HUMAN PITUITARY INTERSTITIAL CELL STIMULATING HORMONE: PRIMARY STRUCTURE OF THE α SUBUNIT

by

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SUMMARY: The primary structure of the α subunit of human ICSH has been deduced from amino acid sequences of tryptic peptides and the compositions of cyanogen bromide fragments as well as the known structure of ovine ICSH-α The proposed structure is shown in Figure 1. It consists of 89 amino acids with the single isoleucine residue in position 22, and the three methionine residues in positions 26, 44 and 68.

INTRODUCTION

There has been a great deal of interest over the past decade in the isolation and characterization of interstitial cell stimulating hormone (ICSH) from human pituitary glands (1-5). It is a glycoprotein with a molecular weight of 26,000. After it was demonstrated (6) that ICSH from ovine pituitaries consists of two non-identical subunits (designated as α and β), the human hormone was also shown to consist of two subunits (7). Several procedures have been developed for the separation of the two subunits of human ICSH(8-10). We wish to report herein the complete amino acid sequence of human ICSH-α and its comparison with the α-subunit of ovine ICSH.

MATERIALS AND METHODS

ICSH was prepared from a side fraction during the isolation of human pituitary growth hormone by procedures as previously described by Papkoff (3) and Hartree (4). Separation of the hormone into its α and β subunits

was effected by modification of the counter current distribution procedure (6) developed earlier for the ovine hormone.

Performic acid oxidation, trypsin digestion and cyanogen bromide (CNBr) cleavage of the reduced and carbamidomethylated hormone (RA-ICSH) were done essentially as described earlier (11). The separation of tryptic glycopeptides was achieved by chromatography on DEAE-cellulose (0.01M NH_4HCO_3, pH 9.0) followed by low voltage electrophoresis on paper (pH 2.5).

Amino acid analyses were performed using the automatic amino acid analyzer. The amino acid sequence of the isolated peptides was determined by the dansyl-Edman method and digestions with carboxypeptidase and leucine aminopeptidase. Details of the above procedures have been given earlier (11).

RESULTS AND DISCUSSION

Table 1 presents the amino acid composition of human ICSH-α and is comparable to that reported by Hartree et al (9). It may be noted that it contains three methionine residues and a single residue of isoleucine. It contains also 22% carbohydrate (10). The NH_2-terminal sequence of oxidized human ICSH-α was found to be H-Val-Glx-Asp- as revealed by the dansyl-Edman procedure. Reactions of oxidized α subunit with carboxypeptidase gave the COOH-terminal sequence: -Tyr-Tyr-His-Lys-SerOH.

Because a very limited amount of ICSH-α was available, the elucidation of its primary structure was derived from the data obtained with the native hormone. It was also necessary to rely on the known amino acid sequence (12, 13) of ovine ICSH-α.

Table 1 gives the amino acid composition of 12 tryptic peptides isolated from tryptic digests of oxidized hormone and assigned to the α subunit. The peptide T1 has the same NH_2-terminal sequence for human ICSH-α

Table 1

Amino Acid Composition of Human ICSH-α Subunit

Amino Acid	Experimental[a]	Theoretical[b]	Human[c] TSH-α	HCG[d]-α
Lysine	5.8	6	4.9	6.8
Histidine	2.5	3	2.7	3.2
Arginine	3.0	3	2.8	3.1
Aspartic acid	5.4	5	4.9	7.0
Threonine	8.5	8	6.7	8.8
Serine	7.8	8	6.2	9.4
Glutamic acid	7.5	9	7.8	11.0
Proline	5.6	6	6.0	8.0
Glycine	4.3	4	4.0	5.0
Alanine	4.2	4	3.7	5.1
Half-cystine	10.2	10	9.0	10.3
Valine	6.4	7	6.2	8.0
Methionine	2.5	3	2.8	2.6
Isoleucine	1.3	1	1.57	1.0
Leucine	4.0	4	4.0	4.0
Tyrosine	4.0	4	4.2	4.0
Phenylalanine	3.5	4	4.3	4.3

[a]Molar ratio based upon 4.0 residues of leucine.

[b]From proposed sequence

[c]See (16); recalculated based upon 4 residues of leucine.

[d]See (17); recalculated based upon 4 residues of leucine.

