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Isolation and primary structure of a novel chromogranin A-derived peptide, WE-14, from a human midgut carcinoid tumour

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The primary structure of a novel human chromogranin A-derived tetradecapeptide, WE-14, possessing N-terminal tryptophanyl (W) and C-terminal glutamyl (E) residues was isolated from a hepatic metastasis of an human ileal carcinoid tumour. Human and bovine WE-14 are structurally identical, while rat, mouse and porcine analogues exhibit 93% homology. WE-14 is flanked by paired basic residues (KR) in all known chromogranin A sequences.

Chromogranin A; WE-14; Sequence analysis; Human

1. INTRODUCTION

The determination of the cDNA sequences of chromogranin A from several species of mammal, revealed the presence of seven conserved pairs of basic amino acids which represent potential proteolytic processing sites [1–5]. These structural characteristics would suggest that chromogranin A may be a precursor of potentially-bioactive peptides. This hypothesis was supported by the discovery of porcine pancreastatin [6], which exhibited a high degree of structural homology with a region of bovine chromogranin A [1]. In addition, there is increasing evidence that chromogranin A undergoes tissue-specific proteolytic processing [7,8] and that chromogranin A-derived peptides exhibit biological activity [9–12]. A comparison of the five known mammalian chromogranin A primary structures revealed that a highly-conserved tetradecapeptide, WE-14 (N-terminal tryptophanyl (W) and a C-terminal glutamyl (E) residue), is flanked by putative paired basic residue (KR) processing sites (Fig. 1). In this study, we report the isolation and primary structure of WE-14 from a hepatic metastasis of a human ileal carcinoid tumour.

2. MATERIALS AND METHODS

2.1. *Radioimmunoassay*

Antisera were raised to the conserved C-terminal hexapeptide of WE-14, designated KELTAE [8], incorporating an N-terminal tyrosyl residue to facilitate radioiodination. Radioiodinated tracer was prepared using Iodogen and the product was resolved by reverse-phase HPLC. One rabbit (R635) produced a sensitive antiserum which was employed in radioimmunoassay at a final dilution of 1:12,000. The assay was carried out in a 40 mmol/l sodium acetate buffer, pH 6.0, containing 0.2% (w/v) bovine serum albumin, in a total volume of 300 μl comprising 100 μl antiserum (1/4000), 100 μl tracer (100 Bq; 2 pg) and 100 μl of synthetic peptide standard (1–250 pg/assay tube) or sample. After addition of all reactants, assays were incubated for 24 h at 4°C. Bound and free peptides were separated by dextran-coated charcoal and centrifugation. Pellets were counted on a Nuclear Enterprises NE 1600 gamma counter. The cross-reactivity of the antiserum was assessed with a wide range of gastro-entero-pancreatic peptide hormones. No cross-reactivity was detected.

2.2. *Peptide isolation*

The hepatic metastasis was obtained at laparotomy from a 60-year-old male with a primary ileal carcinoid tumour. Tissue was snap-frozen in liquid nitrogen and stored at −80°C prior to extraction. Tumour tissue (9 g) was homogenised in the extraction medium (ethanol/0.7 mol/l HCl; 3:1 (v/v); 8 ml/g tissue), stirred overnight at 4°C and centrifuged to remove tissue debris. Ethanol was removed from the supernatant under reduced pressure. Peptides in the remaining acidic solution were concentrated using Sep-Pak C-18 cartridges (Waters Associates, Milford, MA, USA). Bound peptides were eluted with acetonitrile and the eluant was lyophilised. The lyophylisate was reconstituted in 2 ml of 2 M acetic acid prior to gel permeation chromatography (Sephadex G-50 fine, 90 × 1.6 cm column, flow rate 10.4 ml/h, mobile phase 2 M acetic acid, fraction size 3 ml). Fractions were subjected to KELTAE radioimmunoassay and peak-immunoreactive fractions were purified to homogeneity employing the following sequence of reverse phase HPLC columns: (1) Partisil 10 ODS-3, C-18, 1 × 60 cm (Whatman, Kent, UK); flow rate 3 ml/min; (2) Supelcosil LC-308, C-8, 0.46 × 25 cm (Supelco Inc., Bellfonte, PA, USA), flow rate 1.5 ml/min. The gradients employed were generated using trifluoroacetic acid (TFA)/water and TFA/water/acetonitrile as indicated in Fig. 3a and b. The purification of the KELTAE-immunoreactive peptide was monitored by radioimmunoassay and spectrophotometrically at 214 nm and 380 nm (Figs. 2 and 3).

2.3. *Structural analyses*

Spectrophotometrically-homogenous peptide was dissolved in 0.1% (v/v) aqueous TFA and 1.5 nmol was applied to a nitrocellulose-

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WE-14			
Human	322 KR	WSKMDQLAKELTAE KR	339
Bovine	314 --	---------- --	331
Rat	341 --	--R------- --	358
Mouse	338 --	--R------- --	355
Porcine	311 --	-----R---- --	327

Fig. 1. The primary structure of human WE-14, which is flanked by paired basic amino acid (KR) residues, and the corresponding WE-14 sequences deduced from cDNA analysis of bovine [2], rat [3], mouse [4] and porcine [5] chromogranin A.

covered target which was spin-dried and micro-rinsed prior to ^{252}Cf-plasma desorption mass spectroscopy (Biolon 20K time-of-flight instrument). Spectra were recorded at 16 kV for 10^6 primary fission events. The remaining peptide was subjected to automated Edman degradation (Applied Biosystem 470A gas phase sequencer).

