HUMAN PITUITARY THYROTROPIN: ISOLATION AND CHEMICAL CHARACTERIZATION OF ITS SUBUNITS

by

M. R. Sairam and Choh Hao Li

Hormone Research Laboratory
University of California, San Francisco
San Francisco, California 94122

Received February 1, 1973

SUMMARY. The subunits of human pituitary thyrotropin have been separated and purified by countercurrent distribution and exclusion chromatography. The NH_2-terminal sequence of the β subunit is identical to that of the β subunit of bovine thyrotropin. However, amino acid composition and peptide map of tryptic and chymotryptic digests as well as compositions of tryptic and chymotryptic peptides suggest that the amino acid sequence of the α subunit is identical to that of the α subunit of human interstitial cell stimulating hormone.

INTRODUCTION

Human pituitary thyrotropin was first prepared in highly purified form by Condliffe (1) in 1963. In a recent conference, Pierce et al (2) reported the separation of HTSH[1] into two subunits by column chromatography after treatment with propionic acid as previously described (3) for bovine TSH. This communication presents a countercurrent distribution procedure for the separation of the two subunits of HTSH and chemical data for their characterization. It will be seen that the data are consistent with earlier predictions (4) that the amino acid sequence of HTSH-α is identical to that of human ICSH-α.

---

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

MATERIALS AND METHODS

HTSH was isolated by procedures of Condiffe (1) and Stockell-Hartree (5) with minor modifications from fresh pituitary glands. From 1000 fresh glands, a yield of approximately 20 mg HTSH was obtained.

Amino Acid analyses were performed on the Beckman automatic analyzer according to Spackman et al (6). Other procedures such as dansyl-Edman degradation, performic acid oxidation, enzymic digests and two dimensional paper chromatography-electrophoresis were carried out as previously described (7).

For separation of the subunits, 60 mg of the hormone was dissolved in 10 ml 0.05 M HCl containing 8 M urea (pH 2.8). After 24 hours at 22° C, the protein was recovered by desalting on a column of Sephadex G-25 equilibrated in 0.1 M acetic acid and lyophilization. 50 mg of the treated HTSH was dissolved in 15 ml of the lower phase of the solvent system (8) consisting of 40% $(NH_4)_2SO_4$(w/v):0.2% dichloroacetic acid; n-propanol: ethyl alcohol (60:60:27:33). A small amount (0.2 ml) of 2% dichloroacetic acid was added to facilitate solution, which was further incubated for 2 hours at 40° C. The mixture was then submitted to 9 transfers in centrifuge tubes. The small amount of precipitation that occurred at the interphase on mixing with the upper phase in the first tube was retained along with the lower phase. Under these conditions the subunits of the hormone distributed effectively between the two phases. The lower phases of tubes 0-4 (HTSH-α) and upper phases of tubes 5-9 (HTSH-β) were pooled (see Figure 1), concentrated on a rotary evaporator and dialyzed extensively. The fractions were further purified on Sephadex G-100 equilibrated in 0.05 M ammonium bicarbonate. It was found necessary to chromatograph HTSH-α twice on

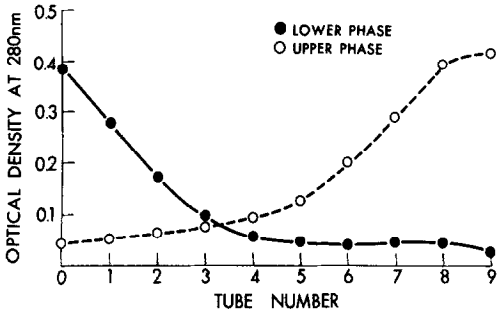

Figure 1: Countercurrent distribution of urea-treated HTSH (50 mg). The lower phases of tubes 0-4 represent the α-fraction and the upper phases of tubes 5-9 represent the β-fraction.

