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# Purification and characterization of islet hormones (insulin, glucagon, pancreatic polypeptide and somatostatin) from the Burmese python, *Python molurus*

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**Abstract**

Insulin was purified from an extract of the pancreas of the Burmese python, *Python molurus* (Squamata:Serpentes) and its primary structure established as: A Chain: Gly-Ile-Val-Glu-Gln-Cys-Cys-Glu-Asn-Thr[10]-Cys-Ser-Leu-Tyr-Glu-Leu-Glu-Asn-Tyr-Cys[20]-Asn. B-Chain: Ala-Pro-Asn-Gln-His-Leu-Cys-Gly-Ser-His[10]-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys-Gly[20]-Asp-Arg-Gly-Phe-Tyr-Tyr-Ser-Pro-Arg-Ser[30]. With the exception of the conservative substitution Phe → Tyr at position B25, those residues in human insulin that comprise the receptor-binding and those residues involved in dimer and hexamer formation are fully conserved in python insulin. Python insulin was slightly more potent (1.8-fold) than human insulin in inhibiting the binding of [[$^{125}$I-Tyr-A14] insulin to the soluble full-length recombinant human insulin receptor but was slightly less potent (1.5-fold) than human insulin for inhibiting binding to the secreted extracellular domain of the receptor. The primary structure of python glucagon contains only one amino acid substitution (Ser[28] → Asn) compared with turtle/duck glucagon and python somatostatin is identical to that of mammalian somatostatin-14. In contrast, python pancreatic polypeptide (Arg-Ile-Ala-Pro-Val-Phe-Pro-Gly-Lys-Asp[10]-Ala-Ser-Val-Asp-Glu-Leu-Ala-Lys-Phe-Tyr[20]-Thr-Glu-Leu-Gln-Gln-Tyr-Leu-Asn-Ser-Ile[30] -Asn-Arg-Pro-Arg-Phe.$NH_2$) contains only 35 instead of the customary 36 residues and the amino acid sequence of this peptide has been poorly conserved between reptiles and birds (18 substitutions compared with alligator and 20 substitutions compared with chicken). © 1997 Elsevier Science B.V.

*Keywords:* Snake; Pancreas; Squamata; Sepentes; Receptor; HPLC

## 1. Introduction

The snakes are the most rapidly evolving group of reptiles and most modern genera belong to families whose major radiation has occurred since the beginning of the Miocene [1]. It is generally accepted that snakes evolved from lizards and the recent identification of the mid-Cretaceous fossil *Pachyrhachis problematicus* as a snake with legs suggests that mosasauroids (a group of extinct marine lizards) and snakes are sister taxa [2]. The pythons (family Boidea), in contrast to the more advanced snake families such as Colubridae, Elapidae and Viperidae, retain many of the primitive features of the earliest snakes, such as vestiges of the pelvic girdle and hind limb [3]. The reptilian endocrine pancreas has been studied using immunocytochemical methods and by light and electron microscopy and cells containing insulin-, glucagon-, somatostatin- and avian pancreatic polypeptide (PP)-like immunoreactivities have been identified in the pancreata of snakes [4,5], alligator [6], crocodile [7] and in several species of lizards [8–10] and turtles [10–12].

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Despite the importance of reptiles in phylogeny, there are been relatively few studies devoted to the structural characterization of reptilian neuroendocrine peptides. The primary structures of reptilian insulins are known only for a crocodilian, the American alligator *Alligator mississipiensis* [13], a chelonian, the red-eared turtle *Psuedemys scripta* [14], and for two species of snakes, the rattlesnake *Crotalus atrox* [15] and the colubrid snake *Zoacys dhumndades* [16]. The amino acid sequence of PP is known for sixteen species of mammal and ten species of bird (reviewed in [17]) but our knowledge of reptilian PP sequences is confined to the alligator [13]. The primary structure of a reptilian glucagon is known only for the turtle, *P. scripta* [14] and a reptilian somatostatin only for the turtle [14] and the alligator [18].

The present article describes the purification, structural characterization and receptor-binding properties of insulin from the Burmese python, *Python molurus* together with the determination of the primary structures of glucagon, pancreatic polypeptide and somatostatin from this species.

