Purification and expression of a processing protease on β-alanine-oxoglutarate aminotransferase from rat liver mitochondria

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Abstract GABA[arrow beta]AlaAT convertase is an endopeptidase that processes brain-type 4-aminobutyrate aminotransferase (GABA AT; EC 2.6.1.19) to liver-type β-alanine-oxoglutarate aminotransferase (β-AlaAT I) in rats. Its molecular mass was 180 kDa as determined by gel filtration. A subunit molecular mass of 97 652 Da was measured using MALDI-TOF MS. The N-terminal sequence of the purified GABA[arrow beta]AlaAT convertase was SRVEVSKVLILGSGGLSIGQAGEFDYSGS-QAV- and was identical to residues 418–449 of carbamoyl-phosphate synthetase I (CPS I; EC 1.2.1.27) purified from rat liver. The subunit molecular mass and the N-terminal amino acid sequence suggested that GABA[arrow beta]AlaAT convertase was the 418–1305 peptide of CPS I. An expression vector containing the coding region of the 418–1305 peptide of rat CPS I was transfected into NIH3T3 cells and the extract of the cells showed GABA[arrow beta]AlaAT convertase activity.

Keywords: 4-Aminobutyrate aminotransferase; 4-Aminobutyrate; Mitochondrial processing peptidase; β-Alanine aminotransferase; β-Alanine; Carbamoyl-phosphate synthetase

1. Introduction

β-Alanine-oxoglutarate aminotransferase (β-AlaAT I) catalyzes the transamination of β-alanine, which is mainly formed in liver cytosol by the reductive degradation of uracil via dihydrouracil and *N*-carbamoyl-β-alanine [1–3], and transported into mitochondria [4]: where β-alanine is further metabolized to malonate semialdehyde by β-AlaAT I or D-3-aminoisobutyrate-pyruvate aminotransferase (β-AlaAT II; EC 2.6.1.40) in the mitochondrial matrix [4,5].

β-AlaAT I purified from rat liver also catalyzes the transamination of 4-aminobutyrate (GABA) [6] and L-β-aminoisobutyrate [7] in addition to β-alanine, while β-AlaAT II is not active for GABA [8]. It is well known that GABA is a major inhibitory transmitter in many invertebrate systems and also in the vertebrate central nervous system. Enzyme activity of β-AlaAT I is mainly distributed in the mitochondrial matrix of the brain [9], liver [4], and kidney [5] in rats. The immunological and kinetic properties show that β-AlaAT I from rat liver closely resembles those of GABA aminotransferase (GABA AT) from the rat brain [6]. Rat liver β-AlaAT I mRNA and rat brain GABA AT mRNA are transcripts from the same gene [10]. However, the N-terminal amino acid of rat liver β-AlaAT I is Val [6], while that of rat brain GABA AT has been identified as I1e [11] by the dansyl method. The amino acid sequence predicted from rat liver cDNA suggests that the precursor to β-AlaAT I (pre-GABA AT) consists of a liver-type mature enzyme of 466 amino acid residues, with 34 residues attributed to the leader peptide. The mature form of GABA AT from the brain has an ISQAAAK-peptide additional to the N-terminus sequence of liver-type mature-β-AlaAT I [10] (Scheme 1).

The liver-type mature enzyme and the brain-type mature enzyme were named mature-β-AlaAT I and mature-GABA AT, respectively. Brain-type GABA AT is cleaved to liver-type β-AlaAT I protein when incubated with fresh mitochondrial extract from rat liver [10]. These results suggest the existence of an enzyme, which converts the brain-type mature-GABA AT to the liver-type mature-β-AlaAT I. This paper describes the purification of the enzyme GABA[arrow beta]AlaAT convertase from rat liver mitochondria.

2. Materials and Methods

2.1. Materials

All reagents were of analytical grade and were purchased from Nacalai Tesque (Kyoto, Japan). DEAE–Sepharose CL-6B and Sephacryl S-200 were obtained from Amersham Biosciences (Uppsala, Sweden). Hydroxyapatite was obtained from the Seikagaku Corporation (Tokyo, Japan) Cibacron blue F3G-A-Sepharose 4B was prepared by binding Cibacron blue F3G-A to Sepharose 4B according to Rinderknecht et al. [12] and was used to remove adenine nucleotide specific binding proteins. The *Escherichia coli* strain TOP 10, pSE420 expression vector and the polymerase chain reaction (PCR) primers were obtained from Invitrogen Corp. (CA, USA). Restriction enzymes