3. RESULTS

3.1. *Peptide isolation*

KELTAE immunoreactivity was abundant in the crude-tissue extract (74 μg/g) and a single immunoreactive peptide was resolved by gel permeation chromatography (Fig. 2). Sequential reverse phase HPLC of the single peak immunoreactive gel permeation fraction resolved a single spectrophotometrically-homogeneous peptide (Fig. 3a and b). Approximately 10 nmol of immunoreactive peptide was purified to apparent homogeneity.

3.2. *Structural analyses*

Plasma desorption mass spectroscopy detected a single ion with a calculated molecular mass of 1649.5 Da, which compares favourably with the expected molecular mass of 1649.9 Da. Automated Edman degradation established unequivocally the sequence of residues 2 to 14. A non-identified PTH amino acid was observed in cycle 1 which might represent an oxidized tryptophan residue or another tryptophan derivative generated in the sequencer (Table I). Spectrophotometric monitoring of the peptide during isolation indicated a 280 nm absorbance consistent with the presence of a tryptophan residue. The molecular mass is consistent with this assignation as is the primary structure of WE-14 previously established by human chromogranin A cDNA analysis.

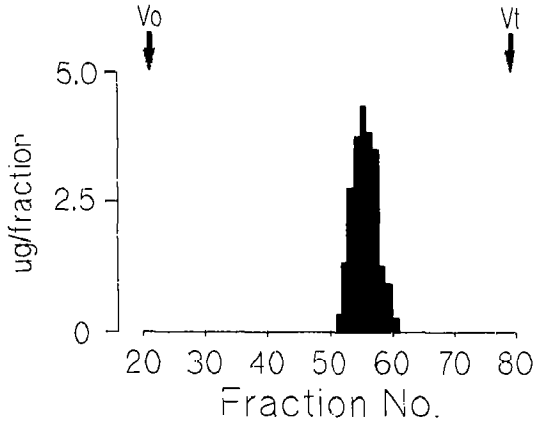

Fig. 2. Gel permeation chromatogram of WE-14 immunoreactivity in extracts of the hepatic metastasis of the human ileal carcinoid. The column was calibrated with Blue dextran (Vo) and potassium dichromate (Vt).

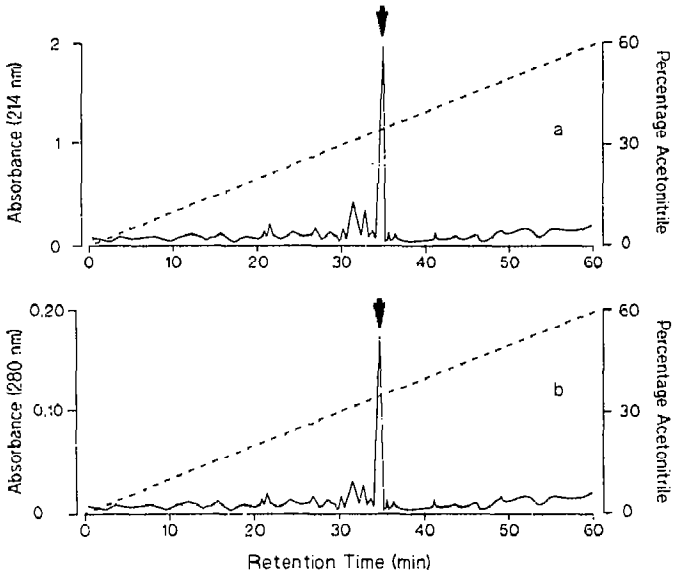

Fig. 3. Final reverse phase HPLC (Supelcosil LC-308) profile of WE-14 immunoreactivity (arrow) monitored at (a) 214 nm and (b) 280 nm.

Table I

Amino acid sequence of human WE-14

Cycle No	PTH amino acid	Yield (pmol)
1	(Trp)*	–
2	Ser	387
3	Lys	829
4	Met	1352
5	Asp	588
6	Gln	938
7	Leu	878
8	Ala	913
9	Lys	524
10	Glu	475
11	Leu	654
12	Thr	279
13	Ala	435
14	Glu	112

*A non-identified PTH amino acid was seen in cycle No. 1, which may represent an oxidized tryptophan or another tryptophan-derivative generated in the sequencer

4. DISCUSSION

Sequence analysis of peptides derived from in vivo [7] and in vitro [13] processing of bovine chromogranin A have demonstrated that cleavage occurred at the pairs of basic residues, 314–315 and 330–331, flanking bovine WE-14, further supporting the proteolytic cleavage of human WE-14. The human and bovine peptides are homologous. Human, rat, mouse and porcine WE-14 exhibit 93% homology. The rat and mouse peptides are identical and differ from the human by a single amino acid substitution (Lys for Arg at residue 3), while similarly, the human and porcine peptides differ by a single substitution (Gln for Arg at residue 6). Unlike other chromogranin A-derived peptides, such as pancreastatin [7,14,15] and chromostatin [12], WE-14 is flanked, both N-terminally and C-terminally by typical processing sites and exhibits a high degree of inter-specific conservation of primary structure. Such factors would suggest that this peptide has a potential physiological role in mammals.

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