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Regulation of Glutaminase Levels in *Escherichia coli*

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Nitrogenous metabolites, cyclic adenosine 3':5'-monophosphate (cAMP), and the stage of culture growth all influence the levels of glutaminase A in *Escherichia coli*, but no variables in culture conditions alter the levels of glutaminase B. Growth of *E. coli* on culture media containing glucose and excess ammonia results in a rise in the level of glutaminase A as the cultures enter stationary phase; this rise is abolished by ammonia limitation. cAMP or glycerol reduce the level of glutaminase A. In mutants deficient in cAMP receptor protein, glutaminase A levels are unchanged by cAMP, but they are still susceptible to regulation by ammonia. We consider glutaminase B to be a constitutive enzyme, since its levels appear independent of nutritional conditions.

Since the amide nitrogen of glutamine is used in the synthesis of amino acids, amino sugars, nucleotides, and cofactors, the enzymes which synthesize and degrade glutamine play a central role in cellular nitrogen metabolism (23). In recent years there have been extensive studies of the enzyme glutamine synthetase which catalyzes the synthesis of glutamine.

L-glutamate + NH_3 + adenosine 5'-triphosphate → L-glutamine + adenosine 5'-diphosphate + P_i

L-glutamine + water → L-glutamate + NH_3

Escherichia coli contain two distinct enzymes which catalyze the hydrolytic deamidation of L-glutamine (8, 21). These isoenzymes have been designated glutaminases A and B. Since glutaminase A is active below pH 5 while glutaminase B is active above pH 7, the level of each enzyme can be readily determined in crude extracts.

Glutaminase A has been previously studied by Meister et al. (15) and more recently by Hartman (8, 9) and Varrichio (28). Their studies indicate the levels of glutaminase A can fluctuate with the stage of growth and the composition of the culture media. Our work has demonstrated that glutaminase A levels are regulated by cyclic adenosine 3':5'-monophosphate (cAMP) and nitrogenous metabolites (20).

Much less is known about the factors which regulate the levels of glutaminase B (8, 20). It is of considerable interest to examine the regulation of glutaminase B, since both glutaminase B and glutamine synthetase are active at neutral pH so that their actions are in direct opposition.

In this communication we report on the regulation of glutaminase A and B levels in *Escherichia coli* during various stages of growth and under several states of nutrition.

MATERIALS AND METHODS

Materials. cAMP was obtained from Sigma, St. Louis, Mo., and P-L Biochemicals, Milwaukee, Wis. The L-[U-^{14}C]glutamine was obtained from New England Nuclear and purified over Dowex 1-Cl. The remaining chemicals were of the purest grades commercially available.

Bacterial growth. *E. coli* B wild type was the gift of Martin Gellert. *E. coli* strain K-12-1100 and two mutant strains, -5333(*crp*) and -5336(*cya*), all requiring thiamine, were the gift of Ira Pastan (12, 20). All cultures were started from single colonies isolated on MacConkey agar. Both the *crp* and *cya* mutants were easily identified since they could not ferment lactose. The bacteria were grown in a liquid minimal media containing 1.7 mM $MgSO_4$, 43 mM NaCl, 14 mM K_2SO_4, 150 mM potassium phosphate, pH 7.4, 30 mM glucose, and 40 mM NH_4Cl, unless otherwise noted. Under these conditions the pH of the media did not drop below 6.7 during the growth. The cells were grown for four generations on minimal media and then a 1:10 inoculum was used for each 50-ml culture which was grown at 37 C in a 300-ml triple baffle Bellco side arm flask on a New Brunswick gyrotory shaker oscillating 100 times/min. Larger cultures of 200 ml were grown in 2,000-ml triple baffle Bellco flasks. Growth was measured using a Klett Summerson colorimeter equipped with a no. 66 filter. A galvanometric reading of 100 Klett units corresponded to an absorbancy of 0.200 as measured on a Gilford 240 spectrophotometer at 660 nm.

