PORCINE LUTEINIZING HORMONE. THE AMINO ACID SEQUENCE OF THE α SUBUNIT

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1. Introduction

Luteinizing hormone β subunits from ovine (O-LH β) and bovine (B-LH β) origins were shown to be completely homologous [1,2]. We recently reported [3] that porcine luteinizing hormone β subunit (P-LH β) exhibits 15 amino acid replacements when compared to B-LH β and O-LH β.

In order to see whether such a difference does also exist between amino acid sequences of porcine and ovine LH α subunits [4,5], we present here the primary structure of P-LH α and compare it to O-LH α.

2. Experimental

P-LH α was prepared as previously described [6]. Methods used for reduction and alkylation of the protein with iodoacetic acid, CNBr cleavage, tryptic hydrolyses, end group analyses and sequence determinations have also been described elsewhere [2,7].

When tryptic peptides were too large to achieve complete determination of their amino acid sequence, they were submitted to digestion with papain (Boehringer, 30 U/mg). This was performed under nitrogen in a 0.2 M pyridine acetate buffer, pH 6.0, containing 0.03 mM mercaptoethanol and 0.5 mM cysteine with an enzyme–substrate ratio of 1:12 at 37° for 18 hr.

The tryptic hydrolysate of the reduced and carboxy-methylated protein was submitted to gel filtration on Sephadex G-50 (fine, Pharmacia) (column 188 × 3.4 cm) equilibrated with 0.05 M ammonium bicarbonate buffer, pH 8.5. The largest peptides, eluted in the first peak, were further purified by chromatography on QAE-Sephadex A-25 (Pharmacia), as previously described [2]. Peptides from the other peaks were separated by preparative high voltage electrophoresis and paper chromatography [2,3].

The peptides obtained by papain digestion were purified by gel filtration on Sephadex G-15 (Pharmacia) (column 0.9 × 85 cm) equilibrated with 0.05 M ammonium bicarbonate buffer, pH 8.5. These peptides were further purified by high voltage electrophoresis.

3. Results and discussion

Fig.1 shows the amino acid sequence of porcine luteinizing hormone α subunit. The amino acid sequence of the tryptic peptides (Ti) of the reduced and carboxymethylated protein have been established by NH_2-terminal sequential degradation and COOH-terminal end group determination. The large T5 peptide was further digested by papain in order to determine its complete sequence.

The tryptic glycopeptides T9 and T12 were found resistant to papain digestion. The failure of the papain cleavage was probably due to the masking effect of the polysaccharide prosthetic groups of these peptides. In addition, there was only partial conversion of cystine to carboxymethyl-cysteine in those regions of the LH α molecule. Consequently, those parts of the sequence shown in brackets in fig.1 could not be determined.

Unspecific partial cleavage with trypsin occurred at the Ser–Lys (23–24) bond. The cleavage of the

