J. Biochem. 128, 101–106 (2000)

Molecular Characterization of a Novel Gamma-Glutamyl Transpeptidase Homologue Found in Rat Brain[1]

Tsuyoshi Yamaguchi,* Nobuyuki Takei,* Kazuaki Araki,* Kyoko Ishii,* Takashi Nagano,* Tomio Ichikawa,† Toshiro Kumanishi,† and Hiroyuki Nawa*,[2]

*Department of Molecular Neurobiology and †Department of Molecular Pathology, Brain Research Institute, Niigata University, Niigata 951-8585

Received April 3, 2000; accepted April 27, 2000

A cDNA clone for a novel homologue to γ-glutamyl transpeptidase (γ-GTP), termed GTPH, was isolated from a rat brain expression cDNA library using antisera against total brain synaptosomal fractions. The cloned GTPH consists of 641 amino acid residues (78 kDa) and exhibits structural similarity with a conventional type of γ-GTP that is predominantly expressed in the liver: They share significant amino acid homology (33% identity, 73% similarity) spanning over the entire sequence. RNA analyses revealed that GTPH mRNA expression is found only in the nervous system, including all brain regions, eyes and peripheral ganglia, and increases during development. Endogenous GTPH protein is a membrane-bound glycoenzyme and migrates as 90–100 kDa in polyacrylamide gels. Taken together, GTPH is a novel form of a γ-GTP-like molecule expressed exclusively in the nervous system.

Key words: brain, γ-glutamyl transpeptidase, glutamyl moiety, glutathione, membrane enzyme.

Gamma-glutamyltranspeptidase (γ-GTP) [(γ-glutamyl)-peptide:amino acid γ-glutamyl transferase, EC 2.3.2.2] is a membrane bound glycoenzyme that transfers the γ-glutamyl moiety of glutathione to various amino acids, glutathione itself or water (*1*). This enzyme, therefore, plays a crucial role in a metabolic pathway, termed the γ-glutamyl cycle, that is involved in the synthesis and degradation of glutathione. This enzyme is found mainly in tissues having secretory or absorptive functions such as kidney, jejunum and pancreas. In the brain, the concentration of glutathione is very high (~2 mM) and the γ-GTP activity is concentrated in the choroid plexus and blood capillaries (*2*), but also detected in glial cells and cultured neurons (*3–5*). Previous biochemical analyses have revealed that there are at least four forms of γ-GTP-like enzymes in the brain (*6*), although the molecular natures of these enzymes are not clear. In addition, there are many related enzymes, including γ-GTP splice variants, as well as γ-glutamyl leukotrienase (GGT-rel) (*7–9*). They exert similar γ-GTP activities but more efficiently on other substrates. Here, we identified a novel homologue of γ-GTP that is specifically expressed only in the nervous system. The primary structure and expression patterns of the new molecule are characterized by molecular analyses in comparison with those of γ-GTP.

[1] This work was supported by the Japanese Society for the Promotion of Science (RFTF-96L00203) and a Grant-in-Aid for Scientific Research (MESSC) GenBank accession number: AF244973

[2] To whom correspondence should be addressed. Tel. +81-25-227-0613, Fax. +81-25-227-0815, E-mail: hnawa@bri.niigata-u.ac.jp

Abbreviations: GGT-rel, γ-glutamyltranspeptidase related enzyme; GTPH, γ-glutamyltranspeptidase homologue, γ-GTP, γ-glutamyltranspeptidase; SSC, saline sodium citrate.

MATERIALS AND METHODS

Immunoscreening—Antisera were produced in chickens by immunizing synaptic/extracellular matrix fractions. A lamda gt11 expression library carrying adult rat brain cDNAs (Clontech) was probed with chicken antisera (1:1,000 dilution) followed by alkaline phosphatase–conjugated anti-chicken IgY (Jackson Immuno. Lab) as described by Snyder *et al.* (*10*). Immunoreactivity was visualized with chromogenic substrates; nitrotetrazolium and 5-bromo-chloro-3-indolyl phosphate (Wako Chemical). A total of 480,000 plaques were screened and 198 positive clones were identified. To classify the cDNA clones isolated, we first attempted to deduce the nucleotide sequences of the PCR products obtained using phage DNA as a template. cDNA inserts were amplified from two PCR primers located around an *Eco*RI cloning site, GACTCCTGGAGCCCGTC and GACCAACTGGTAATGGT, subcloned into pGEM-T vector (Promega), and sequenced by a combination of a Ready Reaction Cycle Sequencing kit and 310 Genetic Analyzer (both Applied Biosystems).

