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Purification and Properties of Bovine Pituitary Follitropin

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A reproducible procedure was developed for the purification of follitropin from frozen bovine pituitary glands. The method involved precipitation with $(NH_4)_2SO_4$ and acetone, followed by ion-exchange column chromatography on CM-cellulose and DEAE-cellulose and gel filtration on Sephadex G-100. A specific radioligand-receptor assay for follitropin was used to locate the activity in eluates after column chromatography and gel filtration. The potency of the highly purified bovine follitropin as measured by Steelman–Pohley bioassay was 164 times that of NIH-FSH-S1 standard preparation. The yield of bovine follitropin was 2.9mg/kg of frozen pituitary glands. Electrophoretically, bovine follitropin was more acidic in nature and migrated further towards the anode than lutropin and thyrotropin. The elution volume of bovine follitropin by gel filtration on Sephadex G-100 was very similar to that of bovine lutropin. The amino acid composition of bovine follitropin was similar to that of sheep and human follitropin, being rich in lysine, aspartic acid, threonine, serine, glutamic acid and half-cystine.

Follitropin (follicle-stimulating hormone, FSH) has been purified from pituitary extracts of a few species, including human (Roos & Gemzell, 1964; Saxena & Rathnam, 1967; Reichert *et al.*, 1968; Peckham & Parlow, 1969; Ryan *et al.*, 1971; Amir & Parlow, 1972; Stockell-Hartree, 1975), sheep (Papkoff *et al.*, 1967*a*; Cahill *et al.*, 1968; Hermier & Jutisz, 1969; Sherwood *et al.*, 1970) and horse (Braselton & McShan, 1970). At present, only human follitropin has been chemically characterized for primary amino acid sequence (Shome & Parlow, 1974*a*,*b*; Rathnam & Saxena, 1975).

The existence of bovine pituitary follitropin has been indicated by the experiments of Nalbandov & Casida (1940), and the content of follitropin in the bovine pituitary gland was low (Ellis, 1958, 1961). So far, there has been only one report on the partial purification of bovine follitropin (Reichert & Jiang, 1965). The present paper describes the preparation of highly purified bovine pituitary follitropin. In the isolation, a radioligand-receptor assay for follitropin (Cheng, 1975*a*,*b*) was used to locate the hormonal activity in eluates after ion-exchange column chromatography and gel filtration on Sephadex G-100. The potency of purified bovine follitropin was 164 times that of sheep NIH-FSH-S1 (FSH-S1 standard distributed by the National Institutes of Health, Bethesda, MD, U.S.A.).

Materials and Methods

Materials

Purified human follitropin (LER-1575C), sheep follitropin (LER-1818-2), human growth hormone (HS1652C), sheep prolactin (P-S10) and reference standard of sheep follitropin (NIH-FSH-S1) were from the National Institutes of Health. Purified bovine lutropin (luteinizing hormone) (potency 2.0 times that of NIH-LH-S1) and bovine thyrotropin (thyroid-stimulating hormone) (potency 30i.u./mg) were gifts from Dr. J. G. Pierce (U.C.L.A, Los Angeles, CA, U.S.A.). Frozen bovine pituitary glands were from Canada Packers, Winnipeg, Manitoba, Canada. Bovine serum albumin (fraction V) was from Miles Laboratories, Kankakee, IL, U.S.A. $Na^{125}I$ (carrier-free) was from New England Nuclear Corp., Boston, MA, U.S.A. CM-cellulose (Cellex CM) and DEAE-cellulose (Cellex D) were from Bio-Rad Laboratories, Mississauga, Ont., Canada. Cross-linked dextran gel, Sephadex G-100, was from Pharmacia (Canada), Quebec, Canada. All other reagents and chemicals were reagent grade from either Fisher Scientific Co., Fair Lawn, or J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A.

Protein determination

Distribution of proteins in eluates after fractionation on ion-exchange column chromatography and

gel filtration on Sephadex G-100 was monitored by absorbance at 278 nm by a Beckman Instruments Inc. (Palo Alto, CA, U.S.A.) model 25 spectrophotometer.

Radioligand-receptor assays

To monitor the distribution of follitropin activity in eluates after ion-exchange column chromatography and gel filtration on Sephadex G-100, a sensitive and specific radioligand-receptor assay for follitropin, utilizing particulate bovine testicular plasma membranes as receptor and ^{125}I-labelled human follitropin as tracer, was used as described previously (Cheng, 1975*a*). Contamination with other pituitary glycoprotein hormones in preparations of purified bovine follitropin was determined by radioligand-receptor assays specific for lutropin by using plasma membranes from bovine corpora lutea and ^{125}I-labelled bovine lutropin (Cheng, 1976), and for thyrotropin by using membranes from pig thyroid and ^{125}I-labelled bovine thyrotropin (Verrier *et al.*, 1974). For monitoring other pituitary hormonal activities, radioligand-receptor assays specific for prolactin (Shiu *et al.*, 1973) and for growth hormone (Tsushima & Friesen, 1973) were used.

