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Changes in the Levels of Translatable Glutaminase mRNA during Onset and Recovery from Metabolic Acidosis†

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Received October 2, 1986; Revised Manuscript Received January 7, 1987

ABSTRACT: The amount of the mitochondrial glutaminase present within rat kidney is increased 5-fold during chronic metabolic acidosis. This adaptive response is due to a corresponding increase in the relative rate of glutaminase synthesis. Poly(A+) RNA was purified from the kidneys of control, 7-day acidotic, and 2-day recovered rats and then fractionated by electrophoresis on a low melting temperature agarose gel. Translation of the fractionated RNA in a rabbit reticulocyte lysate yields a 72 000-dalton protein that is specifically precipitated by anti-glutaminase IgG. The level of this protein is at least 3-fold greater in the translation products of the fractionated poly(A+) RNA derived from the acidotic vs. control or recovered rats. Therefore, the 72 000-dalton product of translation is the apparent precursor to the 68 000- and 65 000-dalton proteins that are contained in the mitochondrial glutaminase. From its relative electrophoretic mobility, the size of the glutaminase mRNA was estimated to be approximately 6.5 kilobases. The relative levels of translatable glutaminase mRNA were determined by using unfractionated poly(A+) RNA prepared from rats at various times following onset and recovery from acidosis. The observed increase occurred gradually, requiring 7 days to reach a maximal induction of 4.2-fold. The increase could be due to the increased transcription of a stable mRNA ($t_{1/2} \sim 3$ days). However, 2 days of recovery was sufficient to return the level of translatable glutaminase mRNA to normal. Thus, the selective inactivation or the altered stability of the glutaminase mRNA must also contribute to the regulation of the glutaminase gene expression.

Plasma glutamine is an important substrate for oxidative metabolism and the biosynthesis of many nitrogen-containing compounds (Haussinger & Sies, 1984). The catabolism of glutamine occurs primarily in the small intestine, brain, liver, and kidney, where it is initiated by a mitochondrial glutaminase (Kovacevic & McGivan, 1983). The hepatic glutaminase is a unique isoenzyme that has a high K_M for glutamine and is dependent upon NH_4^+ ions (Patel & McGivan, 1984). The glutaminases contained in the three other tissues exhibit similar kinetics (Haser et al., 1985), require a polyvalent anion, and are structurally and immunologically related (Curthoys et al., 1976). However, only the renal glutaminase activity exhibits an adaptive increase in response to metabolic acidosis (Tong et al., 1986).

During normal acid–base balance, the rat kidney extracts very little, if any, of the plasma glutamine (Squires et al., 1976; Hughey et al., 1980). Renal extraction is increased rapidly following the onset of acute acidosis and is sustained during chronic acidosis. The initial increase in renal catabolism of glutamine results primarily from changes in the concentration of metabolites and H^+ that regulate flux through glutaminase, glutamate dehydrogenase, and α-ketoglutarate dehydrogenase (Tannen & Sastrasinh, 1984). The resulting increase in ammoniagenesis provides an expendable cation that facilitates the excretion of acids and conserves Na^+ and K^+ ions. The initial changes in renal metabolites and in plasma pH are largely compensated during chronic acidosis (Parry & Brosnan, 1978). The increased renal catabolism of glutamine is sustained by the gradual increase in the relative rate of glutaminase synthesis (Tong et al., 1986). The increased rate of synthesis reaches a plateau within 5 days that is 5.3-fold greater than normal. The apparent half-life for glutaminase degradation is unaltered during acidosis. As a result, the total renal glutaminase activity is increased approximately 5-fold within 7 days after the onset of acidosis.

In this study, renal poly(A+) RNA was purified from control, acidotic, and recovered rats and translated in a rabbit reticulocyte lysate. Specific immunoprecipitation procedures were used to identify the primary translation product of the mitochondrial glutaminase and to further characterize the mechanisms responsible for the regulated expression of the renal glutaminase.

