

Isolation and Primary Structure of β -Endorphin and β -Lipotropin
from Bovine Pituitary Glands

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SUMMARY: Bovine pituitary β -endorphin has been isolated and its amino acid sequence shown to be identical with that of residues 61-91 in the ovine β -lipotropin structure. In addition, the primary structure of bovine β -lipotropin was found to consist of 91 amino acids, rather than 93 amino acids as previously reported.

β -Endorphin (β -EP), a 31-amino acid peptide with opiate activity, has been isolated from camel (1), porcine (2,3), human (4,5), and sheep (4) pituitary glands. The amino acid sequences of camel (1), human (5), and sheep (6) β -EP have also been determined and are identical to the sequence of the COOH-terminal residues 61-91 of β -lipotropin (β -LPH)(7-9). Herein we describe the isolation and primary structure of β -EP from bovine pituitaries and the amino acid sequence of bovine β -LPH.

Results and Discussion

β -LPH and β -EP were isolated by procedures previously described for ovine (7) and human (5) preparations, respectively, from fresh bovine pituitary glands. From 500 glands, 20 mg β -LPH and 10 mg β -EP were obtained. Amino acid analyses, as determined by the method of Moore (10) revealed the following compositions:

β -LPH: Lys_{9.6}, His_{2.1}, Arg_{4.6}, Asp_{4.5}, Thr_{3.8}, Ser_{4.8}, Glu_{16.7}, Pro_{5.5}, Gly_{7.7}, Ala_{13.1}, Val_{2.3}, Met_{2.1}, Ile_{1.7}, Leu_{5.7}, Tyr_{2.7}, Phe_{2.9}, Trp₍₁₎; β -EP: Lys_{4.7}, His_{0.9}, Asp_{2.1}, Thr_{3.0}, Ser_{2.0}, Glu_{3.2}, Pro_{1.1}, Gly_{3.1}, Ala_{2.3}, Val_{0.9}, Met_{0.9}, Ile_{2.0}, Leu_{2.0}, Tyr_{0.9}, Phe_{1.9}.
The NH₂-terminal sequences of β -LPH and β -EP, determined by dansyl-

Table 1

Amino Acid Composition (Molar Ratio) and NH₂-Terminal Residue
of Tryptic Peptides Isolated from Bovine β -Lipotropin

Peptide Number ^a	Composition	NH ₂ -terminal residue
T1	Lys _{2.2} His _{0.7} Asp _{1.0} Ala _{1.0}	Asx
T4	Lys _{1.0} His _{1.0} Asp _{1.0} Ala _{1.0}	Asx
T5	Lys _{1.1} Asp _{1.0}	Asx
T6,T7	His _{0.9} Arg _{1.0} Glu _{1.1} Met _{0.3} Phe _{0.9}	Met
T8	Gly _{0.9} Glu _{1.0}	Gly
T9	Lys _{1.0} Asp _{1.0} Ser _{0.9} Pro _{0.7} Gly _{1.2} Tyr _{0.9}	Asx
T10	Arg _{1.0} Glu _{2.2} Ala _{1.0} Leu _{1.1}	Leu
T11	Arg _{1.0} Thr _{1.0} Glu _{2.1} Gly _{1.0} Leu _{1.1}	Glx
T12	Trp _{1.0} Lys _{1.0} Ser _{0.7} Pro _{2.2} Gly _{1.0}	Trp
T13	Lys _{1.0} Asp _{1.0} Ala _{1.0} Ile _{1.3}	Asx
T14	Arg _{1.0} Ser _{0.9} Glu _{3.2} Pro _{0.9} Gly _{1.5} Ala _{5.2}	Gly
T15	Lys _{1.0} Thr _{1.0} Ser _{1.1} Glu _{1.3} Gly _{2.1} Met _{1.1} Tyr _{0.5} Phe _{0.9}	Tyr
T16	Lys _{1.0} Thr _{1.8} Ser _{0.8} Glu _{1.3} Pro _{1.2} Val _{0.8} Leu _{2.0} Phe _{1.3}	Ser
T17	Glu _{2.0} Ala _{0.8} Leu _{1.1} Tyr _{0.8}	Ala
TA	Lys _{0.7} Glu _{5.4} Gly _{1.4} Ala _{4.7} Val _{0.8} Leu _{1.7} Tyr _{0.7}	Ala

^a T2, free lysine; T3, free arginine; TA obtained from tryptic digest of CB-1 fragment.

Edman procedure (11,12) are, respectively: H-Glu-Leu-Thr-Gly- and H-Tyr-Gly-Gly-Phe-.

For structure determination, tryptic digests of β -LPH and β -EP were separated by two-dimensional paper chromatography-electrophoresis as reported (13). The eluates from each spot were submitted to amino

Table 2

Amino Acid Composition (Molar Ratio) and NH₂-Terminal Residue
of Tryptic Peptides Isolated from Bovine β -Endorphin

Peptide Number ^a	Composition	NH ₂ -terminal residue
T3	Lys _{0.9} His _{1.1} Asp _{1.0} Ala _{1.0}	Asx
T6	Glu _{1.0} Gly _{0.7}	Gly
T7	Lys _{1.0} Asp _{0.9} Ala _{1.1} Ile _{2.0}	Asx
T8	Lys _{1.0} Thr _{0.9} Ser _{0.9} Glu _{1.2} Gly _{1.8} Met _{1.0} Tyr _{0.8} Phe _{1.0}	Tyr
T9	Lys _{1.0} Thr _{2.0} Ser _{0.9} Glu _{1.2} Pro _{0.9} Val _{1.0} Leu _{2.2} Phe _{1.0}	Ser