Extraction and precipitation of pituitary follitropin

Frozen pituitary glands were thawed, washed with several volumes of ice-cold 0.9% NaCl, minced and homogenized with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY, U.S.A.) at a concentration of 4ml of ice-cold water/g of tissue. The pH of the suspension was adjusted to 6.0–6.5 by 2M-HCl or 2M-NaOH. After stirring for 3–4h at 4°C for extraction of proteins, the supernatant was obtained after centrifugation at 12000*g* (Beckman, J-21B refrigerated centrifuge, JA-14 rotor) for 20min at 4°C. The residue was resuspended in 2ml of ice-cold water/g of the original tissue and stirred for another 2–3h in the cold. After centrifugation, the supernatants were pooled for further concentration of follitropin activity.

Fractions precipitated between 25 and 45% saturation, and between 45 and 70% saturation with $(NH_4)_2SO_4$ were obtained by addition of the solid salt and centrifugation (12000*g*, 20min) after allowing the mixtures to stand for 2–3h at 0°C. The precipitate obtained at 25% saturation contained none of the hormonal activities tested for, the second fraction contained the bulk of the lutropin and thyrotropin, and the third fraction contained most of the follitropin activity in the extract. The precipitate was re-dissolved, dialysed against water, and the clear diffusate was centrifuged and then freeze-dried.

The freeze-dried powder was dissolved in 2% (w/v) NaCl solution at a concentration of 10mg/ml, and the pH of the solution was adjusted to 4.1 by 1M-HCl. A reddish-brown precipitate was formed and removed by centrifugation. The pH of the clear supernatant was immediately re-adjusted to 6.5–7.0 by 1M-NaOH. To the supernatant, 1vol. of cold acetone was added to precipitate a substantial amount of inactive proteins, which was removed by centrifugation. Another 2vol. of cold acetone was added to the remaining supernatant to make a final concentration of 75% acetone to precipitate the follitropin activity. After centrifugation, the precipitate was re-dissolved, dialysed against water and freeze-dried.

CM-cellulose column chromatography

For column chromatography, the freeze-dried material was dissolved in the starting buffer at a concentration of 25mg/ml, and the pH of the solution was re-adjusted with either 1M-HCl or 1M-NaOH, and the protein solution was then dialysed overnight against the starting buffer at 4°C.

The follitropin activity was first separated from most of the contaminating lutropin and thyrotropin activities by CM-cellulose chromatography at pH6.0 (0.01M-sodium phosphate buffer) as described by Condliffe & Bates (1956). Under these conditions, the bulk of the follitropin was in the unadsorbed fractions, which were pooled, dialysed and freeze-dried. Follitropin was then fractionated from other contaminant proteins by adsorbing on a CM-cellulose column at pH5.4, equilibrated with 5mM-ammonium acetate, and was eluted by a stepwise gradient of increase concentrations of ammonium acetate at pH5.4. The active fractions were pooled, dialysed and freeze-dried.

DEAE-cellulose column chromatography

The ion-exchanger was equilibrated in 5mM-sodium glycinate buffer, pH9.5. Under these conditions, the small amount of remaining lutropin as contaminant was unadsorbed and eluted with the solvent front as described by Condliffe *et al.* (1959); the adsorbed follitropin was eluted subsequently with a gradient of increasing ionic strength as used by Pierce *et al.* (1971) for the isolation of bovine thyrotropin. The gradient was generated in a Varigrad apparatus with nine chambers (Buchler Instruments Inc., Fort Lea, NJ, U.S.A.). The concentrations (M) of sodium glycinate buffer, pH9.5, were: chamber 1, 0.005; 2, 0.05; 3, 0.10; 4, 0; 5, 0.10; 6, 0; 7, 0.20; 8, 0.25; 9, 0.15. Elution was completed by further addition of 0.15M-buffer. To locate the activity, the eluates were assayed by a radioligand-receptor assay for follitropin, and the appropriate fractions were pooled, dialysed and freeze-dried.

Follitropin was further purified by adsorbing on a DEAE-cellulose column in 0.04M-Tris/HCl buffer, pH8.2 (Fontaine & LeBelle, 1965), and was eluted by generating a continuous gradient of 0–0.2M-NaCl in the Tris/HCl buffer. The eluates were assayed with a

radioligand-receptor assay to locate follitropin activity. The appropriate fractions were pooled, dialysed and freeze-dried.

Gel filtration on Sephadex G-100

Bovine follitropin was finally purified by gel filtration on Sephadex G-100 in 0.5% NH_4HCO_3 buffer, pH8.2. The appropriate fractions of bovine follitropin were located again by a radioligand-receptor assay and pooled and freeze-dried.