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Abbreviations: GABA, 4-aminobutyrate; β-AlaAT I, β-alanine-oxoglutarate aminotransferase; GABA AT, GABA aminotransferase; β-AlaAT II, (*R*)-3-amino-2-methylpropionate-pyruvate aminotransferase [(*R*)-β-aminoisobutyrate-pyruvate aminotransferase]; pre-GABA AT, precursor of GABA AT; CPS I, carbamoyl-phosphate synthetase I; PMSF, phenylmethylsulfonylfluoride; PCR, polymerase chain reaction

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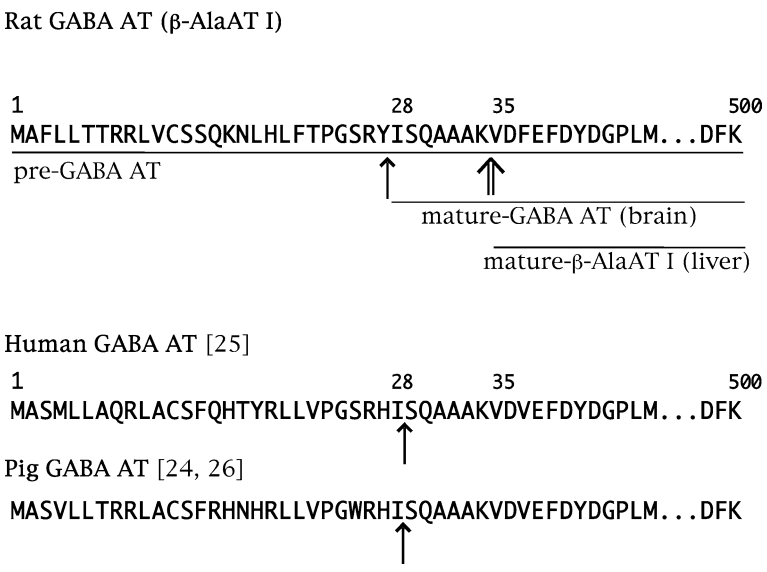

Scheme 1. Processing of rat liver β-AlaAT I. Alignment of the N-terminal amino acid sequence of GABA AT from rats. Single arrow (↑) indicates the putative processing site cleaved by mitochondrial processing peptidase. Double arrow (⇑) indicates the processing site of rat liver GABA[arrow beta]AlaAT convertase.

were the products of New England Biolabs Inc. (MA, USA). A QuickChange site-directed mutagenesis kit was obtained from STRATAGENE (CA, USA), TransFast™ Transfection Reagent and pCI-neo expression vector were obtained from Promega Corp. (WI, USA). NIH3T3 cells were obtained from Riken (Tsukuba, Japan). The molecular mass markers for SDS–PAGE were a product of Invitrogen Corp. (CA, USA).

2.2. Preparation of mitochondrial extract

Fresh livers (40 g) from male Wistar ST strain rats were homogenized using Potter-Elvejem homogenizer in 10 vol. (w/v) of 0.25 M sucrose in respect to the original liver mass. The homogenate was centrifuged for 10 min at 500×*g*. The supernatant was centrifuged again for 10 min at 8000×*g* to obtain pellets of mitochondria. The mitochondria were suspended in a minimum volume of 0.25 M sucrose. They were then put in a tube with a prepared sucrose density gradient from 1.15 to 1.24 g/ml and centrifuged for 180 min at 82 000×*g*. After centrifugation, the mitochondrial fractions were recovered and resuspended in 0.25 M sucrose containing 10 mM potassium phosphate, pH 7.5, 1 mM EDTA, and 2 mM 2-mercaptoethanol. The mitochondrial suspension was sonicated with Microson™, Model XL2000 (Misonix Incorporated, New York, USA). The sonicated suspension was centrifuged for 15 min at 10 000×*g*. The supernatant was used as the mitochondrial extract.

2.3. Preparation of mature-GABA AT from rat brain

Mature-GABA AT from rat brain was purified according to the methods previously described [6].

