Primary Amino Acid Sequence of Follicle-stimulating Hormone from Human Pituitary Glands

I. α SUBUNIT*

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PREMILA RATHNAM AND BRIJ B. SAXENA†

From the Division of Endocrinology, Department of Medicine, Cornell University Medical College, New York City, New York 10021

Follicle-stimulating hormone of a high state of physicochemical and biological purity was isolated from acetone-preserved human pituitary glands. The follicle-stimulating hormone was dissociated into α and β subunits by treatment with 8 M urea and the subunits were separated by ion exchange chromatography on DEAE-Sephadex A-25. The subunits were freed of undissociated or reassociated follicle-stimulating hormone by gel filtration on Sephadex G-100.

For the establishment of the primary amino acid sequence, the α subunit was reduced and either carboxyamidomethylated or S-aminoethylated prior to a thermolytic or a tryptic digestion. Each digest was gel filtered on a column of Sephadex G-50 to separate the glycopeptides from the peptides. The glycopeptides and the peptides were purified further by sequential gel filtration on Sephadex G-25: G-13, G-10, and Bio-Gel P-2 and were isolated by high voltage electrophoresis at pH 6.35, and 2. The purity of the isolated peptides was ascertained further by amino acid analysis. The amino acid sequences of the peptides were determined by Edman degradation followed by subtractive amino acid analysis. COOH-terminal sequences were established by digestion with carboxypeptidases A and H. The primary amino acid sequence of human follicle-stimulating hormone-α is identical to that of human chorionic gonadotropin-α and differs from that of human luteinizing hormone-α in having the tripeptide Ala-Pro-Asx at the NH₂-terminal end.

Highly purified FSH† from human pituitary glands, suitable for structural studies, has been prepared by various investigators (1-6). The insufficient yields of FSH, and particularly of its subunits, however, have handicapped the determination of the primary structure. The availability of human pituitary glands from the National Pituitary Agency (Baltimore, Md.) has permitted the development of procedures for the isolation of anterior pituitary hormones of high physicochemical purity in quantities sufficient for structural studies.

FSH, similar to LH (7-11), TSH (12-17), and HCG (18, 19), has been shown to consist of a hormone-non-specific α subunit and a hormone-specific β subunit (20-22). This paper describes the isolation of the subunits of FSH and the primary amino acid sequence of the α subunit.

MATERIALS AND METHODS

Isolation of FSH

The pituitary acetone powder from 15,000 glands was extracted as described earlier (4) with 35% ethanol containing 10% ammonium acetate adjusted to pH 6.1 with 1 N acetic acid, in a tissue to solvent ratio of 1:15 (w/v). The extract was made 87% in ethanol to obtain 30 g of a precipitate designated as “crude glycoprotein.” All the procedures were performed at 4°C.

Gel Filtration on Sephadex G-100—Batches of 7 g of glycoprotein fraction were fractionated by ascending exclusion chromatography on five columns (10 x 100 cm) of Sephadex G-100 (Pharmacia, Piscataway, N. J.) equilibrated with 0.1 M ammonium bicarbonate at pH 8.0 (Fig. 1). The eluates from this and subsequent columns were monitored for absorption at 280 nm by the aid of a Uvicord recorder and collected in a refrigerated fraction collector (LKB Instruments, Stockholm, Sweden). Hormone fractions obtained during various purification steps were characterized by analytical disc electrophoresis in polyacrylamide gels at pH 8.0 (23), by bioassay of FSH (24), and by bioassay of LH (25), as described earlier (4). Gel filtration on Sephadex G-100 columns removed approximately 70% of inert material from the crude glycoprotein fraction. A total of 10 g of glycoprotein containing FSH, LH, and TSH were obtained.

Ion Exchange Chromatography—The glycoprotein fraction (10 g) containing FSH, LH, and TSH was fractionated by ion exchange
chromatography on a column (10 × 100 cm) of carboxymethyl Sephadex C-50 (Fig. 2).

