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β-ENDORPHIN: PRIMARY STRUCTURE OF THE HORMONE FROM THE OSTRICH PITUITARY GLAND

Ryno J. Naudé, David Chung and Choh Hao Li

Hormone Research Laboratory
University of California
San Francisco, CA 94143

and

Willem Oelofsen

Department of Biochemistry
University of Port Elizabeth
Port Elizabeth, Republic of South Africa

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SUMMARY

The amino acid sequence of β-endorphin from the ostrich pituitary has been determined. It consists of 31 amino acids with high opiate receptor-binding activity. The proposed sequence is as follows: H-Tyr-Gly-Gly-Phe-Met-Ser-Ser-Glu-Arg-Gly-Arg-Ala-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Val-Lys-Ser-Ala-Tyr-Lys-Lys-Gly-Gln-OH. When compared with the primary structures of other known β-endorphins, it is the first instance that residues in positions 6, 9, 10, 11, 12 and 25 are different.

Among the naturally occurring opioid peptides, only β-endorphin is active when injected intravenously in both experimental animals (1,2) and human subjects (3,4). It was isolated and sequenced from camel pituitary glands (5). Human (6), bovine (7) and ovine (8,9) β-EPs have also been isolated and sequenced. However, β-EPs from porcine (10,11), rat (12) and ostrich (13) pituitary glands have been obtained but their amino acid sequences have not been determined. In this communication, we report the complete amino acid sequence of the

Abbreviations: β-EP, β-endorphin (subscript os for ostrich); CMC, carboxymethylcellulose; HPLC, high performance liquid chromatography

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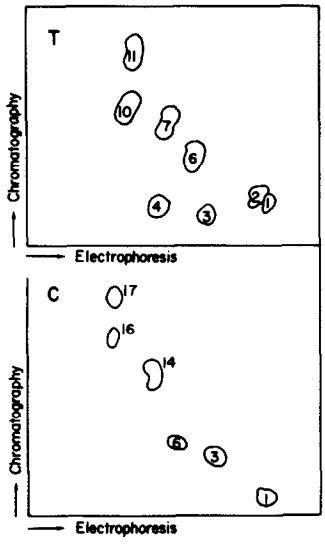

Figure 1. Peptide maps of tryptic (T) and chymotryptic (C) digests of β_{os}-EP

ostrich β-EP. This represents the first report on the primary structure of β-EP from an avian species.

MATERIALS AND METHODS

β-Endorphin from ostrich pituitary glands was isolated by the procedure previously described (13) with minor modifications.

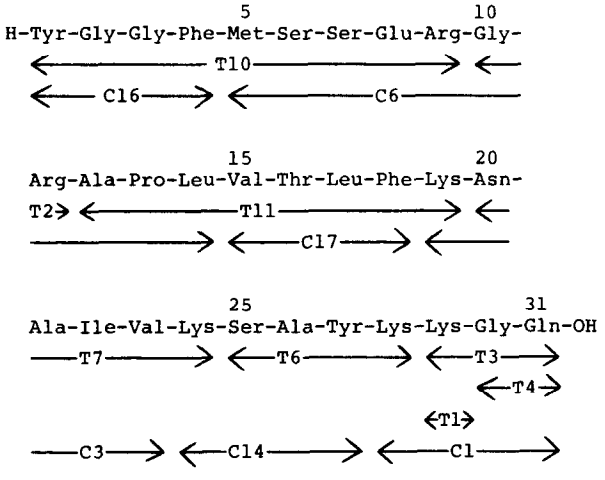

Figure 2. Complete amino acid sequence of β_{os}-EP

Table 1

Amino acid composition (molar ratio)

and NH_2-terminal residues of tryptic peptides[a]

Amino acid	T2	T3	T4	T6	T7	T10	T11
Lys		0.8		0.8	1.1		0.9
Arg	1.1					0.8	
Asx					0.9		
Thr							1.1
Ser				0.7		1.8	
Glx		1.1	1.1			0.9	
Pro							0.9
Gly	0.9	1.1	0.9			2.0	
Ala				1.1	1.2		0.9
Val					0.8		1.0
Met						0.8	
Ile					0.8		
Leu							2.0
Tyr				0.9		1.1	
Phe						1.2	1.0
NH_2-terminal residue	Gly	Lys	Gly	Ser	Asn	Tyr	Ala

[a]See Figure 1; T1, free lysine

After CMC chromatography of fraction D, peak 8 was chromatographed on Sephadex G-25 (fine) in 0.1 M acetic acid. The material in the second peak was submitted to HPLC on a reverse-phase column (Altex; 4.6 x 250 mm) packed with Partisil-10 ODS-2. A 10-25% linear gradient of n-propanol in pyridine acetate buffer (1.0 M pyridine-0.5 M acetic acid, pH 5.5) was used (20 ml-20 ml). Sampling and detection were carried out as described (14).