To obtain the full-length cDNA clone of GTPH, the embryonic rat brain cDNA library constructed with lamda Zap vector (*11*) was rescreened with the 1.6 kb cDNA insert of GTPH obtained above. Twenty positive clones were isolated from 100,000 plaques and converted into plasmids by infecting helper phage (Stratagene). Sequence analysis was done by the automated DNA sequencer as described above.

RNA Analysis—Total RNA was extracted from Sprague-Dawley rats (SLC, Shizuoka) by the Guanidium-CsCl method (*12*). RNA samples (20 μg) were denatured in 50% formamide, 6% formaldehyde, 20 mM 3-(*N*-morpholino)propanesulfonic acid buffer (pH 7.0), 1 mM EDTA at 65°C for 10 min and separated on a 1% formaldehyde-agarose gel.

RNA in the gel was visualized with ethidium bromide to confirm the equal loading of each sample, after which the gel was washed extensively with water, then transferred onto a nylon membrane (Paul Scienctific) with 10× saline-sodium citrate (SSC) buffer and fixed by exposure to ultraviolet light. Alternatively, a preblotted RNA membrane (Multiple tissue blot; Clontech) was used. A ^{32}P-labeled cDNA probe to GTPH (*Eco*R1–*Not*I, the 2.5 kb fragment of rat GTPH; ~5 × 10^9 cpm/μg) was generated using a Random primed DNA labeling kit (Boehringer Mann.). The probe was hybridized to filters for 12–18 h at 42°C in 50% formamide, 5× SSC, 5× Denhardt's solution, and 1% SDS, then washed with 0.1× SSC, 0.1% SDS at 55°C, and the film was exposed.

In Situ Hybridization—Synthetic oligoDNAs were used to detect GTPH mRNA *in situ* (*13*). Fifty base sense and anti-sense oligoDNAs corresponding to the middle region of the open reading frame (nucleotide residues 1069–1119) were labeled with terminal deoxynucleotide transferase. An oligoDNA probe (300 pg/μl) was incubated with 3 MBq of α-^{35}S-dATP (NEN, ~3,000 Ci/mmol) and terminal deoxynucleotide transferase (13 units; Takara Biomed., Japan) for 120 min. The labeled oligoDNA probes were purified by passage through a Sephadex G-50 column. Brains of Wister rats (SLC, Shizuoka) were dissected at embryonic day 18 or postnatal 90 days, and frozen in isopentane/dry ice. Sagittal sections of the brain (10 μm thick) were obtained with a cryostat and dried. Sections were treated with 4% paraformaldehyde in phosphate buffered saline and then with 0.25% acetic anhydrite in 0.1 M Tris buffer (pH 8.0). Sections were treated with the hybridization solution [50% formamide, 5× SSC, 5× Denhardt's solution, 1 mM EDTA, 0.1 M DTT, and 0.5 mg/ml denatured salmon sperm DNA] followed by the solution containing labeled oligoDNA (~10^7 dpm/ml). After overnight hybridization at 42°C, the sections were washed with 0.1× SSC at the same temperature for 2 h and dehydrated with ethanol. Sections were first processed for a BAS 2000 phosphoimager and then exposed to Hyperfilm β-Max films (Amersham).

Antibody Production—A cDNA fragment (nucleotide residues 1736–2000) for a carboxyl terminal region (88 amino acids) of GTPH was subcloned into a His-tagged expression vector, pET16b (Novagen). Recombinant protein was produced in *Escherichia coli*, BL21 strain, and purified on a nickel-conjugated affinity column (Invitrogen). The partially purified protein was subjected to SDS-PAGE, and a protein band migrating at an expected size (12 kDa) was recovered. The recombinant protein (~0.5 mg) including the gel, was emulsified with Freund's complete adjuvant and used to immunize rabbits every 2 weeks for 3 months (*14*). The antiserum titer was estimated by enzyme immunoassay using the recombinant protein used as an antigen. Serum batches showing higher titers were subjected to antigen-affinity chromatography. The affinity column was prepared from 1 mg of the recombinant protein and 1ml bed of Affi-Gel 10 (BioRad). Purified anti-GTPH antibodies were used for immunochemical analyses.

