# MECHANISM OF THE ENZYMATIC INACTIVATION OF GLUTAMINE SYNTHETASE FROM E.COLI [+)]

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In previous papers (1,2) it has been shown that purified glutamine synthetase (GS) from E.coli is enzymatically inactivated according to the equation:

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

The inactivating enzyme as well as the active form (GSa) and the inactive form (GSb) of glutamine synthetase have been purified (1,3). In contrast to the different activity in the glutamine synthetase reaction other properties of GSa and GSb are not different. These include: γ-glutamyl transferase activity (2), sedimentation in the ultracentrifuge, behavior in gel filtration, ion exchange chromatography, and electrophoresis on polyacrylamide and agar-gel (3). The following mechanisms have been proposed for the inactivating reaction (3,4): glutamylation, amidation, phosphorylation or adenylation of the enzyme, proteolytic elimination of amino acids or peptides, conformational changes. In this paper evidence is presented for the binding of the adenine part of ATP to

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glutamine synthetase. This binding is catalyzed by the inactivating enzyme in the presence of ATP, $Mg^{2+}$ and glutamine.

Material and Methods. $^{14}C$-ATP was purchased from Schwarz Bioresearch, Orangeburg, N.Y. (Catalogue No.1422-06; Lot No.6701; spec.act. 90 mC/mmole). All other chemicals were analytical grade. The enzymes were isolated from E.coli B. Growth conditions and the determination of enzyme activities are described elsewhere (1,5). Glutamine synthetase was purified according to Liess (3). The inactivating enzyme was purified as described earlier (1). Unless otherwise stated, radioactivity was determined according to Mans and Novelli (6). Protein was determined by the biuret method (7). All enzyme preparations were concentrated by precipitation with ammonium sulfate (60% sat.) and dialyzed for 12 hours. All incubations were done at 37°. Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer model 3003.

Results. ORD-measurements as well as measurements with the "fluorescent probe" (8) showed no difference between GSa and GSb suggesting a similar conformation of the two proteins. The ultraviolet absorption spectra of GSa and GSb however, are different. The maximum in the region of 260 nm as well as the minimum in the region of 240 nm are shifted to shorter wave lengths for GSb. The difference spectrum (GSb minus GSa) has its maximum at 256 nm, suggesting that the difference between GSa and GSb consists in a nucleotide linked or adsorbed to the protein of GSb. Since ATP is an obligate component of the inactivating system, we investigated, if adenosine, AMP, ADP or ATP are bound to glutamine synthetase in the interconversion of GSa to GSb.

No incorporation of $^{32}P$ from $\gamma$-$^{32}P$-labelled ATP into GS was found (9). Radioactivity from $^{14}C$-labelled ATP is however incorporated into glutamine synthetase during inactivation of GSa to GSb (Table I). In a comparison of the

Table I

$^{14}C$-ATP-incorporation into glutamine synthetase during inactivation.

| assay | total protein-bound radioactivity (cpm) | moles $^{14}C$-ATP bound to protein | $^{14}C$-ATP/mole GS |
|---|---|---|---|
| complete | $2,1.10^{5}$ | $19 .10^{-9}$ | 3,2 |
| - inactivating enzyme | $9,9.10^{3}$ | $0,9.10^{-9}$ | 0,2 |

Incubation mixture (total volume 1,0 ml): 40000 units GS ($6.10^{-9}$ moles); 23 units (1) inactivating enzyme; 50 µmoles $MgSO_4$; 1,0 µmole glutamine; 10 µmoles ATP; $1,1.10^{8}$ cpm $^{14}C$-ATP; 100 µmoles Tris-HCl-buffer, pH 8,0. Incubation time: 30 minutes. The reaction was stopped by adding 4,0 ml of a saturated ammonium sulfate solution. The protein was centrifuged, dissolved in 1,0 ml of 0,05 M Tris-HCl buffer pH 8,0 (containing 15 mg of ATP per ml), purified by reprecipitating four times with ammonium sulfate and again dissolved in buffer as described above. The solution was dialyzed over night against 0,05 M Tris-HCl buffer pH 8,0.

