THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 48, Issue of November 26, pp. 34053–34058, 1999
Printed in U.S.A.
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Sequence, Purification, and Cloning of an Intracellular Serine Protease, Quiescent Cell Proline Dipeptidase*

(Received for publication, April 5, 1999, and in revised form, September 13, 1999)

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We recently observed that specific inhibitors of post-proline cleaving aminodipeptidases cause apoptosis in quiescent lymphocytes in a process independent of CD26/dipeptidyl peptidase IV. These results led to the isolation and cloning of a new protease that we have termed quiescent cell proline dipeptidase (QPP). QPP activity was purified from CD26$^-$ Jurkat T cells. The protein was identified by labeling with [^{3}H]diisopropylfluorophosphate and subjected to tryptic digestion and partial amino acid sequencing. The peptide sequences were used to identify expressed sequence tag clones. The cDNA of QPP contains an open reading frame of 1476 base pairs, coding for a protein of 492 amino acids. The amino acid sequence of QPP reveals similarity with prolylcarboxypeptidase. The putative active site residues serine, aspartic acid, and histidine of QPP show an ordering of the catalytic triad similar to that seen in the post-proline cleaving exopeptidases prolylcarboxypeptidase and CD26/dipeptidyl peptidase IV. The post-proline cleaving activity of QPP has an unusually broad pH range in that it is able to cleave substrate molecules at acidic pH as well as at neutral pH. QPP has also been detected in nonlymphocytic cell lines, indicating that this enzyme activity may play an important role in other tissues as well.

There are relatively few enzymes that have the ability to cleave proline-containing peptide bonds. These include exopeptidases such as dipeptidyl peptidase IV (CD26/DPPIV),[1] dipeptidyl peptidase II (DPPII), and prolylcarboxypeptidase (PCP, angiotensinase C; Ref. 1). CD26/DPPIV is a ubiquitously expressed molecule found on the cell membrane and in a secreted form (2, 3). CD26 was recently shown to cleave dipeptides off the amino terminus of chemokines such as regulated on activation, normal T cell expressed and secreted, stromal-derived factor 1, and macrophage derived chemokine, altering the biological activity of these molecules (4–6). DPPII and PCP are both found in lysosomes. DPPII has a similar substrate specificity to CD26/DPPIV but is only active at acidic pH (7). PCP, however, is a post-proline cleaving activity that liberates amino acids from the carboxyl terminus of proteins (8).

We recently observed that inhibitors of post-proline cleaving aminodipeptidases cause apoptosis in quiescent lymphocytes but not activated or transformed lymphocytes (9). This apoptosis is not mediated by CD26, because CD26$^-$ and CD26$^+$ cells both undergo apoptosis in response to the addition of these inhibitors (9). Closer analysis revealed an intracellular post-proline cleaving aminodipeptidase activity that was functional at neutral and acidic pH.

In this paper we report the purification and sequence of a post-proline cleaving aminodipeptidase that we have termed quiescent cell proline dipeptidase (QPP), according to its functional properties. The post-proline cleaving activity was purified 1000-fold by following the cleavage of the reporter substrates Ala-Pro-7-amino-4-trifluoromethylcoumarin (AFC) and Gly-Pro-paranitroanilide (pNA). The active-site serine containing protein was identified by labeling with [^{3}H]diisopropylfluorophosphate (DFP). Peptide sequencing of this protein provided us with four peptides, which were used to identify cDNAs from the Expressed Sequence Tag (EST) data base. The QPP cDNA contains an open reading frame of 1476 base pairs coding for a 492-amino acid protein. This protein has strong sequence homology with PCP but little similarity to CD26/DPPIV. We show that the QPP cDNA codes for a fully functional enzyme with Ala-Pro-AFC cleaving activity. Unlike the reported activity of DPPII and DPPIV (7), however, QPP is active at both acidic and neutral pH. This enzyme may play a role in the regulation of the large number of proteins that contain a conserved amino-terminal Xaa-Pro motif (1).

