SUMMARY

The specific activity of glutamine synthetase in cultured Chinese hamster cells is inversely related to the concentration of glutamine in the surrounding solution. Enzyme specific activity increases 8- to 10-fold when glutamine is removed from serum-free F12 growth media. The induction of glutamine synthetase activity occurs only after glutamine removal and not after the removal of other amino acids (methionine, leucine, or isoleucine). The analysis of the glutamine-mediated decrease in glutamine synthetase activity has been simplified by the finding that depression proceeds in nutrient-free buffered saline solution (111 mM NaCl, 5.4 mM KCl, and 30 mM Tricine (pH 7.4)). Under these conditions, 0.1 mM cyanide blocks glutamine-mediated depression. The cyanide inhibition is reversed by the addition of 1.0 mM glucose which suggests that ATP is required for depression. Glutamine-mediated depression is temperature-dependent, occurring between 25 and 45° with an optimum rate at 37°. Studies of the time course of induction and depression as a function of glutamine concentration suggest that glutamine regulates the rate at which the enzyme is either modified or degraded. We have employed an antibody prepared against homogeneous Chinese hamster liver glutamine synthetase to measure the amount of glutamine synthetase protein in extracts of cells containing induced or depressed levels of enzyme activity. A highly sensitive immunoprecipitation procedure enables quantitation of nanogram amounts of glutamine synthetase protein. Glutamine synthetase in cell extracts containing induced levels of enzyme activity possesses the same molecular specific activity (ratio of activity to antigenicity) as homogeneous Chinese hamster liver glutamine synthetase. The molecular specific activity of glutamine synthetase is almost the same in extracts of cells

Glutamine synthetase (EC 6.3.1.2) catalyzes the ATP-dependent synthesis of glutamine from glutamate and ammonia. The enzyme plays a major role in the metabolism of nitrogen because the amide group of glutamine is required for the synthesis of several amino acids and nucleotides. The complex mechanisms which control glutamine synthetase in bacteria have been studied in detail (1-3). Regulation includes reversible enzymatic adenylation of specific tyrosine residues and feedback inhibition by various metabolites which incorporate the amide group of glutamine. In contrast to bacterial systems, the mechanisms by which glutamine synthetase is regulated in eucaryotes are poorly understood. Several investigators (4-9) have reported an inverse correlation between glutaminyltransferase activity in cells in tissue culture and the glutamine concentration in the supporting media. Glutaminyltransferase, measured by the capacity to form γ-glutamylhydroxamate, is usually assumed to be synonymous with glutamine synthetase activity.

We have studied the control and regulation of glutamine synthetase in Chinese hamster cells grown in tissue culture (10-12). Chinese hamster cells are ideally suited for biochemical and genetic studies because they can be grown easily, are pseudodiploid, and have a generation time of less than 12 hours. Using a sensitive radioisotope assay for glutamine synthetase which avoids potential problems of the γ-glutamylhydroxamate assay (13, 14), we have purified and characterized the enzyme from Chinese hamster liver. We have shown that the enzyme in Chinese hamster tissue culture cells possesses properties identical to the liver enzyme. Although glutamine synthetase isolated either from Chinese hamster liver or from Chinese hamster tissue culture cells is not inhibited by glutamine, the specific activity of the enzyme in tissue culture cell extracts is inversely related to the concentration of glutamine in the growth media. Removal of glutamine from the media results in a 10-fold increase in glutamine synthetase activity over a 24- to 48-hour period ("induction"). The return of glutamine produces a rapid drop in en-
zyme activity to its initial level ("depression").1 Actinomycin D has little effect on induction or depression, indicating that regulation does not occur at the level of messenger RNA synthesis. Cycloheximide blocks induction but not depression, indicating that protein synthesis (perhaps of new enzyme) is required for increase but not for decrease of enzyme activity.

