INDEPENDENT GENETIC REGULATION OF GLUTAMINE SYNTHETASE AND ITS INACTIVATING (ADENYLATING) ENZYME IN *E. COLI*

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1. Introduction

Recent reports from this laboratory have described a rapid, enzyme mediated inactivation of glutamine synthetase in *Escherichia coli* B [1–3]. The inactivation occurs when cells are exposed to 10^{-4} M NH_4^+ ions or 10^{-3} M glutamine. *In vitro*, only glutamine and some analogues can initiate the inactivation process [4]. The inactivating enzyme catalyzes an ATP-dependent adenyllylation of active glutamine synthetase [5, 6]. Reactivation of glutamine synthetase is caused by metabolite controlled, enzymatic deadenylylation [7–9]. In addition to enzymatic inactivation and reactivation, glutamine synthetase is also regulated by repression of its synthesis. Repression appears when *E. coli* are grown on media containing NH_4^+ ions [1, 10]. Very probably glutamine, formed from ammonia, is the intracellular corepressor. The question whether the synthesis of the inactivating, i.e. adenylylating, enzyme is genetically controlled together with, or independently of glutamine synthetase, is the subject of this report.

2. Results and discussion

A mutant of *E. coli* K12 (obtained from Dr. F. Lingens) was shown to be lacking in glutamine synthetase by its inability to grow in media containing 0.5% nutrient broth; or M63 [11] media containing ammonium sulfate plus 10^{-3} M of one of the following amino acids: asparagine, aspartate, alanine, glutamate. However, if media were supplemented with 10^{-3} M glutamine, the cells grew.

Table 1
Activities of glutamine synthetase and glutamine synthetase inactivating enzyme in *E. coli* K12 wild type and glutamine auxotroph mutant.

	Wild type		Mutant	
NH_4^+ added to the growth medium	10^{-3} M	6×10^{-2} M	10^{-3} M	6×10^{-2} M
Glutamine synthetase	35	5	<2	<2
Inactivating enzyme	2.5	2.8	3.0	3.6

The mutant and the parent K12 strain from which it was derived were grown in M 63 media [11] containing 0.5% glycerol, 2×10^{-3} M glutamine and NH_4^+ ions as indicated. Glutamine was sterilized by millipore filtration. NH_4^+ ions were added as $(NH_4)_2SO_4$. Cultures were grown to early or mid-log phase and extracts were prepared as previously described [1]. Glutamine synthetase was assayed with the γ-glutamyl-NH_2OH-transferase test as described by Mecke et al. [1]. Inactivating enzyme was determined as described by Wulff et al. [3]. One unit equals 10% inactivation per mg protein. The data given represent the average from 2 experiments.

Table 1 shows the glutamine synthetase activity (as measured by the γ-glutamyl transferase test [1], and the activity of the inactivating enzyme of the K12 parent strain and the mutant after growth on high and low ammonium plus glutamine. In the presence of low concentrations of NH_4^+ ions the wild type cells contain high activities of glutamine synthetase, i.e. transferase. In agreement with previous results [1] high concentrations of NH_4^+ ions repress the synthesis of glutamine synthetase and therefore only low

activities are observed. In the mutant no glutamine synthetase activity as measured by the transferase test can be detected in cells grown on media containing either high or low concentrations of NH_4^+ ions. Malic dehydrogenase, glutamate dehydrogenase (NADP), and glutamate oxalacetic transaminase were assayed for control purposes. The control enzyme activities of both the mutant and the wild type were not appreciably affected by the different growth conditions.

With both strains the activities of the inactivating enzyme found were essentially the same after growth on high and low ammonium concentrations – conditions which result in greatly different levels of glutamine synthetase (table 1). It would thus appear that the synthesis of the inactivating enzyme is not affected by the nitrogen source in the medium. Therefore, inactivation can take place whenever the concentration of the initiating metabolite, presumably glutamine, becomes high enough. The rate of inactivation is variable, depending on the concentration of the initiating metabolite and of inhibiting metabolites competing with glutamine [12].

The second important point is that the levels of inactivating enzyme in the mutant strain were not significantly different from those in the wild strain. This shows that the mutation causing loss of glutamine synthetase activity had no effect on synthesis and activity of inactivating enzyme. The inactivating enzyme must therefore be genetically independent from glutamine synthetase. This could mean control by different genes, or perhaps the synthesis of the inactivating enzyme is not controlled in any manner, and its activity is controlled only by glutamine and other allosteric effectors.

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