Isolation and Characterization of a Prenylcysteine Lyase from Bovine Brain*

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Prenylated proteins contain one of two isoprenoid lipids, either the 15-carbon farnesyl or the 20-carbon geranylgeranyl, covalently attached to cysteine residues at or near their C terminus. The cellular abundance of prenylated proteins, which can comprise up to 2% of total cellular protein, raises the question of how cells dispose of prenylcysteines produced during the normal turnover of prenylated proteins. We have identified and characterized a novel enzyme, which we term prenylcysteine lyase, that is capable of cleaving the thioether bond of prenylcysteines. The enzyme was isolated from bovine brain membranes and exhibits an apparent molecular mass of 63 kDa. The enzyme did not require NADPH as cofactor for prenylcysteine degradation, thus distinguishing it from cytochrome P450- and flavin-containing monooxygenases that catalyze S-oxidation of thioethers. Purified prenylcysteine lyase shows similar kinetics in utilization of both farnesylcysteine and geranylgeranylcysteine as substrates, although V max is 2-fold higher with the former compound. Interaction of prenylcysteine substrates with the enzyme requires that they possess a free amino group; N-acetylated prenylcysteines and prenyl peptides are not substrates. These findings suggest that prenylcysteine lyase is a specific enzyme involved in prenylcysteine metabolism in mammalian cells, most likely comprising the final step in the degradation of prenylated proteins.

In eukaryotic organisms, a specific subset of proteins is subject to a post-translational lipid modification termed prenylation (1, 2). Two distinct isoprenoids are involved in the process, the 15-carbon farnesyl and 20-carbon geranylgeranyl, and they are attached to cysteine residues at or near the C terminus of proteins they modify. Isoprenoid attachment has been shown to be crucial for the biological function of prenylated proteins (3, 4). Geranylgeranylated proteins comprise 80±% of prenylated proteins (5, 6).

One important property of protein prenylation is that, unlike many other post-translational modifications, it is a stable modification of proteins (7). The average half-life of a prenyl protein in a mammalian cell line has been determined to be roughly 20 h, and furthermore, prenylated proteins can comprise up to 2% of total cellular protein (7, 8). These features of prenylation raise a metabolic challenge for cells, in that the prenylcysteines produced upon the turnover of prenylated proteins would somehow need to be disposed of. This situation is similar to that which apparently exists in the metabolism of another group of lipid-modified proteins, these being proteins subject to S-acylation, generally by the fatty acyl group, palmitoyl. A lysosomal enzyme termed palmitoyl-protein thioesterase has recently been found to remove the S-acyl group from cysteine-containing molecules thought to be the degradation products of S-acylated proteins (9). Interestingly, genetic defects in palmitoyl-protein thioesterase are linked to forms of the neurodegenerative disorder infantile neuronal ceroid lipofuscinosis (10).

We recently identified a potential route for cellular disposal of prenylcysteines, that being active transport by the cell-surface transporter P-glycoprotein (11, 12). However, most normal tissues do not express appreciable levels of this transporter (13, 14). Prenylated proteins, on the other hand, are ubiquitously expressed in animal cells (2). Moreover, mice in which the gene encoding P-glycoprotein was disrupted were phenotypically normal except for their increased sensitivity to certain drugs (15). Thus, although P-glycoprotein can recognize prenylcysteines as substrates, its ability to function as a metabolic mechanism for disposal of these metabolites may be quite limited.

If transport is not the major route for disposal of prenylcysteines, a logical route would be one of further metabolism. This could also be advantageous, since it could allow re-utilization of the degradation products by cells. In this case, the best mechanism would seem to be cleavage of the thioether bond that links the isoprenoid to cysteine, so that cells could recover both cysteine and the isoprenoid. Several enzymes have been described that are capable of acting on cysteine S-conjugates and xenobiotic compounds containing thioether bonds, the most prominent of these being one termed β-lyase that cleaves at the β-carbon of cysteine to produce pyruvate, ammonia, and stably modified thiols (16). β-Lyase has been found in liver, kidney, and gut microflora, where it plays an important role in the metabolism of glutathione S-conjugates (17). In addition, thioether bonds can be subject to enzymatic S-oxidation to form their sulfoxide counterpart, which can then proceed to an elimination-type reaction that eventually cleaves the C–S bond. Cytochrome P450- and flavin-containing monooxygenases can catalyze this type of S-oxidation using NADPH as cofactor (18, 19). Additionally, a flavin-containing monooxygenase isolated from pig liver can carry out S-oxidation of farnesylcysteine.
Prenylcysteine Degradation