Sephadex G-100 to obtain the final product. The yields of the α and β subunits were 13 mg and 8 mg respectively.

For two dimensional chromatography-electrophoresis of HTSH-α, 2 mg each of the performic acid oxidized material were digested separately with trypsin and chymotrypsin in 0.5 M NH_4HCO_3 for 90 mins. at 37° C with an enzyme:substrate ratio of 1:30. After chromatography-electrophoresis, 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 and put in a test tube containing 1 M NH_4OH. After 24 hours the eluate was air dried and the amino acid composition determined.

RESULTS AND DISCUSSION

It is evident from Figure 1 that the countercurrent procedure is effective in separating the α and β subunits of HTSH. Table 1 presents the amino acid composition of HTSH and its subunits. These values are in agreement with the preliminary data reported by Pierce et al (2). There are significant differences in the compositions between the α and β subunits. The NH_2-terminal amino acid sequence of HTSH-α was found to be H-Val-Glx-Asx-Cys- whereas the sequence of the β subunit was H-Phe-Cys-Ile-Pro-Thr-Glu-Tyr-Met-.

TABLE 1

Amino Acid Composition (Molar Ratio) of Human TSH and Its Subunits

Amino Acid	HTSH	HTSH-α*	HTSH-β
Lysine	12.2	5.1 (5.8)	7.8
Histidine	5.6	2.5 (2.5)	3.0
Arginine	8.0	2.9 (3.0)	5.9
Aspartic acid	16.1	5.5 (5.4)	11.8
Threonine	18.3	8.2 (8.5)	12.7
Serine	12.4	7.6 (7.8)	6.5
Glutamic Acid	19.4	9.4 (7.5)	9.6
Proline	17.0	6.2 (5.6)	10.7
Glycine	12.4	4.6 (4.3)	6.0
Alanine	13.5	4.6 (4.2)	7.8
Half-cystine	20.6	9.2 (10.2)	14.0
Valine	14.3	6.6 (6.4)	5.4
Methionine	4.4	2.3 (2.5)	2.1
Isoleucine	10.0	1.4 (1.3)	9.7
Leucine	12.0	4.0 (4.0)	8.0
Tyrosine	13.8	3.7 (4.0)	11.6
Phenylalanine	9.0	3.8 (3.5)	5.0
Tryptophan	0.0	0.0 (0.0)	0.0

* Values in parentheses for human ICSH-α, taken from (4).

It may be noted that the NH_2-terminal sequence of HTSH-β is identical with that of bovine TSH-β (9) while the sequence of HTSH-α is identical with that of human ICSH-α (4). The carboxypeptidase A digestion of HTSH-α liberated tyrosine, serine, lysine, and histidine; under the same condition, these same amino acids were released from human ICSH-α. In the case of HTSH-β, digestion with carboxypeptidase A released only tyrosine, serine and lysine; histidine was not detected.

As pointed out earlier (2,4), the amino acid content of HTSH-α is remarkably similar to that of the α subunit of human ICSH (see Table 1). The two

