OVINE FOLLICLE STIMULATING HORMONE: PREPARATION AND CHARACTERIZATION OF ITS SUBUNITS

Harold Papkoff and Margareta Ekblad

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

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SUMMARY. Ovine follicle stimulating hormone (FSH) can be dissociated into its subunits and the subunits isolated by treating the hormone with 1 M CH_3CH_2COOH followed by gel filtration. The subunits, α and β, are individually of low biological activity; recombination of the subunits restores an appreciable amount of FSH activity. The subunits have been characterized by disc electrophoresis, amino acid analysis and sugar content. In addition it was found that combination of interstitial cell stimulating hormone (ICSH, LH) subunit CII with FSH-β generates ICSH activity and ICSH-subunit CI with FSH-β generates FSH activity.

INTRODUCTION

Studies on the pituitary glycoprotein hormones, ICSH and thyrotropin (TSH) have shown that they each consist of two chemically dissimilar subunits (1-5). In the case of bovine TSH, it was also shown that one of the subunits was similar, if not identical to the CI subunit of ICSH (6). The remaining pituitary glycoprotein hormone, FSH, is shown by the studies reported here to also consist of subunits. We have previously reported that when purified ovine FSH is examined in the ultracentrifuge, the sedimentation coefficient drops from 3.04S in 0.1 M N_2HCO_3 to 1.54S when examined at pH 1.3, suggestive of a dissociation reaction (7). We show in these studies that the methodology employed for the preparation of the TSH subunits by Pierce and Liao (6) can be applied to FSH as well.

METHODS AND MATERIALS

Ovine FSH was prepared by a modification (to be published) of the previously described procedure (7). Gel filtration experiments were run at 4°. All chemicals employed were reagent grade. FSH activity was determined by the Steelman-Pohley assay (8) and ICSH activity by the ovarian ascorbic acid depletion (OAAD) test (9). Multiple dose assays were performed with standard preparations as reference and the results analyzed by standard statistical methods. The standard gonadotropin preparations were a gift of the Endocrinology Study Section of the National Institutes of Health. Sugar analyses were standard techniques we have previously employed (5). Other techniques of analysis will be cited in the text.

RESULTS AND DISCUSSION

Dissociation and preparation of the subunits of FSH. Ovine FSH was dissolved in 1 M CH_3CH_2COOH at a concentration of 10 mg/ml and allowed to stand at room temperature for 24 hr, after which the acid solution was lyophilized. The dried product was dissolved in 0.5 ml of 0.05 M NH_4HCO_3 and applied to a Sephadex G-100 column equilibrated with 0.05 M NH_4HCO_3. A typical pattern is shown in Figure 1. It is seen that three major peaks are now observed in contrast to the single peak obtained with native FSH which has not been treated with CH_3CH_2COOH. Re-chromatography of each of the fractions (Fig. 1) indicated that the first peak, αp, is a transformation product of the second peak, α, while the most retarded peak, β, does not display this behavior. FSH-α can be almost completely converted to FSH-αp by repeated treatment with CH_3CH_2COOH followed by gel filtration and it appears likely that αp is a polymeric form of α.

