Functional and Structural Characterization of Peptidylamidoglycolate Lyase, the Enzyme Catalyzing the Second Step in Peptide Amidation

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ABSTRACT: Carboxy-terminal amidation is a prevalent posttranslational modification necessary for the bioactivity of many neurohormonal peptides. We recently reported that in addition to peptidylglycine α-monooxygenase (PAM), a second enzyme, which we now call peptidylamidoglycolate lyase (PGL), functions in the enzymatic formation of amides [Katopodis et al. (1990) *Biochemistry 29*, 4551]. The monooxygenase first catalyzes formation of the α-hydroxyglycine derivative of the glycine-extended precursor, and the lyase subsequently catalyzes breakdown of the PAM product to the amidated peptide and glyoxylate. We report here the first primary sequence data for PGL, which establish that it is part of the putative protein precursor which also contains PAM. We also show that PAM and PGL activities are colocalized in the secretory granular fraction of neurointermediate pituitary as would be expected for enzymes sharing the same precursor. Time course studies of the amidation reaction using purified soluble pituitary PAM and PGL indicate that both enzymes are essential for enzymatic amidation. Finally, PGL has no effect on the substrate or inhibition kinetics of PAM, and purified pituitary PAM has an acidic pH optimum consistent with its known localization in secretory granules.

Peptide neurohormones are critical for intercellular communication both in the central nervous system and in the periphery. Peptides are synthesized as biologically inactive precursors which are then subjected to a series of modifications in order to produce the bioactive forms. One of the most common posttranslational modifications, essential for bioactivity, is carboxy-terminal amidation. Early work provided evidence that glycine-extended peptides are the precursors of amidated peptides (Suchanek & Kreil, 1977), and an enzymatic activity which catalyzes the transformation of glycine-extended peptides to the deglycine amides and glyoxylate was subsequently identified (Bradbury et al., 1982) and shown to require ascorbate and molecular oxygen and to be stimulated by copper (Eipper et al., 1983). An amidating enzyme, referred to either as peptidylglycine α-amidating monooxygenase (PAM, EC 1.14.17.3) or as α-amidating enzyme (α-AE), has been purified to homogeneity from various sources (Murthy et al., 1986; Kizer et al., 1986; Mizuno et al., 1986; Mehta et al., 1988), and while the molecular weights of the isolated enzymes and characteristics such as maximum velocities and reported pH optima vary, they all exhibit the same cofactor requirements.

Work aimed at elucidating the mechanism of action of PAM showed that the enzyme is capable of direct hydroxylation at the α-position (Bradbury & Smyth, 1987; Katopodis & May 1990) and strongly implicated the presence of an α-hydroxyglycine intermediate in the amidation pathway (Young & Tambourini, 1989). It was considered, however, that the complete amidation reaction was catalyzed at the active site of PAM. Only recently has it been recognized that the product of the monooxygenase reaction is not the amide, but rather another stable molecule identified as the α-hydroxyglycine derivative of the substrate (Tajima et al., 1990; Katopodis et al., 1990; Takahashi et al., 1990; Suzuki et al., 1990).

Several investigators identified a protein factor which was initially thought to stimulate PAM or alter its pH optimum (Noguchi et al., 1989; Perkins et al., 1990a). We recently isolated a novel enzyme from neurointermediate pituitary, distinct from PAM and α-AE, and we demonstrated that this enzyme catalyzes dealkylation of α-hydroxyglycine derivatives to produce the corresponding amide and glyoxylate (Katopodis et al., 1990). This finding provided direct evidence for the sequence shown in Figure 1 for the physiological carboxy-terminal amidation of glycine-extended peptides, with the monooxygenase and the new enzyme functioning sequentially to produce the amide product. In this scheme, the monooxygenase catalyzing the first step in the amidation reaction is peptidylglycine α-monooxygenase[1] (PAM). While in our initial report we referred to the second enzyme as HGAD (α-hydroxyglycine amidating dealkylase), the systematic name peptidylamidoglycolate lyase (PGL; IUB Nomenclature Committee draft recommended name; subgroup 4.3.2) is directly analogous to the known enzyme ureidoglycolate lyase (EC 4.3.2.3), which catalyzes the conversion of ureidoglycolate to urea and glyoxylate (Trijbels & Vogels, 1967).

