PORCINE LUTEINIZING HORMONE. THE AMINO ACID SEQUENCE OF THE β SUBUNIT*

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1. Introduction

At the former International Symposium on Protein and Polypeptide Hormones, Ward et al. [1] presented a paper outlining slight modification in the complete primary structure of ovine luteinizing hormone subunit (O-LHβ) as compared with their original results [2].

Simultaneously [3], we presented our data concerning the structure of the bovine chain (B-LHβ). A single point of discrepancy was apparent [3, 4] in comparing both sequences which was later cleared on re-examination of the ovine structure [5]. In contrast to the complete homology between O-LHβ and B-LHβ, specific differences were expected for the corresponding porcine subunit (P-LHβ) in view of its composition [6].

In this report, the complete primary structure of the reduced and carboxymethylated P-LHβ is presented with comparison to our results concerning the bovine chain (B-LHβ).

2. Experimental

P-LHβ was prepared as described elsewhere [6]. Methods used for amino acid and sugar analyses, reduction and alkylation with iodoacetic of the protein, CNbr cleavage, tryptic and chymotryptic hydrolyses, end group analyses and sequence determinations have also been described [4, 7].

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Where impracticable on a quantitative basis, due to material limitation, the determination of carboxy-terminal residues was qualitatively performed as follows: the peptide (5 nmoles) was dissolved in 100 μl of 0.1 M Tris-HCl buffer, pH 8.0. Digestions were conducted with carboxypeptidases A and B, at 37°, and stopped by freezing at −25°. Carboxypeptidase A, as a crystalline suspension (46.1 mg/ml, 25 U/mg) was diluted 100 times in 10% lithium chloride, and was added in 10 μl samples for each digestion. Carboxypeptidase B, as a solution at 6.1 mg/ml, 105 U/mg, was also diluted 100 times in 10% lithium chloride, and was used in 50 μl samples. The digestion mixture was freeze-dried, the free amino acids being subsequently labelled with dansyl chloride [8].

Dansyl-arginine (DNS-Arg) was identified by electrophoresis (30 min, 40 V/cm) on polyamide layer (Cheng-Chin Trading Co.) in pH 1.9 buffer (acetic acid:formic acid:water; 120:28:1852) with DNS-Arg (Calbiochem, KIT SAA-4) as reference.

Peptide digestions (Pepsin, Worthington 2530 U/mg) were carried out at pH 2.0 in 0.01 M HCL for 4 hr at 37°, with an enzyme to peptide ratio of 1:100 by weight. The reaction was stopped by addition of pyridine until the solution reached pH 5.

Tryptic peptides from the reduced and carboxymethylated protein were first fractionated by gel filtration on Sephadex G-50 (Fine, Pharmacia) (columns 188 × 3.4 cm) using 0.05 M ammonium bicarbonate buffer. Final purification was achieved by preparative paper chromatography (n-butanol:acetic acid:water; 4:1:5, upper phase) and high voltage paper electrophoresis [4].

The fragments from CNbr cleavage of the reduced and carboxymethylated protein were purified on QAE-Sephadex A-25 developed with ammonium acetate buffers at pH 6.0 using a linear gradient from 0.02 M to 0.70 M in acetate.

3. Results and discussion

Fig. 1 gives the complete amino acid sequence of porcine luteinizing hormone β subunit that has been deduced from data on the composition and both NH_2-terminal and COOH-terminal sequences of tryptic peptides (T_i) of the reduced and carboxymethylated protein.

The T_3 peptide was further digested with both pepsin and chymotrypsin, and the T_7 peptide with chymotrypsin alone. This was necessary for elucidation of the complete structure, including the sequence of 4 residues, His Cys, Gly, Pro (fig. 1) not determined for bovine LHβ [4].