## 2. Materials and methods

### 2.1. Tissue extraction

Pancreas was removed from three subadult specimens (one male; 1.6–2.2 m in total length) immediately after sacrifice. Tissue (4.1 g) was frozen in liquid nitrogen and stored at −70°C. The animals had been fasting for 56, 227 and 355 days. The frozen tissue was homogenized with ethanol/0.7 M HCl (3:1; 200 ml) using a Waring blender and stirred for 2 h at 0°C as previously described [14]. After centrifugation (4000×g for 30 min), ethanol was removed from the supernatant under reduced pressure. After a further centrifugation (4000×g for 30 min.), the extract was pumped at a flow rate of 2 ml $min^{-1}$ through 3 Sep-Pak C18 cartridges (Waters Associates, Milford, MA) connected in series. Bound material was eluted with acetonitrile/water/trifluoroacetic acid (70.0:29.9:0.1) and freeze-dried.

### 2.2. Radioimmunoassay

Insulin-like immunoreactivity was measured by using an antiserum raised against porcine insulin as described previously [19]. PP-like immunoreactivity was measured by radioimmunoassay using antiserum PP221 directed against the COOH-terminal region of human PP [20]. The antiserum requires the presence of an $\alpha$-amidated COOH-terminal residue in PP for reactivity. Glucagon-like immunoreactivity was measured using an antiserum raised against porcine glucagon that is directed towards a site within the C-terminal region of glucagon [21]. Somatostatin-like immunoreactivity was measured with antiserum that is directed towards a site in the central cyclic region of somatostatin-14 [22].

### 2.3. Purification of python insulin

The pancreatic extract, after partial purification on Sep-Pak cartridges, was redissolved in 0.1% (v/v) trifluoroacetic acid/water (5 ml) and injected on to a 25×1 cm Vydac 218TP510 $C_{18}$ reversed-phase HPLC column (Separations Group, Hesperia, CA) equilibrated with 0.1% trifluoroacetic acid at a flow rate of 2 ml $min^{-1}$. The concentration of acetonitrile in the eluting solvent was raised to 21% over 10 min and to 49% over 60 min with linear gradients. Absorbance was measured at 214 nm and 280 nm and individual peaks were collected by hand. The peak designated I in Fig. 1 (containing insulin-like immunoreactivity) was rechromatographed on a 25×0.46 cm Vydac 214 TP54 $C_4$ reversed-phase 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 42% over 40 min using a linear gradient. Python insulin was purified to near homogeneity, as assessed by peak symmetry, by chromatography on a 25×0.46 cm Vydac 219TP54 phenyl column under the same conditions used for the $C_4$ column.

### 2.4. Purification of python glucagon, pancreatic polypeptide and somatostatin

The peak designated G (containing glucagon-like immunoreactivity), the peak designated PP (containing pancreatic polypeptide-like immunoreactivity) and the peak

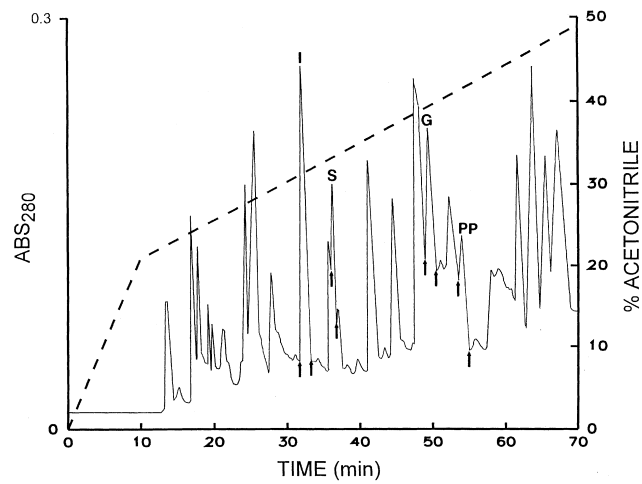


Fig. 1. Purification by reversed-phase HPLC on a semipreparative Vydac $C_{18}$ column of the islet hormones in an extract of python pancreas after partial purification on Sep-Pak cartridges. The peak designated I contained insulin, peak G contained glucagon, peak PP contained pancreatic polypeptide and peak S contained somatostatin. These peptides were purified further on analytical HPLC columns. The concentration of acetonitrile in the eluting solvent is shown by a broken line and the arrows show where peak collection began and ended.

designated S (containing somatostatin-like immunoreactivity) (Fig. 1) were rechromatographed on the analytical Vydac $C_4$ and Vydac phenyl columns under the same conditions used for the purification of insulin.

