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The primary structure of a PYY-related peptide from chicken intestine suggests an anomalous site of cleavage of the signal peptide in preproPYY

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Although the amino acid sequence of members of the pancreatic polypeptide (PP)-family of regulatory peptides has been poorly conserved during vertebrate evolution, the overall length of the peptides (36 amino acid residues) has remained constant. Nucleotide sequence analysis of cloned cDNAs and/or genomic fragments has shown the PP-related sequence immediately follows the signal peptide in the prepropeptides. A peptide tyrosine–tyrosine (PYY)-related peptide with 37 residues has been isolated from the chicken intestine, and its primary structure was established as: Ala-Tyr-Pro-Pro-Lys-Pro-Glu-Ser-Pro-Gly[10]-Asp-Ala-Ala-Ser-Pro-Glu-Glu-Ile-Ala-Gln[20]-Tyr-Phe-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn[30]-Leu-Val-Thr-Arg-Gln-Arg-Tyr.CONH$_2$. The presence of an additional alanine residue at the NH$_2$-terminus of the peptide suggests that the site of cleavage of the signal peptide in chicken preproPYY is different from the site of cleavage in other PP-family prepropeptides.

Peptide tyrosine–tyrosine; Chicken intestine; Signal peptide cleavage; Preprohormone

1. INTRODUCTION

In mammals, peptide tyrosine–tyrosine (PYY) is a hormone, produced primarily by endocrine cells in the ileum and colon, that is released into blood in response to various nutritional stimuli [1]. The primary structure of PYY has been established for the human, pig, dog and rat (reviewed in [2]). Sequence similarity of the peptides [3] and the cDNAs encoding the corresponding biosynthetic precursors [4] has suggested that PYY is homologous to pancreatic polypeptide (PP), produced in the pancreatic islets, and neuropeptide Y (NPY) distributed throughout the central and peripheral nervous systems. All mammalian members of this family of peptides (often referred to as the PP-family) comprise 36 amino acid residues, but sequence similarity between PYY and NPY is only moderate, and between PYY and PP, it is poor [3].

The structure of PYY-related peptides in intestinal tissue from lower vertebrates has been established for the frog, *Rana ridibunda* [5], trout *Oncorhynchus mykiss* [6] and lamprey *Petromyzon marinus* [7], and it has been proposed that the PP-family of peptides identified in the pancreas of teleost, elasmobranch and holostean fish are the piscine equivalent of PYY rather than mammalian PP [6]. These lower vertebrate peptides also comprise 36 amino acid residues. In this study, we describe the purification of a PYY-related peptide from the intestine of the chicken and show that its amino acid sequence is extended, relative to other PP-family peptides, by an additional residue.

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2. MATERIALS AND METHODS

2.1. *Tissue extraction*

Small intestine (650 g) was taken from 20 adult White Leghorn chickens and immediately transported on ice to the laboratory. The tissue was homogenized with 8 vols. of ethanol/0.7 M HCl (3:1 v/v) using a Waring blender and peptides were isolated using Sep-pak C-18 cartridges (Waters Associates) as previously described [5–7]. Bound material was eluted with 70% (v/v) acetonitrile/water and lyophilized. PYY-related peptides were detected using antiserum 8999 that was raised against the cysteine-extended COOH-terminal hexapeptide of pig NPY but reacts strongly with pig PYY [8].