Materials and Methods

Guanidinium thiocyanate and sodium *N*-laurylsarcosine were purchased from Fluka. DEAE Affi-Gel Blue and reagents for polyacrylamide gel electrophoresis were products of Bio-Rad. ^{14}C-Labeled protein molecular weight standards

† This investigation was supported in part by Research Grant AM 16651 from the National Institutes of Health.
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0006-2960/87/0426-2773$01.50/0

and λ DNA–*Hin*dIII fragments were purchased from Bethesda Research Laboratories. [^{35}S]Methionine (specific activity 800 Ci/mmol) was obtained from New England Nuclear. Oligo(dT)–cellulose type II was purchased from Collaborative Research. Calf liver tRNA and creatine phosphokinase were products of Boehringer Mannheim. Low melting temperature agarose was purchased from FMC. Methylmercury hydroxide was obtained from Alfa. Glass fiber filters and 0.025-μm membranes (type VS) were purchased from Whatman and Millipore, respectively. Sodium dodecyl sulfate was obtained from Gallard/Schlesinger. All other biochemicals were obtained from Sigma.

Male Sprague-Dawley rats (200–250 g) were purchased from Zivic–Miller and maintained on Purina Rat Chow. Acidosis was induced by providing 1.5% NH_4Cl as the sole source of drinking water. After drinking the NH_4Cl solution for 5 days, a set of acidotic rats was given water and allowed to recover. The glutaminase was purified from rat brain (Haser et al., 1985), and specific anti-glutaminase IgG was prepared (Tong et al., 1986) as described previously.

Total RNA was isolated from the kidneys of control, metabolic acidotic, and recovered rats according to the procedure of Chirgwin et al. (1979). Poly(A+) RNA was isolated by chromatography on oligo(dT)–cellulose (Aviv & Leder, 1972). Approximately 10 mg of total RNA was heated to 70 °C for 1 min and immediately diluted into 40 mL of 0.5 M NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA),[1] 0.1% SDS, and 10 mM Tris-HCl, pH 7.4. The diluted sample was then applied to a 3-mL oligo(dT)–cellulose column that was preequilibrated with the dilution buffer. The initial eluant was reapplied to the column and after being washed with 40 mL of dilution buffer minus SDS, the bound poly(A+) RNA was eluted with 1 mM EDTA and 10 mM Tris-HCl, pH 7.4.

Poly(A+) RNA was further fractionated on a 1% low melting temperature agarose gel containing 5 mM methylmercury hydroxide (Weislander, 1979). Approximately 50 μg of poly(A+) RNA was denatured by heating with 10 mM methylmercury hydroxide for 1 min at 65 °C, loaded onto the gel, and electrophoresed at 1.5 V/cm for 16 h. The gel was then soaked for 1 h in 10 mM 2-mercaptoethanol. Lanes containing total RNA were stained with ethidium bromide, and the 18S and 28S RNAs were used as rough molecular weight guides. The gel was cut into slices approximately 3 mm in width. The RNA in each slice was extracted by melting the agarose in 0.5 mL of 0.5 M ammonium acetate. An equal volume of phenol was added, and the samples were centrifuged in a Beckman Microfuge for 5 min. The aqueous phase was removed, and the poly(A+) RNA was precipitated by addition of 10 μg of calf liver tRNA and 2 volumes of ethanol.

The amount of the fractionated poly(A+) RNA recovered was determined by hybridization with [^{3}H]poly(U) (Milcarek et al., 1974). Appropriate amounts of fractionated poly(A+) RNA were incubated in 10 μL of [^{3}H]poly(U) (specific activity 7.0 × 10^5 cpm/μg) in the presence of 2× SSC. Hybridization was carried out for 10 min at 42 °C. Pancreatic ribonuclease was added to a final concentration of 20 μg/mL, and the samples were incubated for 15 min at 4 °C. Following the addition of 10 μg of tRNA, RNase-resistant RNA was precipitated with trichloroacetic acid and collected on a glass fiber filter. The average size of poly(A+) RNA is about 2000 nucleotides, 200 nucleotides of which are poly(A). Therefore, the amount of poly(A+) RNA was estimated by multiplying the equivalent value derived from a poly(A) standard curve (0.1–2.0 ng) by a factor of 10.

