HUMAN LUTEINIZING HORMONE THE AMINO ACID SEQUENCE OF THE β SUBUNIT

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1. Introduction

In the last few years the primary structure of several glycoprotein hormones of animal origin such as ovine, bovine and porcine luteinizing hormones* [1–7] and bovine thyroid-stimulating hormone [8] were elucidated. Recently, Bahl et al. [9] presented the complete sequences of the subunits α and β of human chorionic gonadotropin. Different laboratories are now focusing their efforts on the elucidation of the primary structure of LHα and β subunits of human origin.

This task is made difficult by the scarcity and heterogeneity of human pituitaries and difficulties encountered in obtaining pure subunits in good yield [10, 11]. We recently described a method for the dissociation of human LH and the isolation of its subunits in excellent yield [12]. The high degree of purity of our preparations allowed us to approach the elucidation of the subunit sequences.

At the last Laurentian Hormone Conference, Papkoff presented the complete structure of human-LH α [13]. Simultaneously, Ward [14], Shome [15] and we [16] presented preliminary data concerning the primary structure of human-LHβ subunit. We propose here an amino acid sequence for the human LHβ chain and its relationships to the β subunits of pituitary gonadotropins from other species and to that of human chorionic gonadotropin.

* *Abbreviations:*
LH : Luteinizing hormone.
TSH : Thyroid-stimulating hormone.
HCG: Human chorionic gonadotropin.

2. Experimental

2.1. *Methods*

Human LHβ was prepared as previously described [12]. The protein was reduced by dithiothreitol and alkylated by recrystallized iodoacetic acid according to Koningsberg [17]. Methods of amino acid analysis and tryptic hydrolysis have been described previously [5–7]. Thermolysin (Calbiochem) digestions were carried out at pH 8.2 in 0.125 M ammonium bicarbonate and 0.01 M calcium acetate for 2 hr at 37° with a weight ratio of enzyme to peptide of 1/100. Tryptic peptides from the reduced and carboxymethylated protein were first fractionated on a Sephadex G-50 fine (Pharmacia) column (2 × 200 cm) using 0.05 M ammonium bicarbonate as developing buffer.

Further purification was achieved by gel filtration on Sephadex G-25 superfine (Pharmacia) and ion exchange chromatography on Dowex 50 × 2 (Bio-Rad) using pyridine–acetate buffers. Separations of pep-

tides were also performed on a peptide analyzer (Technicon) equipped with the Ultrograd 11300 (LKB). Final purification was achieved by preparative electrophoresis [5, 6] or by preparative paper chromatography using n-butanol–pyridine–acetic acid–water (30:20:6:24) system or pyridine–n-propanol–water (20:37.5:30) system [18]. Amino-terminal amino acid sequences were determined according to Gray [19]. Dansyl amino acids were identified by thin-layer chromatography on polyamide sheets [20]. Digestions with carboxypeptidases A and B were used to elucidate carboxy-terminal amino acids of proteins [6].

Due to limitation of material, carboxy-terminal residues were also identified by the selective tritiation method described by Holcomb et al. [21]. Tritiated and non tritiated amino acids were isolated by electrophoresis (formic acid–acetic acid–water; 20:75:100, pH 1.5) followed by chromatography (n-butanol–pyridine–acetic acid–water; 30:20:6:24). Elution of amino acids was performed with 0.01 M ammonium hydroxide, the eluate was lyophilized and the residue dissolved in a scintillation mixture (5 g PPO, 0.1 g dimethyl POPOP per ℓ of toluene). The radioactivity was measured with a Packard Model 3380 liquid scintillation spectrometer.