2.4. Assay of GABA[arrow beta]AlaAT convertase activity

GABA[arrow beta]AlaAT convertase activity was determined by measuring the rate of conversion from mature-GABA AT to the mature-β-AlaAT I liver form. The standard reaction mixture contained 0.3 μg of purified mature-GABA AT, 10 mM potassium phosphate, pH 7.0, containing 0.5 mM EDTA and 2 mM 2-mercaptoethanol in a final volume of 12 μl. The incubation was carried out for 3 h at 25 °C. The reaction was terminated with the addition of 2% SDS, then analyzed using SDS–PAGE (7.5% gel). The immunoblot analysis was conducted by the method of Towbin et al. [13]. Rabbit polyclonal antibody against rat liver β-AlaAT I has been previously prepared [6]. The densities of bands corresponding to mature-GABA AT and mature-β-AlaAT I were determined using an image analyzer (Fujifilm FLA-2000, Tokyo, Japan). One unit of enzyme activity was defined as the amount that produced a 50% conversion of mature-GABA AT to mature-β-AlaAT I under the standard assay conditions. The enzyme activity was proportional with reaction time until about 90% of the product was formed.

2.5. Purification procedure of GABA[arrow beta]AlaAT convertase

All subsequent steps were performed at approximately 4 °C. Purification was started from the mitochondrial extract from 40 g of rat liver. The precipitate obtained at 50% ammonium sulfate saturation was dissolved in a minimum volume of buffer A (10 mM potassium phosphate, pH 7.5, containing 0.5 mM EDTA, 2 mM 2-mercaptoethanol, and 0.1 mM phenylmethylsulfonylfluoride (PMSF)) and desalted via dialysis against buffer A overnight at 4 °C. After centrifugation, the supernatant was applied to a DEAE–Sepharose CL-6B column (2.0×16 cm, flow rate 24 ml/h) equilibrated with buffer A containing 10% (v/v) glycerol. The column was washed with about 120 ml of buffer A and eluted with buffer A containing 100 mM KC1, followed by 100–350 mM linear gradient KC1 in buffer A. The active fractions were concentrated using a Centricon Plus 20 (Amicon Corp. MA, USA). The enzyme preparation was applied to a Cibacron blue F3G-A-Sepharose 4B column (1.5×8.5 cm, flow rate 12 ml/h) equilibrated with buffer B (10 mM potassium phosphate, pH 7.0, containing 0.5 mM EDTA, 2 mM 2-mercaptothanol, 0.1 mM PMSF, and 10% (v/v) glycerol). GABA[arrow beta]AlaAT convertase passed through the column with buffer B. The eluate was applied to a hydroxyapatite column (1.0×6.4 cm, flow rate 6 ml/h) equilibrated with buffer B, followed by buffer B-buffer C (100 mM potassium phosphate, pH 7.0, containing 0.5 mM EDTA, 2 mM 2-mercaptoethanol, 0.1 mM PMSF, and 10% (v/v) glycerol) linear gradient. The active fraction was concentrated to about 1.0 ml using Centricon Plus 20. The enzyme was applied to a Sephacryl S-200 column (1.5×60 cm, flow rate 10 ml/h) equilibrated with buffer C. The active fraction was concentrated to about 1.0 ml, then stored at 4 °C.

2.6. Inhibition of GABA[arrow beta]AlaAT convertase activity by an antiserum against carbamoyl-phosphate synthetase I

Rabbit polyclonal antibody against rat liver carbamoyl-phosphate synthetase I (CPS I) was prepared previously [14,15]. The proteins of the rabbit serum were precipitated with 50% $(NH_4)_2SO_4$ and dialyzed in 10 mM potassium phosphate, pH 7.0. The solution was diluted with the above buffer and was used as anti-CPS I.

GABA[arrow beta]AlaAT convertase (0.5 μg) was incubated with anti-CPS I [14,15] for 1 h at 25 °C. GABA AT was added to the above reaction mixture and incubated for 3 h at 25 °C.

The processing reaction was stopped by the addition of 2% SDS. Immunoblot analysis was performed using SDS–PAGE with rabbit β-AlaAT I antibody and the inhibition of GABA[arrow beta]AlaAT convertase activity was analyzed.