An extension of these studies is reported in this paper. The analysis has been simplified by our observation that induction of glutamine synthetase proceeds in serum-free media, and that glutamine-mediated depression can occur in nutrient-free saline solution. The concentration of glutamine appears to regulate the rate at which glutamine synthetase is either modified or degraded. This process appears to require ATP and is temperature-dependent. Furthermore, increase in glutamine synthetase activity occurs only upon glutamine removal and is not a general feature of amino acid starvation. An immunological assay has been employed to determine the fate of glutamine synthetase protein following glutamine-mediated depression. Using goat antibody against homogeneous Chinese hamster liver glutamine synthetase, we can measure nanogram amounts of glutamine synthetase protein by immunoprecipitation. We find that glutamine synthetase protein is either degraded or converted to a less antigenic form in extracts of cells containing depressed levels of enzyme activity.

**EXPERIMENTAL PROCEDURES**

**Cell Growth Conditions**—Chinese hamster V79 cells are grown in Falcon 100-mm tissue culture plates in Ham's F12 medium containing or lacking glutamine as previously described (11). Indicated experiments are performed in the absence of serum. Cells are detached from the plates by incubating for 9 min at 37° in a Tricine (N-tris(hydroxymethyl)methylglycine)-buffered trypsin solution (0.025% trypsin, 30 mM Tricine (pH 7.5), 5.5 mM KCl, 141 mM NaCl, 5.5 mM glucose, and 5 mg per liter of phenol red) and sedimented by centrifugation in a clinical centrifuge for 5 min at top speed. The media are withdrawn, and the cells are lysed by suspending in Tricine buffer (50 mM Tricine (pH 7.5), 50 mM KCl, 20 mM MgCl₂, 1.0 mM EDTA, and 10 mM dimethyl sulfoxide) and freezing in Dry Ice. The suspension is thawed in a 37° water bath. Cell nuclei, membranes, and mitochondria are removed by centrifugation at 20,000 × g for 30 min. Extracts containing 10 to 15 mg per ml of protein are prepared from cells suspended at a density of 1.4 × 10⁶ cells per ml of Tricine buffer.

**Cell Suspension Assay for Depression**—Factors affecting depression of glutamine synthetase by glutamine are determined with Chinese hamster cells in suspension buffer (141 mM NaCl, 5.4 mM KCl, and 30 mM Tricine (Sigma), pH 7.4). Chinese hamster cells grown to a density of 3 × 10⁶ cells per 100-mm dish are plated in 12-m1 glass conical centrifuge tubes at 37° in a water bath.

**Glutamine Synthetase Specific Activity**—Glutamine synthetase activity is determined by a sensitive technicon autoanalyzer (11) which measures the conversion of [14C]glutamate to [14C]glutamine. A standard assay reaction mixture of 50 μl is incubated at 37° for 30 min and contains 500 nmo1 of [14C]glutamate at a specific activity of 500 to 600 cpm per nmo1 (counting efficiency of 78%). The background is generally between 150 to 250 cpm and is reproducible to 1 to 2% within a set of assays. Cell extracts prepared as described above are assayed for protein content by the method of Lowry et al. (19).

**Preparation of Glutamine Synthetase Antibody—**Glutamine synthetase used to prepare anti-glutamine synthetase antiserum is purified to apparent homogeneity from Chinese hamster liver as previously described (10). A 70-pound goat is immunized by multiple injections of homogeneous Chinese hamster liver glutamine synthetase (along with Freund's complete adjuvant when glutamine synthetase is injected subcutaneously or intradermally) Day 0 (50 μg subcutaneous), Day 28 (10 μg intradermal), Day 61 (15 μg intradermal), and Day 75 (5 μg intravenous). On Day 84 the goat is exsanguinated and the blood (1.6 liters) is allowed to clot overnight at 5°. The serum is freed of debris by centrifugation at 8,000 × g for 30 min. The supernatant is removed and subjected to additional centrifugation at 20,000 × g for 30 min yielding 900 ml of serum. The antibody is precipitated with ammonium sulfate (0 to 33% saturation), washed once with 40% ammonium sulfate, and then precipitated a second time with ammonium sulfate (0 to 33% saturation). The precipitate is suspended in 0.025% trypsin, 30 mM Tricine (pH 7.5), 5.3 mM KCl, 141 mM NaCl, 5.5 mM glucose, and 1% ammonium hydroxide and is dialyzed overnight against 50 mM Tricine, pH 8.0, and then precipitated a second time with ammonium sulfate (0 to 33% saturation). The precipitate is suspended in 50 mM Tricine, pH 8.0, and subjected to dialysis overnight against 50 mM Tricine, pH 8.0, at 5°. The dialysate (160 ml) containing the anti-glutamine synthetase antibody is stored at -20°. Control antibody, obtained by bleeding the goat prior to injection with glutamine synthetase, is treated in a similar manner. Ammonium sulfate is dialyzed with ammonium sulfate as described above.