Cell Culture—PC12 cells were obtained from Riken Cell Bank (Japan). Cells were maintained in Dulbecco's modified Eagle's medium containing 2 mM purified glutamine and 10% fetal bovine serum (*15*). A full size of GTPH cDNA was subcloned into a eukaryotic expression vector, pCMV-tag1 (Stratagene), and transfected into PC12 cells by a conventional calcium phosphate method (*16*). Recombinant clones were selected in 60 mm dishes with 0.6 μg/ml G418 (Gibco BRL) for 7 weeks and more than 10 clones in a dish were mixed and pooled.

Subcellular Fractionation—Subcellular fractionation was carried out according to Carlin *et al.* (*17*). In brief, whole brains from postnatal day 10 rats were disrupted in a glass-Teflon homogenizer with 10× volume of Solution A [0.32 M sucrose and 1 mM $NaHCO_3$ containing 1 mM $MgCl_2$, 0.5 mM $CaCl_2$]. Crude homogenates were centrifuged at 1,400 ×*g* for 10 min at 4°C and separated into supernatant (S1) and a pellet (P1). The S1 fraction was centrifuged again at 13,800 ×*g* for 10 min and yielded fractions S2 and P2.

Immunoblot Analysis—Immunoblotting was performed with protein extracted from cell culture and brain tissues. Diluted samples were precipitated and concentrated with 10% trichloroacetic acid. The protein was denatured with sample buffer [20 mM Tris (pH6.8), 2% SDS and 0.7 M β-mercaptoethanol], separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE), and blotted onto a nitrocellulose membrane (Schleicher and Schull). Immunoblots were treated with rabbit affinity purified anti-GTPH antibodies. Immunoreactivity was visualized by chemiluminescence reaction (ECL kit; Amersham). For deglycosylation, protein in the P2 fraction (~10 mg/ml) was denatured with denaturing buffer [1 M Tris (pH 8.6), 1% SDS, and 0.21 M β-mercaptoethanol], diluted 10-fold with 5% Nonidet P-40 (BDH Chemicals, England), and treated with glycopeptidase F (50 units/mg protein) (Takara Biomed.) overnight at 37°C. The deglycosylated products were analyzed by immunoblotting as described above.

RESULTS

Immunoscreening—We screened approximately 480,000 independent phage clones with chicken anti–synaptic protein antisera and obtained 198 positive clones. The cDNA inserts of all these clones were subjected to nucleotide sequencing and database searching. Homology searching in the GenBank Database revealed that more than 98% (*i.e.* 195 clones) of the genes encoded the well-known proteins that are particularly enriched in synaptic fractions (*18*), such as neurofilament H, synapsin I and microtubule-associated protein-2 (data not shown). This result confirmed the specificity of the antibodies that indeed recognize such synaptic proteins. Three clones out of the 198 positive clones did not show any homology to the characterized genes, and thus we decided to determine the complete nucleotide sequences of their cDNAs. One of the three, which is most abundant in the brain, was characterized in the present study.

To elucidate the entire coding region of this novel gene, the rat brain cDNA library (~1×10^5 plaques) was re-screened and approximately two hundred clones (~0.2%) gave positive signals. Among them, the largest cDNA clone was isolated and sequenced. It consisted of 2,587 bp and contained the largest open reading frame that encodes 641 amino acids (Fig. 1). This cDNA presumably covers almost the entire region of the mRNA because the size of the mRNA was calculated to be 2.6 kb by RNA blotting analysis (see below). To further confirm whether the largest reading frame is indeed translated into protein, we carried

