CHARACTERIZATION OF A GLUTAMINE SYNTHETASE INACTIVATING ENZYME FROM ESCHERICHIA COLI [+)]

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In E. coli, the synthesis of glutamine synthetase (E.C. 6.3.1.2.) is repressed when the cells are grown in a medium containing NH_4^+ ions (3,6). Addition of NH_4^+ to derepressed cells in vivo does not only cause repression but also a rapid irreversible inactivation of the enzyme (3). This inactivation can also be shown in cell free crude extracts from E. coli where glutamine synthetase is rapidly inactivated in the presence of glutamine, ATP, and Mg^{2+} (4). Therefore, the inactivation in intact cells by NH_4^+ might be provoked by the formation of glutamine which, in the presence of ATP and Mg^{2+}, causes inactivation. Since only the glutamine synthetase activity is inactivated whereas most of the glutamyl transferase activity of the enzyme remains unchanged, only part of the catalytic site is altered.

In this paper it will be shown that the inactivation of glutamine synthetase not only requires ATP, Mg^{2+}, and glutamine, but also the presence of an enzyme. It is proposed that this enzyme be called "glutamine synthetase inactivating enzyme".

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Methods. The conditions of growth, the preparation of extracts, the determination of enzyme activities, and the definition of units are described elsewhere (3). The activity of the inactivating enzyme was determined as follows: in a total volume of 1.0 ml were incubated at 37° 0.001 M glutamine, 0.01 M ATP, 0.05 M Mg^{2+}, 250 units of glutamine synthetase, and the enzyme solution. The reaction was started by the addition of ATP. After 5, 10, and 15 min samples of 0.1 ml were assayed for glutamine synthetase.

One unit of the inactivating enzyme is defined as the amount which inactivates 20% of the glutamine synthetase activity in 10 minutes under the conditions chosen.

Results. The previous experiments concerning the inactivation of glutamine synthetase by ATP, Mg^{2+}, and glutamine were performed with crude extracts or partially purified enzyme (4). Now, we have purified glutamine synthetase by a procedure outlined by Woolfolk and Stadtman (6). About 50-fold purification from derepressed cells was obtained as shown in Table I. Precipitation with acetone or ammonium sulfate, chromatography on DEAE-cellulose, as well as centrifugation in sucrose gradients gave no further increase of the specific activity. The enzyme showed a single peak in the analytical ultracentrifuge. Compared with extracts from repressed cells, the specific activity of the enzyme had increased about 500-fold.

In contrast to the experiments with crude extracts, incubation of purified glutamine synthetase with ATP, Mg^{2+}, and glutamine did not lead to any inactivation (see Fig. 1). Therefore, a factor which is required for the inactivation had been eliminated during the purification procedure. This

Table I

Purification of glutamine synthetase from E. coli.

Procedure	specific activity	total units	ratio Transferase/ Synthetase	purification
crude extract	158	197000	5,0	1
streptomycine supernatant	160	181000	-	1
acid fraction I (pH 7.0-5.0)	16	2200	-	-
acid fraction II (pH 5.0-4.4)	1570	160000	6.8	10
heat denaturation	7800	234000	7.3	49

Growth conditions and preparation of crude extract as described by Mecke and Holzer (3) were used, except 0.028 M D-glucose was used as carbon source and 0.015 M L-glutamate as nitrogen source. 300 ml of crude extract were precipitated with 75 ml 0.074 M streptomycine sulfate. The supernatant (pH 7.0) was adjusted to pH 5.0 with 0.1 N acetic acid and the precipitate ("acid fraction I") centrifuged. The supernatant was then adjusted to pH 4.4. The precipitate ("acid fraction II") containing the enzyme was dissolved in 20 ml of 0.1 M Tris/HCl buffer pH 8.0, mixed with 2.0 ml 0.1 M $MgSO_4$ and heated for 10 minutes at 60°.

factor is a component of the protein fraction which precipitates between pH 7.0 and 5.0 ("acid fraction I"). Addition of this fraction which was free of glutamine synthetase to the incubation mixture, caused a rapid inactivation of glutamine synthetase. Acid fraction I does not inactivate glutamine synthetase without ATP or Mg^{2+} or glutamine. The inactivating activity was lost after heating the fraction for 5 min at 100°. Dialysis did not change the activity of the inactivating component.

