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Isolation and Characterization of Aminotripeptidase from Monkey Brain[1]

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Aminotripeptidase [EC 3.4.11.4] was purified from monkey brain by a five-step procedure comprising extraction from brain homogenate, ammonium sulfate fractionation, DEAE-cellulose chromatography, hydroxylapatite chromatography, and Sephadex G-200 gel filtration. A purification of 1,100-fold over the homogenate was achieved and the yield was 12%. The purified enzyme appeared to be homogeneous on polyacrylamide gel electrophoresis at pH 8.9. The amino acid composition of the enzyme resembled that of the pig kidney enzyme. The molecular weight of the enzyme was estimated to be about 65,000 by gel filtration on Sephadex G-200 and 70,000 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The pH optimum for L-leucyl-glycyl-glycine was about pH 7.5. The enzyme hydrolyzed only tripeptides to yield the NH_2-terminal residues as free amino acids and the residual dipeptides. The enzyme did not show activities of arylamidase or carboxypeptidases A and B. The enzyme was inhibited by PCMB, *o*-phenanthroline, and bestatin. The inhibition by bestatin was competitive and the K_i value was calculated to be 5×10^{-7} M.

Various aminopeptidases and arylamidases have been detected in mammalian brain tissues (*1*). These enzymes are thought to be involved in protein metabolism in the central nervous system, and some are assumed to participate in the inactivation and degradation of neuropeptides in brain tissues (*2*, *3*).

Preliminary results reported from our laboratory indicated that monkey brain possessed two aminopeptidase activities using L-leucyl-glycyl-glycine as a substrate (*4*). One of these was identified as an arylamidase which has been purified and characterized previously (*5*). The other aminopeptidase is presumably an aminotripeptidase in view of the following properties: active only towards tripeptide, a molecular weight of about 70,000, appropriate amino acid composition, and inactivation by PCMB and *o*-phenanthroline.

Aminotripeptidase has been partially purified from calf thymus (*6*) and horse erythrocytes (*7*). The first purification and characterization of this enzyme was accomplished by Chenoweth *et al.* using pig kidney (*8*). More recently, the enzyme has been purified from rabbit intestinal mucosa

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Abbreviations: SDS, sodium dodecyl sulfate; PCMB, *p*-chloromercuribenzoate; TPCK, α-*N*-tosyl-L-phenylalanine chloromethyl ketone; TLCK, α-*N*-tosyl-L-lysine chloromethyl ketone; DFP, di-isopropyl phosphorofluoridate; BPB, Bromphenol blue; IAA, iodoacetic acid; NEM, *N*-ethylmaleimide.

(*9*). The purification of this enzyme from brain tissues has only been carried out from bovine brain (*10*).

The present paper describes the purification of aminotripeptidase from monkey brain together with various characteristics such as amino acid composition, the effects of various compounds, and substrate specificity.

EXPERIMENTAL PROCEDURE

Material—Japanese monkey (*Macaca fuscata fuscata*) was anesthetized with ketamine hydrochloride and killed by exsanguination. The brain was immediately removed and stored at −20°C until use. L-Leucyl-glycyl-glycine,[2] leucyl-glycine, di-glycine, leucine amide, met^5-enkephalin, leu^5-enkephalin, carbobenzoxy-glycyl-phenylalanine, and benzoyl-glycyl-arginine were obtained from the Protein Research Foundation, Osaka, Japan. Di-alanine, tri-alanine, tetra-alanine, penta-alanine, tri-leucine, di-leucine, phenylalanyl-glycine, L-amino acid oxidase (Type I), horseradish peroxidase (Type II), and *o*-dianisidine (3,3′-dimethoxy benzidine) were obtained from Sigma Chemical Co., St. Louis, Missouri, U.S.A. Bestatin was kindly supplied by Drs. H. Umezawa and T. Aoyagi. Diethylaminoethyl cellulose (DE-32), hydroxylapatite, and Sephadex G-200 were purchased from Whatman Biochemical Ltd., England, Seikagaku Kogyo Co., Tokyo, Japan, and Pharmacia Fine Chemicals, Uppsala, Sweden, respectively. Polyamide layer sheets were products of Chen Chin Trading Co., Ltd., Taipei, Taiwan. All other chemicals were of reagent grade.

