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Studies on the Isolation and Chemical Properties of Porcine Follicle-Stimulating Hormone[1] (39260)

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(Introduced by A. Clark Griffin)

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Porcine follicle-stimulating hormone (pFSH) from pituitary glands has been most extensively studied by Steelman and coworkers (1-3). These workers report several methods (including pancreatin digestion and chromatography on DEAE-cellulose and hydroxyl apatite) for isolation of the most biologically active forms of pFSH prepared to date. In this paper we present a procedure for preparation of pFSH of comparable potency from acetone-dried pituitary glands using ethanol–acetate extraction followed by chromatography on QAE-Sephadex, then Sephadex G-100. The sialic acid and amino acid composition of the isolated pFSH are reported. To date, no amino acid composition of a highly purified preparation of pFSH of comparable biological activity has been documented.

Materials and methods. Preparation of pFSH. Porcine pituitary glands were dried by successive washes with acetone at 4° for 2 days. The dried glands were first homogenized in acetone with a Waring blender, then by a Tekmar homogenizer, Super Dispax System, Model SD-45 (Tekmar Company, Cincinnati, Ohio). The final homogenate was dried with a vacuum pump to an acetone powder. Using a modified Koening and King procedure (4), the acetone powder was allowed to extract at 4° for 24 hr in pH 5.0 ethanol–acetate buffer (40% ethanol and acetate at 0.5 ionic strength) containing 0.02% phenylmethylsulfonylfluoride (PMSF) added as a solid immediately at the start of the extraction. The extract was centrifuged and the supernatant rendered 80% with respect to ethanol. After 24 hr at 4°, the 80% ethanol precipitate was collected at the centrifuge, dissolved in 0.12 *M* NH_4HCO_3, dialyzed, and lyophilized. This dried gonadotropin-rich fraction was dissolved in 0.12 *M* NH_4HCO_3 (40 mg gonadotropin fraction/ml buffer), heated at 60° for 3 min, cooled, and centrifuged. The supernatant (labeled S_2) was lyophilized to dryness. Chromatography of S_2 on QAE-Sephadex (A50) was accomplished with a stepwise addition of pH 5.5 ammonium acetate buffers (0.01 *M*, 0.1 *M*, 0.25 *M*) followed by final column elution with 0.50 *M* NH_4HCO_3, pH 8.0. The FSH biological activity of each fraction was measured by the method of Steelman and Pohley (5) with the precautions detailed by Jacobs and Ward (6). Relative potency was evaluated by the slope-ratio analysis (5), using 150 and 450 μg NIH-FSH-P-1 as standard and each unknown in three dose concentrations, at least two of which were appropriate for optimal responses with respect to the standard. Porcine activity was detected in Fractions 2 and 3 eluted with 0.1 and 0.25 *M* ammonium acetate. Fraction 2 was chromatographed on Sephadex G-100 in 0.12 *M* NH_4HCO_3, pH 8.0, for isolation of the pFSH submitted to chemical analyses.

Chemical analyses. Sialic acid was determined by the thiobarbituric acid method of Warren (7).

Amino acid analyses were carried out on a Beckman/Spinco Model 120C Amino Acid Analyzer equipped with a Durram DC-2a single column (8). The analyzer was equipped with a Glenco automatic sample loader (Model 400), a Glenco differential absorbance monitor (Model 56V), and an Autolab System AA computing integrator. Samples, weighed on a Cahn Electro-Balance, were hydrolyzed *in vacuo* with 6 *N*

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HCl at 110° for 24 and 72 hr. Threonine and serine were corrected for decomposition by extrapolation to zero time. Performic acid oxidations as described by Mueller *et al.* (9) were performed for determination of cysteine and cystine as cysteic acid and of methionine as the sulfone. Performic acid oxidized samples were hydrolyzed for 24 hr as for the other samples prior to analysis. The tryptophan content was determined by ion exchange chromatography of alkaline hydrolysates (10).

Results. Table I. The modified Koening and King extraction of acetone dried glands using PMSF and heat treatment yields a gonadotropin-rich fraction (S_2) assaying 1.99 × NIH-FSH-P1. Chromatography of S_2 on QAE Sephadex separates pFSH from pLH (in fraction 1) and further elutes a biologically active pFSH in fractions 2 and 3. A twofold increase in biological activity of pFSH in fraction 2 is observed, while the pFSH in fraction 3 is not significantly changed in potency from S_2. Submission of pFSH fraction 2 to Sephadex G-100 yields pFSH with a Ve/Vo of 1.32 and assaying 15 × NIH-FSH-P-1, thus accomplishing a 7.6-fold increase in potency relative to the S_2 starting material. The sialic acid composition of all the fractions designated in Table I did not change appreciably.

Table II. The amino acid composition of a highly purified preparation of pFSH from Sephadex G-100 (see Table I) expressing high biological activity is reported. The analyses were carried out on 24- and 72-hr hydrolysates with the appropriate corrections for serine and threonine. For valine and isoleucine the higher (72 hr) values have been tabulated since intermediate dipeptides of these amino acids are known to be resistant to hydrolysis. Because tryptophan is destroyed during acid hydrolysis, separate alkaline hydrolysates were analyzed yielding the reported 0.79 residue tryptophan/100 residues. The amino acid analysis is characterized by high lysine, aspartic acid, and glutamic acid. Significant amounts of threonine, proline, and half cystine are noted.