EXPERIMENTAL PROCEDURES

Materials—The peptidase inhibitors Lys-thiazolidide, Lys-piperidide, and Val-boro-Pro (VbP) were provided by R. Snow (Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT). L-125 was provided by J. T. Welch (State University New York, Albany, NY; see Fig. 1). Frozen pellets of Jurkat cells were provided by R. Barton (Boehringer Ingelheim). All chromatography media and Ficoll-Hypaque were purchased from Amersham Pharmacia Biotech. Aim V cell culture medium was purchased from Life Technologies. [^{3}H]DFP and [^{3}H]Enhance were purchased from NEN Life Science Products. Ala-Pro-AFC and AFC were purchased from Enzyme Systems Products (Dublin, CA). Protein concentrations were determined using the Coomassie Plus protein assay reagent purchased from Pierce, and centrifugal concentrators were purchased from Ambion (Austin, TX). Rapid amplification of cDNA

* This work was supported by National Institutes of Health Research Grants AI36696 and AI43469 (to B.T.H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF154502.

‡ Supported by National Institutes of Health Training Grant T32AR07570.

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[1] The abbreviations used are: DPPIV, dipeptidyl peptidase IV; DPPII dipeptidyl peptidase II; QPP, quiescent cell proline dipeptidase; VbP, Val-boro-Pro; PCP, prolylcarboxypeptidase; PBMC, peripheral blood mononuclear cell; AFC, amino-4-trifluoromethylcoumarin; pNA, paranitroanilide; DFP, diisopropylfluorophosphate; EST, Expressed Sequence Tag; RACE, rapid amplification of cDNA ends; S-110, 110,000 × *g* supernatant; PAGE, polyacrylamide gel electrophoresis; HEPBS

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athon cDNA amplification kit, and human leukocyte Marathon-Ready cDNA was purchased from CLONTECH. DNA sequencing and oligonucleotide synthesis was performed at the protein analysis facility (Tufts University). The TA cloning vector pCR2.1 was purchased from Invitrogen (Carlsbad, CA). All EST clones were purchased from ATCC. All additional reagents were purchased from Sigma.

Preparation of a Soluble Fraction of Ala-Pro-AFC Cleaving Activity—Human peripheral blood mononuclear cells (PBMCs; ~4.3×10^8 cells) were isolated from 450 ml of whole blood by Ficoll-Hypaque gradient. The PBMCs were washed three times in cold phosphate-buffered saline and resuspended in 7 ml of ice-cold lysis buffer (0.02 M Tris, pH 7.8, 4 μg/ml aprotinin, 8 μg/ml leupeptin, 8 μg/ml antipain, 5 mM EDTA) and lysed by Dounce homogenization. The homogenate was centrifuged at $1000 \times g$ for 10 min at 4 °C. The supernatant was removed and centrifuged at $45{,}000 \times g$ for 20 min at 4 °C. The resulting supernatant was removed and centrifuged at $110{,}000 \times g$ for 1 h at 4 °C. The $110{,}000 \times g$ supernatant (S-110) was used as a source of soluble cellular proteins. For preparation of S-110 from Jurkat cells, a 68 g (wet weight) frozen pellet of these cells was subjected to the same homogenization and centrifugation procedure used to prepare S-110 from PBMCs. For preparation of S-110 from 293T human fibroblasts expressing QPP cDNA, ~1.8×10^8 cells were lysed in 5 ml of ice-cold lysis buffer containing 0.02 M phosphate-buffered saline, pH 7.4, instead of 0.02 M Tris and then subjected to the same homogenization and centrifugation procedure used to prepare S-110 from PBMCs.

Purification of the Soluble Ala-Pro-AFC-Cleaving Activity—30 ml of Jurkat S-110 (corresponding to 17 g of cells) was dialyzed (molecular mass cutoff, 2 kDa) overnight at 4 °C against 4 liters of 50 mM acetic acid, titrated to pH 4.5 with NaOH. The protein sample was clarified by centrifugation at $1000 \times g$ for 10 min at 4 °C. The clarified supernatant was concentrated on a Centricon 50 membrane to ~10 ml. The concentrated sample was loaded onto a 3-ml HiTrap SP-Sepharose column and equilibrated with 50 mM acetate, pH 4.5 (start buffer). The column was washed with 10 column volumes of start buffer and eluted with a linear 0–300 mM NaCl gradient in start buffer. 0.5-ml fractions were collected and assayed for cleavage of Gly-Pro-pNA. Active fractions were pooled and concentrated to ~1 ml on a Centricon 50 membrane and then to ~0.2 ml on a Microcon 30 membrane. The concentrated material was loaded onto a Superdex 12 gel filtration column and equilibrated with 50 mM acetate, pH 4.5, 150 mM NaCl. The column was eluted with the same buffer, and 0.5-ml fractions were collected and assayed for Gly-Pro-pNA cleavage. Active fractions were pooled and used as a purified preparation of the activity. The soluble Ala-Pro-AFC cleaving activity of the QPP cDNA-transfected 293T human fibroblasts was partially purified by using a gel filtration column and then an ion exchange column, in a similar manner as above. CD26/DPPIV was purified from pig kidney as described previously (10).