Determination of Protein—Protein was determined according to Lowry *et al.* (*11*). Bovine serum albumin was used as a standard.

Assay of Enzymatic Activities—Enzyme assay using the hydrolysis of leucyl-glycyl-glycine was conducted by the method of Nicholson and Kim (*12*) with a slight modification. The reaction mixture contained 0.1 ml of appropriately diluted enzyme and 0.75 ml of 50 mM Tris-HCl buffer, pH 7.5, containing 500 nmol of leucyl-glycyl-glycine, 100 μg of L-amino acid oxidase, 50 μg of *o*-dianisidine, and 10 μg of horseradish peroxidase. The mixture was incubated at 37°C for 20 min. The reaction was stopped by adding 0.3 ml of 50% sulfuric acid. The absorbance at 530 nm was measured using a Hitachi 124 spectrophotometer. One unit of aminopeptidase activity was defined as the appearance of absorbance at 530 nm equivalent to 1.0 optical density in 20 min at 37°C. In the study of substrate specificity, the reaction was carried out as described above using various peptide substrates. Enzyme assay for arylamidase was carried out as described previously (*5*).

Polyacrylamide Gel Electrophoresis—Polyacrylamide gel electrophoresis was carried out in 7.5% cross-linked polyacrylamide gel according to the method of Ornstein (*13*) and Davis (*14*). Polyacrylamide gel electrophoresis in the presence of SDS was carried out according to the method of Weber and Osborn (*15*). Protein was stained with Coomassie brilliant blue.

Determination of Molecular Weight—The molecular weight of the enzyme was determined by gel filtration on a Sephadex G-200 column (1.7×100 cm) as described by Andrews (*16*). The elution buffer was 0.01 M potassium phosphate buffer, pH 7.0, containing 0.1 M NaCl. Standard proteins were γ-globulin (160,000), serum albumin (68,000), and sperm whale myoglobin (17,200). The void volume was determined with blue dextran. The molecular weight was also determined by electrophoresis in polyacrylamide gel containing SDS. Standard proteins in this case were serum albumin, ovalbumin (43,500), and sperm whale myoglobin.

Amino Acid Analysis—Amino acid analyses were performed with a Hitachi 835 amino acid analyzer according to the procedure of Spackman *et al.* (*17*). The protein sample (33 μg) was hydrolyzed with 1.0 ml of 6 N HCl at 110°C for 24, 48, and 72 h in evacuated sealed tubes. Half-cystine was determined as cysteic acid after performic acid oxidation (*18*). The content of tryptophan was determined spectrophotometrically by the method of Goodwin and Morton (*19*).

pH Optimum for Leucyl-Glycyl-Glycine—The pH optimum of the enzyme for leucyl-glycyl-glycine was determined in 0.1 M potassium phosphate buffer (from pH 5.5 to 7.0) and in 0.1 M Tris-HCl buffer (from pH 7.0 to 9.0) using 0.1 μg of the enzyme.

[2] The constituent amino acids of the peptides used were all of L-configuration.

Effect of Temperature—The enzyme, 0.1 ml (0.1 μg), was pretreated at various temperatures for 15 min and portions were transferred to an ice bath. The residual enzyme activity was determined as described above. The enzyme activity after treatment at 37°C under the same conditions was taken as 100%.

Effects of Various Compounds—Portions of 50 μl (0.1 μg) of the enzyme were mixed with 50 μl of various compounds dissolved in 0.1 M Tris-HCl buffer, pH 7.5. After incubation at 37°C for 15 min, the residual enzyme activity was determined as described above.

