Subcellular Location of Chicken Brain Glutamine Synthetase and Comparison with Chicken Liver Mitochondrial Glutamine Synthetase*

(Received for publication, May 20, 1983)

Darwin D. Smith, Jr. and James W. Campbell
From the Department of Biology, Rice University, Houston, Texas 77251

Chicken brain glutamine synthetase has been found to be localized in the cytosolic fraction of this tissue in contrast to its mitochondrial location in chicken liver. Despite this difference in subcellular distribution, the enzyme from brain exhibits the same molecular weight during sodium dodecyl sulfate-polyacrylamide gel electrophoresis, has the same isoelectric point, and is immunologically cross-reactive. In addition, the immunochemically isolated primary translation products for glutamine synthetase from brain and liver mRNA are the same size. These data suggest that cytosolic brain and mitochondrial liver glutamine synthetase may be derived from the same gene.

Ammonia detoxification and glutamate recycling in neural tissue is mediated primarily by the enzyme glutamine synthetase (EC 6.3.1.2) (1). Immunohistochemical studies indicate that this enzyme is distributed among the glial cells in rat brain (2, 3) and rat and chick retina (4, 5). A cytosolic subcellular location of glutamine synthetase has been found in rat brain (6) and rat liver (7). However, contrary to the situation in ureotelines, glutamine synthetase is mitochondrially localized in the liver of uricotoles such as the chicken and snake (8). Recent studies in this laboratory have demonstrated that antibodies raised against the purified chicken mitochondrial enzyme are cross-reactive with both mitochondrial and cytosolic glutamine synthetases spanning the phylogenetic tree from shark to human (9). In addition, theoretical calculations using the amino acid residue content for the various glutamine synthetases indicate as high as 80% sequence homology between the cytosolic mammalian enzymes and the mitochondrial chicken enzyme. Since such immunological and sequence relatedness between enzymes located in distinctly different compartments is unusual, studies were initiated to determine if glutamine synthetase exists as a mitochondrial or cytosolic enzyme in the chicken brain. The following is a summary of the subcellular localization of chicken brain glutamine synthetase and a comparison with the purified mitochondrial enzyme.

EXPERIMENTAL PROCEDURES

Adult White Leghorn hens were maintained on a Purina chicken scratch grain diet for brain fractionation and brain mRNA isolation. Chickens were fed a United States Biochemical Corporation chicken diet supplemented with 60% protein for liver mRNA isolation.

Hens brains were prepared as 10% (w/v) homogenates in 5 mM HEPES, 250 mM sucrose, and 1 mM EDTA near 0 °C and fractionation was as described (8) with the modification of sedimenting all mitochondria at 13,300 × g. Glutamine synthetase, cytochrome oxidase, lactate dehydrogenase, and glucose 6-phosphatase were assayed essentially as described (8) with the exception that the glutamine synthetase reaction mixture contained the ATP regeneration system recommended by Vorhaben et al. (10). Glutamate dehydrogenase activity was monitored by the oxidation of NADH at 340 nm as described (11).

The final volume of activity is defined as 1 µmol of product produced per min at room temperature for lactate dehydrogenase, cytochrome oxidase, and glutamate dehydrogenase and at 37 °C for glucose 6-phosphatase. One unit of glutamine synthetase activity is defined as 1 µmol of product produced per h at 37 °C. Protein was determined by the method of Bradford (12).

Immunoblotting was performed according to the method of Towbin et al. (13) using rabbit anti-chicken liver mitochondrial glutamine synthetase IgG (9), horseradish peroxidase conjugated goat anti-rabbit IgG and 4-chloro-1-naphthol (Sigma) (14). Samples were electrophoresed in 10% SDS-PAGE slab gels, electrophoretically transferred to nitrocellulose (Schleicher and Schuell, BA-85) and proteins were stained with Amido black. Isoelectric focusing was done in 5% polyacrylamide tube gels containing 2% ampholytes (Bio-Rad) (15). Detection of glutamine synthetase activity in isoelectric focusing gels was as described (16).

RNA was isolated from chicken liver and brain via the guanidine HCl extraction technique of Deelely et al. (17) and mRNA was affinity purified from this fraction by oligo(dT) chromatography (18). Translation of the mRNA utilized a cell-free rabbit reticulocyte system (19) containing 0.5 mM spermidine, 8 mM creatine phosphate, 25 µM amino acids minus methionine, 2 mM dithiothretiol, 20 mM HEPES, pH 7.4, 0.65 mM magnesium acetate, 80 mM potassium chloride, 20 µl of micrococcal nuclease-treated reticulocyte lysate, 25 µM hemin, 15 µg of creatine phosphokinase, 50 µCi of [35S]methionine (American Corp.), 10 µg/ml of each of the protease inhibitors leupeptin, bestatin, antipain, chymostatin, and pepstatin (Sigma) and 2-4 µg of mRNA in a final volume of 50 µl. Incubation was at 30 °C for 1 h.

