia coli* is located at the *glnA* site, a position corresponding to 77 min on the Taylor map (2, 8). We have shown that mutations in the *glnA* site can result in several distinct phenotypes. Some *K. aerogenes* mutants are devoid of glutamine synthetase activity and are unable to produce histidase in the presence of glucose. Other *K. aerogenes* mutants are similarly devoid of glutamine synthetase activity, but produce histidase in the presence of glucose even in media containing an excess of ammonia, conditions under which cells with normal glutamine synthetase fail to produce histidase. Some of these mutants produce high levels of material capable of reacting with anti-glutamine synthetase serum whether or not ammonia is present in the medium, but in one such mutant no material antigenically related to glutamine synthetase could be found. Finally, some *K. aerogenes* mutants with lesions in the *glnA* site contain a high level of glutamine synthetase whether grown with an excess of ammonia or on a growth rate-limiting nitrogen source; they also produce histidase under both conditions. These mutants are unable to produce glutamate dehydrogenase.

These findings could best be explained by the assumption that glutamine synthetase cannot only activate the synthesis of histidase (7, 9), but can also regulate the synthesis of glutamine synthetase itself. In that case, mutations in the structural gene for glutamine synthetase might be expected to affect either the catalytic or the regulatory properties of glutamine synthetase or both. Furthermore, we would expect the introduction of a *glnA*$^+$ gene coding for normal glutamine synthetase into strains with mutations in the *glnA* site to restore not only the ability to produce the enzyme, but also normal regulation of the synthesis of glutamine synthetase, and consequently normal regulation of the synthesis of histidase. This paper describes the effects of the introduction of an F′ *glnA*$^+$ episome of *E. coli* on the formation of glutamine synthetase, glutamate dehydrogenase, and histidase in *K. aerogenes*.

MATERIALS AND METHODS

Bacterial strains. Strains of *K. aerogenes* and *E. coli* used in this study are listed in Table 1. The merodiploid strains of *K. aerogenes* and *E. coli* carrying the F′14 episome were isolated by the plate mating procedure previously described (8), and were maintained by growth in selective medium. The presence of the episome in purified clones and in cells to be assayed for enzyme activity was determined after two cycles of nonselective growth by observing segregation of the episome and the reappearance of previously complemented markers.

Strain MK267, a glutamine auxotroph containing the *glnA51* mutation, was isolated from a mutagenized culture of strain MK189 by J. E. Brenchley. Strains MK189 and MK267 contain the *asm-200* mutation, which has been previously shown to abolish the activity of glutamate synthase and to affect the rate of synthesis of glutamine synthetase and of histidase (1). To examine the effect of the *glnA51* mutation alone on the synthesis of other enzymes it was necessary to replace the *asm-200* mutation with the wild-type allele. This was accomplished by first transferring the F′14 episome from strain AB1206 into strain MK267 by selecting glutamine-independ-

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TABLE 1. *List of K. aerogenes and E. coli strains and their characteristics*

Strain	Relevant genotype	Source
Klebsiella aerogenes		
MK53	Prototroph	(6)
MK104	*glnA6*[a]	(7)
MK189[b]	*asm-200*[c]	(1)
MK267[b]	*glnA6 asm-200*	(2)
MK912[b]	F'14/*glnA51 asm-200*	AB1206 × MK267
MK913[b]	F'14/*glnA51*	PW52·MK53 + MK912
MK921	F'14/*glnA6*	AB1206 × MK104
MK924[b]	*glnA51*[a]	Gln⁻ segregant of MK913
MK980	*glnA4 glnB3 ilv-4*	Derivative of MK94 (7)
MK981	F'14/*glnA4 glnB3 ilv-4*	AB1206 × MK980
MK9000	Prototroph	P1[s] of MK53 (8)
MK9011	*glnA6 ilvA1*[a]	(8)
MK9021	*glnA10 ilvA1 rha-1*[a]	(8)
MK9028	*glnA4 ilvA1*[a]	(8)
MK9129	*glnA4 glnB3 ilvA3*	Derivative of MK94 (7)
MK9141	F'14/*glnA10 ilvA1 rha-1*	AB1206 × MK9021
MK9142	F'14/*ilvA1 metB6 rha-1*	AB1206 × MK9271
MK9143	F'14/*glnA6 ilvA1*	AB1206 × MK9011
MK9170	F'14/*glnA4 ilvA1*	AB1206 × MK9028
MK9171	F'14/*glnA4 glnB3 ilvA3*	AB1206 × MK9129
MK9271	*ilvA1 metB6 rha-1*	(8)
Escherichia coli K-12		
LS519	*metB rha*	L. Soll
LS5191	*glnA200 metB rha*[a]	B. Tyler
FLS519	F'14/*metB rha*	AB1206 × LS519
FLS5191	F'14/*glnA200 metB rha*	AB1206 × LS5191
AB1206	F'14	B. Bachmann
E. coli W	Prototroph	This laboratory