### 2.5. Structural characterization

Python insulin (approximately 3 nmol) was reduced (dithiothreitol) and pyridylethylated (4-vinylpyridine) as previously described [14]. The derivatized A-chain and B-chain were separated by reversed-phase HPLC on a Vydac $C_4$ column under the conditions used for the purification of insulin (Fig. 2A).

Amino acid compositions were determined in duplicate by precolumn derivatization with phenylisothiocyanate using an Applied Biosystems model 420A derivatizer followed by reversed-phase HPLC with an Applied Biosystems model 130A separation system. Hydrolysis (24 h at 110°C in 5.7 M HCl) of approximately 1 nmol of peptide was carried out. Cysteine and tryptophan residues were not determined. The primary structures of the peptides were determined by automated Edman degradation in an Applied Biosystems model 471A sequenator modified for detection of phenylthiohydantoin amino acid derivatives under gradient elution conditions. Mass spectrometry was performed on a Voyager RP MALDI-TOF instrument (Perspective Biosystems Inc., Framingham, MA). The accuracy of the mass determinations was within 0.02%.

### 2.6. Insulin binding studies.

Competitive binding studies were carried out using both the secreted recombinant extracellular domain and the soluble full-length recombinant human insulin receptor, expressed in 293EBNA cells (an adenovirus transformed human kidney cell line expressing EBV nuclear antigen) [23]. Porcine insulin binds to a single population of binding sites with a dissociation constant $K_d$ of 0.46 nM in the secreted extracellular domain and to two population of binding sites in the full-length receptor with $K_d$ values of 2.8 pM and 0.51 nM. The abilities of python insulin (purity >98%) and human insulin to inhibit the binding of [3-[$^{125}$I]iodotyrosine-A14] human insulin (specific radioactivity 74 TBq mmol$^{-1}$; Amersham Corporation, Arlington