Bioassay for follitropin

The human choriogonadotropin-augmentation method of Steelman & Pohley (1953) was used for bioassay of the purified bovine pituitary follitropin. Sprague–Dawley rats of 25 days old were used, and the potency was expressed in terms of NIH-FSH-S1 standard.

^{125}I labelling

Bovine follitropin was ^{125}I-labelled with 1.0mCi of ^{125}I per 5μg of hormone by the enzymic method with lactoperoxidase as described previously (Cheng, 1975*a*). Bovine lutropin and thyrotropin, 5μg each, were iodinated with 0.5mCi of ^{125}I by the chloramine-T method (Hunter & Greenwood, 1962). The ^{125}I-labelled hormones were separated from free ^{125}I by gel filtration on Sephadex G-100 in 25mM-Tris/HCl, pH7.2.

Polyacrylamide-gel electrophoresis

Polyacrylamide-gel electrophoresis was performed by the method of Davis (1964). Gels (0.4cm×6.0cm) of 7.5% (w/v) polyacrylamide were used. Samples (50 or 100μg) were introduced in 40% (w/v) sucrose solution. The gels after electrophoresis were stained by 1% (w/v) Amido Black 10B solution in 7% (v/v) acetic acid.

Determination of molecular weight by gel filtration

The molecular weight of purified bovine follitropin was determined by gel filtration as described by Andrews (1964). A Sephadex G-100 column (1.4cm×200cm) equilibrated with 0.1M-sodium phosphate buffer containing 0.1M-NaCl at pH7.0 was used. The column was calibrated for elution volumes of ovalbumin, chymotrypsinogen A, myoglobin and cytochrome *c* with mol.wts. of 45000, 25000, 17800 and 12400 respectively.

Amino acid analysis

Samples (500μg each) of purified bovine follitropin were hydrolysed with 6M-HCl in evacuated, sealed tubes at 110°C for 22h. Amino acid analyses were performed with a Beckman model 120C amino acid analyser.

Results

Extraction and precipitation of bovine pituitary follitropin

Data on the recovery of materials and follitropin activity at different steps of purification are summarized in Table 1. About 70% of the total follitropin in the crude extract was recovered in the fraction precipitated by 45–70%-satd. $(NH_4)_2SO_4$ (Table 1). Follitropin activity was concentrated further by adjusting the pH of the extract to 4.1, and precipitation of proteins by adding acetone to the solution to give concentrations of 50 and 75% (v/v) acetone (Table 1).

Step-gradient chromatography on CM-cellulose

The straw-coloured 50–75%-acetone precipitate was dissolved in 0.01M-sodium phosphate buffer, pH6.0, and fractionated on a CM-cellulose column in the same buffer. Under these conditions, the unadsorbed fractions containing the bulk of the follitropin activity were pooled, dialysed and freeze-dried (fraction FSH-CM-I). The adsorbed fractions,

Table 1. *Recovery of follitropin from 1kg of frozen bovine pituitary glands*

The procedure used to obtain each fraction is described in the Materials and Methods section. The protein yields are dry weights of freeze-dried materials. Relative potencies are determined by radioligand-receptor assay with reference to human follitropin (LER-1575C). The percentage of follitropin recovered in each fraction was calculated with reference to the original crude extract as 100%. Values are the mean of two separate extractions of 4.5kg each of frozen bovine pituitary glands.

Fraction	Yield of protein (mg)	Relative potency (μg/mg)	Total activity (mg)	Recovery (%)
Crude extract	52000–58000	0.5	27.50	100
45–70%-satd.-$(NH_4)_2SO_4$ precipitate	6400–7000	2.8	18.76	68.2
50–75% (v/v)-acetone precipitate	750–800	14.0	10.85	39.5
FSH-CM-I	560–620	16.6	9.79	35.6
FSH-CM-II	19–23	201.9	4.24	15.4
FSH-DE-I	7.6–8.1	380.0	2.98	10.8
FSH-DE-II	4.1–4.6	580.0	2.52	9.2
FSH	2.7–3.2	780.0	2.30	8.4

