Regulation of Glutamine Synthetase by Dexamethasone in Hepatoma Tissue Culture Cells*

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In certain lines of hepatoma tissue culture (HTC) cells, glutamine synthetase (EC 6.3.1.2) specific activity is increased 2.5- to 3-fold by the addition of glucocorticoids to the growth media. Actinomycin D blocks both the induction and deinduction of glutamine synthetase by glucocorticoids, suggesting a requirement of RNA synthesis for both processes.

Using an antisera raised against purified rat liver glutamine synthetase, we have precipitated radiolabeled glutamine synthetase from HTC cells. Electrophoresis of the immunoprecipitates on sodium dodecyl sulfate-acrylamide gels isolates the subunit of glutamine synthetase and permits the radioactivity in the glutamine synthetase band to be quantitated. Using this technique, we have investigated the effect of dexamethasone, a synthetic glucocorticoid, on the rates of synthesis and degradation of glutamine synthetase.

Dexamethasone (10^-7 M) increases the rate of synthesis of glutamine synthetase 2- to 3-fold but has no effect on the rate of glutamine synthetase degradation. The rates of total cell protein synthesis and degradation are not significantly affected by dexamethasone. The presence of actinomycin D at the time of removal of dexamethasone from induced cells prevents the fall in the induced rate of synthesis of glutamine synthetase normally seen when the inhibitor is removed from the culture medium.

The regulation of glutamine synthetase by dexamethasone has been compared to the regulation of another dexamethasone-inducible enzyme in HTC cells, tyrosine aminotransferase, and been found to be similar in all parameters studied.

Glutamine synthetase [L-glutamate:ammonia ligase (ATP), EC 6.3.1.2] plays a central role in the metabolism of living cells because of the multiplicity of metabolic pathways which require glutamine (1, 2). In Escherichia coli, the importance of glutamine synthetase is underscored by the multiple levels of control which regulate its activity. These include feedback inhibition by small molecules (3, 4), adenylylation of the enzyme subunit polypeptide catalyzed by a "cascade" of regulatory enzymatic activities (1), and transcriptional control, possibly by glutamine synthetase itself (5, 6). There is also evidence that the glutamine synthetase polypeptide itself acts as a regulatory element in the control of several other enzymes (5).

Elucidation of the regulation of glutamine synthetase in eukaryotes is less advanced. Evidence has been presented that sheep brain, rat liver (7, 8), and Chinese hamster liver (9) glutamine synthetases are directly regulated by metabolic compounds. In addition, glucocorticoids (10-14), glutamine (11-13, 15-17), and cAMP (18, 19) have been reported to regulate the level of glutamine synthetase activity in animal cells. In chick embryo neural retina, glucocorticoids cause an increase in the rate of synthesis of glutamine synthetase (14).

In this report, we describe further investigations of the mechanism of steroid induction of glutamine synthetase. Employing a rabbit antibody raised against purified rat liver glutamine synthetase (20), we have examined the effect of dexamethasone, a synthetic glucocorticoid, on the rates of synthesis and degradation of glutamine synthetase in hepatoma cells. The control of glutamine synthetase and tyrosine aminotransferase is compared and found to be remarkably similar.

MATERIALS AND METHODS

Chemicals—All chemicals were from Sigma or Mallinckrodt and were of the highest quality available unless otherwise specified. [15N]Methionine (330 Ci/mmol) was obtained from New England Nuclear.

Media and Growth of Cells—The cells used in this study are subclones of a hepatoma tissue culture line derived from Morris hepatoma 7285C. GM22 is a glutamine prototrophic subclone selected by growth in soft agar without glutamine (12). ST3 is a glutamine auxotrophic subclone, picked under nonselective conditions from soft agar. Cells were grown in spinner and monolayer in Swim’s S7 medium as described (22, 23), except that 10% calf serum (Gibco) was used as a serum supplement.

Preparation of Cell Extracts—Cells were grown to a density of 5 × 10^6 cells/ml. About 10^7 cells were centrifuged at 160 g for 5 min and the pellet was resuspended by gentle shaking. The cells were washed once in 0.15 M NaCl and resuspended in 1 ml of the same. Cells were sonicated with a Bronwill Biosonik Sonicator (20% output; two 10-s bursts at 4°C) and centrifuged at 160 × g for 5 min at 4°C, and the supernatant was used for enzyme assays.