Cell-free protein synthesis was carried out by using a micrococcal nuclease treated rabbit reticulocyte lysate that was prepared by Dr. R. Jagus according to the method of Pelham and Jackson (1976). Assays were carried out in a total volume of 55–60 μL containing 40 μL of reticulocyte lysate, 2.5 μL of 2 M KCl and 10 mM magnesium acetate, 2.5 μL of a 2–5 mM mixture of the 19 common amino acids minus methionine, 2.5 μL of 0.2 M phosphocreatine, 0.5 μL of 200 units/mL creatine phosphokinase in 100 mM KCl and 50% glycerol, 1 μL of 1 mM hemin in ethylene glycol, 5 μL of [^{35}S]methionine (10 mCi/mL), and appropriate amounts of poly(A+) RNA which was denatured with 2.5 mM methylmercury hydroxide. Pretreatment of the poly(A+) RNA with the denaturant significantly increased the amount of 72 000-dalton protein obtained in the specific immunoprecipites in the in vitro translations. After being incubated at 30–32 °C for 30 min, 5 μL of 100 mM methionine was added, and the translates were microdialyzed against 25 mL of 85 mM Tris-HCl (pH 7.4), 11 mM $MgCl_2$, 0.1 mM EDTA, and 0.1% Nonidet P-40 (TME-40) for 15 min using a 0.025-μm membrane. A 200-μL aliquote of TME-40 containing 10 mM methionine was added, and the sample was centrifuged for 1 h at 210000g_{max} in a 50 Ti rotor. The supernatant was incubated with 1 μL of rabbit anti-glutaminase IgG (~20 units/mL) at room temperature for 30 min and then at 4 °C for 60 min. A 10-μL aliquot of a protein A–Sepharose suspension (2 mg/mL) was added, and the sample was incubated at 4 °C for 30 min with shaking. The immunoprecipitate was collected by centrifugation at 10000g for 2 min and washed 4 times with 1 mL of 0.15 M NaCl, 85 mM Tris-HCl (pH 7.4), 11 mM $MgCl_2$, 0.1 mM EDTA, and 0.1% Nonidet P-40. The final precipitate was subjected to 10% polyacrylamide gel electrophoresis in the presence of SDS (Laemmli, 1970) and analyzed by fluorography (Chamberlain, 1979).

The incorporation of [^{35}S]methionine into total protein was determined by precipitation with trichloroacetic acid. A 2-μL aliquot of the translation products was diluted with 1 mL of H_2O and 3 mL of 10% trichloroacetic acid. The sample was incubated on ice for 30 min and then heated at 90 °C for 15 min. It was then cooled to 4 °C and filtered through glass fiber filters. The filters were washed twice with 5% trichloroacetic acid, rinsed with 70% ethanol, and dried before the radioactivity was determined. The incorporation into glutaminase was determined by densitometric tracing of the 72 000-dalton protein band on the fluorogram and was corrected for background. The relative level of translatable glutaminase mRNA was calculated as the ratio of the densitometric tracing vs. the [^{35}S]methionine incorporation into total protein. Incorporation of [^{35}S]methionine in the nuclease-treated lysate was totally dependent upon the presence of exogenous poly(A+) RNA. The addition of 2 μg of isolated rat renal poly(A+) RNA typically produced a 12–15-fold stimulation of incorporation compared to the background. With the concentrations used in this study, the incorporation of [^{35}S]methionine into total protein and the relative intensity of the 72 000-dalton protein band were proportional to the quantity of poly(A+) RNA added.

RESULTS

Equivalent amounts of poly(A+) RNA isolated from the kidneys of a control and a 7-day acidotic rat were translated in a rabbit reticulocyte lysate. The translation products were incubated with anti-glutaminase IgG, and the resulting im-

[1] Abbreviations: SDS, sodium dodecyl sulfate; SSC, 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; kb, kilobase(s).