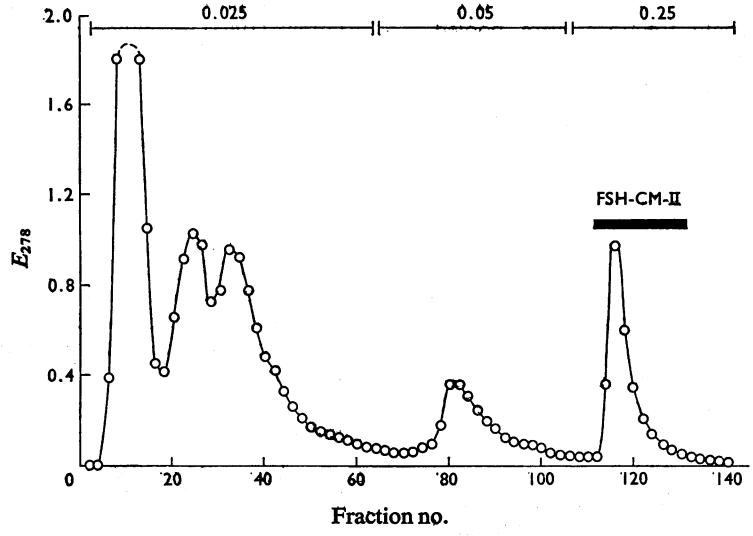


Fig. 1. *Chromatography of the FSH-CM-I fraction on a CM-cellulose column* (3 *cm* × 18 *cm*) *in* 5 *mM-ammonium acetate, pH* 5.4

The material (2.6 g) was dissolved in 150 ml of buffer and applied to the column. A stepwise elution with 0.025, 0.05 and 0.25 M-ammonium acetate was used to fractionate the proteins as indicated. Fractions (15 ml) were collected at a flow rate of 70 ml/h. The appropriate fractions (FSH-CM-II) were pooled as indicated by the bar.

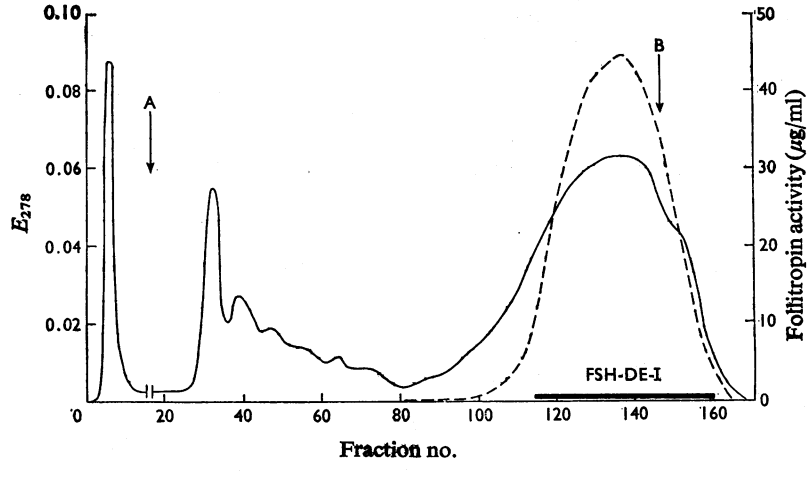


Fig. 2. *Chromatography of the FSH-CM-II fraction on a DEAE-cellulose column* (1.8 *cm* × 15 *cm*) *in* 5 *mM-sodium glycinate, pH* 9.5

The material (95 mg) was dissolved in 20 ml of buffer and applied to the column. After elution of the unadsorbed material, the column was eluted with a continuous gradient (arrow A) of increasing ionic strength generated by a nine chamber Varigrad apparatus of 150 ml per chamber (see the Materials and Methods section), and the elution was finished by applying more 0.15 M-sodium glycinate, pH 9.5 (as indicated by arrow B). Fractions (10 ml) were collected at a flow rate of 50 ml/h. Protein was monitored by E_{278} measurements (——) and follitropin activity (– – – –) was measured by radioligand-receptor assay. Appropriate fractions (FSH-DE-I) were pooled as indicated by the bar.

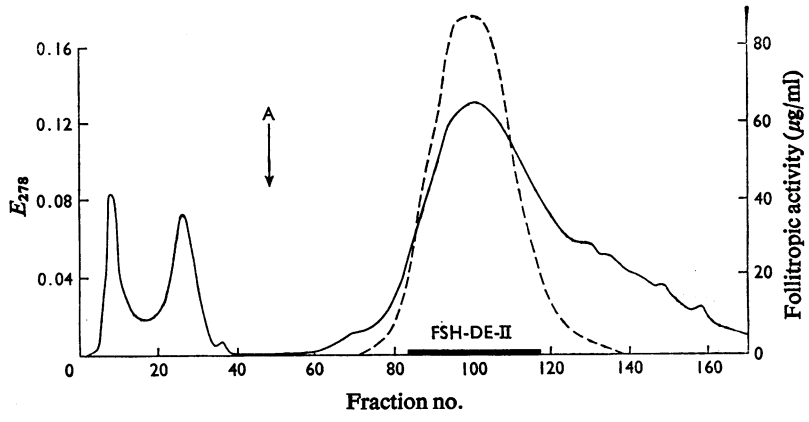


Fig. 3. *Chromatography of the FSH-DE-I fraction on a DEAE-cellulose column* (1.4cm×10cm) *in* 0.04M-*Tris/HCl, pH*8.2

The material (35.3mg) was dissolved in 4ml of 0.02M-Tris/HCl and applied to the column. After elution of the unadsorbed material with 0.04M-Tris/HCl, a linear gradient of increasing molarity of NaCl (obtained by mixing 300ml each of 0.04M-Tris/HCl and buffer containing 0.2M-NaCl), as indicated by arrow A, was applied. Fractions (5ml) were collected at a flow rate of 20ml/h. Appropriate fractions (FSH-DE-II), monitored by radioligand-receptor assay (----), were pooled as indicated by the bar. ——, E_{278}.