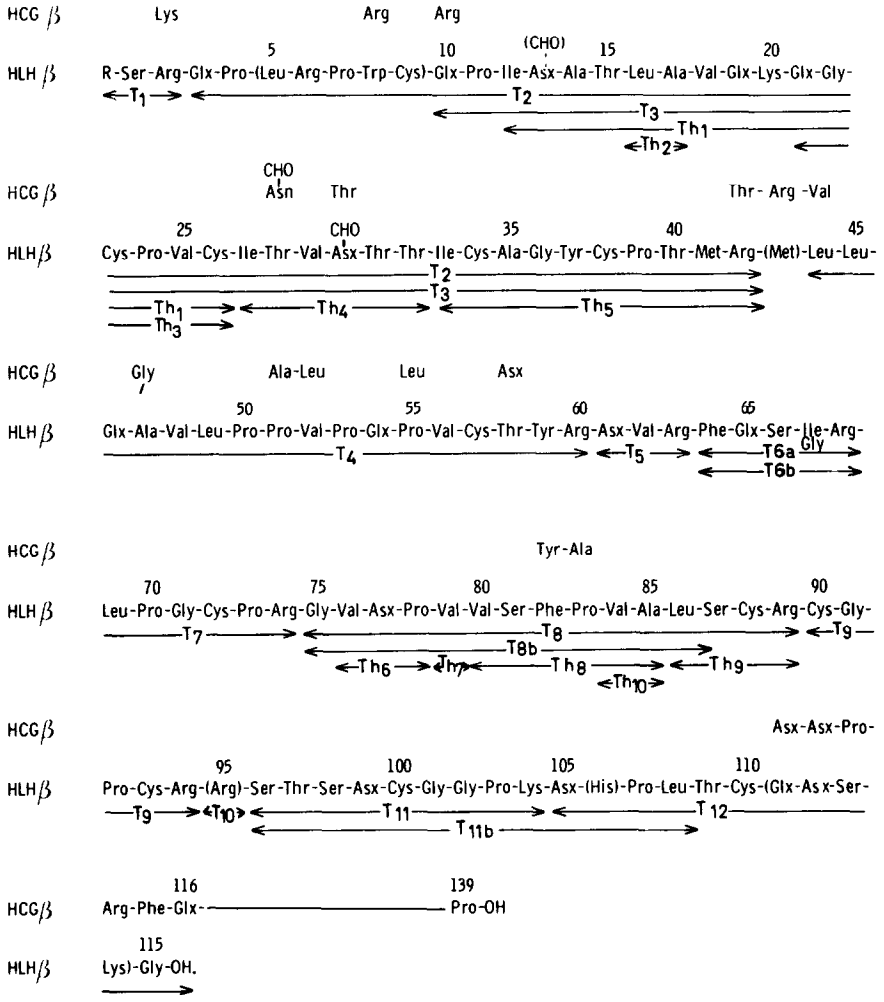

Fig. 1. The amino acid sequence of human luteinizing hormone β subunit. T_i indicates tryptic peptides; Th_i, peptides resulting from thermolysin digestion of tryptic peptides. CHO symbolizes polysaccharide prosthetic group; R, an unidentified acyl group. Parts of sequences shown in parentheses represent gaps in the elucidation of sequence. Non identical residues in HCGβ are indicated above the LHβ sequence.

Tryptophan containing peptides were identified by staining with Erlich reagent [22].

3. Results and discussion

The amino acid sequence of human luteinizing hormone β subunit (fig. 1) was established according to both NH_2 - and COOH-terminal sequences of tryptic peptides (T_i) of the reduced and S-carboxymethylated protein. As previously described for other proteins the arginine–proline bond (6–7) was found to be resistant to tryptic hydrolysis. The lysine–glutamic acid bond (20–21) also exhibited an unexpected stability toward trypsin. Unusual cleavages were observed at leucine–serine bond (86–87) and leucine–threonine bond (108–109).

After partial elucidation of their sequence, the large tryptic peptides were further digested with thermolysin. The resulting peptides (Th_i) were analysed for their composition and submitted to sequential degradations.

Both tryptic and thermolytic peptides were ordered on the basis of their homology with bovine LHβ and HCGβ sequences.

A most difficult task was the elucidation of the COOH-terminal sequence of human LHβ subunit. Indeed, T12 was assigned as the COOH-terminal tryptic peptide, its composition and NH_2-terminal sequence being similar to the corresponding sequence of LHβ from other species or to that of HCGβ. Due to severe limitation of material, this peptide could not be submitted to further cleavage, so that a gap in the elucidation of its sequence (Glx–Asx–Ser–Lys) could not be filled. Moreover, selective tritiation revealed several labeled amino acids, glycine being predominant. This possibly indicates a COOH-terminal heterogeneity of the protein, as seen in the case of ovine, bovine and porcine LHβ [1, 5, 6].

No free amino-terminal residue was identified on the reduced and carboxymethylated human LHβ by the dansyl labeling technique. The NH_2-terminal peptide T1 had an electrophoretical mobility at pH 3.7 similar to the blocked NH_2-terminal peptide of the bovine and porcine β subunits. Nevertheless, a trace of serine was observed by the dansyl method applied to this peptide. This observation suggests that the blocked NH_2-terminal residue could be unmasked during the various steps of the isolation of this peptide.

In our work, sequence 5–9 was not determined. Recently Ward [14] accomplished the determination of 26 steps from the NH_2-terminal end of the molecule and showed that positions 5 to 9 corresponded to a Leu–Arg–Pro–Trp–Cys sequence. The amino acid compositions of both T2 and T3 peptides in our work were indeed in agreement with these data.

Our sequence of human LHβ differs in three respects from the provisional data proposed by Shome and Parlow [15]. We found leucine and valine residues, respectively, in positions 44 and 52 whereas Shome placed valine and leucine residues. The most important discrepancy occurs in the 28–30 portion of the molecule where we assigned the sequence Thr–Val–Asx instead of Asn–Val–Thr as reported by Shome and Parlow [15]. As in both cases, the Asx residue is a presumed site of attachment for a polysaccharide side chain, it would be important to look for additional information, concerning this part of the sequence. The 28–30 sequence as defined by Shome and Parlow [15] was identical to that found in HCGβ chain by Bahl et al. [9].