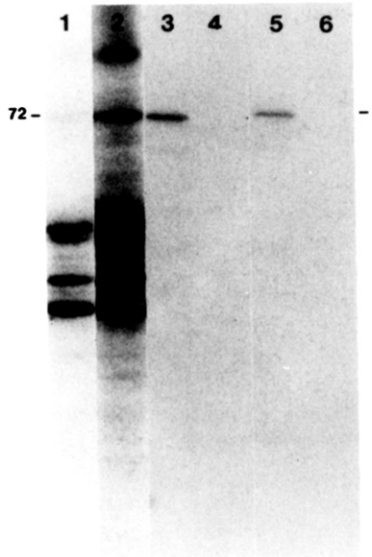

FIGURE 1: Identification of the in vitro translation product of glutaminase mRNA. The products of the in vitro translation of isolated renal poly(A+) RNA were precipitated with anti-glutaminase IgG and subjected to SDS–polyacrylamide gel electrophoresis. The poly(A+) RNA was isolated from a control (lane 1) or 7-day acidotic rat (lane 2). The acidotic poly(A+) RNA was further fractionated by electrophoresis on a low melting temperature agarose gel (lanes 3–6). The sample in lane 4 was precipitated by using preabsorbed antibodies. The immunoprecipitates shown in lanes 5 and 6 were obtained following the addition of 0.006 and 0.06 units of purified brain glutaminase, respectively.

munoprecipitates were analyzed by SDS–polyacrylamide gel electrophoresis (Figure 1, lanes 1 and 2). The two precipitates contain a protein (M_r 72 000) that is slightly larger than the peptides that are associated with the native glutaminase (Haser et al., 1985). The relative intensity of this band is significantly increased in the sample obtained following translation of the poly(A+) RNA preparation isolated from the acidotic rat. To determine if the 72 000-dalton protein is the translation product of the glutaminase mRNA, the total poly(A+) RNA isolated from a chronically acidotic rat was fractionated on a low melting temperature agarose gel. The poly(A+) RNA recovered from a slice of the gel was enriched approximately 25-fold in the mRNA that yields the 72 000-dalton protein (see below). When the in vitro translation products of this poly(A+) RNA fraction were immunoprecipitated with rabbit anti-glutaminase IgG, the 72 000-dalton protein was the dominant band present on the fluorogram (Figure 1, lane 3). However, antibodies that were preabsorbed with purified rat brain glutaminase were unable to precipitate the 72 000-dalton protein (Lane 4). When increasing amounts of purified brain glutaminase were added to the products of the in vitro translation, the precipitation of the 72 000-dalton protein was reduced (lanes 5 andd 6). The addition of 0.06 unit of purified brain glutaminase was sufficient to completely inhibit precipitation. Therefore, the 72 000-dalton protein is immunologically related to the glutaminase and is probably the initial translation product of the glutaminase mRNA.

The fractions from the low melting temperature agarose gel that contain the glutaminase mRNA were identified by the presence of 72 000-dalton protein in the immunoprecipitates of the in vitro translation products. The translation of the RNA extracted from each slice resulted in a similar extent of incorporation of [^{35}S]methionine into total protein. Therefore, the intensity of the 72 000-dalton band reflects the relative abundance of the glutaminase mRNA. On the basis of this analysis, the glutaminase mRNA migrates more slowly than the 28S mRNA. Compared to the mobility of the *Hin*dIII fragments of λ DNA, the size of the glutaminase mRNA was estimated to be approximately 6.5 kb.

Table I: Comparison of the Relative Level of Translatable Glutaminase mRNA in Fractionated Poly(A+) RNA Obtained from Control, Chronic Acidotic, and Recovered Rats[a]

poly(A+) RNA	[^{35}S]methionine incorporation: total protein (cpm × 10^{-6})	[^{35}S]methionine incorporation: glutaminase (units)	relative level of glutaminase mRNA (units/cpm × 10^{5})
control	1.36	85	6.3
	1.27	85	6.7
7-day acidotic	1.22	225	19.0
	1.60	260	16.0
2-day recovered	1.42	60	4.2
	1.32	60	4.5

[a] Renal poly(A+) RNAs isolated from control 7-day acidotic, and 2-day recovered rats were fractionated on a low melting temperature agarose gel in the presence of methylmercury hydroxide. The amounts of the poly(A+) RNA recovered in the fraction that were enriched in glutaminase mRNA were measured by poly(U) hybridization. Aliquots containing 34 ng of poly(A) equivalents were used for in vitro translation. The incorporation into total protein was determined by precipitation with trichloroacetic acid and was corrected for background that occurred in the absence of added poly(A+) RNA. The incorporation into glutaminase was determined by the densitometric tracing of the 72 000-dalton protein contained in the immunoprecipitates that were subjected to SDS–polyacrylamide gel electrophoresis and fluorography.

