INVOLVEMENT OF PROTEIN SYNTHESIS IN THE POST-TRANSLATIONAL CONTROL OF GLUTAMINE SYNTHETASE ACTIVITY OF CULTURED HEPATOMA CELLS

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SUMMARY

Glutamine accelerates the degradation of glutamine synthetase in hepatoma tissue culture (HTC) cells. Preincubation of HTC cells in low glutamine with cycloheximide or emetine for 30 min prevents the decay of glutamine synthetase activity elicited by adding high concentrations of glutamine. However, when cycloheximide or emetine are added with or after high concentrations of glutamine the decay of glutamine synthetase activity is not immediately inhibited but proceeds for 1-3 hr. These observations suggest that the modulation of glutamine synthetase activity by glutamine involves a labile polypeptide factor which is synthesised both in the presence and absence of glutamine and is stabilized by glutamine.

Previous investigations showed that glutamine stimulates the disappearance of glutamine synthetase (L-glutamate: ammonia ligase (ADP), EC 6.3.1.2) activity in hepatoma tissue culture (HTC) cells (1, 2). More recent evidence indicates that when HTC cells are treated with glutamine, glutamine synthetase is initially converted into a modified form and is subsequently degraded (3). This is a new type of regulatory mechanism whereby the degradation of an enzyme is accelerated by one of its products. Glutamine apparently modulates glutamine synthetase activity by a similar mechanism in Chinese hamster (4) and L cells (5).

Earlier experiments in this (2) and other laboratories (5) showed that high concentrations of cycloheximide or puromycin inhibited the glutamine-induced decay of glutamine synthetase. These findings suggested that some factor synthesized by the protein synthesis apparatus may be required for the degradation of glutamine synthetase. The aim of the present investigation was to obtain more insight into the mechanism of

action of protein synthesis inhibitors on the glutamine-stimulated decay of glutamine synthetase in HTC cells.

MATERIALS AND METHODS

HTC cells, subclone GM 22-5 (2), were grown in suspension in Swim's 77 medium, modified to contain 0.5 g $NaHCO_3$ per liter, 0.05 M Tricine, 10% (v/v) calf serum and 2 mM glutamine. "Induction medium" was the same medium containing 0.2 mM (instead of 2 mM) glutamine, 0.5 mM asparagine and 10% (v/v) dialyzed calf serum. High levels of glutamine synthetase were "induced" in cells by the following treatment. Cells were suspended at a density of 2 x 10^5 cells/ml in "induction medium" containing 0.5 μM dexamethasone and incubated for 48 hr ("induced cells").

Preparation of cell extracts and assays of glutamine synthetase and protein were performed as described previously (2, 6).

RESULTS AND DISCUSSION

Preincubation of induced cells with high concentrations of cycloheximide or emetine abolished the glutamine-stimulated decay of glutamine synthetase activity (Fig. 1). In experiments not shown here the

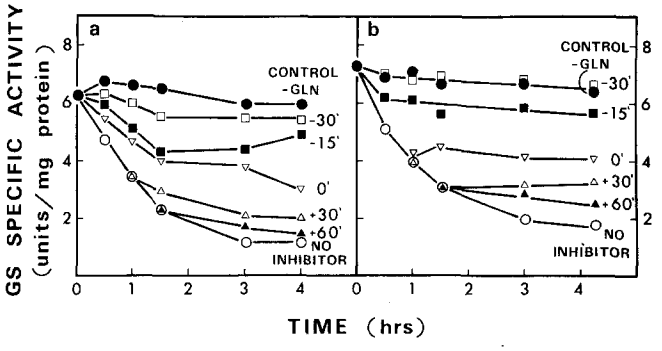


Fig. 1. Time-dependent effect of emetine or cycloheximide on the glutamine-stimulated decay of glutamine synthetase. "Induced" cells were resuspended in a fresh batch of induction medium containing 0.5 μM dexamethasone at a density of 5 x 10^5 cells/ml. Portions of 30 ml were placed in 125 ml Erlenmeyer flasks and incubated for 1 hr in a rotatory shaking bath at 37° before adding 5 mM glutamine to all flasks, except the control with low glutamine. The time of addition of glutamine was the zero time of the experiment. a) Emetine (10^{-5} M) or b) cycloheximide (4 x 10^{-4} M)were added at the following times: 30 min before glutamine (□); 15 min before glutamine (■); with glutamine (▽) 30 min after glutamine (△) 60 min after glutamine (▲). Controls without inhibitor: with 5 mM glutamine (O) and with 0.2 mM glutamine (●, control -GLN) were also included.