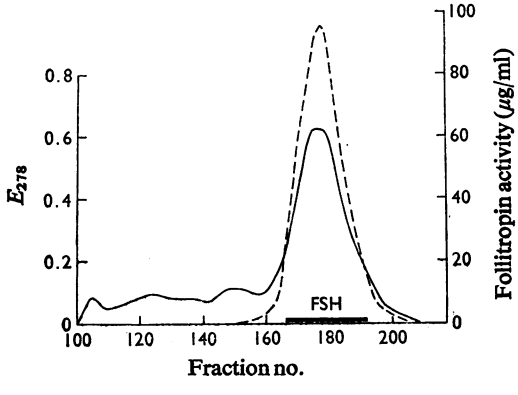


Fig. 4. *Gel filtration of the FSH-DE-II fraction on Sephadex G-100 in* 0.5% NH_4HCO_3

The material (19.6mg) was dissolved in 3ml of buffer and applied to the column (2.5cm×300cm). Fractions (4.5ml) were collected at a flow rate of 25ml/h. Appropriate fractions (FSH, 8.2mg), monitored by radioligand-receptor assay (----), were pooled as indicated by the bar. ——, E_{278}.

containing most of the lutropin and thyrotropin activities, were eluted with 0.1M-sodium phosphate buffer/0.1M-NaCl, pH6.0.

Follitropin (fraction FSH-CM-I) was further purified by adsorbing on a CM-cellulose column at pH5.4 (Fig. 1). Large peaks corresponding to proteins without activity were obtained after eluting with 0.025M-ammonium acetate. Another protein with low activity was eluted with 0.05M-ammonium acetate. Over 60% of the follitropin activity applied to the column was eluted with 0.25M-ammonium acetate (Fig. 1). These fractions (FSH-CM-II) were pooled, dialysed and freeze-dried.

Continuous-gradient chromatography on DEAE-cellulose

The follitropin fraction (FSH-CM-II) was adsorbed on a DEAE-cellulose column at pH9.5, and eluted with a continuous gradient of increasing ionic strength of sodium glycinate buffer, pH9.5, generated by a Varigrad apparatus with nine chambers (Fig. 2). The appropriate fractions (FSH-DE-I) were pooled, dialysed and freeze-dried.

Finally, the follitropin fraction (FSH-DE-I) was again adsorbed on a DEAE-cellulose column at pH8.2, and eluted with a gradient of increasing concentration of NaCl from 0 to 0.2M. The distribution of proteins and follitropin activity (fraction FSH-DE-II) is shown in Fig. 3.

Gel filtration on Sephadex G-100

Fig. 4 shows the elution pattern of proteins and follitropin activity of the freeze-dried material (fraction FSH-DE-II) after gel filtration on Sephadex G-100. The peak of follitropin activity was coincident with the major protein peak. The appropriate fractions (fraction FSH) were pooled and freeze-dried as the final product of purified bovine pituitary follitropin.

Table 2. *Contaminations of lutropin, thyrotropin, prolactin and growth hormone in highly purified bovine follitropin preparation as assessed by specific radioligand-receptor assays*

Values are means of two separate determinations. Reference standards: bovine lutropin (potency 2.0 times that of LH-S1-N2H) and bovine thyrotropin (potency 30i.u./mg) (both from Dr. J. G. Pierce); human growth hormone (HS1652C); sheep prolactin (NIH-P-S10). N.D., not detectable.

Bovine follitropin sample (ng/ml)	Hormonal activities tested			
	Lutropin (ng/ml)	Thyrotropin (ng/ml)	Prolactin (ng/ml)	Growth hormone (ng/ml)
100	N.D.	N.D.	N.D.	N.D.
1000	1.4	1.7	N.D.	N.D.
10000	11.8	13.6	N.D.	N.D.
100000	124.6	156.2	8.8	10.2

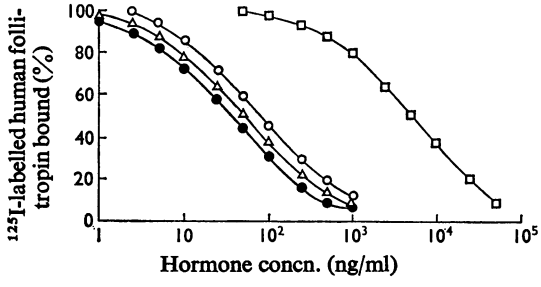


Fig. 5. *Dose–response curves for purified bovine, sheep and human follitropin in a radioligand-receptor assay with ^{125}I-labelled human follitropin as tracer*

Purified bovine follitropin (△); purified sheep follitropin, LER-1818-2 (○); purified human follitropin, LER-1575C (●); sheep follitropin reference standard, NIH-FSH-S1 (□).