Substrate Specificity—The hydrolysis of various peptides by the enzyme was studied by the L-amino acid oxidase method at pH 7.5 as described above. The arylamidase activity of the enzyme was determined as described previously (*5*). The amount of the enzyme used was 0.1 μg for each assay.

K_m *Determination*—The K_m values for leucyl-glycyl-glycine and phenylalanyl-glycyl-glycine were determined by the method of Lineweaver and Burk (*20*). The substrate concentration used in this study ranged from 6×10^{-4} M to 3×10^{-5} M. The amount of the enzyme used was 0.1 μg for each assay.

K_I *Value for Bestatin*—The K_I value for bestatin was determined by the method of Dixon (*21*).

Analysis of Hydrolysis Products of Peptides—Samples of 10 μl (0.32 μg) of the enzyme were mixed with 10 μl (5 nmol) of various peptides dissolved in 0.1 M potassium phosphate buffer, pH 7.5. The mixture was incubated at 37°C for 60 min. The enzyme reaction was terminated by heating for 5 min in boiling water. The dansylation of hydrolysis products and polyamide layer chromatography of dansyl (DNS-) amino acids were carried out as described previously (*3*).

RESULTS

Purification of the Enzyme—An aminotripeptidase from monkey brain was purified by a five-step procedure. All operations were carried out at 0–4°C.

Step 1. Extraction: One Japanese monkey brain (99 g) was homogenized in a Waring blender for 5 min with 300 ml of 2% NaCl, and the homogenate was dialyzed against 2% NaCl for 3 h. The dialyzed solution (370 ml) was centrifuged at 30,000 × *g* for 60 min. The sediments were resuspended in 200 ml of 2% NaCl and centrifuged at 30,000 × *g* for 60 min. The two supernatants were combined.

Step 2. Ammonium sulfate fractionation: Solid $(NH_4)_2SO_4$ was added to the extract (450 ml) and the precipitates between 40 and 70% saturation were collected by centrifugation at 10,000 × *g* for

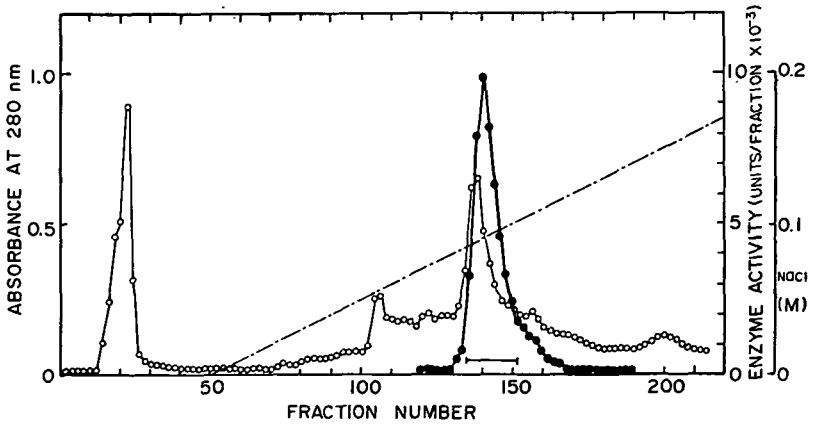

Fig. 1. Chromatography of aminotripeptidase on DEAE-cellulose. The enzyme solution from step 2) was applied to a column (3 × 40 cm), and the adsorbed enzyme was eluted with a linear gradient of NaCl as described in the text. The flow rate was 30 ml/h and fractions of 10 ml were collected. ○, absorbance at 280 nm; ●, enzyme activity; —·—, NaCl concentration.

30 min. The precipitates were dissolved in 50 ml of 0.01 M Tris-HCl buffer, pH 8.0, and the solution was dialyzed overnight against the same buffer.

Step 3. Chromatography on DEAE-cellulose: The dialyzed solution (84 ml) was applied to a DEAE-cellulose column (3×40 cm) previously equilibrated with 0.01 M Tris-HCl buffer, pH 8.0. The column was washed with 400 ml of the equilibrating buffer and adsorbed proteins were eluted with a linear gradient from 0 to 0.2 M NaCl. The elution pattern is shown in Fig. 1. The fractions (135–152) containing peptidase activity were pooled.

