HUMAN PITUITARY THYROTROPIN:

PRIMARY STRUCTURE OF THE HORMONE SPECIFIC β SUBUNIT

by

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Received June 14,1973

Summary: The primary structure of the hormone specific β subunit of human pituitary thyrotropin has been deduced from the composition and complete or partial amino acid sequences of the tryptic and chymotryptic peptides. It consists of 112 amino acid residues with the single oligosaccharide moiety which is assumed to be linked to the asparagine residue at position 23.

Human pituitary thyrotropin has recently been shown to consist of two dissimilar subunits (1, 2). In an earlier communication (2) we presented evidence derived from the compositions and partial sequences of the tryptic and chymotryptic peptides of HTSH-α[1] that its primary structure is identical to that of human ICSH-α. This communication presents the complete amino acid sequence of HTSH-β. It will be seen that the primary structure of the human hormone is highly homologous to that of the bovine hormone whose amino acid sequence has also been recently elucidated (3).

MATERIALS AND METHODS

HTSH was isolated from fresh pituitary glands by minor modifi-

[1] Abbreviations used: TSH, Thyrotropin; HTSH, Human thyrotropin; HTSH-α, α subunit of HTSH; HTSH-β, β subunit of HTSH; ICSH-α, α subunit of interstitial cell stimulating hormone.

cations of the procedures of Stockell-Hartree (4) and Condliffe (5). The β subunit was obtained by the counter current distribution procedure developed recently for the human hormone (2). Amino acid analyses were performed on the Beckman automatic analyzer according to Spackman et al (6). Other procedures such as enzymic digests, two dimensional paper chromatography-electrophoresis, dansyl-Edman degradation and performic acid oxidation were carried out as previously described (7).

The tryptic digest (22 mg) was fractionated on a Sephadex G-50 (2.6 x 70 cm) column in 0.01 M NH_4OH. Several discrete fractions were obtained and recovered by lyophilization. Each of these peptide fractions were subjected to two dimensional paper chromatography-electrophoresis. After the electrophoretic run, the paper was thoroughly air dried and lightly sprayed with 0.1% ninhydrin in 95% alcohol and allowed to develop at room temperature; the purple spots were each cut out into small pieces and put in a test tube containing 1 M NH_4OH for elution. After 24 hours the eluate was air dried, amino acid composition determined and sequenced. It may be mentioned that the total amount of HTSH-β employed in this investigation was approximately 25 mg (less than 2 μM).

RESULTS AND DISCUSSION

A total of 17 tryptic peptides were isolated and their compositions are given in Table 1. These tryptic peptides were sequenced and the following results were obtained: T2, Phe-Cya-Ile-Pro-Thr-Glu-Tyr; T3, Met-Thr-His-Ile-Glu-Arg; T5, Glu-Cya-Ala-Tyr-Cya-Leu-Thr-Ile-Asn-Thr-Thr-Ile-Cya-Ala-Gly-Tyr-Cya-Met-Thr-Arg; T6, Asp-Ile-Asx-Gly-Lys; T7, Leu-Phe-Leu-Pro-Lys; T8, Tyr-Ala-Leu-Ser-Gln-Asp-

Table 1

Amino acid composition (Molar Ratio) of tryptic peptides isolated from oxidized HTSH-β