**Ouchterlony Analysis—**Ouchterlony double diffusion precipitation reactions are performed on immunodiffusion plates purchased from Hyland on glutamine synthetase antibody. Precipitation of glutamine synthetase activity by anti-glutamine synthetase antibody is carried out in microcentrifuge tubes (4 × 55 mm, Markson Science, Inc.). The immunoprecipitation mixture of 100 μl contains anti-glutamine synthetase antibody, Tricine buffer, cell extract, rabbit anti-goat γ-globulin antibody, and control goat antibody to keep the total goat antibody concentration constant. First, cell extract is added to a mixture containing glutamine synthetase antibody, Tricine buffer, and control antibody and mixed vigorously (about 1 s) on a Vortex mixer. The mixture is incubated for 24 hours at 3°. Next, 5 μl of diluted rabbit-anti-goat γ-globulin antibody are added and the mixture is mixed and incubated for 15 min at 37°. The enzyme-antibody complex is sedimented by centrifugation at 40,000 × g for 20 min in adaptors which we have built to use in a Beckman type 40 rotor. Due to the large size of the Chinese hamster liver glutamine synthetase (13.8 S), a gradient of enzyme activity begins to form under the centrifugation conditions used to sediment the antigen-antibody complexes. Following centrifugation, we find it necessary to remove and mix the whole supernatant in order to obtain a representative sample of the residual activity. The supernatant is withdrawn with a drawn-out Pasteur pipette. After mixing, a sample of the supernatant (20 μl) is removed and assayed for glutamine synthetase activity as described above.

**RESULTS**

**Amino Acid Specificity in Regulation of Glutamine Synthetase**—To determine if the increase in glutamine synthetase activity in cultured Chinese hamster cells is specific for the removal of glutamine or a general feature of amino acid deprivation, we have examined enzyme activity in cells growing in the absence of the essential amino acids methionine, isoleucine, or leucine. Glutamine synthetase activity is not increased by removing any of these amino acids from media containing glutamine (Fig. 1). Moreover, enzyme induction by removal of glutamine (Fig. 2) occurs in the absence of these amino acids, but to a lesser extent.

**Effect of Glutamine Concentration on Induction and Depression**—The effect of glutamine concentration on the rate of glutamine synthetase induction in cells initially containing depressed levels of enzyme is determined by measuring the enzyme specific activity at various times after replacing media containing 1.0 mM glutamine with serum-free media containing different concentra-
Cells grown in media lacking glutamine for 30 hours are detached with trypsin and first incubated for 60 min at 37° in suspension buffer (1), or in suspension buffer containing 0.1 mM KCN (2), 1.0 mM glucose (3), or 0.1 mM KCN and 1.0 mM glucose (4). After the first incubation either glutamine is added to a final concentration of 1.0 mM or no addition is made and the cell suspensions undergo a second incubation for 60 min at 37°. The specific activity of glutamine synthetase in the cells is determined as described under "Experimental Procedures." In these experiments, induced glutamine synthetase specific activities are 0.76 to 0.82 milliunits per mg and depressed specific activities are 0.33 to 0.39 milliunits per mg.

The effect of glutamine concentration on the rate of glutamine synthetase depression in cells initially containing induced levels of enzyme is determined in an analogous experiment. Enzyme specific activity is measured at various times after replacing media lacking glutamine with serum-free media containing different concentrations of glutamine (Fig. 4). The initial rate of depression increases with increasing glutamine concentration. Regardless of whether the cells initially have induced or depressed levels of glutamine synthetase, a steady state enzyme level is eventually reached which is determined only by the glutamine concentration.