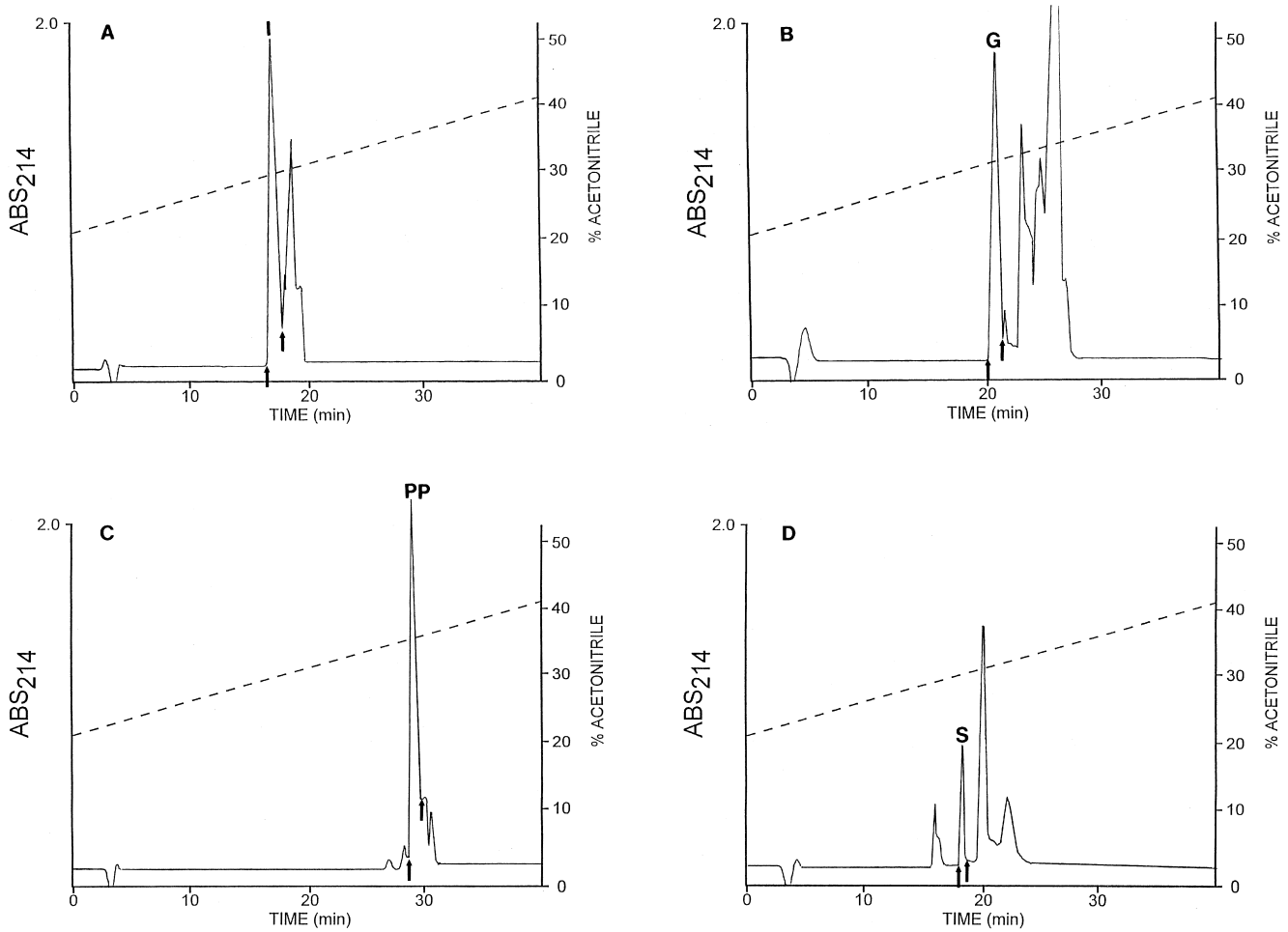


Fig. 2. Purification by reversed-phase HPLC on an analytical Vydac $C_4$ column of python (A) insulin I, (B) glucagon G, (C) pancreatic polypeptide PP and (D) somatostatin S. The arrows show where peak collection began and ended.

Heights, IL) to the soluble forms of the insulin receptor were determined using a procedure previously described in detail [23,24]. All determinations were performed in triplicate.

## 3. Results

### 3.1. Purification of python insulin

The crude extract of python pancreas appeared to contain only a very low concentration of insulin-like immunoreactivity (approximately 1 pmol $g^{-1}$ tissue weight), measured with an antiserum raised against pig insulin, and the immunoreactivity in serial dilutions of the extract did not diminish in parallel with the pig insulin standard curve in radioimmunoassay. The insulin-like immunoreactivity in the extract, after partial purification on Sep-Pak cartridges, was eluted from a semipreparative Vydac $C_{18}$ reversed-phase HPLC column (Fig. 1) as the single prominent and well-resolved uv-absorbing peak designated I. This material was rechromatographed on an analytical Vydac $C_4$ column (Fig. 2A) and the peptide was separated from a later-eluting impurity. Python insulin was purified to near homogeneity, as assessed by symmetrical peak shape, by a final chromatography on analytical Vydac phenyl column (chromatogram not shown). The final yield of pure peptide (determined by amino acid analysis) was 24 nmol.

### 3.2. Purification of python glucagon, pancreatic polypeptide and somatostatin

Chromatography on an analytical Vydac $C_4$ column of the peaks containing glucagon (Fig. 2B), pancreatic polypeptide (Fig. 2C) and somatostatin (Fig. 2D) resulted in good separation of the hormone-containing peaks from non-immunoreactive material. The peptides were purified to near homogeneity by a final chromatogramy on an analytical Vydac phenyl column (data not shown). The yields of purified material were glucagon 14 nmol, pancreatic polypeptide 11 nmol and somatostatin 1 nmol.