Enzyme Assays—Glutamine synthetase activity was measured by the γ glutamyltransferase assay (12). A 1 ml reaction mixture contained 120 mM L-glutamine, 40 mM imidazole-HCl, 10 mM Na2AsO3, 50 mM ATP, 1.2 mg/ml bovine serum albumin, 30 mM NH4OH, final pH 6.0, and up to 500 μg of protein from the cell extract. The reaction ingredients were made up in two solutions: I contained glutamine, imidazole-HCl, Na2AsO3, and ATP, and II contained MnCl2 and NH4OH. These were stored at 4°C and were stable for up to 6 months. The enzymatic reaction was allowed to proceed for up to 75 min and was stopped by the addition of 0.75 ml of 0.51 M trichloroacetic acid, 0.8 M HCl, and 0.063 M FeCl3. Denatured protein was removed by centrifugation at 50 × g for 2 min in a Sorvall GLC-3 centrifuge. The absorbance at 500 nm of the supernatant was then measured in a Zeiss spectrophotometer and compared with the absorbance obtained in a standard curve with known amounts of γ-glutamyl hydroxamate. One unit of glutamine synthetase activity is defined as the amount of enzyme which produces 1 pmol of γ-glutamyl hydroxamate per h. Glutamine synthetase was obtained from rabbits. This antiserum was assayed as described (22).

Immunological Techniques—Antiserum against purified rat liver glutamine synthetase was obtained from rabbits. This antiserum was purified by ammonium sulfate precipitation and DEAE-cellulose chromatography (20). Sheep antiserum to rabbit IgG was a generous gift from Dr. Patricia Jones. It was purified by a 30 to 40% ammonium sulfate fractionation followed by dialysis overnight against 10 mM NaH2PO4/Na2HPO4 and 50 mM NaCl, pH 7.5.

Antibody-Antigen Reaction—Precipitin tests were performed by adding increasing volumes of rabbit IgG to a fixed concentration of sheep anti-rabbit IgG in a 1-ml reaction mixture containing 0.15 M NaCl. After incubation overnight at 4°C, the reaction mixtures were centrifuged at 10,000 × g for 5 min at 4°C and the pellets were resuspended by gentle shaking. The cells were incubated with glutamine concentrations fixed as noted in individual experiments. The final protein concentration was adjusted to a value between 5 and 8 mg/ml with bovine serum albumin. The reaction was allowed to proceed for 2 h at 4°C. IgG antiserum was then added, usually 2-fold in excess of the equivalence point of the glutamine synthetase antiserum added previously. The total volume was 1 ml. After overnight incubation at 4°C, the reaction mixtures were centrifuged at 30,000 × g for 10 min, and the supernatants were assayed for glutamine synthetase activity.

Labeling of Protein for SDS-PAGE—5 × 10^6 cells were plated on a 100-mm plastic dish. After the first freeze, the pellets may be stored at -60°C for up to 2 months without loss of glutamine synthetase activity. Extracts were centrifuged at 30,000 × g for 10 min to remove denatured protein. The supernatant was removed with a Clinac micropipetter holding a 50-μl glass tip and transferred to a disposable glass test tube (10 × 75 mm). The extracts are adjusted to 50 μl with 0.15 M NaCl and 42.2 μl of a solution containing equal parts of 0.1 M ATP, pH 7.0, 0.1 M MnCl2, 10 mM L-[3H]methionine, and 1 mM NH4OH was added. Glutamine synthetase stability at 0°C requires the substrates to be present. The substrates, however, prevent the formation of the glutamine synthetase-antiglutamine synthetase complex unless diluted 20-fold or more. The extracts, therefore, are brought to room temperature, sealed with parafilm, and heated in a 64°C water bath for 5 min. This step results in a 3- to 4-fold purification with no loss of glutamine synthetase activity. After cooling in an ice bath, the samples were transferred to polystyrene test tubes (12 × 75 mm) and centrifuged at 30,000 × g for 10 min to remove denatured protein. The supernatant was recovered, transferred to 5-ml Pyrex conical glass tubes, and diluted with cold 0.15 M NaCl to 0.9 ml. A 50-μl sample was taken for protein determination, and the glutamine synthetase activity of nonlabeled samples prepared in the same manner was determined. In order to determine the amount of glutamine synthetase antiserum required to be 1.5 to 2.0 times in excess of the glutamine synthetase activity was determined and added to all labeled samples.

