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)

Robert Underwood, Murali Chiravuri, Henry Lee, Tracy Schmitz, Alisa K. Kabcenell‡, Kurt Yardley§, and Brigitte T. Huber¶

From the Department of Pathology, Program in Immunology, Tufts University School of Medicine, Boston, Massachusetts 02111 and the Department of Immunological Diseases, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut 06877

We recently observed that specific inhibitors of post-proline cleaving aminopeptidases 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 [3H]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 aminopeptidases 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 mem- 

* 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.

† Supported by National Institutes of Health Training Grant T32AR07570.

‡ Supported by National Institutes of Health Training Grant T32AR07570.

¶ To whom correspondence should be addressed: Dept. of Pathology, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111. Tel.: 617-636-6905; Fax: 617-636-0449; E-mail: bhuber@opal.tufts.edu.

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; PAGE, polyacrylamide gel electrophoresis; HEPBS, N-(2-hydroxyethyl)piperazine-N′-(4-butanesulfonic acid).

form (2, 3). CD26 was recently shown to cleave dipeptidases 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 aminopeptidases 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 aminopeptidase activity that was functional at neutral and acidic pH.

In this paper we report the purification and sequence of a post-proline cleaving aminopeptidase 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 [3H]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). t-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. [3H]DFP and [3H]Enhance were purchased from Amersham Pharmacia Biotech. Aim V cell culture medium was purchased from Life Technologies. [3H]DFP and [3H]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 Amicon (Austin, TX). Rapid amplification of cDNA ends (RACE) polymerase chain reaction was performed using the Mar-
qpp, an amino-terminal proline-specific dipeptidase

athan cdna amplification kit, and human leukocyte marathoneady cdna was purchased from clontech. dna sequencing and oligonucleotide synthesis was performed at the protein analysis facility (tufts university). the ta cloning vector pcrr2.1 was purchased from invitrogen (carlsbad, ca). all est clones were purchased from atcc. all additional reagents were purchased from st. louis biotechnology products.

Preparation of a Soluble Fraction of Ala-Pro-AFC Cleaving Activity—Human peripheral blood mononuclear cells (pbmc's; ~4 × 10⁶ cells) were isolated from 450 ml of whole blood by ficoll-hypaque gradient. the pbmc's were washed three times in cold phosphate-buffered saline and resuspended in 7 ml of ice-cold lysis buffer (0.2 m triis, ph 7.4, 4 µg/ml aprotinin, 8 µg/ml leupeptin, 500 µm sodium fluoride, 100 µm sodium pyrophosphate, and 50 µm sodium 3-mercaptopropionylglycine) and lysed by dounce homogenization. the homogenate was centrifuged at 1000 g for 10 min at 4 °c. the supernatant was removed and centrifuged at 45,000 g for 20 min at 4 °c. the resulting supernatant was removed and centrifuged at 110,000 g for 1 h at 4 °c. the 110,000 × 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 pbmc's. for preparation of s-110 from 293t human fibroblasts expressing qpp cDNA, centrifugation procedure used to prepare s-110 from pbmc's. 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 pbmc's. for preparation of s-110 from 293t human fibroblasts expressing qpp cDNA, ~1.8 × 10⁶ cells were lysed in 5 ml of ice-cold lysis buffer containing 0.02 m phosphate-buffered saline, ph 7.4, and subjected to the same homogenization and centrifugation procedure used to prepare s-110 from pbmc's.

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 kD) overnight at 4 °c against 4 liters of 50 m m acetate acid, titrated to ph 4.5 with naoh. the protein sample was clarified by centrifugation at 1000 × 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 hi-trap sp-sepharose column and equilibrated with 50 m m acetate, ph 4.5 (start buffer). the column was washed with 10 column volumes of start buffer and eluted with a linear 0-300 m m 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 equilibrated with 50 m m acetate, ph 4.5. the eluate was concentrated and the buffer was exchanged with 50 m m sodium phosphate, ph 7.5 and neutralized by the addition of 1 ml of 0.5 m m ethylenediaminetetraacetic acid (edta) and 15 m l was used as template for a nested amplification, using 0.2 m m primer ap2, and 0.2 m m nested qpp-primer ap2. the reaction was boiled and separated by sdspage. after running, the gel was stained with 5 g of total hwpes, ph 7.5, and incubated at room temperature for 60 min. sdsl loading buffer was added, and the reaction was boiled and separated by sdspage. the eluate was concentrated 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 m m acetate, ph 4.5, 150 m m sodium chloride. the eluate was collected 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 alma-pro-aFC-cleaving activity of the qpp cdna-transfected 293t human fibroblasts was partially purified on 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).