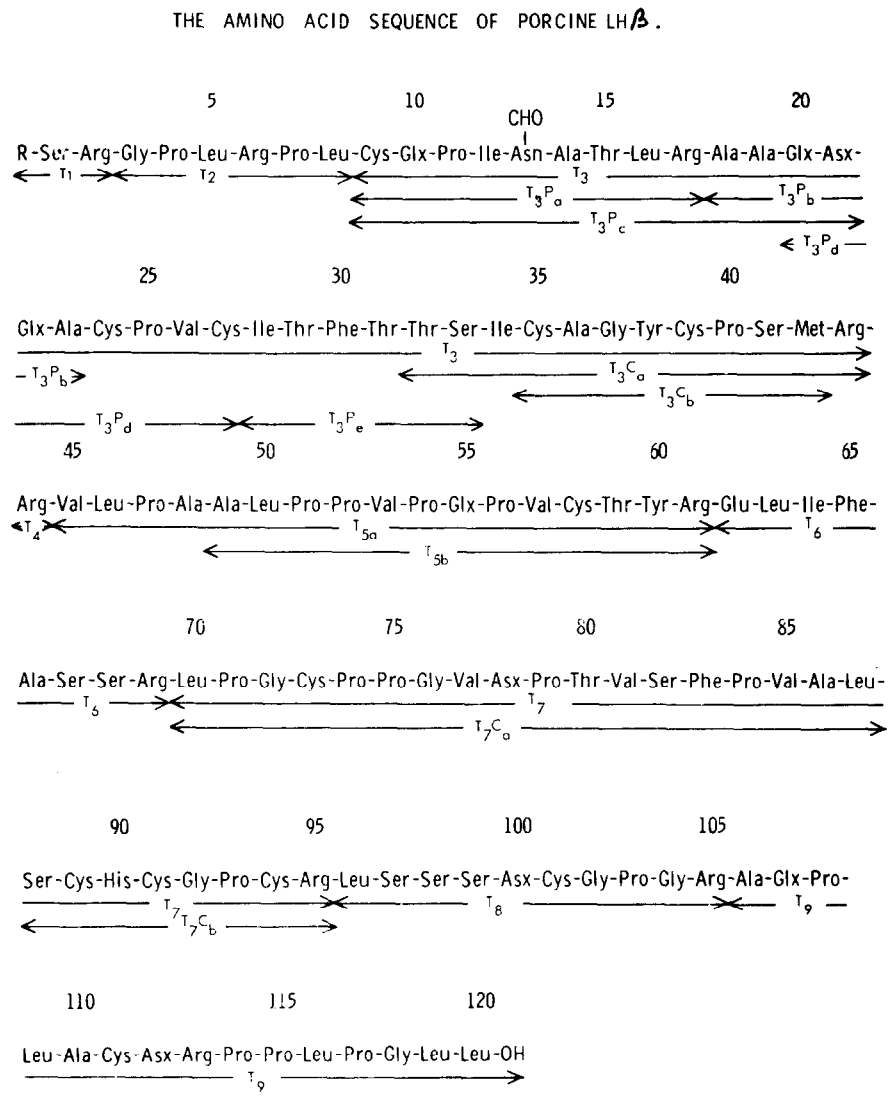

Fig. 1. T_i indicates tryptic peptides; T_iC_j, peptides resulting from chymotrypsin digestion of tryptic (T_i) peptides; T_iP_k, peptides resulting from pepsin digestion of T_i. CHO symbolizes the polysaccharide prosthetic group; R, an unidentified acyl group.