2.2. *Purification of the peptide*

The extract was redissolved in 1% (v/v) trifluoroacetic acid/water (20 ml) and chromatographed on a (90×5 cm) Biogel P-10 (fine) column (Bio-Rad) equilibrated with 1 M acetic acid at a flow rate of 72 ml/h. Fractions (12 ml) were collected and the presence of PYY-like immunoreactivity was determined by radioimmunoassay at an appropriate dilution. Immunoreactive fractions were pooled (total volume 120 ml) and pumped at a flow rate of 2 ml/min onto a (250×10 mm) Vydac 218TP510 (C-18) column (Separations Group) equilibrated with 0.1% (v/v) trifluoroacetic acid/water. The concentration of acetonitrile in the eluting solvent was raised to 21% (v/v) over 10 min, held at this concentration for 30 min and then raised to 49% (v/v) over 60 min using a linear gradient. Absorbance was measured at 214 and 280 nm and 2 ml fractions were collected. The fraction denoted by the bar (Fig. 2A) was rechromatographed on a (250×4.6 mm) Vydac 214TP54 (C-4) column equilibrated with acetonitrile/water/trifluoroacetic acid (21.0.78.9:0.1) at a flow rate of 1.5 ml/min. The concentration of acetonitrile in the eluting solvent was raised to 49% (v/v) over 50 min using a linear gradient. The fraction denoted by the bar (Fig. 2B) was rechromatographed firstly on a (250×4.6 mm) Vydac 219TP54 phenyl column and then on a (250×4.6 mm) Vydac 218TP54 (C-18) column under the same conditions of chromatography used with the C-4

column except that the concentration of acetonitrile was raised to 42% (v/v) over 40 min.

2.3. *Structural characterization*

The primary structure of the peptide was determined by automated Edman degradation, and its amino acid composition was determined in duplicate by pre-column derivatization with phenylisothiocyanate using the methods and instrumentation described previously [5–7]. In order to demonstrate that the COOH-terminal residue in chicken PYY-related peptide is α-amidated, peptide (approx. 2 nmol) in 0.2 M ammonium bicarbonate (50 μl) was digested for 16 h at 37°C with 1-tosylamide-2-phenylethyl chloromethyl ketone-treated trypsin (Sigma) at a substrate/enzyme ratio of 50:1. The α-amidated tyrosine residue in the reaction mixture was derivatized with phenylisothiocyanate using an Applied Biosystems model 420A derivatizer and identified by reverse-phase HPLC [9].

3. RESULTS

3.1. *Purification of chicken PYY-related peptide*

PYY-like immunoreactivity in the intestinal extract was eluted from a Biogel P-10 gel permeation column as a single broad major peak with the same elution volume as pig neuropeptide Y. The fractions containing maximum immunoreactivity were pooled and chromatographed on a semi-preparative C-18 column (Fig. 1A). PYY-like immunoreactivity was associated only with the prominent peak denoted by the bar. Chromatography of this material on an analytical C-4 column (Fig. 1B) showed that PYY-like immunoreactivity was associated with the major peak in the chromatogram, as delineated by the arrows. Successive chromatographies of this fraction on an analytical Vydac phenyl column (Fig. 1C) and an analytical Vydac C-18 column (Fig. 1D). led to the purification to apparent homogeneity of the chicken PYY-related peptide. The final yield of pure material was approximately 11 nmol.

3.2. *Peptide characterization*

The primary structure of the chicken PYY-related peptide was determined by automated Edman degradation. Unambiguous assignment of amino acid phenylthiohydantoin derivatives was possible for 37 cycles of operation of the sequencer and the primary structure of the peptide is shown in Table I. The amino acid composition of the peptide was established as: Asx 1.7 (2), Glx 4.7 (5), Ser 2.8 (3), Gly 1.2 (1), His 0.9 (1), Arg 2.7 (3), Thr 1.0 (1), Ala 5.0 (5), Pro 5.2 (5), Tyr 3.9 (4), Val 1.1 (1), Ile 2.1 (2), Leu 2.3 (2), Phe 1.2 (1), Lys 1.1