[3H]dPf Labeling—5 mg (total protein) of purified alma-pro-aFC cleaving activity was mixed with [3H]dPf (specific activity 8.4, Ci/mmol) at a final concentration of 12 mCi in 50 m m hwpes, ph 7.5, and incubated at room temperature for 60 min. sdsl loading buffer was added, and the reaction was boiled and separated by sdspage. the eluate was concentrated 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 m m acetate, ph 4.5, 150 m m sodium chloride. the eluate was collected 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 alma-pro-aFC-cleaving activity of the qpp cdna-transfected 293t human fibroblasts was partially purified on 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).

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 m l and neutralized by the addition of 5 m l of 100 mm tris, ph 7.8. sdsl loading buffer was added, and the sample was boiled before separation by sds-page. after running, the gel was fixed with 10% acetic acid and 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 was washed was decanted, and the gel slice was snap-frozen in N₂.

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 fastat3 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 bluescript at ecoRI-XhoI (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 x polymerase chain reaction buffer, 0.2 m m dNTPs, 0.2 m m primer AP1, 0.5 ng of adapter-ligated human leukocyte cdna, 1 x KlenTaq polymerase mix, and 0.2 m m qpp-specific primer BBE1R (ACTCTGGCCGCTAAAGTCCGCCGTT). the 5' and 3' race products were prepared from this 50-fold enriched cDNA using a 90-10 reaction with 0.2 m m deoxyadenosine triphosphate (dATP), 0.2 m m deoxycytidine triphosphate (dCTP), 0.2 m m deoxyguanosine triphosphate (dGTP), 0.2 m m deoxythymidine triphosphate (dTTP), and 0.2 m m diphosphorylated pNA at a final concentration of 12 mM in 50 m m hwpes, ph 7.5. the reaction was boiled and separated by sdspage. after running, the gel was stained with 5 g of total hwpes, ph 7.5, 0.1 m m EDTA, and 15 m l was used as template for a nested amplification, using 0.2 m m primer AP2, and 0.2 m m nested qpp-specific primer BBE2R (CCCGAGGCGCTGCCACAGCTAAAGG). 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 NolI and MstII. the 5' race product was excised from pCR2.1 by digestion with NolI and MstII. the 5' race product and bluescript containing the 3' sequences of EST 69230 were gel purified and ligated together, generating a full-length cdna in bluescript.

Transfection of qpp into 293t fibroblasts—qpp cdna was polymers and digestion-amplified with primers containing XhoI and EcoRI restriction sites using DeepVet polymerase (New England Biolabs). this was cloned into the pC1-neo expression vector (Promega) and transfected into 293t fibroblasts using the calcium phosphate method (11). lysates from transient transfecants were assayed for alma-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 pbmc's and jurkat cells using the trizol kit (Life Technologies). 20 µg of total RNA per lane was loaded from each sample. 32P-labeled qpp cdna was used to probe the Northern blot.

Results

Novel Intracellular DPPiv-like Activity in Lymphocytes—functional analyses revealed that culturing pbmc's 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 pbmc's was prepared. this fraction contained proteolytic activity that cleaved the cd26/dppiv substrate alma-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. data can be seen in Tables I, II, and III. qpp has a signature Kᵣ for vBp, whereas Kᵣ for VbP, whereas kᵣ for vBp, whereas kᵣ for VbP, whereas kᵣ for vBp, whereas kᵣ for vBp, whereas kᵣ for vBp. because pbmc's contain cd26' cells, and a large quantity of blood would be required to isolate enough pbmc's to purify the activity, the soluble fraction of cd26' jurkat T cells was used as a source for alma-pro-aFC cleaving activity. we analyzed the Kᵣ values of the purified alma-pro-aFC cleaving activities from
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. 2A). 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-cleaving 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 $\approx$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 inhibitors or natural substrates of QPP activity. Cellular substrates could compete with Ala-Pro-AFC, thus acting as competitive inhibitors. Similar to CD26/DPPIV, which cleaves amino-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. 2B).