**Zone Electrophoresis**—A batch of 0.8 g of the FSH fraction obtained from the CM-Sephadex C-50 column was purified further by preparative zone electrophoresis (Fig. 3a) on a column (10 × 60 cm) of cellulose in 0.05 M Tris-HCl buffer of pH 8.2 (catalog No. #8400; 60A; LKB Instruments, Stockholm, Sweden).

The FSH fraction (0.5 g) recovered from the first zone electrophoresis was again subjected to electrophoresis on the same column under similar conditions to isolate FSH (Fig. 3b). The residual FSH present in the LH and TSH fraction from CM-Sephadex C-50 (Fig. 2) also was recovered during the isolation of LH and TSH by isoelectric focusing (26). The FSH isolated by the zone electrophoresis exhibited a high degree of purity as demonstrated by disc electrophoresis and immunoelectrophoresis, as described earlier (4). Bioassay of purified FSH revealed that this preparation was virtually free of LH (27), TSH (28), GH (29), and ACTH (30), and PRL activity (31), as reported earlier (4).

**Isolation of Subunits of FSH**

A batch of 178 mg of FSH isolated from 15,000 pituitary glands was dissociated with urea and the subunits were separated by ion exchange chromatography as in the procedure described for HCG (18, 19). The FSH was dissolved in 10 ml of 0.04 M ammonium acetate, pH 7.5 containing 8 M cyanate-free urea (Sequenal grade, Pierce Chemical Co., Rockford, Ill.) and incubated for 1 hour at 40°. The incubated material was applied to a column (2 × 22 cm) of DEAE-Sephadex A-25 equilibrated with 0.1 M ammonium bicarbonate buffer. A voltage of 250 volts was applied for 96 hours, after which the column was eluted at a flow rate of 28 ml/hour. Aliquots of 20 ml/tube were collected.

The FSH fraction (0.5 g) recovered from the first zone electrophoresis to re-electrophoresis, under similar conditions.

**Amino Acid Sequence of FSH-a**

**Amino Acid Analysis and Terminal Amino Acid Sequence of FSH-a**—Three aliquots of 50 µg of FSH-a were sealed under vacuum in Pyrex glass tubes in twice glass-distilled 5.7 M HCl and were hydrolyzed at 110° for 24, 48, and 72 hours. The hydrolysates were concentrated to a small volume under reduced pressure and lyophilized. The material was dissolved in a small amount of water and lyophilized again to remove residual HCl. The samples were dissolved in 50 µl of 0.2 M citrate buffer of pH 2.2. The amino acid analyses of 20-µl aliquots of the hydrolysates were performed on a Durrum amino acid analyzer. The NH₂- and COOH-terminal analyses were performed by the dansyl procedure of Gray and Hartley (32) and by carboxypeptidase A and B digestion, respectively. The amino acids were identified by thin layer chromatography on polyamide plates (33) and by direct amino acid analysis after separation of the free amino acids (34).

An aliquot of 3 mg of protein (approximately 300 nm) of the reduced and carboxymethylated α subunit was also examined by a Beckman Automatic Sequencer (model 890C) for the amino acid sequence at the NH₂-terminus end. The thiazolinone derivatives were converted to phenylthiohydantoins by heating at 80° for 10 min in 1.0 M cyanate-free urea (Sequenal grade, Pierce Chemical Co., Rockford, Ill.) and isolated by electrophoresis on cellulose plates (33). The amino acid analysis was performed by thin layer chromatography on polyamide plates (33) and by direct amino acid analysis after separation of the free amino acids (34).
HCl and extracting the products into ethyl acetate. The phenylthiodyantoin derivatives were identified with a Perkin-Elmer model 900 gas chromatograph employing the system reported by Pissano and Bronzert (51).

Reduction and Carboxamidomethylation of FSH-α—A batch of 30 mg of FSH-α was dissolved in 2 ml of 1.0 M Tris-HCl buffer at pH 8.2 containing 8% urea and 2% EDTA. Forty milligrams of diithiothreitol were added, and the solution was allowed to stand for 1 hour at room temperature. Ninety-eight milligrams of iodoacetamide were then added, and the solution was allowed to stand in the dark at 0 °C for 1 hour. The solution was gel filtered in the dark on a column (2.5 x 50 cm) of Sephadex G-25 (course) and the column was eluted with 0.1 M NH₄HCO₃ at pH 8.2. The unretarded protein fraction containing reduced and carboxamidomethylated FSH-α was lyophilized.