[a] The phenotypes conferred by mutations in the *glnA* site in the haploid state are as follows: *glnA4*, constitutive synthesis of glutamine synthetase, CN^R (see text for details); *glnA6*, requires glutamine for growth, CN^S; *glnA10*, requires glutamine for growth, CN^R; *glnA51*, requires glutamine for growth, CN^R; *glnA200*, requires glutamine for growth.

[b] These strains are *hutC*⁺; all other *K. aerogenes* strains contain the *hutC515* mutation (6).

[c] Strain MK189 *(asm-200)* requires high concentrations of NH_3 or glutamate or glutamine for growth.

ent exconjugants. The merodiploid strain MK912 has a phenotype similar to that of strain MK189; it is unable to grow in a glucose medium containing histidine as the nitrogen source. A transductant able to grow in this medium was selected after infection of strain MK912 with the generalized transducing phage PW-52 grown on the wild strain, MK53. This transductant, strain MK913, was grown in nonselective medium and a glutamine-requiring segregant was obtained. This haploid strain, MK924, contains only the *glnA51* mutation and was used in several of the experiments described below.

Growth media and the cultivation of bacteria. The rich medium used was LB or LBgln, which have been previously described (8). The minimal medium used was W salts (7) supplemented with 0.4% glucose as the source carbon and energy. Nitrogen sources (at a final concentration of 0.2%), used where indicated, included: ammonium sulfate, glutamine, or histidine. Solutions of glutamine (Calbiochem, A grade) and histidine (Sigma) were prepared immediately before use and were sterilized by filtration. When required, amino acid supplements were added at a final concentration of 100 μg/ml. Media used for the growth of *E. coli* strains were supplemented with vitamin B_1 (10 μg/ml).

Bacteria for enzyme assays were grown in 250 ml of the appropriate minimal medium in a 2-liter Fernback flask at 30 C with vigorous aeration. Inocula were grown aerobically at 30 C to saturation in the appropriate medium; the cells were harvested by centrifugation, washed once with sterile saline (0.85% NaCl), and diluted 1:100 into fresh medium. The cells were harvested by centrifugation when the cell density reached about 5×10^8 cells/ml (100 Klett units). The cells were washed once with saline, and the cell pellets were stored at −20 C until extracts were prepared. When cultures of merodiploid strains were grown, samples were removed before harvesting and examined for the presence of the episome.

Cell extracts. Cell extracts were prepared by sonic oscillation as previously described (7) in a buffer containing 10 mM imidazole (pH 7.15), 10 mM $MnCl_2$ and 2 mM β-mercaptoethanol. Cell extracts were stored at 4 C and assayed for enzyme activity within 3 days of preparation. Protein concentrations were determined by the method of Lowry et al. (4), with crystalline bovine serum albumin serving as the standard.

Enzyme assays. The assay procedures and conditions for glutamine synthetase (transferase activity), glutamate dehydrogenase, and histidase were as previously described (1). The amount of glutamine synthetase antigen was determined by the procedure described by DeLeo and Magasanik (2) using antiserum prepared against the glutamine synthetase of *K. aerogenes*. Enzyme activity is expressed as nanomoles of product formed per minute per milligram of protein. One unit of cross-reacting material (CRM) blocks the precipitation of 1 unit of wild-type glutamine synthetase by antiserum.

RESULTS

It has previously been shown that the level of glutamine synthetase in *E. coli* depends on the source of nitrogen (5). Cells growing on a poor source of nitrogen, such as proline, are richer in glutamine synthetase than cells growing on a good source of nitrogen, such as ammonia. This effect is illustrated in Table 2. In cells of two wild strains of *E. coli* grown in a medium containing glutamine as the only source of nitrogen, the level of glutamine synthetase was four to five times as high as in cells grown in a medium containing glutamine and ammonia. The levels of glutamine synthetase antigen correspond to the levels of enzyme, indicating that

the increased enzyme activity reflects an increase in the number of enzyme molecules.

A glutamine-requiring mutant, strain LS5191, failed to produce the enzyme. Introduction of F'14, an episome previously shown to carry the *glnA* site (8), restored glutamine independence and the ability to produce the enzyme. In this strain, FLS5191, the levels of glutamine synthetase in cells grown in the absence or presence of ammonia corresponded to the levels found in similarly grown cells of the wild strain.