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The cells were harvested at three different times during growth: mid logarithmic, early stationary and late stationary. Early stationary phase and late stationary phase were defined as 2 to 3 h and 10 to 14 h, respectively, after logarithmic growth had ceased.

The generation time was altered by the type of nitrogen source used and not by the concentration of the nitrogen source (Table 1). *E. coli* B cells had generation times of 0.9, 2.0, and 4.0 h when growing in a glucose-minimal salts medium containing NH_4Cl, glutamine, or glutamate, respectively. The maximum culture density observed in the stationary phase of growth was dependent upon the level of nitrogen source provided. Cultures containing 4 mM NH_4Cl exhibited a maximum Klett value of 130 in stationary phase, whereas cultures containing 40 mM NH_4Cl showed a value of 240. Addition of NH_4Cl to stationary phase cultures grown on 4 mM NH_4Cl resulted in further growth, whereas addition of NH_4Cl to those grown on 40 mM NH_4Cl did not result in further growth. Clearly, the maximum cell density in the 4 mM NH_4Cl cultures was limited by the availability of NH_4Cl but no conclusions can be drawn about the availability of NH_4^+ during the logarithmic phase of growth. Cultures grown in 10 mM glutamine or glutamate showed a maximum cell density in stationary phase of 210 Klett units, whereas cultures grown in 100 mM glutamine or glutamate exhibited Klett values of 310 and 300, respectively.

Harvest was accomplished by cooling the bacterial culture with ice and sedimenting cells by centrifugation at 10,000 × *g* for 5 min at 4 C. The cells were washed with distilled water, recentrifuged, and frozen in liquid nitrogen.

Preparation of extracts. The frozen cells were later thawed in a 37 C water bath with 1 ml of 10 mM imidazole-chloride, pH 7.1, and sonicated with a Branson sonifier equipped with a microtip. The cells were given three 15-s periods of sonication. During each period the temperature was maintained at less than 17 C. The extracts were centrifuged at 45,000 × *g* for 30 min at 4 C and the supernatants (5 to 20 mg of protein/ml) were assayed for enzymatic activities.

Assays. Glutaminases A (pH 5) and B (pH 7) were assayed by measuring the [^{14}C]glutamine formed from L-(U-^{14}C)glutamine as described previously (20, 21). Identical results were obtained when glutaminases A and B were assayed measuring the NH_3 produced as described in another communication (20).

Asparaginases I and II were assayed by measuring the [^{14}C]aspartate-formed L-[U-^{14}C] asparagine (21). The [^{14}C]aspartate was isolated by chromatography on Dowex-1-Cl and the radioactivity was measured in Bray solution with a Beckman LS 250 liquid scintillation counter. The asparaginase I assay solution (0.1 ml) contained 30 mM L-asparagine, specific activity 0.1 mCi/mmol, and 50 mM tris(hydroxymethyl)aminomethane-chloride, pH 7.8. The asparaginase II assay solution (0.1 ml) contained 0.1 mM L-asparagine and 50 mM tris(hydroxymethyl)aminomethane-chloride pH 7.8 (26). The blanks contained no enzyme and all samples were incubated at 37 C for 5 min.

Protein was determined by the biuret method, with crystalline bovine serum albumin as standard (6).

TABLE 1. *Dependence of generation times and extent of growth on nitrogen sources for Escherichia coli B*

Nitrogen source[a]	Concn (mM)	Generation time (h)	Maximum cell density in stationary phase (Klett units)
NH_4Cl	4	0.9	130
	40	0.9	240
Glutamine	10	2.0	210
	100	2.0	310
Glutamate	10	4.0	210
	100	4.0	300

[a] Culture medium: 11 mM glucose and minimal salts. See Materials and Methods for details.

RESULTS

Effect of nitrogen supply on levels of glutaminases A and B. The regulation of glutaminase levels in *E. coli* was studied by examining the influence of nutritional conditions and of culture growth. Extensive studies with glutamine synthetase from *E. coli* indicated that nitrogen limitation resulted in derepression of enzyme synthesis and a low state of adenylylation, whereas nitrogen excess resulted in a repression of enzyme synthesis and a high state of adenylylation (27, 30). These extremes of nitrogen metabolism were examined using low and high levels of ammonium chloride as the nitrogen source.