Digestion of Reduced and Carboxamidomethylated FSH-α Subunit with Thermolysin—The reduced and carboxamidomethylated FSH-α (30 mg of protein) was dissolved in 3.2 ml of 1.0 M ammonium bicarbonate and digested with 0.37 ml of a 1 mg/ml solution of thermolysin (Calbiochem, San Diego, Calif.) in 1% NH₄HCO₃, at 37 °C. At the end of 10 min, another aliquot of 0.37 ml of the thermolysin solution was added. After 2 hours, the digest was taken to pH 4.0 with acetic acid to terminate the enzymatic action.

Separation of Thermolytic Peptides of FSH-α by Gel Filtration on Sephadex G-50—The thermolytic digest was applied to a column (1.5 x 250 cm) of Sephadex G-50 (superfine). The column was developed with 0.1 M ammonium bicarbonate at a flow rate of 18 ml/hour. The eluate was monitored at 280 and 206 nm (Uvicord III, LKB Instruments, Stockholm, Sweden) to locate peptides. Aliquots of 5 ml were collected and pooled into fractions as shown in Fig. 5.

Isolation of Peptides—Diagram 17 shows the further purification of the fractions obtained from the Sephadex G-50 column. Fraction I (III+IV) consisted of undigested material.

Fraction II consisted of a mixture of glycopeptides. It was further fractionated by gel filtration on columns (1 x 100 cm) of Sephadex G-25 and G-15, and on a column (1 x 30 cm) of Bio-Gel-P-2 (BioRad Laboratories, Richmond, Calif.). All gel filtration columns were eluted with 0.1 M ammonium bicarbonate buffer containing 5% propanol. Two glycopeptides were isolated by two-dimensional high voltage electrophoreses at pH 5.3 and pH 6.

All high voltage electrophoreses were performed in Fiberglas tanks containing a coolant in which papers are suspended (Savant Instruments, Hicksville, N. Y.). Voltages of 4,000, 4,000, and 2,500 volts were applied for pH 2 (2% formic acid/8% acetic acid), pH 3.5 (acetic acid/pyridine/water, 100:10:2,890), and pH 6 (acetic acid/pyridine/water, 100:10:890), respectively, with time of electrophoresis varying from 1.5 to 3 hours.

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The fraction (III+IV) was divided into the following subfractions: A, V, and VI+VII. Peptide A was isolated from Fraction II consisting of a mixture of glycopeptides. It was further fractionated by gel filtration on columns (1 x 100 cm) of Sephadex G-25 and G-15, and on a column (1 x 30 cm) of Bio-Gel-P-2. The peptide-containing fractions recovered from these columns were finally purified on Whatman No. 1 paper by two-dimensional high voltage electrophoresis at pH 2, and/or pH 3.5, and/or pH 6. Guide strips (0.5 cm) were cut and stained with 0.1% ninhydrin in acetone to locate the peptides. The peptides were eluted from the paper using 5% pyridine in water. Aliquots of the peptide fractions were hydrolyzed and subjected to amino acid analysis. The peptides showing impurities were purified further.

Reduction and S-Aminoethylation of FSH-α—A batch of 30 mg of FSH-α was dissolved in 3 ml of 8% urea containing 0.3 mg of EDTA and 1.0 M Tris buffer at pH 8.6. Thirty micrograms of β-mercaptoethanol were added and the solution was allowed to stand for 4 hours at room temperature with constant stirring. Ethanethioline (36) then was added in five portions of 0.04 ml each at 10-min intervals. The reaction of the sulfhydryl groups was followed with nitroprusside reagent (37). The entire solution was dialyzed for 24 hours against distilled water and lyophilized.