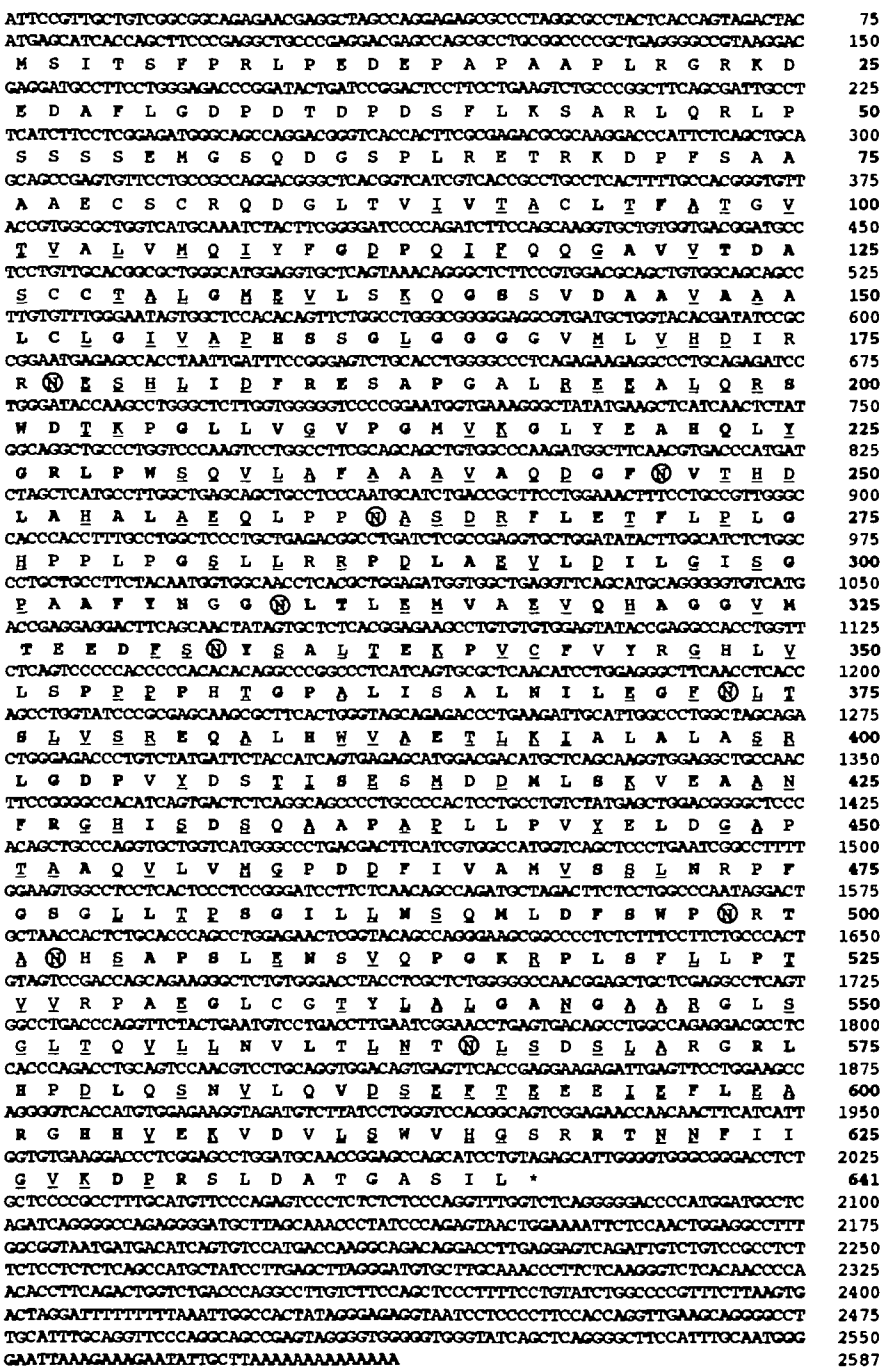

Fig 1. **Nucleotide sequence and deduced amino acid sequence of rat GTPH cDNA.** Circled asparagine residues denote potential *N*-linked glycosylation sites. Identical amino acids between GTPH and γ-GTP are indicated in bold letters and structurally similar ones are underlined.

out an *in vitro* translation experiment. mRNA, which was synthesized from the cDNA plasmid with T7 RNA polymerase, produced a 78 kDa protein in rabbit reticulolysates. The agreement in the molecular sizes of the *in vitro* translation product and the calculated one confirms the accuracy about our assumption of this open reading frame.