Peptide No. *	Composition **	Total Residues
T1	$His_{0.9}Arg_{1.1}Cya_{0.96}Met_{0.9}Thr_{2.0}Glu_{2.0}Pro_{1.2}Ile_{2.1}Tyr_{0.98}Phe_{1.1}$	13
T2	$Cya_{1.0}Thr_{0.9}Glu_{1.0}Pro_{1.4}Ile_{0.9}Tyr_{0.6}Phe_{0.7}$	7
T3	$His_{0.8}Arg_{1.0}Met_{1.0}Thr_{0.6}Glu_{1.2}Ile_{1.1}$	6
T5***	$Arg_{1.0}Cya_{4.0}Asp_{1.2}Met_{0.8}Thr_{3.5}Glu_{1.1}Gly_{1.1}Ala_{1.9}Ile_{1.8}Leu_{1.1}Tyr_{1.6}$	20
T6	$Lys_{1.1}Asp_{1.9}Gly_{1.3}Ile_{1.0}$	5
T7	$Lys_{1.0}Pro_{1.1}Leu_{2.0}Phe_{1.0}$	5
T8	$Arg_{0.8}Cya_{1.0}Asp_{1.0}Thr_{0.9}Ser_{0.9}Glu_{1.0}Ala_{0.9}Val_{1.0}Leu_{1.1}Tyr_{1.6}$	11
T9	$Arg_{1.0}Asp_{0.92}Ile_{0.95}Tyr_{1.0}Phe_{1.1}$	5
T10	$His_{0.8}Cya_{1.0}Thr_{0.9}Glu_{1.0}Pro_{3.0}Gly_{1.0}Ala_{1.0}Val_{1.6}Ile_{0.97}Leu_{1.03}Tyr_{0.9}$	14
T11	$Lys_{1.1}Cya_{1.0}Ser_{1.8}Pro_{1.1}Ala_{1.0}Val_{1.0}Leu_{1.0}Tyr_{1.0}Phe_{1.0}$	10
T12	$Lys_{1.0}Cya_{0.9}Gly_{0.8}$	3
T13	$Lys_{1.8}Cya_{1.7}Ser_{1.7}Pro_{1.1}Gly_{1.4}Ala_{1.1}Val_{1.2}Leu_{1.0}Tyr_{1.0}Phe_{1.0}$	13

*T4 is free agrinine

**Met determined as Methionine Sulfone

***T5 contains carbohydrates

Table 1 (cont'd)

T14	$Lys_{0.9}His_{0.8}Cya_{1.9}Asp_{3.2}Thr_{1.0}Ser_{0.9}Glu_{1.1}$ $Ala_{1.0}Ile_{2.1}Tyr_{0.7}$	14
T15	$Lys_{1.6}His_{1.0}Cya_{2.5}Asp_{3.4}Thr_{1.1}Ser_{1.2}Glu_{1.4}$ $Gly_{1.1}Ala_{1.2}Ile_{2.0}Tyr_{0.8}$	17
T16	$Lys_{2.1}Cya_{1.1}Asp_{0.9}Thr_{1.6}Glu_{0.9}Pro_{1.0}Tyr_{0.7}$	9
T17	$Ser_{0.8}Tyr_{1.0}$	2

Val-Cys-Thr-Tyr-Arg; T9, Asp-Phe-Ile-Tyr-Arg; T10, Thr-Val-Glu-Ile-Pro-Gly-Cya-Pro-Leu-His-Val-Ala-Pro-Tyr; T11, Phe-Ser-Tyr-Pro-Val-Ala-Leu-Ser-Cya-Lys; T12, Cya-Gly-Lys; T14, Cya-Asx-Thr-Asx-Tyr-Ser-Asx-Cya-Ile-His-Glu-Ala-Ile-Lys; T16, Thr-Asn-Tyr-Cya-Thr-Lys-Pro-Gln-Lys; T17, Ser-Tyr. It has been shown previously (2) that the NH_2-terminal sequence of the oxidized HTSH-β is Phe-Cya-Ile-Pro-Thr-Glu-Tyr-Met and the COOH terminal is Lys-Ser-Tyr. Thus, peptide T2 must be located at the NH_2-terminus and T17 at the COOH terminus.

In order to align the tryptic peptides the following chymotryptic peptides were obtained by two dimensional chromatography-electrophoresis of the chymotryptic digest (1.5mg): C2, Met-(Thr, $His_1Ile_1Glu_2Arg_2Cya_1$ Ala_1)Tyr; C4, Cya-($Met_1Thr_1Arg_1Asp_2Ile_1Gly_1Lys_1$)Leu; C6, Phe-($Leu_1$ Pro_1Lys_1)-Tyr; C8, Arg-Asx-Phe-Ile-Tyr; C9, Arg-Thr-($Val_1Glu_1Ile_1$ $Pro_2Gly_1Cya_1$)-Leu; and C13, Ser-Cya-($Lys_3Cya_3Gly_1Asp_4Thr_2Tyr_1Ser_1$ $Ile_2His_1Glu_1Ala_1$)-Tyr. From these data it is possible to formulate the complete amino acid sequence of HTSH-β as shown in Figure 1. It may be noted that there is only one bond between residues 74-75 that is lacking