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. 4A), 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 purified activity (Fig. 4B), and then an aliquot of the purified activity was incubated with $[^3]H$DFP and analyzed by SDS-PAGE and radiofluorography. As can be seen in Fig. 4A, a
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. 5A). The peptide sequences were used as virtual probes to search translations of the EST database. 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. Furthermore, the QPP cDNA contains the consensus sequence for the active-site serine residue of serine-type proteases, Gly-Xaa-Ser-Xaa-Gly (Fig. 5A). As Fig. 5B 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 database (Fig. 5C). 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. 6A). 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 activity than cells transfected with the pCIneo vector alone (Fig. 6B). Recombinant QPP exhibits a pH profile similar to that of native QPP (Fig. 3B). The discrepancy between the units of
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. 6C). These results show that the cloned cDNA is full-length and encodes an active QPP protease, the activities and characteristics of which mimic the native QPP.

### 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 a $[^3H]$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 database 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.
tered throughout the primary structure of the protein but are brought into close proximity in the properly folded enzyme, forming the active site. Identification of the positions of these amino acids either experimentally or by comparison of the sequences of homologous enzymes is useful for the classification of the serine proteases into families, which are further grouped into clans. The members of a clan are groups of families thought to have common ancestry (16, 17), preferably identified by similarities in tertiary structure. However, the tertiary structure of most enzymes is unknown; therefore, the order of the catalytic residues in the sequence is commonly used. The catalytic residues in the sequences of QPP and PCP are ordered serine, aspartic acid, and histidine. This arrangement and the homology of QPP and PCP (42% amino acid identity over their entire open reading frame) place QPP in the serine-type peptidase clan SC, family S28, with PCP. Recently, DPPII was assigned to this family, based on its amino-terminal sequence similarity to PCP and specificity for prolyl bonds (17).

As mentioned earlier, QPP and DPPII may be closely related despite certain differences in physical properties.

In terms of substrate specificity, QPP resembles CD26/DPPIV and DPPII. QPP and CD26/DPPIV, however, have dissimilar cDNA structures and no significant amino acid homology. Furthermore, a detailed analysis of QPP and CD26/DPPIV activity revealed differences in their inhibitor profiles, indicating differences in the catalytic sites of the two proteases. Before obtaining the QPP cDNA, we had assumed that QPP and CD26/DPPIV had evolved from a common ancestral gene. Although this is clearly not the case, our results indicate that QPP is related to DPPII and PCP.

It is interesting to note that QPP is able to cleave the substrate Ala-Pro-AFC over a broad pH range, from acidic to neutral pH. Typically, enzymes do not exhibit activity over such a wide pH range. There are a few exceptions, however, including cathepsin B, a cysteine protease that catalyzes toxin proteolysis in endosomes, which is active from acidic to neutral pH (18). Recent results show that QPP may indeed localize to the endosomal and lysosomal compartment and a secretory pH (18). Recent results show that QPP may indeed localize to the endosomal and lysosomal compartment and a secretory pH (18). Recent results show that QPP may indeed localize to the endosomal and lysosomal compartment and a secretory pH (18).

Although QPP is expressed in resting and activated PBMCs as well as Jurkat cells, we have observed that blocking QPP leads to cell death exclusively in resting PBMCs (9). It is likely that QPP has many substrates that are processed in the cell, only some of which are necessary for survival of resting PBMCs. Activated PBMCs and transformed cells are very different from resting cells in both gene expression and cell cycle progression and may not produce the same substrates as resting cells or may not require the products of QPP for survival. Efforts are under way to identify the natural substrates of QPP as well as to elucidate the mechanisms of this cell death pathway.

Proteases cleave substrates at little energy cost to the cell and may be important mediators of homeostasis in metabolically inactive quiescent cells. We have recently observed that post-proline aminopeptidase inhibitors cause apoptosis in quiescent cells and that QPP is a likely target of these inhibitors. A large number of signal molecules have a highly conserved Xaa-Pro motif on the amino terminus, whereas there are relatively few enzyme activities that have the ability to cleave peptide bonds containing proline (1). The isolation and cloning of QPP will help us understand the role of post-proline cleavage in the regulation of proteins with an amino-terminal Xaa-Pro motif.

Acknowledgments—We thank the following people for providing us with valuable reagents: R. Snow for VβP, Lys-thiazolidide, and Lys-piperidide; J. T. Welch for L-125; and R. Burton for Jurkat cell pellets. We also thank Jaison Paliakkava for assistance in protein purification and Julie Anselmo, Lia Kim, and Suzanne Hurta for excellent technical assistance. Partial support for initial protein purification was provided by Boehringer Ingelheim Pharmaceuticals, Inc.

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\* M. Chiravuri, F. Agarraberes, S. Hurta, K. Yardley, H. Lee, and B. T. Huber, manuscript in preparation.