Digestion of Reduced and Ae-FSH-α with Trypsin—Five milliliters of water were added to the Ae-FSH-α. The pH of the suspension was adjusted to 8.0. Trypsin (0.7 mg, Worthington Biochemical Corp., Freehold, N. J.) was treated with tosyl-L-phenylalanyl chloromethane (38) and dissolved in 200 μl of 4 mM HCI-20 mM CaCl₂ before addition to the Ae-FSH-α. The temperature was maintained at 37° and a pH of 8 was maintained. The substrate was completely solubilized after 6 hours when another aliquot of 0.4 mg of the trypsin in 100 μl of 4 mM HCI-20 mM CaCl₂ was added. The solution was kept overnight in an oven at 37°. The total alkali uptake and the number of bonds cleaved were calculated.

Gel Filtration of Tryptic Peptides on Sephadex G-50—The tryptic digest was applied to a column (1.5 x 250 cm) of Sephadex G-50 (superfine), equilibrated in 0.1 M ammonium bicarbonate containing 5% propanol. The column was eluted with the same solvent as described earlier for the thermolytic peptides. Fractions were pooled as shown in Fig. 6.
I Instruments. Palo Alto, Calif. 1. Five microliters of a freshly prepared peptide was derivatized prior to Edman degradation. The derivatized peptide then was subjected to Edman degradation. The reaction was shown to be 0.5 or less by subtractive amino acid analysis.

Amino Acid Analysis of Peptides—Peptides were hydrolyzed in 200 μl of 6 N HCl containing 0.2% (w/v) phenol and 0.1% (v/v) mercaptoacetic acid in evacuated, sealed tubes (10 x 75 mm Pyrex No. 9820) at 110° for 20 hours (39). The HCl was removed under vacuum in a desiccator. The amino acid analysis was performed on a Durrum amino acid analyzer (model D-500, Durrum Instruments, Palo Alto, Calif.). The yields of the peptides were calculated from the amino acid analysis.

NH₂-terminal Analysis of Peptides—Aliquots of 5 to 10 nmol of peptides were analyzed by the dansyl procedure of Gray and Hartley (32). The Dns-amino acids were identified by thin layer chromatography on polyamide sheets (Cheng Chin Trading Co., Taipei, Taiwan; obtained through Gallard Schlesinger Chemical Co., Long Island, N. Y.) by the methods of Woods and Wang (33).

COOH-terminal Analysis of Peptides with Carboxypeptidases A and B—Peptides (2 to 3 nmol) were digested with 5 to 10 μl of a 15% solution of carboxypeptidases A and B in 0.1 M NH₄HCO₃, pH 8.0, at 37° (39). Suitable aliquots of enzymatic digest were taken at 5, 10, 30, and 60 min. The aliquots were dried under nitrogen, dissolved in pH 2.2 citrate buffer, and applied directly to the amino acid analyzer.

Sequential Degradation of Peptides—Appropriate quantities of peptides, to give 2 to 5 nmol of peptide/cycle, were subjected to Edman degradation, in test tubes (10 x 75 mm), by the method of Salmikow et al. (39).

For the coupling reaction, the peptide was dissolved in 100 μl of pH 8.6 buffer and 5 μl of phenylisothiocyanate was added. The coupling reaction was carried out at 45° under nitrogen for 30 min. The mixture then was dried over NaOH and P₂O₅ at 60° in a desiccator. The amino acid analysis was performed on a Durrum amino acid analyzer (model D-500, Durrum Instruments, Palo Alto, Calif.). The yields of the peptides were calculated from the amino acid analysis.

Digestion of Peptides with Proteolytic Enzymes—As shown in the Appendices A and B, peptides were subjected to further digestion with trypsin, chymotrypsin, or DAP I. For trypsin or chymotrypsin digestion, 0.1 to 0.2 μmol of peptide dissolved in 50 to 100 μl of enzyme solution (0.5 to 1 mg/ml of enzyme in 1% NH₄HCO₃) was incubated at 37° for 1 to 3 hours (39).

For DAP I, the procedure of Callahan et al. (42) was used with the following modification. The peptide was applied to Whatman No. 1 paper after incubation with DAP I and the released dipeptides were separated by electrophoresis at pH 2 for 2 hours at 4,000 volts. The paper then was dried and immersed in a solution of 1% ninhydrin in acetone to visualize the peptides. After development of the color overnight, the peptides were cut out and eluted with 5.7 N HCl containing 0.2% (w/v) phenol and 0.1% (v/v) mercaptoacetic acid (39) and hydrolyzed for 20 hours at 110°. The hydrolysates were dried and analyzed directly to identify amino acids.