[^{3}H]DFP Labeling—5 mg (total protein) of purified Ala-Pro-AFC cleaving activity was mixed with [^{3}H]DFP (specific activity 8.4, Ci/mmol) at a final concentration of 12 mM in 50 mM HEPES, pH 7.5, and incubated at room temperature for 60 min. SDS loading buffer was added, and the reaction was boiled and separated by SDS-polyacrylamide gel electrophoresis (PAGE). A control reaction without [^{3}H]DFP was run in parallel on the same gel. The control lane was silver-stained, and the labeled lane was equilibrated first in [^{3}H]Enhance and then 3% glycerol. The gels were dried, and the labeled gel was exposed to film.

Enzymatic Assays—DPPIV-like activity was assayed by fluorogenic or chromogenic assays. In the fluorescence assay, peptidase activity was measured by monitoring the accumulation of AFC liberated from the substrate Ala-Pro-AFC for 1 min, using a Perkin-Elmer fluorescence spectrometer (excitation, 400 nm; emission, 505 nm). Assays were carried out in 50 mM HEPES, pH 7.5, containing 10 μM Ala-Pro-AFC. In the chromogenic assay, pNA liberated from the substrate Gly-Pro-pNA (1 mM) was measured by absorbance at 410 nm. Plates were read on an MR700 plate reader (Dynatech Inc.). The K_m for the cleavage of Ala-Pro-AFC was determined by assaying a standard amount of activity at several concentrations of substrate in 50 mM HEPES, pH 7.5. K_m was calculated by standard transformation of the Henri-Michaelis-Menten equation. The K_i for VbP was determined by assaying a standard amount of Ala-Pro-AFC cleaving activity and several concentrations of inhibitor.

pH profile—Purified QPP was added to 150 μl of 20 μM Ala-Pro-AFC in one of the following buffers: 170 mM cacodylate buffer (pH range, 4.0–7.0, in increments of 0.5), 50 mM HEPES (pH range, 6.5–8.5, in increments of 0.5), and 50 mM HEPBS (pH range, 7.5–9.0, in increments

Preparative SDS-PAGE—To prepare QPP for tryptic digestion and amino acid sequence analysis, the active fractions from the Superdex 12 column were concentrated to ~60 μl and neutralized by the addition of 5 μl 100 mM Tris, pH 7.8. SDS loading buffer was added, and the sample was boiled before separation by SDS-PAGE. After running, the gel was fixed in 50% methanol, 10% acetic acid for 15 min and then stained with Coomassie Blue R-250. The 55-kDa band was excised and washed with water and with 50% high-performance liquid chromatography grade acetonitrile. The final wash was decanted, and the gel slice was snap-frozen in N_2.

Data Base Searches and Sequence Comparisons—Peptide sequences were used to identify homologous proteins and EST clones using BLAST at the National Center for Biotechnology Information. Searches of Swissprot for proteins with homology to QPP were performed using FASTA3 at the European Bioinformatics Institute. Multiple alignment of homologous sequences was performed using CLUSTAL W at the European Bioinformatics Institute.

Cloning of QPP—EST 69230 was supplied in pBluescript at *Eco*RI-*Xho*I (ATCC). The 5′ end of the QPP cDNA was isolated from human leukocyte cDNA, using the Marathon 5′ RACE system. The primary amplification mixture contained 1 × polymerase chain reaction buffer, 0.2 mM dNTPs, 0.2 mM primer AP1, 0.5 ng of adapter-ligated human leukocyte cDNA, 1 × KlenTaq polymerase mix, and 0.2 mM QPP-specific primer BBE1R (ACTCTGGCCCTCAAAGTCCGCCGTG). The products of this reaction were diluted 50-fold with 10 mM Tricine-KOH, pH 8.5, 0.1 mM EDTA, and 15 μl was used as template for a nested amplification, using 0.2 mM primer AP2, and 0.2 mM nested QPP-specific primer BBE2R (GCCGAGGCCTGCCACAGCTAGAACG). A prominent band of 600 base pairs was excised, extracted from the gel, and TA-cloned into pCR2.1. Several clones were isolated and sequenced. All contained ~200 base pairs of 3′ sequence that overlapped with EST 69230. To assemble a full-length cDNA, EST 69230 was digested with *Not*I and *Mst*II. The 5′ RACE product was excised from pCR2.1 by digestion with *Not*I and *Mst*II. The 5′ RACE product and pBluescript containing the 3′ sequences of EST 69230 were gel purified and ligated together, generating a full-length cDNA in pBluescript.