(FC)\(^1\) (20). The significance of this reaction in the metabolism of prenylated proteins, however, remains to be established. The ability of cytochrome P450- and flavin-containing monoxygenases to protect cells from toxic metabolites and xenobiotics is dependent on their abilities to recognize a broad spectrum of substrates. On the other hand, a cellular enzyme responsible for the prenylcytysteine metabolism would need to be quite specific, since it must distinguish those molecules from the functional prenyl proteins and peptides in cells. It is not clear whether the cytochrome P450- or flavin-containing monoxygenases possess the substrate specificity required for this catalytic process.

In this study, we report the isolation and characterization of a novel enzyme, which we have termed prenylcytysteine lyase (PCLase). This enzyme, purified from bovine brain membranes, cleaves the thioether bond of prenylcytysteine in a fashion that results in free cysteine production. The biochemical properties of PCLase are distinct from that of \(\beta\)-lyase and cytochrome P450- and flavin-containing monoxygenases. PCLase recognizes both farnesylcytysteine and geranylgeranylcysteine with high affinity and specificity, suggesting its involvement in the metabolism of prenylated proteins in mammalian cells.

### Experimental Procedures

#### Synthesis of Radialabeled FC and Geranylgeranylcysteine (GGC)—Synthesis of \(^{35}\)S-labeled prenylcysteines was performed by a modification of the procedure used for synthesis of the nonradioabeled compounds (12). In a typical synthesis reaction, 500 \(\mu\) Ci of lyophilized \(^{35}\)S-cysteine (1075 Ci/mmol, American Radiolabeled Chemicals, St. Louis, MO) was mixed with 50 nmol of either farnesyl bromide or geranylgeranyl chloride in 100 \(\mu\) l of 4 mM ammonium hydroxide in methanol. The reaction was stirred at 4 °C for at least 4 h, and the products were purified by reverse-phase HPLC as described. Unlabeled prenylcysteines and prenyl peptides were synthesized as described previously (12).

#### Measurement of PCLase Activity—Degradation of prenylcytysteines was assayed by incubating the radiolabeled isoprenoid, typically at a concentration of 1 \(\mu\)M and specific activity 100–200 cpm/pgmol, with the material to be assayed (e.g., membranes, column fractions) in 100 \(\mu\) l of 20 mM Tris-HCl, pH 7.7. At the end of incubation, the reaction was quenched by addition of 200 \(\mu\)l ice-cold 20 mM Tris-HCl, pH 7.7, and the diluted reaction mixture was extracted twice with 200 \(\mu\)l of n-butyl alcohol. Following a brief centrifugation to separate the organic and aqueous phases, the aqueous phase containing the radiolabeled product was removed and radioactivity determined by liquid scintillation spectrometry. For the experiments in which the degradation of unlabeled prenylcysteines was assayed (Fig. 8), reactions were assayed by reverse-phase HPLC. Reaction mixtures were injected onto a 0.4 \(\times\) 25-cm C\(_18\) reverse-phase HPLC column (Phenomenex) equilibrated in 10% acetonitrile containing 0.1% trifluoroacetic acid. The column was then developed with a linear gradient to 100% acetonitrile, 0.1% trifluoroacetic acid over 40 min; the absorbance at 210 nm was monitored.

#### Purification of PCLase from Bovine Brain—Bovine brain membranes were prepared as described. The membrane fractions were then suspended in 20 mM Tris-HCl, pH 7.7, 2 mM EDTA, 2 mM diithiothreitol, 0.5% Triton X-100, and a mixture of protease inhibitors (21). The mixture was gently stirred at 4 °C for 1 h and then centrifuged at 100,000 \(\times\) g for 90 min. The supernatant, designated the detergent extract, was removed and immediately subjected to the chromatographic steps described below.