Preparation of [3H]Methionine-Labeled Samples for Sodium Dodecyl Sulfate Gels—Immunoprecipitates formed during the overnight incubation at 4°C were washed and centrifuged 3 times in 0.15 M NaCl at 990 × g for 20 min, and then in glass-disked water at 2100 × g for 10 min. The pellet was then resuspended in 50 to 500 μl of sample buffer (10% (w/v) glycerol, 5% (v/v) 2-mercaptoethanol, 2.3% (w/v) SDS, and 0.0025% Triton HCl, pH 6.8). This solution was either stored immediately at -90°C, or heated in boiling water for 1 to 2 min to disaggregate proteins. Twenty to forty microliters containing no more than 40 μg of protein were applied to each of the gel wells. Protein determinations of the extract in sample buffer were made using the method of Lowry et al. (24) using bovine serum albumin (Sigma) as a standard. Tyrosine aminotransferase was assayed as described (22).

Quantitation of Gel Bands—The optical density of the image on x-ray film increases linearly with respect to radioactivity from 0.5 to 3.2 (27). All data reported here were either on the linear portion of the curve or were corrected using a standard curve.

Identification of Radiolabeled Glutamine Synthetase on Gels—This is described in the supplemental material.

1This section (including Figs. 1S and 4S and Plates 1S and 2S) is presented in miniprint at the end of this paper. Full size photocopies
RESULTS

Effect of Dexamethasone on the Specific Activity of Glutamine Synthetase—Dexamethasone increases the specific activity of glutamine synthetase in a dose-dependent response (Fig. 1) after a 12-h exposure of cells to the steroid. Tyrosine aminotransferase specific activity assayed from cells treated identically is also shown (Fig. 1) to increase significantly. Both enzymes are induced to half-maximal activities by \(5 \times 10^{-8}\) M dexamethasone. Since the accumulation of dexamethasone-bound steroid receptor in the nucleus follows this same dose-response curve (28), the induction of glutamine synthetase and tyrosine aminotransferase likely involves the same steroid receptor molecule. At \(10^{-7}\) M dexamethasone, on optimal dose of steroid, increased specific activity of glutamine synthetase is seen 2 h after the steroid is added to the growing cells. Maximum activity is seen after 10 to 12 h of growth with the steroid.

If dexamethasone is washed out of cells previously grown for 12 h with dexamethasone, a decline of glutamine synthetase activity to the basal level occurs (Fig. 2a). The kinetics of the decline in specific activity are variable but usually occur with a half-time of 4 to 6 h. The continued presence of dexamethasone is required for the maintenance of glutamine synthetase activity at the induced level.

Actinomycin D (0.1 μg/ml) inhibits RNA synthesis by 98% in HTC cells (29), and inhibits dexamethasone induction of glutamine synthetase in excess of 90%. 2 × 10^{-4} M cycloheximide, which inhibits protein synthesis by more than 97% (13, 30) also inhibits the dexamethasone induction of glutamine synthetase in excess of 90%. These results suggest that both RNA and protein synthesis are required for glutamine synthetase induction, confirming the results of Kulka and Cohen (13).

The effect of actinomycin D on tyrosine aminotransferase “deinduction” is central to the analysis of proposed post-transcriptional mechanisms of steroid action in HTC cells (31, 32). Actinomycin D added to induced cultures at the time of dexamethasone removal prevents the decline in enzyme activity and the decline in the relative rate of synthesis of tyrosine aminotransferase that occurs when actinomycin D is not added to the culture medium. If 0.5 μg/ml of actinomycin D is added to one of two sets of induced cultures washed free of dexamethasone, glutamine synthetase specific activity remains elevated in the actinomycin D-treated cultures but not in the untreated cultures (Fig. 2b). This result suggests that RNA synthesis is required for the fall in glutamine synthetase activity that occurs when dexamethasone is removed from the medium.

Immunoprecipitation of Glutamine Synthetase—We next wanted to study the rates of synthesis and degradation of glutamine synthetase. Rabbit antiserum raised against purified rat liver glutamine synthetase (20), together with sheep anti-rabbit IgG, was used in an indirect immunoprecipitation to assay hepatoma glutamine synthetase. Precipitin curves of anti-glutamine synthetase were used to determine optimum conditions for the immunoprecipitation studies.