Amino Acid Primary Structure—Using the open reading frame, we analyzed the primary structure of the translation product with the GenBank Database. BLAST sequence homology search revealed identical amino acids between the sequences of this clone and rat γ-GTP [EC 2.3.2.2] at 182 positions (32%), and structurally homologous amino acids at 406 positions (73%) in the overlapping regions of the 556-residue sequence (Fig. 1) (*6*, *19*, *20*). Only the amino-terminal region, consisting of 90 amino acid residues, showed no structural homology. With the given structural homology, therefore, we named this novel protein gamma-glutamyltranspeptidase homologue (GTPH). The amino terminal region of GTPH is enriched in hydrophobic amino acids and presumably represents a signal peptide sequence for its secretion. GTPH also contains many putative N-linked glycosylation sites in its middle portion, as γ–GTP does.

Spatial and Temporal Expression of GTPH mRNA—To study the tissue distribution of GTPH mRNA, we carried out RNA blotting. Among the rat tissues examined, a signal

for GTPH mRNA was detected at a position coresponding to 2.6 kb only in the brain (Fig. 2). The testis also gave a very faint signal, but the size of the signal was larger than that of the GTPH we characterized. During development, the expression of GTPH mRNA in the brain gradually increases and reaches a plateau at postnatal 40 days (Fig. 3). *In situ* hybridization analysis revealed a more detailed distribution of the GTPH mRNA: GTPH mRNA expression was detected in all the nervous system including the eye and peripheral ganglia at embryonic day 18 (Fig. 4). In adults, it was found in all regions of the brain, but most enriched in the hippocampus and cerebellum. These results suggest that the GTPH mRNA is fairly abundant and expressed exclusively in the nervous system.

Biochemical Features of the GTPH Protein—An antibody was raised against the carboxyl terminal region of the GTPH protein and its specificity was tested by immunoblotting. Total protein was extracted from PC12 cells transfected with the expression vector pCMV-tag1 (Stratagene) carrying GTPH cDNA. Transformants gave a strong immunoreactive signal migrating around 90 kDa (Fig. 5). Although parental PC12 cells, as well as those transfected with pCMV-tag1 vector carrying luciferase cDNA (a control

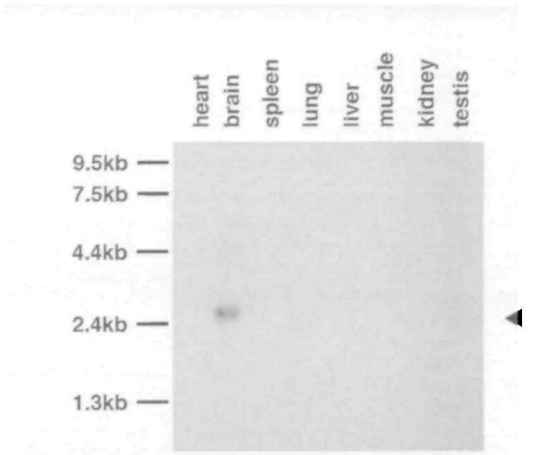

Fig 2 **Tissue distribution of GTPH mRNA.** RNA blotting was done using a commercial pretransferred sheet (Clontech; Rat Multiple tissue blot) Each lane of this blot carries 2 μg polyA+RNA prepared from the tissues of adult rats. RNA hybridization was performed under stringent conditions with a full-length GTPH cDNA as a probe A 2 6 kb signal was detected only in the brain

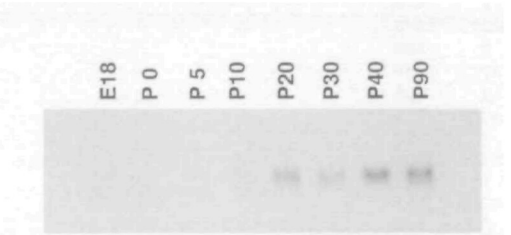

Fig 3 **Developmental increase in GTPH mRNA in rat brain.** RNA was extracted from whole rat brain at various developmental stages (E18, embryonic day 18, P0; postnatal day 0, P5, P10, P20, P30, P40, and P90) Ten micrograms of poly(A)-enriched RNA was separated in a 1% agarose gel containing formamide and formaldehyde and transferred to a nylon membrane. Hybridization was carried out as described above

