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Identification of a peptide arising from the specific post-translation processing of secretogranin II

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The biological role of secrotogranin II is unknown but it has been suggested that the protein may function as a precursor of one or more biologically active neuroendocrine peptides. We have isolated a 33 amino acid-residue peptide from the brain of the frog *Rana ridibunda* that shows strong (82%) homology with human presecretogranin II-(182–204)-peptide. This region of secretogranin II has also been very strongly conserved in the rat and bovine proteins. Analysis of the nucleotide sequence of the mammalian secretogranin II cDNAs indicates that the peptide sequence is flanked by two Lys-Arg dibasic residue processing sites. It is proposed, therefore, that this fragment represents a specific product of the post-translational processing of secretogranin II and, by analogy with peptides derived from chromogranin A, may be important in the regulation of neurosecretion.

Secretogranin II; Chromogranin; Frog brain; Post-translational processing

1. INTRODUCTION

Secretogranin II (previously termed chromogranin C) is an acidic protein containing sulphated tyrosine residues that was identified independently in the anterior pituitary [1] and in chromaffin granules of the adrenal medulla [2] and subsequently shown to be present in most tissues of the neuroendocrine system (reviewed in [3]). The complete primary structure of secretogranin II may be deduced from the nucleotide sequence of cloned DNA complementary to human [4], rat [5] and bovine [3] secretogranin II mRNAs. In secretory vesicles, secretogranin II is proteolytically cleaved in a tissue specific manner to a range of fragments of intermediate size [6]. In chromaffin granules, for example, 50% of the protein is processed to as yet uncharacterized peptides. The biological function of secretogranin II is unknown and it has been speculated that its action may be mediated through such peptide fragments [3,4]. Neuroendocrine peptides are generally generated from precursor proteins by proteolytic cleavage at the site of pairs (or groups) of basic amino acids [7]. The sequence of secretogranin II contains nine such dibasic pairs (Lys-Arg and Arg-Lys), conserved across species, that represent potential recognition sites for a processing enzyme. In a recent study, we have purified an α-MSH release-inhibiting factor (melanostatin) from an extract of frog brain [8]. A systematic search of side-fractions from this purification for other potential neuroendocrine peptides has led to the identification of a fragment of secretogranin II that has arisen by proteolytic cleavage at dibasic residue processing sites.

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2. MATERIALS AND METHODS

2.1. *Tissue extraction*

The extraction of whole brain (94.5 g) from 1200 specimens of adult *Rana ridibunda* has been described previously [8]. Peptides were isolated from the extract using Sep-Pak C18 cartridges (Waters Associates). Bound material was recovered by elution with 70% (v/v) acetonitrile/water and freeze-dried.

2.2. *Purification of the peptide*

The brain extract was redissolved in 1% (v/v) trifluoroacetic acid (10 ml) and chromatographed on a (100×2.5 cm) column of Sephacryl S-100 (Pharmacia) equilibrated with 1 M acetic acid at a flow rate of 120 ml/h. Fractions (10 ml) were collected and absorbance was monitored at 280 nm. Fractions with K_{av} between 0.24 and 0.39, containing melanostatin activity, were pooled and 50% of the total volume was pumped at a flow rate of 2 ml/min onto a (250×10 mm) Vydac 218TP54 C18 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 raised to 49% over 60 min using a linear gradient. Absorbance was monitored at 214 nm and 280 nm, and peaks were collected manually without using a fraction collector. The peak denoted by the arrow (Fig. 1) was re-chromatographed on a (250×10 mm) Ultrapore RPSC C3 column (Beckman) 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% (v/v) over 45 min using a linear gradient.

2.3. *Characterization of the peptide*

The amino acid composition of the peptide was determined by pre-column derivatization with phenylisothiocyanate and its primary structure was determined by automated Edman degradation using procedures previously described [8].

3. RESULTS

3.1. *Purification of the secretogranin fragment*

The elution profile on a semi-preparative Vydac C18 column of the extract of frog brain, after partial purification by gel permeation chromatography, is shown in Fig. 1. The prominent peak denoted by M contained melanostatin [8]. All the major peaks in the chromatogram were re-chromatographed on a semi-preparative Ultrapore C3 column. The elution profile of the fraction containing the peak denoted by the arrow is shown in Fig. 2. The peak denoted by the bar contained the secretogranin II fragment. The peptide was eluted from an analytical Vydac C4 column as a sharp and symmetrical peak (chromatogram not shown) and the yield of pure peptide was approximately 4 nmol.