Anti-glutamine synthetase was used to titrate glutamine synthetase from cells grown in medium containing 0.2 mM and 2 mM glutamine, in the presence and absence of 10^{-7} M dexamethasone. The glutamine concentration in the culture medium regulates glutamine synthetase specific activity (12, 13) and concentration (33). The results were normalized to the data with the greatest activity (0.2 mM glutamine plus dexamethasone). The immunoprecipitation curves were plotted as shown in Fig. 3. Each of the four extracts has the same equivalence point, suggesting that the enzyme in each extract is antigenically indistinguishable from the other. The data also show that the precipitation of glutamine synthetase by rabbit anti-rat liver glutamine synthetase is reproducible and effective in precipitating glutamine synthetase activity in solution. The interaction of glutamine synthetase with antiglutamine synthetase does not significantly affect glutamine synthetase catalytic activity. The decrease in supernatant glutamine synthetase activity with addition of antiglutamine synthetase thus is a true reflection of immunoprecipitation.

Effect of Dexamethasone on the Synthesis of Glutamine Synthetase—The requirement of protein synthesis for glutamine synthetase induction might be due to the synthesis of another protein which then modified pre-existing glutamine synthetase, as has been suggested for the mechanism of steroid induction of alkaline phosphatase in HeLa cells (34). Alternatively, it might reflect the synthesis of glutamine synthetase itself. To determine whether the synthesis of glutamine synthetase increases in the presence of dexamethasone, cultures grown in the presence or absence of dexamethasone were incubated for various lengths of time in labeling medium, containing [\(^{35}\)S]methionine, with and without dexamethasone. Immunoprecipitates were prepared and subjected to electrophoresis as described under “Materials and Methods.” The glutamine synthetase peak on tracings of the gel patterns was cut out, weighed, and normalized to the amount of protein applied to the gel. The data from these experiments are shown in Fig. 4. In cells grown in the presence of dexamethasone, the rate of incorporation of isotope into glutamine synthetase from induced cells is 2 to 2.5 times greater than in cells grown in the absence of dexamethasone. Dexamethasone does not significantly influence the rate of isotope incorporation into total protein, however (inset, Fig. 4) (35).

In this experiment, an average of 2 × 10^{6} cpm were incorporated into 1 mg of soluble protein in 30 min. The immunoprecipitate from 1 mg of protein was 20 μg, 2% of the total protein, and contained 4 × 10^{5} cpm. By equating the counts per min in the immunoprecipitate with the total area under the gel tracing, the extent of incorporation was determined to be about 4,000 cpm in induced glutamine synthetase and 1,200 cpm in uninduced glutamine synthetase. Glutamine synthetase thus contains 0.2% and 0.06% of the isotope incorporated.

**Fig. 1.** Dose-response curve of dexamethasone (Dex) effect on glutamine synthetase (GS) and tyrosine aminotransferase (TAT) in GM22 cells. Cells grown in growth medium were given various concentrations of dexamethasone. Twelve hours later, glutamine synthetase and tyrosine aminotransferase specific activities from each set of cultures were determined as described under "Materials and Methods."
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Fig. 2. A, effect of dexamethasone (Dex) removal on glutamine synthetase activity. Cells grown 12 h in growth medium containing 10⁻⁷ M dexamethasone were centrifuged at 160 x g for 5 min at 25°C, the supernatant decanted, and the cell pellet resuspended in growth medium, either: Δ, with dexamethasone; or ○, without dexamethasone. Glutamine synthetase activity was determined at various times.

B, effect of 0.5 mg/ml of actinomycin D (AMD) on glutamine synthetase activity following dexamethasone removal. The protocol in A was followed except that cells were resuspended in growth medium containing one of the following: ○, no additions; □, 10⁻⁷ M dexamethasone; Δ, 10⁻⁷ M dexamethasone plus 0.5 µg/ml of actinomycin D; or △, 0.5 µg/ml of actinomycin D. Glutamine synthetase activity was determined at various times.

Effect of Dexamethasone on the Degradation of Glutamine Synthetase—We then investigated the effect of dexamethasone on the rate of degradation of glutamine synthetase. Induced and uninduced cells were labeled for 35 min and then incubated in growth medium without isotope, with or without dexamethasone, for varying lengths of time. The label remaining in glutamine synthetase was determined as above and plotted, and the half-time of degradation was calculated. The results (Fig. 5) show that the half-time of degradation of glutamine synthetase from cells grown in the presence or absence of dexamethasone is about 1.75 h. Thus, dexamethasone appears to have no effect on the rate of degradation of glutamine synthetase. The degradation of total protein is also not significantly influenced by growth in dexamethasone (inset, Fig. 5).

