EFFECTS OF GLUTAMINE ON THE TWO CATALYTIC ACTIVITIES OF GLUTAMINE SYNTHETASE IN CULTURED MOUSE CELLS STRAIN L

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The regulation of glutamine synthetase (GS) provides a system particularly amenable to detailed studies of cellular control mechanisms. Both the transferase (γ-glutamyl transfer) and the synthetase (acyl activation) activities of GS appear to reside within the same enzyme molecule and both may be measured by the hydroxamate method, the synthetase being generally lower than the transferase activity[1,2]. Apart from other types of control, previous reports have shown that the transferase activity in animal cells is repressed by glutamine[3,4,5]. Inasmuch as glutamine is the end product of the synthetase activity, elucidation of its mechanism of action is of primary importance for understanding the framework within which control by other substances can be exerted on the GS system. The application of an improved method of preparation and detection (by the hydroxamate principle) of GS activities in cell-free extracts of L cells[6] permits a clear unambiguous resolution of the variations of the two catalytic activities under the influence of glutamine, even under conditions of limited GS formation. These variations in a well-defined system and the mechanism thereof are the subject of the present report.

METHOD

A subline of NCTC clone 929 mouse cells strain L was serially subcultured at weekly intervals in rotating (0.5 rpm) 250 ml milk dilution bottles at 37° containing 40 ml of an entirely synthetic medium[5] devoid of antibiotics or serum. Cell-free extracts were prepared by freezing and thawing in

0.04 M imidazole[6].

The assay mixture for the transferase activity (pH optimum 7.25)[6] contained, in a final volume of 0.4 ml, 25 mM imidazole 0.1 mM ADP, 0.025 mM $MnCl_2$, 25 mM KH_2AsO_4, 0.1 M hydroxylamine, 40 mM L-glutamine and 0.1 ml cell-free extract (1.5 mg protein, modal value). The assay mixture for the synthetase activity (pH optimum 6.70)[6] contained, in a final volume of 0.4 ml, 25 mM imidazole, 10 mM ATP, 20 mM $MgCl_2$, 1.8 mM Cleland's reagent[7], 10 mM creatine phosphate, 0.25 I.U. creatine phosphate kinase, 0.1 M hydroxylamine, 40 mM L-glutamate and 0.1 ml cell-free extract (1.5 mg protein, modal value). Prior to mixing, reagents were adjusted to the pH optimum of the respective reaction. After incubation at 37° C for 20 min. (transferase) or 60 min. (synthetase) the reaction was terminated by adding 0.4 ml of a 10% $FeCl_3 \cdot 6\ H_2O$ solution in 0.7 N HCl and 0.2 N trichloacetic acid. Absorbance was measured at 505 mμ (γ-glutamylhydroxamate: $A_{M,505}$=974). Specific activities are expressed as nmoles γ-glutamylhydroxamate formed per hour per mg protein of cell-free extract. Protein was determined according to Lowry et al[8].

RESULTS AND DISCUSSION

"Normal" growth medium, containing 2.4 mM glutamine, is added to the cells twice during the weekly subculture interval: at the time of planting and 4 days thereafter, at the time of refeeding. The rapid utilization of glutamine by L cells is obviously accountable for the relatively high levels of GS encountered just prior to refeeding[5]. If the cells are refed with glutamine-free medium, both GS activities remain high and slowly increase with time, the transferase being 5 times higher than the synthetase activity (Fig. 1a,b). On the other hand, after refeeding with normal medium, the GS activities drop within 24 hours to a low level (4-5 times lower than that attained, respectively, in the absence of glutamine) and, contrary to previous findings[5], remain at this level (Fig. 1a,b) although (a) during this period both the intra- and extracellular concentrations of glutamine rapidly decline[5] and (b) the action of glutamine is concentration dependent (Table 1).

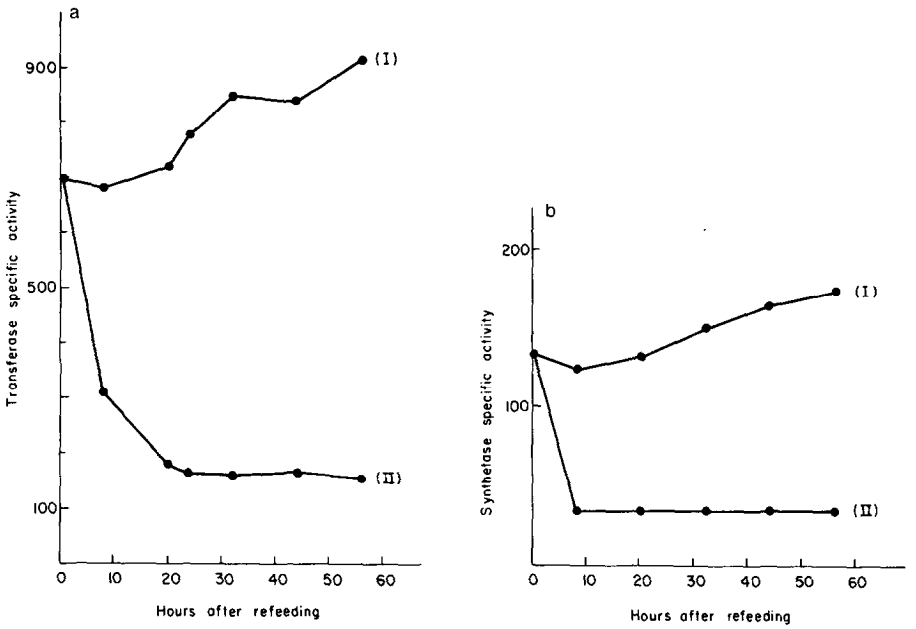

Fig. 1 GS spec. activities of L cells after refeeding at the end of the 4th day with glutamine-free (I) or with normal (II) medium. (a) transferase activity. (b) Synthetase activity.