3.2. *Structural characterization*

Amino acid analysis of the secretogranin fragment indicated a total of 33 amino acids excluding possible tryptophan and cysteine residues. The following composition was obtained: Asx 3.0, Glx 8.4, Ser 2.0, Gly 3.2, Thr 3.0, Ala 2.1, Pro 1.1, Tyr 0.9, Val 1.6, Ile 0.8, Leu 3.8, Phe 1.0, Lys 2.0 (residues/mol peptide). The primary structure of the peptide was determined by automated Edman degradation. Unambiguous assignment of phenylthiohydantoin amino acid derivatives was possible for 33 cycles of operation of the sequenator and the proposed amino acid sequence of the peptide is shown in Table I. 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 peptide was identified as a fragment of secretogranin II (corresponding to residues (182–204) of human presecretogranin II) using the FASTA Protein Sequence Data Base [9].

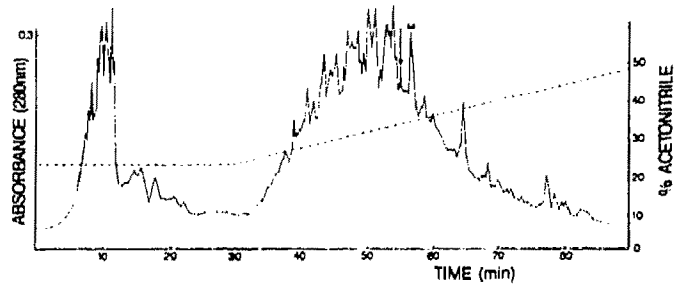

Fig. 1. Reversed-phase HPLC on a semi-preparative Vydac C-18 column of an extract of frog brain after partial purification by gel permeation chromatography. The arrow shows the peak containing the secretogranin II fragment and the peak containing melanostatin is denoted by M. (- - -) shows the concentration of acetonitrile in the eluting solvent.

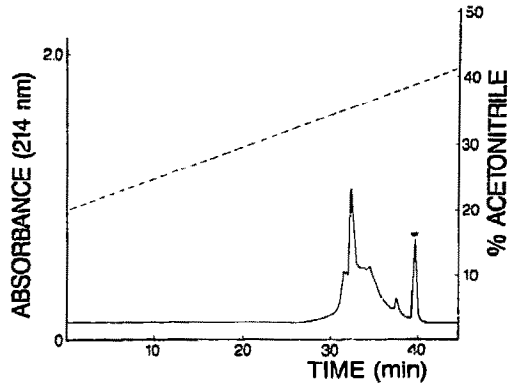

Fig. 2. Purification to near homogeneity of a fragment of frog secretogranin II on a semi-preparative Ultrapore C-3 column. The peak containing the fragment is shown by the bar.

4. DISCUSSION

This study has shown that secretogranin II is present in neuroendocrine tissue of amphibia as well as mammals and that the protein is proteolytically processed to a smaller fragment. Table I shows a comparison of the amino acid sequence of the frog secretogranin II fragment with the sequences of corresponding regions of human, rat and bovine secretogranins (predicted from the nucleotide sequences of cDNAs) [3–5]. Evolutionary pressure to conserve this region of the protein has been very strong, consistent with a possible regulatory role for the peptide. In the mammalian secretogranins, the peptide fragment sequence is flanked by pairs of basic amino acids and conformational analysis indicates that these processing sites, as is the case with many prohormones, are located adjacent to regions of the protein with a high probability of β-turn formation [10].

Chromogranin A, like secretogranin II, contains 9 dibasic residue potential processing sites and post-translational processing can generate biologically active peptides. Pancreastatin, a peptide first isolated from porcine pancreas that inhibits insulin release in rats [11] was subsequently shown to represent a fragment of chromogranin A [12]. More recently a second fragment of chromogranin A, chromostatin, was found to inhibit catecholamine secretion from adrenal chromaffin cells [13]. The post-translational processing of chromogranin B has been studied less extensively but a fragment of unknown function, comprising chromogranin B-(420–493)-peptide, has been shown to have a widespread distribution in the central nervous system of mammals [14]. Further studies are clearly warranted to determine whether the secretogranin II fragment isolated in this study has effects on neuroendocrine secretion.

Table I

A comparison of the amino acid sequence of a fragment of frog secretogranin II with the predicted sequences of the corresponding region of human, bovine and rat secretogranin II

Frog		TNEIV	EGQYT	PQSLA	TLQSV	FQELG	KLKGQ	ANN
Human	[KR]	-----	-E---	-----	--E--	-----	--T-P	N-Q[KR]
Rat	[KR]	-----	-E---	-----	--E--	-----	--T-P	S-Q[KR]
Bovine	[KR]	-----	-E---	--N--	--E--	-----	--T-P	NSQ[KR]

(-) denotes residue identity. The boxed residues are putative sites of post-translational processing of secretogranin II.

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