Transfection of QPP into 293T Fibroblasts—QPP cDNA was polymerase chain reaction-amplified with primers containing *Xho*I and *Eco*RI restriction sites using DeepVent polymerase (New England Biolabs). This was cloned into the pCI-neo expression vector (Promega) and transfected into 293T fibroblasts using the calcium phosphate method (11). Lysates from transient transfectants were assayed for Ala-Pro-AFC cleaving activity, as described above. Stable lines of 293T cells were used as a source of recombinant QPP for analysis of pH optima and inhibitor analysis.

Northern Analysis—Total RNA was isolated from resting PBMCs and Jurkat cells using the TRIZOL kit (Life Technologies). 20 μg of total RNA per lane was loaded from each sample. ^{32}P-labeled QPP cDNA was used to probe the Northern blot.

RESULTS

Novel Intracellular DPPIV-like Activity in Lymphocytes—Functional analyses revealed that culturing PBMCs with CD26/DPPIV inhibitors led to apoptosis in resting lymphocytes (9). CD26/DPPIV, a T cell surface molecule, was excluded as a target for this death-inducing activity, because both $CD26^+$ and $CD26^-$ lymphocytes were equally sensitive to apoptosis induction in the presence of the DPPIV inhibitors (9). To search for a novel DPPIV-like activity, a soluble fraction of PBMCs was prepared. This fraction contained proteolytic activity that cleaved the CD26/DPPIV substrate Ala-Pro-AFC (data not shown). The activity was inhibited by VbP (Ref. 12; see Fig. 1 for structure) in the micromolar range but only partially inhibited by millimolar concentrations of serine- and cysteine-protease inhibitors with broad specificity (Table I). We analyzed the ability of various DPPIV inhibitors to block QPP activity. As can be seen in Table I, Lys-thiazolidide and Lys-piperidide inhibit QPP with similar K_i values to VbP, whereas L-125 (see Fig. 1. for structure) does not.

Because PBMCs contain $CD26^+$ cells, and a large quantity of blood would be required to isolate enough PBMCs to purify the

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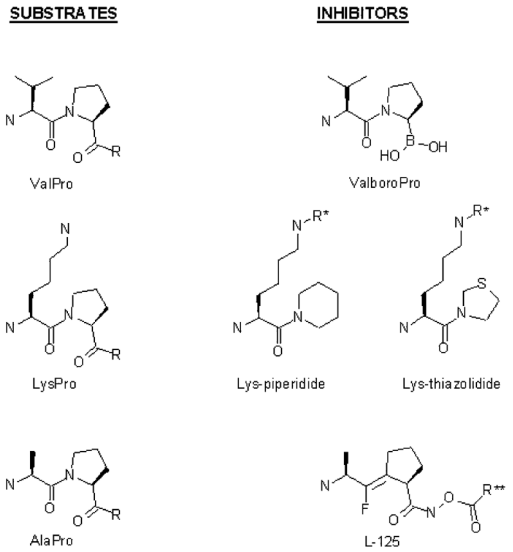

FIG. 1. **Inhibitors of post-proline aminodipeptidases.** *R*, a polypeptide; *R**, (4-NO2)-Z; *R***, (4-NO2)-C6H4.

TABLE I
Inhibition profile of QPP

Inhibitor	Concentration	% Inhibition
		%
VbP	1 μM	85
Antipain	1 mM	0
Leupeptin	1 mM	12
Phenymethylsulfenyl fluoride	1 mM	36
Benzamidine	1 mM	35
N-ethylmaleimide	1 mM	27
Iodoacetimide	1 mM	24

CD26/DPPIV inhibitors	K_i (nM)	
	CD26	QPP
	nM	
VbP	2	125
Lys-thiazolidide	ND[a]	145
Lys-piperidide	ND	300
L-125	188	>1000

[a] ND, not determined.