Kinetics of Glutamine Synthetase Induction and Deinduction—The time course for the increase in the rate of glutamine synthetase synthesis after dexamethasone addition was determined. A set of cultures was pulsed with [⁴⁰S]methionine for 30 min after having been exposed to dexamethasone for various lengths of time. The results, shown in Fig. 6 (solid line), indicate that a new steady state rate of glutamine synthetase synthesis is reached after about a 4-h exposure to dexamethasone. The prompt increase in the rate of synthesis of glutamine synthetase is consistent with the idea that the synthesis of other gene products is not first required for the subsequent increase in the rate of glutamine synthetase synthesis. Glutamine synthetase specific activity (hatched line) follows after a lag of 30 to 40 min.

The time course for the fall of the rate of glutamine synthetase synthesis after dexamethasone removal is shown in Fig. 6 (dashed line). The prompt increase in the rate of synthesis of glutamine synthetase is consistent with the idea that the synthesis of other gene products is not first required for the subsequent increase in the rate of glutamine synthetase synthesis. Glutamine synthetase specific activity (hatched line) follows after a lag of 30 to 40 min.

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Fig. 3. Equivalence titrations varying antibody. Cells were grown for 24 h in growth medium containing: ○, 2 mM glutamine; △, 2 mM glutamine plus 10⁻⁷ M dexamethasone; Δ, 0.2 mM glutamine; and ○, 0.2 mM glutamine plus 10⁻⁷ M dexamethasone. Extracts were then prepared and analyzed as described under "Materials and Methods," and the results were normalized to the control value of the 0.2 mM glutamine plus 10⁻⁷ M dexamethasone extract. GS, glutamine synthetase.

tase activity following dexamethasone removal. The protocol in A was followed except that cells were resuspended in growth medium containing one of the following: ○, no additions; □, 10⁻⁷ M dexamethasone; Δ, 10⁻⁷ M dexamethasone plus 0.5 µg/ml of actinomycin D; or △, 0.5 µg/ml of actinomycin D. Glutamine synthetase activity was determined at various times.

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7 (hatched line). Cells were pulsed for 30 min with [35S]methionine at various times after removal of dexamethasone. The rate of glutamine synthetase synthesis falls with a half-time of roughly 3 to 4 h. The continued presence of dexamethasone is thus required for the elevated rate of glutamine synthetase synthesis.

Actinomycin D stabilization of glutamine synthetase specific activity (Fig. 2b) could be due to either a maintenance of the rate of synthesis or to a decrease in the rate of degradation or both. To determine which is the case, cells grown with dexamethasone were removed to dexamethasone-free media. 0.5 μg/ml of actinomycin D was added to one of two sets of these induced cultures, while the other culture received no additions after removal to the dexamethasone-free media. Fig. 7 (solid line) demonstrates that glutamine synthetase synthesis continued for at least 6 h in the presence, but not in the absence, of actinomycin D. Thus, maintenance of the specific activity of glutamine synthetase under these conditions is due to the maintenance of the rate of glutamine synthetase synthesis, and RNA synthesis appears to be required for deinduction.

Comparison of Glutamine Synthetase and Tyrosine Aminotransferase Regulation by Dexamethasone—Dexamethasone regulates the levels of both glutamine synthetase and tyrosine aminotransferase in HTC cells. It is of interest to compare the regulation of the two enzymes because the two may share a common mechanism of control responsive to glucocorticoids. Accordingly, a comparison of several parameters of steroid control of glutamine synthetase and tyrosine aminotransferase by dexamethasone is presented in Table I. Using the parameters listed in Table I, no differences in the regulation of the two enzymes by dexamethasone were found, suggesting the regulation follows similar or identical pathways.

FIG. 5. Effect of dexamethasone on the degradation of glutamine synthetase. Cells grown as in Fig. 4 were incubated in [35S]methionine as in Fig. 4 for 35 min, then washed and resuspended in growth medium with or without 10⁻⁷ M dexamethasone, for various lengths of time. The cells were then processed as in Fig. 4. Δ, plus dexamethasone; 0, minus dexamethasone. Inset, the rate of degradation of general protein in the same cells. The procedure in inset, Fig. 4, was followed.