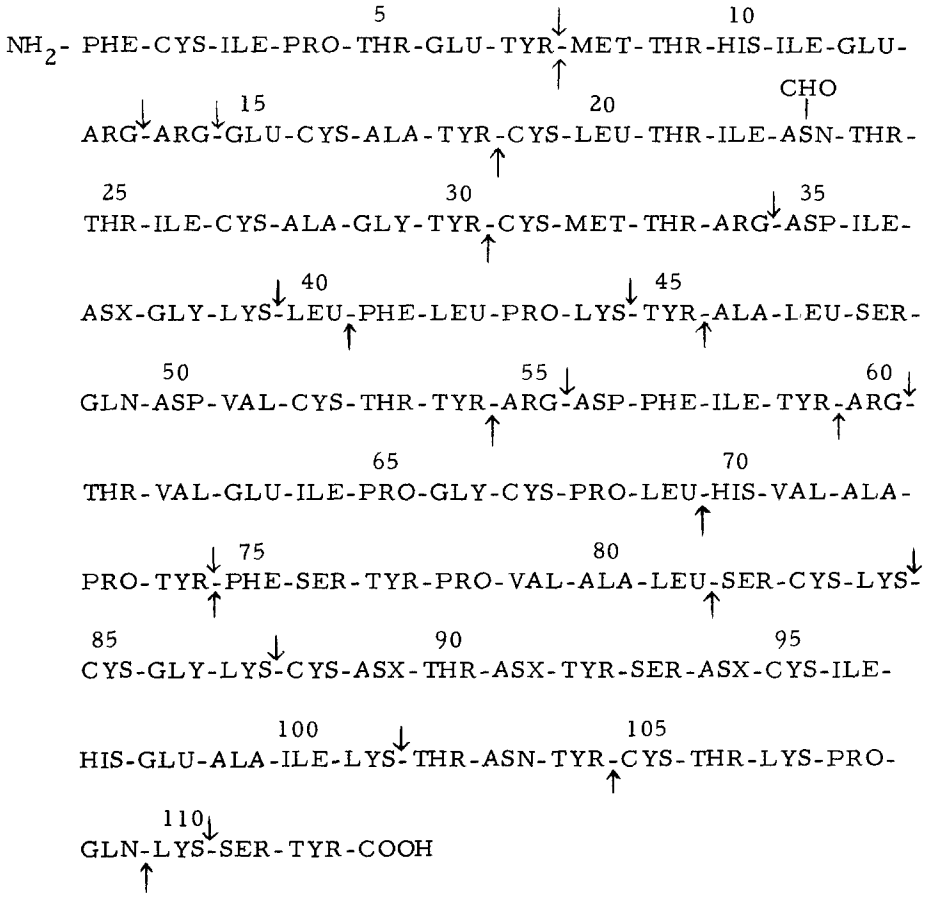

Figure 1: Amino acid sequence of the β Subunit of HTSH
↓ Tryptic cleavage ↑ Chymotryptic cleavage

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the required overlap. This is because of the extreme susceptibility of the tyrosyl-phenylalanine bond to both chymotryptic and tryptic cleavages. The single oligosaccharide unit is most probably covalently linked to Asn_{23} as in the case of the bovine TSH-β (3). The amino acid composition ($Lys_7His_3Arg_5Asp_9Thr_{11}Ser_5Glu_7Pro_7Gly_4Ala_6Cys'_{12}Val_4Met_2Ile_9Leu_6Tyr_{11}Phe_4$) as computed from the proposed structure is in good agreement with the experimental values (2).

A comparison of the proposed primary structure of HTSH-β

(Figure 1) with that proposed for the bovine TSH-β (3) reveals a high degree of conservation. All the amino acid residues in the two subunits may be arranged without the introduction of any gaps in the sequence. If we assume that the disposition of the amide or acid forms of Asx residues in the two species are the same, there are only 11 residue positions that are different. However, nine of these changes can be considered to be highly acceptable replacements (8). In addition, there are 113 amino acid residues in the bovine TSH-β (3) and 112 in HTSH-β (Figure 1). It should also be noted that the COOH-terminal methionine residue in the bovine TSH-β (3) is not present in HTSH-β.

ACKNOWLEDGMENTS

We thank Jean Knorr and Daniel Gordon for excellent technical assistance. This work was supported in part by the United States Public Health Service Grant A-6097 from the National Institute of Arthritis and Metabolic Diseases. The authors also thank the National Pituitary Agency in Baltimore for the supply of fresh human pituitary glands.

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