Characteristics of Glutamine-mediated Depression—The experiments with cyanide described in Table I suggest that glutamine-mediated depression requires energy. Cyanide blocks the accumulation of ATP by oxidative phosphorylation and permits the dissipation of cellular ATP pools. Cells with induced levels of enzyme are incubated in suspension buffer containing cyanide, and then glutamine is added. At cyanide concentrations of 0.1 mM and above (Line 2), the addition of glutamine does not depress glutamine synthetase activity. The effect of cyanide is reversed by the addition of glucose (Line 4) which permits cells to synthesize ATP via glycolysis. Glucose by itself (Line 3) has no effect on glutamine synthetase activity either in the presence or in the absence of glutamine.

Glutamine-mediated depression is temperature-dependent (Fig. 5). Depression occurs between 25 and 45° with a maximum rate at 37°. Depression does not occur at 0°.

Depression in the cyanide and temperature experiments is measured at 60 min after glutamine addition, and therefore the depression is less than the 8- to 10-fold observed after long term incubations in glutamine (cf. Fig. 4).

Characterization of Glutamine Synthetase Antibody—The Ouchterlony double-diffusion technique demonstrates the specificity of the antigen-antibody reaction. A single precipitin band forms when homogeneous Chinese hamster liver glutamine synthetase or a Chinese hamster liver extract is tested with the goat anti-glutamine synthetase antibody (Fig. 6).

The amount of anti-glutamine synthetase antibody
Fig. 5. Effect of temperature on depression. Cells grown in media lacking glutamine for 24 hours are detached with trypsin and incubated in suspension buffer for 5 min at the indicated temperatures. After the first incubation, either glutamine is added to a final concentration of 1.0 mM or no addition is made and the cells are incubated further for 60 min at the indicated temperatures. The ratio of the enzyme activity in cells incubated without glutamine to that in cells incubated with glutamine is presented as a function of temperature. The specific activity of glutamine synthetase in cells incubated without glutamine is 1.1 milliunits per mg from 0 to 40° and it increases 2-fold from 40 to 50° due to denaturation of other proteins.

Fig. 6. Ouchterlony double diffusion precipitin analysis of goat anti-glutamine synthetase antibody with Chinese hamster glutamine synthetase. The inner well contains 5 μl of anti-glutamine synthetase antibody (0.7 mg). The outer wells contain either 5 μl of 30 to 50% ammonium sulfate fraction from Chinese hamster liver (A, B = 0.15 mg) or homogeneous Chinese hamster liver glutamine synthetase (C, D = 7 μg).

required to precipitate half the glutamine synthetase activity can be used to quantify the amount of glutamine synthetase protein. In Fig. 7, the unprecipitated glutamine synthetase activity is measured as a function of added anti-glutamine synthetase antibody. Each curve represents a different initial concentration of enzyme. As the concentration of the anti-glutamine synthetase antibody increases, more glutamine synthetase precipitates and less is found in the supernatant. Under our experimental conditions, a small amount of glutamine synthetase activity (approximately 0.1 to 0.2 nmol of glutamine per 30 min) remains unprecipitated even at high anti-glutamine synthetase antibody concentrations. This unprecipitated enzyme may be attributed to incomplete precipitation of the enzyme-antibody complex by the rabbit anti-goat γ-globulin antibody. Apparently, in our assay conditions the anti-goat γ-globulin antibody is not in large excess. Ten-fold higher amounts of anti-goat γ-globulin antibody than used in these experiments reduces the unprecipitable glutamine synthetase 2- to 3-fold. Nevertheless, the data in Fig. 8 indicate that the assay is satisfactory, and the routine use of higher levels of rabbit anti-goat γ-globulin antibody was impractical.

The data in Fig. 7A are obtained with glutamine synthetase from extracts of Chinese hamster cells having induced levels of enzyme activity. Analogous curves are obtained using homogeneous Chinese hamster liver glutamine synthetase (Fig. 7B). The concentration of antibody for 50% precipitation is defined as that amount at which the enzyme activity remaining in the supernatant is the average of the initial and final levels.