It is somewhat surprising that, in strain FLS519 carrying a normal *glnA*$^+$ gene both on the chromosome and on the episome, the levels of glutamine synthetase were not significantly higher than in strains carrying a single *glnA*$^+$ gene: there appears to be no effect of gene dosage on the rate of synthesis of this enzyme.

We have previously shown (see also Table 3) that in cells of *K. aerogenes* whose growth rate is limited by the nitrogen source, the level of glutamate dehydrogenase is low (1). As seen in Table 2, this is apparently not the situation in cells of *E. coli*; the level of glutamate dehydrogenase was the same in cells grown with or

TABLE 2. *Levels of glutamine synthetase and of glutamate dehydrogenase in strains of E. coli*[a]

Expt	Strain	Relevant genotype	Nitrogen source	Enzyme activity (nmol/min per mg of protein)	
				Glutamine synthetase	Glutamate dehydrogenase
1	W	*glnA*$^+$	N, G[b]	200 (200)[c]	ND[d]
2			G	1,020 (1,020)	ND
3	LS519	*glnA*$^+$	N, G	310	865
4			G	1,350	830
5	LS5191	*glnA200*	N, G	0	795
6			G	0	830
7	FLS519	F'*glnA*$^+$/*glnA*	N, G	300	1,030
8			G	1,745	1,030
9	FLS5191	F'*glnA*$^+$/*glnA200*	N, G	200	720
10			G	1,540	815

[a] Cells were grown and extracts were prepared and assayed as described in Materials and Methods.
[b] N, Ammonium sulfate; G, glutamine.
[c] Values in parentheses are the amounts of glutamine synthetase antigen determined as described by DeLeo and Magasanik (2).
[d] ND, Not determined.

TABLE 3. *Effect of F'glnA*$^+$ *of E. coli on the levels of glutamine synthetase, glutamate dehydrogenase, and histidase in K. aerogenes*[a]

Expt	Strain	Relevant genotype	Nitrogen source	Enzyme activity (nmol/min per mg protein)		
				Glutamine synthetase	Glutamate dehydrogenase	Histidase
1	MK9271	*glnA*$^+$	N, G[b]	240	420	55
2			N	230	475	65
3			G	1,060	245	460
4			H	1,000	30	650
5	MK9011	*glnA6*	N, G	0	565	ND
6			G	0	285	37
7	MK9143	F'*glnA*$^+$/*glnA6*$^+$	N, G	105	410	65
8			N	275	340	65
9			G	1,500	235	205
10			H	1,345	110	260
11	MK9142	F'*glnA*$^+$/*glnA*$^+$	N, G	115	270	70
12			N	330	390	85
13			G	910	320	105
14			H	1,300	25	370

[a] Cells were grown and extracts were prepared and assayed as described in Materials and Methods.
[b] N, Ammonium sulfate; G, glutamine; H, histidine.

without an excess of ammonia. Additional experiments (not shown) indicate that even severe nitrogen limitation, achieved by supplying *E. coli* with glutamate as sole source of nitrogen, did not bring about a significant reduction in the level of this enzyme. In *K. aerogenes*, as in *E. coli*, the level of glutamine synthetase depends on the nitrogen source of the medium. As shown in Table 3, in strain MK9271 (*glnA*$^+$), the enzyme level was three- to fourfold higher in cells grown in media containing histidine or glutamine as sole source of nitrogen than in media containing ammonia.

It has previously been shown that glutamine synthetase activates the formation of histidase (7). This effect is apparent in the results presented in Table 3: the level of histidase in the wild strain, MK9271, was much greater when the cells were grown in a medium containing glutamine as the only nitrogen source than when grown in a medium containing ammonia as well as glutamine; in the glutamine-requiring mutant, MK9011, whose inability to produce glutamine synthetase is apparently due to a small deletion in *glnA* (8), the level of histidase was low even when the cells were grown with glutamine as the nitrogen source (Table 3, experiment 6).

In contrast to its activating effect on histidase formation, glutamine synthetase has an inhibitory effect on glutamate dehydrogenase formation in *K. aerogenes* (1). Cells of the strain with normal glutamine synthetase contained only a very low level of glutamate dehydrogenase when grown in a medium containing histidine as the only source of nitrogen (Table 3, experiment 4). In this medium, the growth rate was more restricted by the nitrogen source than in the other three media used in these experiments (6).

