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 γ-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 showed significant amino acid sequence homology to several catalytic metallopeptidases including a glutamate carboxypeptidase II also known as N-acetyl-aspartyl-α-glutamate carboxypeptidase or as prostate-specific membrane antigen and expressed glutamate carboxypeptidase activity. Expression of the PGCP cDNA in COS-1 cells, followed by Western blotting and metabolic labeling showed that PGCP is synthesized as 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-α-glutamate carboxypeptidase (NAALADase)1 (8, 9) hydrolyzes the neuromodulator N-acetyl-1-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 prostatic cancer metastasis (reviewed in Ref. 15). NAALADase is a 94-kDa transmembrane protein, homologous both to the transferrin receptor and the bacterial catalytic 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, catalytic 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 β-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 β-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 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 neural 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 purified by guest on August 31, 2017 http://www.jbc.org/ Downloaded from

[1] The abbreviations used are: NAALADase, N-acetyl-aspartyl-α-glutamate carboxypeptidase or glutamate carboxypeptidase II; PSMA, prostate-specific membrane antigen; CathA, cathepsin A/protective protein; CathD, cathepsin D; FPLC, fast protein liquid chromatography; TBS, Tris-HCl-buffered saline; PAGE, polyacrylamide gel electrophoresis; AMC, 7-amido-4-methylcoumarin; FA, 3-(2-furyl)acryloyl; βNA, β-naphthylamide; pNA, p-nitroanilide; Suc, succinyl; Z, benzyl-oxycarbonyl; EST, expressed sequence tag; PGCP, plasma glutamate carboxypeptidase; CAPS, 3-(cyclohexylamino)propanesulfonic acid.
rified 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 immunologic characterization 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 conformational affinity A-Sepharose (21). The preparation was passed through a p-aminophenyl-β-thiogalactopyranoside-agarose affinity column to separate the β-galactosidase-Catha-sialidase of PGCP, from which the HindIII-EcoRI and cloned into pBluescript-PGCP, from which the HindIII-EcoRI cassette had been excised, and the insert of the resulting construct (pBluescript-PGCP) was completely sequenced on each strand as above.

**RNA Isolation and Northern Blot**—Human skin fibroblasts from normal controls were cultured to confluency in Eagle’s minimal essential medium (Mediatech, Washington, D. C.) supplemented with 10% fetal bovine serum and antibiotics. Total RNA was isolated from the cell pellet or from human placenta tissue by ultracentrifugation in a CsCl₂ gradient as described by Maniatis et al. (32).

Purified RNA was analyzed by Northern blot as described (32). A HindIII/NcoI PGCP cDNA fragment from clone 31259 was labeled with [³²P]dCTP using the Oligolabeling Kit (Amersham Pharmacia Biotech) and used as a hybridization probe.

**Antibodies**—Rabbit polyclonal antibodies against PGCP were prepared as follows. A 577-kilobase pair fragment of PGCP cDNA spanning codons 127–320 was obtained by EcoRI/BglII digestion. After blunting with Klenow fragment DNA polymerase, the fragment was inserted into the Smal site of pGEX-2T vector (Amersham Pharmacia Biotech). The resulting plasmid was expressed in Escherichia coli 801-C to produce PGCP fusion protein. The protein was purified from the bacteria homogenate by affinity chromatography on glutathione-Sepharose (Amersham Pharmacia Biotech) as described by the manufacturer. Purified fusion protein (1 mg), homogeneous by SDS-PAGE analysis, was used to immunize a rabbit. IgG fraction purified from the antisera by ammonium sulfate fractionation was passed through a recombinant glutathione S-transferase-Sepharose column to absorb the anti-glutathione S-transferase-specific antibodies. The resulting antibody preparation was used in a dilution of 1:10,000 or 1:15,000 for Western blotting, 1:500 for immunoelectron, and 1:200 for immunofluorescence microscopy.

**Western Blotting**—Cellular or tissue homogenates, concentrated cellular medium, blood serum, or purified preparation of PGCP, was subjected to SDS-PAGE and electrotransferred to a NITRIGATION MEM nitrocellulose membrane (Micron Separations Inc., Westboro, MA). The detection of protein bands cross-reacting with anti-PGCP antibodies was performed using the BM Chemiluminescence kit (Boehringer Mannheim) in accordance with the manufacturer’s protocol.

**Immunoelectron Microscopy**—Cultured human skin fibroblasts were transfected with pCMV-PGCP expression vector using Lipofectamine (Life Technologies Inc.) in accordance with the manufacturer’s protocol. 24, 48, and 72 h after transfection, different peptide activities and control β-hexosaminidase activity were assayed in cell homogenates and medium.