Total poly(A+) RNA preparations isolated from the kidneys of control, 7-day chronic acidotic, and 2-day recovered rats were similarly fractionated on low melting temperature agarose gel. The amounts of enriched mRNA recovered from the agarose gel were quantitated by poly(U) hybridization, and equivalent aliquots were used for in vitro translation (Table I). The relative level of translatable glutaminase mRNA was 3-fold higher in acidotic rats than in control rats, and it decreased to a value slightly below the control within 2 days of recovery. These results are similar to the observed changes in relative rates of glutaminase synthesis (Tong et al., 1986) and thus support the conclusion that the 72 000-dalton protein is the initial translation product of the glutaminase mRNA. However, the amounts of RNA recovered from the low-melting gels were too low to accurately determine if the glutaminase mRNA was enriched to the same extent in each preparation of fractionated mRNA. Therefore, the changes in relative levels of translatable glutaminase mRNA were also analyzed by using unfractionated poly(A+) RNA.

Poly(A+) RNA preparations were purified from the kidneys of rats that were sacrified at various times following onset and recovery from acidosis and used for in vitro translation. The resulting immunoprecipitates contain different amounts of the 72 000-dalton protein (Figure 2). By 7 days, the relative level of translatable glutaminase mRNA is increased 4.2-fold compared to that observed with the control poly(A+) RNA. However, recovery from acidosis results in a rapid return to the control level of translatable glutaminase mRNA. The observed changes in the relative levels of translatable glutaminase mRNA correlate well with the alterations in the relative rates of glutaminase synthesis that occur during metabolic acidosis.

The half-life of the glutaminase mRNA can be calculated from the increase in the relative level of translatable glutaminase mRNA, if one assumes that the adaptation is due solely to a rapid increase in the rate of transcription (K_S) and that following induction the new rate of transcription remains constant. Under these conditions, the rate of synthesis of the

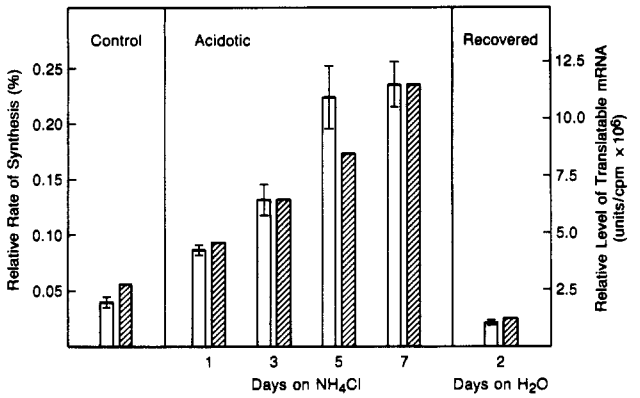

FIGURE 2: Comparison of the effect of metabolic acidosis on the relative level of translatable glutaminase mRNA and the relative rate of glutaminase synthesis. The relative levels of translatable glutaminase mRNA (slashed bars) were calculated from the relative intensity of the 72 000-dalton protein produced by in vitro translation of isolated poly(A+) RNA divided by the [^{35}S]methionine incorporated into total protein. Each point is the mean of duplicate determinations carried out using two separate RNA preparations. The relative rates of glutaminase synthesis (open bars) were taken from the study of Tong et al. (1986).

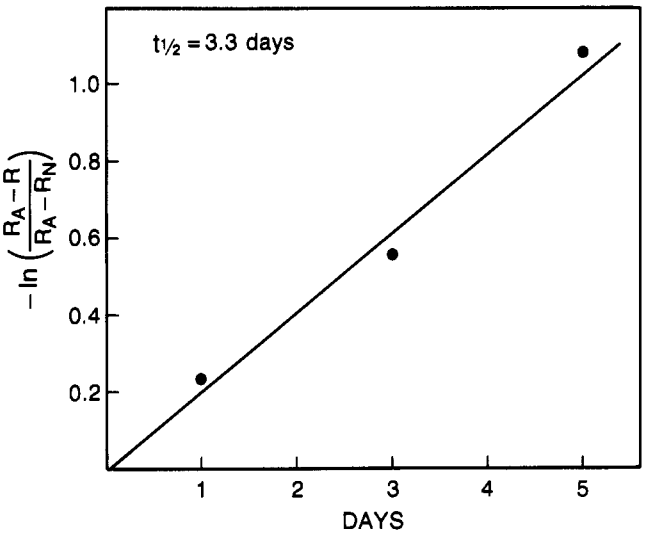

FIGURE 3: Estimation of the half-life of renal glutaminase mRNA. The increase in the relative level of translatable glutaminase mRNA (R) during onset of acidosis was plotted according to the equation which describes the approach to steady-state conditions. R_N and R_A are the mean relative levels of translatable glutaminase mRNA in control and fully induced chronic acidotic rats, respectively.