The introduction of the F'*glnA*$^+$ episome from *E. coli* into strain MK9011 (*glnA6*), restored to this strain the ability to grow without glutamine and to produce glutamine synthetase. The expression of the glutamine synthetase determined by the *glnA*$^+$ gene of *E. coli* in strain MK9143 was regulated in the same manner as that of the *glnA*$^+$ gene of *K. aerogenes* in strain MK9271. Furthermore, the glutamine synthetase determined by the *E. coli glnA*$^+$ gene was capable of stimulating the synthesis of histidase and inhibiting the synthesis of glutamate dehydrogenase (Table 3, experiments 7 through 10).

A strain carrying a functional *glnA*$^+$ gene of *K. aerogenes* on the chromosome and a functional *glnA*$^+$ gene of *E. coli* on the episome did not differ significantly in its production of the three enzymes from strains carrying a single *glnA*$^+$ gene (Table 3, experiments 11 through 14). In this case too, no gene dosage effect was observed.

Mutations in the *glnA* site resulting in the inability to produce active glutamine synthetase can have different effects on the ability of the cell to regulate the synthesis of histidase and of glutamate dehydrogenase. We have already shown (Table 3) that a strain with the *glnA6* mutation failed to produce histidase when grown in a medium containing glutamine as the only source of nitrogen. This was also true for another strain carrying the *glnA6* mutation (Table 4, experiments 3 and 4); here we show that this also failed to produce material antigenically related to glutamine synthetase.

On the other hand, strain MK924, (*glnA51*) contained glutamine synthetase antigen at a high level, but no active enzyme in a medium containing glutamine and ammonia (Table 4, experiment 5; see also reference 2). Cells of this strain, grown in a medium containing ammonia and glutamine, had a very low level of glutamine dehydrogenase, but a high level of histidase. It appears that the enzymatically inactive glutamine synthetase, present in these cells even when they are grown with an excess of ammonia, was capable of stimulating the synthesis of histidase and of inhibiting the synthesis of glutamate dehydrogenase.

Cells of strain MK9021 (*glnA10*) had a low level of glutamate dehydrogenase and a high level of histidase when grown in a medium containing ammonia and glutamine; however, in these cells no material antigenically related to glutamine synthetase was detected.

In all three strains, the introduction of an episome carrying the normal *glnA*$^+$ gene of *E. coli* restored normal control of the three enzymes. It is of particular interest that in the case of strain MK913 (F'*glnA*$^+$/*glnA51*), the level of glutamine synthetase antigen did not exceed the level of active enzyme, even in cells grown with histidine as the only source of nitrogen (Table 4, experiments 10 and 11). This level was approximately the same as that found in similarly grown cells carrying a single normal *glnA*$^+$ gene either on the chromosome or on the episome (see Tables 2–4).

We can conclude that the phenotypes determined by mutations *glnA6*, *glnA51*, and *glnA10* are in all respects recessive to the phenotype determined by the *E. coli glnA*$^+$ gene.

There is still another phenotype that can arise from a mutation in the *glnA* site: high level of glutamine synthetase and of histidase and a low level of glutamate dehydrogenase in

TABLE 4. *Effect of F'glnA⁺ of E. coli on enzyme levels in glutamine auxotrophs of K. aerogenes*[a]

Expt	Strain	Relevant genotype	Nitrogen source[b]	Enzyme activity (nmol/min per mg protein)			
				Glutamine synthetase		Glutamate dehydrogenase	Histidase
				Catalytic activity	Antigen		
1	MK53	+	N, H	195	220	675	40
2			H	950	800	25	265
3	MK104	*glnA6*	N, H, G	0	0	566	37
4			G	0	ND	286	40
5	MK924	*glnA51*	N, H[c], G	0	930	22	314
6	MK9021	*glnA10*	N, G	0	0	40	221
7			G	0	0	18	314
8	MK921	F'*glnA⁺*/*glnA6*	N, H	150	145	336	52
9			H	960	800	13	290
10	MK913	F'*glnA⁺*/*glnA51*	N, H[c]	220	190	396	49
11			H	1,070	940	38	294
12	MK9141	F'*glnA⁺*/*glnA10*	N	310	ND	219	82
13			H	1,240	ND	16	425

[a] Cells were grown and extracts were prepared and assayed as described in Materials and Methods.
[b] N, Ammonium sulfate; H, histidine, G, glutamine.
[c] L-Histidine (0.2%) was added to the medium to induce the *hut* operons.