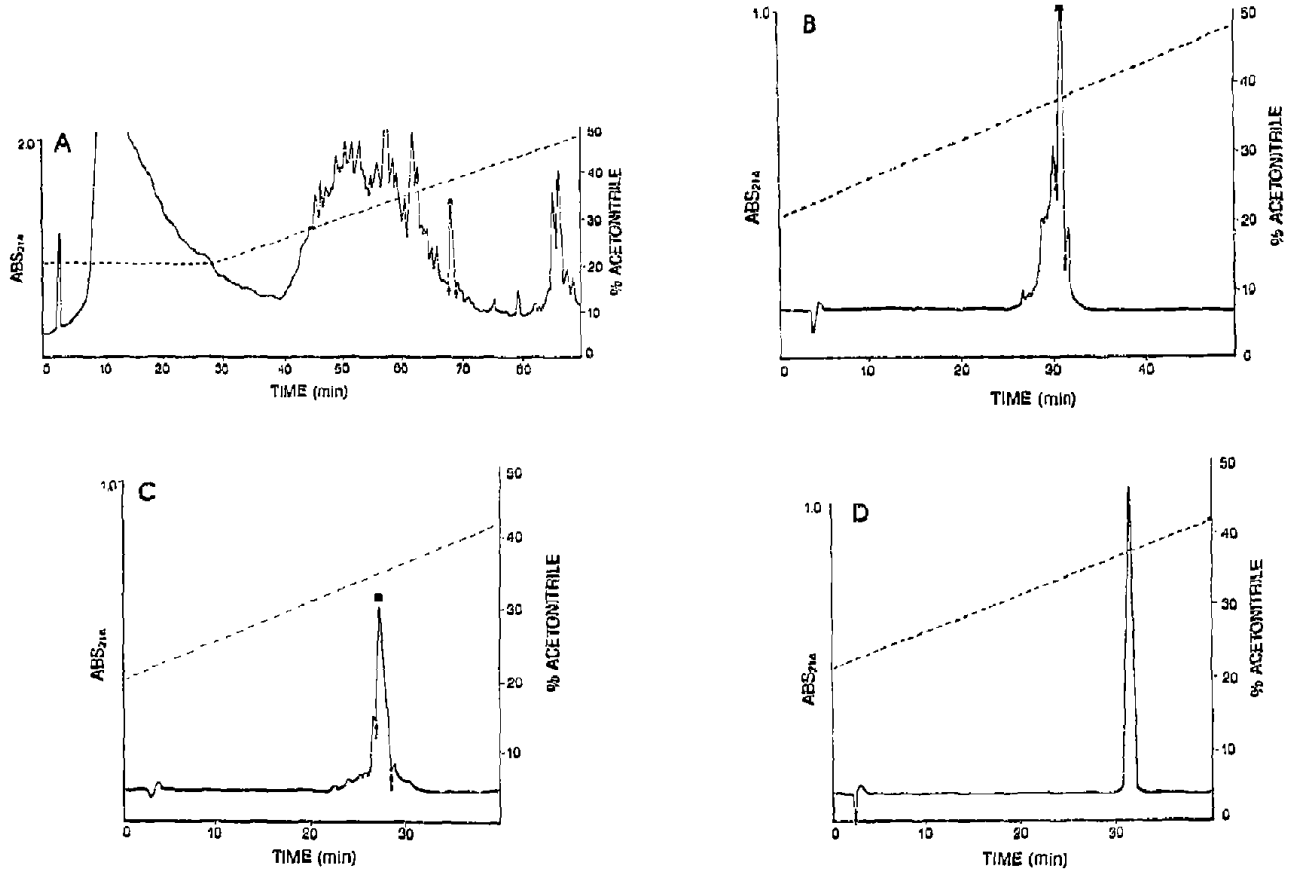

Fig. 1. Successive reverse-phase chromatographies on (A) a semi- preparative Vydac C-18 column, (B) an analytical Vydac C-4 column, (C) an analytical Vydac phenyl column, and (D) an analytical Vydac C-18 column, of an extract of chicken intestine after partial purification by gel-permeation chromatography. The fractions containing the chicken PYY-related peptide are denoted by the bars, and the arrows indicate when peak collection began and ended. The dashed line shows the concentration of acetonitrile in the eluting solvent.