The detergent extract was diluted 2.5-fold with 20 mM Tris-HCl, pH 7.7, reducing the Triton X-100 concentration to 0.2%, and loaded on a 200-ml DEAE-Sephael column equilibrated in 20 mM Tris-HCl, pH 7.7, containing 0.2% Triton X-100. The column was washed with 500 ml of 20 mM Tris-HCl, pH 7.7, containing 0.1% Triton X-100, and protein eluted with a 600-ml linear gradient of the same buffer containing 0–400 mM NaCl. PCLase eluted from this column in a symmetrical peak of activity centered at 200 mM NaCl.

The peak fractions from DEAE-Sephael column were combined and supplemented with 1 mM potassium phosphate, pH 7.7, to a final concentration of 5 mM. This sample was then loaded on a 80-ml column of phenyl-Sepharose (Pharmacia Biotech Inc.) previously equilibrated in 20 mM Tris-HCl, pH 7.7, 5 mM potassium phosphate, 150 mM NaCl, and 0.1% Triton X-100. The column was then washed with 300 ml of the same buffer, except that Triton X-100 was replaced by 0.4% CHAPS, and activity was eluted with a 500-ml linear gradient of 0.5–300 mM potassium phosphate, pH 7.0, containing 0.4% CHAPS. PCLase eluted from this column in a symmetrical peak of activity centered at 70 mM potassium phosphate.

The peak fractions from hydroxylapatite column were combined, diluted 2-fold with water, and supplemented with NaCl to a final concentration of 500 mM. This pool was then loaded on a 80-ml column of phenyl-Sepharose (Pharmacia Biotech Inc.) previously equilibrated in 20 mM Tris-HCl, pH 7.7, 5 mM potassium phosphate, 150 mM NaCl, and 0.2% CHAPS. The column was washed with 150 ml of the same buffer, followed by a 100-ml wash with 20 mM Tris-HCl, pH 7.7, 100 mM NaCl, and 0.6% CHAPS. PCLase was then eluted with 20 mM Tris-HCl containing 0.6% CHAPS.

The active fractions from phenyl-Sepharose column were combined and loaded on a Mono Q HR 5/5 column (Pharmacia) equilibrated with 0.6% CHAPS and 20 mM Tris-HCl, pH 7.7. After washing the column with 3–5 ml of the same buffer, the activity was eluted with a 20-ml linear gradient of 0–500 mM NaCl in the same buffer. Eluate from the gradient was collected in 0.5-ml fractions and fractions 19–25, which contained the activity peak, were combined.

The Mono Q pool was diluted 2-fold with 20 mM Tris-HCl buffer, and NaCl was added to the final concentration of 1 M. This sample was then loaded on a Phenyl-Sepharose HR 5/5 column (Pharmacia) equilibrated with 0.2% CHAPS, 1 mM NaCl, and 20 mM Tris-HCl, pH 7.7. The column was washed with 3–5 ml of the same buffer, and a 10-ml linear gradient that decreased NaCl to zero and increased CHAPS to 0.6% was applied to the column. At the end of gradient, the elution was continued with additional 10 ml wash of 20 mM Tris-HCl, pH 7.7, containing 0.6% CHAPS. Fractions of 0.5-ml volume were collected, and activity peak was identified in fractions 28–30.