For structure determination, a tryptic digest of 2.3 mg β_{os}-EP was fractionated by two-dimensional paper chromatography-electrophoresis as described (15). The eluates from each spot were submitted to amino acid analysis on a Beckman 119C analyzer according to Spackman et al. (16); NH_2-terminal and sequence analyses were carried out by the dansyl-Edman procedure as

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                    1                   5                  10
          Human: H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-
Camel, bovine, ovine: H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-
        Porcine: H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-
       Ostrich: H-Tyr-Gly-Gly-Phe-Met-Ser-Ser-Glu-Arg-Gly-

                              15                  20
                Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-
                Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-
                Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-
                Arg-Ala-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-

                              25                      31
                Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu-OH
                Ala-Ile-Ile-Lys-Asn-Ala-His-Lys-Lys-Gly-Gln-OH
                Ala-Ile-Val-Lys-Asn-Ala-His-Lys-Lys-Gly-Gln-OH
                Ala-Ile-Val-Lys-Ser-Ala-Tyr-Lys-Lys-Gly-Gln-OH
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Figure 3. Primary structures of β-EP from ostrich, human, camel, bovine, ovine and porcine pituitary glands

described (17,18). To align the tryptic peptides, 1 mg β_{os}-EP was digested by chymotrypsin and the digest was fractionated by peptide mapping as described for the tryptic digest. The chymotryptic peptides were submitted to amino acid (16) and NH_2-terminal residue analyses (17).

The opiate receptor-binding assay was performed according to the procedure recently described (19,20) using rat brain membrane preparations.

RESULTS AND DISCUSSION

From 780 g adenohypophyses, 5.6 mg β_{os}-EP were obtained. In comparison with synthetic camel β-EP (21), results (IC_{50} in nM) of the assay are as follows: camel, 0.10; ostrich, 0.037. Thus, β_{os}-EP is almost three times more active than the camel hormone.

Figure 1 presents the peptide maps of the tryptic and chymotryptic digest of β_{os}-EP. The amino acid compositions and NH_2-terminal residues of these tryptic and chymotryptic peptides are shown in Tables 1 and 2. In Table 3, the sequence analyses of the tryptic peptides are shown. From the data in Tables 1, 2 and 3, the amino acid sequence of β_{os} is shown in Fig. 2. When the structure of β_{os}-EP is compared with mammalian hormones 22 sequence positions are identical (Fig. 3). It is striking

Table 2

Amino acid composition (molar ratio) and NH_2-terminal residues of chymotryptic peptides

Amino acid	C1	C3	C6	C14	C16	C17	Ostrich β-EP	
							Acid[a]	Enzyme[b]
Lys	1.8	1.3		0.8			4.0	4.0
Arg			1.8				2.0	2.2
Asp		1.0					1.0	
Asn								
Gln								5.8[c]
Thr						1.0	1.0	
Ser			1.7	1.1			3.0	
Glu	1.0		1.1				2.0	1.2
Pro			0.9				0.9	0.9
Gly	1.1		1.3		2.0		4.0	4.0
Ala		0.6	1.2	1.3			3.3	2.7
Val		0.9				1.0	1.6	2.0
Met			0.5				1.0	1.0
Ile		0.8					0.6	1.0
Leu			1.2			1.1	2.0	2.2
Tyr				1.0	0.9		1.8	2.1
Phe					0.9	1.0	1.9	2.1
NH_2-terminal residue	Lys	Lys	Met	Lys	Tyr	Val		

[a] Taken from (13).

[b] Total enzymic digest was carried out as described (22).

[c] Corresponds to sum of Asn + Gln + Thr + Ser.

that residues in positions 6, 9, 10, 11, 12 and 25 of the β_{os}-EP structure are different from other β-EPs. In addition, all known β-EPs do not contain arginine and β_{os}-EP has two arginine residues in positions 9 and 11. The high opiate receptor-binding

Table 3

Sequence analyses on tryptic peptides

Peptides[a]	
T11	Ala-Pro-Leu-Val-Thr-Leu-Phe-Lys
T10[c]	Tyr-Gly-Gly-Phe-Met-Ser-Ser-Glu-Arg
T7[c]	Asn-Ala-Ile-Val-Lys
T6	Ser-Ala-Tyr-Lys
T4[c]	Gly-Gln
T3[c]	Lys-Gly-Gln
T2	Gly-Arg

[a]See Table 1 for amino acid analysis

[b]→, dansyl-Edman procedure

[c]The determination of Asp/Asn and Glu/Gln was deduced from the mobility on paper electrophoresis at pH 7

activity of β_{os}-EP may be due to the presence of these two arginines.

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