Purification, cDNA Cloning, and Expression of a New Human Blood Plasma Glutamate Carboxypeptidase Homologous to N-Acetyl-aspartyl-\(\alpha\)-glutamate Carboxypeptidase/Prostate-specific Membrane Antigen*

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We describe the identification, cDNA cloning, and biochemical characterization of a new human blood plasma glutamate carboxypeptidase (PGCP). PGCP was co-purified from human placenta with lysosomal carboxypeptidase, cathepsin A, lysosomal endopeptidase, cathepsin D, and a \(\gamma\)-interferon-inducible protein, IP-30, using an affinity chromatography on a Phe-Leu-agarose column. A PGCP cDNA was obtained as an expressed sequence tag clone and completed at 5'-end by rapid amplification of cDNA ends polymerase chain reaction. The cDNA contained a 1623-base pair open reading frame predicting a 541-amino acid protein, with five putative Asn glycosylation sites and a 21-residue signal peptide. PGCP was a 62-kDa precursor, which is processed to a 56-kDa mature form containing two Asn-linked oligosaccharide chains. The mature form of PGCP was secreted into the culture medium, which is consistent with its intracellular localization in secretion granules. In humans, PGCP is found principally in blood plasma, suggesting a potential role in the metabolism of secreted peptides.

Cellular and secreted carboxypeptidases are important in the generation, processing, and inactivation of different vertebrate neuropeptides (for recent reviews, see Refs. 1–7). In particular, an important brain glutamate carboxypeptidase II (EC 3.4.17.21) also called N-acetyl-aspartyl-\(\alpha\)-glutamate carboxypeptidase (NAALADase)\(^1\) (8, 9) hydrolyzes the neuropeptide, N-acetyl-l-aspartyl-l-glutamate, releasing glutamate, the dominant excitatory neurotransmitter/neuromodulator of the mammalian central nervous system (10, 11). NAALADase is also required for the intestinal uptake of folate and is responsible for the resistance of some tumors to methotrexate (12). Cloning of NAALADase (12–14) showed that it is identical to the so-called prostate-specific membrane antigen (PSMA), which is strongly expressed in prostate cancer and serves as a marker for detection of prostate cancer metastasis (reviewed in Ref. 15). NAALADase is a 94-kDa transmembrane protein, homologous both to the transferrin receptor and the bacterial cocatalytic zinc metallopeptidases (16). Recent cloning of the human ileal membrane dipeptidyl peptidase, I100 (17), the catalytic domain of which is homologous to NAALADase/PSMA, revealed that in mammals, cocatalytic zinc metallopeptidases may be represented by multiple enzymes with different substrate specificity.

We describe a new human peptidase of the NAALADase/PSMA family, a 56-kDa blood plasma glycoprotein termed PGCP, which was affinity-co-purified with the lysosomal carboxypeptidase, cathepsin A (Catha; EC 3.4.16.1). CathA forms a high molecular weight complex with \(\beta\)-galactosidase (EC 3.2.1.23), sialidase (neuraminidase; EC 3.2.1.18), and N-acetylgalactosamine-6-sulfate sulfatase (EC 3.1.6.4) that is essential for their function in the lysosome (18–20). About two-thirds of lysosomal CathA is not complexed with \(\beta\)-galactosidase and sialidase (21) and can be excreted into plasma from platelets (22) and lymphoid cells (23). Our recent studies on CathA substrate specificity (24, 25) indicated that CathA is the major carboxypeptidase in human tissues able to cleave hydrophobic amino acid residues, including the amidated ones. In \textit{vitro}, CathA can inactivate regulatory peptides like endothelin, substance P, bradykinin, and angiotensin I (22, 23, 26–29), suggesting that it can be involved in the regulation of central and cardiovascular functions. Our recent studies propose that CathA may be associated with the metabolism of peptides implicated in memory consolidation (30).