PBMCs and Jurkat T cells and found them to be similar. Both PBMC and Jurkat activities are inhibited by VbP with a K_i of 125 nM (Fig. 2*A*). From these studies we concluded that the Ala-Pro-AFC cleaving activity found in the soluble fraction of PBMCs and Jurkat cells is attributable to the same enzyme, and we used Jurkat cells as a source of the activity for purification.

Biochemical Isolation of QPP—The soluble Ala-Pro-AFC-cleavage activity, termed QPP, was purified from the soluble fraction of Jurkat cells by the removal of an acid-insoluble, denatured fraction, followed by column chromatography on SP-Sepharose and Superose 12. At each step fractions were assayed for cleavage of the chromogenic substrate Gly-Pro-pNA. Active fractions were combined and further purified. The Gly-Pro-pNA cleaving activity eluted as a single peak from all chromatography columns, and this scheme provided a 1000-fold purification of the activity with 27% yield (Table II). Acid precipitation removed ~75% of the bulk protein with a 131% recovery of Ala-Pro-AFC-cleaving activity. This increased activity was most likely attributable to the removal of an acid-insoluble inhibitor. However, from the purification it is impossible to distinguish whether the cytosolic fraction contained

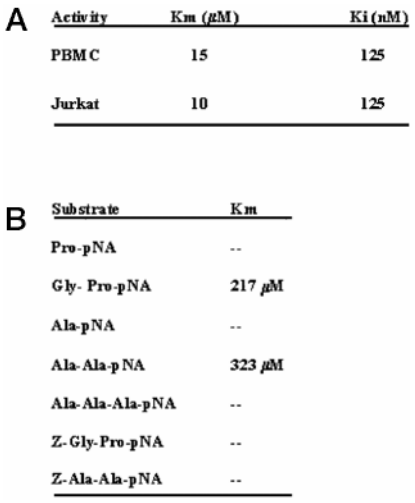

FIG. 2. **Biochemical analysis of Ala-Pro-AFC cleaving activity.** *A*, kinetic analyses. K_m and K_i values of the Ala-Pro-AFC cleaving activity purified from the cytosol of PBMCs and Jurkat cells, respectively, were compared, using Ala-Pro-AFC as substrate and VbP as inhibitor. *B*, substrate specificity of the Ala-Pro-AFC cleaving activity for various alanine-and proline-containing peptide-pNA substrates was tested using purified Jurkat QPP. - -, undetectable activity; *Z,* benzyl blocking group on the amino terminus that blocks QPP-mediated cleavage of the substrates.

TABLE II
Purification of QPP

Fraction	Protein	Activity[a]	Specific activity	Purification	Yield
	mg	*mU*	*mU/mg*	*fold*	*%*
S-110	480	1119	2		100
Acetate Supernatant	109	1466	13	7	131
SP Sepharose	5	994	199	100	89
Superdex-12	0.15	301	2007	1004	27

[a] Units defined as the cleavage of 1 μmol of substrate/min.

no-terminal dipeptides when the penultimate amino acid is proline or, to a lesser extent, alanine (13), the purified QPP activity is an amino dipeptidase that degrades substrates with prolyl and, to a lesser extent, alanyl residues in the penultimate position. Purified QPP activity is devoid of amino peptidase activity and does not cleave model substrates with blocked amino termini (Fig. 2*B*).

The Ala-Pro-AFC cleaving activity of purified QPP is active over a broad pH range, from acidic to neutral pH (pH 5.0–7.5; Fig. 3). The Ala-Pro-AFC-cleaving activity is clearly detectable from pH 4.0–7.5. When incubated in 170 mM cacodylate buffer, a peak of maximum activity was detected at an acidic pH of 5.5, whereas a similar amount of activity was seen at pH 7.0 with HEPES buffer. The Ala-Pro-AFC cleaving activity was lower (69%) at pH 7.0 with the cacodylate buffer than the HEPES buffer, and this may be attributable to the fact that this pH is out of the range of the buffering capacity of cacodylate buffer. In both HEPES and HEPBS buffers the activity clearly drops off at pH 8.0 and is completely undetectable by pH 8.5.