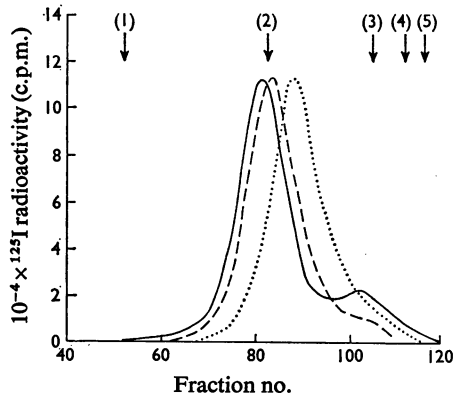


Fig. 6. *Determination of molecular weights for ^{125}I-labelled bovine follitropin, lutropin and thyrotropin by gel filtration on a Sephadex G-100 column (1.4cm×200cm)*

Sodium phosphate buffer (0.1M) at pH7.0, containing 0.1M-NaCl, was used as eluting solvent. ——, Follitropin; – – – –, lutropin; ····, thyrotropin; The standards are: 1, Blue; Dextran 2, ovalbumin; 3, chymotrypsinogen A; 4, myoglobin; 5, cytochrome *c*.

Bioassays and radioligand-receptor assays performed with purified bovine follitropin

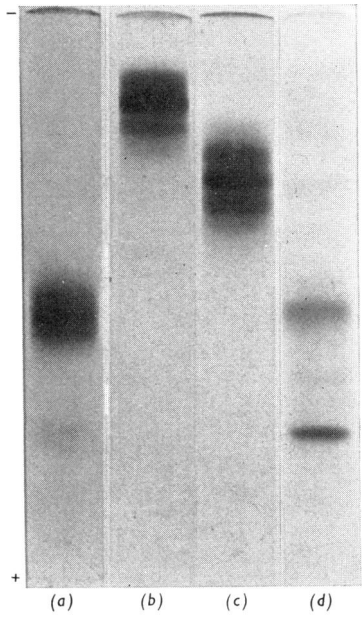
By using the Steelman–Pohley (1953) bioassay, the relative potency of purified bovine follitropin was found to be 164 times that of NIH-FSH-S1 with 95%-confidence limits of 126–188.

The bovine follitropin preparation was assessed for contamination by pituitary protein and glycoprotein hormones by radioligand-receptor assays for lutropin, thyrotropin, growth hormone and prolactin. Purified bovine follitropin was practically free from growth hormone and prolactin at concentrations of 100μg/ml, and was contaminated with approx. 0.1–0.2% each of lutropin and thyrotropin as monitored by radioligand-receptor assays (Table 2).

By radioligand-receptor assay, the purified bovine follitropin was found to be 120 times the potency of the sheep follitropin (NIH-FSH-S1) reference standard. This preparation of bovine follitropin was also demonstrated to be more active than the purified sheep follitropin (LER-1818-2). The slope of all these dose–response curves was almost identical (Fig. 5). The difference in degree of inhibition might reflect the degree of purity of the different preparations of follitropin, or species difference interacting with the receptor molecule.

Polyacrylamide-gel electrophoresis

Electrophoretically, purified bovine follitropin was homogeneous and gave a rather diffuse protein band, which migrated further towards the anode in gels at pH8.9 than did lutropin and thyrotropin, as shown in Plate 1.



EXPLANATION OF PLATE 1

Polyacrylamide-gel electrophoresis of purified preparations of bovine follitropin, lutropin and thyrotropin

Samples of 100 μg per gel were used, and electrophoresis was performed at 2 mA per gel for 1 h. (*a*) Bovine follitropin after Sephadex G-100 purification (see Fig. 4); (*b*) purified bovine lutropin; (*c*) purified bovine thyrotropin; (*d*) crude bovine follitropin from the National Institutes of Health.

Table 3. *Comparison of amino acid compositions of bovine follitropin with human and sheep follitropin, and bovine lutropin and thyrotropin*

Values for bovine follitropin were expressed in number of residues/mol, assuming the total weight of amino acid residues was approx. 22000 g/mol, based on the reported sequence of human follitropin. Similarly, the amino acid composition for sheep follitropin from Papkoff *et al.* (1967*a*) was re-adjusted for comparison on the same basis. The number of residues of amino acids for human follitropin (Shome & Parlow, 1974*a*,*b*), and bovine lutropin and thyrotropin (Pierce *et al.*, 1971) were obtained from reported sequence data. Values were not corrected for destruction or incomplete liberation during hydrolysis.