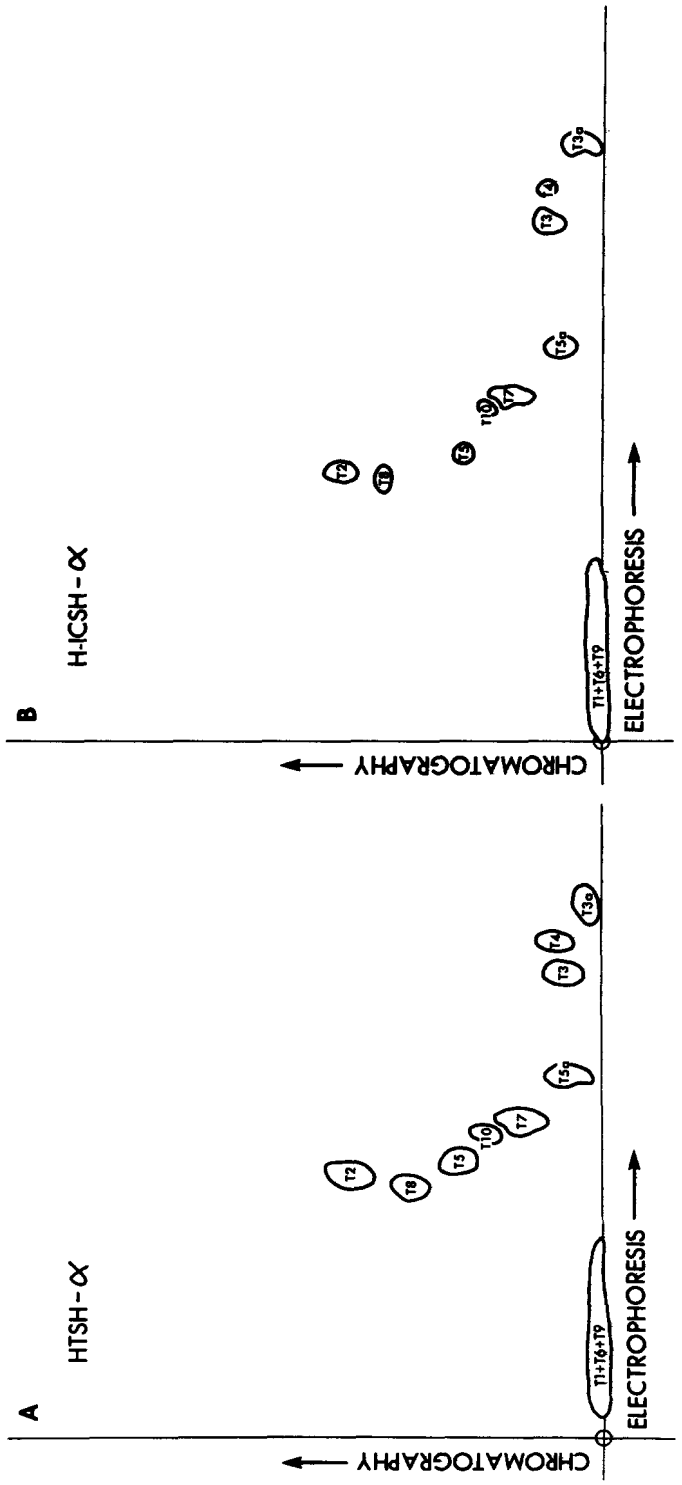

Figure 2: Two dimensional chromatography-electrophoresis patterns of tryptic digests of (A) oxidized HTSH-α and (B) oxidized human ICSH-α. Chromatography in solvent system n-butanol/acetic acid/water (4/1/5 by volume) and electrophoresis in pH 2.0 formic acid-acetic acid buffer for 60 mins. at 2000 volts.

TABLE 2

Amino acid composition (molar ratio) of peptides isolated from the Tryptic and chymotryptic maps of oxidized HTSH-α