For purification of CathA, we designed an affinity column by coupling a specific substrate of CathA, Phe-Leu, to an epoxy-activated agarose matrix. Analysis of the protein fraction pu...
rifed from human tissues using this column revealed a novel 56-kDa glycoprotein, PGCP. In this paper, we report cloning of PGCP cDNA; characterization of its primary structure, enzymatic activity, expression, and metabolism; and its immunolocalization in human tissues and cells, which altogether suggest that PGCP is a novel glutamate carboxypeptidase secreted into the blood plasma.

**EXPERIMENTAL PROCEDURES**

**Purification of PGCP from Human Placenta**—A crude glycoprotein fraction was purified from 10 human placentas (~5 kg of wet tissue weight) using a combination of affinity chromatography. The preparation was passed through a p-aminophenyl-β-thiogalactopyranoside-agarose affinity column to separate the β-galactosidase-CathA-sialidase complex and then applied onto a 20-ml Phe-Leu-agarose affinity column, which specifically binds CathA and cathepsin D (CathD) (21). The column was washed with 20 volumes of 20 mM sodium acetate buffer, pH 4.75, containing 0.15 M NaCl, and eluted with 5 volumes of 0.1 M Tris-HCl buffer, pH 7.5, containing 5 mM benzoylcarbonyl-Phe-Leu. The eluate was concentrated using an Amicon stirred cell with a PM-10 membrane, dialyzed against 10 mM Tris-HCl buffer, pH 7.5, centrifuged at 10,000 × g for 10 min, applied to an FPLC Mono Q column (Amersham Pharmacia Biotech) equilibrated with the same buffer, and eluted with a linear NaCl gradient from 0 to 0.5 M. The 0.075–0.1 M NaCl fractions, which contained cathepsin D and PGCP, were pooled, concentrated, and applied to a FPLC Superose 12 gel filtration column (Amersham Pharmacia Biotech), and then eluted in 20 mM sodium acetate buffer, pH 5.2, containing 0.15 M NaCl and 0.02% (v/v) NaN3. Eluted fractions were analyzed by SDS-PAGE. Fractions corresponding to the first protein peak, containing only PGCP, were pooled, concentrated, and stored at −30 °C. The molecular masses of the eluted proteins were determined using the following molecular mass standards (Amersham Pharmacia Biotech): blue dextran (~2,000 kDa), ferritin (440 kDa), catalase (232 kDa), aldolase (158 kDa), ovalbumin (43 kDa), chymotrypsinogen (25 kDa), and ribonuclease A (13.7 kDa). A similar procedure was used for the purification of PGCP from mouse liver.

**N-terminal Amino Acid Sequencing of Proteins**—SDS-PAGE was performed as described by Laemmli (31) on an 11% acrylamide gel. Protein bands were stained with Coomassie Blue, the proteins were electrotransferred to Immobilon TM-P membrane (Millipore Corp.) in a Trans-Blot cell (Bio-Rad) for 2.5 h at 4 °C in 10 mM CAPS buffer, pH 11, 10% (v/v) methanol at a current of 480 mA. After staining with Coomassie Blue R-250, the bands were excised, and amino acid sequences were determined in an Applied Biosystems 470 A gas phase sequencer. Sequences were analyzed using the BLAST network service of the National Center for Biotechnology Information (Bethesda, MD).

**EST Clone of PGCP**—The dbEST was screened with the NH2-terminal sequence of PGCP, which contained deduced amino acid sequence identical to the NH2-terminal sequence of PGCP (GenBank™ accession number R18560), which was deduced from total human placenta RNA using this column revealed a novel 56-kDa glycoprotein, PGCP. In this paper, we report cloning of PGCP cDNA; characterization of its primary structure, enzymatic activity, expression, and metabolism; and its immunolocalization in human tissues and cells, which altogether suggest that PGCP is a novel glutamate carboxypeptidase secreted into the blood plasma.