Table 2. Amino Acid Composition (Molar Ratio) of Tryptic Peptides of Oxidized Human ICSH Assigned to the α-Subunit

	Peptide No.[a]									
Amino Acid	T1	T2	T3	T3a	T5	T5a	T6[b]	T7	T8	T9[c]
Lysine			1.0 (1)	2.0 (2)	1.1 (1)	2.0 (2)	1.1 (1)		1.1 (1)	0.8 (1)
Histidine										3.0 (3)
Arginine	0.8 (1)	1.0 (1)						0.9 (1)		
Cysteic acid	4.9 (5)						2.0 (2)			2.7 (3)
Aspartic acid	2.1 (2)						0.9 (1)	1.0 (1)		1.1 (1)
Methione sulfone	1.0 (1)				0.9 (1)	1.0 (1)			1.0 (1)	
Threonine	1.1 (1)	1.0 (1)			0.6 (1)	0.8 (1)	2.1 (2)		1.0 (1)	1.7 (2)
Serine	1.8 (2)		0.8 (1)	0.8 (1)			1.9 (2)	1.1 (1)		1.1 (1)
Glutamic acid	6.0 (6)				1.1 (1)	1.0 (1)	1.0 (1)			1.1 (1)
Proline	3.9 (4)	1.9 (2)								
Glycine	2.0 (2)								1.8 (2)	
Alanine	1.0 (1)	1.0 (1)					0.95 (1)			1.0 (1)
Valine	0.9 (1)				1.0 (1)	1.0 (1)	1.9 (2)		1.9 (2)	1.2 (1)
Isoleucine	0.9 (1)									
Leucine	1.8 (2)	1.1 (1)			1.0 (1)	0.9 (1)				
Tyrosine		1.1 (1)						1.0 (1)		1.5 (2)
Phenylalanine	2.7 (3)								1.0 (1)	
No. of Residues	32	7	2	3	6	7	12	4	8	16
NH_2-Terminal	Val	Ala	Ser	Ser	Thr	Lys	Asp	Ser	Val	Val
COOH-Terminal	Arg	Arg	Lys	Lys	Lys	Lys	Lys	Arg	Lys	Lys

[a] T4, free lysine; T10, free serine

[b] Containing carbohydrates

[c] 72 hr hydrolysis; containing carbohydrates

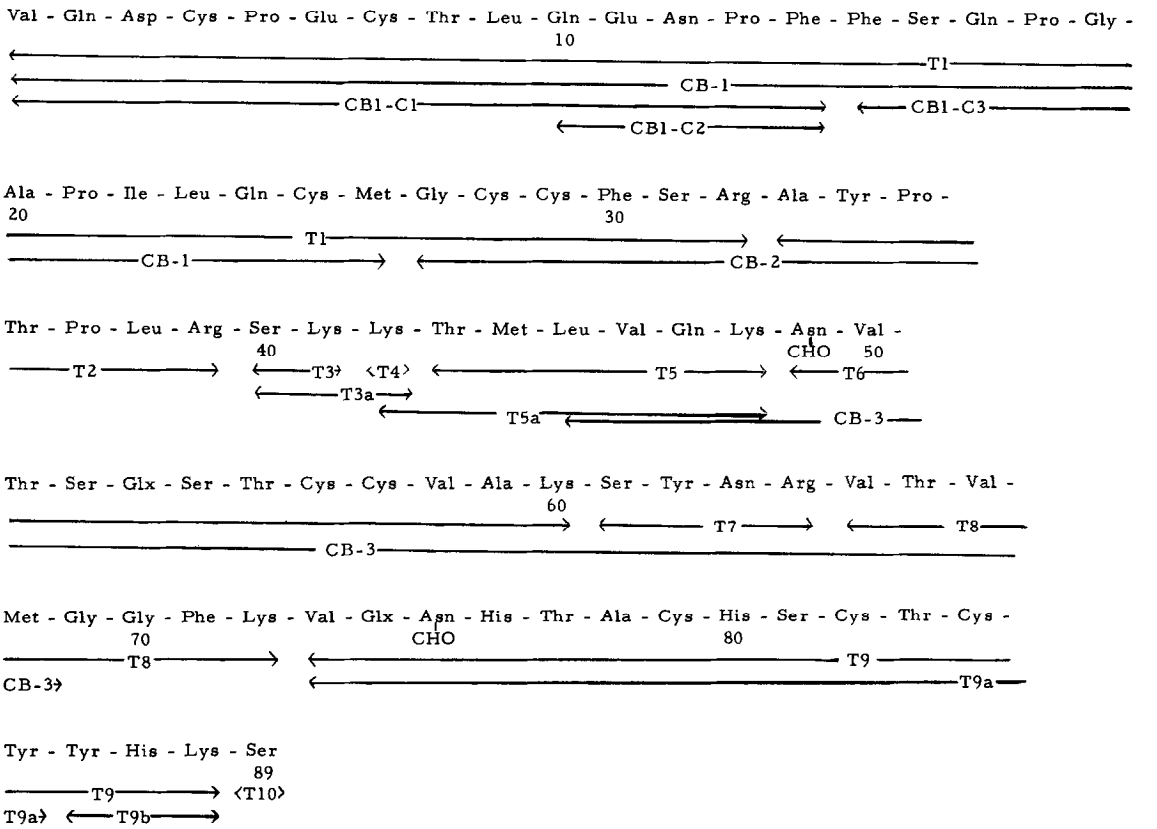

Fig. 1. Amino Acid Sequence of the Human ICSH-α Subunit

and hence must be at the NH_2-terminus. The glycopeptide T9, which has identical COOH-terminal sequence as human ICSH-α, must be at the COOH-terminus. The determination of the sequence of the other peptides and comparison with the sequence of the ovine ICSH-α (12, 13) enables us to propose the complete sequence of the human ICSH-α as shown in Figure 1. Additional evidence for the assignment of these peptides was provided by the composition of T2, T3, T5, T7, T8, T10 which were obtained from a peptide map of a tryptic digest of oxidized human ICSH-α. The sequence of the first 35 amino acid residues is in complete agreement with that recently reported by Inagami et al (14).