**Immunofluorescent Microscopy**—Cultured human skin fibroblasts of normal controls were fixed on the glass slides with acetone/methanol (4:1) at ~20 °C, washed in phosphate-buffered saline, blocked for 1 h with 2% (w/v) bovine serum albumin in phosphate-buffered saline, incubated with anti-PGCP antibodies at a final dilution of 1:200 for 1 h at room temperature, washed with phosphate-buffered saline, and further incubated for 30 min with rhodamine-conjugated goat anti-rabbit IgG at a dilution of 1:100. Epifluorescent microscopy was performed using a Zeiss Axiophot microscope.

**Immunoglobulin Microscopy**—Cultured human skin fibroblasts were detached from the culture dishes with a rubber policeman, washed with Hank’s balanced salt solution, and fixed in 4% paraformaldehyde, 0.5% glutaraldehyde in 50 mM phosphate buffer, pH 7.5. The cell pellets were dehydrated in methanol and embedded in Lowicryl K45 as described previously (34, 35).

Ultrathin Lowicryl sections were mounted on 300-nm Formvar-coated nickel grids (Polysciences, Inc., Warrington, PA). Each section was incubated for 15 min in 20 mM Tris-HCl-buffered saline (TBS), containing 0.1% (v/v) Tween 20 as well as 15% (v/v) goat serum and for 30 min with anti-PGCP antibodies diluted 1:500 in TBS. The sections were then washed four times with TBS containing 0.05% (v/v) Tween 20, incubated for 15 min in TBS containing 15% (v/v) goat serum, and
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).

**Metal labeling**—48 h after transfection with pCMV-PGCP expression vector, COS-1 cells grown to confluency in 75-cm² culture flasks (~10⁶ cells) were washed twice with Hanks’ balanced salt solution and chased at 37 °C in Eagle’s minimal essential medium supplemented with [35S]methionine (DuPont), 0.1 mCi/ml. The radioactive medium was then removed, and the cells were washed twice with Hanks’ 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, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, 5 μg/ml leupeptin, and 0.1 mM a-toluenesulfonyl fluoride. The lysate was collected and centrifuged at 13,000 × g for 10 min to remove the cell debris.

**Immunoprecipitation, Electrophoresis, and Detection of PGCP**—CathA activity was measured according to the method of Barret (36) with bovine hemoglobin (Sigma) as a substrate; CathD activity was measured with Ac-Asp-Glu (Sigma) substrate as follows.

\[
\text{CathA activity} = \left( \frac{V}{V_o} \right) \times 100
\]

Where \(V\) is the rate of the reaction with substrate and \(V_o\) is the rate of the reaction with buffer alone.

**Immunoprecipitation Column Purification of PGCP**—Immunoprecipitation gel was prepared by coupling of 25 mg of anti-PGCP rabbit antibodies to 3 ml of BrCN-activated Sepharose 4B (Sigma) using the manufacturer’s protocol. The PGCP-containing fraction of human plasma proteins obtained by ammonium sulfate precipitation (33–50% saturation) from 30 ml 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 Tris-HCl buffer.

Enzyme Activity Assays—CathA activity was measured as described by Pshehetskyy et al. (24) with benzoyloxycarbonyl-Phe-Leu as a substrate; CathD activity was measured according to the method of Barret (36) with bovine hemoglobin (Sigma) as a substrate; and β-hexosaminidase activity was measured using 4-methylumbelliferyl-glucosaminide (37). Aminocephosphitase, dipetidyl peptidase, endopeptidase, carboxypeptidase, and esterase activities were assayed fluorometrically or spectrophotometrically as described previously using the following substrates (all Bachem or Sigma): H-Ala-AMC, H-Arg-AMC, H-Glu-AMC, H-Leu-AMC, H-Lys-AMC, H-Phe-AMC, H-Gly-Phe-βNA, Z-Arg-Arg-βNA, benzoyl-tyr-Arg-βNA, Z-Gly-Gly-Leu-βNA, Suc-Phe-Leu-βNA, Suc-Leu-Tyr-AMC, Z-Phe-βNA, FA-Phe-Ala, FA-Phe-Arg, FA-Phe-Glu, FA-Phe-Gly, FA-Phe-Phe, and FA-Glu-Tyr. Glutamate carboxypeptidase activity was measured with Ac-Asp-Glu (Sigma) substrate as follows. The 100 μl of incubation mixture contained 1 mM Ac-Asp-Glu and 1–5 μg of sample protein in 100 mM Tris-HCl buffer, pH 7.5, containing 1 mM dithiothreitol, and 0.02% (v/v) bromphenol blue. The proteins were denatured by boiling for 5 min, and 50 μl of each sample was subjected to SDS-PAGE according to Laemml (31). The molecular weights were determined with [14C]-labeled protein markers (Amersham Pharmacia Biotech). The gels were fixed in acetic acid/isopropyl alcohol/water (10/50/40), soaked for 30 min in Amplify™ solution (Amersham Pharmacia Biotech), vacuum-dried at 60 °C, and analyzed by autoradiography.
mM ZnCl$_2$. 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 mmol of substrate/min. Proteins were assayed according to Bradford (38) with bovine serum albumin (Sigma) as a standard.