Fig. 7. Immunoprecipitation titration of glutamine synthetase. A, an extract of cells containing an induced level of glutamine synthetase (2.6 milliunits per mg) is diluted to four different initial concentrations of glutamine synthetase. The 50% precipitation point is the amount of antibody at the average of the initial and final enzyme activities. The 50% precipitation points (given in nanomoles of [14C]glutamine formed in a 30-min assay) for the different initial concentrations of glutamine synthetase are: 0.48, 0.35 (○—○); 0.71, 0.42 (□—□); 0.66, 0.54 (△—△); 1.39, 0.74 (●—●). B, homogeneous Chinese hamster liver glutamine synthetase is diluted to three different initial concentrations of enzyme. The initial and 50% precipitation points (given in nanomoles of [14C]glutamine formed in a 30-min assay) for the different initial concentrations of glutamine synthetase: are 1.32, 0.71 (○—○); 2.01, 1.06 (□—□); 2.70, 1.40 (△—△).

Fig. 8. The 50% precipitation point as a function of the initial activity of glutamine synthetase. Data from Fig. 7A (○); data from a separate similar experiment (△); data from Fig. 7B (□). The 50% precipitation point is defined as that amount at which the enzyme activity remaining in the supernatant is the average of the initial and final levels. The amount of glutamine synthetase protein corresponding to the initial activity is based on the known specific activity of homogeneous glutamine synthetase, 9.5 units per mg (10).
**Fig. 9. Immunoprecipitation titration of glutamine synthetase**

FIG. 9. Immunoprecipitation titration of glutamine synthetase from extracts of cells containing depressed levels of enzyme. The data fall on the same line, glutamine synthetase in extracts of Chinese hamster cells having induced levels of glutamine synthetase activity and from reactions with homogeneous Chinese hamster liver glutamine synthetase. Since both sets of data fall on the same line, glutamine synthetase in extracts of Chinese hamster cells with induced levels of activity must have the same relative amounts of activity and antigenicity as those in Chinese hamster liver.

We can define the molecular specific activity as the ratio of activity to antigenicity. The molecular specific activity is a measure of the activity of an enzyme molecule. The data in Fig. 8 suggest that the immunoprecipitation reaction can be used to compare the molecular specific activity of glutamine synthetase in extracts of cells with induced levels of enzyme activity with that in extracts of cells with depressed levels of enzyme activity.

**Measurement of Glutamine Synthetase Protein in Cell Extracts Containing Depressed Levels of Glutamine Synthetase Activity**—

The data in Fig. 9 illustrate the results of an immunoprecipitation experiment with extracts of cells which have been grown in the presence of glutamine for short (2 hours) and long (24 hours) periods of time. Both extracts require somewhat more anti-glutamine synthetase antibody for precipitation than do extracts containing induced levels of enzyme at the same initial activity. The calibration curve (Fig. 8) is redrawn in the inset in Fig. 9 along with the data obtained from the immunoprecipitation experiments with the extracts of cells containing depressed levels of enzyme. Also shown are the data that would be expected if glutamine-mediated depression resulted from a modification of glutamine synthetase to a less catalytic but antigenically identical form.

**Discussion**

Glutamine synthetase specific activity in cultured Chinese hamster cells is inversely related to the concentration of glutamine in cell media. Our attempts to demonstrate and to characterize glutamine-mediated depression of enzyme activity in cell-free extracts have not been successful. We have sought to better characterize enzyme regulation in vivo as a prelude to more systematic studies in vitro.

The current work demonstrates that both induction by glutamine removal and depression by glutamine addition occur in the absence of serum. Hence, glutamine-mediated regulation can be studied in defined media. Furthermore, our observation that depression can occur in a saline solution has enabled us to examine potential factors affecting glutamine depression. Cell suspensions rather than monolayers are employed in these latter studies to facilitate thorough washing of cells and removal of glutamine. The slower rates of depression observed under these conditions compared to those found in previous studies (11) may be due to the absence of media components which affect the rate but not the extent of depression.

Induction of glutamine synthetase results from glutamine removal specifically and is not caused by general amino acid deprivation. Starvation for methionine, leucine, or isoleucine does not increase enzyme activity when glutamine is present. Moreover, removal of any of these three essential amino acids does not prevent partial induction when glutamine is simultaneously removed. We previously have shown that cycloheximide blocks induction implying that protein synthesis is required for increase in enzyme activity (11). The removal of an essential amino acid (e.g. methionine) decreases the rate of incorporation of another essential amino acid (e.g. [3H]leucine) by approximately 50% (data not shown), indicating that protein synthesis continues at a decreased rate during essential amino acid starvation. Since pools of methionine and leucine are low in Chinese hamster cells (data not shown), the necessary amino acids for synthesis of glutamine synthetase during essential amino acid starvation are probably provided by protein turnover. The increase in glutamine synthetase specific activity is not due to selected cell survival or accelerated degradation of non-glutamine synthetase protein since viable cell survival is 100% and total protein before and after induction is nearly identical.