Characterization studies of FSH-αp, α and β. Due to the small

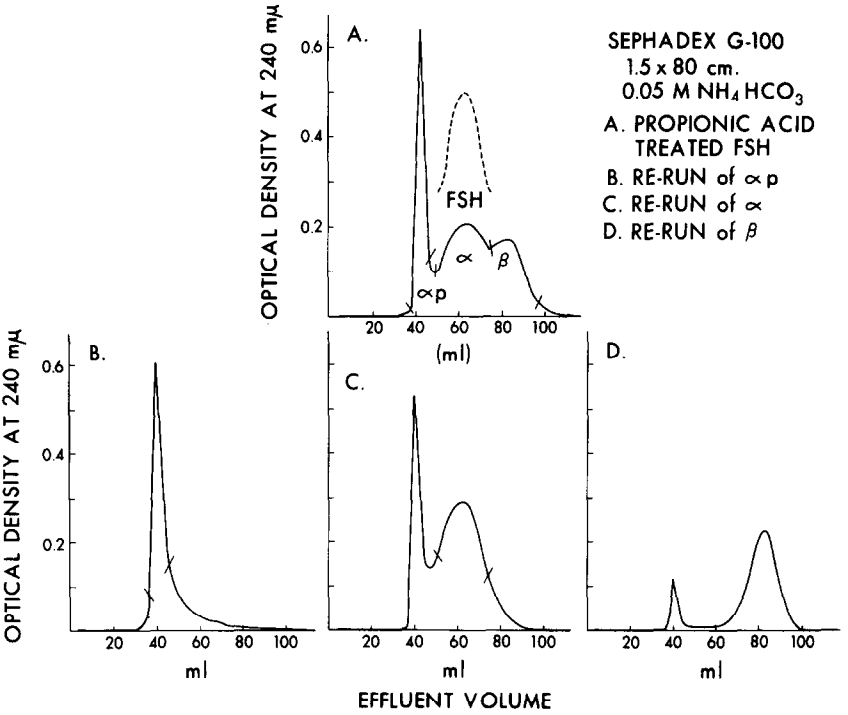

Fig. 1 Gel filtration of ovine FSH treated with propionic acid.

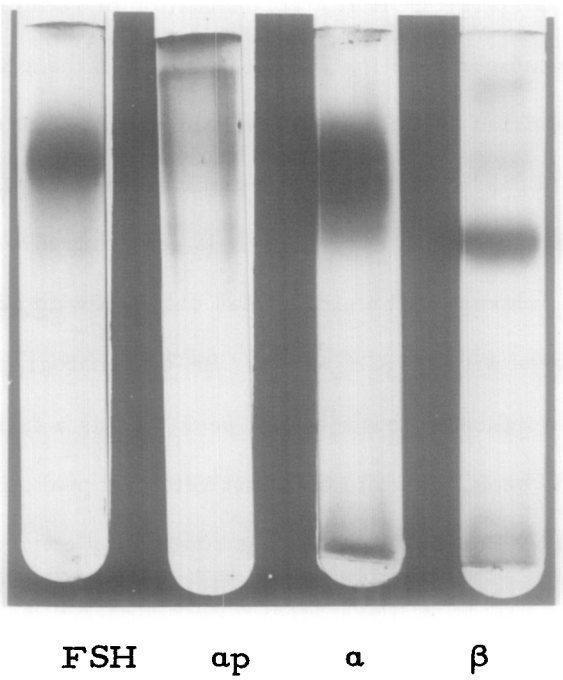

Fig. 2 Disc electrophoresis patterns of ovine FSH and its subunits.

amounts of the FSH subunits available, only limited characterization studies have thus far been attempted. The materials have been analyzed by the disc electrophoresis technique in 12% polyacrylamide gel at pH 8.3. The patterns obtained are seen in Figure 2. It is evident that FSH-α and FSH-β possess different electrophoretic properties relative to each other and to the native hormone.

Table I

CARBOHYDRATE CONTENT* OF OVINE FSH AND SUBUNITS

	FSH	αp	α	β	ICSH-CI	ICSH-CII
Hexose	5.7	2.8	2.8	13.8	8.3	4.3
Hexosamine	4.5	1.8	2.3	7.1	8.6	5.8
Sialic Acid	2.8	0.6	0.8	3.7	-	-

*g/100 g glycoprotein

Carbohydrate analyses are shown in Table I. FSH-αp and FSH-α are virtually identical and possess much less carbohydrate than FSH-β. In this respect, the FSH subunits resemble those of ICSH (1, 4, 5) where the ICSH-CI has approximately 2 x the sugar content of ICSH-CII.

Samples for amino acid analysis were prepared by hydrolysis in constant boiling HCl at 110° in sealed, evacuated tubes for 20 h. Analysis was by the method of Spackman et al. (10) in a Beckman Model 120B analyzer. The results are shown in Table II. Comparison of FSH-α with FSH-β show few major differences in amino acid composition. In the main, major differences are noted in the contents of lysine, threonine, cystine and leucine.

For comparative purposes, the composition of the ICSH subunits are also listed in Table I and it can be seen that ICSH-CI has a similar composition to FSH and the FSH subunits.

Table II

AMINO ACID COMPOSITION* OF OVINE FSH SUBUNITS

Amino Acid	FSH	αp	α	β	ICSH-CI	ICSH-CII
Lysine	8.6	8.2	10.5	7.8	9.6	1.9
Histidine	3.1	2.4	3.2	3.1	2.9	2.8
Arginine	4.2	4.4	4.5	4.0	2.9	6.6
Aspartic	9.7	11.2	11.2	10.0	6.7	4.7
Threonine	7.3	5.3	6.2	9.5	9.6	4.7
Serine	6.1	6.5	5.7	7.5	5.8	4.7
Glutamic	11.3	11.8	12.6	11.0	9.6	5.7
Proline	5.9	5.6	5.6	6.7	7.7	18.9
Glycine	5.2	5.6	4.7	4.9	4.8	5.7
Alanine	7.8	8.2	8.3	8.3	7.7	6.6
1/2 Cystine	6.1	3.0	4.9	8.0	9.6	9.4
Valine	6.7	6.8	5.5	6.3	5.8	7.6
Methionine	1.1	0.4	0.3	0.3	3.9	1.9
Isoleucine	3.1	3.5	2.7	3.6	1.9	3.8
Leucine	7.3	10.2	9.0	4.8	1.9	11.3
Tyrosine	3.3	2.2	2.7	3.8	4.8	0.9
Phenylalanine	4.1	5.0	4.2	3.8	4.8	2.8