In view of these recent findings, it is now clear that elucidation of the structure and enzymology of PGL and clarification of its catalytic relationship to PAM are important goals in efforts to understand the metabolism and regulation of neuropeptide amidation. We now report the first structural data for PGL. We also demonstrate that pituitary PAM and PGL are colocalized within the same organelle. Finally, we establish that sequential action of both soluble pituitary PAM and PGL is required for formation of the final amide product.

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[1] The deletion of the word amidating reflects the actual catalytic competence of this enzyme, and this name parallels that of dopamine β-monooxygenase (DBM, EC 1.14.17.1), which is also a copper- and ascorbate-dependent monooxygenase and with which PAM shares many structural (Southan & Kruse, 1989) and reactivity similarities (Katopodis & May, 1990).

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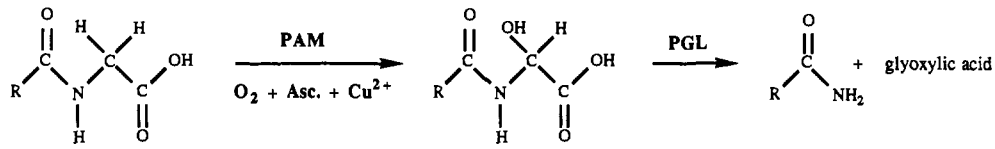

Glycine-extended peptide a-hydroxyglycine derivative Amidated peptide

FIGURE 1: Peptide carboxy-terminal amidation is a two-step process. The monooxygenase, PAM, forms the α-hydroxyglycine derivative of the substrate peptide, and then PGL catalyzes the dealkylation step to form the amide product and glyoxylate. The α-hydroxyglycine intermediate decomposes in aqueous solution at high pH; it is, however, stable at the physiological pH of secretory granules (5.5–6), and PGL is required for formation of amide product.

Materials and Methods

Materials. Frozen bovine pituitaries were from Pel Freeze Biologicals, bovine liver catalase (65 000 units/mg) was from Boehringer Mannheim, α-hydroxyhippuric acid was from Aldrich and was recrystallized before use, and TNP-D-Tyr-Val-Gly was synthesized as previously reported (Katopodis & May, 1990).

Enzyme Assays and Purification. PGL activity was assayed as described previously (Katopodis et al., 1990), by measuring the conversion of α-hydroxyhippuric acid to benzamide. PGL was purified to homogeneity by using a modification of our previous procedure (Katopodis et al., 1990). Neurointermediate tissue from 50 frozen pituitaries was homogenized in 500 mL of 50 mM Tris-HCl, pH 8.0, and fractionated with ammonium sulfate. The 40–70% pellet was dissolved in 50 mM potassium phosphate, pH 7.0, applied on a 15-mL chelating Sepharose column charged with Cu^{2+}, and equilibrated with 50 mM potassium phosphate/500 mM NaCl, pH 7.0. Elution was over 14 h with a 350-mL gradient to 30 mM imidazole, pH 7.0. Active fractions, after concentration, were separated on a Sephadex G-75 column (1.6 × 100 cm) at a flow rate of 9 mL/h. Fractions were pooled according to PGL activity, applied to a Mono Q HR 5/5 column equilibrated with 30 mM Tris-HCl, pH 8.5, and eluted with a 60-mL gradient, over 2 h, to 300 mM NaCl buffer. PGL activity was pooled, and the solution was made 1.4 M in ammonium sulfate and applied on a phenyl-Superose HR 5/5 column equilibrated with 30 mM Tris-HCl, pH 7.0, and 1.4 M ammonium sulfate. Elution was performed by using a 60-mL gradient, over 2 h, from 1.0 to 0.4 M ammonium sulfate. Active fractions were pooled and concentrated to 0.2 mL by using a Centricon 10. The material was applied on a Superose 12 HR 10/30 column, equilibrated with 100 mM ammonium bicarbonate, and eluted at a flow rate of 0.2 mL/min. PGL activity eluted in a symmetrical peak coincident to the major protein peak. The enzyme was stored at −20 °C in 50% glycerol containing 1 mg/mL BSA.