TABLE 5. *Effect of F'glnA⁺ of E. coli on enzyme levels of K. aerogenes strains with the glnA4 mutation*[a]

Expt	Strain	Relevant genotype	Nitrogen source[b]	Enzyme activity (nmol/min per mg of protein)		
				Glutamine synthetase	Glutamate dehydrogenase	Histidase
1	MK9028	*glnA4*	N, G	1,530	45	410
2			N	1,400	110	640
3			G	1,860	50	450
4			H	1,440	40	635
5	MK9129	*glnA4 glnB3*	N, G	1,640	10	325
6			N	1,450	15	500
7			G	1,620	10	475
8			H	1,680	15	425
9	MK9170	F'*glnA⁺*/*glnA4*	N, G	635	295	80
10			N	800	350	90
11			G	2,190	140	390
12			H	1,760	35	370
13	MK9171	F'*glnA⁺*/*glnA4 glnB3*	N, G	350	395	110
14			G	1,450	465	150

[a] Cells were grown and extracts were prepared and assayed as described in Materials and Methods.
[b] N, Ammonium sulfate; G, glutamine; H, histidine.

cells growing in a medium containing an excess of ammonia. Such mutants are isolated as frequent pseudorevertants of a mutant with a defect in the *glnB* site that leads to inability to produce active glutamine synthetase (7).

The results of experiments summarized in Table 5 show these effects of the *glnA4* mutation. They also show that the expression of the three enzymes in strains carrying the *glnA4* mutation is not affected by the state of the *glnB* gene: irrespective of the medium, the cells had high levels of glutamine synthetase and of histidase, and a low level of glutamate dehydrogenase, whether they carry the normal (strain MK9028), or the defective (strain MK9129) *glnB* gene.

Introduction of the F'*glnA⁺* of *E. coli* affects strains MK9028 and MK9129 differently. Strain MK9170 (F'*glnA⁺*/*glnA4*) grows well in a medium devoid of glutamine, but strain MK9171 (F'*glnA⁺*/*glnA4 glnB3*) requires glutamine (Fig. 1). (Its growth in glutamine-free medium after a lag of 5 h may be due to the selection of revertants.) Thus, the introduction of the normal *glnA⁺* gene of the episome has overcome the suppression by the *glnA4* gene on *glnB3*,

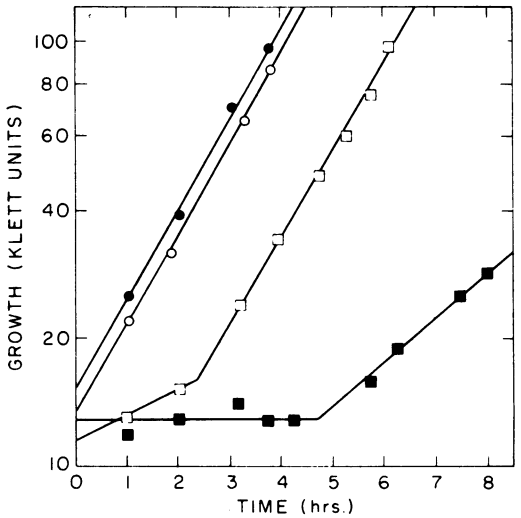

FIG. 1. *Growth of strains MK9170 and MK9171 in the presence and absence of glutamine. The strains were grown overnight in minimal medium containing glutamine. The cells were washed once with saline and diluted into fresh medium containing ammonia and glutamine (strain MK9170) [○]; strain MK9171 [□]) or ammonia (strain MK9170 [●]; strain MK9171 [■]).*

and restored the glutamine-dependent phenotype of the original *glnB3* mutant.

The enzyme levels of strain MK9170 are presented in Table 5. It can be seen that the presence of the normal *glnA*$^+$ gene of *E. coli* leads to a reduction of the glutamine synthetase levels in cells grown in ammonia containing media; however, the level of glutamine synthetase was distinctly higher than that found in cells that do not carry the *glnA4* mutation (see Tables 3 and 4). Nevertheless, cells of strain MK9170 (*glnA*$^+$/*glnA4*) grown with an excess of ammonia contained a high level of glutamate dehydrogenase and a low level of histidase. It is apparent that the introduction of the *glnA*$^+$ gene of *E. coli* has restored normal regulation of these enzymes.

It is surprising, in view of the inability of strain MK9171 to grow in the absence of glutamine, to find that cells of the strain grown in media containing glutamine alone or glutamine and ammonia as sources of nitrogen had levels of glutamine synthetase as high as those found in similarly grown cells of the wild type. However, in contrast to cells of the wild type, cells of strain MK9171 did not contain a high level of histidase when grown with glutamine as the only source of nitrogen (compare Tables 3 and 5).