Table 1

	Concentration of glutamine in medium (mM)[a]				
	0	0.5	1.0	2.0	2.4[b]
Transferase Spec. activity	805	618	493	308	173

[a]At the end of the 4th day, L cells were refed with medium containing the indicated concentrations of glutamine and were incubated for 24 hours, after which transferase spec. activities were determined.

[b]Concentration of glutamine in normal medium.

Whereas the depressive effect of glutamine is established within 24 hours (Fig. 2-Ia,II), the increase in GS activity following withdrawal of glutamine is markedly slower (Fig. 2-IIa) and is eliminated by cycloheximide (Fig. 2-IIb). Thus, it involves de novo protein synthesis, as Paul & Fottrell[4] had described. The long half-life of GS observed in the absence of glutamine (Fig. 2-A) is drastically shortened in its presence (Fig. 2-II,Ia). The withdrawal of glutamine results in a 40% increase in GS activity within 24 hours, while during an equal time period glutamine decreases the GS level by 80%. Hence, apart from interfering with GS synthesis, glutamine obvious-

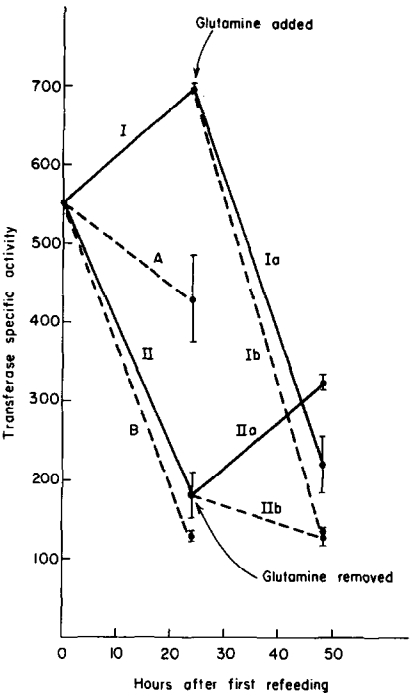

Fig. 2 Effect of cycloheximide (10 µg/ml) on GS activity. L cells were refed at the end of the 4th day with glutamine-free (group I) or with normal (group II) medium where they were incubated for 24 hours, after which cultures of group I were placed either in normal medium (Ia) or in normal medium containing added cycloheximide (Ib) and cultures of group II were placed either in glutamine-free medium (IIa) or in glutamine-free medium containing added cycloheximide (IIb). Second incubation lasted for 24 hours. Also, at the end of the 4th day replicate cultures were refed with glutamine-free medium containing added cycloheximide (A) or with normal medium containing added cycloheximide (B). Vertical bars indicate range of values obtained from two separate experiments.

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ly promotes GS destruction. Cycloheximide does not interfere with the depressive action of glutamine and, in fact, enhances it (Fig. 2-Ib,B). Identical results, not reported here, were obtained with another protein synthesis inhibitor, p-fluorophenylalanine (1 mM). Thus, the action of glutamine does not require protein synthesis. The rise in GS activity following refeeding on the 4th day with glutamine-free medium (Fig. 2-I) is decreased, but not completely eliminated, by 8-azaguanine or by 5-bromo-2'-deoxyuridine (Table 2), thereby indicating a slow rate of message synthesis and degradation. Neither of the two base analogues interferes with the depressive action of glutamine (Table 2), which, therefore, does not appear to depend on RNA synthesis.

Table 2

Effect of nucleic acid antagonists on glutamine synthetase.

Glutamine concentration in medium (mM)	Inhibitor[1] None	8-azaguanine (1mM)	5-bromo-2'-deoxy uridine (0.1 mM
0	791 ± 10	667 ± 22	660 ± 18
2.4	173	201	176

[1] L cells were refed on the 4th day with medium containing the combinations shown in the table and were incubated for 24 hours, after which transferase spec. activities were determined.

Glutamine is, thus, shown here to equally depress both catalytic activities of GS in L cells by two mechanisms, neither of which requires protein or RNA synthesis: (a) a slowly elicited (obviously due to the slow synthesis and degradation of the enzyme) inhibition of GS synthesis; and (b) a rapidly elicited increase in the rate of destruction (inactivation) of preexisting enzyme or complements of it. The difference in the rapidity of appearance of the two glutamine effects may account for the steady net level at which GS remains upon prolonged incubations with glutamine (Fig. 1a,b) even though the concentration of the latter during this period gradually declines.

The present findings do not offer definitive evidence as to the site(s) at which glutamine inhibits GS synthesis. It may be noted, however, that, contrary to the translational inhibitors, the nucleic acid base analogues do not potentiate the depressive action of glutamine (Table 2). Since the base analogues can decrease GS synthesis in the absence of glutamine, one might expect that, if their sites of action were different from that of glutamine, their effect would be cooperative to, rather than masked within, that of glutamine. It is, thus, tentatively suggested, as a basis for further study, that the inhibitory effect of glutamine on GS synthesis may occur at the nucleic acid level, at a stage preceding that of translation.

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SUMMARY

When cultured mouse cells strain L are incubated in the presence of glutamine (normally a component of their growth medium) both the transferase (γ-glutamyl transfer) and the synthetase (acyl activation) activities of glutamine synthetase are equally depressed, the transferase being on the whole 5 times higher than the synthetase activity. Whereas the depressive action of glutamine is established within 24 hours, the increase in enzymatic activity following withdrawal of glutamine is markedly slower. The action of glutamine involves two mechanisms, neither of which requires protein or RNA synthesis: (a) inhibition of the synthesis of glutamine synthetase; and (b) promotion of destruction of preexisting enzyme or complements of it.

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