PAM activity was assayed with the tripeptide TNP-D-Tyr-Val-Gly as described earlier (Katopodis & May, 1988). Product detection was performed at 344 nm on a C8 reversed-phase column using a mobile phase of 56% water/0.1% trifluoroacetic acid/44% acetonitrile at a flow rate of 1.5 mL/min. In this manner, both TNP-D-Tyr-Val-α-hydroxyGly and TNP-D-Tyr-Val-NH_2 were quantitated simultaneously. The identity of TNP-D-Tyr-Val-α-hydroxyGly was confirmed by NMR analysis. Enzymatically produced material was purified by preparative HPLC and the FT NMR spectrum obtained. As expected, the doublet centered around 3.9 ppm, arising from the glycine hydrogens of the substrate, was not present; instead, consistent with the presence of a hydroxy group on the terminal glycine, a singlet which integrated to one proton was apparent at 5.4 ppm.

Purification of neurointermediate pituitary PAM B (the most abundant form of pituitary PAM) was performed as described elsewhere (Murthy et al., 1986), with several minor modifications. The 20–40% ammonium sulfate pellet was used, the ethanol precipitation step was omitted, and the final gel filtration column was replaced with a Superose 12 column, using conditions identical with those described above for PGL. The final purified PAM obtained in this manner contained a single band by SDS–PAGE corresponding to a molecular mass of 39 kDa, and we thus believe it to be PAM B (Murthy et al., 1986).

Sequence Analysis. Automated Edman degradation chemistry was used to determine the NH_2-terminal protein sequence (Hunkapiller et al., 1983). An Applied Biosystems Inc. Model 470A gas-phase sequencer was employed for the degradations using the standard sequencer cycle 03CPTH. The respective PTH-aa derivatives were identified by reversed-phase HPLC analysis in an on-line fashion employing an Applied Biosystems Inc. Model 120A PTH analyzer fitted with a Brownlee 2.1-mm i.d. PTH-C18 column. N-Terminal sequence of the purified protein was obtained through 22 positions. Following reduction and alkylation with iodoacetic acid, PGL was digested overnight at 37 °C with endoproteinase Glu-C (1:20) and endoproteinase Lys-C (1:20). The subsequent digestion mixture was applied to an Aquapore Butyl C4 microbore (2.1-mm i.d.) reversed-phase column, and the chromatography was developed over 120 min using a gradient from 0% to 70% acetonitrile in H_2O/0.1% TFA. The flow rate was maintained at 0.150 mL/min. Fragments were characterized by sequence analysis and FAB-MS. Mass spectral data were collected with a ZAB-SE mass spectrometer from VG Instruments.

The total amino acid composition of PGL was obtained following vapor-phase 6 N HCl hydrolysis at 115 °C for 24 h (Moore & Stein, 1963). The analysis was performed after postcolumn derivatization of the hydrolysates using ninhydrin. A Beckman Model 6300 autoanalyzer was employed for the actual determinations.

Subcellular Fractionation. Neurointermediate tissue from a single fresh bovine pituitary was homogenized in 8 mL of isotonic buffer (250 mM sucrose/20 mM Tris-HCl, pH 7.4). After centrifugation (10 min at 400*g*), the supernatant was layered onto a 2-mL isotonic Percol solution (1.08 g/mL diluted with isotonic buffer) and centrifuged (30 min at 10000*g*). The resulting band at the interface of the sucrose and Percol solutions was layered on 10 mL of isotonic Percol and centrifuged (30 min at 60000*g*). The contents of the centrifuge tube were collected into fractions of ca. 0.3 mL each. PGL and PAM activities of 20-μL aliquots of each fraction were determined in 20-min assays. Protein was measured by the Coomassie brilliant blue binding assay. Aliquots of 30 μL each were assayed for fumarase (Kanarek & Hill, 1964) and NADPH-dependent cytochrome *c* reductase (Omura et al., 1967), marker enzymes for mitochondria and rough endoplasmic reticulum.