#### Thin-layer Chromatography (TLC)—Analysis of degradation products of \(^{35}\)S-labeled prenylcysteines was accomplished by spotting the reactions with 50 \(\mu\)l of methanol, followed by addition of 100 nmol of unlabeled cysteine and spotting of 10 \(\mu\l) of this mixture onto a thin-layer silica gel plate (Kieselgel 60 F254). Plates were developed in either n-propyl alcohol/acetic acid/H\(_2\)O (60:10:30) or n-propyl alcohol/ammonia hydroxide/H\(_2\)O (60:30:10) as indicated in the appropriate figure legend. Analysis of degradation product of \(^{14}\)CFC was accomplished by spotting the reactions by flash-freezing in liquid nitrogen and, after thawing, spotting the 30-\(\mu\l) reaction mixtures onto a silica gel plate with a predesorvent zone (LKB6, Whatman). The plate was developed in hexane/tetrahydrofuran (3:1). In both cases, plates were dried ENHANCE (NEN Life Science Products) and then processed for fluorographic exposure to detect radiolabeled compounds. Detection of standard compounds was confirmed in parallel with ninhydrin spray (Sigma) for the cysteine standard or exposure to iodine vapor the trans-trans-farnesol standard (Aldrich).

#### General Methods—Protein concentration was determined by the Amido Black staining procedure (24) using bovine serum albumin as the standard.
Identification of a Prenylcysteine Lyase Activity—To study the degradation of prenylcysteines in mammalian cell extracts, 
\(^{35}\text{S}\)SFC (1 \(\mu\text{M}\)) was incubated with either a bovine brain membrane fraction (10 \(\mu\text{g}\) of protein) or with buffer alone (Buffer). Reaction mixtures were incubated at 37 °C for specified times before termination by addition of 50 \(\mu\text{M}\) of methanol. Aliquots of 10 \(\mu\text{L}\) were processed by silica gel TLC and the plate developed in n-propyl alcohol/acetic acid/water (60:10:30). Visualization was either by fluorographic analysis (lanes 1–4) or ninhydrin staining (lane 5) as described under “Experimental Procedures.” Lane 1, incubation with buffer alone for 20 min; lanes 2–4, incubation with bovine brain extract for 5, 10, and 20 min, respectively; lane 5, cysteine standard.

A rapid and sensitive enzymatic assay was developed to measure PCLase activity. This assay takes advantage of the hydrophilic property of 
\(^{35}\text{S}\)S-labeled cysteine product, which can be readily separated from the substrate 
\(^{35}\text{S}\)SFC by a butanol extraction procedure. Analysis of PCLase activity in bovine brain membranes using this procedure showed a time-dependent increase in radioactivity in aqueous phase, while this activity was not seen when 
\(^{35}\text{S}\)SFC was incubated with buffer alone (Fig. 1). Inclusion of NADPH in the reaction mixture did not influence the activity (Fig. 2). Incubation with NADPH-regenerating system, consisting of 0.4 \(\text{mM}\) NADPH, 0.4 \(\text{mM}\) glucose 6-phosphate, and 1 unit of glucose-6-phosphate dehydrogenase, was included in the assays.

RESULTS AND DISCUSSION

Identification of a Prenylcysteine Lyase Activity—To study the degradation of prenylcysteines in mammalian cell extracts, 
\(^{35}\text{S}\)S-labeled versions of the two compounds of interest, FC and GGC, were synthesized (see “Experimental Procedures”). Analysis of mixtures in which 
\(^{35}\text{S}\)SFC was incubated with extracts derived from bovine brain revealed generation of a hydrophilic product in a time-dependent fashion, while this product was not seen if 
\(^{35}\text{S}\)SFC was incubated with buffer alone (Fig. 1). Furthermore, the hydrophilic product produced by the degradation of 
\(^{35}\text{S}\)SFC comigrated in the thin-layer chromatography system employed with an authentic cysteine standard (Fig. 1). These data suggested that a cleavage of the thioether bond of FC had occurred, releasing cysteine or a cysteine-like product that retained the 
\(^{35}\text{S}\) label. The hydrophilic nature of 
\(^{35}\text{S}\)-labeled product also indicated that 
\(^{35}\text{S}\)SFC was not metabolized by a known carbon-sulfur \(\beta\)-lyase, since this enzyme would have produced a thiol-containing product of much greater hydrophobicity, as the 
\(^{35}\text{S}\) would remain attached to the prenyl group. The component in the bovine brain extract that catalyzed the degradation of FC was thus tentatively designated as a prenylcysteine lyase or PCLase.

