THE SUBUNITS OF BOVINE THYROTROPIN. THEIR ISOLATION AND COMPARISON WITH THE SUBUNITS OF LUTEINIZING HORMONE

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1. Introduction

Ultracentrifugation in a dissociating medium and countercurrent distribution have demonstrated that luteinizing-hormone** is made of two non-identical subunits of similar molecular weight [1–3]. Several attempts to dissociate thyrotropin (thyroid-stimulating hormone**) into subunits by the above procedures had previously failed to provide similar evidence of quaternary structure [4, 5].

In this report, a procedure for the dissociation of TSH into subunits is described. The isolation and partial characterization of the individual polypeptide chains has allowed comparison with the subunits of LH, referring to the recently demonstrated homology between the two hormones [6]. The effect of dissociation and reassociation of the subunits of TSH on the thyroid-stimulating potency has been investigated as well as hybridization of complementary subunits from both hormones.

2. Materials and methods

2.1. *Hormone preparations*

Bovine TSH and LH were purified from a crude extract (Ambinon Organon, 0.5 IU/mg) by sequential chromatography on carboxymethyl-cellulose and diethylaminoethyl-(DEAE)-cellulose as described by Bates and Condliffe [7], with some modifications.

TSH, recovered from DEAE-cellulose by elution with 0.5 M sodium chloride was finally chromatographed and rechromatographed on Sephadex G-100 (Pharmacia) in 0.05 M ammonium bicarbonate. The terminal material, eluted as a unique symmetrical peak, exhibits a specific activity of 50 IU/mg as determined by bioassay in mice [8] using the international standard as reference.

LH, recovered from the fraction not adsorbed on DEAE-cellulose, was further purified by the gel filtration procedures used for TSH. The terminal preparation eluted, as a unique symmetrical peak, exhibits a specific activity of 3 LH U/mg, as determined by the ovarian ascorbic acid depletion test of Parlow [9] with NIH LH S15 as a standard.

2.2. *Isolation of subunits*

TSH (1 g) was dissolved in 0.1 M sodium acetate, pH 4.8, 8 M urea and charged on a 2.5 × 37 cm column of SE-Sephadex C-25 (Pharmacia) equilibrated with the same buffer. After collection of the non-adsorbed fraction, 0.3 M sodium acetate was applied, pH and urea concentration being kept constant. The eluted material was desalted by gel filtration on Sephadex G-25 (Pharmacia) in 0.05 M ammonium bicarbonate, and lyophilized. The dry powder (500 mg) was dissolved in 10 ml and immediately submitted to gel filtration on Sephadex G-100, 0.05 M ammonium

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** *Abbreviations:*
LH : Luteinizing hormone
TSH: Thyroid stimulating hormone.

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bicarbonate (5 X 100 cm column, Pharmacia). Besides an eluted material which was discarded, two well-resolved peaks were eluted. The main central portions of each were selected and lyophilized. The first peak exhibits a marked asymmetry due to a material of higher molecular weight; the asymmetry is not modified by repeated gel filtration. At the top of the peak, the calculated V_e is identical to that given for native TSH, under similar experimental conditions. The preparation corresponding to the peak eluted first has been called TSH II while that corresponding to the second has been called TSH I. The separation of LH subunits was performed by chromatography on SE-Sephadex C-25, as described elsewhere [10]. The subunits of LH were called CI and CII, as suggested by others [5].

The individual subunits of TSH and LH were analyzed by electrophoresis in urea-starch gel, made 0.1 M with mercaptoethanol, as previously described [11] and studied for their amino acid [12] and sugar [13] compositions, peptide maps [14], and thyroid-stimulating activity. Free sulfhydryl groups have been determined according to Ellman [15].

Molecular weights were determined by low speed sedimentation equilibrium of 0.5% protein solutions in a Spinco model E ultracentrifuge [16].

3. Results and discussion

3.1. *Characterization of the subunits*

Fig. 1 gives the electrophoretic patterns of the individual subunits of TSH and LH. Electrophoretic heterogeneity is observed for the subunits of both hormones as already noted for bovine LH subunits [5].

The molecular weights of TSH, LH and their subunits are given in table 1. Contrary to LH, TSH is only partially dissociated in 8 M urea. LH CI and CII have very similar molecular weights, in agreement with earlier results [3]. The molecular weight of TSH I is comparable to that of the LH subunits and accounts for half the molecular weight of native TSH. The recorded molecular weight for TSH II appears to be twice that of TSH I.