The level of glutaminase A is markedly changed by the stage of growth and the concentration of ammonia (Table 2). The level of glutaminase A increases 10-fold as the organisms enter stationary phase if the culture

TABLE 2. *Effects of growth and ammonia on the levels of glutaminases A and B in Escherichia coli B*[a]

Enzyme	NH_4Cl in culture media[b] (mM)	Stage of growth		
		Logarithmic (units/mg)	Early stationary (units/mg)	Late stationary (units/mg)
Glutaminase A	40	0.038	0.387	0.468
	4	0.042	0.060	0.067
Glutaminase B	40	0.070	0.065	0.061
	4	0.067	0.079	0.053

[a] One unit of enzyme is the amount that catalyzes conversion of 1.0 μmol of substrate to product per minute under standard assay conditions.

[b] Culture medium: 11 mM glucose and minimal salts.

contains 40 mM NH_4Cl and 11 mM glucose. There was a small but significant increase in the level of glutaminase A later in stationary phase as evidenced by the 20% higher value in late stationary cultures compared to early stationary cultures. When the concentration of NH_4Cl is reduced to 4 mM, the level of glutaminase A is low and no increase is seen as the bacteria enter stationary phase. The level of glutaminase B is not altered by the stage of growth or the level of ammonia. Nitrogen limitation in cultures containing 4 mM NH_4Cl was demonstrated by an increase in optical density of the cultures upon the addition of more NH_4Cl. Addition of NH_4Cl to the cultures containing 40 mM NH_4Cl did not result in an increase in the optical density of the cultures.

The levels of glutaminases A and B extracted from cultures grown on glutamate or glutamine as nitrogen sources are depicted in Table 3. The level of glutaminase A during logarithmic growth is approximately fivefold higher with L-glutamate compared to NH_4Cl (Table 2). As the organisms entered stationary phase, the level of glutaminase A rose 30% in the 100 mM glutamate cultures, but did not change in the 10 mM glutamate cultures. The stationary phase glutaminase A levels for organisms grown on high or low concentrations of L-glutamate lie between the values observed for glutaminase A in *E. coli* grown on high and low concentrations of NH_4Cl (Table 2).

Growth on L-glutamine showed a different pattern of control for glutaminase A. The levels of glutaminase A during logarithmic growth were two- to threefold higher with L-glutamate. As the organisms which were growing on 100 mM L-glutamine entered stationary phase the levels of glutaminase A increased 10-fold and continued to rise slightly. A fivefold increase in glutaminase A was observed with a lower level of L-glutamine (10 mM). The highest levels of glutaminase A were found during stationary phase with growth on glutamine. These values represent a 30-fold increase over the levels of glutaminase A found in *E. coli* logarithmically growing on NH_4Cl. As illustrated, the levels of glutaminase B were unaffected by L-glutamate and L-glutamine in the culture media.

Logarithmic growth on NH_4Cl + glutamate as the nitrogen sources gave a level of glutaminase A equal to that observed with NH_4Cl alone, and in stationary phase the levels of glutaminase A increased approximately fivefold to that observed with glutamate alone.

Effect of cAMP on levels of glutaminases A and B. Since glutaminases A and B probably function as catabolic enzymes, we investigated the influence of cAMP on the levels of these enzymes. Extensive studies on the function and mechanism of action of cAMP have revealed an elegant system of generalized control for catabolic enzyme synthesis in microorganisms (3, 18). The addition of 5 mM cAMP to culture media with 40 mM NH_4Cl reduced the level of glutaminase A 40 to 50% in logarithmic phase cells and approximately 80% in stationary phase cells (Table 4). *E. coli* grown on 6 mM NH_4Cl showed low levels of glutaminase A as expected and these levels were further diminished 50 to 60% by cAMP during all stages of growth. cAMP had no effect on the levels of glutaminase B.

