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ISOLATION AND CHARACTERIZATION OF δ-MELANOTROPIN, A NEW PEPTIDE FROM BOVINE PITUITARY GLANDS

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A new melanotropin (MSH) was isolated from bovine pituitary extract by means of gel filtration, ion exchange chromatography, high performance liquid chromatography and paper electrophoresis. Amino terminal analysis, amino acid composition and tryptic hydrolysis were performed on the purified peptide. The peptide was found to contain the amino acid sequence of γ-MSH, a theoretical segment of the proopiomelanocortin molecule. However, the new peptide differs from the γ-MSH in several major ways, thus it is designated a bovine δ-MSH or δ_b-MSH.

The precursor molecule proopiomelanocortin (POMC) from which corticotropin and β-lipotropin are systematically cleaved has been identified by cDNA sequencing as a protein with 265 amino acid residues (1). The NH_2-terminal portion of this molecule contains the tetrapeptide sequence His-Phe-Arg-Trp which occurs in the primary structure of α- and β-MSH. Thus, Nakanishi et al. (1) hypothesized that POMC may contain a third melanotropin and named it γ-MSH with 12 amino acid residues (see Fig. 3). In this report, we describe the isolation and characterization of a 28 residue peptide from bovine pituitary that contains the His-Phe-Arg-Trp sequence. The new peptide is apparently derived from the NH_2-terminal portion of the POMC molecule.

MATERIALS AND METHODS

Acid acetone powder from 300 g bovine pituitaries was prepared as described previously (2). The powder (9 g) was

Abbreviations: MSH, melanotropin, melanocyte-stimulating hormone; HPLC, high performance liquid chromatography; POMC, proopiomelanocortin; CMC, carboxymethylcellulose

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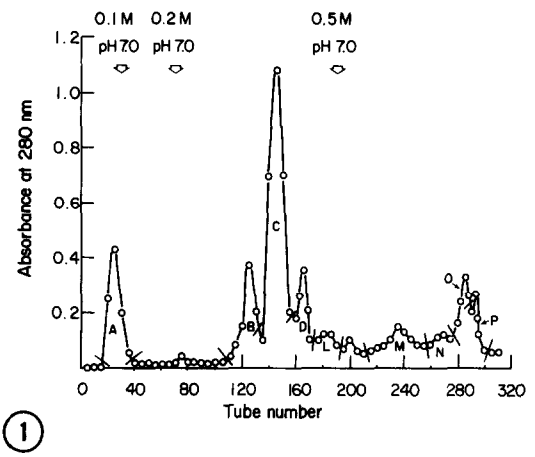
dissolved in distilled water (900 ml) and adjusted to pH 3.0; NaCl was added to a saturation of 0.06. The resulting precipitate was removed and the supernatant lyophilized and then desalted through a Sephadex G-10 column (3.5 x 30 cm, 0.1 M acetic acid). The V_e/V_o = 1 fraction (designated fraction D) was lyophilized, redissolved in 0.01 M NH_4OAc and fractionated by ion exchange chromatography in a CMC (Whatman CM 52) column as previously described (3). Fractions were pooled and lyophilized. Fraction O (Fig. 1) from the CMC column was further purified by HPLC using an Alltech Vydac C-6000 (C_{18}, 300 Å porosity) column with a 10→50% linear gradient of propanol-2 in 0.1% trifluoroacetic acid at a flow rate of 0.5 ml/min. The absorbance at 210 nm was recorded in a Heathkit Model SR-255-B (Benton Harbor, MI) recorder. The content of the major peak at 22 min as seen by HPLC (see Fig. 2) was recovered and analyzed by electrophoresis on Whatman 3 MM paper in collidine/acetic acid buffer (pH 7.0) at 400 V. The positions of the resulting bands and the presence of tryptophan were determined by staining a guide strip with ninhydrin and the Ehrlich reagent. The Erhlich positive peptide was eluted from the paper with 0.1 M acetic acid and NH_2-terminal analysis performed with the dansyl procedure (4,5). Amino acid composition was analyzed in an automatic amino acid analyzer (Beckman model 119C) according to the method of Spackman (6).

Trypsin (Worthington Biochemical) digestion was performed in 0.2 M NH_4OAc buffer (pH 8.5) at 37° for 4 hr with an enzyme/substrate ratio of 1:50. The tryptic peptides were intially separated by paper electrophoresis at pH 7.0 as described above and finally in pyridine/acetic acid buffer (pH 3.7) at 400 V. The peptides were detected by spraying lightly with ninhydrin and then eluted from paper with 0.1 M acetic acid. Aliquots were then submitted to amino acid and NH_2-terminal residue analyses.

Digestions with carboxypeptidase (Worthington Biochemical) were carried out in pH 8.5 Tris buffer (0.05) for 2 and 8 hr at 37° with an enzyme/substrate ratio of 1:25. The digest was submitted to amino acid analyses.

RESULTS AND DISCUSSION

Acid acetone extraction of 300 g of bovine pituitaries yielded 9 g of crude extract and 914 mg of fraction D. Figure 1 is the chromatogram resulting from ion exchange chromatography of fraction D through a CMC column; each fraction was isolated and examined by HPLC. It was found that fraction O (19.6 mg) contained the desired peptide. Separation of 4 mg of fraction O by HPLC yielded a major peak (0.65 mg) at 22 min as shown in Fig. 2. Paper electrophoresis of the material gave two components, one of which was Ehrlich positive. The amount of the purified tryptophan-containing peptide was 212 µg. The NH_2-terminal residue analysis of the peptide showed that Lys was the