PCLase activity was found in both particulate and cytosolic fractions prepared from the bovine brain extract (data not shown). While it is not yet clear whether these activities are from the same enzyme, we elected to focus on the particulate activity since, considering the hydrophobic nature of prenylcysteines, we felt it would be the more relevant activity. In addition, in extracts derived from a Chinese hamster ovary cell line, PCLase activity was found predominantly associated with the particulate fraction (data not shown).

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\(^{35}\text{S}\)SFC was incubated with buffer alone (Fig. 2). Inclusion of NADPH in the reaction mixture did not influence the activity (Fig. 2), distinguishing the PCLase-catalyzed reaction from that of S-oxidation catalyzed by P450 and flavin-containing oxygenases. Taken together, these results indicate that PCLase is a novel enzyme that represents a

FIG. 1. Prenylcysteine degradation by bovine brain extract. 
\[^{35}\text{S}\]FC (1 \(\mu\text{M}\)) was incubated in 50 \(\mu\text{L}\) of 50 \(\text{mM}\) Tris-HCl, pH 7.7, either alone (Buffer) or with bovine brain extract (10 \(\mu\text{g}\) of protein). Reaction mixtures were incubated at 37 °C for specified times before termination by addition of 50 \(\mu\text{M}\) of methanol. Aliquots of 10 \(\mu\text{L}\) were processed by silica gel TLC and the plate developed in n-propyl alcohol/acetic acid/water (60:10:30). Visualization was either by fluorographic analysis (lanes 1–4) or ninhydrin staining (lane 5) as described under "Experimental Procedures." Lane 1, incubation with buffer alone for 20 min; lanes 2–4, incubation with bovine brain extract for 5, 10, and 20 min, respectively; lane 5, cysteine standard.

FIG. 2. PCLase activity in bovine brain membranes. 
\[^{35}\text{S}\]FC (1 \(\mu\text{M}\)) was incubated with either a bovine brain membrane fraction (10 \(\mu\text{g}\) of protein) in 100 \(\mu\text{L}\) of 50 \(\text{mM}\) Tris-HCl, pH 7.7 (Membrane) or the buffer alone (Buffer) and the reactions conducted at 37 °C for the indicated times. At the end of incubation, reactions were terminated by addition of 200 \(\mu\text{L}\) of ice-cold 50 \(\text{mM}\) Tris-HCl, pH 7.7, and subjected to 200-µl butanol extraction as described under "Experimental Procedures." The aqueous phase was removed for scintillation counting. For reaction mixtures containing NADPH, a NADPH-regenerating system, consisting of 0.4 \(\text{mM}\) NADPH, 0.4 \(\text{mM}\) glucose 6-phosphate, and 1 unit of glucose-6-phosphate dehydrogenase, was included in the assays.

standard. SDS-PAGE was performed under standard conditions and proteins visualized by staining with either Coomassie Blue or silver.

FIG. 3. Chromatography of PCLase on Mono Q resin. The pool from the phenyl-Sepharose chromatography step in the PCLase purification was applied to a Mono Q HR 5/5 anion-exchange column and chromatography performed as described under "Experimental Procedures." A, aliquots of 2 \(\mu\text{L}\) of the indicated fractions were assayed for PCLase activity as described in the legend to Fig. 2. The NaCl gradient is also shown (-- -- -- –). B, aliquots of 15 \(\mu\text{L}\) of fractions 17–29 were subjected to SDS-PAGE analysis. Resolved proteins were detected by staining with Coomassie Blue dye. Fractions 19–23 were pooled for subsequent purification by phenyl-Superose.

that retained the 
\(^{35}\text{S}\) label. The hydrophilic nature of 
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new mechanism for cleavage of thioether bonds in mammalian cells.