The difference in composition between TSH I and II (table 2) rules out the possibility that TSH II corresponds to an undissociated fraction of the hormone. Under the above conditions of gel filtration, TSH II associates as a dimer together with higher aggregates. In 8 M urea, pH 4.8, sedimentation equilibrium reveals an almost homogeneous solution of a material probably representing the dimer of TSH II. The compositions of both LH and TSH subunits are compared in

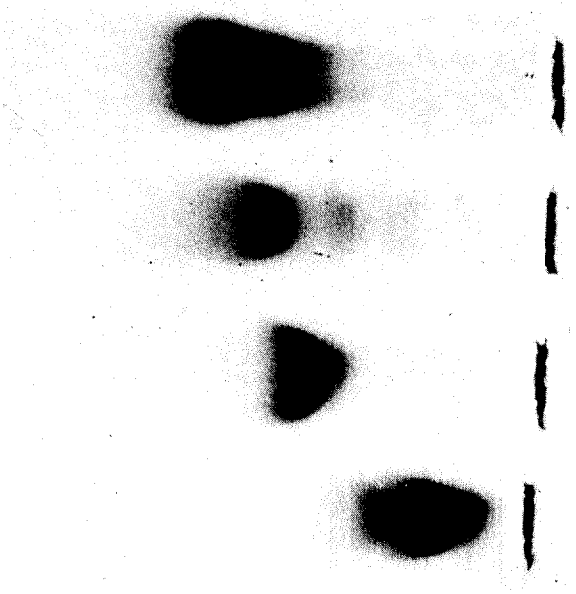

Fig. 1.

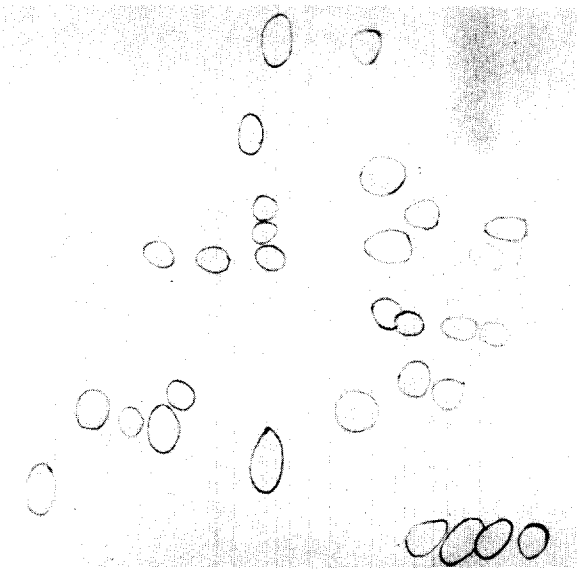

Fig. 2a.

Table 1
Molecular weights of bovine TSH and LH and their subunits.

Preparations	Buffers	Molecular weights
TSH	0.1 M sodium acetate	32,000*
TSH	0.1 M potassium chloride, 8 M urea	20,000–25,000**
TSH	0.1 M sodium acetate, pH 4.8, 8 M urea	19,000–23,000**
TSH I	0.1 M sodium acetate, pH 4.8, 8 M urea	15,000*
TSH II	0.1 M sodium acetate, pH 4.8, 8 M urea	33,000*
LH	0.1 M potassium chloride, 8 M urea	33,000*
LH	0.1 M potassium chloride, 8 M urea	14,500*
LH CI	0.1 M sodium acetate, pH 4.8, 8 M urea	14,500*
LH CII	0.1 M sodium acetate, pH 4.8, 8 M urea	13,500–14,500**

* Extrapolated to $C = O$ from plots of $\frac{1}{M}$ versus C.
** Range of values obtained from the meniscus to the bottom of the column.

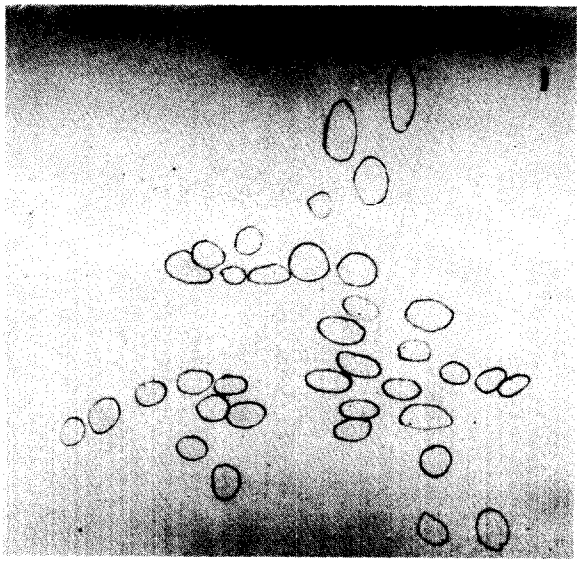

Fig. 2b.