### 3.3. Structural characterization

The primary structures of the pyridylethylated A-chains and B-chains of python insulin were determined by automated Edman degradation and the results are shown in Table 1. It was possible to identify without ambiguity phenylthiohydantoin derivatives of amino acids for 21 cycles of operation during sequence analysis of the A-chain and for 30 cycles in the case of the B-chain. The results of amino acid analysis demonstrated that the python insulin had the following composition: Asx 5.0 (5), Glx 6.9 (7), Ser 4.0 (4), Gly 4.1 (4), His 2.1 (2), Arg 2.4 (2), Thr 1.1 (1), Ala 2.1 (2), Pro 2.3 (2) Tyr 4.7 (5), Val 2.4 (3), Ile 0.4 (1), Leu 6.3 (6) Phe 1.2 (1) (mol of residue/mol of peptide). The values in parentheses show the number of residues predicted from the proposed structure. Agreement between the results of Edman degradation and amino acid composition analysis was good demonstrating that the full sequences of the peptides had been obtained. These data indicated that the insulin was $>98\%$ pure.

The primary structures of python glucagon, PP and somatostatin-14 were determined without ambiguity by Edman degradation and the results are shown in Table 1. In the case of somatostatin, no phenylthiohydantoin derivatives were detected during cycles three and fourteen, consistent with the presence of a cystine bridge in the peptide. The amino acid composition of python glucagon [Found: Asx 3.7 (4), Glx 2.9 (3), Ser 2.7 (3), Gly 1.1 (1), His 0.9 (1), Arg 2.1 (2), Thr 3.2 (3), Ala 1.0 (1), Tyr 1.9 (2), Val 1.0 (1), Met 0.7 (1), Leu 1.9 (2), Phe 1.8 (2), Lys 1.0 (1) (mol of residue/mol of peptide)] and python PP [Found: Asx 4.1 (4), Glx 4.1 (4), Ser 2.0 (2), Gly 1.3 (1), Arg 3.2 (3), Thr 1.2 (1), Ala 3.1 (3), Pro 3.0 (3) Tyr 2.1 (2), Val 2.0 (2), Ile 2.0 (2), Leu 3.2 (3) Phe 2.9 (3), Lys 2.2 (2) (mol of residue/mol of peptide)] are consistent with their proposed structures. The presence of a C-terminally $\alpha$-amidated residue in python PP is suggested by its reactivity with an antiserum directed against the COOH-terminal hexapeptide region of human PP that requires an $\alpha$-amidated residue for recognition. This conclusion was confirmed by the results of MALDI-TOF mass spectrometry. The observed molecular mass of python PP was $4083.6 \pm 0.8$ a.m.u. compared with a calculated mass of 4084.0 for the $\alpha$-amidated form of the peptide.

### 3.4. Receptor-binding properties of python insulin

The abilities of python insulin and human insulin to inhibit binding of $^{125}$I-labelled human insulin to the soluble full-length human insulin receptor are compared in Fig. 3A. The mean concentration of python insulin producing a 50% inhibition of binding was 0.032 nM (range 0.031–0.033 nM). The corresponding value for human insulin, in incubations carried out at the same time and under identical conditions, was 0.058 nM (range 0.055–0.062 nM). The mean concentrations producing 50% inhibition of binding of labelled insulin to the secreted extracellular domain were: python insulin 0.64 nM (range 0.62–0.67 nM) and human insulin 0.44 nM (range 0.43–0.45 nM) (Fig. 3B).

## 4. Discussion

The primary structure of python insulin is compared with the structure of human insulin and with other known reptilian insulins in Fig. 4. Those residues on the surface of insulin that are considered to comprise the receptor-binding region (A1–A3, A5, A19, A21, B22–B26) [25] have