Step 4. Chromatography on hydroxylapatite: The pooled fractions containing peptidase activity from step 3) were dialyzed against 0.01 M potassium phosphate buffer, pH 6.5. The dialyzed solution was applied to a column of hydroxylapatite (2× 35 cm) equilibrated with 0.01 M potassium phosphate buffer, pH 6.5. The column was washed with 200 ml of the equilibrating buffer, and adsorbed proteins were eluted with a linearly increasing phosphate concentration from 0.01 to 0.2 M (Fig. 2). The fractions (60–78) containing peptidase activity were pooled.

Step 5. Gel filtration on Sephadex G-200: The pooled fractions containing peptidase activity from step 4) were concentrated to a small volume (16 ml) by ultrafiltration in a Diaflow MC-2A ultrafiltration cell with a reservoir (Bio Engineering Co.) using a G-05 T membrane. The solution was further concentrated by dialysis against 0.01 M potassium phosphate buffer, pH 7.0, containing 40% sucrose (final volume, 2.9 ml). The dialyzed solution was applied to a column (2.5×135 cm) of Sephadex G-200 equilibrated with 0.01 M potassium phosphate buffer, pH 7.0. The elution pattern is shown in Fig. 3. The fractions (84–102) containing peptidase activity were concentrated by ultrafiltration as described above and stored at −20°C. The yield and the purification factor of the enzyme preparation at each purification step are summarized in Table I. The enzyme was purified about 1,100-fold with a yield of 12%. From one monkey brain, 0.91 mg of protein was obtained.

Characterization of the Enzyme—Purity of the enzyme preparation: The purified enzyme was examined by analytical polyacrylamide gel electrophoresis at pH 8.9. As shown in Fig. 4, a single protein band was seen when a gel was stained with Coomassie brilliant blue.

Molecular weight: The molecular weight of the enzyme was determined by gel filtration and SDS-polyacrylamide gel electrophoresis. The results are shown in Fig. 5. The molecular weight

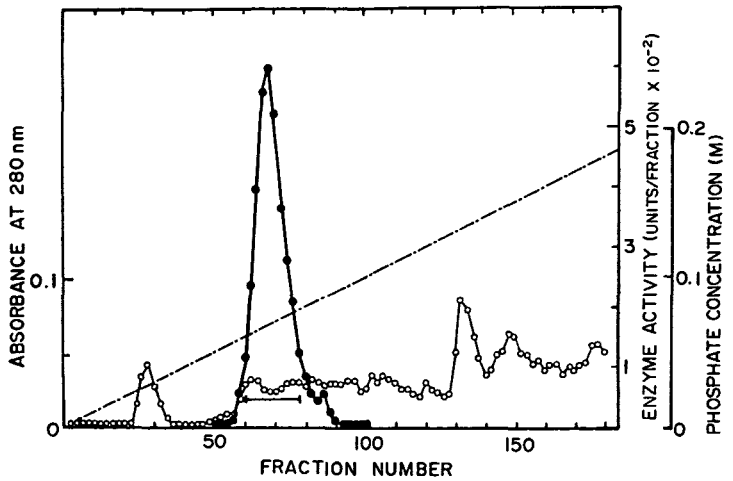

Fig. 2. Chromatography of aminotripeptidase on hydroxylapatite. The enzyme solution from step 3) was applied to a column (2×35 cm), and the adsorbed enzyme was eluted with a linear gradient of potassium phosphate as described in the text. The flow rate was 18 ml/h and fractions of 10 ml were collected. ○, absorbance at 280 nm; ●, enzyme activity; —·—, phosphate concentrantion.

TABLE I. Summary of the purification procedure for aminotripeptidase from monkey brain.