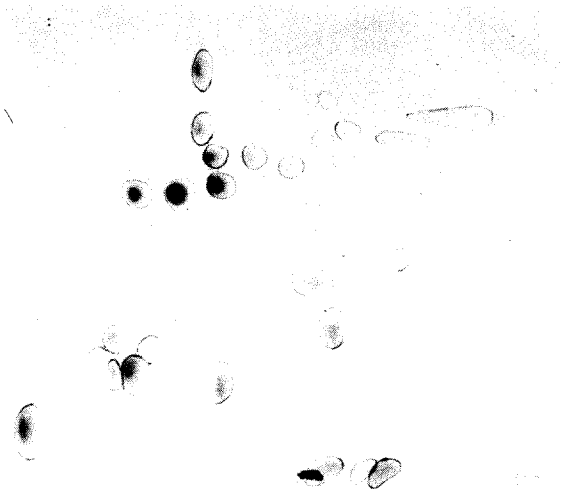

Fig. 2c.

Table 2
Composition of the subunits of bovine TSH and LH.

	TSH_{II}	TSH_{I}	LH C_{I}	LH C_{II}
	Residues per 100 residues*			
Amino acids				
Lysine	10.7	9.8	9.8	2.2
Histidine	3.3	2.7	3.0	2.2
Arginine	4.1	3.4	3.8	6.2
Aspartic acid	9.9	7.7	6.0	4.5
Threonine	4.5	9.0	9.1	6.3
Serine	5.0	5.4	6.4	6.9
Glutamic acid	14.2	8.2	8.5	5.9
Proline	5.1	6.2	8.3	16.3
Glycine	4.0	4.0	4.6	6.1
Alanine	7.4	6.4	7.5	7.1
½ Cystine	5.0	10.5	10.2	9.8
Valine	5.2	5.3	5.2	6.3
Methionine	0.9	3.2	3.7	2.5
Isoleucine	3.3	4.3	2.2	3.4
Leucine	10.2	3.5	3.3	9.2
Tyrosine	3.1	6.7	4.2	1.9
Phenylalanine	4.2	3.7	4.1	2.6
*Sugars***				
Total amount*** (μg/mg)	53	126	152	109
Fucose (μmole/mg)	–	0.046	0.037	0.060
Mannose (μmole/mg)	–	0.270	0.463	0.232
Galactose (μmole/mg)	0.023	–	traces	–
Glucose (μmole/mg)	0.307	0.081	traces	–
N-Acetyl-glucosamine (μmole/mg)	–	0.210	0.288	0.191
N-Acetyl-glucosamine (μmole/mg)	–	0.098	0.112	0.120

* Hydrolyses were performed for 24, 48 and 96 hr at 110°.

Values for serine, valine and isoleucine are corrected. Compositions are expressed as mean of duplicate analyses.

** Arabinose is used as internal standard for neutral sugar analyses instead of glucose, as described in the original assay method [13].

*** Values expressed per mg without correction for moisture and ash.

table 2. LH subunits differ markedly in their composition, as described previously [3]; the dissimilarities between TSH I and II are also striking considering their threonine, glutamic acid, cystine, methionine, leucine and tyrosine contents. No free sulfhydryl groups have been detected in either material. The total sugar content is higher in TSH I than in TSH II. Moreover, in the sugar portion of TSH II, glucose is the only carbohydrate present in significant amount. The sugar compositions for LH CI and CII agree with those presented by others [5].

The peptide maps of TSH I and TSH II, together with their respective amino acid compositions, make it clear that those subunits represent different proteins (fig. 2). The common patterns between the maps of LH CI and CII are of questionable significance, since they could be due to some degree of cross contamination (fig. 2). Several peptides appear to be com-

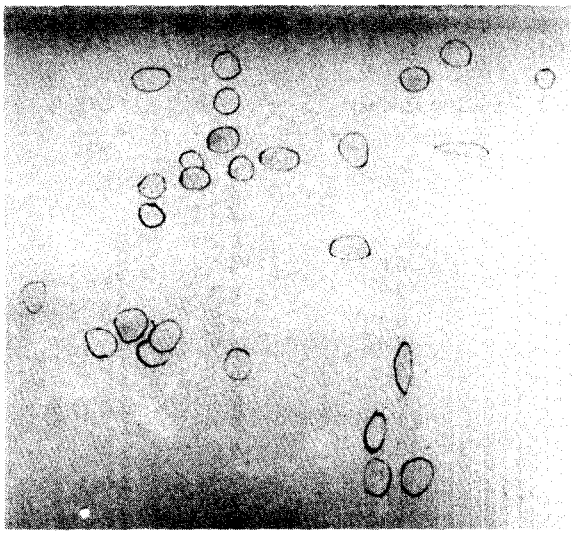

Fig. 2d.

mon to TSH I and LH CI, in agreement with similarities in the patterns of their amino acid compositions (fig. 2). TSH and LH CI thus appear as possible homologous proteins, although certainly not identical.