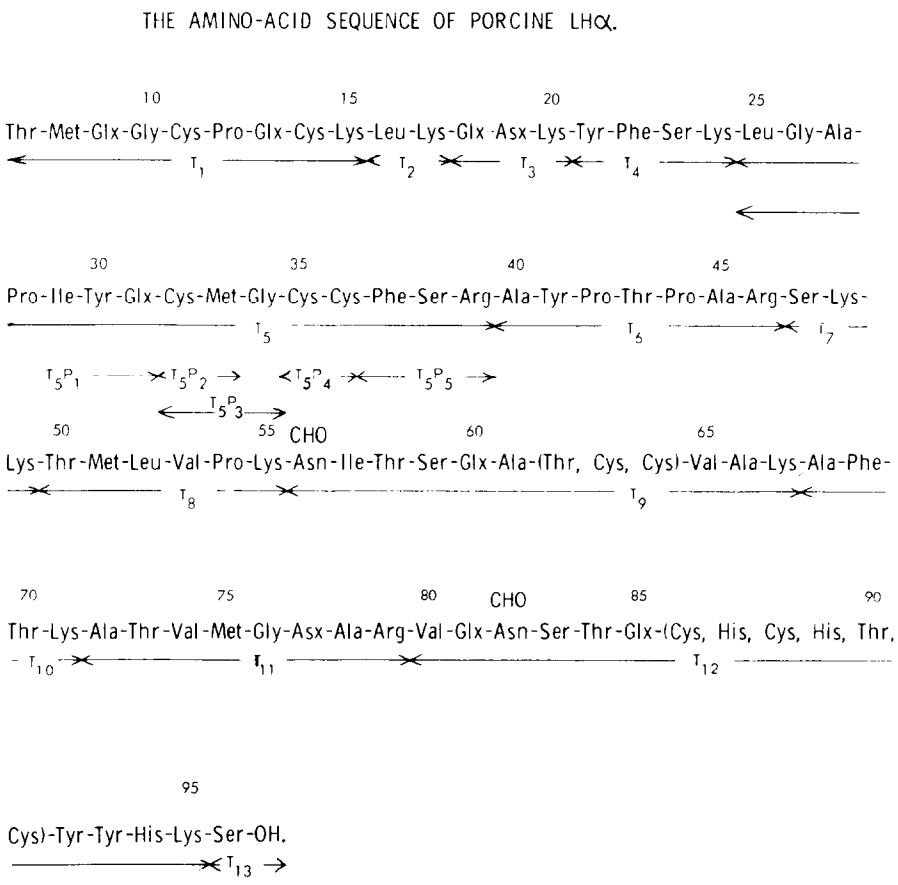

Fig. 1. The numbering of the residues is that of the ovine LH α subunit. Ti indicates tryptic peptides; TiPj, peptides resulting from papain digestion of tryptic (Ti) peptides. CHO symbolizes the polysaccharide prosthetic group.

Lys–Glx (17–18) bond was not complete and as a result, peptides Leu–Lys (16–17), Glx–Asx–Lys (18–20), and Leu–Lys–Glx–Asx–Lys (16–20) were isolated. Similarly, the tryptic hydrolyses of the Lys–Lys (48–49) and Lys–Thr (49–50) peptide bonds were only partial, with the peptides Ser–Lys (47–48) and Lys–Thr–Met–Leu–Val–Pro–Lys (49–55) being found in addition to T7 and T8.

The primary structure of P-LH α was reconstructed by ordering the tryptic peptides by analogy with the ovine sequence [4,5]. This alignment is compatible with composition and partial amino acid sequence of the five cyanogen bromide fragments (7–8, 9–33, 34–51, 52–75 and 76–96).

The primary structures of porcine LH α and ovine LH α are compared in fig.2. Six residues are missing at the NH_2-terminal of the P-LH α molecule with reference to the ovine sequence. This point merits discussion. Indeed, in a previous paper [6] we reported that phenylalanine was the most abundant NH_2-terminal residue for P-LH α. For the porcine LH α material used in the present study and obtained from a source other than that referred to [6], a Thr–Met sequence was found at the NH_2-terminal end, and T1 was the unique tryptic peptide corresponding to that portion of the molecule. Similar NH_2-terminal heterogeneity was described by Liu et al. [5] for ovine LH α and by Pierce et al. [8] for bovine LH α and TSH α. It must be noted however that human LH α [9] is devoid of the NH_2-terminal heptapeptide of ovine LH α. This part of the α subunit can thus vary to a large extent, without major alteration of the biological activity.