Results and Discussion

A typical PGL purification from bovine neurointermediate pituitary is summarized in Table I. The enzyme obtained

Table I: Purification of PGL from Bovine Neurointermediate Pituitary[a]

fraction	vol (mL)	total protein (mg)	PGL act. nmol/h	PGL act. nmol mg^{-1} h^{-1}	yield (%)	purification (x-fold)
cell supernatant	800	912	121.3×10^3	0.13×10^3	100	1
40–70% ammonium sulfate	50	174	67.5×10^3	0.39×10^3	56	3
chelating Sepharose pool	20	46.4	40.7×10^3	0.88×10^3	34	7
G-75 pool	11	11.3	42.5×10^3	3.8×10^3	35	29
MonoQ pool	16	6.6	41.6×10^3	6.3×10^3	34	48
phenyl-superose pool (after concn)	0.7	0.36	23.8×10^3	66×10^3	20	507
Superose 12 pool	0.8	0.04	10.4×10^3	200×10^3	8.6	1538

[a] Data are from a single isolation using 50 pituitaries (16 g of dissected tissue). Activity was measured as nanomoles of benzamide produced by using the α-hydroxyhippuric acid assay (Katopodis et al., 1990). Protein was quantitated by the method of Bradford.

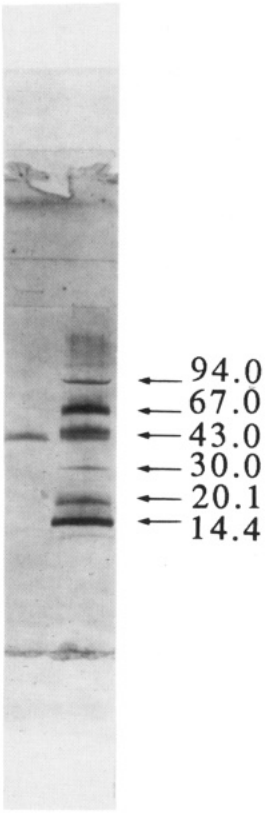

FIGURE 2: SDS–polyacrylamide gel electrophoresis was performed under reducing conditions on 8–25% gradient gels. Lane A contains purified PGL, and lane B contains molecular weight standards of the indicated weights ($\times 10^{-3}$). The gel was first stained with Coomassie blue and subsequently with silver.

from the final gel filtration column is homogeneous as judged by SDS–PAGE (Figure 2). Gas-phase automated Edman analysis of the purified PGL provided the N-terminal sequence of the enzyme to 22 positions. Additional sequence information was obtained from internal fragments produced through an enzymatic digestion employing endoproteinase Glu-C and endoproteinase Lys-C. This digestion yielded several fragments which were analyzed by using Edman degradation sequence analysis and mass spectral molecular ion determination (Figure 3).

Studies on the structure of the amidating enzyme gene in bovine intermediate pituitary have identified a cDNA which codes for a ca. 108-kDa predicted precursor protein, the amino portion of which corresponds to isolated soluble PAM (Eipper et al., 1987). The PAM domain is followed by a 430 amino acid intragranular domain, and subsequent membrane-spanning and cytoplasmic domains. When the PGL sequence data are compared to the structure of this translated bovine cDNA, it is clear that the PGL-derived sequences correspond to the intragranular domain, with the N-terminal residue of the

Table II: Total Compositional Analysis of Purified Bovine Pituitary PGL[a]

residue	experimental no. of AAs	theoretical no. of AAs	% of theoretical
N/D	37.86	39	97.1
T	16.65	16	104.0
S	24.28	24	101.2
Q/E	28.85	37	78.0
P	26.34	26	101.3
G	30.41	30	101.4
A	15.53	14	110.9
C	3.12	4	78.1
V	29.94	30	99.8
M	4.01	4	100.2
I	13.35	14	95.3
L	28.4	26	109.2
Y	11.76	9	130.6
F	22.42	21	106.8
H	14.61	13	112.4
K	16.91	18	93.9
R	9.99	11	90.8