One explanation for these results is that the glutamine synthetase in strain MK9171 is always highly adenylylated. This would have two consequences; a lack of adequate capacity to synthesize glutamine for the needs of the cell and thus a glutamine requirement, and an inability to activate the synthesis of histidase (9) or to repress the synthesis of glutamate dehydrogenase. To examine this possibility, we measured the biosynthetic activity of glutamine synthetase in strains with the genotypes *glnA*$^+$ and F'*glnA*$^+$/*glnA4 glnB3* (a different strain isolate with the same genotype as strain MK9171). The assay used in this experiment was the "biosynthetic assay" (R. A. Bender, K. A. Jonssen, A. D. Resnik, M. Blumenberg, F. Foor, and B. Magasanik, manuscript in preparation), which measures the formation of γ-glutamylhydroxamate from glutamate and hydroxylamine at the expense of adenosine 5'-triphosphate. This assay is more indicative of the ability of glutamine synthetase to form glutamine in the cell than the transferase activity measured in the presence of Mg^{2+}. It is seen in Table 6 that although transferase activity in the two strains was very similar, the biosynthetic activity (the ability to form glutamine) was markedly lower in strain MK981; it is likely that this accounts for the inability of this strain and of strain MK9171 to grow in the absence of glutamine.

DISCUSSION

We have previously shown that the glutamine synthetase of *K. aerogenes*, in addition to its enzymatic function, can regulate the synthesis of enzymes involved in nitrogen metabolism: it inhibits the synthesis of glutamate dehydrogenase and it activates the synthesis of histidase. This activation reflects the stimulation of the transcription of *hut*-specific deoxyribonucleic acid by the non-adenylylated glutamine synthetase (9).

We have now shown that the glutamine synthetase of *E. coli* can also exert these regulatory functions in the cytoplasm of *K. aerogenes*.

TABLE 6. *Comparison of glutamine synthetase transferase and biosynthetic activity in a wild-type and a merodiploid strain of K. aerogenes*[a]

Strain	Relevant genotype	Glutamine synthetase (nmol/min per mg protein)	
		Transferase activity	Biosynthetic activity
MK9000	+	200	110
MK981	F'*glnA*$^+$/*glnA4 glnB3*	230	16

[a] The cells were grown to 100 Klett units in minimal medium containing ammonia and glutamine. The cells were harvested and assayed by the procedure of Bender et al. (manuscript in preparation).

The introduction of an F′ episome carrying the functional structural gene for glutamine synthetase, *glnA*$^+$ of *E. coli*, into a mutant of *K. aerogenes* lacking glutamine synthetase because of a small deletion in the gene, *glnA6* (8), restores to this organism not only the ability to produce glutamine synthetase, but also the ability to regulate the synthesis of glutamate dehydrogenase and of histidase. This finding is particularly interesting because in its own cytoplasm, the glutamine synthetase of *E. coli* fails to regulate the synthesis of glutamate dehydrogenase, and lacks the opportunity to regulate the synthesis of histidase, since *E. coli* does not carry the *hut* genes.

The fact that the glutamine synthetase of *E. coli* so closely resembles in regulatory activities the enzyme from *K. aerogenes*, made it possible for us to investigate the nature of mutations in the *glnA* site of *K. aerogenes* that result in altered regulation of the synthesis of histidase and glutamate dehydrogenase by observing the effects of the introduction of the episome carrying the functional *glnA*$^+$ gene of *E. coli*.

All mutations of the *glnA* site we have examined so far affect not only glutamine synthetase, but also the regulation of histidase and of glutamate dehydrogenase. According to the effects on regulation, the mutants can be divided into two classes: those with the Cn^S phenotype and those with the Cn^R phenotype (7). The former produce glutamate dehydrogenase at a high level and histidase at a low level in an ammonia-deficient medium; the latter produce glutamate dehydrogenase at a low level and histidase at a high level in an ammonia-rich medium. Mutants carrying the *glnA6* mutation are Cn^S (Table 3, experiment 6 and Table 4, experiment 4), and mutants carrying mutations *glnA51*, *glnA10*, and *glnA4* are Cn^R (Table 4, experiments 5 and 6; and Table 4, experiments 1, 2, 5, and 6).