inhibitory effect of cycloheximide on the glutamine-stimulated fall of glutamine synthetase activity was shown to be reversible. The time of preincubation with inhibitor required to completely inhibit the effect of glutamine varied considerably (from 10-30 min) from experiment to experiment. Fig. 1 shows that when the inhibitor was added with glutamine at zero time the activity of glutamine synthetase fell for 1-1.5 hr and then levelled off. Since protein synthesis was inhibited immediately (0-3 min) after adding cycloheximide (Fig. 2), the lag in the effect of the inhibitor on the glutamine-induced decay of glutamine synthetase activity was not due to delayed inhibition of protein synthesis. Fig. 1 also shows that when cycloheximide or emetine were added 30 or 60 min after glutamine, glutamine synthetase specific activity continued to fall for a further 1-3 hr. The above results suggest that the modulation of glutamine

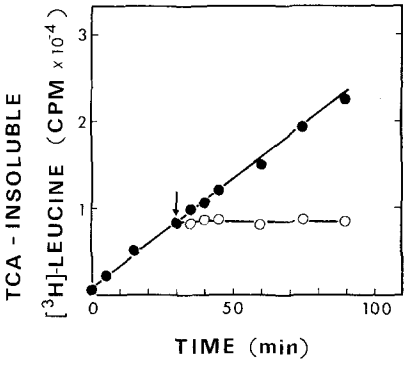


Fig. 2. Time course of inhibition of protein synthesis by cycloheximide. Cells were "induced" and resuspended in fresh induction medium containing 0.5 μM dexamethasone at a density of 4 x 10^5 cells/ml. Portions of 15 ml were preincubated in 50 ml Erlenmeyer flasks, for 1 hr in a rotatory shaking bath at 37°. At the beginning of the experiment 5 mM glutamine and 6.5 μCi of [^{3}H]-leucine were added. After 30 min (arrow) 4 x 10^{-4} M cycloheximide was added to one of the flasks. Duplicate samples of 0.5 ml were removed at intervals and each placed in a tube containing 5% (w/v) trichloroacetic acid at 0° with mixing. The precipitate was washed twice with 1.0 ml of cold 5% trichloroacetic acid. After heating for 20 min at 95° with 5% trichloroacetic acid the samples were filtered on Whatman No. 1 filter discs and the precipitate washed successively with 5% trichloroacetic acid, acetone: ether (1:1) and ether. After drying, the filters were counted in 4 ml of toluene scintillation fluid. Each point is mean of duplicate samples: ●, no inhibitor; O, 4 x 10^{-4} M cycloheximide.

synthetase activity by glutamine involves a labile polypeptide factor synthesized by the ribosomes both in the presence and in the absence of glutamine. When synthesis of the factor is inhibited in the absence of glutamine, it is destroyed in 30 min or less, but if glutamine is present, it survives for 1-3 hr.

Further evidence for the participation of a labile polypeptide factor in the regulation of glutamine synthetase activity is presented in Fig. 3. Cells were incubated for 1 hr with glutamine and when transferred to a medium without glutamine containing cycloheximide. Glutamine was then added at different times. When glutamine was added concomitantly with cycloheximide, the decay of glutamine synthetase activity continued for a further 1.5-2 hr. When glutamine was added 30 min after cycloheximide glutamine synthetase activity decreased only slightly. When added 1 hr after cycloheximide, glutamine no longer stimulated the inactivation of glutamine synthetase. These results