[a] The compositional analysis results were based on the translated "PAM c-DNA" beginning at the established N-terminus of PGL and extending through K342 (excluding tryptophan residues which are destroyed during hydrolysis). The experimental values were calculated by dividing picomoles per residue into each residue's actual picomole yield. The theoretical values were obtained by summing the number of each residue present in the translated "PAM c-DNA" beginning at the established N-terminus of PGL through K342.

isolated form of PGL representing amino acid 478 of the translation product and the internal fragments from PGL representing additional sequences located within this intragranular domain (see Figures 3 and 4). There is complete identity between the obtained N-terminal and fragment sequences with the corresponding regions of the cDNA translated sequence. Thus, it is clear that bovine pituitary PGL is indeed the protein encoded by the intragranular domain of the PAM-encoding cDNA.

PAM-encoding mRNAs similar to that from bovine pituitary have been identified in human thyroid carcinoma (Glauder et al., 1990), rat atrium (Stoffers et al., 1989), and frog skin, and in the latter case isolated soluble PAM also corresponds to the amino portion of the putative precursor protein (Oshuye et al., 1988). In all cases, there is high homology both in the PAM and in the intragranular domains, suggesting a conserved physiological role for both portions of the precursor protein. Comparison of the sequences of the PGL fragments to those predicted by PAM-encoding cDNAs isolated from rat atrium and frog skin shows a high degree of homology. A computer-based sequence search (FASTA) of the NBRF, SWISS-PROT, and the translated GENBANK sequence databases (Pearson & Lipman, 1988; Sidman et al., 1988; Bilofski & Burks, 1988) did not show any other significant identity finds.

The cDNA-derived sequence shows several pairs of dibasic residues located near the expected C-terminus of PGL.

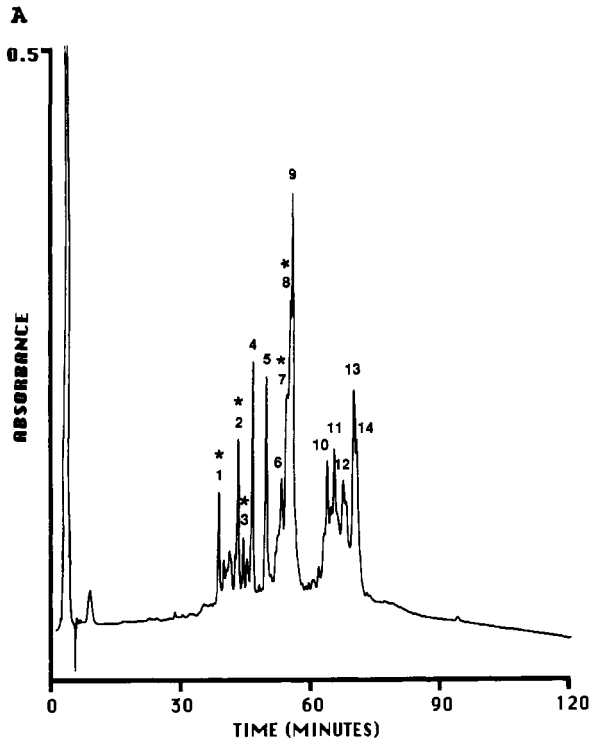

FIGURE 3: (A) Enzymatic digestion of PGL and HPLC analysis of the fragments were performed as indicated under Materials and Methods. On the basis of molecular ions greater than 1200 daltons, several fragments were selected for sequence analysis. These are denoted by asterisks (F1, F2, F3, F7 and F8). Additional fragments were not sequence analyzed. (B) Gas-phase sequence analysis results for PGL as compared to the translated 3′ half of the bovine pituitary PAM-encoding cDNA sequence (Eipper et al., 1987). Approximately 15% of the observed yield represented a species missing the N-terminal leucine residue, thereby suggesting a ragged NH_2-terminus. The sequences obtained from fragments of F1, F2, F3, F7, and F8 are shown, and, where determined, the corresponding molecular ions obtained from FAB-MS are designated. Note that both F7 and F8 yielded partial, mixed sequence. The dibasic residue sites are designated by asterisks.