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**N-terminal Amino Acid Sequencing of Proteins**—SDS-PAGE was performed as described by Laemmli (31) on an 11% acrylamide gel. Protein bands were stained with Coomassie Blue. For NH2-terminal sequencing, the proteins were electrotransferred to Immobilon™-P membrane (Millipore Corp.) in a Trans-Blot cell (Bio-Rad) for 2.5 h at 4 °C in 10 mM CAPS buffer, pH 11, 10% (v/v) methanol at a current of 480 mA. After staining with Coomassie Blue, the proteins were electrotransferred to Immobilon™-P membrane (Millipore Corp.) in a Trans-Blot cell (Bio-Rad) for 2.5 h at 4 °C in 10 mM CAPS buffer, pH 11, 10% (v/v) methanol at a current of 480 mA. After staining with Coomassie Blue, the proteins were electrotransferred to Immobilon™-P membrane (Millipore Corp.) in a Trans-Blot cell (Bio-Rad) for 2.5 h at 4 °C in 10 mM CAPS buffer, pH 11, 10% (v/v) methanol at a current of 480 mA. After staining with Coomassie Blue, the proteins were electrotransferred to Immobilon™-P membrane (Millipore Corp.) in a Trans-Blot cell (Bio-Rad) for 2.5 h at 4 °C in 10 mM CAPS buffer, pH 11, 10% (v/v) methanol at a current of 480 mA. After staining with Coomassie Blue, the proteins were electrotransferred to Immobilon™-P membrane (Millipore Corp.) in a Trans-Blot cell (Bio-Rad) for 2.5 h at 4 °C in 10 mM CAPS buffer, pH 11, 10% (v/v) methanol at a current of 480 mA. After staining with Coomassie Blue, the proteins were electrotransferred to Immobilon™-P membrane (Millipore Corp.) in a Trans-Blot cell (Bio-Rad) for 2.5 h at 4 °C in 10 mM CAPS buffer, pH 11, 10% (v/v) methanol at a current of 480 mA. After staining with Coomassie Blue, the proteins were electrotransferred to Immobilon™-P membrane (Millipore Corp.) in a Trans-Blot cell (Bio-Rad) for 2.5 h at 4 °C in 10 mM CAPS buffer, pH 11, 10% (v/v) methanol at a current of 480 mA. After staining with Coomassie Blue, the proteins were electrotransferred to Immobilon™-P membrane (Millipore Corp.) in a Trans-Blot cell (Bio-Rad) for 2.5 h at 4 °C in 10 mM CAPS buffer, pH 11, 10% (v/v) methanol at a current of 480 mA. After staining with Coomassie Blue, the proteins were electrotransferred to Immobilon™-P membrane (Millipore Corp.) in a Trans-Blot cell (Bio-Rad) for 2.5 h at 4 °C in 10 mM CAPS buffer, pH 11, 10% (v/v) methanol at a current of 480 mA. After staining with Coomassie Blue, the proteins were electrotransferred to Immobilon™-P membrane (Millipore Corp.) in a Trans-Blot cell (Bio-Rad) for 2.5 h at 4 °C in 10 mM CAPS buffer, pH 11, 10% (v/v) methanol at a current of 480 mA.
for 30 min with colloidal gold (10 nm)-conjugated goat anti-rabbit IgG (Zollinger Inc., Montréal, Québec, Canada). The sections were washed two times with TBS containing 0.05% (v/v) Tween 20 and two times with distilled water and counterstained with uranyl acetate followed by lead citrate (34, 35). Normal rabbit serum was used as a control. Electron micrographs were taken using a Philips 400 electron microscope (Philips Electronics, Toronto, Ontario, Canada).