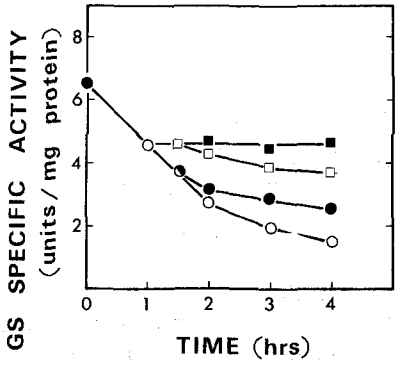


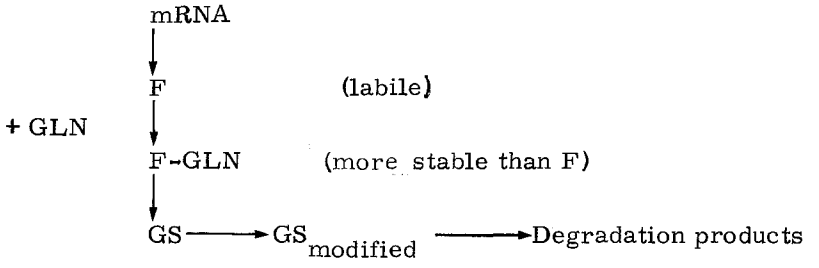
Fig. 3. Effect of cycloheximide on the glutamine-stimulated decay of glutamine synthetase activity in cells preincubated with glutamine. "Induced cells were preincubated for 1 hr at a density of 4 x 10^5 cells/ml in fresh induction medium containing 0.5 μM dexamethasone. After removal of a zero time sample 5 mM glutamine was added and incubation was continued for a further 1 hr. The cells were then sedimented and resuspended in induction medium (0.2 mM glutamine) containing 0.5 μM dexamethasone. Portions of 30 ml were transferred to 125 ml Erlenmeyer flasks. Cycloheximide (4 x 10^{-4} M) was added to each flask except for the control without inhibitor. Glutamine was added: O, immediately after resuspension in induction medium (control); ●, together with cycloheximide; □, 30 min after cycloheximide; ■, 60 min after cycloheximide.

indicate that the hypothetical factor requires the presence of glutamine for activity and is rapidly destroyed in the absence of the latter.

Actinomycin D does not decrease the initial rate of inactivation of glutamine synthetase in high glutamine (2). Decay of glutamine synthetase activity is, however, arrested after incubation of cells for 5 hr or more with the inhibitor. Thus, RNA synthesis is not required to maintain the initial rate of decay of glutamine synthetase in presence of high glutamine.

Since three different inhibitors of protein synthesis, namely puromycin (2), cycloheximide and emetine have similar effects, it seems likely that these inhibitors act specifically by inhibiting polypeptide synthesis and not by some other mechanism. In addition, the fact that the effect of cycloheximide is reversible makes it unlikely that this inhibitor acts non-specifically by causing irreversible damage to the cells.

The following model could account for the observations of this and previous papers:



It is proposed that a labile factor (F) is required for the breakdown of glutamine synthetase (GS). Glutamine binds to F and increases its stability. The complex of F with glutamine is somehow involved in the modification which precedes the breakdown of glutamine synthetase (3).

Earlier work in other laboratories has suggested the possible involvement of labile polypeptide factors in the degradation of cellular proteins. Protein synthesis inhibitors have been shown to inhibit the degradation of prelabelled cellular proteins in serum-deprived tissue culture cells (7-10). Epstein et al. (10) have suggested that the break-

down of slowly-degraded proteins in HTC cells requires the continuous formation of a product of protein synthesis. The specific breakdown of tyrosine amino transferase in liver in vivo and in hepatoma cells cultured in serum-free medium is inhibited by protein synthesis inhibitors (7, 11, 12). Of particular relevance to our observations are the experiments of Kenney and his associates (12) which show that cycloheximide inhibits tyrosine aminotransferase degradation in cultured hepatoma cells only after a lag of 1-2 hr. These investigators suggest that some cellular polypeptide component with a lifetime of 1-2 hr may be required for tyrosine aminotransferase breakdown. There is no evidence to indicate whether the putative polypeptide factor required for tyrosine aminotransferase degradation is specific for the enzyme or is a general factor involved in protein degradation. The hypothetical factor (F) involved in the glutamine-stimulated degradation of glutamine synthetase is probably specific, since it is stabilized by glutamine. It is tempting to suggest that the hypothetical factor carries the glutamine-binding site involved in regulation of glutamine synthetase activity (6).

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REFERENCES

1. Kulka, R.G., Tomkins, G.M., and Crook, R.B. (1972) J. Cell Biol. 54, 175-179.
2. Kulka, R.G., and Cohen, H. (1973) J. Biol. Chem. 248, 6736-6743.
3. Arad, G., Freikopf, A., and Kulka, R.G. (1976) Cell 8, 95-101.
4. Milman, G., Portnoff, L.S., and Tiemeier, D.C. (1975) J. Biol. Chem. 250, 1393-1399.
5. Barnes, P.R., Hersh, R.T., and Kitos, P.A. (1974) In Vitro 9, 230-238.
6. Freikopf, A., and Kulka, R.G. (1975) Eur. J. Biochem. 56, 483-492.
7. Hershko, A., and Tomkins, G.M. (1971) J. Biol. Chem. 246, 710-714.
8. Hershko, A., Mamont, P., Shields, R., and Tomkins, G.M. (1971) Nature New Biol. 232, 206-211.
9. Gelehrter, T.D., and Emanuel, J.R. (1974) Endocrinology 4, 676-684.
10. Epstein, D., Elias-Bishko, S., and Hershko, A. (1975) Biochemistry 14, 5199-5204.
11. Kenney, F.T. (1967) Science 156, 525-527
12. Barker, K.L., Lee, K.L., and Kenney, F.T. (1971) Biochem. Biophys. Res. Commun. 43, 1132-1138.