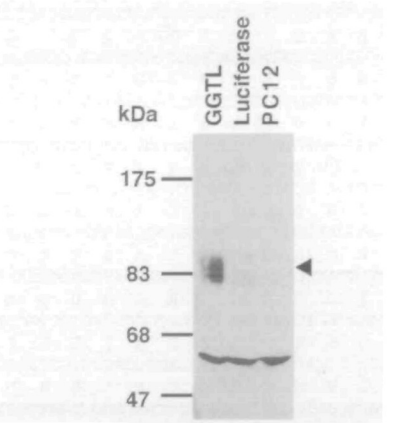

Fig 5 **Overexpression of GTPH protein in PC12 cells.** Rat GTPH cDNA was subcloned into a eukaryotic expression vector, pCMV-Tag1 (Stratagene), and transfected into PC12 cells by the calcium phosphate method. Recombinant PC12 cells carrying the GTPH gene were selected with Genetisin (G418). A vector containing the luciferase gene was also transfected as a control Protein samples were prepared from PC12 cell lines carrying GTPH, luciferase, or nothing, separated by 8% SDS-PAGE, and blotted onto a nitrocellulose membrane. The membrane was probed with affinity purified anti-GTPH antibodies. Strong immunoreactive bands between 80–90 kDa were seen only in the lane corresponding to GTPH-transfected PC12 cells, whereas a 60 kDa band was detected in all samples with the same intensity. The 60 kDa protein is presumably cross-immunoreactive to the antibodies but unrelated to GTPH

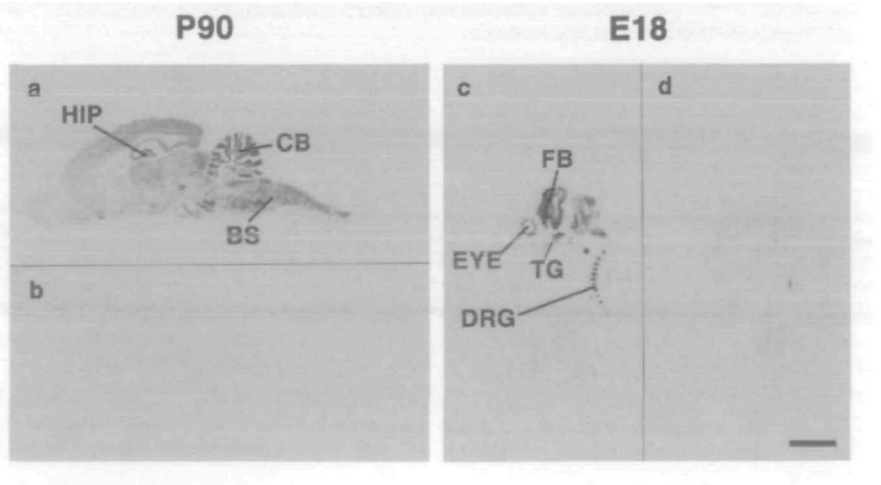

Fig. 4. ***In situ* hybridization of GTPH mRNA in rat brain.** Parasagittal brain sections from embryonic and adult rats were prepared at embryonic day 18 (E18) (c, d) or postnatal day 90 (P90) (a, b) Sections were hybridized with 50 mer synthetic oligodeoxynucleotide probes corresponding to the sense (b, d) and antisense (a, c) strands of GTPH mRNA (nucleotide residues 1070–1119). BS, brain stem; CB, cerebellum; DRG, dorsal root ganglia; FB, forebrain; HIP, hippocampus, TG, trigeminal ganglia.

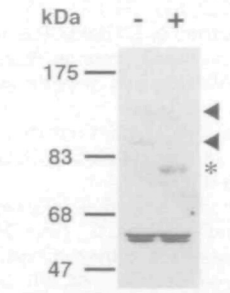

Fig. 6. **Deglycosylation of GTPH protein.** Protein in the brain P2 fraction was treated with (+) or without (–) endopeptidase F which cleaves *N*-acetylglucosamine from *N*-linked glycoproteins. The reaction products were analyzed by immunoblotting using anti-GTPH antibodies. After deglycosylation, the intensities of both the 110 and 90 kDa bands were reduced significantly and new immunoreactivity appeared at 78 kDa

gene), showed a faint band with a similar size of 90 kDa, this signal presumably represented the endogenous GTPH protein in this cell line. Smaller bands located around 40–50 kDa were common among all the PC12 samples and might reflect the immuno-crossreactivity of the antibody. The size (~90 kDa) of the signal appearing on immunoblots was larger than that of the *in vitro* translation products, presumably because of post-translational modifications such as glycosylation. To study *N*-linked glycosylation of the GTPH protein, the membrane fraction of P2 prepared from the rat brain was treated with glycopeptidase and subjected to immunoblotting (Fig 6). Immunoblotting revealed the immunoreactivity to GTPH in the P2 membrane fraction but not in the S2 soluble fraction (data not shown). After glycopeptidase treatment, the GTPH immunoreactivity disappeared from the original migration sites on SDS-PAGE and shifted to the same size as the *in vitro* translation product (~78 kDa). All these results suggest that the GTPH protein is heavily glycosylated and bound to organelle membranes or the cytoplasmic membrane.