For 48 h after transfection with pCMV-PGCP expression vector, COS-1 cells grown to confluence in 75-cm² culture flasks (~10⁶ cells) were washed twice with Hank’s balanced salt solution and then incubated for 2 h in methionine-free Dulbecco’s modified Eagle’s medium (Life Technologies) supplemented with 1-glutamine and sodium pyruvate, and for 40 min in 5 ml of the same medium supplemented with [35S]methionine (DuPont), 0.1 μCi/ml. The radioactive medium was then removed, and the cells were washed twice with Hank’s balanced salt solution and chased at 37°C in Eagle’s minimal essential medium supplemented with 20% (v/v) fetal calf serum. At the times indicated in the figures, the cells were placed on ice, washed twice with ice-cold phosphate-buffered saline, and then lysed for 30 min on ice in 1 ml of radioimmunoprecipitation assay buffer, containing 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% (v/v) Nonidet P-40, 0.05% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, 5 μg/ml leupeptin, and 0.1 μg/ml a-toluenesulfonyl fluoride. The lysate was collected and centrifuged at 13,000 x g for 10 min to remove the cell debris.

Immunoprecipitation, Electrophoresis, and Detection of PGCP—1.0 ml of lysate was incubated for 4 h with preimmune serum at a final dilution of 1:20. Then the pellet obtained from 300 μl of Pansorbin cells (Calbiochem) was added, and the resulting suspension was incubated for 2 h at 4°C, followed by centrifugation for 10 min at 13,000 x g. Supernatants were incubated overnight with the anti-PGCP antibodies in a 1:100 final dilution and then transferred to 100 μl of serum was loaded on the immunoaffinity column. The column was washed by 10 ml of 50 mM sodium phosphate buffer, pH 7.5, containing 0.4 M NaCl, and eluted with 5 ml of 0.2 M glycine buffer, pH 2.3. The pH of collected 1-ml fractions was immediately adjusted to 7.5 by 1 M HCl. The indicated gel filtration fractions were analyzed by SDS-PAGE as described. The protein bands are identified on the right of the gel: 31-kDa polypeptide chain of CathD (CathD31 and CathD14), γ-interferon inducible protein, IP-30, and PGCP.

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Metabolic Labeling—48 h after transfection with pCMV-PGCP expression vector, COS-1 cells grown to confluence in 75-cm² culture flasks (~10⁶ cells) were washed twice with Hank’s balanced salt solution and then incubated for 2 h in methionine-free Dulbecco’s modified Eagle’s medium (Life Technologies) supplemented with 1-glutamine and sodium pyruvate, and for 40 min in 5 ml of the same medium supplemented with [35S]methionine (DuPont), 0.1 μCi/ml. The radioactive medium was then removed, and the cells were washed twice with Hank’s balanced salt solution and chased at 37°C in Eagle’s minimal essential medium supplemented with 20% (v/v) fetal calf serum.

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mM ZnCl₂. After 1–15 h of incubation at 37 °C, samples were incubated for 3 min at 100 °C and mixed with 0.9 ml of 100 mM Tris-HCl buffer, pH 8.0, containing 1 mM NAD⁺ (Sigma) and 0.25 units/ml of bovine glutamate dehydrogenase (Boehringer Mannheim). After a 1-h incubation at 37 °C, the concentration of glutamate was measured spectrophotometrically at 340 nm using the calibration curve obtained with 1–200 nmol of glutamate. One unit of enzyme activity corresponds to a conversion of 1 μmol of substrate/min. Proteins were assayed according to Bradford (38) with bovine serum albumin (Sigma) as a standard.

RESULTS AND DISCUSSION

Purification and NH₂-terminal Sequencing of PGCP—The fraction of crude glycoproteins of human placenta retained by the Phe-Leu-agarose affinity column contained four major proteins. We separated these proteins by FPLC ion exchange chromatography using Mono Q column (Fig. 1) and identified three of them by NH₂-terminal sequencing as cathepsin D (CathD) (peak I eluted at NaCl concentration between 0.057 and 0.1 M), CathA (peak II, 0.1–0.17 M NaCl), and a so-called IP-30 (39), a 30-kDa glycoprotein induced in hematopoietic cells in response to γ-interferon, the biological function of which is unclear (peak III, 0.27–0.35 M NaCl). Peak I in addition to 31- and 15-kDa subunits of CathD also contained a 56-kDa glycoprotein, further termed PGCP. NH₂-terminal amino acid sequence of PGCP, (G)DVAKAIINLAVYGKAQ(N)RSYERLALLVDTVG did not match any of proteins in the Non-Redundant GenBank™ data base (all nonredundant GenBank™ cDNA sequence translations, plus all entries from the Protein Data Bank, Swiss-Prot.