The mutants of the second class are not necessarily incapable of producing enzymatically active glutamine synthetase. Strains MK9028 and MK9129, carrying the *glnA4* mutation, differ from the wild-type organism by producing glutamine synthetase at a high level even in the presence of ammonia. Mutants carrying the *glnA51* mutation produce no active enzyme, but glutamine synthetase antigen is present at a high level even in the presence of ammonia. Neither enzyme nor antigen could be detected in mutants carrying the *glnA10* mutation. However, the fact that these mutations are interspersed at the *rha*-proximal end of the *glnA* site has suggested to us that they all result in the formation of an altered *glnA* product (8). We assume that this product may have enzymatic as well as antigenic activity (*glnA4*), only antigenic activity (*glnA51*), or neither (*glnA10*), but that its production is no longer controlled by the presence of ammonia in the medium. This product activates the synthesis of histidase and inhibits the synthesis of glutamate dehydrogenase, resulting in the Cn^R phenotype. The Cn^S phenotype associated with the *glnA6* mutation is a manifestation of the absence of such a *glnA* product, even in cells grown in an ammonia-deficient medium.

The dominance of the normal *glnA*$^+$ gene over *glnA51* and *glnA10* is illustrated in experiments 10 to 13 of Table 4. It can be seen that the merodiploid cells have lost the characteristics associated with the *glnA51* and *glnA10* mutations: active glutamine synthetase is present at a low level when ammonia is in excess and at a high level when no ammonia is added to the medium; the histidase level is low, and the glutamate dehydrogenase level is high, in cells grown in the presence of ammonia. These are the results expected from the assumption that the product of the normal *glnA*$^+$ gene lowers the expression of both the episomal *glnA* gene and of the mutated chromosomal *glnA* gene in cells grown with an excess of ammonia. It is somewhat unexpected that in the case of strain MK913 (F′*glnA*$^+$/*glnA51*), grown in the absence of ammonia (Table 4, experiment 11), the level of glutamine synthetase antigen does not exceed the level of active enzyme. This finding seems to imply that the chromosomal *glnA51* gene is not expressed, even in the absence of ammonia. However, we must consider the possibility of subunit mixing: it is possible that dodecameric glutamine synthetase composed of a mixture of subunits arising from the expression of *glnA51* and the normal *glnA*$^+$ gene has normal enzymatic activity and can regulate the expression of *glnA*. The fact that no gene dosage effect can be seen is in agreement with an observation recorded in Table 2; strain FLS519, carrying two normal *glnA*$^+$ genes, does not produce twice as much glutamine synthetase as strains LS519 and FS5191, which carry only one *glnA*$^+$ gene.

The organism carrying the *glnA4* mutation (Table 5) was isolated as a revertant to glutamine independence of a glutamine-requiring mutant with a lesion, *glnB3*, in a site unlinked to *glnA* (8). We have previously shown that cells carrying only the *glnB3* mutation produce very little glutamine synthetase and have the Cn^S phenotype, namely, high glutamate dehydrogenase and low histidase when grown in an ammonia-deficient medium (1, 7). Very recently we have found that the *glnB3* mutation results in a defect in a protein, P_{III}, required

for the deadenylylation of glutamine synthetase (3). We interpret this finding as an indication that the adenylylated form of glutamine synthetase, which predominantes in cells growing with an excess of ammonia, is responsible for the repression of glutamine synthetase. Derepression would require deadenylylation, made impossible by the *glnB3* mutation. We can, however, not exclude an alternative interpretation: the unadenylylated glutamine synthetase, which cannot be produced as result of the *glnB3* mutation, may be an essential activator of the synthesis of glutamine synthetase.

It is of particular interest that strains carrying the *glnA4* mutation have a high level of glutamine synthetase even when grown with an excess of ammonia and have the Cn^R phenotype (7). The product of the *glnA4* gene apparently has lost the ability to prevent expression of the *glnA* gene or always activates its expression. Consequently, glutamine synthetase is produced irrespective of the state of the *glnB* gene and of the presence or absence of ammonia (Table 5, experiments 1 through 8). This observation does not prove that this glutamine synthetase cannot be adenylylated. In fact, we have shown previously that the glutamine synthetase activity in mutants carrying the *glnA4* mutation can be partially inhibited by Mg^{2+}, an indication of the presence of adenylylated enzyme (7). We assume, rather, that the *glnA4* glutamine synthetase fails to have its regulatory properties altered by adenylylation. Thus, this adenylylated enzyme would resemble the nonadenylylated enzyme in its inability to repress glutamine synthetase, or its ability to activate its synthesis as well as in its ability to repress glutamate dehydrogenase and to activate the synthesis of histidase.

The behavior of cells carrying *glnA4* on the chromosome and normal $glnA^+$ on the episome shows that the effects of the *glnA4* glutamine synthetase are recessive to those of the wild-type enzyme of the episome. A comparison of cells of strain MK9028 (*glnA4*) and of strain MK9170 (F'$glnA^+$/*glnA4*) growing with an excess of ammonia shows that introduction of the normal *glnA* gene has restored normal regulation of glutamate dehydrogenase and of histidase. Thus, as far as these characters are concerned, the phenotype of normal $glnA^+$ is completely dominant over the phenotype of *glnA4*.