Table 1
Automated Edman degradation of the A-chain and B-chain of insulin, Glucagon and pancreatic polypeptide (PP) from the python

| Cycle No | A-Chain | B-Chain | Glucagon | PP | Somatostatin |
|---|---|---|---|---|---|
| 1 | Gly(991) | Ala(1342) | His(1012) | Arg(672) | Ala(371) |
| 2 | Ile(801) | Pro(1296) | Ser(194) | Ile(947) | Gly(297) |
| 3 | Val(879) | Asn(1104) | Gln(1152) | Ala(913) | (Cys)N.D. |
| 4 | Glu(611) | Gln(816) | Gly(1052) | Pro(795) | Lys(195) |
| 5 | Gln(835) | His(619) | Thr(354) | Val(852) | Asn(126) |
| 6 | Cys(632) | Leu(677) | Phe(586) | Phe(742) | Phe(136) |
| 7 | Cys(654) | Cys(670) | Thr(217) | Pro(678) | Phe(141) |
| 8 | Glu(409) | Gly(814) | Ser(95) | Gly(821) | Trp(58) |
| 9 | Asn(608) | Ser(72) | Asp(639) | Lys(895) | Lys(74) |
| 10 | Thr(186) | His(311) | Tyr(636) | Asp(846) | Thr(21) |
| 11 | Cys(293) | Leu(383) | Ser(69) | Ala(739) | Phe(55) |
| 12 | Ser(31) | Val(443) | Lys(438) | Ser(109) | Thr(20) |
| 13 | Leu(194) | Glu(357) | Tyr(507) | Val(704) | Ser(9) |
| 14 | Tyr(198) | Ala(353) | Leu(360) | Asp(533) | (Cys)N.D. |
| 15 | Glu(162) | Leu(308) | Asp(527) | Glu(507) | |
| 16 | Leu(129) | Tyr(323) | Thr(187) | Leu(629) | |
| 17 | Glu(80) | Leu(330) | Arg(347) | Ala(668) | |
| 18 | Asn(107) | Val(331) | Arg(439) | Lys(697) | |
| 19 | Tyr(94) | Cys(231) | Ala(342) | Phe(523) | |
| 20 | Cys(46) | Gly(283) | Gln(423) | Tyr(776) | |
| 21 | Asn(39) | Asp(299) | Asp(434) | Thr(244) | |
| 22 | | Arg(254) | Phe(184) | Glu(288) | |
| 23 | | Gly(245) | Val(245) | Leu(415) | |
| 24 | | Phe(200) | Gln(270) | Gln(395) | |
| 25 | | Tyr(154) | Trp(17) | Gln(449) | |
| 26 | | Tyr(259) | Leu(158) | Tyr(499) | |
| 27 | | Ser(23) | Met(86) | Leu(375) | |
| 28 | | Pro(96) | Asn(71) | Asn(281) | |
| 29 | | Arg(61) | Thr(13) | Ser(49) | |
| 30 | | Ser(15) | | Ile(370) | |
| 31 | | | | Asn(211) | |
| 32 | | | | Arg(288) | |
| 33 | | | | Pro(157) | |
| 34 | | | | Arg(221) | |
| 35 | | | | Phe(17) | |

The figures in parentheses show yields of PTH-amino acids (pmol). N.D. not detected.

been retained in python insulin with the exception of the conservative substitution Phe→Tyr at position B25. Similarly, those residues in human insulin involved in dimer formation (B12, B16, B20, B24, B26 and B28) [25] and those residues involved in hexamer formation (B6, B10, B14, B17, B18, A13, A14) [25] are fully conserved in python insulin. It is not surprising, therefore, that affinity of python insulin for the human insulin receptor is not appreciable different from that of human insulin. Python insulin was slightly less potent (1.5-fold) than human insulin for inhibiting the binding of radiolabelled human insulin to the secreted extracellular domain of the recombinant human insulin receptor but slightly more potent (1.8-fold) than human insulin in inhibiting binding to the soluble full-length receptor. This result contrasts with the observation that insulin from the salamander *Amphiuma tridactylum* is appreciably (approximately 5-fold) more potent than porcine insulin in the same system [24]. Amphiuma insulin contains a histidine residue at position A8, compared with a glutamic acid residue in python insulin (Fig. 4), and it is believed that this residue, by forming stabilizing structural motifs in the insulin molecule that are of critical importance for receptor recognition, is responsible for the observed increase in binding affinity [26]. In particular, the helical regions at N-terminus of A-Chain and in the central region of the B-chain are believed to be stabilized. The primary structure of insulin has not been particularly well conserved among the reptiles (Fig. 4). Python insulin contains five amino acid substitutions compared with the more highly derived rattlesnake [15] and colubrid snake [16], seven substitutions compared with a chelonian, the red-eared turtle [14] and nine substitution compared with a crocodilian, the American alligator [13]. However, the unusual substitution (Phe→Tyr) at position B25 and a Glu residue at position A8 are found in all three snake insulins.