2.7. Expression of GABA[arrow beta]AlaAT convertase in mammalian cells

GABA[arrow beta]AlaAT convertase (the peptide of CPS I 418–1305) cDNA was amplified using the PCR method with the forward primer 5′-AAGAAATTCATGTCTCGAGTCGAGGTTTCCAA-3′ and the reverse primer 5′-AAGAATTCTTACTTAATGGCAA-CATAGTCAGAG-3′ containing EcoR I sites, respectively. The PCR-generated fragment GABA[arrow beta]AlaAT convertase cDNA was then cloned into the EcoR I site of pCI-neo for the mammalian cell expression vector (pCI-neo-convertase). The constructs were transfected into NIH3T3 cells using TransFast™ Transfection Reagent following the manufacturer's protocol. NIH3T3 cells were harvested 48 h after transfection using centrifugation for 2 min at 300×*g*. The precipitate was suspended with 200 μl of 10 mM potassium phosphate, pH 7.0, containing 0.5 mM EDTA, 2 mM 2-mercaptoethanol, 0.1 mM PMSF and 10% glycerol. After sonication, the suspension was centrifuged for 20 min at 15 000×*g*. The supernatant was used for the assay of GABA[arrow beta]AlaAT convertase activity. The pCI-neo expression vector was transfected to NIH3T3 cells as a control.

2.8. Expression of the pre- and mature-GABA ATs in E. coli

The coding region of GABA AT cDNA was amplified using the PCR method with the forward primer 5′-TAGCGGCCGCA-TGGCCTTCTTGTTGACTAC-3′ and the reverse primer 5′-GAC-TCGAGTTACTTGAAGTCTGCTAAGA-3′ containing *Nco*I and *Xho*I sites, respectively. The PCR generated fragment was then cloned into the *Nco*I and *Xho*I sites of pSE420. The resulting plasmid pSE420-pre-GABA AT contained the nucleotides that contributed to a 500 amino acid residue. *E. coli* TOP 10 strain was transformed with the ligation mixture. pSE420-pre-GABA AT clones were selected in the ampicillin-resistant transformants. The construction of pSE420-mature-GABA AT was performed using a QuickChange site-directed mutagenesis kit following the manufacturer's protocol. *E. coli* transformed with pSE420-pre-GABA AT or pSE420-mature-GABA AT were cultured for 48 h at 29 ° C, then harvested using centrifugation for 5 min at 800×*g* [16,17]. The precipitate was washed with 10 mM potassium phosphate, pH 7.5, containing 1.0 mM EDTA, 2 mM 2-mercaptoethanol, and 50 μM pyridoxal phosphate, then harvested for 5 min at 800×*g*. After sonication of the harvested cells, the suspension was centrifuged for 20 min at 10 000×*g*. The supernatant containing the recombinant pre-GABA AT and mature-GABA AT was used as a substrate of GABA[arrow beta]AlaAT convertase.

2.9. Determination of molecular masses

The subunit molecular mass of purified GABA[arrow beta]AlaAT convertase was determined by Sephacryl S-200 chromatography with thyroglobulin (670 kDa), gammaglobulin (158 kDa), ovalbumin (44 kDa) and myoglobin (17 kDa)as the markers. The molecular masses of the GABA[arrow beta]AlaAT convertase, mature-GABA AT and mature-β-AlaAT I were determined with MALDI-TOF MS, Voyager DE-STR (Boston, MA, USA).

3. Results

3.1. Purification of GABA[arrow beta]AlaAT convertase

The purification steps and the specific activity were shown in Table 1. GABA[arrow beta]AlaAT convertase could be separated from mature-β-AlaAT I, which is basally expressed in liver, by DEAE–Sepharose chromatography. Therefore, we could estimate GABA[arrow beta]AlaAT convertase activity after the DEAE–Sepharose chromatography step.