Compared with other glycoprotein hormones, human LHβ showed a higher degree of holomogy with HCGβ than with LHβ subunits of animal origins (ovine, bovine and porcine). Indeed 86 positions of human LHβ and HCGβ are identical. Nevertheless, a striking difference exists between the pituitary and chorionic gonadotropin β subunits, the latter exhibiting an additional COOH-terminal portion of 24 amino acids. Seventy six positions were found to be identical in bovine LHβ (or ovine LHβ) and human LHβ.

It is interesting to note that the sequence variability already observed in bovine LHα [23] was also present in the sequence of human LHβ. Indeed, we isolated a peptide T6b similar in sequence to T6a fragment except that isoleucine is replaced by a glycine residue. Furthermore, Ward recently reported [14] sequence variability in positions 8, 13 and 15 of human LHβ, tryptophan, asparagine and threonine being replaced by valine, arginine and isoleucine residues, respectively. It is striking to note that variability in positions 13 and 15 implies that a possible recognition site for carbohydrate linkage might exist in that portion of human LHβ structure. LHβ subunits from other species possess only one carbohydrate unit on the asparagine 13, whereas polysaccharide prosthetic

groups are located at positions 13 and 28 in HCGβ.

4. Conclusion

The amino acid sequence of human LHβ was established using only 3 μmoles of reduced and S-carboxymethylated protein. Trypsin and thermolysin were used for protein and peptide cleavages, respectively. Only a single gap in the sequence remains to be filled between positions 111–114.

It was shown that human LHβ HCGβ exhibit 86 identical residues within their common sequence (positions 1–112) the extent of homology being less with LHβ subunits from ovine, bovine and porcine species.

As for bovine LHα subunit, sequence variability was also demonstrated for human LHβ.

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References

[1] D.N. Ward and W.K. Liu, in: Protein and Polypeptide Hormones. Proceedings of the International Symposium, Liège 1971, part 1, ed. M. Margoulies, ICS 241 (Excerpta Medica, Amsterdam), p. 80.
[2] W.K. Liu, H.S. Nahm, C.M. Sweeney, W.M. Lamkin, H.N. Baker and D.N. Ward, J. Biol. Chem. 247 (1972) 4351.
[3] W.K. Liu, H.S. Nahm, C.M. Sweeney, G.N. Holcomb and D.N. Ward, J. Biol. Chem. 247 (1972) 4365.
[4] H. Papkoff, M.R. Sairam and C.H. Li, J. Am. Chem. Soc. 93 (1971) 1531.
[5] G. Maghuin-Rogister and A. Dockier, FEBS Letters 19 (1971) 209.
[6] G. Maghuin-Rogister and G. Hennen, FEBS Letters 23 (1972) 225.
[7] G. Maghuin-Rogister, Y. Combarnous and G. Hennen, FEBS Letters 25 (1972) 57.
[8] T.H. Liao and J.G. Pierce, J. Biol. Chem. 246 (1971) 850.
[9] O.P. Bahl, R.B. Carlsen, R. Bellisario and N. Swaminathan, Biochem. Biophys. Res. Commun. 48 (1972) 416.
[10] A. Stockell-Hartree, M. Thomas, M. Braikewith, E.T. Bell, D.W. Christie, G.V. Spaull, R. Taylor and J.G. Pierce, J. Endocr. 51 (1971) 169.
[11] L.E. Reichert, A.R. Midgley, G.D. Niswender and D.N. Ward, Endocrinology 87 (1970) 634.
[12] J. Closset, G. Hennen and R.M. Lequin, FEBS Letters 21 (1972) 325.
[13] M.R. Sairam, H. Papkoff and C.H. Li, Biochem. Biophys. Res. Commun. 48 (1972) 530.
[14] D.N. Ward, Communication at the Laurentian Hormone Conference (1972).
[15] B. Shome and H. Parlow, Discussion, Laurentian Hormone Conference (1972).
[16] R.M. Lequin, Discussion, Laurentian Hormone Conference (1972).
[17] W. Koningsberg, in: Methods in Enzymology, ed. W. Hirs, Vol. XXV (Academic Press, New York, 1967) p. 185.
[18] D.N. Ward, C.M. Sweeney, G.N. Holcomb, W.M. Lamkin and M. Fujino, Proceedings of the Third International Congress of Endocrinology, Mexico 1968 (Excerpta Medica Series 184) p. 385.
[19] W.R. Gray, in: Methods in Enzymology, ed. W. Hirs, Vol. XI (Academic Press, New York, 1967) p. 469.
[20] K.R. Woods and K.J. Wang, Biochem. Biophys. Acta 133 (1967) 369.
[21] G.N. Holcomb, S.A. James and D.N. Ward, Biochemistry 7 (1968) 1291.
[22] R.E. Canfield and C.B. Anfinsen, in: Proteins, ed. H. Neurath, Vol. 1 (Academic Press, New York, 1963) p. 319.
[23] G. Maghuin-Rogister and G. Hennen, European J. Biochem. 21 (1971) 489.