Fig. 3. Nucleotide sequence of PGCP cDNA and predicted amino acid sequence. The putative signal peptide is boxed, and the NH₂-terminal amino acid sequence of the purified protein is underlined. Potential glycosylation sites are marked with black dots, and the terminal stop codon is marked with an asterisk.
PGCP and CathD were resolved on a Superose 12 FPLC gel filtration column. Eluted fractions were analyzed for CathD activity and by SDS-PAGE (Fig. 2). Using a Superose 12 column, the molecular mass of PGCP (peak I at Fig. 2) was estimated as 120 kDa, suggesting that PGCP may form homodimers of 56-kDa subunits.

cDNA and mRNA of PGCP and Its Chromosomal Localization—A search in the dbEST data base showed that the NH2-terminal amino acid sequence of PGCP had a 100% identity with a deduced amino acid sequence of an EST clone 30142 (GenBankTM accession number R18560). From this cDNA fragment, which we extended by 5' rapid amplification of cDNA ends polymerase chain reaction, we derived a cDNA that we predict contains the entire PGCP coding sequence (see “Experimental Procedures”). This cDNA, confirmed by sequencing of several independent clones, contains a 1623-base pair open reading frame starting from a potential ATG initiation codon several independent clones, contains a 1623-base pair open reading frame starting from a potential ATG initiation codon and ends polymerase chain reaction, we derived a cDNA that we predict contains the entire PGCP coding sequence (see “Experimental Procedures”). This cDNA, confirmed by sequencing of several independent clones, contains a 1623-base pair open reading frame starting from a potential ATG initiation codon and ends polymerase chain reaction, we derived a cDNA that we predict contains the entire PGCP coding sequence (see “Experimental Procedures”). This cDNA, confirmed by sequencing of several independent clones, contains a 1623-base pair open reading frame starting from a potential ATG initiation codon and ends polymerase chain reaction, we derived a cDNA that we predict contains the entire PGCP coding sequence (see “Experimental Procedures”). This cDNA, confirmed by sequencing of several independent clones, contains a 1623-base pair open reading frame starting from a potential ATG initiation codon, was used to screen the dbSTS library at GenBankTM with an accession number R18560 for the EST clone revealed the identity of PGCP with an unidentified EST transcript WI-6164 (similar to Cda01e07, or D8S1377E) encoded on human chromosome 8 between D8S257 and D8S270 markers (8q22.2 locus).

Expression and Metabolic Labeling of PGCP—To study the synthesis and processing of PGCP, we cloned its full-length cDNA in the pcMV expression vector (33) and expressed it in COS-1 cells. 48 h after transfection, the total cell homogenate and concentrated cell medium were studied by Western blot using antibodies against a recombinant PGCP-glutathione S-transferase fusion protein. We detected a 56-kDa protein that cross-reacted with anti-PGCP antibodies in the homogenates of COS-1 cells transfected with pcMV-PGCP expression vector but not in untransfected control cells or cells transfected with pcMV-β-galactosidase vector (Fig. 5a). The molecular size of the expressed protein was similar to that of PGCP purified from human placenta (Fig. 5a, lane 1), suggesting that in COS-1 cells PGCP is properly processed and glycosylated. Western blot analysis of the culture medium (Fig. 5b) showed that the majority of expressed PGCP is secreted. The treatment of the cells with 10 mM NH4Cl (19) did not increase the secretion of PGCP, suggesting that mannose 6-phosphate receptors are not involved in the trafficking of this protein.

30-h pulse-chase experiments (Fig. 5c) demonstrated that PGCP is initially synthesized as a 62-kDa polypeptide, with a molecular weight consistent with that of the PGCP precursor. During a 6-h chase, the precursor is completely processed to a 56-kDa mature form similar to that detected by Western blotting in the cell lysates and in culture medium (Fig. 5, a and b).