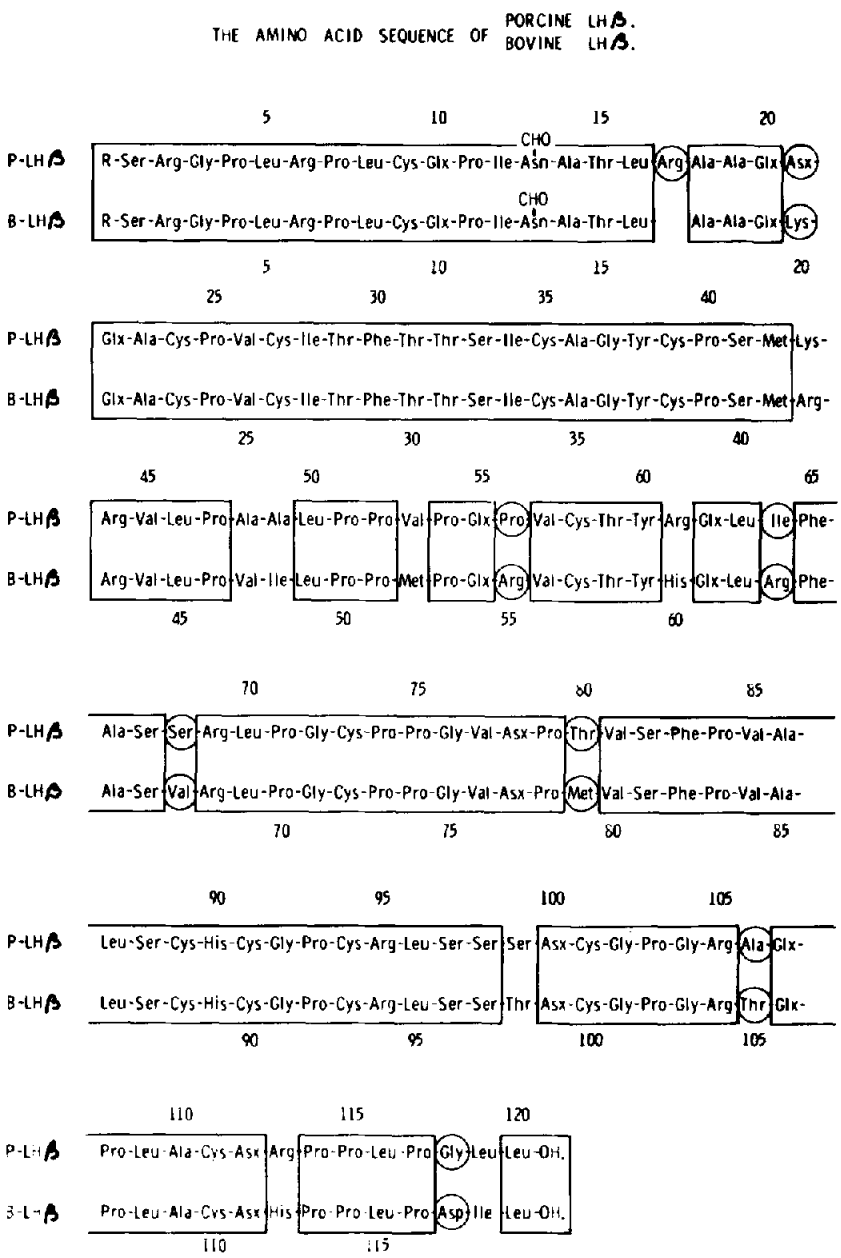

Fig. 2. Both sequences are disposed in order to show their homology. Numbering of the residues are, respectively, the upper one for P-LHβ and lower for B-LHβ. Identical residues are enclosed in rectangles. Circles enclose residues whose side chain function is is changed compared to that of corresponding residue in the bovine sequence. CHO and R have the same significance as in fig. 1.

Our data concerning that portion of the porcine sequence agrees with those of Ward and Liu [1] for O-LHβ. Ordering the tryptic peptides was done on the basis of the bovine sequence [4] and from the compositions and partial sequences of both CNbr fragments (1–42 and 43–120) of the reduced and S-carboxymethylated porcine LHβ. A unique polysaccharide prosthetic group was identified, linked at the Asx occupying position 13 in the porcine chain as in the case of both O-LHβ [1, 2] and B-LHβ [3, 4].

As previously observed for other proteins, we found arginyl–proline bonds (6–7 and 113–114) resistant to trypsin digestion. The arginyl–alanine bonds in position 17–18 showed an unexpected stability toward trypsin. This could be due to the vicinity of the polysaccharide prosthetic group. Trypsin also cleaved the peptide bond between the two alanine residues in positions 48–49 which allowed the complete sequence elucidation of T5. Another unusual cleavage, already observed in the case of the bovine chain [4], was between leucine and carboxymethylcysteine in positions 8 and 9. Our results concerning the carboxy-terminal residues of P-LHβ are consistent with the fact that not all the polypeptides possess a Leu–Leu carboxyterminal sequence, about 50% of them lacking either one or both leucines. The NH_2-terminal end of porcine subunit is protected by an acyl-group. Those particularities of the NH_2 and COOH terminal ends in the porcine chain were also observed in the case of ovine [1] and bovine LHβ [4, 9] subunits. A striking point in the comparison of the porcine and bovine sequences is an additional Arg (position 17) in the porcine β subunit. Taking into account the respective phylogenetic positions* of the porcine and of the bovine species, a deletion appears as the most probable mutational event to explain the shortening of the bovine chain by one residue. For appreciation of the degree of homology, this additional Arg residue must be disregarded as shown in fig. 2. In that case, 15 substitutions are readily noted; 14 of the 15 amino acid replacements are consistent with the change of a single nucleotide in the DNA codon. A substitution of Ile (bovine) by Ala (porcine) requires a two base mutation and is not frequently observed [10].

* As determined from the structural informations concerning cytochrome *c* and α and β chains of haemoglobin [11].

The amino acids occupying in the bovine chain positions 20, 63, 67, 79, 105 and 117, are replaced in the porcine sequence by residues in which the side chain function must induce modifications in interactions with the surrounding thus creating possible conformational changes. A prolyl residue in position 56 of the porcine LHβ is found in place of Arg in the bovine sequence (position 55 for B-LHβ). In view of the rigidity of proline's ring, this part of the polypeptide would be expected to take up the folding usually caused by this amino acid.

The significance of these observed phenomena will be examined at several levels of molecular organization, including the conformation of the isolated chains (bovine and porcine β subunits) in solution, and the specific changes induced by combination of the β-chain with the α subunit for both species.

The data of our physico-chemical studies will be precisely interpreted on the basis of chemical structure, as, in addition to that of P-LHβ, we have now determined the primary structure of porcine luteinizing hormone** α subunit.

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