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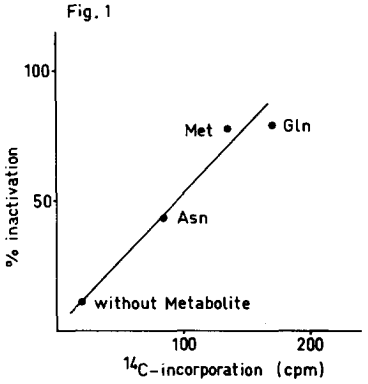
$^{14}C$-incorporating activity with the GS-inactivating activity in the presence of several (allosteric?) effectors, the incorporated radioactivity was proportional to the activity of the inactivating enzyme (Fig. 1). We conclude that the incorporation of radioactivity is caused by the inactivating enzyme.

Further evidence for the identity of the previously described GS-inactivating reaction with the $^{14}C$-incorporating activity is presented in Fig. 2. Inactivating enzyme was incubated with GS, ATP, $Mg^{2+}$ and glutamine, aliquots were taken at the indicated times and assayed for enzymatic



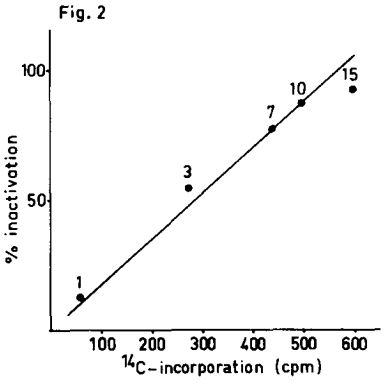




Fig. 1: Inactivation of glutamine synthetase compared with binding of $^{14}C$ from $^{14}C$-ATP to the enzyme protein.

Incubation mixture (total volume 0,10 ml): 2400 units GS; 2,3 units inactivating enzyme; 5 µmoles $MgSO_4$; 0,1 µmole of the effector; 1 µmole ATP; $1,9.10^6$ cpm $^{14}C$-ATP; 10 µmoles Tris-HCl buffer, pH 8,0. After different incubation times samples of 5 µl were removed and assayed for GS-activity as well as for protein bound radioactivity. The figure shows the values after 5 minutes of incubation.

Fig. 2: Comparison of the kinetics of inactivation of GS and of the binding of $^{14}C$ from $^{14}C$-ATP to the enzyme protein.

Incubation conditions were the same as described in Fig. 1. The effector is glutamine. The radioactivity per assay (0,10 ml) is $2,4.10^6$ cpm. The numbers in the figure give the time of incubation in minutes.

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activity. The ratio of GS-inactivation and $^{14}C$-incorporation remained constant during the reaction which was completed in 15 minutes.

Discussion. The mechanism of regulation of glutamine synthetase from E.coli by an "inactivating enzyme" is similar to the mechanism of regulation of mammalian glycogen phosphorylase by a phosphorylating enzyme as discussed earlier (1,2,5). For phosphorylase the controling effector is

cyclic 3',5'-AMP, for GS glutamine and several other metabolites, replacing glutamine or competing with glutamine are the controlling effectors. In both systems ATP is the compound reacting with the enzyme and causing the structural change. Phosphorylase however is phosphorylated, whereas in glutamine synthetase the adenine containing part of ATP (without the γ-P) is bound to the enzyme.

Kingdon and Stadtman (10) described two preparations of glutamine synthetase with different sensitivities to feedback effectors. Very recently Stadtman et al. [+] (11,12) described the enzymatical interconversion of one form to the other by incubation with ATP and glutamine. Stadtman et al. clearly demonstrated that the interconversion of the two forms of glutamine synthetase, different in feedback sensitivity, is an "adenylylation" of glutamine synthetase by ATP. Our data demonstrate the parallelism of the inactivation of glutamine synthetase and the incorporation of $^{14}C$ label from ATP into glutamine synthetase. They establish the identity of the system studied by Stadtman et al. with the inactivating system.

Summary. The previously described "glutamine synthetase inactivating enzyme" from E.coli catalyzes the incorporation of $^{14}C$ into glutamine synthetase in the presence of $^{14}C$-labelled ATP, $Mg^{2+}$ and glutamine. A comparison of glutamine with other stimulating effectors (methionine, asparagine) in the inactivating system and in the $^{14}C$ incorporating system shows parallel effects in both reactions.

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[+] We thank Dr.Stadtman for sending us copies of the manuscripts prior to publication.

Furthermore the ratio of $^{14}C$-incorporation and glutamine synthetase inactivation is constant during the course of the reaction. It is concluded that adenylylation is the mechanism of the inactivation of glutamine synthetase.

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