Glutamine synthetase was immobilized (20) from the translation mixtures containing at least 1 × 10⁶ cpm by dilution to 1 ml final volume with 10 mM Tris-HCl, pH 7.4, 0.1% SDS, 0.05% Triton X-100, 150 mM NaCl, 10 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride. This solution was then precleared with 20 µl of 10% (w/v) Staphylococcus aureus cells (The Enzyme Center) incubated at 4 °C for 15 min followed by removal of the cells by centrifugation. Anti-glutamine synthetase antiserum (5-10 µl) was then added to the supernatant solution and incubated for 1 h at room temperature and at 4 °C overnight. Fifty µl of 10% S. aureus cells were then added and the mixture was incubated at room temperature for 1 h after which the cells were washed with the dilution buffer 3 times and the cells were then prepared for SDS-PAGE. The radiolabeled proteins were visualized by impregnation of the cells with Enhance (New England Nuclear) and fluorography (21).

RESULTS AND DISCUSSION

Table I illustrates the subcellular distribution of glutamine synthetase in chicken brain. The majority of the activity (76%) is localized in the cytosolic fraction which is characterized by the soluble marker enzyme lactate dehydrogenase; 64% of the lactate dehydrogenase was present in this fraction.

azinethanesulfonic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
Table 1

Subcellular distribution of chicken brain glutamine synthetase

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein mg/g tissue</th>
<th>Glutamine synthetase units/g</th>
<th>Lactate dehydrogenase units/g</th>
<th>Cytochrome oxidase units/g</th>
<th>Glutamate dehydrogenase units/g</th>
<th>Glucose 6-phosphotase units/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>74.0</td>
<td>384.7</td>
<td>141.9</td>
<td>38.3</td>
<td>14.5</td>
<td>14.7</td>
</tr>
<tr>
<td>Nuclear</td>
<td>18.2</td>
<td>49.0 (13)*</td>
<td>33.7 (20)</td>
<td>7.2 (24)</td>
<td>6.3 (43)</td>
<td>3.0 (39)</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>17.0</td>
<td>20.1 (5)</td>
<td>22.9 (13)</td>
<td>7.1 (39)</td>
<td>8.5 (56)</td>
<td>2.5 (32)</td>
</tr>
<tr>
<td>Microsomal</td>
<td>7.0</td>
<td>22.1 (6)</td>
<td>5.4 (3)</td>
<td>1.0 (3)</td>
<td>0.1 (1)</td>
<td>1.1 (2)</td>
</tr>
<tr>
<td>Soluble</td>
<td>33.1</td>
<td>287.6 (76)</td>
<td>107.9 (64)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
<td>0.0 (0)</td>
</tr>
<tr>
<td>Total recovered</td>
<td>75.3</td>
<td>378.8 (100)</td>
<td>169.9 (100)</td>
<td>29.6 (100)</td>
<td>14.7 (100)</td>
<td>7.7 (100)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses are the percentages of recovered activity.

Fig. 1. Immunoblot of glutamine synthetase from chicken brain cytosol. Proteins from chicken brain cytosol (100 μg), chicken liver mitochondria (100 μg), and purified chicken liver mitochondrial glutamine synthetase (5 μg) were electrophoresed in a 10% SDS-PAGE slab gel, electrophoretically transferred to nitrocellulose, and probed with anti-mitochondrial glutamine synthetase IgG. Lane 1, brain cytosol; lane 2, liver mitochondria; lane 3, purified glutamine synthetase.

These values compare favorably with those found in the rat brain in which 62% of the glutamine synthetase activity and 56% of the lactate dehydrogenase activity were confined to the cytosol (6). Only 5% of the glutamine synthetase activity is found associated with the chicken brain mitochondrial fraction. Cytochrome oxidase, a membrane bound mitochondrial enzyme, had 73% of its activity localized in the mitochondria while 56% of the glutamate dehydrogenase activity was mitochondrially associated. Glutamate dehydrogenase, a soluble mitochondrial matrix protein, was not found in the cytosolic fraction, which indicates that the mitochondria remained intact during fractionation. It should be noted that a difficulty in the subcellular fractionation of brain tissue is the considerable myelin membrane content which causes trapping of some subcellular components (6). This problem is illustrated by the contamination of the nuclear fraction with mitochondrial and microsomal enzymes. Synaptosome formation may also trap cytosolic enzymes which could contaminate mitochondrial and nuclear fractions.

Since the data demonstrate chicken brain glutamine synthetase to be a cytosolic protein, it might be expected that the protein would be a structurally distinct isozyme from the liver mitochondrial form. Ouchterlony immunodiffusion and rocket electrophoresis (data not shown) indicated that the chicken brain enzyme was cross-reactive with antibodies elicited against the purified mitochondrial protein. Therefore, these antibodies were used to probe proteins transferred from 10% SDS-PAGE gels to nitrocellulose. Fig. 1 shows an immunoblot of electrophoresed proteins from brain cytosol (lane 1), liver mitochondria (lane 2), and purified mitochondrial glutamine synthetase (lane 3). Within the limits of resolution of the gel system, chicken brain glutamine synthetase has the same molecular weight as the purified and unpurified liver mitochondrial enzyme, which is 42,000 (15).