^a T2, free lysine.

acid (10) and NH₂-terminal sequence analyses (11,12) and the results summarized in Tables 1 and 2. β -LPH was also reacted with CNBr (14) in 70% formic acid and the reaction mixture separated by paper electrophoresis at pH 6.7. The two main CNBr fragments were analysed for amino acid and NH₂-terminal residue composition: CB1: Lys_{2.7}, Arg_{2.7}, Asp_{1.3}, Thr_{1.1}, Ser_{2.0}, (Glu + Hse)_{13.1}, Pro_{1.9}, Gly_{4.5}, Ala_{11.2}, Val_{1.1}, Leu_{3.9}, Tyr_{1.8}(NH₂-terminal residue, Glu); CB2: Lys_{6.9}, His_{1.8}, Arg_{2.2}, Asp_{3.1}, Thr_{2.8}, Ser_{2.7}, (Glu + Hse)_{4.8}, Pro_{3.0}, Gly_{3.7}, Ala_{2.3}, Val_{1.3}, Met_{0.2}, Ile_{1.2}, Leu_{2.3}, Tyr_{1.2}, Phe_{3.0}(NH₂-terminal residue, Glu).

From these data and the homologous relationship to the primary structures of ovine (7,8) and camel (1) hormones, the amino acid sequences of β -LPH and β -EP are proposed as shown in Figures 1 and 2. It is evident that the primary structure of bovine β -LPH is identical with that of the ovine hormone (7,8). This is expected, as Lohmar and Li (15) have shown that the hormone is indistinguishable from ovine β -LPH on the basis of its amino acid composition, NH₂-terminal analysis, molecular weight, and biological activity. Since camel (1)

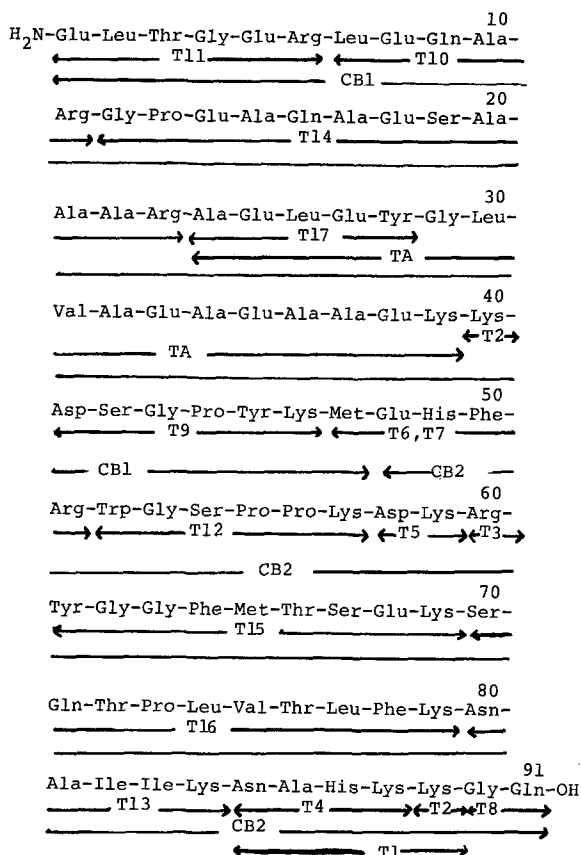


Fig. 1 Proposed amino acid sequence of bovine β -lipotropin.

and human (5) β -EP have primary structures corresponding to ovine β -LPH-(61-91) and human β -LPH-(61-91), respectively, it is to be expected that the amino acid sequence of bovine β -EP is identical to that of bovine β -LPH-(61-91) as shown in Figures 1 and 2.

In a preliminary note, Pankov (16) proposed the complete amino acid sequence of β_b -LPH with 93 amino acid residues. Interestingly, he found (16) an additional dipeptide, Ala-Glu, in positions 36 and 37, and glycine in position 16. Since β -LPHs from ovine (7,8), porcine (17-19), and human (9) pituitary glands consist of 91 amino acids, it is reasonable to assume that the bovine hormone also has 91 amino

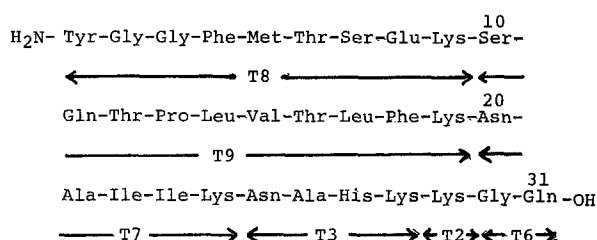


Fig. 2 Proposed amino acid sequence of bovine β -endorphin.

acids. There is no evidence in our work that residue position 16 in bovine β -LPH is glycine.

After Hughes, Kosterlitz *et al.* (20) isolated two opiate peptides, met- and leu-enkephalin, from porcine brains and showed the amino acid sequence of met-enkephalin to be the same as β -LPH-(61-65). Subsequently, Simantov and Snyder (21) obtained these two enkephalins from bovine brains. Various investigators (22, 23) have proposed that the leu-enkephalin is possibly derived from [Leu⁶⁵]- β -LPH. We have attempted in the present work to search for the leucine analog of bovine β -LPH without success*.

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*While this manuscript was in preparation, Crine *et al.* (24) detected the presence of β -EP in bovine pituitary slices in *in vitro* biosynthetic studies using sheep pituitary homogenate as carrier and ³⁵S-methionine.

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