Determination of Amides—In most cases, amides were assigned by the hydrolysis of peptides with either carboxypeptidases or with leucine aminopeptidase and chromatographic measurement of glutamine or asparagine. Ten- to 25-nmol aliquots of peptides were hydrolyzed with 12.5 μg of leucine aminopeptidase for 40 to 48 hours at 37° in 25 μl of 0.1 M Tris-HCl and 0.0025 M MgCl₂, pH 8.6. The hydrolysates were lyophilized and the amino acids released were determined with an amino acid analyzer. Since asparagine and glutamine emerge with serine, their presence was indicated by the appearance at an enlarged set of peaks and the disappearance of the corresponding aspartic or glutamic acid peak as compared to an acid hydrolysate. Alternatively, the assignment of amides was based on the behavior of the parent peptides on paper electrophoresis at pH 6.0. If more than one possible amide occurred in a given peptide, the mobilities relative to aspartic acid of the peptides obtained before and after successive removal of residues by Edman degradation were compared (8, 39).

Assignment of Carbohydrate Side Chains—Twenty nanomoles each of the glycopeptides T-8 and T-13, identified by the presence of amio-

Fig. 6 Gel filtration of a tryptic digest of reduced and S-aminoethylated PS-H on a column (1.5 x 250 cm) of Sephadex G-50.
sugars in the amino acid analyses, were dissolved in 100 μl of 1% solution of pronase in 1% ammonium bicarbonate, pH 8.0, and were incubated at 37° for 24 hours. An aliquot of 30 μl of carboxypeptidase A and an aliquot of 20 μl of carboxypeptidase B (1 mg/ml solutions in 0.1 M ammonium bicarbonate) were added and the mixture was incubated for a further 24 hours. The sample was gel-filtered on a column (0.5 x 30 cm) of Sephadex G-25 (superfine) to remove enzymes and free peptides and amino acids. The column eluate was monitored at 206 nm (Univox LKB Instruments, Stockholm, Sweden). The fraction containing the glycopeptide was pooled, lyophilized, and further purified by electrophoresis at pH 6.0. An acid hydrolysate of the purified glycopeptide was examined in the amino acid analyzer for the single amino acid residue attached to the carbohydrate moiety (44).

RESULTS AND DISCUSSION

Yield and Activity—The yield and activity of FSH and its purified subunits are summarized in Table I. From 15,000 human pituitary glands, a yield of 178 mg of highly purified and physicochemically homogenous FSH, representing a 40,000-fold purification of the fresh tissue was achieved. The purified FSH showed a biopotency of 335 ± 10 NIH-FSH-S1 units/mg and little contamination with other anterior pituitary hormones. After dissociation of FSH with urea, purification and isolation of the subunits by ion exchange chromatography on DEAE-Sephadex A-25 and gel-filtration on Sephadex G-100, respectively, a yield of 30 mg of FSH-a, 40 mg of FSH-α, and a total of 75 mg of “undissociated FSH” was obtained. The undissociated FSH was retreated with urea to recover more subunits.

Amino Acid Analysis of FSH-a—The amino acid composition obtained by analyses of 24-, 48-, and 72-hour acid hydrolysates of FSH-a is compatible with the amino acid sequence (Table III). The amino acid composition, however, showed a few differences from that of a 24-hour hydrolysate of an earlier preparation of FSH-a (20), which was not purified by gel filtration on Sephadex G-100. The methionine content reported for the earlier preparation was higher due to the addition of a peak in the region of methionine sulf oxide to methionine. The amino acid composition of FSH-a and the recovery of 3 residues of methionine in sequence determinations indicates that the peak for methionine sulf oxide was an artifact and was erroneously added to the methionine in our earlier report (20). The values of serine, tyrosine, and cysteine in the earlier preparation were not extrapolated to zero time and were lower due to partial destruction during hydrolysis.