*20 h. hydrolysis; Results are calculated as residues/100 residues analyzed

Bioassay studies of the FSH subunits. The results of two types of bioassay experiments are shown in Tables III and IV. In the first, the Steelman-Pohley assay (8) for FSH is employed to determine the potency of the FSH subunits. In Table III it is seen that FSH-α and FSH-β are relatively inactive (2.4-4.7 x NIH-FSH-S1) compared to the starting material (37.0 x NIH-FSH-S1). Activity can be regenerated by recombining the subunits, although full recovery was not achieved (25-50% of initial). This degree

Table III

BIOLOGICAL ACTIVITY OF THE SUBUNITS OF OVINE FSH

Preparation	Specific Activity*	95% C. L.	Precision, λ
Ovine FSH	37.2	30.9 - 43.0	0.060
FSH-αp	<1.2		
FSH-α	~2.4		
FSH-β	~4.7		
FSH-α + FSH-β**	Exp. 1 10.9	7.1 - 14.3	0.098
	10.3	5.3 - 14.9	0.145
	Exp. 2 11.5	5.8 - 16.5	0.140
	Exp. 3 21.7	10.7 - 39.1	0.188
	22.8	17.8 - 31.0	0.100
	Exp. 4 13.8	12.6 - 16.3	0.050
FSH-β + ICSH-CI**	16.6	10.1 - 22.9	0.125
	18.6	9.8 - 35.7	0.188
FSH-β + ICSH-CII**	7.6	5.8 - 9.6	0.089

*Steelman-Pohley HCG augmentation test; expressed in terms of NIH-FSH-S1.

**1 mg/ml of each in pH 5.4, 0.1 μ acetate at r.t. for 20 h.; Activity of ICSH subunits not determined; precursor ICSH < 0.005 x NIH-FSH-S1.

Table IV

GENERATION OF ICSH (LH) ACTIVITY FROM FSH-β AND ICSH-CII

Preparation	Specific Activity*	95% C. L.	Precision, λ
ICSH-CII	0.071	0.048 - 0.130	0.150
FSH-α + ICSH-CII**	0.08	0.06 - 0.10	0.105
FSH-β + ICSH-CII**	0.20	0.12 - 0.28	0.126
	0.21	0.16 - 0.27	0.097
	0.21	0.14 - 0.29	0.122

*Ovarian ascorbic acid depletion test; expressed in terms of NIH-LH-S1.
**1:1 of each subunit incubated in pH 5.4, 0.1 μ acetate at r.t. for 20 h.; Activity of FSH subunits not determined; precursor FSH had an activity of 0.034 x NIH-LH-S1.

of activation, however, is highly significant in view of the inertness of the individual subunits. It was of interest to determine whether addition of the ICSH subunits to the FSH subunits would result in the generation of FSH activity. Addition of either ICSH-CI or ICSH-CII to FSH-α was without effect. However, the addition of ICSH-CI to FSH-β resulted in a highly significant generation of FSH activity (18 x NIH-FSH-S1). Table III also shows a smaller, but possibly significant generation of FSH activity with ICSH-CII and FSH-β.

In the second test, the ICSH subunits are combined with the FSH subunits and tested for ICSH activity. It is seen (Table IV) that significant ICSH activity (0.2 x NIH-LH-S1) can be generated by the combination of ICSH-CII and FSH-β. This is a somewhat remarkable observation insofar as it suggests that the FSH-β subunit possesses structural features which

can give rise to either FSH or ICSH activity, depending upon the subunit with which it is combined. Combining ICSH-CI with either FSH-α or FSH-β did not result in the generation of significant ICSH activity. The low level of activity observed with FSH-α and ICSH-CII (Table IV) is not significant We have recently reported (11) that ICSH activity (0.55 x NIH-LH-S1) can be generated from native FSH and ICSH-CII.

In conclusion, we have shown that ovine FSH can be dissociated into subunits which differ in their carbohydrate and amino acid content. They are individually inactive, but recombination results in the re-gain of biological activity.

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