FIG. 6. Kinetics of the dexamethasone (Dex) induction of glutamine synthetase (GS) rate of synthesis. Cells grown in growth medium were exposed to 10⁻⁷ M dexamethasone for various lengths of time. The cells were then washed in 0.15 M NaCl and resuspended in labeling medium with 170 μCi/ml of [35S]methionine and 10⁻⁷ M dexamethasone (except zero time cultures, which did not receive dexamethasone) for 30 min. Extracts were prepared as in Fig. 4. 25 μl was taken from each for glutamine synthetase assays, and immunoprecipitates were made and analyzed as in Fig. 4. Δ, glutamine synthetase peak weight; A, glutamine synthetase specific activity.

FIG. 7. Effect of the removal of dexamethasone (Dex), in the presence and absence of actinomycin D (AMD), on the rate of synthesis of glutamine synthetase (GS). Cells were grown in growth medium containing 10⁻⁷ M dexamethasone for 12 h. They were then washed in 0.15 M NaCl and resuspended in growth medium without dexamethasone and either with (Δ) or without (0) 0.5 μg/ml of actinomycin D for various times. Cells were labeled and immunoprecipitates were prepared and analyzed as described in Fig. 6.

TABLE I

Comparison of some parameters of the regulation of glutamine synthetase and tyrosine aminotransferase by dexamethasone

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glutamine synthetase</th>
<th>Tyrosine aminotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of synthesis (% of total)</td>
<td>Induced: 0.2</td>
<td>Induced: 0.5 (36)</td>
</tr>
<tr>
<td></td>
<td>Uninduced: 0.06</td>
<td>Uninduced: 0.05 (36)</td>
</tr>
<tr>
<td>Rate of degradation (half-time)</td>
<td>Induced: 1.75 h</td>
<td>Induced: 4 h (44)</td>
</tr>
<tr>
<td></td>
<td>Uninduced: 1.75 h</td>
<td>Uninduced: 4 h (44)</td>
</tr>
<tr>
<td>Induction</td>
<td>Extent: 2-3×</td>
<td>Extent: 8-10× (35)</td>
</tr>
<tr>
<td>RNA synthesis required</td>
<td>Yes</td>
<td>Yes (28)</td>
</tr>
<tr>
<td>Protein synthesis required</td>
<td>Yes</td>
<td>Yes (29)</td>
</tr>
<tr>
<td>Effect on enzyme rate of synthesis</td>
<td>Inc. 2-3×</td>
<td>Inc. 10× (36)</td>
</tr>
<tr>
<td>Effect on enzyme rate of degradation</td>
<td>None</td>
<td>None (44)</td>
</tr>
<tr>
<td>Kinetics (half-time)</td>
<td>Specific activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4-5 h</td>
<td>4-5 h (37)</td>
</tr>
<tr>
<td>Rate of synthesis</td>
<td>2-3 h</td>
<td>3 h (36)</td>
</tr>
<tr>
<td>Deinduction</td>
<td>RNA synthesis required for:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Specific activity decrease</td>
<td>Yes (38)</td>
</tr>
<tr>
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<td>Rate of synthesis decrease</td>
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<td>Protein synthesis required</td>
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<td></td>
<td>Specific activity</td>
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<td>4-6 h</td>
<td>3-6 h (39)</td>
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<tr>
<td>Rate of synthesis</td>
<td>4 h</td>
<td>2.5 h (39)</td>
</tr>
<tr>
<td>Dose-response curve (half-maximal dose)</td>
<td>5 × 10⁻¹⁷ M</td>
<td>5 × 10⁻¹⁷ M (40, -7)</td>
</tr>
</tbody>
</table>

* Rate in cells grown in medium supplemented with 10% calf serum.
* The absolute rate of tyrosine aminotransferase synthesis decreases; the rate relative to that of cellular protein synthesis does not (38). In some experiments, glutamine synthetase synthesis in the absence of actinomycin D falls slightly after dexamethasone removal (R. B. Crook, unpublished observations).
* A second mode of control, mediated by glutamine, exists for glutamine synthetase in HTC cells (32). The absence of protein synthesis alters this mode, thus affecting glutamine synthetase specific activity.
* This paper.

DISCUSSION

The increase in the rate of synthesis of glutamine synthetase induced by dexamethasone is consistent with effects of corti-
corticosteroids reported on other functions in various systems (41, 43). The requirement for RNA synthesis which precedes the increase in glutamine synthetase synthesis suggests the possibility that glutamine synthetase mRNA must increase in concentration before the synthesis of glutamine synthetase itself increases. Models to explain the induction of glutamine synthetase by dexamethasone which propose an increase in another RNA species (such as a tRNA) which increases the translation of glutamine synthetase mRNA cannot be ruled out, however.