Table I

| Fraction          | Yield (mg) | Specific activity (units/mg) |
|-------------------|------------|-----------------------------|
| Glycoprotein      | 35,000     | 1.05 (0.01-1.21)            |
| FSH               | 178        | 335 ± 10^a                  |
| FSH α             | 30         | None                        |
| FSH β             | 40         | Trace                       |
| Undissociated FSH | 75         |                             |

* FSH-S3; LH-S1; TSH-S1; BGH-10 (bovine growth hormone); PRL (National Institutes of Health standards); ACTH (Parke Davis).
* Obtained from 15,000 human pituitary glands.
* Confidence limits, 95%.
* Standard error.

Table II

| Amino acid analysis of FSH-a from human pituitary glands |
|----------------------------------------------------------|
| Amino acid | Acid hydrolysis: 24 hr | Acid hydrolysis: 48 hr | Acid hydrolysis: 72 hr | Average or extrapolated to zero time (46) | Residues from sequence data | Residues from sequence data |
|------------|------------------------|------------------------|------------------------|-------------------------------------|----------------------------|----------------------------|
|            | 1 mol/mol protein      | 1 mol/mol protein      | 1 mol/mol protein      |                                     |                            |                            |
| Aspartic acid | 6.3 6.1 6.4           | 6.4 6.6 6.4            | 6.4 6.6 6.4            | 6 6                                  |                            |                            |
| Threonine   | 8.0 7.8 7.8            | 7.9 8.3 8.5            | 7.9 8.3 8.5            | 8 8                                  |                            |                            |
| Serine      | 6.9 6.3 6.6            | 6.7 6.5 6.7            | 6.7 6.5 6.7            | 6 6                                  |                            |                            |
| Glutamic acid | 9.4 9.2 9.5           | 9.4 9.2 9.5            | 9.4 9.2 9.5            | 9 9                                  |                            |                            |
| Proline     | 7.4 7.4 7.5            | 7.4 7.4 7.5            | 7.4 7.4 7.5            | 7 7                                  |                            |                            |
| Glutamic acid | 5.4 5.2 5.4           | 5.2 5.0 5.2            | 5.2 5.0 5.2            | 5 4                                  |                            |                            |
| Alanine     | 5.2 4.9 5.2            | 5.1 4.9 5.1            | 5.1 4.9 5.1            | 5 5                                  |                            |                            |
| Half-Cystine | 9.5 9.7 9.8           | 9.8 9.9 9.9            | 9.8 9.9 9.9            | 9 9                                  |                            |                            |
| Valine      | 7.1 7.2 7.5            | 7.3 7.5 7.6            | 7.3 7.5 7.6            | 7 7                                  |                            |                            |
| Methionine  | 2.4 2.4 2.4            | 2.2 2.3 2.2            | 2.2 2.3 2.2            | 2 2                                  |                            |                            |
| Isoleucine  | 1.5 1.6 1.6            | 1.5 1.6 1.6            | 1.5 1.6 1.6            | 1 1                                  |                            |                            |
| Leucine     | 4.5 4.3 4.6            | 4.4 4.4 4.4            | 4.4 4.4 4.4            | 4 4                                  |                            |                            |
| Tyrosine    | 3.6 3.9 3.7            | 3.7 3.9 3.7            | 3.7 3.9 3.7            | 4 4                                  |                            |                            |
| Phenylalanine | 3.9 3.8 4.0         | 4.0 3.9 4.0            | 4.0 3.9 4.0            | 4 4                                  |                            |                            |
| Histidine   | 2.1 2.2 2.4            | 2.3 2.5 2.4            | 2.3 2.5 2.4            | 3 3                                  |                            |                            |
| Lysine      | 5.1 5.5 5.8            | 5.5 5.6 5.8            | 5.5 5.6 5.8            | 5 5                                  |                            |                            |
| Arginine    | 3.3 3.4 3.1            | 3.1 3.3 3.1            | 3.1 3.3 3.1            | 3 3                                  |                            |                            |

* Calculated molecular weight for protein moiety from sequence data.
* Calculated on the basis of total micrograms of residues recovered using a molecular weight of 10,326.