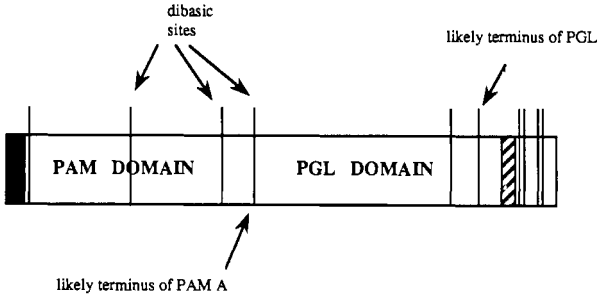

FIGURE 4: Schematic representation of the predicted precursor protein to both PAM and PGL. The figure is based on sequencing data from bovine pituitary (Eipper et al., 1987), frog skin (Oshuye et al., 1988), rat heart (Stoffers et al., 1989), and human thyroid carcinoma (Glauder et al., 1990). The dark area represents the putative signal sequence, and the hatched area represents a putative membrane-spanning domain.

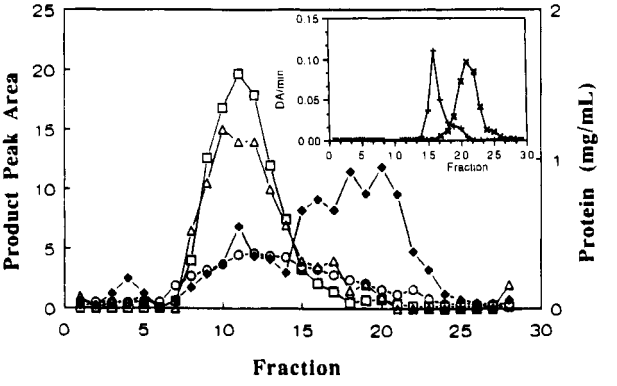

FIGURE 5: Subcellular localization of PAM and PGL. PAM activity was measured with TNP-D-Tyr-Val-Gly as a substrate, and both TNP-D-Tyr-Val-α-hydroxyGly (○) and TNP-D-Tyr-Val-NH_2 (□) were quantitated. The relative ratios of these two detected products are sensitive to the presence of PGL, and their sum represents total PAM activity. PGL was assayed independently by measuring turnover of α-hydroxyhippuric acid to benzamide (△), a reaction which is independent of the presence of PAM. Protein (♦) was measured by the Coomassie brilliant blue binding assay. The inset shows the fumarase (+) and cytochrome *c* reductase (×) activities of the same fractions.

Considering each dibasic residue site within that region as a potential C-terminal processing site, the experimentally derived composition (Table II) correlates best with a theoretical composition of PGL which terminates at K341-K342 (Figure 3B).

If indeed soluble PAM and PGL are excised from a common precursor protein within the secretory vesicle, the two enzymes should be colocalized within the same organelle. Accordingly, bovine neurointermediate pituitary tissue homogenate was fractionated on a Percoll density gradient, and fractions were assayed for both PAM and PGL. As shown in Figure 5, both enzymatic activities are indeed colocalized within the secretory granular fraction of bovine pituitary.

A physiological rationale for the colocalization of PAM and PGL is provided by the time courses of TNP-D-Tyr-Val-Gly amidation shown in Figure 6. It is evident that when highly purified PAM and PGL are used in the experiment, conversion of TNP-D-Tyr-Val-Gly to TNP-D-Tyr-Val-NH_2 exhibits an absolute requirement for both enzymes. In the absence of PGL, only the α-hydroxyglycine peptide, and not the final amide product, accumulates. The rate of nonenzymatic breakdown of the α-hydroxyglycine peptide in the assay solution increases markedly with pH in the alkaline region (Tajima et al., 1990; see Figure 7B). As expected for a nonenzymatic reaction, the very small background rate of amide formation evident in panel A of Figure 6 is neither