Further evidence for the structural similarity of chicken brain glutamine synthetase and its mitochondrial counterpart is illustrated in Fig. 2. Isoelectric focusing of brain cytosol glutamine synthetase. Purified mitochondrial glutamine synthetase (80 μg), gel 1; brain cytosol (300 μg), gel 2; and liver mitochondrial protein (300 μg), gel 3, were electrophoresed in 5% polyacrylamide gels containing 2% (w/v) ampholytes ranging from pH 3 to 10. The gels were stained for activity as described under "Experimental Procedures."
brain and liver glutamine synthetase is presented in Fig. 2, in which the isoelectric focusing patterns of the cytosolic and mitochondrial enzymes are compared. The chicken brain enzyme (gel 2) has the same isoelectric point as the purified (gel 1) and unpurified (gel 3) liver mitochondrial enzyme (pI = 6.1) as shown by activity staining. This has been further substantiated (data not shown) by crossed immunoelectrophoresis of similar isoelectric focusing gels into agarose gels containing anti-glutamine synthetase IgG in which the activity staining and immunoprecipitin lines corresponded and were the same for the enzyme from both sources.

The high degree of structural and compositional homology of the cytosolic brain glutamine synthetase with its liver synthetase subunit is initially synthesized as its 42,000-dalton translation products as shown in Fig. 3. Current studies in this laboratory (22) indicate that the chicken liver glutamine synthetase subunit is initially synthesized as its 42,000-dalton mature form, while most mitochondrial matrix proteins are synthesized as higher molecular weight precursors (23, 24). Chicken liver glutamine synthetase is an octameric protein with eight identical 42,000-dalton subunits (15).

Comparison of the immunoprecipitated translation products, from both liver mRNA (lane 6) and brain mRNA (lane 5) shows that the glutamine synthetase primary translation products are the same size. The controls of nonimmune antiserum in lane 3 and competition with unlabeled mitochondrial glutamine synthetase in lane 4 confirm the identity and similarity of the brain glutamine synthetase in lane 5.

Glutamine synthetase of adult chicken brain thus appears to be a cytosolic enzyme which is identical to the adult chicken liver mitochondrial enzyme based on the criteria of SDS-PAGE, isoelectric focusing, immunological reactivity, and competition with unlabeled mitochondrial translation products. This extent of homology is considered unusual for isoymes located in different subcellular compartments (25). One might expect that a difference would be observed in the primary translation products as in the case of chicken aspartate aminotransferase (26) and chicken phosphoenolpyruvate carboxykinase (27). The mitochondrial and cytosolic forms of the two proteins are coded for by different genes and are not immunochemically cross-reactive. Although the subcellular fractionation studies for glutamine synthetase in other tissues has not been reported, Soh and Sarkar (28) mention that during the preparation of polysomes from cultured chick embryonic retina the mitochondrial fraction had little glutamine synthetase activity while the cytosolic and polysomal fractions contained significant activity. Since the purified enzyme from adult retina has been shown to also have a molecular weight of 42,000 (29), it may be possible that glutamine synthetase from retina is cytosolic as shown here for the brain enzyme.

Existence of a cytosolic glutamine synthetase in the chicken with physical properties the same as that of the mitochondrial enzyme suggests that the two are encoded for by the same gene and that their ultimate subcellular destination is tissue-specific and post-translationally determined by either subtle changes in protein structure or modification of mitochondrial competence for sequestering glutamine synthetase. Studies discerning this subcellular targeting problem in the chicken may help explain the molecular evolutionary divergence of glutamine synthetase with regard to ureotelism and uricotelism and also delineate some of the features necessary for import of mitochondrial proteins.

REFERENCES

Fig. 3. Translation in vitro of chicken brain mRNA. Cell-free translation products labeled with [35S]methionine were electrophoresed and fluorographed as described under "Experimental Procedures." Lane 1, no added RNA; lane 2, total translation products from brain mRNA; lane 3, immunoprecipitation of brain mRNA products using nonimmune antiserum; lane 4, immunoprecipitation using monospecific anti-liver glutamine synthetase in the presence of competing levels (50 µg) of unlabeled, purified glutamine synthetase; lane 5, immunoprecipitation as in lane 4 omitting the unlabeled glutamine synthetase; lane 6, immunoprecipitation of glutamine synthetase programmed by chicken liver mRNA.
Subcellular location of chicken brain glutamine synthetase and comparison with chicken liver mitochondrial glutamine synthetase.
D D Smith, Jr and J W Campbell


Access the most updated version of this article at http://www.jbc.org/content/258/20/12265

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/258/20/12265.full.html#ref-list-1