These differences were resolved by the performance of amino acid analysis after 24, 48, and 72 hours of hydrolysis of highly purified FSH-a. The amino acid analysis of FSH-a as reported by Reichert and Ward (21) has a lower proline, valine, and cysteine content and a higher tyrosine and glutamic acid content.

NH₂ and COOH-terminal Analyses—Alanine was found to be the major NH₂-terminal amino acid present. A predominant alanine NH₂-terminal peptide was also recovered from both digests of Ae-FSH-a confirming that FSH-a is similar to HCG-a (41) at the NH₂-terminal. The data obtained with the automatic Sequencer indicated the NH₂-terminal sequence of FSH-a to be Ala Pro Arg Val Glu Aep.
The COOH-terminal analyses (Table III) showed 1 mol of serine, lysine, and histidine and 2 mol of tyrosine per mol of protein in 6 hours, which is consistent with the sequence of Tyr-Tyr-His-Lys-Ser obtained from tryptic and thermolytic peptides of FSH-α (Fig. 7).

Amino Acid Sequence of FSH-α—Purification and amino acid composition of the thermolytic and the tryptic peptides are given in Diagrams 1 and 2 and Tables IV and VI, respectively. The amino acid sequence of each peptide is given in Tables V and VII. The complete sequence of FSH-α is given in Fig. 7. The experimental details are presented in Appendices A and B.

During the tryptic digestion of Ae-FSH-α, an uptake of 18 mol of alkali/mol of protein was obtained, which is in close agreement with the total of 17 tryptic peptides obtained (Table VII).

The amino acid composition, terminal residues, and amino acid sequences of peptides (Table V) suggested that during thermolytic digestion, partial cleavage of FSH-α occurred at positions 73-74 (Gln-Phe), 76-77 (Lys-Val), and 87-88 (Cys-Tyr), resulting into two overlapping peptides, Th-13a and Th-13b (Table V). Similarly, peptides Th-4a, Th-6a, and Th-6b with overlapping sequences were also obtained in a much lower yield. Similarly, no significant cleavage occurred between Cys/Ae-Pro, and between Lys/Lys-bonds in peptides T-1 and T-6.

The alignment of amino acid sequences of the tryptic and thermolytic peptides of FSH-α did not permit overlaps between positions 28-29, 32-33, 60-61, and 67-68; however, the arrangement shown in Fig. 7 is almost certainly the correct one since it results in identical sequences of FSH-α and hCG-α (41), and homologous sequences when FSH-α is compared to human, ovine, and bovine LH-α (19, 49-51) and bovine TSH-α (52). Moreover, the validity of this arrangement is supported by the observation that it would confirm the sequence given in the preliminary report of Shome and Parlow (50).

Assignment of Carbohydrate Side Chains—Glycopeptides Th-13 and Th-8, following extensive proteolytic digestion and removal of the enzymes, peptides, and amino acids, showed only aspartic acid on amino acid analysis, indicating that the carbohydrate moiety is linked to the asparagine at positions 52 and 78. This is also suggested by glycopeptide Th-8, which contains only asparagine that can be linked to the carbohydrate moiety. Linkages of the carbohydrate moiety to asparagine, serine, or threonine are commonly found in glycoproteins.

![Fig. 7. Amino acid sequence of FSH-α. The solid lines below represent peptides recovered and sequenced.](http://www.jbc.org/)

| Table III | Amino acids released from FSH-α by digestion with carboxypeptidases A and B |
|-----------|--------------------------------------------------------------------------------|
| Time      | Ser | Lys | His | Tyr |
| 5 min     | 1.0 | 0.7 | 0.6 | 1.2* |
| 30 min    | 1.0 | 1.0 | 0.7 | 2.0 |
| 2 hours   | 1.2 | 1.1 | 0.9 | 2.2 |
| 4 hours   | 1.3 | 1.1 | 0.8 | 2.1 |
| 6 hours   | 1.2 | 1.1 | 0.8 | 2.2 |

* Values corrected for losses during experimental procedure.

* Protein determined by the method of Lowry et al. (48), using FSH-α as standard.