Glutamine-mediated regulation of glutamine synthetase specific activity could operate by affecting either the rate of enzyme synthesis or the rate of enzyme degradation. During induction, enzyme specific activity linearly increases after an initial lag at a rate which is independent of the glutamine concentration. In contrast, the addition of glutamine to cells with induced activity results in an exponential decrease in activity at an initial rate which is dependent upon glutamine concentration. Similar results have been obtained by Kulka and Cohen (9) in steroid-treated hepatoma tissue culture cells. We conclude that glutamine either directly or indirectly regulates the rate of depression of glutamine synthetase, and does not affect the rate of enzyme synthesis. Depression could occur either by degradation or by modification to produce an enzyme with a lower molecular specific activity.

Stadtman and co-workers have found that covalent adenylylation (promoted by glutamine) controls the activity of *Escherichia coli* glutamine synthetase (2). The adenylylated enzyme possesses lower specific activity and displays different susceptibilities to inhibition by metal ions and cellular metabolites. Although induced and depressed Chinese hamster cell glutamine synthetase...
appear to be identical by several catalytic and physical criteria (12), the existence of two enzyme species differing in their molecular specific activities was not precluded.

Regulation by specific degradation or inactivation by a variety of mechanisms is well documented in eucaryotic cells (16-20). The speed and apparent specificity in the response of glutamine synthetase to glutamine are similar to class-specific proteinase now implicated in enzyme regulation by Katsumu, Holzer, and their co-workers (21-25). Conformation appears to be an important factor in determining the susceptibility of protein structure to cleavage and degradation. Proteins undergo constant fluctuations in their three-dimensional structures (26, 27). As some conformations are more labile than others, the relative stabilization of a particular conformation by binding some component or by univalent modification could modulate the half-life of the molecule (24, 25, 28, 29).

Our studies relating glutamine synthetase activity and ATP (10-12) may also reflect the involvement of conformational equilibria in enzyme regulation. We have previously shown that ATP protects Chinese hamster glutamine synthetase from inactivation by heat or the sulfhydryl-specific reagent N-ethylmaleimide. In addition, ATP also potentiates inactivation by the glutamine analogs 6-diazo-5-oxo-L-norleucine or 5-diazo-4-oxo-L-norvaline. The experiments with cyanide reported in this paper indicate that ATP may be essential for glutamine-mediated depression of glutamine synthetase. Thus, in the presence of ATP, glutamine or some component proportional to glutamine concentration could bind to glutamine synthetase and enhance its susceptibility to degradation.

Immunoprecipitation of specific enzyme protein can be used to determine if a change in specific activity of an enzyme results from a corresponding change in level of the enzyme protein (30). The immunoprecipitation method most often used involves the addition of increasing amounts of enzyme activity to a constant amount of antibody. The antibody-antigen complex is removed by centrifugation and the residual activity left in the supernatant is measured. We find that this immunoprecipitation titration technique is not satisfactorily reproducible at the low glutamine synthetase concentrations present in Chinese hamster cell extracts. The concentration of glutamine synthetase in extracts of Chinese hamster cells with induced levels of enzyme is 10- to 100-fold less than that employed in other immunoprecipitation studies (30-39). Moreover, the concentration of glutamine synthetase in extracts of cells with depressed levels of enzyme is 10-fold lower.

When the amount of enzyme is kept constant and the amount of antibody is varied, a linear calibration curve can be obtained (Fig. 8) which relates the amount of glutamine synthetase protein to the amount of antibody needed to precipitate half the enzyme activity. As little as 2 ng of glutamine synthetase protein can be detected.

