Amino Acid Composition and Molecular Weight of Human Follicle Stimulating Hormone[1] (36311)

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Conflicting reports in regard to the biological potency of purified human pituitary follicle stimulating hormone (FSH) preparations have appeared in the literature (1–4). The potencies of such preparations have ranged from 45 to about 500 times NIH-FSH-SI. Reports on the chemical composition of FSH similarly contain significant discrepancies (1–5). Studies of human FSH, therefore, continue to be of interest.

We have obtained a homogenous human FSH preparation, by employing a series of simple column fractionation steps, and have examined some of its physicochemical characteristics.

Methods, Results and Discussion. Acetone-dried human pituitary powder was extracted and purified according to the procedure of Hartree (6). The crude material was further purified on a column of DEAE-C (2), followed by fractionation on Sephadex G-100, and then rechromatography on DEAE-Sephadex (4). Final purification was effected by absorption onto sulfoethyl Sephadex (7). Inactive material was removed by stepwise elution with acetate buffer, ph 4, ionic strength 0.03, and phosphate buffer, pH 6, ionic strength 0.03. FSH eluted with 0.01 *M* $NA_2\ HPO_4$, was exhaustively dialyzed against distilled water at 4° and stored frozen at −70°.

Biological assay for FSH was performed by the method of Steelman and Pohley (8), as modified (9). Biological assay for LH was performed by the method of Parlow (10). All solutions used for bioassay were thawed and appropriately diluted immediately prior to injection. The dosage was based on the quantity of FSH determined either by the Lowry and co-workers' method (11) or by obtaining the dry weight of the freshly lyophilized material. The value from the former procedure corresponds to 78% of the dry weight (12).

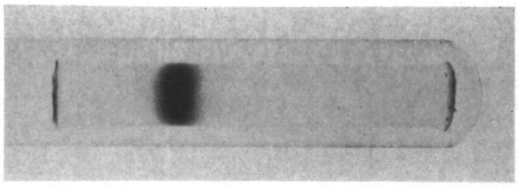

Figure 1.

The purified FSH has a biologic potency of 120X NIH-FSH-S1/mg. LH contamination was 0.24X NIH-LH-S1. The FSH preparation was homogeneous by the criterion of polyacrylamide gel electrophoresis, in which it moved as a single broad band at pH 8.3 (Fig. 1).

The Stokes' radius of FSH was calculated from gel filtration experiments as described by Siegel and Monty (13). A linear calibration graph was obtained when the experimentally determined K_{av} values of reference proteins (nonenzymatic protein mol wt markers, Mann Research Laboratories) were plotted against their known Stokes' radii. The FSH was labeled with ^{131}I and eluted from Sephadex G-100. The fractions were assayed for radioactivity in an automatic gamma counter. The value for Stokes' radius, obtained from the experimentally determined K_{av} value was 32.0 Å which is in the agreement with the value of 32.2 Å found earlier

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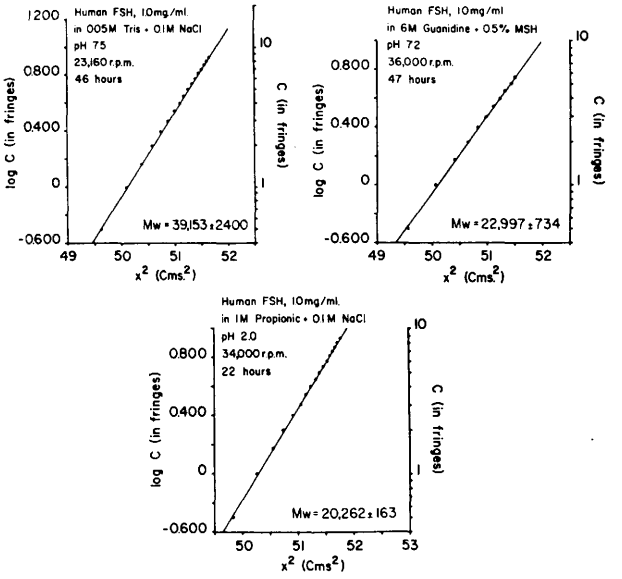

FIGURE 2.

by similar technique (12).

The weight-average molecular weight was determined in the Spinco Model E ultracentrifuge at 20° by the high speed equilibrium method of Yphantis (14). A partial specific volume ($\bar{V}$) of 0.696 ml/g was used for these calculations (15). The results plotted as log C versus r^2 (where C is the protein concentration in fringes and r is the distance from the axis of rotation) yielded rectilinear graphs for the native FSH as well as the dissociated species (Fig. 2).

Samples of FSH were dissolved and equilibrated in 0.05 *M* Tris buffer (ph 7.5), containing 0.1 *M* sodium chloride at 4° for 48 hr. Ultracentrifugation was performed for 46 and 70 hr at 23,150 and 27,690 rpm. The apparent weight-average molecular weight was calculated as 36,300 ± 2240.

Separate samples of the hormone were also equilibrated with 1 *M* propionic acid in 0.1 *M* NaCl. The weight-average molecular weight at 22 hr and 34,000 rpm was found to be 18,700 ± 130, indicating the dissociation of the molecule into subunits of half its original size.

Behavior of FSH in guanidine HCl was also examined. Ultracentrifugation studies were carried out in 6 *M* guanidine HCl containing 0.5% mercaptoethanol (pH 7.2) at 23, 47, and 71 hr, and 34,000 and 36,000 rpm. The molecular weight value under these conditions was 20,000 ± 645.

TABLE I. Amino Acid Composition of Human FSH.

Residues/36,000 mol wt.

Amino acid	Time of hydrolysis (hr): 24	48	72
Lysine	9.0	10.1	9.6
Histidine	4.2	4.5	4.9
Arginine	6.1	6.8	5.9
Aspartic acid	11.6	12.6	12.6
Threonine	15.1	15.6	14.9
Serine	11.6	12.3	10.6
Glutamic acid	15.0	16.4	16.5
Proline	9.6	10.0	10.4
Glycine	9.4	11.9	10.6
Alanine	8.3	9.0	9.9
Half cysteine	14.3	15.6	16.6
Valine	10.7	11.6	11.5
Methionine	2.4	2.5	2.7
Isoleucine	5.4	5.9	6.2
Leucine	7.9	8.8	8.8
Tyrosine	8.2	9.0	8.9
Phenylalanine	—	6.4	6.7

Samples of human FSH (0.5–0.6 mg) were dissolved in 6 *N* hydrochloric acid (0.5–0.6 ml) and hydrolyzed in sealed, evacuated tubes

at 110° for 24, 48, and 72 hr. The amino acid analysis was performed on a Beckman 120C amino acid analyzer. Norleucine was added as an internal standard prior to hydrolysis and the data shown in Table I have been corrected for norleucine recoveries. No other correction factors have, however, been applied. It is noteworthy that the molar ratios shown in Table I bear close resemblance to the data published earlier by Roos (2).

Summary. Examination of the physicochemical characteristics of homogenous human FSH, prepared in our laboratory, revealed a molecular weight of 36,300; dissociation in 1 *M* propionic acid into subunits with a molecular weight of 18,700; and an amino acid composition similar to that previously reported by Roos (2).

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