Fig. 5. Expression and biogenesis of PGCP in COS-1 cells. a, Western blot of homogenates of COS-1 cells harvested 48 h after transfection with pcMV-PGCP (lanes 2 and 3) or with pcMV-β-galactosidase (lanes 4 and 5) or of homogenates of nontransfected cells (lanes 6 and 7). Lane 1 contained 0.5 μg of purified PGCP. Protein samples (20 μg for lanes 2, 4, and 6 and 10 μg for lanes 3, 5, and 7) were subjected to SDS-PAGE, transferred to a nitrocellulose membrane, and stained with rabbit anti-PGCP antibodies as described. b, Western blot of culture medium of COS-1 cells 48 h after transfection with pcMV-PGCP. Cells were cultured in the presence (lane 1) or in the absence (lane 2) of mannose 6-phosphate. 20 μg of total protein were applied on each lane. c, fluorographs of pulse labeling and chase of PGCP in COS-1 cells 48 h after transfection with pcMV-PGCP expression vector. Lane 1, pulse; lanes 2–5, chase for 1, 3, 6, and 22 h, respectively. Molecular mass values of PGCP precursor (62 kDa) and its mature form (56 kDa) are shown.
analysis showed that the protein is unequally expressed in human tissues (Fig. 6a). PGCP was abundant in placenta and kidney; low in muscles, liver, and skin fibroblasts; and undetectable in brain or white blood cells. Since the highest level of PGCP was found in total blood, we performed a Ficoll fractionation of blood and found that the protein is localized exclusively in plasma (Fig. 6a).

To characterize PGCP detected in blood plasma, we developed its rapid purification procedure using an immunoaffinity column. The fraction of blood serum proteins obtained by ammonium sulfate precipitation (33–50% saturation) was loaded on the immunoaffinity column containing anti-PGCP antibodies coupled to BrCN-activated Sepharose 4B. The preparation eluted from the column with 0.2 M glycine buffer, pH 2.3,
contained only a 56-kDa protein that strongly cross-reacted with anti-PGCP antibody and had an affinity to the Phe-Leu-agarose column. Endoglycosidase F treatment of PGCP preparations purified from human placenta using a Phe-Leu-agarose column and from human blood using an immunoaffinity column equally reduced their molecular mass to 52 kDa (Fig. 6b), close to that predicted for the mature deglycosylated PGCP, suggesting that the processing and glycosylation of PGCP secreted into blood serum is similar to that of PGCP purified from placenta.

The intracellular distribution of PGCP was studied in skin cultured fibroblasts by both immunohistochemistry and immunoelectron microscopy. Fluorescent microscopy of fibroblasts revealed a peripheral punctate intracellular staining consistent with localization of PGCP in a vesicular compartment (Fig. 7, upper panel). Indeed, immunoelectron microscopy of fibroblasts with anti-PGCP antibody (Fig. 7, lower panel), revealed heavily labeled electron lucent vesicles of approximately 150 nm in diameter that are located close to the plasma membrane. Taking into the account that PGCP is found in blood plasma and that most of PGCP from transfected COS-1 cells was secreted into the culture medium, the results of the immunofluorescent and immunoelectron microscopy can be interpreted as the localization of PGCP in secretory granules.

Amino Acid Sequence Homology of PGCP to Amino- and Carboxypeptidases—The homology search in GenBank Non-Redundant data base and SwissProt data bases using the complete amino acid sequence of PGCP revealed significant homology with human, rat, and mouse NAALADase/PSMA as well as with bacterial zinc aminopeptidases from Aeromonas proteolytica (V. proteolyticus, VIBR) and S. griseus (SGAP) or NAADase/prostate-specific membrane antigen from humans (PSMAHUM) and rat (PSMARAT). The figure shows the central part of the deduced “catalytic domain” of PGCP (amino acids 255–425, ~30% of the total sequence) and aligning residues of PSMAHUM, PSMARAT, VIBR, and SGAP that demonstrated the highest identity score as is shown in Table I. Alignment was performed using the Lipman-Pearson algorithm and a BLOSUM62 matrix by ProteinManager™ software package for sequence analysis (ACD Inc.). Identical amino acids are colored. Hyphens represent gaps introduced to optimize the alignment. Active site residues are indicated with the following symbols: *, nucleophyl; †, zinc-binding residues, * , residues of the substrate-binding pocket. Numbers refer to the positions of amino acids.