Pancreatic polypeptide is a member of family of structurally-related regulatory peptides that, in tetrapods, comprise three members: PP localized to endocrine cells primarily in the pancreatic islets but also occurring in the intestine, neuropeptide tyrosine (NPY) synthesized in neurons of the central and peripheral nervous systems and

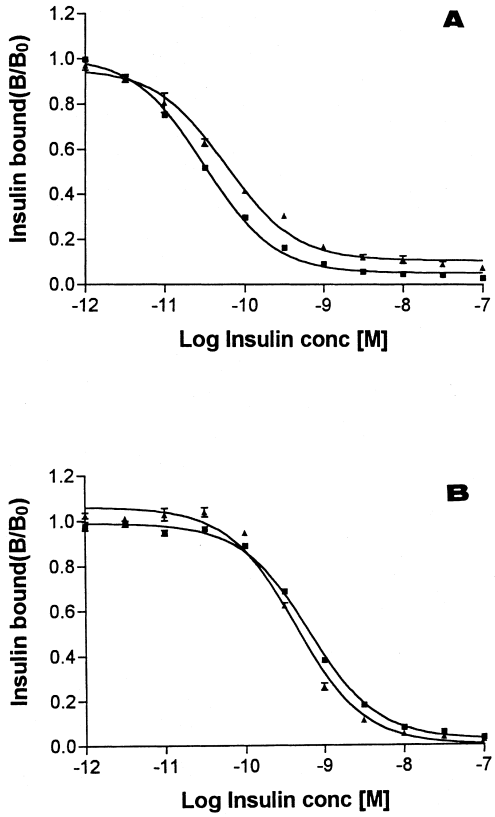


Fig. 3. Comparison of the abilities of python and human insulins to inhibit the binding of [$^{125}$I-Tyr-A14] human insulin to (A) the full-length and (B) the secreted extracellular domain of the recombinant human insulin receptor. Data are presented as the $B/B_0$ ratio where B is $^{125}$I-labelled insulin specifically bound at the indicated insulin concentration and $B_0$ is the binding in the absence of added insulin. Each point represents mean of three independent experiments with python (■—■) and human (▲—▲) insulins.

A-Chain

| | |
|---|---|
| Python | GIVEQ CCENT CSLYE LENYC N |
| Human | ----- --TSI ----Q ----- - |
| Rattlesnake | ----- ----- ----Q ----- - |
| Colubrid snake | ----- ----- ----- ----- - |
| Turtle | ----- --H-- ----Q ----- - |
| Alligator | ----- --H-- ----Q ----- - |

B-Chain

| | |
|---|---|
| Python | APNQH LCGSH LVEAL YLVCG DRGFY YSPRS |
| Human | FV--- ----- ----- ----- E---F -T-KT |
| Rattlesnake | ----R ----- ----- F-I-- E---- ----- |
| Colubrid snake | ----R ----- ----- F-I-- E---- ----T |
| Turtle | -A--- ----- ----- ----- E---F ---KA |
| Alligator | -A--R ----- --D-- ----- E---F ---KG |

Fig. 4. Comparison of the primary structures of python insulin with the corresponding peptides from the human and from other reptiles. (-) denotes residue identity.

in chromaffin cells of the adrenal medulla and peptide tyrosine (PYY) localized primarily to endocrine cells in the lower intestinal tract [17,27]. The structural similarity between the peptides has led to the suggestion that the family has arisen from successive duplications of an ancestral gene that have taken place before, or concomitant with the appearance of the amphibia [17,27]. Although the physiological role of PP is unclear, interest in the peptide has been stimulated by the recent identification and cloning of a novel human receptor with a strong selectivity for the ligand [28].