In another communication (20) we have reported that 3.6 mM cAMP gave a half-maximal decrease in the level of glutaminase A and more than 60% of this decrease occurred within one generation. In addition, mixing experiments gave expected average values suggesting that inhibition of the enzyme was not an explanation for the effect of cAMP. The diminution of

TABLE 3. *Effects of glutamate and glutamine on the levels of glutaminases A and B in Escherichia coli B*

Enzyme	Nitrogen source[a] (mM)		Stage of growth		
			Logarithmic (units/mg)	Early stationary (units/mg)	Late stationary (units/mg)
Glutaminase A	L-Glutamate	(100)	0.183	0.301	0.271
		(10)	0.193	0.176	0.199
	L-Glutamine	(100)	0.122	1.357	1.592
		(10)	0.072	0.332	0.677
Glutaminase B	L-Glutamate	(100)	0.105	0.080	0.120
		(10)	0.066	0.062	0.070
	L-Glutamine	(100)	0.068	0.071	0.067
		(10)	0.090	0.072	0.089

[a] Culture medium: 11 mM glucose and minimal salts.

TABLE 4. *Glutaminase levels in Escherichia coli K-12-1100: effects of growth and CAMP*

Enzyme	NH_4Cl[a] (mM)	cAMP (mM)	Stage of growth		
			Logarithmic (units/mg)	Early stationary (units/mg)	Late stationary (units/mg)
Glutaminase A	40	0	0.078	0.466	0.531
		5	0.049	0.103	0.198
	6	0	0.076	0.075	0.093
		5	0.039	0.028	0.047
Glutaminase B	40	0	0.058	0.060	0.054
		5	0.059	0.056	0.051
	6	0	0.080	0.073	0.065
		5	0.076	0.068	0.067

[a] Culture media contained 11 mM glucose and minimal salts.

glutaminase A levels by cAMP was not observed with other nucleotides and was prevented by chloramphenicol.

Because *E. coli* cells grown on glycerol have an intracellular cAMP content approximately 100 times higher than those grown on glucose (12), glutaminase levels in cells grown on glycerol or glucose in the presence and absence of cAMP were compared (Table 5). cAMP had no effect on the level of glutaminase A in cells grown on glycerol plus excess ammonia but decreased the level nearly 40% in cells grown on glycerol plus limiting ammonia. In general, the level of glutaminase A in cells grown on glycerol was equal to that found in cells grown on glucose + cAMP irrespective of the ammonia concentration. Neither glycerol nor cAMP altered the levels of glutaminase B.

E. coli K-12 deficient in adenyl cyclase have low levels of endogenous cyclic AMP (19). The levels of glutaminase A in these cells grown on excess and limiting ammonia were similar to those observed in wild-type *E. coli* at all three stages of growth that were examined. Addition of cAMP to the culture media reduced glutaminase A levels 60 to 80% during all stages of growth (Table 6). The levels of glutaminase B in the adenyl cyclase mutants were not influenced by cAMP. Thus, the effects of cAMP on glutaminase A and B levels in these adenyl cyclase-deficient mutants were similar to those observed with wild-type *E. coli*.

cAMP has been shown to bind to cAMP receptor protein (CRP) which regulates the initiation of messenger ribonucleic acid transcription by ribonucleic acid polymerase at the promoter region (3). The glutaminase levels of mutants deficient in CRP were examined as shown in Table 7. The levels of glutaminase A in the CRP mutants were not altered by the addition of cAMP to the culture media. During logarithmic growth the glutaminase A levels in *crp* mutants were fourfold greater than those in wild-type *E. coli*. But in wild-type organisms, a rise in glutaminase A levels was observed when the *crp* mutants entered stationary phase in the presence of excess ammonia. Growth on limiting ammonia prevented this increase of glutaminase A in *crp* mutants as they entered stationary phase. Throughout stationary phase the levels of glutaminase A in the *crp* mutant were consistently greater than those observed for wild-type and adenyl cyclase-deficient *E. coli*. Again, glutaminase B was unaltered by cAMP.