Amino acid	Human follitropin	Sheep follitropin	Bovine follitropin			Bovine lutropin	Bovine thyrotropin
			Expt. 1	Expt. 2	Mean		
Lysine	13	14.9	13.8	14.6	14.2	12	19
Histidine	6	5.5	5.1	4.9	5.0	6	6
Arginine	8	8.0	8.3	8.7	8.5	11	7
Aspartic acid	15	16.6	16.3	16.9	16.6	11	15
Threonine	22	17.2	20.4	18.8	19.6	16	20
Serine	15	13.3	12.3	13.0	12.7	14	11
Glutamic acid	20	18.5	19.6	19.9	19.8	14	15
Proline	11	16.9	12.0	10.0	10.0	28	14
Glycine	10	10.9	10.3	10.3	10.3	11	8
Alanine	10	13.6	13.5	12.6	13.1	15	13
Cystine (half)	22	14.7	17.0	17.9	17.5	22	22
Valine	13	11.6	13.1	10.2	12.7	13	11
Methionine	4	2.4	3.7	4.2	4.0	7	9
Isoleucine	7	7.8	7.2	7.2	7.2	7	8
Leucine	9	9.8	9.3	10.2	9.8	14	6
Tyrosine	11	8.6	10.9	8.6	10.3	7	16
Phenylalanine	7	7.9	6.9	6.3	6.6	8	9

Determination of molecular weight by gel filtration on Sephadex G-100

^{125}I-labelled bovine follitropin, lutropin and thyrotropin were applied separately on to a pre-calibrated column of Sephadex G-100 for determination of molecular weight. Fig. 6 depicts the elution patterns of the ^{125}I-labelled hormones and the protein markers. The elution volumes of ^{125}I-labelled bovine follitropin and lutropin were very similar, whereas that of thyrotropin was retarded slightly further (Fig. 6).

Amino acid composition

Table 3 shows the amino acid composition of bovine follitropin, which is rich in lysine, aspartic acid, threonine, glutamic acid and half-cystine. However, the presence of tryptophan in bovine follitropin has not been determined.

Discussion

Follitropin activity has been demonstrated in extracts of bovine pituitary glands (Nalbandov & Casida, 1940), but the content of follitropin in this species was shown to be very low (Ellis, 1958, 1961). There has been only one report (Reichert & Jiang, 1965) on the purification of bovine pituitary follitropin, and the potency of this partially purified preparation was only 3.1 times that of NIH-FSH-S1 standard. In the present studies, a reproducible and relatively simple procedure, utilizing a radioligand-receptor assay for follitropin (Cheng, 1975*a*,*b*), has been developed for the preparation of highly purified follitropin from bovine pituitary glands. The specific activity of this preparation was measured to be 164 times that of NIH-FSH-S1 standard. When the potency of this preparation was determined by radioligand-receptor assay, utilizing ^{125}I-labelled human follitropin as tracer and unlabelled human follitropin as standard, this preparation of bovine follitropin was only 120 times that of NIH-FSH-S1 standard (Fig. 5). However, in a radioligand-receptor assay system using ^{125}I-labelled bovine follitropin, the biological activity of purified bovine follitropin was shown to be even higher than that of purified human follitropin (LER-1575C), being 210 times that of NIH-FSH-S1 standard (K.-W. Cheng, unpublished work).

One major problem in the purification of bovine follitropin was the fractionation and recovery of the small amounts of follitropin present in the pituitary glands from relatively large quantities of lutropin and to a lesser degree thyrotropin. Because of the quantity of lutropin, a very small percentage of this hormone remaining in a small fraction of follitropin recovered in a purification step became a major contaminant in the fraction. Accordingly, several steps of different experimental conditions were used

to isolate follitropin largely free from contamination by lutropin and thyrotropin. These were ion-exchange column chromatography on CM-cellulose at pH6.0 (Condliffe & Bates, 1956), and DEAE-cellulose at pH9.5 (Condliffe *et al.*, 1959), and at pH8.2 (Fontaine & LeBelle, 1965) by the established procedures for the purification of bovine lutropin and thyrotropin. Another useful step in separating bulk of contaminating inert proteins from follitropin activity was column chromatography on CM-cellulose in ammonium acetate, pH5.4 (Fig. 1). However, it should be pointed out that a specific radioligand-receptor assay (Cheng, 1975*a*) is almost essential for monitoring the distribution of follitropin activity in eluates after continuous-gradient ion-exchange column chromatography (Figs. 2 and 3). Though the follitropin activity was found in the major protein peak, the distribution of activity in the eluates was not exactly identical with that of the protein distribution. To obtain highly purified bovine follitropin, a specific radioligand-receptor assay should therefore be used as a routine in these steps of this procedure of isolation.

Electrophoretically, follitropin was more acidic than lutropin and thyrotropin, and migrated further towards the anode (Plate 1). This acidic nature of bovine follitropin is probably due to the presence of sialic acid in the molecule; presence of sialic acid has been reported in purified follitropin preparations of sheep (Papkoff *et al.*, 1967*a*; Cahill *et al.*, 1968) and human (Papkoff *et al.*, 1967*b*; Reichert *et al.*, 1968).