Fraction	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Yield (%)	Purification factor
Homogenate	8,680	26,984	3.1	100	1
30,000 × *g* supernatant	2,318	24,557	10.6	91	3.4
Precipitates at 40–70% $(NH_4)_2SO_4$	1,047	24,261	23.2	90	7.5
DEAE-cellulose	100.5	11,041	110	41	35.5
Hydroxylapatite	4.8	8,294	1,728	31	557
Sephadex G-200	0.91	3,120	3,429	12	1,106

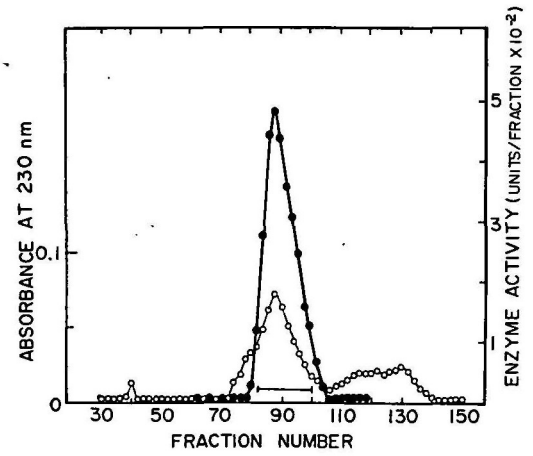

Fig. 3. Chromatography of aminotripeptidase on Sephadex G-200. The enzyme solution from step 4) was concentrated to a small volume and applied to a column (2.5 × 135 cm) with 0.01 M potassium phosphate buffer, pH 7.0. The flow rate was 12 ml/h and fractions of 5 ml were collected. ○, absorbance at 230 nm; ●, enzyme activity.

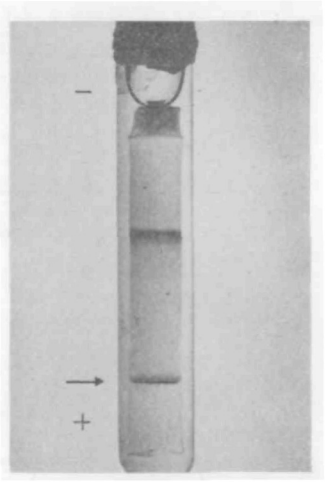

Fig. 4. Disc electrophoresis of the purified enzyme. The enzyme preparation (16 μg) was layered on a column of 7.5% polyacrylamide gel, pH 8.9. The arrow indicates the position of BPB.

of the enzyme was determined to be 65,000 by gel filtration. The enzyme migrated as a single band on SDS-polyacrylamide gel in the presence of β-mercaptoethanol, the migration distance corresponding to a molecular weight of 70,000. The results suggest that the enzyme is composed of a single polypeptide chain.

Amino acid composition: The amino acid compositions of the enzymes from monkey brain and pig kidney are presented in Table II. The compositions of the two enzymes are very similar. The significant differences are that the monkey brain enzyme contains less methionine, tyrosine, and leucine, and more aspartic acid and glycine.

pH optimum: The effect of pH on the rate of hydrolysis of leucyl-glycyl-glycine by the enzyme is shown in Fig. 6. The maximal activity was observed at pH 7.5.

Effects of various compounds: The effects of various compounds on the enzyme activity are presented in Table III. Bestatin, an inhibitor of various aminopeptidases, showed strong inhibition. Pepstatin, an inhibitor of acid proteases such as cathepsin D, leupeptin, an inhibitor of thiol proteases such as papain and cathepsin B, and puromycin, an arylamidase inhibitor, did not show any appreciable effects. As indicated in Fig. 7, inhibition of the enzyme by bestatin was competitive and the K_I value was calculated to be 5×10^{-7} M. Among various chelating agents, *o*-phenanthroline showed strong inhibition of the