Table I

A comparison of the amino acid sequences of peptides related to chicken PYY-related peptide

Chicken PYY		A YPPKP ESPGD AASPE EIAQY FSALR HYINL VTRQR Y
Chicken NPY		--S-- D---E D-PA- DM-R- YAS-- ----- I---- -
Chicken PP		G-SQ- TY--- D-PV- DLIRF YND-Q Q-L-V ---H- -
Human PYY		--I-- -NA-E D---- -LNR- YAS-- --L-- ----- -
Rat PYY	[...LVDA]	--A-- -A--E D---- -LSR- YAS-- --L-- ----- -
Frog PYY		----- -N--E D---- -MTK- LT--- ----- ----- -
Trout PYY		----- -N--E D-P-- -LAK- YT--- ----- I---- -

(–) denotes residue identity. The residues in parentheses represent the region at the COOH-terminus of the signal peptide, as deduced from the nucleotide sequence of the corresponding cDNA.

(1) residues/mol peptide. The values in parentheses show the values predicted from the proposed structure. The agreement between the sequence analysis and the amino acid composition data was good, demonstrating that the full sequence of the peptide had been obtained. The presence of an α-amidated COOH-terminal tyrosine residue was demonstrated by chromatographic analysis of the products of digestion of chicken PYY-related peptide with trypsin. After treatment of the reaction mixture with phenylisothiocyanate, the retention time of the phenylthiocarbamyl derivative of the COOH-terminal tyrosine residue of chicken PYY-related peptide was 17.00 min compared with retention times of 16.99 min and 12.85 min for the retention times of the derivatives of L-tyrosinamide and L-tyrosine, respectively.

4. DISCUSSION

This study has described the first isolation and structural characterization of a PYY-related peptide from a bird, chicken, and complements earlier studies describing the characterization of chicken PP [10] and the cloning and nucleotide sequence analysis of the cDNA encoding chicken NPY [11]. As shown in Table I, evolutionary pressure to conserve the amino acid sequence of the chicken PP-family peptides has been weak, with only 13 invariant residues between the three molecules. Chicken PYY-related peptide is the only member of the PP-family yet described that does not contain 36 amino acid residues. A plausible explanation for this observation is the hypothesis that a mutation in the signal peptide region of chicken preproPYY has necessitated an alternative site of cleavage by the signal peptidase. The sites of cleavage in prepropeptides may be predicted with a high degree of accuracy by the (−3,−1) rule of von Heijne [12]. This rule states that the residue in the −1 position must be small, the residue in the −3 position must not be aromatic, charged or large and polar, and proline must be absent from positions −3 through +1. The amino acid sequence of rat preproPYY may be predicted from the nucleotide sequence of the gene [13]. As shown in Table I, the residue at the −1 position in rat preproPYY (alanine) is the same as the additional residue at position +1 in chicken PYY-related peptide. We may speculate, therefore, that an amino acid substitution at position −3 to a 'forbidden' residue in chicken preproPYY has led to an alternative site of signal peptide cleavage. The replacement of the otherwise strongly conserved Asp^{11} and Tyr^{21} residues in the PP-family peptides by Ala and Phe, respectively, in the chicken peptide is also a novel structural feature.

The physiological role of the PYY-related peptide in the chicken, and the effect of the additional alanine residue on the biological properties of PYY, are unknown. A recent study has suggested that changes in the structure of PYY in its NH_2-terminal region may alter the receptor-binding specificity of the ligand [14]. An extract of fresh dog colon contains approximately 40% of the metabolite, PYY-(3–36)-peptide, and this peptide is a selective agonist for the Y_2 receptor. Des[Ala1-Tyr2-Pro3]PYY was not detected in the extract of chicken intestine, or the equivalent peptide in extracts of the frog [5] or trout intestines [6]. The chicken PYY-related peptide contains a proline residue at position 4 (Table I), and it is suggested that the presence of this residue may inhibit the dipeptidyl aminopeptidase responsible for generating the metabolite. For example, a peptide with the NH_2-terminal sequence Xaa-Pro-Pro-... is not a substrate for the intestinal brush border enzyme, dipeptidyl aminopeptidase IV [15].

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