The activity eluted from gel filtration with an apparent molecular size of 120 kDa. SDS-PAGE revealed the presence of several polypeptides in the purified preparation but no polypeptide of 120 kDa (Fig. 4*A*), indicating that the native enzyme may be multimeric or exist as a complex. The catalytic polypeptide was identified using the irreversible inhibitor of serine-type proteases DFP. First, DFP was shown to inhibit the

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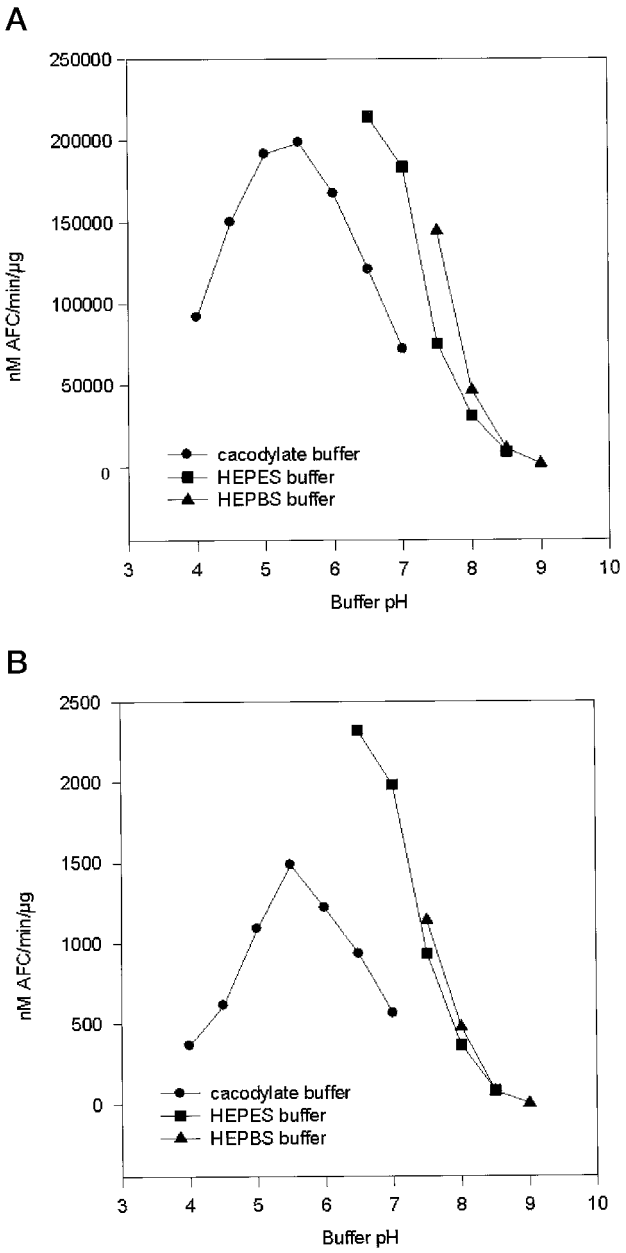

FIG. 3. **pH profile of purified QPP.** Ala-Pro-AFC cleaving activity was assayed using 170 mM cacodylate, 50 mM HEPES, or 50 mM HEPBS buffer. *A*, native QPP purified from Jurkat cells. *B*, recombinant QPP expressed in 293T human fibroblasts.

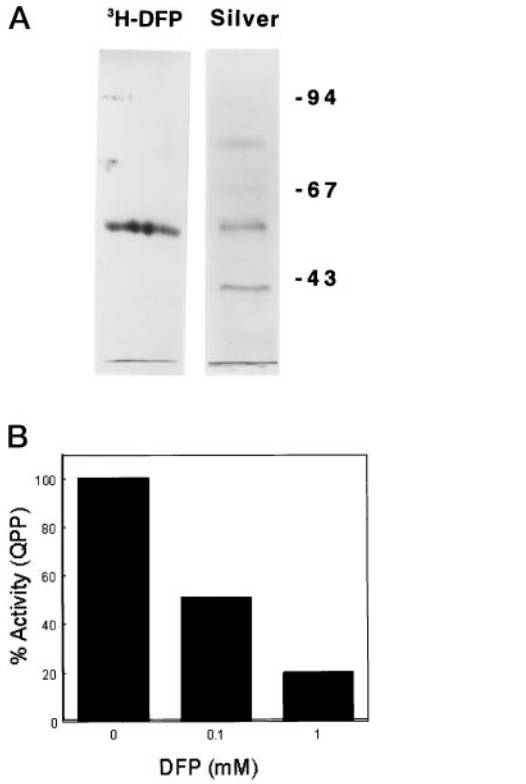

FIG. 4. **Purification of QPP.** *A*, silver stain and [^{3}H]DFP labeling of active site serine of 1000-fold enriched fraction run on SDS-PAGE and visualized by autoradiography. *B*, inhibition profile of QPP Ala-Pro-AFC cleaving activity by DFP. Untreated QPP showed an activity of 2.03×10^3 nM min^{-1} μg^{-1}.

single polypeptide of 58 kDa was labeled compared with three bands seen by silver staining. The corresponding band was excised from a Coomassie Blue-stained gel and submitted for tryptic digestion and amino acid sequence analysis (Harvard Microchemistry Facility).