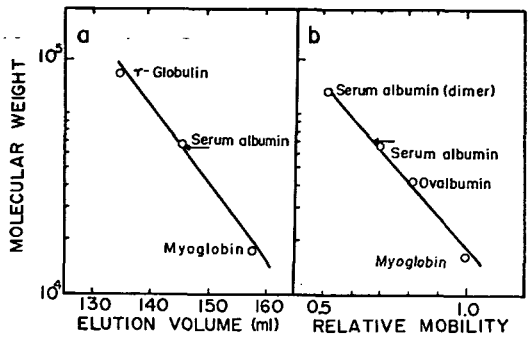

Fig. 5. Molecular weight determination of the enzyme by Sephadex G-200 chromatography (a) and SDS-polyacrylamide gel electrophoresis (b). Standard proteins used are γ-globulin, serum albumin, ovalbumin, and sperm whale myoglobin. The arrows indicate the position of the enzyme.

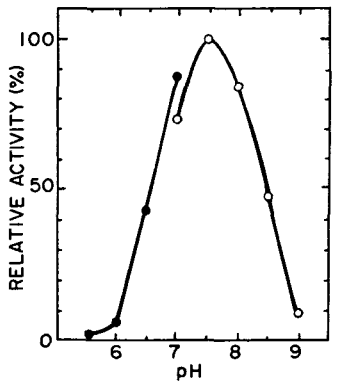

Fig. 6. Effect of pH on the enzyme activity. The enzyme activity was measured in 0.1 M potassium phosphate buffer from pH 5.5 to 7.0 and in 0.1 M Tris-HCl buffer from pH 7.0 to 9.0. The maximal activity was taken as 100%.

enzyme. EDTA and EGTA inhibited the enzyme only slightly. Divalent metal ions, Mn^{2+}, Mg^{2+}, Ca^{2+}, and Co^{2+} did not have any appreciable effects. Zn^{2+} showed inhibition of the enzyme. Complete inhibition of the enzyme was observed by PCMB. NEM showed 38% inhibition, while iodoacetic acid inhibited the enzyme only slightly. TPCK and DFP had slight effects on the activity, but TLCK showed 37% inhibition.

Effect of temperature: The effect of temperature on the activity is presented in Fig. 8. The enzyme was gradually inactivated at above 37°C and at 55°C, it was completely inactivated.

TABLE II. Amino acid compositions of monkey brain aminotripeptidase and pig kidney aminotripeptidase.

Amino acid	Number of residues per molecule of protein	
	Monkey brain aminotripeptidase[a]	Pig kidney aminotripeptidase (*22*)
Lys	37.0 (37)	41
His	13.6 (14)	19
Arg	22.5 (23)	23
Asp	65.0 (65)	52
Thr	30.4[b] (30)	38
Ser	48.3[b] (48)	50
Glu	73.1 (73)	71
Pro	40.3 (40)	38
Gly	58.9 (59)	32
Ala	47.2 (47)	40
Cys/2	17.1[c] (17)	12
Val	40.8 (41)	40
Met	4.8 (5)	10
Ile	25.3 (25)	25
Leu	59.9 (60)	71
Tyr	12.7 (13)	21
Phe	22.5 (23)	28
Trp	15.6[d] (16)	18
Total	636	629

[a] The values were calculated assuming the molecular weight of aminotripeptidase to be 70,000. Except for threonine, serine and tryptophan, each value is an average of values obtained for three different periods of hydrolysis. The values in parentheses are nearest integers. [b] Values extrapolated to zero time of hydrolysis. [c] Estimated as cysteic acid by the method of Moore (*18*). [d] Determined by the method of Goodwin and Morton (*19*).

Substrate specificity: Table IV summarizes the relative rates of hydrolysis of various peptides by the enzyme. It hydrolyzed only tripeptides. Among the tripeptides tested, leucyl-glycyl-glycine was hydrolyzed most rapidly. K_m values for leucyl-glycyl-glycine and phenylalanyl-glycyl-glycine were determined to be 0.25 mM and 0.33 mM, respectively. The enzyme did not hydrolyze various amino acid β-naphthylamides, carbobenzoxy-glycyl-phenylalanine, or benzoyl-glycyl-argi-

TABLE III. Effects of various compounds on aminotripeptidase activity. The purified enzyme (0.1 μg) was preincubated with each compound at the indicated concentration for 15 min at 37°C before incubation for 20 min with leucyl-glycyl-glycine.