Isolation of PCLase—Several detergents were tested for their ability to solubilize PCLase activity from bovine brain membrane, and Triton X-100 was found to be quite efficient in this regard (not shown). Chromatography of a Triton X-100 extract on an anion-exchange resin resulted in PCLase activity eluting as a single, broad peak centered at a NaCl concentration of 0.2 M (not shown). Further chromatography on hydroxylapatite provided additional enrichment of activity and allowed for exchange of the detergent from Triton X-100 to CHAPS, which was found to be critical for the hydrophobic chromatography step utilizing phenyl-Sepharose (not shown). The final steps in the purification procedure involved the use of high-resolution fast protein liquid chromatography utilizing Mono Q anion-exchange followed by phenyl-Superose resins. The elution profile of PCLase from the Mono Q resin is shown in Fig. 3A. Visualization of the protein profile across the peak of activity by staining of SDS-PAGE gels revealed a prominent 63-kDa protein that coeluted with PCLase activity (Fig. 3B). In the final purification step on phenyl-Superose, the elution profile of the 63-kDa protein matched precisely with enzymatic activity (Fig. 4). While a poorly resolved 47-kDa band was occasionally observed in this profile, its appearance did not correlate with the activity profile as well as the 63-kDa protein. From these data, we concluded that the 63-kDa protein purified by this procedure is PCLase.

The purification of PCLase is summarized in Table I. The procedure described above resulted in a 2500-fold purification compared with the activity in the bovine brain membrane starting material. Typical overall yields in the procedure were 5–10%. Both the Mono Q and phenyl-Superose pools could be stored at 4 °C for several weeks without a significant decline in activity.

### Table I

| Step                  | Protein Specific activity |
|-----------------------|--------------------------|
|                       | mg | nmol/mg protein h−1 |
| Total membrane        | 6000 | 0.7 |
| Triton X-100 extract  | 1400 | 1.9 |
| DEAE                  | 270 | 3.1 |
| Hydroxylapatite       | 76.4 | 9.7 |
| Phenyl-Sepharose      | 8.7 | 107 |
| Mono Q                | 1.7 | 250 |
| Phenyl-Superose       | 0.16 | 2500 |

Prenylcysteine degradation by purified PCLase to determine fate of the isoprenoid. [35S]FC (10 μM) was incubated in 20 mM Tris-HCl, pH 7.7, containing 1 mM Zwittergent 3–14 with 390 ng of either heat-inactivated (lane 1) or native (lanes 2–4) PCLase in a final volume of 100 μl. Aliquots of 30 μl were removed following incubation at 37 °C for the indicated times and reactions terminated by flash-freezing. The aliquots were processed by silica gel TLC using hexane:tetrahydrofuran as the solvent as described under “Experimental Procedures” and the plate processed for fluorographic analysis. The migration position of farnesol (FOH) determined by chromatography of the unlabeled compound that was detected by staining with ninhydrin is indicated.
activity. For longer term storage, flash-freezing the purified protein after inclusion of 50% glycerol in the sample has been found to be quite effective in preserving activity.

Characterization of PCLase—Purified PCLase can process both FC and GGC as substrates (Fig. 5). After incubation of either compound with the enzyme, a 35S-labeled product was identified that comigrated with cysteine on silica TLC under both acidic (Fig. 5A) and basic (Fig. 5B) conditions. We also attempted to identify the isoprenoid product of the reaction by subjecting [14C]FC, labeled in the farnesyl group, to enzymatic digestion and analyzing the product by silica TLC. When this reaction mixture was analyzed by reverse-phase HPLC, no product could be recovered, even though disappearance of the substrate could be readily monitored (results not shown). This suggested that the isoprenoid product was substantially more hydrophobic than farnesol, which was the product we expected (see below). The isoprenoid product was, however, readily detected when the degradation of [14C]FC was analyzed by silica TLC using a hexane-tetrahydrofuran solvent system, and its behavior in this system was consistent with its being much more hydrophobic than farnesol (Fig. 6).

Steady-state kinetic analysis of PCLase revealed typical Michaelis-Menten behavior using both FC and GGC as substrates (Fig. 7, see also Table II). Processing of both substrates by the enzyme occurred with similar apparent $K_m$ values, 0.69 $\mu$M for FC and 0.84 $\mu$M for GGC, but the $V_{max}$ for FC degradation was roughly 2-fold greater than that for GGC. For comparison, the reported $K_m$ for FC utilization by a pig liver flavin containing monooxygenase is 23 $\mu$M (20) or about 30-fold higher than for utilization of this prenylcysteine by PCLase. The kinetic constants for the two reactions catalyzed by PCLase are summarized in Table II.