TABLE I. Isolation of Porcine FSH.

Fraction	Sialic acid (%)	Relative potency × NIH-FSH-P-1 (CL)[b]
S_2[a]	1.90	1.99 (1.86–2.13)
QAE-Sephadex of S_2		
pFSH fraction 2	1.85	4.23 (3.51–4.96)
pFSH fraction 3	2.04	1.45 (0.94–1.96)
G-100 Sephadex on fraction 2		
pFSH fraction	2.10	15.12 (13.85–16.38)

[a] S_2 = Gonadotropin-rich fraction from modified Koenig and King extraction with 0.02% PMSF followed by 60° heat treatment (see text).
[b] 95% CL, confidence limits.

TABLE II. Amino Acid Composition of pFSH.[a]

Amino acid	Protein (μmole/mg)	Residues/100 residues
Lysine	.418	8.12
Histidine	.147	2.86
Arginine	.208	4.04
Aspartic acid	.358	6.96
Threonine[b]	.438	8.51
Serine[b]	.360	6.99
Glutamic acid	.448	8.70
Proline	.386	7.50
Glycine	.339	6.60
Alanine	.383	7.44
Half cystine[c]	.424	8.24
Valine	.271	5.27
Methionine[c]	.088	1.71
Isoleucine	.175	3.41
Leucine	.300	5.82
Tyrosine	.222	4.32
Phenylalanine	.138	2.69
Tryptophan[d]	.041	0.79

[a] Values are averaged from 24- and 72-hr hydrolysates.
[b] Corrected for decomposition during hydrolysis. See text.
[c] Estimation from performic acid oxidized sample. See text.
[d] Estimation from alkaline hydrolysates. See text.

Discussion. A rather facile procedure for isolation of pFSH and the analysis of the sialic acid and amino acid composition of the fraction with the highest biological activity has been presented. Addition of PMSF to the initial extraction medium was for the purpose of inactivating serine-active center proteases presumably preventing hydrolysis of pFSH in the pituitary extract (11, 12). The 60° heat treatment was intended to inactivate neuraminidase activity released from the pituitary (13). The necessity of retaining sialic acid for full expression of biological activity by FSH has been reported (14, 15). Without these precautions, the comparable fractions seldom exceeded 3–4

units per milligram biological activity. The most active product from the present procedure by contrast had a potency of 15 × NIH-FSH-P-1.[2] This biological activity is compatible with that reported for the highest potency pFSH prepared to date (2). Disc gel electrophoresis in 7.5% acrylamide at pH 8.4 in Tris-HCl buffer indicated the pFSH from G-100 Sephadex contained one major broad component with a minor component of slightly lower mobility. The biological activity was associated with the major component.

To date, no amino acid composition of a highly purified preparation of pFSH of a comparable biological activity has been reported. The present porcine FSH preparation is characterized by a high content of acidic amino acids, hydroxyamino acids, lysine, and cystine. This composition compares closely with analyses of highly purified ovine FSH reported from the laboratories of Jutisz *et al.* (16), Cahill *et al.* (17), Papkoff *et al.* (18), and Sherwood *et al.* (19). The former two reports show seven to eight residues of leucine/100 residues, while the latter two reports indicate four to five residues of leucine/100 residues. In this analysis in Table II the pFSH contains six residues of leucine/100 residues. By comparison to porcine LH (20), the porcine FSH analysis here exhibits a *higher* content of lysine, aspartic, and glutamic acids than pLH. Conversely the pFSH composition in Table II indicates slightly *lower* alanine, methionine, and leucine composition than pLH. The most significant difference on a residue/100 residue basis is in comparison of the proline content; pFSH contains approximately one-half the proline content of pLH.

Currently studies are in progress to elucidate the physiochemical properties in addition to extending the chemical characterization of pFSH.

Summary. Porcine follicle-stimulating hormone (pFSH) has been prepared from acetone dried pituitary glands. The acetone powder was initially extracted in pH 5 ethanol–acetate buffer (40% ethanol and acetate at 0.5 ionic strength) containing 0.02% PMSF. The gonadotropin rich fraction, obtained in the 80% ethanol precipitate, was dissolved in 0.12 *M* NH_4HCO_3, heated at 60° for 3 min, cooled, and centrifuged. The supernatant was lyophilized for chromatography on QAE-Sephadex, then Sephadex G-100. The pFSH obtained has a biological activity of 15 × NIH-FSH-P-1 and 2.1% sialic acid. The amino acid analysis is characterized by high lysine, aspartic acid, and glutamic acid with notable amounts of threonine, proline, and half-cystine.

[2] Under the conditions of our assay, NIH FSH-P-1 had a relative potency of 0.45 compared to NIH FSH-S9. This can only be regarded as a gross approximation since the assay response parameters of the porcine and ovine hormones are significantly different. The value is added for comparative purposes only, at the request of a referee.

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