glutaminase mRNA (R) at any time (t) during induction can be described by the equation:

$$-\ln [(R_A - R)/(R_A - R_N)] = K_D t$$

where K_D is the first-order rate constant for mRNA degradation and R_N and R_A are the rates of glutaminase mRNA synthesis in the normal steady state and in the acidotic steady state, respectively. The slope determined by linear regression analysis of a plot of $-\ln [(R_A - R)/(R_A - R_N)]$ vs. t yields an estimate of the rate constant for mRNA degradation of 0.21 day^{-1} (Figure 3). This value corresponds to a half-life for the glutaminase mRNA of 3.3 days. However, within 2 days of recovery, the increased level of translatable glutaminase mRNA has decreased 9-fold. Therefore, either the glutaminase mRNA is selectively inactivated or its rate of degradation is increased significantly during recovery from acidosis. Alternatively, the initial assumption that the increase in glutaminase activity is due solely to an increase in transcription may be incorrect. The increase in the level of translatable glutaminase mRNA may result from the stabilization of glutaminase mRNA or from a combination of increased transcription and decreased degradation. Reversal of the changes in the two processes could account for the rapid decrease in translatable glutaminase mRNA during recovery.

Discussion

Immunoblot analyses have established that the glutaminase present in rat brain and kidney consists of 68 000- and 65 000 dalton proteins that are structurally and immunologically related (Shapiro et al., 1987). The rat renal glutaminase is asymmetrically associated with the internal surface of the mitochondrial inner membrane (Shapiro et al., 1985). Many of the proteins contained in this membrane are initially translated on cytoplasmic ribosomes as precursors that contain an N-terminal extension of 20–35 amino acids (Schatz & Butow, 1983; Douglas et al., 1986). Following membrane translocation, the targeting segment is removed by a proteinase that is present within the mitochondrial matrix.

In vitro translation of the fractionated rat renal poly(A+) RNA yields a single 72 000-dalton protein that is specifically precipitated with anti-glutaminase IgG. The immunoprecipitates of the translation products obtained with unfractionated poly(A+) RNA contained additional proteins. However, an identical pattern of proteins, minus the 72 000-dalton protein, was precipitated when the translation products were incubated with antibodies in which the anti-glutaminase IgG had been preabsorbed with purified rat brain glutaminase (data not shown). Therefore, the 72 000-dalton protein is the apparent precursor to the two proteins that are contained in the renal and brain mitochondrial glutaminase. In order to further characterize the difference between the two mitochondrial proteins and to determine their functional significance, we have already initiated a study of the biosynthesis and processing of the mitochondrial glutaminase.

Densitometric tracing was required to quantitate the amount of the glutaminase synthesized by in vitro translation. In acidotic rats, the glutaminase constitutes 0.2% of the total protein synthesized (Tong et al., 1986). If 0.2% of the mRNA translated in vitro were glutaminase mRNA, it should have been feasible to directly quantitate the amount of the glutaminase produced. However, direct counting of the immunoprecipitate of the in vitro translation product was not possible because the precipitate frequently contained additional proteins. Attempts to cut the 72 000-dalton protein from an SDS–polyacrylamide gel and directly determine radioactivity were not successful due to the low level of radioactivity associated with the 72 000-dalton band. Therefore, the relative amount of the glutaminase synthesized in the rabbit reticulocyte lysate was less than the relative rate of glutaminase synthesis observed in vivo. This may be due to the large size of the glutaminase mRNA.

