STUDIES ON THE PRIMARY STRUCTURE OF HUMAN β-LIPOTROPIC HORMONE

G. CSEH, E. BARÁT, A. PATTHY and L. GRÁF
Research Institute for Pharmaceutical Chemistry, Budapest, Hungary

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1. Introduction

The isolation and some characteristics of a lipolytic peptide from human pituitaries were described in our previous paper [1]. On the basis of chemical and biological studies this peptide was assumed to be a species homologue of the β-lipotropic hormone (β-LPH) discovered by Li and coworkers [2]. Meanwhile the isolation and complete sequence of porcine β-LPH has been reported from our laboratory [3, 4]. Recently, we have extended our structural studies to the human β-LPH. In this paper we present some results of this work.

2. Materials and methods

3000 Human pituitaries collected in acetone were prepared according to our procedure [1] and 15 mg of β-LPH was obtained. This amount of the hormone has been used for the investigation of its primary structure.

The homogeneity of the preparation was checked by disc electrophoresis [1] and NH_2-terminal end-group analysis [5]. The subsequent 2 residues at the NH_2-terminus were determined by the Edman–dansyl procedure [5]. To analyze the COOH-terminal residues, β-LPH was hydrolyzed by carboxypeptidase A (Fluka) in 0.05 M $NaHCO_3$ with an enzyme:peptide ratio of 1:20 (w/w) at 37° for 1 and 5 hr and samples were taken out for amino acid analysis on a JEOL (JLC-5AH) amino acid analyzer.

Trypsin cleavage was performed in 0.05 M NH_4HCO_3, pH 7.5, with an enzyme:peptide ratio of 1:50 at 37° for 2 hr. The fragments were isolated from the digest by high voltage paper electrophoresis (40–70 V/cm) at pH 6.5, 5.0 and 2.0, respectively. Further purification of some neutral peptides was carried out by paper chromatography (butanol–pyridine–acetic acid–water, 30:20:6:24).

Homogeneous peptides were eluted from the paper and aliquots were subjected to amino acid analysis after acid hydrolysis in 6 N HCl for 45 hr at 110° in sealed, evacuated tubes. The fragments were sequenced by the Edman–dansyl method [5]. The dansyl amino acid residues were identified by the method of Sajgó [6] and Woods and Wang [7], respectively. In one case dansylated synthetic dipeptide Ile–Ile was used as a reference for the identification.

Table 1
Sequence and amino acid composition of some tryptic fragments of human β-lipotropic hormone.

Peptide	Sequence
n_1	Tyr–Gly–Gly–Phe–Met–Thr–Ser–Glu–Lys
n_4	Lys–Asp–Glu–Gly–Pro–Tyr–Arg
n_5	Lys–Gly–Glu
n_6	Asp–Lys
b_1	Ser–Gln–Thr–Pro–Leu–Val–Thr–Leu–Phe–Lys
b_{21}	Try–Gly–Ser–Pro–Pro–Lys
b_{22}	Asn–Ala–Ile–Ile–Lys
b_{23}	Asn–Ala–Tyr–Lys
b_{24}	Met–Glu–His–Phe–Arg
b_{31}	Asp–Lys–Arg
b_{32}	Leu–Arg
a_1	Asp–Glu–Gly–Pro–Tyr–Arg
a_{21}	Glx–Gly–Asx–Gly–/1 His, 3 Asp, 1 Ser, 3 Glu, 2 Pro, 2 Gly, 4 Ala, 1 Val, 2 Leu/–Lys
a_3	Gly–Glu

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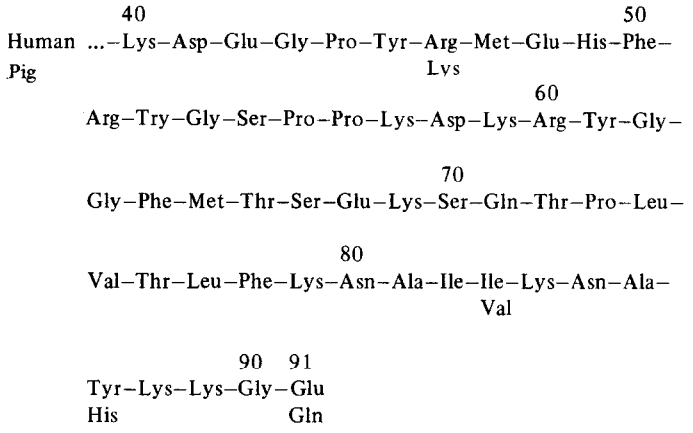

Fig. 1. Comparison of the COOH-terminal sequences of human and porcine β-lipotropic hormones.