TABLE 5. *Effects of glycerol and cAMP on the levels of glutaminases A and B in Escherichia coli K-12-1100*[a]

cAMP (mM)	Carbon source	NH_4Cl (mM)	Glutaminase A (units/mg)	Glutaminase B (units/mg)
0	Glycerol	40	0.091	0.063
5	(60 mM)	40	0.100	0.075
0	Glucose	40	0.466	0.060
5	(30 mM)	40	0.103	0.056
0	Glycerol	6	0.032	
5	(60 mM)	6	0.018	
0	Glucose	6	0.075	
5	(30 mM)	6	0.028	

[a] Cells were grown on a minimal salts medium and harvested in early stationary phase.

Effect of other factors on levels of glutaminases A and B. The experiments presented above indicate that the glutaminase levels in *E. coli* are subject to regulation by nitrogenous metabolites and cAMP, whereas glutaminase B levels are unchanged. As part of an extensive search to find growth conditions which would alter the level of glutaminase B, the effect of

TABLE 6. *Glutaminase levels in Escherichia coli K-12-5336: the effects of growth and cAMP on a mutant deficient in adenylate cyclase*[a]

Enzyme	NH_4Cl (mM)	cAMP (mM)	Stage of growth		
			Logarithmic (units/mg)	Early stationary (units/mg)	Late stationary (units/mg)
Glutaminase A	40	0	0.132	0.482	0.562
		5	0.049	0.090	0.216
	6	0	0.133	0.161	0.140
		5	0.044	0.044	0.027
Glutaminase B	40	0	0.056	0.064	0.064
		5	0.068	0.066	0.049
	6	0	0.078	0.078	0.054
		5	0.056	0.066	0.048

[a] Culture media contained 11 mM glucose and minimal salts.

TABLE 7. *Glutaminase levels in Escherichia coli K-12-5333: the effects of growth and cAMP on a mutant deficient in cAMP receptor protein*[a]

Enzyme	NH_4Cl (mM)	cAMP (mM)	Stage of growth		
			Logarithmic (units/mg)	Early stationary (units/mg)	Late stationary (units/mg)
Glutaminase A	40	0	0.359	0.661	0.659
		5	0.330	0.696	0.700
	6	0	0.322	0.327	0.392
		5	0.359	0.317	0.322
Glutaminase B	40	0	0.085	0.091	0.099
		5	0.073	0.079	0.108
	6	0	0.072	0.052	0.074
		5	0.082	0.065	0.078

[a] Culture media contained 11 mM glucose and minimal salts.

anaerobiosis was examined on the levels of glutaminases A and B. The level of asparaginase II in *E. coli* has been shown to rapidly increase during anaerobiosis, but the mechanism of this increase is unknown (2). Asparaginase I and glutaminases A and B were unchanged (Table 8).

Table 9 summarizes a considerable number of culture conditions which were tested for their influence on glutaminase B levels. None of the nine carbon sources or five nitrogen sources listed had any effect on the level of glutaminase B. Varying the phosphate, chloride, iron, potassium, sodium, and trace metal content of the culture media also had no effect; and thiamine and various nucleotides did not produce any change in the level of glutaminase B. Aeration, pH, temperature, and stage of growth did not alter glutaminase B levels. Since glutaminase B levels appear to be independent of the variables listed above, we conclude that the enzyme is probably constitutive (16).

TABLE 8. *Effects of anaerobiosis on glutaminase and asparaginase levels in Escherichia coli B*[a]

Condition	Enzyme level			
	Glutaminase A (units/mg)	Glutaminase B (units/mg)	Asparaginase I (units/mg)	Asparaginase II (units/mg)
Aerobiosis	0.033	0.062	0.041	0.00045
Anaerobiosis	0.036	0.065	0.040	0.010

[a] Culture media contained 40 mM NH_4Cl, 11 mM glucose, and minimal salts. Logarithmically growing cells were subjected to anaerobiosis by turning off the gyrotory shaker 1 h before harvest.