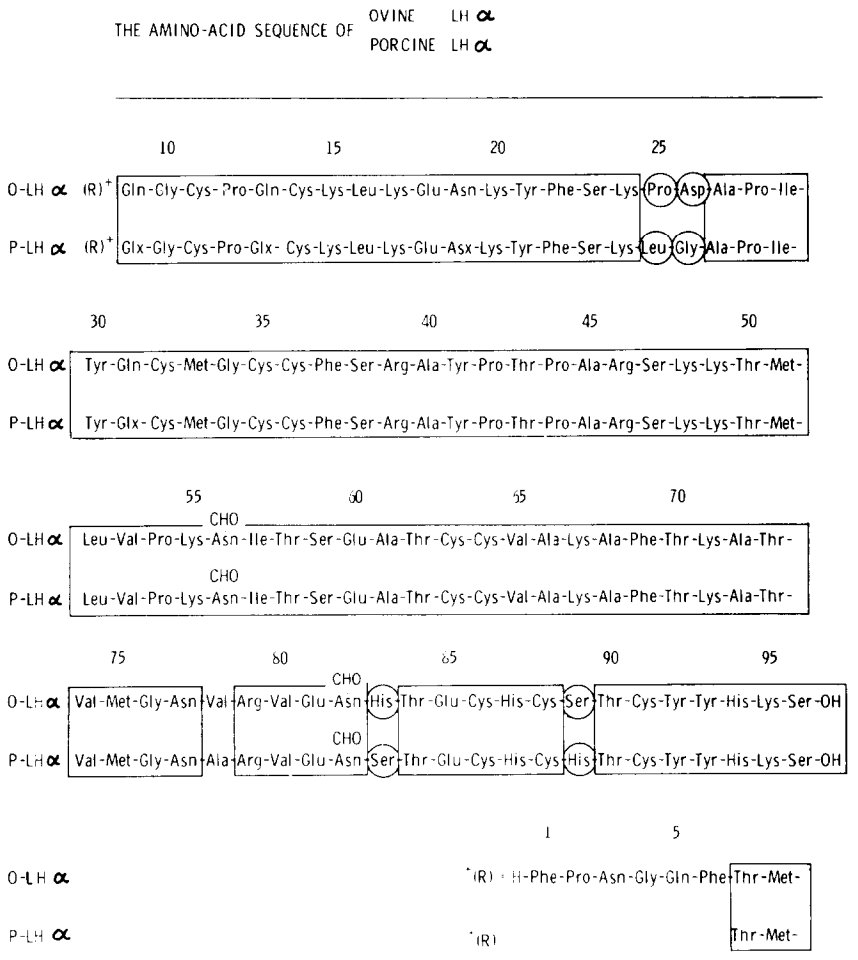

Fig. 2. The numbering of the residues is that of the ovine LH α subunit. Identical residues are enclosed in rectangles. Circles enclose residues whose side chain function is changed compared to that of corresponding residues in the ovine sequence. CHO has the same signification as in fig.1.

It must also be noted that the present P-LH α preparation exhibits heterogeneity in the carboxy-terminal length of its polypeptide chain. Such a phenomenon was previously observed for bovine LH α subunit [7].

Five amino acid replacements are evidenced when the sequences of P-LH α and O-LH α are compared. The Pro ↔ Leu substitution in position 25 corresponds to a 'one base' mutation in the DNA codon, however it is not frequently observed [10]. The Gly ↔ Asp and Val ↔ Ala replacements (position 26 and 78), are frequent and consistent with the change of a single nucleotide. Finally, the Ser ↔ His point mutations at positions 83 and 89 are frequently observed but involve more than one nucleotide replacement in the DNA triplet coding for the amino acids in these positions. It must be noted that four out of the five amino acid substitutions observed, involve modification of the side chain function and, in the case of Pro–Leu point mutation, a change in the folding ofthe polypeptide chain.

It is noteworthy that the sequences of ovine LH α subunit [4,5] and bovine thyroid stimulating hormone α subunit (B-TSH α) [11], are identical. Pierce and Liao [8] have shown that the tryptic

peptides and the cyanogen bromide fragments from bovine LH α have identical amino acid compositions to that of bovine TSH α. Pierce et al.[12] have indicated that the sequence of bovine LH α is probably similar to that of B-TSH α. Complete homology was also found between the amino acid sequences of ovine LH β [1] and bovine LH β [2]. Contrarily, 15 amino acid replacements are noted between bovine (or ovine) and porcine LH β sequences [3]. In the present study, no more than 5 mutations were found between P-LH α and O-LH α (and presumably B-LH α).

This might indicate that a restriction exists in the variability of the LH α subunit by comparison with the β subunit.

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