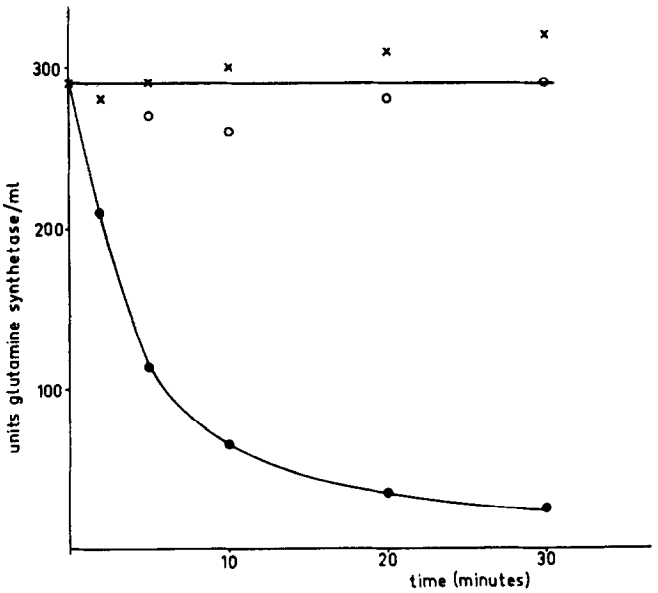

Figure 1: Inactivation of purified glutamine synthetase. Incubation mixture (total volume 1.0 ml; 37°): 290 units glutamine synthetase, 0.001 M glutamine, 0.01 M ATP, 0.05 M Mg^{2+}. x—x no further addition; ●—● addition of 0.45 ml "acid fraction I" (see legend to Table I); o—o addition of 0.45 ml heated "acid fraction I". The reaction was started with ATP. The glutamine synthetase was assayed in samples of 0.1 ml.

The initial velocity of the inactivation is nearly proportional to the amount of the inactivating fraction. Using this property as an assay the inactivating factor was fractionated. Precipitation with acid followed by precipitation with ammonium sulfate gave a 9-fold purification, as shown in Table II. The behavior of the inactivating component during the purification, heat treatment and dialysis shows that this component is an enzyme protein. This enzyme catalyzes the reaction:

$$\text{glutamine synthetase a} \xrightarrow[\text{ATP, } Mg^{2+}\text{, glutamine}]{\text{inactivating enzyme}} \text{glutamine synthetase b}$$

Following the nomenclature in the case of glycogen phospho-

Table II

Purification of the inactivating enzyme from E. coli.

Procedure	specific activity	total units	yield %
crude extract	4	22100	100
streptomycine supernatant	-	-	-
"acid fraction I" pH 7.0 - 5.0	13	8500	38
Ammoniumsulfate fractionation 32.5-37.5% saturation	35	1400	7

"Acid fraction I" (see legend to Table I), dissolved in 20 ml of 0.1 M Tris-buffer, was fractionated with ammonium sulfate. The inactivating enzyme precipitates between 30 and 40% saturation with a yield of 25%. The highest specific activity was obtained between 32.5 and 37.5% saturation. The precipitate was dissolved in 2.5 ml of 0.01 M Tris/HCl buffer pH 7.6 and dialyzed over night against 5 l of the same buffer.

rylase, glutamine synthetase a and glutamine synthetase b designate the active and the inactive glutamine synthetase, respectively.

When a mixture of inactivating enzyme, ATP, Mg^{2+}, and glutamine was incubated for 1 hour and then heated or precipitated by acid, it contained no inactivating activity (Table III). Therefore, the inactivating enzyme does not catalyze the formation of an inactivating metabolite in the presence of ATP, Mg^{2+}, and glutamine. Probably the inactivating enzyme acts directly at the surface of the glutamine synthetase, which becomes irreversibly changed. An acylation, amination or phosphorylation of a group at the catalytic site of the glutamine synthetase might be considered as possible mechanism of inactivation.

Table III

Action of a deproteinized inactivation mixture on glutamine synthetase.

Sample number and deproteinization	Glutamine synthetase (units)	inactivating enzyme (units)	inactivation after 20 min (%)
(1) heat	250	-	< 5
(2) heat	250	10	80
(3) $HClO_4$	250	-	< 5
(4) $HClO_4$	250	10	30

80 units of inactivating enzyme were incubated for 1 hour at 37° with 0.001 M glutamine, 0.01 M ATP, 0.05 M Mg^{2+} (total volume 2.0 ml). Samples (1) and (2) were deproteinized by heating for 10 minutes at 100°; samples (3) and (4) were precipitated with $HClO_4$, the supernatant neutralized with KOH and centrifuged. 0.3 ml samples were incubated (total volume 0.4 ml) with glutamine synthetase (purified as described in Table I) and inactivating enzyme (purified as described in Table II).

The "metabolite-induced enzymatic inactivation" of the glutamine synthetase resembles the enzymatic inactivation of glycogen phosphorylase (1,2). There is also evidence that the activity of phosphofructokinase from yeast is regulated by enzymatic inactivation and reactivation (5). The mechanism of the inactivating reaction, the isolation and characterization of the inactivated glutamine synthetase and the enzymatic reactivation are under further investigation.

Summary. Glutamine synthetase from Escherichia coli is inactivated by an enzyme, which was purified 9-fold. In addition to the inactivating enzyme glutamine as well as ATP and Mg^{2+} are necessary for the inactivation.

References

(1) Cori, G. T., and A. A. Green, J. Biol. Chem. 151, 31 (1943).

(2) Krebs, E. G., and E. H. Fischer, Advanc. Enzymol. 24, 263 (1962).

(3) Mecke, D., and H. Holzer, Biochim. Biophys. Acta in press.

(4) Mecke, D., K. Wulff, and H. Holzer submitted to Biochim. Biophys. Acta.

(5) Vinuela, E., M. L. Salas, M. Salas, and A. Sols, Biochem. Biophys. Res. Commun. 15, 243 (1964).

(6) Woolfolk, C. A., and E. R. Stadtman, Biochem. Biophys. Res. Commun. 17, 313 (1964).