In addition, glutaminase B levels could not be altered by adaptation or mutagenesis of the bac-

TABLE 9. *Independence of glutaminase B levels in Escherichia coli B from variations in culture conditions*[a]

Variables examined			
Carbon sources		pH	
Glucose[b]	(10–30)	5.8–8.0	
Gluconate	(15)	Aeration	
Glycerol	(6–40)	Aerobiosis	
Succinate	(20–40)	Anaerobiosis	
α-Ketoglutarate	(5–50)	Temperature	
L-Glutamate	(10–100)	30 C	
L-Glutamine	(10–100)	37 C	
L-Aspartate	(10–100)	Stage of growth	
L-Asparagine	(10–100)	Logarithmic	
Nitrogen sources		Early stationary	
Ammonia	(4–100)	Late stationary	
L-Glutamate	(10–100)		
L-Glutamine	(10–100)		
L-Aspartate	(10–100)		
L-Asparagine	(10–100)		
Inorganic elements			
Phosphate	(10–150)		
Chloride	(10–100)		
Iron	(5 mg/ml)		
Potassium	(10–100)		
Sodium	(10–100)		
Trace metals[c]			
Organic compounds[d]			
Thiamine	(10 mg/ml)		
3′:5′-cAMP	(0.1–10)		
ATP	(5, 10)		
ADP	(5, 10)		
5′-AMP	(5, 10)		
3′-AMP	(5, 10)		
3′:5′-cGMP	(5, 10)		

[a] Glutaminase B levels were unchanged when various components of the culture media were tested over the ranges noted above.

[b] Concentration range measured in millimolar units.

[c] Trace metal mixture contained 18 μM $CaCl_2$, 248 μM $FeCl_2$, 252 μM $ZnSO_4$, 256 μM $CuSO_4$, 304 μM $CoCl_2$, and 216 μM NaEDTA (ethylenediaminetetraacetic acid).

[d] ADP, adenosine 5′-diphosphate; cGMP, cyclic guanosine 3′:5′-monophosphate.

teria. *E. coli* which were adapted to grow on L-glutamine as the sole source of carbon and nitrogen showed no alteration of glutaminase B levels.

DISCUSSION

The regulation of glutaminase levels in *E. coli* appears to be somewhat different from that observed for many bacterial enzymes. Growth on excess ammonia permits glutaminase A to rise abruptly as the organisms enter stationary phase, whereas ammonia limitation prevents this rise. During logarithmic growth, NH_4Cl, irrespective of concentration, diminished the level of glutaminase A to a greater extent than was observed with other nitrogen sources such as glutamate and glutamine. These observations are similar to those of Varrichio, who also found that logarithmic growth on glutamate or proline produces high levels of glutaminase A which are prevented by chloramphenicol, whereas growth on glutamine results in low levels of the enzyme (28). Our work shows that growth on glutamine results in intermediate levels of glutaminase A during logarithmic phase and very high levels during stationary phase. The rise in glutaminase A levels as the organisms enter stationary phase (8) appears more complex than simply derepression or induction and undoubtedly involves modulation of both synthesis and degradation of enzyme (5, 29).

Control of glutaminase levels in other organisms by nitrogenous metabolites has been reported. In *Pseudomonas* an enzyme with both glutaminase and asparaginase activity is repressed by NH_4Cl, aspartate, and glutamate and is increased by asparagine and glutamine in the culture media (13). A similar enzyme with both activities in *Achromobacteraceae* is induced by glutamate (24). Induction of glutaminases which are not associated with asparaginase activity in *S. cerevisiae* (1) and *Clostridium welchii* (11) by nitrogenous metabolites was not demonstrable. Glutaminase activities in *Pseudomonas* (14) and *S. cerevisiae* (24) rose early during logarithmic growth and were relatively constant during stationary phase. In contrast, the glutaminase of *C. welchii*, like glutaminase A from *E. coli*, increased markedly as the organisms entered stationary phase (8, 11). It is noteworthy that in the glutaminase from *C. welchii* (11) the pH of the culture media could be maintained at 7 throughout growth without altering the regulation of glutaminase levels. Likewise, we observed that the rise of glutaminase A associated with entrance of the organism into stationary phase was not altered by varying the pH from 5.8 to 8. Hartman found that glutaminase A synthesis was increased twofold by allowing the pH of the culture medium to drop from 6.5 to 5.3 as the organisms enter stationary phase (9).