**Fig. 8.** Amino acid sequence alignment of PGCP with homologous peptidases: zinc aminopeptidases from *A. proteolytica* (V. proteolyticus, VIBR) and *S. griseus* (SGAP) or NAADase/prostate-specific membrane antigen from humans (PSMAHUM) and rat (PSMARAT). The figure shows the central part of the deduced “catalytic domain” of PGCP (amino acids 255–425, ~30% of the total sequence) and aligning residues of PCMAHUM, PCMARAT, VIBR, and SGAP that demonstrated the highest identity score as is shown in Table I. Alignment was performed using the Lipman-Pearson algorithm and a BLOSUM62 matrix by ProteinManager™ software package for sequence analysis (ACD Inc.). Identical amino acids are colored. Hyphens represent gaps introduced to optimize the alignment. Active site residues are indicated with the following symbols: *, nucleophyl; †, zinc-binding residues, * , residues of the substrate-binding pocket. Numbers refer to the positions of amino acids.
proteins, we suggest that PGCP also contains two cocrystalline zinc atoms ligated with Asp^202, Glu^337, and His^333 and with His^290, Asp^302, and Asn^353, respectively (marked by triangles in Fig. 8). As in VIBR, SGAP, and NAALADase/PSMA, Asp^202 can be a ligand for both zinc atoms. Catalytic site nucleophile, Glu^336, is also topologically conserved in PGCP and may form a cis-peptide bond with Glu^337 as in the case of other members of the M28 family. In VIBR and SGAP aminopeptidases, the specificity pockets are formed by markedly hydrophobic residues (for example, Met^280, Ile^299, Cys^329, Tyr^331, Cys^333, Met^348, Phe^350, Phe^354, Tyr^357, and Ile^361 in VIBR). In contrast, only two of the corresponding positions are occupied by hydrophobic residues in PGCP (Ile^284 and Leu^290). Similarly to NAALADase, PGCP contains basic residues (Arg^282, Lys^429) in the substrate binding pocket that may contribute to the binding of substrates with negatively charged amino acids.

**Carboxy- and Endopeptidase Activity of PGCP**—In order to verify if PGCP has a peptidase activity as predicted both by its amino acid homology with amino- and carboxypeptidases and its affinity to Phe-Leu-agarose, we tested the preparations of PGCP purified from human plasma, mouse liver, and blood plasma for the enzymatic activity against the number of substrates including those of aminopeptidase (H-Ala-AMC, H-Arg-AMC, H-Glu-AMC, H-Leu-AMC, H-Lys-AMC, and H-Phe-AMC), dipeptidyl peptidase (H-Gly-β-NA and Z-Arg-Arg-βNA), endopeptidase and esterase (benzoyl-β-NA, Suc-Phe-Leu-β-NA, Z-Gly-Gly-Leu-β-NA, Suc-Leu-Tyr-AMC, and Z-Phe-β-NA), and carboxypeptidase (FA-Phe-Ala, FA-Phe-Arg, FA-Phe-Glu, FA-Phe-Gly, FA-Phe-Phe, FA-Phe-Tyr, and Ac-Asp-Glu). The results of these experiments are shown in Fig. 9a).

We observed a significant glutamate carboxypeptidase activity with Ac-Asp-Glu substrate (2.5–4.5 nmol/min/mg of protein) but also a low chymotrypsin-like activity with enz.

**REFERENCES**

cDNA Cloning of Human Blood Plasma Glutamate Carboxypeptidase

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