DISCUSSION

In the present study, we have identified a novel gene that is expressed predominantly in the nervous system. The mRNA level of this gene is markedly high (>0.2%) in adult rat brain as estimated from the frequency of positive clones in the library. The gene product shares structural homology with and similar molecular features to γ–GTP: Both are heavily glycosylated and bound to cellular membranes. Despite these similarities, however, there are several differences between these molecules as well: γ–GTP consists of the two subunits produced from its glycosylated 78 kDa precursor through proteolytic processing (*21*). In contrast, we failed to detect such processed products from the GTPH molecule. The fact that the GTPH protein does not contain the amino acid sequence for proteolytic processing confirms this observation. In agreement, the degree of sequence identity between GTPH and γ–GTP appeared to be lower, 33%, but the similarity was very high (73%) at the amino acid level. However, similar relationships can be seen with another GTP homologue, GGT-rel: The identity between GGT-rel and γ–GTP is less than 40%, even though both enzymes transfer the same glutamyl moiety, but from different core structures. Thus, it can be speculated that GTPH might be involved in the metabolism of the glutamyl moiety of the compounds whose chemical structures are far from cystenyl glycine and leukotriene. Our preliminary experiments confirm that this novel protein, GTPH, fails to perform γ-glutamyltranspeptidation (*1*) from glutathione or a chromogenic substrate, γ-glutamyl-*p*-*N*-ethyl-*N*-hydroxyethyl aminoanilide (data not shown).

There are many di-peptides containing glutamyl moieties that have been identified in the brain, including γ-glutamyl-Asp, γ-glutamyl-Glu, γ-glutamyl-taurine, and γ-glutamyl-4-aminobutyric acid. These have been implicated in modulation of glutamatergic neurotransmission (*22*, *23*). All these di-peptides are presumably synthesized by a transglutamyl reaction to an amino acid. The novel form of GTPH reported here might be involved in any of these reactions and contribute to excitatory neurotransmission. Subsequent experiments will elucidate the enzymatic nature of the novel molecule as well as its physiological roles in the mature brain.

We thank Osamu Saitoh, Tomomi Yamazaki, and Mihoko Kawamura for technical assistance

REFERENCES

1. Tate, S S and Meister, A. (1985) γ-Glutamyl transpeptidase from kidney in *Methods in Enzymology* (Meister, A., ed.) Vol 113, pp 400–419, Academic Press, New York
2. Shine, H D and Haber, B (1981) Immunocytochemical localization of gamma-glutamyl transpeptidase in the rat CNS. *Brain Res.* **217**, 339–349
3. Colom, J and Pitot, H C (1986) Characterization and sequence of a cDNA clone of gamma-glutamyltranspeptidase. *Nucleic Acids Res.* **4**, 1393–1403
4. Richelt, K.L and Poulsen, E. (1992) Gamma-glutamylaminotransferase and transglutaminase in subcellular fractions of rat cortex and in cultured astrocytes. *J Neurochem.* **59**, 500–504
5. Shine, H D , Hert, L., Vellis, J , and Harber, B (1981) A fluormetric assay for γ-glutamyltranspeptidase. demonstration of enzymatic activity in cultured cells of neural origin *Neurochem Res.* **6**, 453–463
6. Reyes, E and Barela, T D (1980) Isolation and purification of multiple forms of gamma-glutamyl transpeptidase from rat brain *Neurochem Res.* **5**, 159–170
7. Harding, C.O., Williams, P., Wagner. E., Chang, D S , Wild, K., Colwell, R E , and Wolff, J A. (1997) Mice with genetic gamma-glutamyl transpeptidase deficiency exhibit glutathionuria, severe growth failure, reduced life spans, and infertility. *J. Biol. Chem* **272**, 12560–12567
8. Carter, B Z , Wiseman, A.L., Orkiszewski, R., Ballard, K.D., Ou, C.N., and Lieberman, M.W (1997) Metabolism of leukotriene C4 in gamma-glutamyl transpeptidase-deficient mice *J. Biol Chem.* **272**, 12305–12310
9. Potdar, P.D., Andrews, K.L , Nettesheim, P., and Ostrowski, L.E (1997) Expression and regulation of gamma-glutamyl transpeptidase-related enzyme in tracheal cells. *Am. J Physiol* **273**, 1082–1089
10. Snyder, M., Elledge, S , Sweetser, D., Young, R.A., and Davis, R W (1987) λgt11· Gene isolation with antibody probes and other applications in *Methods in Enzymology* (Wu, R. and Grossman, L , eds.) Vol 154, pp 107–128, Academic Press, New York
11. Nakajima, Y., Iwakabe, H., Akazawa, C., Nawa, H , Shigemoto, R., Mizuno, N , and Nakanishi, S. (1993) Molecular characterization of a novel retianal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for l-2-amino-4-phos-