Cloning of cDNA Encoding QPP—Four peptides were successfully isolated and sequenced (Fig. 5*A*). The peptide sequences were used as virtual probes to search translations of the EST data base. Four overlapping EST clones were identified and sequenced. The largest clone, EST 69230, contained 1.23 kilobases of sequence including a polyadenylation signal and poly(A) tail and encoded the three peptides obtained from the tryptic digest, GT69, GT103, and GT148. The 5′ end of the cDNA was isolated by RACE polymerase chain reaction from tide sequence of the full-length cDNA encoding QPP was unambiguously determined by sequencing both strands.

The cDNA encodes a protein of 492 amino acids with a predicted molecular mass of 54.3 kDa. It appears to contain the complete open reading frame, because the nucleotide sequence around the initiating methionine conforms to the Kozak consensus (14), and the cDNA contains a polyadenylation signal and poly(A) tail. Furthermore, the QPP cDNA contains the consensus sequence for the active-site serine residue of serine-type proteases, Gly-Xaa-Ser-Xaa-Gly (Fig. 5*A*). As Fig. 5*B* shows, QPP protein bears strong homology to PCP (Ref. 8; 42% identity), particularly at the putative active site residues. It is interesting that these two post-proline cleaving enzymes have strong sequence homology, even though QPP is an aminodipeptidase, whereas PCP is a carboxypeptidase. QPP also shows homology to hypothetical proteins obtained from the *Caenorhabditis elegans* EST data base (Fig. 5 C). There is a remarkable conservation at and around the active-site residues, suggesting an evolutionary link.

QPP cDNA Codes for a Functionally Active Protease—Northern blot analysis of Jurkat T cells and PBMCs shows that QPP is expressed in both of these cell types (Fig. 6*A*). Using a QPP cDNA probe the Northern analysis revealed a band of 1.7 kilobases that corresponds to QPP. To determine whether the QPP cDNA encodes an active protease, we transfected 293T human fibroblasts with QPP cDNA cloned into the pCIneo expression vector. Ala-Pro-AFC cleaving activity was measured from lysates of these samples at neutral pH. We found that extracts of fibroblasts transfected with the QPP cDNA contained severalfold higher specific Ala-Pro-AFC cleaving activ-