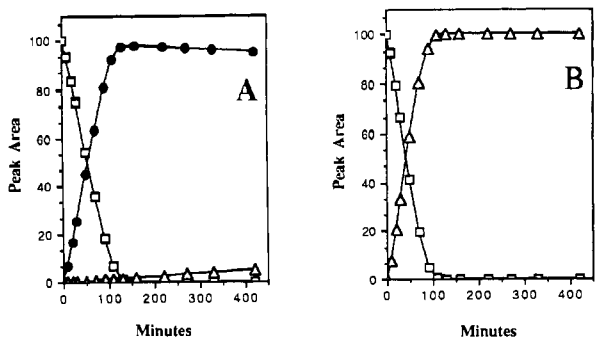

FIGURE 6: Progress curve of PAM turnover. (A) 0.4 μg of purified PAM B was incubated at 37 °C in 4.8 mL of 100 mM NaMES, pH 6.6, containing 30000 units of catalase, 2 mM ascorbate, 4 μM Cu^{2+}, and 52 μM TNP-D-Tyr-Val-Gly. Aliquots were quenched at the indicated times and analyzed by HPLC. (B) A parallel experiment was performed under the same conditions with the addition of 0.5 μg of PGL in the assay mixture. The plots show the integrated areas for TNP-D-Tyr-Val-Gly (□), TNP-D-Tyr-Val-α-hydroxyGly (●), and TNP-D-Tyr-Val-NH_2 (△).

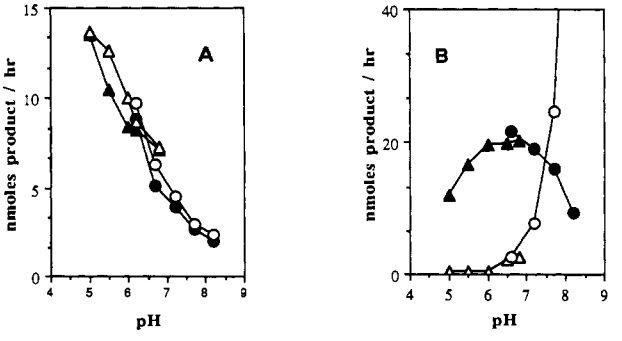

FIGURE 7: (A) pH dependence of PAM in the presence and absence of PGL. Mixtures containing ca. 0.05 μg of purified PAM, 43 μM TNP-D-Tyr-Val-Gly, 2 mM ascorbate, 4 μM Cu^{2+}, and 10000 units of catalase in a total volume of 0.5 mL of (▲, △) 100 mM NaMES or (●, ○) 100 mM Tris-HCl of the indicated pH were incubated at 37 °C for 20 min. The solid symbols represent TNP-D-Tyr-Val-α-hydroxyGly detected when only PAM was present, and the open symbols represent TNP-D-Tyr-Val-NH_2 detected when ca. 0.1 μg of PGL was also added to the assay mixture. (B) pH dependence of PGL. Assays were run using the same buffers as in panel A and 1.0 mM α-hydroxyhippuric acid as a substrate. The open symbols show benzamide formation in the absence of enzyme, and the closed symbols show benzamide formation in the presence of ca. 0.1 μg of purified PGL. The nonenzymatic rate has been subtracted from the enzymatic rate shown.

enhanced by the addition of fresh PAM nor diminished by the addition of 1 mM benzoylacrylic acid, an irreversible inhibitor of PAM (Katopodis & May, 1990). Since secretory granules maintain a pH of ca 5.5–6.0 (Gainer et al., 1985), the rate of nonenzymatic α-hydroxyglycine peptide breakdown under physiological conditions is even lower than this very small background rate evident under our assay conditions (pH 6.6).

There has been considerable disagreement in the literature regarding the pH optimum of PAM (Noguchi et al., 1989). Most literature assays depend on the detection of amide as a measure of PAM activity, and the true pH dependence of PAM has been obscured by the failure to follow formation of the α-hydroxyglycine species, the true product of the monooxygenase reaction. Figure 7A shows the pH profile for the reaction of highly purified PAM with TNP-D-Tyr-Val-Gly in the absence of PGL, where the formation of the α-hydroxyglycine species is being followed, and in the presence of excess PGL, where formation of amide occurs without buildup of the α-hydroxyglycine peptide. Figure 7B shows the pH profile of PGL alone, where the formation of benzamide from α-hydroxyhippuric acid is being monitored; also shown is the pH profile for the nonenzymatic background hydrolysis of α-hydroxyhippuric acid under assay conditions. It is evident that both purified PAM and PGL have acidic pH optima consistent with their colocalization within secretory granules. Furthermore, it is clear that PGL has no influence on the pH sensitivity of PAM turnover.