phonobutyrate. *J. Biol. Chem.* **267**, 13361–13368
12. Maniatis, T., Fritsch, E.F, and Sambrook, J (1991) *Molecular Cloning: A Laboratory Mannual.*, Cold Spring Harbor Press, Cold Spring Harbor, NY
13. Katagiri, T., Feng, X., Ichikawa, T., Usui, H., Takahashi, Y., and Kumanishi, T (1993) Neuron-specific enolase (NSE) and non-neuronal enolase (NNE) mRNAs are co-expressed in neurons of the rat cerebellum: in situ hybridization histochemistry. *Mol Brain Res.* **19**, 1–8
14. Araki, K., Megro, H., Kushiya, E , Takayama, C., Inoue, Y , and Mishina, M. (1993) Selective expression of the glutamate receptor channel delta 2 subunit in cerebellar Purkinje cells. *Biochem. Biophys. Res. Commun.* **197**, 1267–1276
15. Greene, L.A., Aletta, J.M., Rukenstein, A., and Green, S.H. (1987) PC12 pheochromocytoma cells: culture, nerve growth factor treatment, and experimental exploitation in *Methods in Enzymology* (Barnes, D. and Sirbasku, D. A., eds.) Vol. 147, pp 207–216, Academic Press, New York
16. Adachi, A., Kawamura, M , Tokunaga, K., and Sakai, H. (1996) *Methods for HIV/SIV Gene Analysis. Viral Genome Methods*, pp 44–52, CRC Press
17. Carlin, R.K., Grab, D.J., Cohen, R.S., and Siekevitz, P. (1980) Isolation and characterization of postsynaptic densities from various brain regions: Enrichment of different types of postsynaptic densities. *J. Cell Biol.* **86**, 831–843
18. Walch M.J. and Kuruc, N J. (1992) The postsynaptic density; constituent and associated proteins characterized by electrophoresis, immunoblotting and peptide sequencing. *J Neurochem* **59**, 667–678
19. Colom, J. and Pitot, H.C. (1986) Characterization and sequence of a cDNA clone of gamma-glutamyltranspeptidase. *Nucleic Acid Res.* **4**, 1393–1403
20. Laperche, Y., Bulle, F., Aissani, T., Chobert, M N., Aggerbech, M , Hanoune, J., and Guellaen, G. (1986) Molecular cloning and nucleotide sequence of rat kidney gamma-glutamyltranspeptidase. *Proc. Natl. Acad. Sci. USA.* **83**, 937–941
21. Nash, B. and Tattoo, S.S. (1984) *In vitro* translation and processing of rat kidney gamma-glutamyl transpeptidase. *J. Biol. Chem.* **259**, 678–685
22. Li, X., Orwar, O., Persson, J., Sandberg, M , and Jacobson, I. (1993) gamma-L-Glutamyl-L-glutamate is an endogenous dipeptide in the rat olfactory bulb which activates *N*-methyl-D-aspartate receptor *Neurosci. Letter.* **155**, 42–46
23 Verga, V , Janaky, R., and Oja, S.S. (1992) Modulation of glutamate agonist-induced influx of calcium into neurons by gamma-L-glutamyl and beta-L-aspartyl dipeptides. *Neurosci. Lett.* **138**, 270–274