RESULTS AND DISCUSSION

Purification and NH$_2$-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 NH$_2$-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 g-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. NH$_2$-terminal amino acid sequence of PGCP, (G)DVAKAIINLAVYGKAQ(N)RSYERLALLVDTG, 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) and was compared with those from NCBI and EMBL.

**FIG. 3.** Nucleotide sequence of PGCP cDNA and predicted amino acid sequence. The putative signal peptide is boxed, and the NH$_2$-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 (GenBank™ 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 and 147 base pairs of 3'-untranslated region containing a polyadenylation site (Fig. 3). On Northern blots of mRNA from human placenta and fibroblasts, the PGCP cDNA hybridized with a single ~1.7-kilobase pair transcript, indicating that the acquired cDNA was near full-length (Fig. 4).

Analysis of the deduced 541-amino acid sequence of PGCP using a SignalP program (40) predicted that the first 24 amino acids may represent the signal peptide (boxed in Fig. 3), with a polar c-region with a predicted cleavage site between Cys273 and Lys274 (41). The NH₂-terminal sequence of the purified protein (underlined in Fig. 3) starts at Asp45, suggesting that amino acids 25–45 may represent a propeptide not retained in mature PGCP. The sequence contains 5 potential N-glycosylation sites (indicated by black dots in Fig. 3), which is consistent with the binding of PGCP to concanavalin A-Sepharose.

Screening of the dbSTS library at GenBank™ with an accession number R18560 for the EST clone revealed the identity of PGCP with an unidentified STS transcript WI-6146 (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).

Tissue and Cellular Distribution of PGCP—Western blot
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 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,

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 visicular 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* (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: *l*, nucleophile; *μ*, zinc-binding residues, *θ*, 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: *l*, nucleophile; *μ*, zinc-binding residues, *θ*, residues of the substrate-binding pocket. Numbers refer to the positions of amino acids.
proteins, we suggest that PGCP also contains two catalytic zinc atoms ligated with Asp302, Glu336, and His333 and with His290, Asp302, and Asn333, respectively (marked by triangles in Fig. 8). As in VIBR, SGAP, and NAALADase/PSMA, Asp302 can be a ligand for both zinc atoms. Catalytic site nucleophile, Glu336, is also topologically conserved in PGCP and may form a cis-peptide bond with Glu337 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, Met280, Ile299, Cys329, Tyr331, Cys333, Met348, Phe350, Phe354, Tyr357, and Ile361 in VIBR). In contrast, only two of the corresponding positions are occupied by hydrophobic residues in PGCP (Ile254 and Leu269). Similarly to VIBR, SGAP, and NAALADase, PGCP contains basic residues (Arg382, Lys425) 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 aminoo- and carboxypeptidases and its affinity to Phe-Leu-agarose, we tested the preparations of PGCP purified from human placenta, mouse liver, and blood plasma for the enzymatic activity against the number of substrates listed under “Experimental Procedures.” We observed a significant glutamate carboxypeptidase activity toward Ac-Asp-Glu (b, left axis), Z-Phe-pNA (b, left axis), and Z-Gly-Gly-pNA (b, left axis); esterase activity toward Z-Phe-pNA (b, left axis), and glutamate carboxypeptidase activity toward Ac-Asp-Glu (E, right axis). Activity values measured with other substrates listed under “Experimental Procedures” were below the detection level (less than 1 pmol/min/mg).

In contrast to glutamate carboxypeptidase II, PGCP also showed endopeptidase activity with substrates containing hydrophobic amino acid residues at the P1 and P1′ positions similar to that of chymotrypsin or calpain. Although understanding the exact biological function of this new peptidase requires further characterization of its physiological substrates, the high level of PGCP observed in blood serum suggests that the protein may play an important role in the hydrolysis of circulating peptides.

**Acknowledgments**—We are grateful to ACD Inc. (Toronto, Ontario, Canada) for providing the Protein Manager software package for sequence analysis, Dr. R. Kenigsehr for the help in immunocytochemical studies, Dr. G. Mitchell and Dr. L. Ashmarina for helpful advice, and M. Patenaude for excellent secretarial assistance.

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cDNA Cloning of Human Blood Plasma Glutamate Carboxypeptidase

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

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doi: 10.1074/jbc.274.17.11742

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