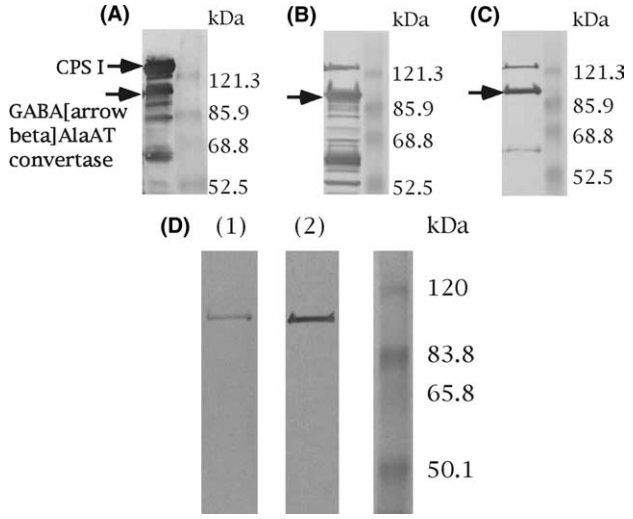

Fig. 1. Gel electrophoresis of purified GABA[arrow beta]AlaAT Convertase. The enzyme of each purification step was analyzed by polyacrylamide gel electrophoresis in the presence of 0.1% SDS and immunoblot analysis was performed with rabbit CPS I antibody. A Bench Mark™ Prestained Protein Ladder(Invitrogen) was used as the molecular mass markers. (A) The mitochondrial extract (total protein 120 μg). (B) After DEAE–sepharose CL-6B chromatography (total protein 100 μg). (C) After hydroxyapatite chromatography (total protein 2 μg). (D) The purified enzyme (total protein 0.5 μg after Sephacryl S-200 chromatography): (1) the gel was stained with Coomassie brilliant blue R-250; (2) the gel was immunoblotted and analyzed with CPS I antibody.

In gel electrophoresis, a GABA[arrow beta]AlaAT convertase band stained with CPS I antibody was found in the rat liver mitochondrial extract (Fig. 1A) and purified to a single band (Fig. 1D-2). The purified GABA[arrow beta]AlaAT convertase showed a single band stained with Coomassie brilliant blue R-250 (Fig. 1D-l).

By comparing it with the reference proteins, the molecular mass of the purified enzyme by Sephacryl S-200 was calculated to be 180 kDa. The subunit molecular mass of the purified GABA[arrow beta]AlaAT convertase was 97 kDa (Fig. 1). The molecular mass of the purified enzyme determined by MALDI-TOF MS was 97 652 Da. These findings suggest that purified GABA[arrow beta]AlaAT convertase from rat liver is organized as a dimer.

3.2. N-terminal amino acid sequence and identification with a segment of CPS I

The N-terminal sequence of the purified GABA[arrow beta]AlaAT convertase determined by dansyl Edman method

Table 1
Purification of GABA[arrow beta]AlaAT convertase from rat liver

Steps	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification (-fold)	Yield (%)
DEAE–Sepharose chromatography	13.69	53.4	3.9	1.0	100
Cibacron blue chromatography	1.87	21.8	11.7	3.0	41
Hydroxyapatite chromatography	0.20	5.3	26.0	6.7	9.9
Sephacryl S-200 chromatography	0.05	4.6	92.0	23.6	8.6

One unit of processing activity is defined as the amount of enzyme that cleaves 50% of mature-GABA AT to mature-β-AlaAT I under the assay condition described in Section 2.

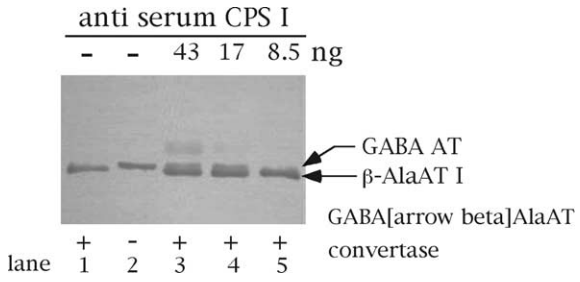

Fig. 2. Inhibition of GABA[arrow beta]AlaAT convertase activity by an antibody against CPSI GABA[arrow beta]AlaAT convertase was preincubated with buffer or anti CPS I serum. After the incubation, GABA[arrow beta]AlaAT convertase activity was assayed with 0.3 μg of purified GABA AT as substrate.

was SRVEVSKVLILGSGGLSIGQAGEFDYSGSQAV- and was the same as the amino acid sequence at positions 418–449 from rat CPS I [18]. When the purified GABA[arrow beta]AlaAT convertase was incubated with the antiserum against rat liver CPS I, GABA[arrow beta]AlaAT convertase activity was inhibited with an antiserum dose dependency (Fig. 2). The purified CPS I (30 μg) did not show any sign of GABA[arrow beta]AlaAT convertase activity under the standard assay conditions.

After incubation of the purified rat brain mature-GABA AT with GABA[arrow beta]AlaAT convertase (Fig. 2, lane 1), the product molecular mass was 52 960 Da by MALDI-TOF MS, which was the same as that of rat liver β-AlaAT I [10].