The data in Fig. 8 show that homogeneous glutamine synthetase from Chinese hamster liver and glutamine synthetase in cell extracts containing induced levels of enzyme display identical immunoprecipitation reactions. Thus, glutamine synthetase in cell extracts containing induced levels of enzyme possess the same molecular specific activity as homogeneous liver glutamine synthetase. Glutamine synthetase in extracts of cells grown for either short (2 hours) or long (24 hours) times in the presence of glutamine and which therefore contain depressed levels of activity have only a slightly lower molecular specific activity. If a glutamine-dependent modification reaction were responsible for the 8- to 10-fold decrease in glutamine synthetase activity, one would expect that the molecular specific activity of glutamine synthetase in extracts of cells containing depressed levels of activity would be 8- to 10-fold less than that for glutamine synthetase in extracts of cells containing induced levels of enzyme (Fig. 9, inset). The fact that we see only a slight decrease in molecular specific activity suggests that most of the glutamine synthetase protein that is present in extracts of cells containing induced levels of enzyme is either degraded upon depression of the enzyme activity or drastically altered in antigenicity. The latter possibility seems unlikely since a protein as large as glutamine synthetase (10) probably has many antigenic sites, only a few of which are likely to be altered by a modification.

A model for glutamine-mediated regulation of Chinese hamster glutamine synthetase which fits our data is illustrated in Fig. 10. Glutamine synthetase enzyme (E) is assumed to be synthesized at a rate independent of the glutamine concentration. Glutamine or some component directly proportional to the glutamine concentration (G) binds reversibly to the enzyme, making it more susceptible to degradation. The model predicts that the molecular specific activities of glutamine synthetase in cultured Chinese hamster cells grown for long times either in the presence or in the absence of glutamine should be the same. If the degradation step is fast, the amount of glutamine synthetase protein will decrease at the same rate as the decrease in activity. Under these conditions, the molecular specific activity also will be nearly the same in cells depressed for short times. Although we find that the molecular specific activity in cells depressed for short and long times is slightly less than that in cells containing induced levels of enzyme, this difference could result from the presence of a small amount of partially degraded but antigenic enzyme molecules.

A mathematical analysis of our model is consistent with the steady state levels of glutamine synthetase observed at different glutamine concentrations (Figs. 3 and 4). The rate of enzyme degradation is given by

\[ \frac{k_1 [Gln]}{k_a + [Gln]} \]

where \( k_1 \) is a rate constant, \( k_a \) is an affinity constant, and \( E \) is the relative fraction of cell protein that is glutamine synthetase. If \( E_I \) is the maximum relative fraction of enzyme protein in cells with induced levels of enzyme activity growing in the absence of glutamine, then:

\[ E = E_I - \frac{k_1 [Gln]}{k_a + [Gln]} \]

The specific activity \( S \) as a function of glutamine concentration is given by:

\[ S = S_I - \frac{k_1 [Gln]}{k_a + [Gln]} \]
responsible for glutamine-mediated degradation of glutamine appears to be inactivated when cells are lysed. Glutamine synthetase activity might occur by a glutamine-activated enzyme-specific protease or by a glutamine-mediated alteration in the enzyme's structure enhancing its susceptibility to degradation. Unfortunately, the molecular components required for depression appear to be inactivated when cells are lysed. Glutamine synthetase is remarkably stable in crude cell extracts in vitro in the presence or absence of glutamine. This equation can be rearranged to give: 

\[ k(S_1 - S) = k_1[Gln]^{-1} + (k_l + 1) \]

As predicted by this model, a plot of \((S_1 - S)^{-1}\) versus \([Gln]^{-1}\) should be a straight line. From Figs. 3 and 4, the specific activities from Figs. 3 and 4 give a straight line (Fig. 11).

In conclusion, the data presented in this paper suggest that the glutamine-mediated depression of glutamine synthetase activity results from degradation of enzyme molecules. Depression might occur by a glutamine-activated enzyme-specific protease or by a glutamine-mediated alteration in the enzyme's structure enhancing its susceptibility to degradation. Unfortunately, the molecular components required for depression appear to be inactivated when cells are lysed. Glutamine synthetase is remarkably stable in crude cell extracts in vitro in the presence or absence of glutamine. In future studies, we will attempt to identify and characterize the molecular mechanisms responsible for glutamine-mediated degradation of glutamine synthetase.

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