To begin to address the structure-activity requirements for utilization of prenylcysteines by PCLase, HPLC analysis of the degradation of specific analogs was performed. Degradation of FC by the enzyme could be readily detected by this method (Fig. 8, A and B), but a farnesylated dipeptide could not be processed under the same condition (Fig. 8, C and D). The enzyme could also not catalyze the degradation of N-acetylated farnesylcysteine (AFC; Fig. 8, E and F), providing further evidence that PCLase cannot remove the prenyl group from prenylated proteins or peptides. These data also indicate that

![Fig. 7. Substrate dependence of PCLase activity. Purified PCLase (35 pg) was incubated with the indicated concentrations of [35S]FC (●) or [35S]GGC (○) in 100 µl of 20 mM Tris-HCl, pH 7.7, and enzymatic activity assayed as described under “Experimental Procedures.” A, substrate saturation curves for PCLase utilization of both FC and GGC. B, double-reciprocal plot of data from A.](image)

![Fig. 8. Substrate specificity of PCLase. Purified PCLase (250 pg; B, D, and F) or control buffer (A, C, and E) was incubated with FC (A and B), the S-farnesylated form of the dipeptide γGlu-Cys (γGlu-F-Cys; C and D), or N-acetylated farnesylcysteine (AFC; E and F), each at a concentration of 10 µM, in 100 µl of 20 mM Tris-HCl, pH 7.7. Reactions were conducted at 37 °C for 60 min and the reaction mixtures then analyzed by C18 reverse-phase HPLC as described under “Experimental Procedures.” The absorbance at 210 nm was monitored.](image)
the free amino group of prenylcysteines plays an important role in substrate interaction with enzyme.

Although progress toward understanding the biochemical mechanisms and biological consequences of protein prenylation has advanced rapidly in the past few years, little is known about the catabolism of prenylated proteins in cells. The degradation of prenylated proteins, however, may have profound effects on the normal cellular processes, since prenylcysteines, the potential metabolites of this process, can seriously perturb a number of these processes (25, 26). In this study, we describe the isolation and characterization of a mammalian enzyme that catalyzes prenylcysteine degradation. Several biochemical properties of this enzyme, termed PCLase, are consistent with a role in catabolism of prenyl proteins. Most importantly, PCLase cleaves the thioether bond of specific prenylcysteines that are the most probable metabolites generated during the turnover of prenyl proteins. Another important property of the enzyme is that its action on a prenylcysteine requires that the cysteine contain a free amino group; this property prevents PCLase from acting on prenyl peptides and requires that the cysteine contain a free amino group; this property of the enzyme is that its action on a prenylcysteine during the turnover of prenyl proteins. Another important property of the enzyme is that its action on a prenylcysteine requires that the cysteine contain a free amino group; this property prevents PCLase from acting on prenyl peptides and requires that the cysteine contain a free amino group; this property of the enzyme is that its action on a prenylcysteine during the turnover of prenyl proteins. Another important property of the enzyme is that its action on a prenylcysteine requires that the cysteine contain a free amino group; this property prevents PCLase from acting on prenyl peptides and requires that the cysteine contain a free amino group; this property of the enzyme is that its action on a prenylcysteine during the turnover of prenyl proteins.

PCLase acts on both FC and GGC to generate a common product that was identified as free cysteine. While we have not yet identified the product containing the isoprenoid moiety, the results clearly indicate that this is not the case. Determination of the precise structure of the isoprenoid product should shed light on what appears to be an unusual mechanism for this enzyme; experiments to this end are currently under way.

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Fig. 9. Potential mechanisms for cellular degradation of prenylcysteines. A number of potential mechanisms for cellular degradation of prenylcysteines and the products that would result are shown. The enzymatic equivalent of acid-catalyzed solvolysis is designated “H + H2O.” The “X” at the terminus of the isoprenoid product of the PCLase reaction signifies the uncertainty of this structure. See text for further details.