Peptide No.*	Composition	Total Residues	Human ICSH-α (residue nos.)**
	Tryptic Peptides		
T1 + T6 + T9	$Lys_{1.9}$ $His_{3.0}$ $Arg_{1.0}$ $Cya_{8.5}$ $Asx_{4.0}$ $Thr_{5.1}$ $Ser_{4.6}$ $Glx_{7.6}$ $Pro_{3.5}$ $Gly_{3.1}$ $Ala_{3.0}$ $Val_{4.4}$ $Ile_{1.0}$ $Lue_{1.5}$ $Tyr_{2.0}$ $Phe_{3.0}$ $Met_{1.8}$	58-61	1-32, 49-60, 73-88
T2	$Ala_{0.9}$ $Tyr_{0.9}$ $Pro_{2.0}$ $Thr_{1.1}$ $Leu_{1.1}$ $Arg_{1.0}$	7	33-39
T3	$Ser_{0.7}$ $Lys_{1.0}$	2	40-41
T3a	$Ser_{0.8}$ $Lys_{2.0}$	3	40-42
T4	Free Lys	1	42
T5	$Thr_{0.7}$ $Met_{1.0}$ $Leu_{1.0}$ $Val_{0.8}$ $Glx_{1.0}$ $Lys_{1.0}$	6	43-48
T5a	$Lys_{2.0}$ $Thr_{0.8}$ $Met_{0.7}$ $Leu_{0.9}$ $Val_{1.0}$ $Glx_{0.9}$	7	42-48
T7	$Ser_{0.6}$ $Tyr_{0.7}$ $Asx_{1.0}$ $Arg_{0.8}$	4	61-64
T8	$Val_{2.0}$ $Thr_{0.8}$ $Met_{1.0}$ $Gly_{2.0}$ $Phe_{1.0}$ $Lys_{1.0}$	8	65-72
T10	Free Ser	1	89
	Chymotryptic Peptides		
C1	$Ser_{0.7}$ $Arg_{0.8}$ $Ala_{1.0}$ $Tyr_{0.8}$ $Pro_{2.0}$ $Thr_{1.0}$ $Leu_{1.2}$	8	31-38
C2	$Arg_{0.7}$ $Ser_{1.0}$ $Lys_{2.0}$ $Thr_{1.0}$ $Met_{1.0}$	6	39-44
C3	$Asx_{0.9}$ $Arg_{1.0}$ $Val_{1.9}$ $Thr_{0.9}$ $Met_{1.0}$	6	63-68
C4	$Gly_{2.0}$ $Phe_{1.2}$	3	69-71
C5	$Tyr_{0.7}$ $His_{1.0}$ $Lys_{1.0}$ $Ser_{1.0}$	4	86-89

*See Figure 2.

**See reference (4).

dimensional chromatography-electrophoresis patterns of the tryptic digest of oxidized HTSH-α and human ICSH-α are indistinguishable as shown in Figure 2. The same is true for the chymotryptic pattern. As summarized in Table 2, the amino acid composition of tryptic and chymotryptic peptides, which could be isolated from finger prints of oxidized HTSH-α, are identical to that obtained from human ICSH-α.

From the above data, it may be assumed that the amino acid sequence of HTSH-α is nearly identical with the α subunit of human ICSH. This assumption is not unreasonable as it has been shown in the bovine pituitary gland that the α subunits of the TSH and ICSH molecules have identical amino acid sequence (10).

ACKNOWLEDGMENT

We thank Jean Knorr and Daniel Gordon for 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.

REFERENCES

1. Condliffe, P. G., Endocrinology 72, 893, 1963.
2. Pierce, J. G., Liao, T. H., Howard, S. M., Shome, B., and Cornell, J. S., Rec. Prog. Hor. Res. 27, 165, 1971.
3. Liao, T. H., and Pierce, J. G., J. Biol. Chem. 245, 3275, 1970.
4. Sairam, M. R., Papkoff, H., and Li, C. H., Biochem. Biophys. Res. Comm. 48, 530, 1972.
5. Stockell-Hartree, A., Biochem. J. 100, 754, 1966.
6. Spackman, D. H., Stein, W. H., and Moore, S., Anal. Chem. 30, 1190, 1958.
7. Li, C. H., Dixon, J. S., Lo, T. B., Schmidt, K., and Pankov, Y., Arch. Biochem. Biophys. 141, 705, 1970.
8. Papkoff, H., and Samy, T. S. A., Biochem. Biophys. Act. 147, 175, 1967.
9. Liao, T. H. and Pierce, J. G., J. Biol. Chem. 246, 850, 1971.
10. Pierce, J. G., Liao, T. H., Carlsen, R. B., and Reimo, T., J. Biol. Chem. 246, 866, 1971.