Compound	Concentration (mM)	Activity (%)
None	—	100
EDTA[a]	5	73
	1	75
EGTA[a]	5	67
	1	75
o-Phenanthroline[a]	5	0
	1	26
PCMB	1	0
	0.1	0
IAA	1	91
NEM	1	62
TLCK	1	63
TPCK	1	81
DFP	1	97
Bestatin	1	0
	0.1	4
Pepstatin	1	105
Leupeptin	1	102
Puromycin	1	103
$MnCl_2$	1	108
$MgCl_2$	1	103
$CoCl_2$	1	116
$CaCl_2$	1	110
$ZnCl_2$	1	2

[a] The enzyme was preincubated for 30 min at 37°C.

nine. These results indicate that the enzyme has no arylamidase or carboxypeptidase A and B activities.

Analysis of hydrolysis products of various peptides: To determine the site of hydrolysis of peptides by the enzyme, polyamide layer chromatography was employed to detect liberated amino acids as their dansyl derivatives. In the cases of di-, tetra-, and penta-alanines, no DNS-alanine was detected. On the other hand, when tri-alanine was used as a substrate, DNS-alanine and DNS-di-alanine were observed. When leucyl-glycyl-glycine, phenylalanyl-glycyl-glycine, and tyrosyl-glycyl-glycine were used as substrates, dansyl derivatives of the NH_2-terminal amino acids and DNS-glycyl-glycine were detected.

TABLE IV. Relative rates of hydrolysis of various peptides by aminotripeptidase and K_m values for peptides. The reactions were carried out as described in the "EXPERIMENTAL" section. The rate of hydrolysis of leucyl-glycyl-glycine was taken as 100%.

Substrate	Relative activity (%)	K_m (mM)
Leucyl-glycyl-glycine	100	0.25
Phenylalanyl-glycyl-glycine	90	0.33
Tyrosyl-glycyl-glycine	31	
Tri-leucine	4	
Leucyl-glycine	0	
Phenylalanyl-glycine	0	
Di-leucine	0	
Leucine amide	0	
Met[5]-, leu[5]-enkephalin	0	
Leucine β-naphthylamide	0	
Alanine β-naphthylamide	0	
Arginine β-naphthylamide	0	
Carbobenzoxy-glycyl-phenylalanine	0	
Benzoyl-glycyl-arginine	0	

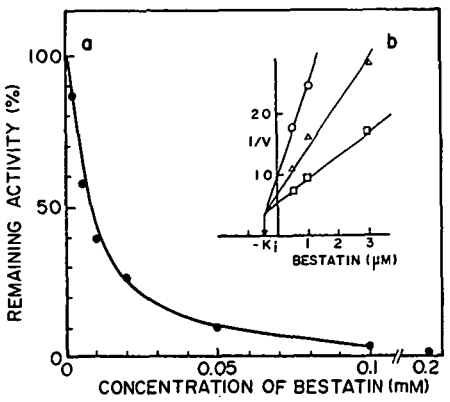

Fig. 7. a) Effect of bestatin on the activity of the enzyme. The activity of enzyme not treated with bestatin was taken as 100%. b) Dixon plot of the inhibition of the enzyme activity by bestatin. The concentration of leucyl-glycyl-glycine was 0.1 mM (○), 0.25 mM (△), or 0.5 mM (□).

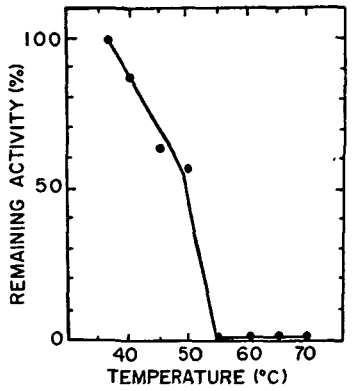

Fig. 8. Effects of temperature on the stability of the enzyme. The activity of enzyme treated at 37°C for 15 min was taken as 100%.