The dominance of the normal $glnA^+$ gene is not as complete with regard to glutamine synthetase. The level of this enzyme in cells of strain MK9170 grown with an excess of ammonia was considerably lower than in similarly grown cells of strain MK9028 (Table 5, experiments 9 and 1); however, it was not as low as the level found in similarly grown cells of strain MK9271, carrying a normal $glnA^+$ gene on the chromosome, or of strain MK9143, carrying a normal $glnA^+$ on the episome (Table 3, experiments 1 and 7).

The suppression of the phenotype of *glnB3*, (Gln^-, Cn^S) by the *glnA4* mutation is complete: essentially the same levels of glutamine synthetase, glutamate dehydrogenase and of histidase were found in cells of strain MK9028 (*glnA4*) and strain MK9129 (*glnA4 glnB3*), whether they were grown with ammonia limitation or excess (Table 5, experiments 1 to 8). However, introduction of the episome carrying normal $glnA^+$ into these strains produces two strains with very different phenotypes. Strain MK9170, (F'$glnA^+$/*glnA4*) has, as previously discussed, a phenotype closely resembling that of a strain carrying a single normal *glnA* gene, whereas strain MK9171 (F'$glnA^+$/*glnA4 glnB3*) has a phenotype that resembles (but not completely) that of the original *glnB3* mutant. Cells of this strain require glutamine for growth (Fig. 1) and contain a high level of glutamate dehydrogenase and a low level of histidase when grown in an ammonia-deprived medium (Table 5, experiment 14). Apparently, the dominance of the normal *glnA* gene over *glnA4* prevents the suppression of *glnB3* by *glnA4*. A completely unexpected observation was the high level of glutamine synthetase in strain MK9171. This glutamine synthetase was highly adenyllyated, as reflected in the very low biosynthetic activity (Table 6). The question remains as to why, in this organism, the highly adenylylated, as reflected in the very not more effective in reducing the expression of the *glnA* gene. We must keep in mind that glutamine synthetase is a dodecamer and that each of the 12 subunits may carry an adenyl group. We cannot even begin to speculate whether every one of the many possible forms or only a specific one has activity as a regulator.

In summary, the results reported in this paper show that in cells merodiploid for *glnA*, the phenotype with regard to the regulation of the synthesis of glutamine synthetase, glutamate dehydrogenase, and histidase of the mutant allele is recessive to the phenotype of the normally functioning $glnA^+$ gene. These findings support the hypothesis that glutamine synthetase regulates the expression of its structural gene, *glnA*.

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LITERATURE CITED

1. **Brenchley, J. E., M. J. Prival, and B. Magasanik.** 1973. Regulation of the enzymes responsible for the glutamate formation in *Klebsiella aerogenes*. J. Biol. Chem. 248:6122-6128.
2. **DeLeo, A. B., and B. Magasanik.** 1975. Identification of the structural gene for glutamine synthetase in *Klebsiella aerogenes*. J. Bacteriol. 121:313-319.
3. **Foor, F., Janssen, K. A., and Magasanik, B.** 1975. Regulation of synthesis of glutamine synthetase by adenylylated glutamine synthetase. Proc. Natl. Acad. Sci. U.S.A. 72:4844-4848.
4. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein determination with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
5. **Mecke, D., and H. Holzer.** 1966. Repression und Inaktivierung von Glutamine Synthetase in *Escherichia coli* durch NH_4^+. Biochim. Biophys. Acta 122:341-351.
6. **Prival, M. J., and B. Magasanik.** 1971. Resistance to catabolite repression of histidase and proline oxidase during nitrogen limited growth of *Klebsiella aerogenes*. J. Biol. Chem. 246:6288-6296.
7. **Prival, M. J., J. E. Brenchley, and B. Magasanik.** 1973. Glutamine synthetase and the regulation of histidase formation in *Klebsiella aerogenes*. J. Biol. Chem. 248:4334-4344.
8. **Streicher, S. L., R. A. Bender, and B. Magasanik.** 1975. Genetic control of glutamine synthetase in *Klebsiella aerogenes*. J. Bacteriol. 121:320-331.
9. **Tyler, B. M., A. B. DeLeo, and B. Magasanik.** 1974. Activation of transcription of *hut* DNA by glutamine synthetase. Proc. Natl. Acad. Sci. U.S.A. 71:225-229.