3.2. *Thyroid-stimulating activity of isolated subunits*

Isolated TSH II has a very low (if any) biological potency (table 3). The specific activity of TSH I accounts for 10% of that of intact TSH. Like TSH II, LH CII is devoid of any significant biological potency, while LH CI exhibits a significant thyroid-stimulating activity, 50 times less than that of TSH I. Thyroid-stimulating activity, as an intrinsic property, is thus not restricted to materials originating from TSH.

3.3. *Reconstitution of TSH from isolated subunits*

TSH II (140 mg), together with an excess of TSH I (310 mg), was dissolved in 17 ml of 0.05 M ammonium bicarbonate and kept for one hr at room temperature. The mixture was applied to a 5 × 100 cm column (Pharmacia) of Sephadex G-50 (Pharmacia),

Table 3
Thyroid-stimulating activity of isolated or co-incubated subunits of TSH and LH.

Preparations*	Concentrations	Biological activities (IU/mg)
TSH	0.1%	50.0
TSH I	0.1%	5.0
TSH II	0.1%	0.005
TSH, reconstituted and isolated by gel-filtration	0.1%	40.0
TSH I + TSH II	0.1% each	35.0
TSH I + TSH II	0.05%(I) and 0.1%(II)	30.0
LH	0.1%	0.007
LH CI	0.1%	0.1
LH CII	0.1%	0.005
LH CI + TSH II	0.1% each	0.8
LH CI + TSH II	0.05%(CI) and 0.1 M (TSH II)	1.2
LH CII + TSH I	0.1% each	4.0

* All preparations were kept in 0.15 M sodium chloride at 4° for 20 hr before bioassay.

and gel filtration was carried out at 4° in 0.05 M ammonium bicarbonate. Two partially overlapping peaks were eluted. The material corresponding to the overlap has not been studied. The amino acid composition and the electrophoretical pattern of the rapidly excluded material are almost identical to those of native TSH, while specific activity represents 80% of that of the native hormone. It is thus reasonable to assume that the present procedure has reconstituted an appreciable amount of TSH from its individual subunits. The material corresponding to the second peak is electrophoretically and biologically identical to TSH I. Table 3 gives the biological potencies of various preparations obtained by co-incubation of the subunits. Co-incubation of TSH I and TSH II restores most of the initial biological potency of intact TSH, as already demonstrated for LH [5]. A specific quaternary organization of TSH subunits is therefore compulsory for full biological efficiency.

Tentative hybridation of TSH I plus LH CII yields a preparation whose thyroid-stimulating activity does not differ significantly from that expected from the individual active subunit itself. LH CI plus TSH II yields preparations markedly more potent than isolated LH CI. This suggests a possible association between complementary subunits originating from different hormones. It must be pointed out that native LH, representing the original association of LH CI plus CII, is practically devoid of thyroid-stimulating activity. Therefore the native association of the thyroid-stimulating subunits CI with its complementary subunit CII cancels the thyrotropic activity of LH CI.

Although thyroid-stimulating activity, as an intrinsic property, is not restricted to the first TSH subunit, only certain combinations result in a highly potent, thyroid-stimulating material. The thyroid-stimulating activity of LH CI, although potentiated by its probable association with TSH II, is not quantitatively comparable to the activity resulting from the association of TSH I and II. It must again be emphasized that, despite the homology of TSH I and LH CI, differences in their respective biological activities correspond to physico-chemical differences.

4. Conclusion

Both LH and TSH are structurally organized according to a very similar model, associating to form two different subunits of identical (or almost identical) molecular weight. While some degree of thyroid-stimulating activity can be attributed to one subunit of TSH, full hormonal activity depends on the integrity of quaternary structure. The common thyroid-stimulating properties of TSH I and LH CI correspond with their chemical homology, quantitative differences of specific activity being related to chemical differences. The structural similarity between TSH I and LH CI subunits is sufficient to allow association between LH CI and TSH II subunits with a resulting increase in thyroid-stimulating activity. On the other hand, the natural association of LH CI with its complementary subunit LH CII results in a suppression of its intrinsic thyroid-stimulating activity. This emphasizes, once more, the dominant role of quaternary structure for specific hormonal activity.

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