DISCUSSION

Aminotripeptidase has been purified to homogeneity from monkey brain with a yield of 12%. The purification of this enzyme has been attempted using various mammalian tissues (*6–10*), and the enzyme has been obtained from *Escherichia coli* (*22*). However, a homogeneous preparation has only been obtained from pig kidney (*8*). In bovine brain tissue, three aminopeptidases have been reported using leucyl-glycyl-glycine as a substrate (*10*). The enzyme of the present study was eluted as a single peak throughout the purification steps and was similar in molecular weight and substrate specificity to E1P1 or E1P2 of bovine brain. The results presented in Table I show that about 50% of the enzyme activity was lost on DEAE-cellulose chromatography. This reflects the loss of arylamidase during DEAE-cellulose chromatography: preliminary observations indicated that the monkey brain arylamidase was eluted at 0.2 to 0.3 M NaCl on DEAE-cellulose chromatography.

The molecular weight of the monkey brain enzyme was determined to be 65,000 by gel filtration and 70,000 by SDS gel electrophoresis. These values are close to those reported for the enzymes from pig kidney (71,100), rabbit intestinal mucosa (50,000), bovine brain (61,500 for E1P1, 85,700 for E1P2), and *E. coli* (80,000).

The amino acid composition has only been reported for the enzyme from pig kidney (*23*). As shown in Table II, the compositions of the enzymes from pig kidney and monkey brain are very similar, though the content of methionine of the monkey brain enzyme is lower. This value may have been somewhat underestimated. Differences are also apparent in the contents of glycine, aspartic acid, tyrosine and leucine.

The optimum pH for the hydrolysis of leucyl-glycyl-glycine was pH 7.5. This value is close to those obtained for the enzymes from pig kidney (pH 7.9), calf thymus (pH 7.9), horse erythrocytes (pH 7.9), bovine brain (pH 6.6–7.8 for E1P1, pH 7.2–8.1 for E1P2), and rabbit intestinal mucosa (pH 7.5).

Among various compounds tested, bestatin, which is a specific inhibitor for various aminopeptidases (*3*, *24*), showed a strong inhibitory effect. The K_I value was calculated to be 5×10^{-7} M, which is close to that of monkey brain arylamidase previously obtained (9×10^{-7} M for leucyl-glycyl-glycine) (*3*). Among chelating agents, *o*-phenanthroline caused strong inactivation of the enzyme, while EDTA and EGTA caused a slight decrease in the enzyme activity. These properties resemble those of the enzymes from calf thymus (*6*), horse erythrocytes (*7*), and pig kidney (*8*). The complete inactivation of the enzyme by PCMB indicates a requirement for free sulfhydryl group(s) for catalytic activity. The same properties have been reported for the enzymes of pig kidney (*8*) and bovine brain (*10*).

As for the substrate specificity, no hydrolysis was observed of dipeptides, amino acid amide, tetrapeptides and pentapeptides. The enzyme hydrolyzed only tripeptides to yield the NH_2-terminal free amino acids and the residual dipeptides. The K_m of 0.25 mM for leucyl-glycyl-glycine is comparable to the K_m of 0.5 mM found for the enzyme from rabbit intestinal mucosa (*9*).

The physiological significance of the enzyme in brain tissues is not yet clear. Recently, however, tripeptidyl aminopeptidase has been identified in the bovine anterior pituitary gland (*25*). This enzyme released NH_2-terminal tripeptides from bovine growth hormone. Tripeptides, His-(Gly, Lys), that show inhibitory effect on neurons have been identified in the cat spinal cord and brain stem (*26*). The enzyme of the present study may be involved in the metabolism of these tripeptides in the brain tissues. Further studies are necessary to clarify these possibilities.

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