The cleavage of 2 adjacent residues of tyrosine at positions 88 and 89 (Fig. 7) is represented by 1.2 residues of tyrosine.
The sequences in the region of attachment of the carbohydrate moieties, viz., "Asn(CH0)-Val-Thr-" and "Asn(CH0)-His-Thr-" with threonine in the third position agrees well with similar sequences obtained for many asparagine-carbohydrate-linked glycoproteins (53).

After the first indication of the subunit nature of LH by Li and Starman (54), and Dola/Loo and Jutiez (55), other glycoprotein hormones, viz., HCG (18, 19), TSH (12-17), LH (7-11), and FSH (20, 56) have been shown to contain a common hormone-nonspecific α subunit and a hormone-specific β subunit. The presence of subunits in human FSH has been confirmed by Reichert and Ward (21) and Parlow and Shome (57).

The primary amino acid sequence of FSH-α is identical with that of HCG-α (41), and differs from that of human LH-α and human FSH-α as reported by Shome and Parlow (50) and Inagami et al. (51) in having the tripeptide Ala-Pro-Asp at the NH2 terminus. The sequence at the NH2 terminus also was confirmed by the results obtained from experiments using the automatic sequenator. The sequence at positions 84 and 85 as Cys-Ser of FSH-α, however, is in agreement with Shome and Parlow (50) and differs from Ser-Cys at these positions reported by Safran et al. for human LH-α (49).

Recombination of subunits of glycoprotein hormones has indicated that the α subunits are interchangeable at all intra- and interspecies levels (58-61). It may be of interest to note that significantly higher FSH activity was recovered during recombination experiments between HCG-α and FSH-β subunits, and between FSH-α and FSH-β subunits, than between human LH-α and FSH-β subunits (61). In addition to this, FSH-α generated a higher HCG-like activity when incubated with HCG-β, as compared to human LH-α (58). The recovery of 81% of FSH activity by Reichert (62) on incubation of human FSH (α, β) in the presence of HCG-α and the 93-fold increase in HCG-like activity, when human FSH (α, β) was incubated in the presence of HCG-β, further demonstrates that biologically active molecules can be formed between human FSH-β and HCG-α, and between human FSH-α and HCG-β. These data further support the identity of FSH-α with HCG-α and suggest an essential nature of the NH2-terminal tripeptide Ala-Pro-Asp.

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Addendum—Since the submission of the manuscript, peptides from a chymotryptic digest of FSH-α have been isolated and sequenced, as shown in Fig. 7. These peptides provide the overlap between positions 28-29, 32-33, 60-61, and 67-68. Keutmann et al.4 have also shown that 60% of hFSH-α chains contain the NH2-terminal sequence of Ala-Pro-Asp-Val-Gln-Asp−, and 30% contain Asp-Val-Gln-Asp−, whereas, only 10% contain Val-Gln-Asp−.

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## Appendix D

### Hypothetical Population

| Table ID | Sample Size | Mean | Standard Deviation | Mean | Standard Deviation |
|---------|-------------|------|--------------------|------|--------------------|
| Table A | 100         | 50   | 10                 | 50   | 10                 |
| Table B | 100         | 50   | 10                 | 50   | 10                 |

### Hypothetical Data

| Table ID | Sample Size | Mean | Standard Deviation |
|---------|-------------|------|--------------------|
| Table C | 100         | 50   | 10                 |
| Table D | 100         | 50   | 10                 |

### Graphical Representation

[Graphical representation of data and relationships]

### Table D.1

| Table ID | Sample Size | Mean | Standard Deviation |
|---------|-------------|------|--------------------|
| Table E | 100         | 50   | 10                 |
| Table F | 100         | 50   | 10                 |

### Table D.2

| Table ID | Sample Size | Mean | Standard Deviation |
|---------|-------------|------|--------------------|
| Table G | 100         | 50   | 10                 |
| Table H | 100         | 50   | 10                 |

### Graphical Representation

[Graphical representation of data and relationships]
Primary amino acid sequence of follicle-stimulating hormone from human pituitary glands. I. alpha subunit.
P Rathnam and B B Saxena

J. Biol. Chem. 1975, 250:6735-6746.

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