The synthesis of a 72 000-dalton protein would require approximately 2 kb of translated mRNA. However, the size of the glutaminase mRNA was estimated to be 6.5 kb. The reticulocyte lysate is more efficient at translating smaller mRNAs. The [^{35}S]methionine incorporation into glutaminase was increased significantly when the isolated poly(A+) RNA was pretreated with methylmercury hydroxide. Therefore, the glutaminase mRNA may have large regions of secondary structure that also contribute to its low efficiency of translation. Alternatively, the presence of regulatory sequences within the 4.5 kb of untranslated RNA may reduce the efficiency of translation of the glutaminase mRNA in a heterologous system. The inclusion of RNasin or of protease inhibitors had no effect on the pattern of peptides or the relative intensity

of the 72 000-dalton protein obtained in the immunoprecipitates (data not shown). Therefore, the degradation of the glutaminase mRNA or of the synthesized glutaminase is not likely to have contributed to the low level of the 72 000-dalton protein produced in the rabbit reticulocyte lysate.

The increase in the amount of the mitochondrial glutaminase during acidosis could be due to alterations at various steps in the synthesis of the active form of the mitochondrial glutaminase. The results presented in this study indicate that the increase in the relative level of translatable glutaminase mRNA is nearly sufficient to account for the observed increase in the relative rate of enzyme synthesis. Therefore, the adaptive increase in glutaminase activity is probably not due to the regulation of a required posttranslational modification or the rate of translocation and proteolysis of the enzyme to the mature mitochondrial form. If the glutaminase mRNA activity is a direct measure of glutaminase mRNA concentration, then regulation of transcription could account for the adaptive increase in glutaminase activity.

The mechanism of induction of rat renal phosphoenolpyruvate carboxykinase in response to acidosis has also been characterized. A significant increase in the relative rate of phosphoenolpyruvate carboxykinase synthesis is observed within 2 h after administration of an acute acid load, and the relative rate of synthesis reaches a plateau after 8 h (Iynedjian et al., 1975). The increased rate of synthesis occurs concomitant with an identical increase in the level of translatable phosphoenolpyruvate carboxykinase mRNA (Iynedjian & Hanson, 1977) and the total amount of phosphoenolpyruvate carboxykinase mRNA measured by hybridization assay (Cimbala et al., 1982). The effects of dexamethasone and of cAMP on the rate of transcription were investigated by using isolated rat renal nuclei (Meisner et al., 1985). The increased rate of transcription accounted for the increases in cytosolic mRNA and the rate of translation of phosphoenolpyruvate carboxykinase. Thus, glucocorticoids and cAMP induce the expression of the phosphoenolpyruvate carboxykinase gene through transcriptional regulation. Similar experiments to determine if increased transcription is the sole mechanism by which onset of acidosis affects an increase in phosphoenolpyruvate carboxykinase have not been reported.

The half-life for glutaminase mRNA during onset of acidosis was estimated to be 3.3 days. If the increased level of glutaminase mRNA is degraded at this rate during recovery, it should take more than 11 days for it to return to normal. Since 2 days of recovery are sufficient to return to control level, the rate of glutaminase mRNA inactivation or degradation must be significantly increased during recovery. Stabilization of the glutaminase mRNA could also be an important factor in its induction during acidosis. The regulation of the glutaminase gene expression is likely to require a transacting factor. This factor could be a nuclear and cytoplasmic protein that undergoes significant changes in conformation in response to a slight decrease in intracellular pH. In one conformation, the protein could act to stimulate transcription and stabilize the glutaminase mRNA. Alternatively, acidosis could stimulate the synthesis or release of a specific hormone which initiates the effect on gene transcription and mRNA stability. In this case, recovery from acidosis must result in the rapid clearance of the hormone. It is also interesting that after 2 days of recovery the relative rate of gluaminase synthesis (Tong et al., 1986) and the relative level of translatable glutaminase mRNA are lower than the values observed in control animals. This could represent an overshoot due to the rapid rate of glutaminase mRNA degradation. The further characterization of this adaptation will require the isolation of a glutaminase cDNA that can be used to quantitate total glutaminase mRNA by hybridization analysis.

ACKNOWLEDGMENTS

We thank Dr. Rosemary Jagus for the rabbit reticulocyte lysate and for her helpful advice and assistance. We also thank Dr. Christine Milcarek for her assistance with the poly(U) hybridization assays.

Registry No. Glutaminase, 9001-47-2.

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