3.3. Expression of recombinant GABA[arrow beta]AlaAT convertase

In order to estimate whether the recombinant peptide is responsible for the GABA[arrow beta]AlaAT convertase activity, an expression vector containing the coding region of the 418–1305 peptide fragment of rat CPS 1 was constructed and transfected into NIH3T3 cells. Fig. 3A shows that the recombinant peptide was detected in NIH3T3 cells transfected with pCI-neo-convertase vector, but not empty pCI-neo vector. The recombinant protein revealed the GABA[arrow beta]AlaAT convertase activity (Fig. 3B, lanes 2, 3). The extract

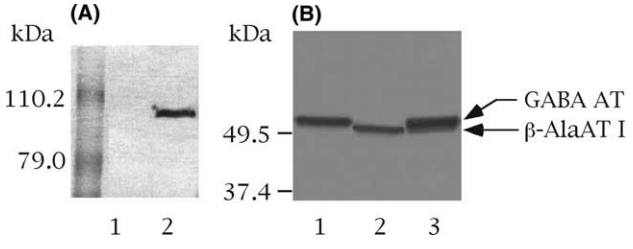

Fig. 3. GABA[arrow beta]AlaAT convertase activity of the recombinant CPS I418–1305. (A) Immunoblot analysis of recombinant GABA[arrow beta]AlaAT convertase with CPS I antibody. Lane 1, 50 μg protein of crude extract of NIH3T3 cells which were transfected with the pCI-neo vectors; lane 2, 50 μg protein of crude extract of NIH3T3 cells which were transfected with the pCI-neo-convertase. (B) GABA[arrow beta]AlaAT convertase activity. GABA[arrow beta]AlaAT convertase activity of each sample was assayed. Lane 1,50 μg protein of NIH3T3 cells transfected with the pCI-neo vector; lane 2, 50 μg protein of crude extract of NIH3T3 cells transfected with the pCI-neo-convertase; lane 3, 20 μg protein of crude extract of NIH3T3 cells transfected with the pCI-neo-convertase.

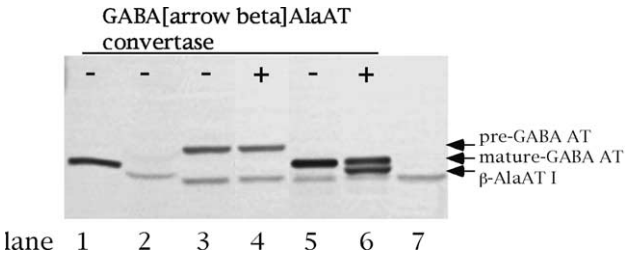

Fig. 4. Processing of GABA AT precursors. The extract from bacteria, which expressed pre-GABA AT or mature-GABA AT, was incubated in the presence or absence of 0.5 μg of purified GABA[arrow beta]AlaAT convertase under the standard assay conditions. Immunoblot analysis was performed with anti-rat β-AlaAT I rabbit serum. Lane 1, 10 μg protein of fresh extract from rat brain; lane 2, 10 μg protein of fresh extract from rat liver; lanes 3 and 4, 15 μg crude extract of pre-GABA AT; lanes 5 and 6, 15 μg crude extract of recombinant mature-GABA AT; lane 7, 30 μg crude extract of the *E. coli* strain TOP 10. The bands of low molecular mass in lanes 3–7 are non-specific bands, which are derived from the *E. coli* strain TOP 10.

from the control cells did not show any sign of enzyme activity (Fig. 3B, lane 1).

3.4. Processing of β-AlaAT I precursor by GABA[arrow beta]AlaAT convertase

The recombinant β-AlaAT I precursors were utilized to confirm the processing properties of GABA[arrow beta]AlaAT convertase. The pre-GABA AT and mature-GABA AT at 1–500 and 28–500, respectively, in Scheme 1, were expressed in *E. coli*. The purified GABA[arrow beta]AlaAT convertase cleaves mature-GABA AT (Fig. 4, lane 6), but not the pre-GABA AT as a substrate (Fig. 4, lane 4). The band of β-AlaAT I in Fig. 4, lane 6 was analyzed using Edman degradation. The N-terminal sequence was VDFEFDY-, which was the same as the N-terminal amino acid sequence of the liver-type mature-β-AlaAT I.