As shown in Fig. 5, the amino acid sequence of PP has been poorly conserved during evolution (eighteen amino acid substitutions between PP from the python and the alligator and eleven substitutions between PPs from two species of amphibia: the bullfrog *Rana catesbeiana* [29] and the amphiuma [30]). This structural diversity has suggested to Larhammar [17] that the PP molecule was in an a state of rapid evolutionary flux during the time when the amphibian and reptilian species diverged from each other. The structure of python PP is particularly unusual in view of the fact that it contains 35 amino acid residues instead of the 36 residues found in all other PP molecules yet described. The strongly conserved residues $Pro^2$ and $Asp^{10}$ are replaced by Arg and Lys respectively but other strongly conserved residues such as $Pro^5$, $Pro^8$, $Gly^9$, $Ala^{12}$,$Leu^{24}$, $Tyr^{27}$, $Arg^{33}$ and $Arg^{35}$ are retained. Python PP, in common with PP from the alligator [13] and from the bullfrog [29] and the European common frog *Rana temporaria* [31], contains a C-terminal $\alpha$-amidated Phe residue rather than the Tyr residue that is present in mammalian PPs.

The amino acid sequence of preproPP may be predicted from the nucleotide sequence of cloned cDNAs for several mammalian species including the human [32]. The signal peptide sequence in the precursor immediately precedes the PP sequence and so the isolation in high yield of a 35-amino-acid residue PP from python pancreas suggest that a mutation in the signal peptide region of the preproPP has necessitated an alternative site of cleavage by the signal peptidase. This hypothesis was proposed to account for the fact that chicken PYY contains 37 amino acid residues [33]. An anomalous site of cleavage of the signal peptide in python preproPP seems a more plausible explanation for the isolation of a truncated PP than the alternative hypothesis that PP has been cleaved by an aminopeptidase during tissue collection and storage because no metabolites of the other islet hormones were identified in this study.

The primary structure of somatostatin-14 has been very strongly conserved during evolution (reviewed in [34]) whereas the structure of glucagon is strongly conserved

Glucagon

| | |
|---|---|
| Python | HSQGT FTSDY SKYLD TRRAQ DFVQW LMNT |
| Human | ----- ----- ----- S---- ----- ---- |
| Chicken | ----- ----- ----- S---- ----- --S- |
| Turtle/Duck | ----- ----- ----- ----- ----- --S- |
| Bullfrog | ----- ----- ----- S---- ----- ---S |

Pancreatic Polypeptide

| | |
|---|---|
| Python | RIAP VFPGK DASVD ELAKF YTELQ QYLNS INRPR F |
| Human | APLE- -Y--D N-TPE QM-Q- AAD-R R-I-M LT--- Y |
| Chicken | GPSQ- TY--D --P-E D-IRF -ND-- ----V VT-H- Y |
| Alligator | TPLQ- KY--D --P-E D-IQF -ND-- ----V VT--- - |
| Bullfrog | APSE- HH--D Q-TP- Q--Q- -SD-Y --ITF -T--- - |
| Amphiuma | APKE- EH--D --SPE Q-EK- -QD-F --IIF -T--- Y |

Fig. 5. Comparison of the primary structures of python glucagon and pancreatic polypeptide with the corresponding peptides from representatives of other vertebrate taxa. (-) denotes residue identity.

only among tetrapods (reviewed in [30]). Consistent with these general trends, python somatostatin is identical in structure to somatostatin-14 isolated from all other species of mammals, birds and reptiles to date. The only molecular variant of somatostatin to be identified in a tetrapod is [$Pro^{2}$,$Met^{13}$]somatostatin-14 isolated from an extract of the brain of the frog *Rana ridibunda* [35]. Python glucagon contains only a single amino acid substitution ($Ser^{16}\rightarrow Thr$) compared with human glucagon that is shared with the identical glucagon from the turtle [14] and the duck [36]. In common with bullfrog glucagon [29], but in contrast to the avian glucagons, the python peptide contains an Asn residue at position 28. The sequence of alligator glucagon has not been determined but its amino acid composition suggests that it is identical to duck/turtle glucagon [13].

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