3. Results and discussion

The amino acid composition of human β-LPH was found to be slightly different from that described previously [1]: Lys_{10} His_2 Arg_5 Asp_9 Thr_4 Ser_5 Glu_{13} Pro_6 Gly_9 Ala_8 Val_3 Met_2 Ile_2 Leu_7 Tyr_3 Phe_3 Try_1. The Edman–dansyl procedure gave the following result for the NH_2-terminus of the molecule: Glx–Gly–Asx. Incubation with carboxypeptidase A resulted in the release of Glu and Gly in nearly equimolar quantities, indicating that these residues are near the COOH-terminus.

From the tryptic digest 4 neutral (n), 7 basic (b), 3 acidic (a) fragments and free Glu and Lys were isolated in homogeneous form. Except for the peptide a_{21}, the complete sequence of these fragments was established (table 1).

Considering the identity of the NH_2-terminal sequence, peptide a_{21} appears to be the NH_2-terminal tryptic fragment of β-LPH. In the course of its Edman degradation, however, some difficulties arose: The partial resistance of the Asp-3 against the removal by phenylisothiocyanate made further analysis impossible. In the cases of deamidated porcine adrenocorticotropic hormone [8] and bovine prolactin [9] similar phenomena were found as a consequence of β-linkage of an aspartic acid which was formed from asparagine followed by glycine in the sequence. On the basis of the above examples an Asn–Gly sequence is assumed to be present at positions 3–4 of the native human β-LPH.

It is very probable, that each pair of fragments n_4–a_1, b_{31}–n_6, and n_5–a_3, respectively, represents homologues originated by alternative cleavages of the polypeptide chain.

The fragments listed in table 1 were compared with the sequence of the porcine β-LPH [4]. It was found that fragments n_1, b_1, b_{21}, b_{24}, b_{31} are identical with the corresponding fragments of porcine hormone and each of fragments n_4, n_5, b_{22}, b_{23} differs only in one amino acid residue from the homologous fragments of porcine β-LPH. This high degree of homology permits a preliminary ordering of fragments of table 1 on the basis of the known structure of porcine β-LPH (fig. 1). The alignment of the tryptic fragments in an order of n_4–b_{24}–b_{21}–b_{31}–n_1–b_1–b_{22}–b_{23}–n_5 gives a continuous portion, related to the 40–91 COOH-terminal part of the porcine β-LPH.

It is worth to mention, this portion comprises the 4 to 22 sequence of the human β-melanophore stimulating hormone (β-MSH) at its NH_2-terminus (positions 40 to 58). Within this overlap human β-LPH contains Arg at position 60 instead of Lys, corresponding to the species difference between the human and porcine β-MSH's [10, 11]. Further species differences from the porcine β-LPH are assigned to the COOH-terminal region (positions 83, 87 and 91) of the molecule. However, the possibility can not be

excluded, that the COOH-terminal glutamic acid is an artifact owing to acidic deamidation of glutamine [12].

The presence of some additional neutral fragments has also been detected in the tryptic digest of the hormone, but the attempts for their isolation have not been successful. These fragments with the dipeptide Leu–Arg (b_{32}) together may originate from the middle-part of the polypeptide chain. It is evident already at this stage of the structure analysis, from the amino acid composition of the NH_2-terminal tryptic peptide and the "lacking" peptides (derived from the difference between the amino acid composition of the entire β-LPH molecule and that of the studied fragments) that the majority of the species differences is cummulated within the NH_2-terminal region of the human β-LPH, as it was also shown in the relation of the ovine and porcine homologues [4]. It must be emphasized, however, that lipolytic activity of the β-LPH seems to be entirely connected with the COOH-terminal region 40 to 91, as was observed in our laboratory on comparison of lipolytic effect of fragments obtained by different enzymatic cleavages of β-LPH [13].

To complete the sequence analysis of the human β-LPH, further investigations are in progress.

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