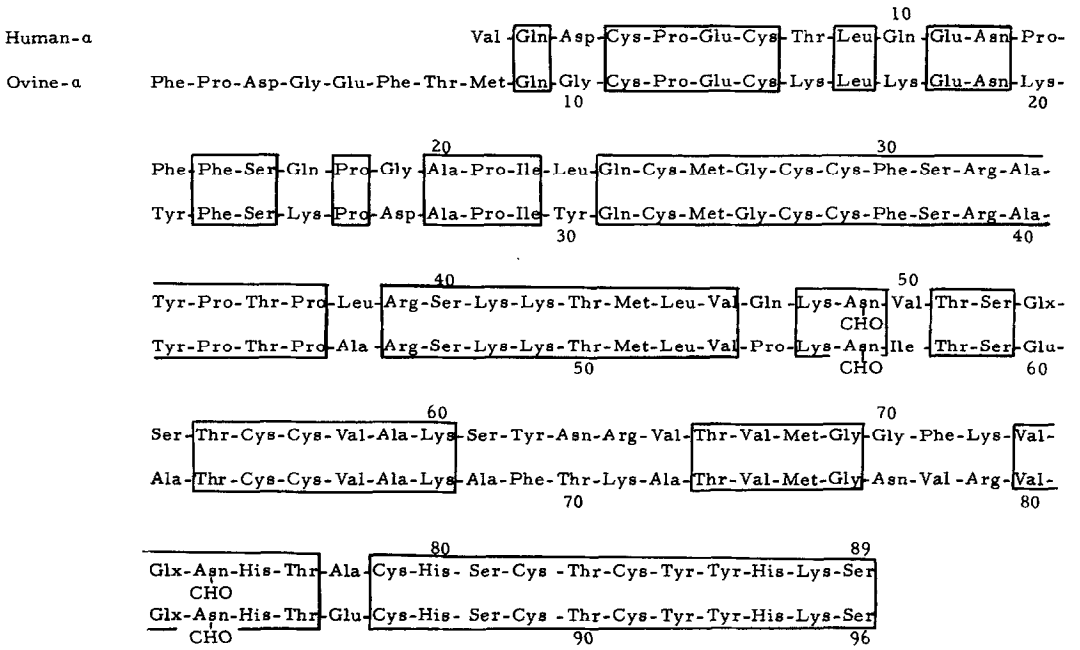

Fig. 2 Comparison of the Amino Acid Sequences of Human ICSH-α and Ovine ICSH-α

The isolation and compositions of 3 CNBr fragments obtained from RA-ICSH provides the necessary overlaps for peptides T1 to T8. It can be seen from Fig. 1 that the overlap between peptides T8 and T9 is missing, but they can be arranged in this order by the data which place the glycopeptide T9 at the COOH-terminus. It should be pointed out that, as in the case of the ovine ICSH-α, the reaction of RA-ICSH with cyanogen bromide was incomplete and thus gave difficulties in isolating the fourth CNBr fragment.

The number of amino acid residues in the proposed primary structure of human ICSH-α (Figure 1) is in good agreement with the composition as recorded in Table 1. Human ICSH is reported to contain 1-2 residue(s) of tryptophan (15), while this amino acid is absent in the ovine hormone (12, 13). Preliminary color tests have indicated that tryptophan is present

in ICSH-β; our data herein reported support this conclusion.

There are 89 amino acid residues in human ICSH-α, whereas ovine α subunit has 96 residues. Approximately 70% of these residues are occupied at identical positions as shown in Figure 2. In addition, 21% of the amino acids can be replaced by a single base change in the codon.

Recent structural investigations on the subunits of ovine ICSH (12, 13) and bovine thyrotropin (TSH) (16) have revealed several interesting features of the two hormones. While the amino acid sequence of the α subunit is virtually identical, a great deal of homology can also be found in the hormonal specific β subunits. The remarkable similarity in the amino acid composition (see Table 1) of human TSH-α (16) and ICSH-α leads to the speculation that the amino acid sequence of human TSH-α will nearly be the same as that of human ICSH-α reported herein. From the amino acid composition (see Table 1) of the α subunit of human chorionic gonadotropin (HCG), it may also be expected that a homology* occurs between HCG-α and human ICSH-α.

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* Indeed, this is the case as revealed to us by Dr. Om P. Bahl (private communication.)

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