Besides control by nitrogenous metabolites and the stage of growth, glutaminase A levels are also regulated by cAMP. In contrast to enzymes which are under catabolite repression (18), glutaminase A levels are increased by

growth on glucose and decreased by cAMP and glycerol. We have recently shown that glutamate synthetase levels in *E. coli* are also decreased by cAMP while glutamate dehydrogenase and glutamine synthetase are increased (28). The molecular mechanism of negative control by cAMP into microorganisms is unknown but it is clear that this system of negative control requires CRP. Although cAMP did not influence the level of glutaminase A in *crp* mutants, NH_4Cl did alter glutaminase A levels in a manner similar to that observed for wild-type bacteria. These observations provide evidence for two distinct and separable systems which control glutaminase A levels in *E. coli*.

The regulatory patterns discussed above for glutaminase A appear strikingly similar to some of those observed for glutamate decarboxylase in *E. coli* (7). Both enzymes have pH optima of 5, both appear in the stationary phase of growth, and both are regulated by the carbon and nitrogen sources in culture media. Like glutaminase A, glucose increases the level of glutamate decarboxylase and glycerol decreases the level. Indeed, these observations would predict that glutamate decarboxylase may be a third enzyme in *E. coli* under negative control by cAMP. In addition, the levels of both glutaminase A and glutamate decarboxylase are increased by growth on glutamate. One difference in the regulation of these enzymes is the 10-fold increase in glutamate decarboxylase which is observed when the pH of the culture media was dropped from 6.8 to 5.8, whereas glutaminase A levels did not fluctuate significantly over this pH range. It is of considerable interest to know if these two enzymes are coordinately controlled since they might possibly function together in the conversion of glutamine to γ-aminobutyrate during stationary phase. The precise metabolic role of glutaminase A remains to be established.

In contrast to the 50-fold fluctuation of glutaminase A brought about by nitrogenous metabolites and cAMP, no growth conditions were found which influenced the level of glutaminase B. Since glutaminase B levels appear independent of the nutritional conditions of the culture media, the enzyme is said to be constitutive (17). The labeling of glutaminase B as constitutive is precarious because the possibility always exists that there is a set of nutritional conditions which alters its level. Models for the control of constitutive enzyme synthesis include: a steady balance between inducers and repressors, the absence of repressor production, or the presence of only a structural gene with a "promotor" for the initiation of transcription (4, 17). In the case of glutaminase B we have no information as to the mechanism of constitutivity.

Recent experimental data on the regulation of glutaminase B activity and on the fluctuation of metabolite levels in *E. coli* suggest that constitutive control of glutaminase B may be very advantageous (10, 22, 25, 30). When NH_4Cl was added to *E. coli* growing on a glucose-proline media, the ammonia was rapidly incorporated into L-glutamine resulting in a 20-fold increase of the amino acid within 1 min; concomitantly, intracellular adenosine 5'-triphosphate (ATP) levels decreased 90% (25). Subsequently, the glutamine level returned to normal over the next 5 min and glutamate and ATP levels increased. The restoration of homeostasis after the near lethal exhaustion of ATP stores may be explained in part by the turning on of glutaminase B which is inhibited by ATP and activated by AMP. The deamidation of glutamine provides glutamate for ATP generation in the tricarboxylic acid cycle and turns off glutamine-dependent biosynthetic pathways which consume ATP. The rapidity of these events and the exhaustion of ATP stores make an inducible glutaminase B unfeasible in this situation and stress the importance of constitutive enzymes.

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