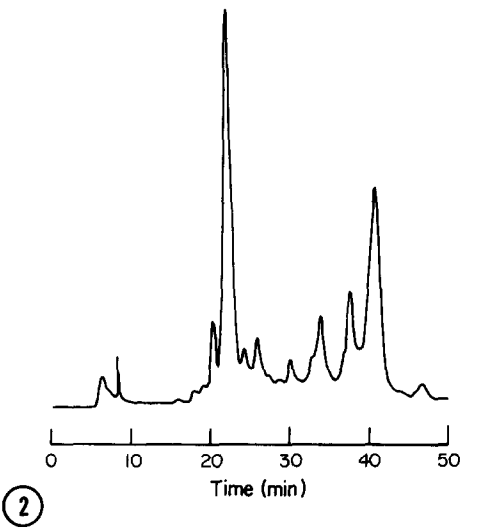


Figure 1: CMC cnromatogram of 705 mg fraction D: Column size, 1.5 x 45 cm; flow rate 12 ml/hr; 4 ml/tube. Elution was performed initially with 0.01 M NH_4OAc buffer of pH 4.6.

Figure 2: Reverse-phase HPLC of 4 mg of fraction O from CMC chromatography (see Fig. 1).

only residue. The COOH-terminal sequence as revealed by carboxypeptidase digest of the peptide was -Ala-Gln-OH. The amino acid composition of the purified peptide is summarized in Table 1. It consists of 28 amino acids and lacks Thr and Pro. In addition, glucosamine was present indicating that the peptide had carbohydrate moiety in its structure. Galactosamine was not detected. The total weight of the purified peptide was 572 µg/300 pituitaries, or approximately 2 µg/pituitary.

The tryptic digest of the peptide separated into 3 major bands after electrophoresis on paper at pH 7.0. The band with the least mobility separated into two bands after electrophoresis on paper at pH 3.7. The amino acid compositions and NH_2-terminal residues of the four tryptic peptides are shown in Table 1. By comparison with the cDNA sequence of the POMC molecule (1) the tryptic peptides are linked as: T2→T1B→T3→T1A. Thus, the amino acid sequence of the purified peptide is proposed as shown in Fig. 3. It may be noted that the proposed sequence has carbo-

Table 1

Amino Acid Composition of the

Purified Peptide and Its Tryptic Fragments

Amino acid	Purified peptide[2] (δ_b-MSH)	Tryptic peptide T1A[2]	T1B	T2	T3
Asp	2.0 (2)	1.4 (1)	1.1 (1)		
Ser	3.4 (4)	3.2 (4)			
Glu	0.9 (1)	1.0 (1)			
Gly	5.5 (6)	4.2 (4)		1.4 (1)	1.0 (1)
Ala	1.5 (2)	1.6 (2)			
Val	1.8 (2)	0.9 (1)		1.0 (1)	
Met	1.0 (1)			0.9 (1)	
Tyr	1.0 (1)			0.9 (1)	
Phe	2.1 (2)			0.9 (1)	0.3 (1)
His	1.0 (1)			0.9 (1)	
Lys	0.9 (1)			0.5 (1)	
Trp[1]	n.d.(1)		n.d.(1)		
Arg	4.3 (4)	0.7 (1)	0.9 (1)	0.9 (1)	1.0 (1)
NH_2-terminal residue	Lys	Arg	Trp	Lys	Phe

[1]Trp was not determined (n.d.) quantitatively but detected by the Ehrlich reagent.

[2]Glucosamine was found to be present in the purified T1A.

hydrate moiety linking to Asn-16 and contains His-Phe-Arg-Trp in residue positions 6-9. Moreover, the theoretical γ-MSH (1) is in residue positions 2-13. The purified peptide may thus be named bovine δ-MSH or δ_b-MSH.

The isolation of a 103-residue glycopeptide with amino acid sequence corresponding to approximately -1 to -105 of the NH_2-terminal portion of the bovine POMC molecule (1) from pig (7) and human (8,9) pituitary glands has been reported. Browne et

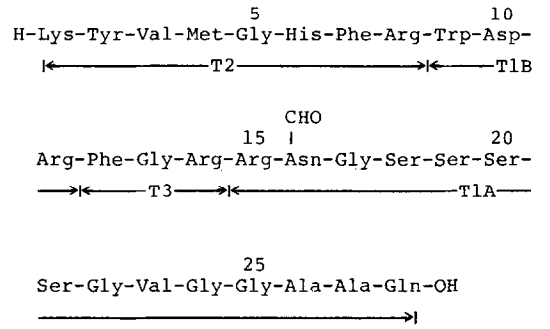


Figure 3: Amino acid sequence of δ_b-MSH

al. (10) described the isolation of a glycopeptide of 25 amino acids with the presence of glucosamine and galactosamine from the neurointermediary lobe of the rat pituitary which contains the γ-MSH sequence. This rat MSH-like peptide of Browne et al. (10) is homologous to the δ_b-MSH structure reported herein with three amino acids deletion in positions 17, 22 and 23 (see Fig. 3) and three amino acid substitutions:

	14	21	26
Bovine:	Arg,	Ser,	Ala
Rat:	Pro,	Ala,	Ser

We may thus designate the Browne MSH-like peptide as the rat δ-MSH or δ_r-MSH.

Preliminary studies on the lipolytic activity of δ_b-MSH in isolated rabbit fat cells showed that it had a minimal activity when compared with equine β-MSH. It is hoped that a sufficient quantity of δ_b-MSH will be available for the investigation of the biological role of the new MSH-like peptide.

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Addendum (7/30/82). After the manuscript has been submitted for publication, a brief report on the isolation of the same MSH peptide came to our attention [F.S. Esch, T. Shibasaki, P. Böhlen, W. B. Wehrenberg and N. C. Ling in Peptides, Synthesis-Structure-Function, D. H. Rich and E. Gross (eds.) 1981 Pierce Chem. Co.].

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