We have previously reported that PGL does not change the kinetic parameters of irreversible inhibitors of PAM (Katopodis et al., 1990). Similarly, we find that with purified PAM the substrate TNP-D-Tyr-Val-Gly exhibits a K_m of 13 ± 3 μM in the presence of PGL and 11 ± 3 μM in the absence of PGL. Thus, the kinetic data obtained so far provide no evidence of kinetic cooperativity between PAM and PGL under in vitro conditions.

Recently, expression of a truncated form of PAM-encoding mRNA containing only the intragranular portion of the PAM gene produced a protein that has catalytic properties similar to PGL (Kato et al., 1990). Also, a protein which increases amide appearance during PAM turnover at pH 6.0 was shown to cross-react with antibodies raised against a synthetic portion of the PAM intragranular domain (Perkins et al., 1990b). These results are clearly consistent with the sequence data presented here.

A second form of PAM-encoding mRNA has also been identified in various tissues (Stoffers et al., 1989; Bertelsen et al., 1990; Glauder et al., 1990). This is similar to pituitary PAM-encoding mRNA with the exception that a ca. 320 nucleotide is missing. The missing segment codes for an amino acid region located between the PAM and PGL domains which contains a dibasic site, the probable signal for endoproteolytic cleavage to separate PAM and PGL. The mature protein coded by such a truncated mRNA may therefore contain both the PAM and PGL active sites in one polypeptide chain. Indeed, there is some evidence that the amidating enzyme purified from rat thyroid carcinoma may indeed represent such a mature polyprotein with both active sites, since it is the product of a truncated mRNA (Beaudry & Bertelsen, 1989), has an acidic pH optimum (Mehta et al., 1988), and catalyzes amide formation from both glycine-extended and α-hydroxyglycine substrates (Young & Tamburini, 1989).

Several different forms of amidating enzyme have been reported from a wide variety of organisms and tissues, and it is conceivable that some of these forms may possess both monooxygenase and lyase activities within the same active site. The results reported here clearly establish that the soluble bovine pituitary PAM used in this work (PAM B, see Materials and Methods) catalyzes only monooxygenation of the glycine-extended substrate and possesses no lyase activity. However, we note that a homogeneous frog enzyme, AE-I, has been described which has a pH optimum for the production of amide products of 6–7 (Mizuno et al., 1986). Interestingly, AE-I has a precursor which does not contain an intragranular domain (Mizuno et al., 1987), and, therefore, tissues that express the AE-I precursor do not simultaneously express PGL in the same mRNA. Therefore, unlike the thyroid carcinoma enzyme, if AE-I indeed possesses lyase activity, it may arise from a single active site which is capable of catalyzing both formation and breakdown of the α-hydroxyglycine species.

A minor form of soluble pituitary PAM (PAM A), which contains a 54 amino acid carboxy-terminal extension absent from PAM B, has also been reported (Eipper et al., 1987). The sequence data in Figure 3 now reveal that this carboxy-terminal extension of PAM A terminates prior to the amino terminus of PGL (see Figure 4). Whether this carboxy-terminal extension confers any additional catalytic capabilities

to PAM A is presently unknown.

Peptide amidation may be both a regulatory and also an activating event in neurohormone biosynthesis. The realization that this reaction proceeds stepwise via a stable α-hydroxyglycine species and that the active sites which catalyze this transformation may be processed either as a single or as two separate proteins raises intriguing questions about the relationship between the various forms of PAM, PGL, α-hydroxyglycine-extended peptides, and amidated peptides. In our in vitro experiments, the presence of equimolar PAM and PGL effectively eliminated any accumulation of α-hydroxyglycine peptide. Under physiological conditions with various neuropeptides, however, steady-state or transient accumulation of α-hydroxyglycine peptides may occur. The important question of whether such species have a biological function remains to be elucidated.

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