The maintenance of the induced level of glutamine synthetase by actinomycin D in the absence of steroid suggests that a new gene product is required to produce the decline in the rate of glutamine synthetase synthesis. A simple model previously advanced in this laboratory to explain the regulation of tyrosine aminotransferase by steroids (32) may be applied to glutamine synthetase as well. This model proposes that in addition to glucocorticoids increasing the concentration of the specific mRNA for glutamine synthetase, a second labile RNA or protein product of that RNA inactivates the glutamine synthetase-specific mRNA. When actinomycin D is added to induced cells and steroid is withdrawn, the labile factor inactivating the mRNA for glutamine synthetase is lost. The concentration of glutamine synthetase-specific RNA therefore remains constant, and the induced rate of glutamine synthetase synthesis is maintained. The phenomenon of actinomycin D maintaining functions in the absence of their inducers is not uncommon (44), regardless of the mechanism by which the influence is effected.

The half-time of degradation of glutamine synthetase of 1.75 h in medium containing 2 mM glutamine places it, along with tyrosine aminotransferase, in a class of proteins distinct from the majority of cellular proteins in HTC cells, which have half-lives averaging 17 h (45).

In HTC cells, the similarity of the regulation by dexamethasone suggests that glutamine synthetase and tyrosine aminotransferase may be regulated by similar, if not identical, mechanisms and that the common intermediate in the induction of both enzymes is the steroid receptor. That both enzymes decline with similar half-times raises the possibility that the labile degrading functions, which depress enzyme synthesis in the absence of inducer, are also similar. The only salient difference between the steroid-mediated inductions of glutamine synthetase and tyrosine aminotransferase is the extent of the induction itself.

Other mechanisms, however, are clearly not shared by glutamine synthetase and tyrosine aminotransferase. A 10-fold reduction in the extracellular glutamine concentration results in a 6- to 8-fold increase in glutamine synthetase specific activity (19, 13, 33), whereas tyrosine aminotransferase activity decreases somewhat.4 The effect on tyrosine aminotransferase is probably due to a decrease in the rate of synthesis observed in general for cellular protein under glutamine stepdown conditions (33). Also, HTC cells when incubated for 24 h with 10-5 M BrdU show a 10-fold reduction in tyrosine aminotransferase specific activity, but an increase in the specific activity of glutamine synthetase.

A third steroid-inducible function, surface factor, which promotes cell adhesion to surfaces, has been studied in HTC cells (46, 47). It appears to be controlled differently from glutamine synthetase and tyrosine aminotransferase in that deinduction occurs in the presence or absence of actinomycin D.

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REFERENCES

Regulation of Glutamine Synthetase in HTC Cells


SUGGESTED MATERIAL

1. Regulation of Glutamine Synthetase by Demethylation

In Hepatocytic Tissue Culture Cells

by

Richard A. Stark, Harueo Hori, Thomas F. Davies, and Gordon M. Tomkins

Specifications of Modified Glutamine Synthetase on Gel

Extracts of cells labeled for 3 h. with [55S]methionine were reacted with glutamine synthetase antisera. The immunoprecipitates were then subjected to electrophoresis in a 10% acrylamide slab gel as described in Materials and Methods. The resulting pattern was of three bands in each lane (Figure 1). A variety of proteins with intermediate, which is present in the immunoprecipitation, and serum from normal rat liver, but not in the serum from rat liver, was also present in the immunoprecipitation, and serum from normal rat liver, but not in the serum from rat liver, was also present in the immunoprecipitation, and serum from normal rat liver, but not in the serum from rat liver, was also present in the immunoprecipitation, and serum from normal rat liver, but not in the serum from rat liver, was also present in the immunoprecipitation, and serum from normal rat liver, but not in the serum from rat liver, was also present in the immunoprecipitation, and serum from normal rat liver, but not in the serum from rat liver, was also present in the immunoprecipitation, and serum from normal rat liver, but not in the serum from rat liver, was also present in the immunoprecipitation, and serum from normal rat liver, but not in the serum from rat liver, was also present in the 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Regulation of glutamine synthetase by dexamethasone in hepatoma tissue culture cells.
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