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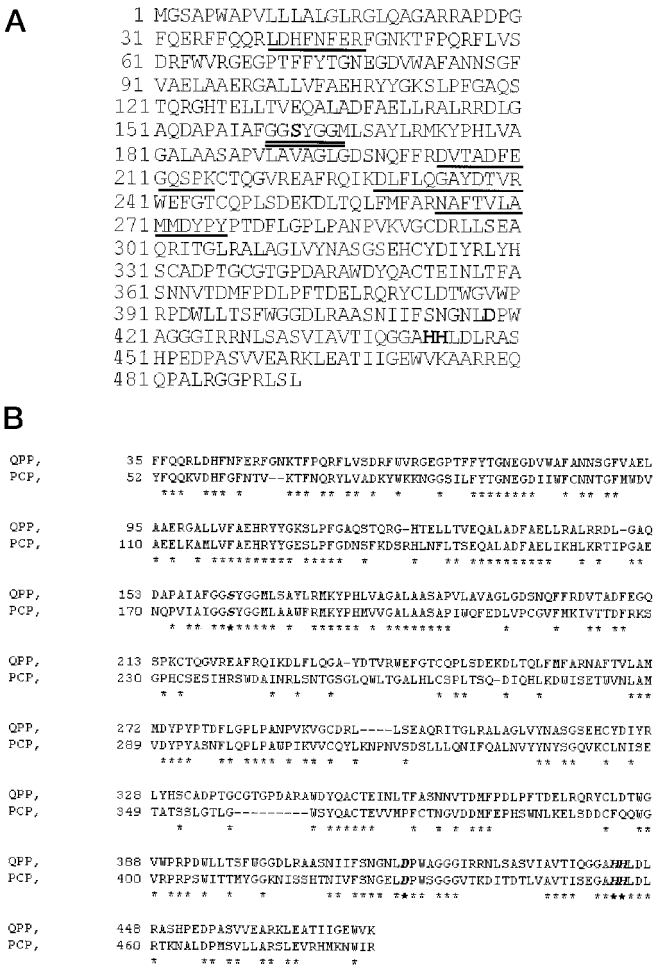
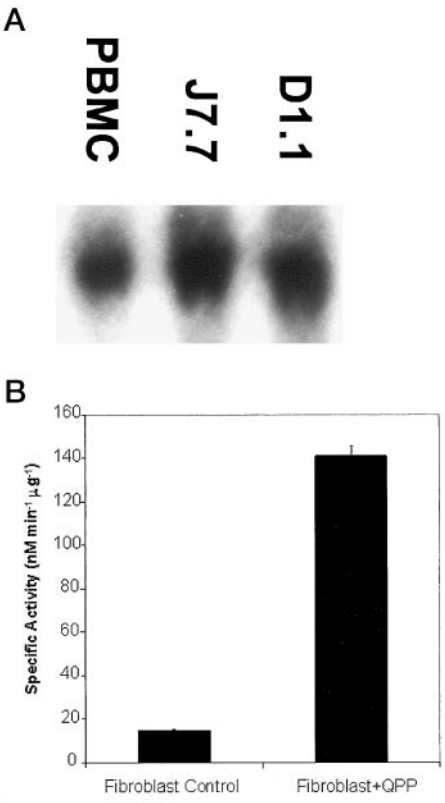


FIG. 5. **QPP sequence and alignments.** *A*, deduced amino acid sequence of QPP, showing tryptic peptide overlap (*underline*) and consensus sequence for the active-site serine residue of serine-type proteases Gly-Xaa-Ser-Xaa-Gly (*double underline*). *B*, sequence alignment of QPP and PCP. *Stars* indicate identity, and active-site residues are shown in *boldface*. *C*, amino acid alignment of the active-site residues for QPP, PCP, and the three *C. elegans* hypothetical proteins YO26, YOG1, and YM67. Sequences around the active-site residues are shown in *boldface*.

specific activity of the pH profiles of the native and recombinant QPP is attributable to the fact that the recombinant QPP was only partially purified. This presence of extraneous proteins results in a decrease in specific activity. However, the general trends of the pH profile mirror those of native QPP. Additionally, recombinant QPP has a similar K_i for VbP as native QPP and exhibits the same level of inhibition with phenylmethylsulfonyl fluoride (Fig. 6*C*). These results show



C

	Native QPP	Recombinant QPP
Ki of VbP (nM)	125	110
% inhibition PMSF (1 mM)	36	40

FIG. 6. **Recombinant QPP is functional and similar to native QPP.** *A*, Northern blot analysis of Jurkat clone J7.7 and PBMCs using a full-length QPP probe. *B*, Ala-Pro-AFC cleaving activity of lysates made from control (vector-transfected) and QPP cDNA-transfected 293T cells. Experiments were done in triplicate, and *error bars* represent S.D. *C*, recombinant QPP has a similar K_i for VbP as native QPP and shows the same level of inhibition with phenylmethylsulfonyl fluoride.

DISCUSSION

QPP was biochemically purified from CD26/DPPIV$^-$ Jurkat cells, sequenced, and cloned. The translated product contains the consensus sequence for the active site of a serine-type protease, in agreement with the aminodipeptidase inhibitor profile. The purified activity eluted from gel filtration chromatography with an apparent molecular size of 120 kDa but ran as a [^{3}H]DFP-labeled band of 58 kDa on SDS-PAGE, indicating that the native enzyme may be oligomeric or exist as a complex. A search of the Swissprot data base for similar proteins produced surprising results: PCP (8) bore significant amino acid sequence homology to QPP, whereas CD26/DPPIV did not. The sequence of QPP also bears similarity to the limited sequence available of porcine DPPII (15). It is interesting to note that there is significant protein homology between human QPP and three *C. elegans* proteins. Such conservation may imply an important role for this gene family.

Alignment of QPP and PCP revealed a striking degree of sequence conservation around the active-site residues of PCP (Fig. 5). Serine-type peptidases catalyze the hydrolysis of pep-

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