4. Discussion

We purified a new type of mature-β-AlaAT I converting endopeptidase, GABA[arrow beta]AlaAT convertase, from rat livers. Most mitochondrial proteins are translated on cytoplasmic ribosomes as larger precursors and are then imported into the organelle [19,20]. The presequences are recognized so as to direct the proteins to mitochondria, then cleaved and lost in the mitochondria [21,22]. The mitochondrial presequences were cleaved and classified as four cleavage site motifs as reported by Gakh et al. [23]: (i) XRX↓X(S/X) (R-2 motif); (ii) XRX(Y/X)↓(S/A/X)X (R-3 motif); (iii) XRX↓(F/L/I)XX(S/T/G)XXXX↓ (R-10 motif); and (iv) XX↓X(S/X) (R-none motif). The amino acid sequence of the coding region of rat GABA AT (β-AlaAT I) contains 500 residues [13] similar to GABA AT from pig [24] and human [25]. Precursors of GABA AT from pig and human have the R-10 motif, but that of rat has the R-2 motif (Scheme 1). Therefore, the mature-GABA AT from pig [24,26] or human [25] was composed of the same 472 amino acid residues, while the mature one from rat [10] was 473. The N-terminal amino acids of β-AlaAT I from rat liver and GABA AT from rat brain are Val [6] and I1e [11], respectively. The N-terminal of rat mature-GABA AT may be further cleaved in the liver (Scheme 1). It was suggested that a new type of liver mature-β-AlaAT I converting endopeptidase,

GABA[arrow beta]AlaAT convertase, cleaves the heptapeptide at the N-terminal of the brain form of GABA AT [10]. The purified GABA[arrow beta]AlaAT convertase could not cleave the rat pre-GABA AT, which is expressed in *E. coli,* but was active for expressed rat mature-GABA AT. We confirmed the previous finding that the mature-β-AlaAT I is proteolytically cleaved in two steps [10]. The first cleavage of the R-2 motif is performed by a mitochondrial processing peptidase, yielding an intermediate sized protein which is the mature GABA AT. The second cleavage is carried out by a mitochondrial endopeptidase of GABA[arrow beta]AlaAT convertase.

The N-terminal amino acid sequences of GABA[arrow beta]AlaAT convertase were the same as positions 418–449 of rat liver CPS I. The subunit molecular mass of GABA[arrow beta]AlaAT convertase was 97 652 Da. The subunit molecular mass of rat liver CPS I is 160 kDa [18]. The precursor of CPS I consists of a 1500 amino acid residue with a 38-amino-termmal leader segment. Southern hybridization analysis of rat genomic DNA suggested that the CPS I gene is present in a single copy [18]. These findings suggest that CPS I may be cleaved between positions 417/418 and 1305/1306 (theoretical molecular mass 97 652 Da).

After the total RNA of rat liver was translated in a rabbit reticulocyte protein-synthesizing-system, the protein CPS I produced was detected as a single band by CPS I antibody [14,15]. Immunoblot analysis with anti-rat CPS I rabbit serum showed a single band with the same positive stain using Coomassie brilliant blue R-250 (Fig. 1D). These findings suggest that GABA[arrow beta]AlaAT convertase is composed of a 98 kDa protein cleaved from CPS I. The 98 kDa protein stained with anti-rat CPS I rabbit serum was found in the rat liver mitochondrial extract (Fig. 1A). The peptide CPS I 418–1305 expressed in NIH3T3 cells shows the GABA[arrow beta]AlaAT convertase activity (Fig. 3). It is well established that CPS I is not distributed in the brain. Therefore, liver-type β-AlaAT I cannot be detected in the brain (Fig. 4, lane 1).

The significance of the existence of GABA[arrow beta]AlaAT convertase is not well documented. Pyrimidine catabolizing enzymes of dihydropyrimidine dehydrogenase, dihydropyrimidinase and β-ureidopropionase are localized mainly in the liver, followed by the kidney in rats [10]. The affinity of β-alanine for β-AlaAT I is significantly higher than that for GABA AT in rats [10]. The β-alanine concentration is about 0.1 mM in the liver [27] and 0.01–0.04 mM in the brain [28,29], Therefore, liver-type mature-β-AlaAT I may be more favorable for β-alanine catabolism than brain-type mature-GABA AT at least.

GABA[arrow beta]AlaAT convertase derived from CPS I was clearly found in the rat liver mitochondrial extract and it is not formed during the course of the purification steps. CPS I in the rat liver has been estimated to make up 15–20% of the total mitochondrial protein [30]. It is well recognized that CPS I functions to convert ammonia, bicarbonate and ATP into carbamoyl phosphate, the initial step in the urea cycle. Native CPS I did not show the GABA[arrow beta]AlaAT convertase activity. As shown in this paper, a fragment of CPS I had an unexpected proteolytic activity and processing activity function towards rat liver β-AlaAT I.

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