The mol.wt. of bovine follitropin was found to be 45000 by gel filtration on Sephadex G-100 (Fig. 6); however, the elution volume of bovine follitropin was very similar to that of bovine lutropin (Fig. 6). By sedimentation analysis, the mol.wt. of bovine lutropin has been reported to be 30000 (Reichert *et al.*, 1969; Reichert, 1971). Similarly, mol.wts. for sheep and human follitropin were demonstrated to be 30000–32000 (Roos & Gemzell, 1964; Papkoff *et al.*, 1967*a*,*b*; Reichert, 1971). In view of these findings, the mol.wt. for bovine follitropin is probably approx. 30000. Further, it is noteworthy that a small peak of radioactivity corresponding to mol.wt. of approx. one-half that of ^{125}I-labelled follitropin was observed after gel filtration on Sephadex G-100 (Fig. 6). This is probably due to dissociation of intact follitropin into its subunits upon ^{125}I-labelling. The presence of subunit structure in bovine follitropin has been demonstrated (K.-W. Cheng, unpublished work).

The amino acid composition of bovine follitropin was similar to that of sheep follitropin (Papkoff *et al.*, 1967*a*) and human follitropin (Shome & Parlow, 1974*a*,*b*), being rich in lysine, aspartic acid, threonine, serine, glutamic acid and half-cystine (Table 3). Bovine follitropin was different from bovine lutropin in having fewer proline, glycine, methionine, leucine and phenylalanine residues, and from bovine thyrotropin in having fewer methionine, tyrosine and phenylalanine residues (Table 3). However, the exact chemical relationships between these glycoprotein hormones within and between species remain to be elucidated.

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References

Amir, S. M. & Parlow, A. F. (1972) *Proc. Soc. Exp. Biol. Med.* **139**, 1120–1122
Andrews, P. (1964) *Biochem. J.* **91**, 222–233
Braselton, W. E., Jr. & McShan, W. H. (1970) *Arch. Biochem. Biophys.* **139**, 45–48
Cahill, C. L., Shetlar, M. R., Payne, R. W., Endecott, B. & Li, Y. T. (1968) *Biochim. Biophys. Acta* **154**, 40–52
Cheng, K.-W. (1975*a*) *J. Clin. Endocrinol. Metab.* **41**, 581–589
Cheng, K.-W. (1975*b*) *Biochem. J.* **149**, 123–132
Cheng, K.-W. (1976) *Biochem J.* **159**, 71–77
Condliffe, P. G. & Bates, R. W. (1956) *J. Biol. Chem.* **223**, 843–852
Condliffe, P. G., Bates, R. W. & Fraps, R. M. (1959) *Biochim. Biophys. Acta* **34**, 430–438
Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404–427
Ellis, S. (1958) *J. Biol. Chem.* **233**, 63–68
Ellis, S. (1961) *Endocrinology* **69**, 554–570
Fontaine, Y. A. & LeBelle, N. (1965) *Curr. Top. Thyroid Res. Proc. Int. Thyroid Conf. 5th* 424–432
Hermier, C. & Jutisz, M. (1969) *Biochim. Biophys. Acta* **175**, 402–408
Hunter, W. M. & Greenwood, F. C. (1962) *Nature (London)* **194**, 495–496
Nalbandov, A. V. & Casida, C. E. (1940) *Endocrinology* **27**, 424–432
Papkoff, H., Gospodarowicz, D. & Li, C. H. (1967*a*) *Arch. Biochem. Biophys.* **120**, 434–439
Papkoff, H., Mahlmann, L. J. & Li, C. H. (1967*b*) *Biochemistry* **6**, 3976–3982
Peckham, W. D. & Parlow, A. F. (1969) *Endocrinology* **85**, 618–622
Pierce, J. G., Liao, T. H., Howard, S. M., Shome, B. & Cornell, J. S. (1971) *Recent Progr. Horm. Res.* **27**, 165–212
Rathnam, P. & Saxena, B. B. (1975) *J. Biol. Chem.* **250**, 6735–6746
Reichert, L. E., Jr. (1971) *Endrocinology* **88**, 1029–1044
Reichert, L. E., Jr. & Jiang, N. S. (1965) *Endocrinology* **77**, 124–127
Reichert, L. E., Jr., Kathan, R. H. & Ryan, R. J. (1968) *Endocrinology* **82**, 109–114
Reichert, L. E., Jr., Rasco, M. A., Ward, D. N., Niswender, G. D. & Midgley, A. R., Jr. (1969) *J. Biol. Chem.* **244**, 5110–5117

Roos, P. & Gemzell, C. (1964) *Biochim. Biophys. Acta* **82**, 218–220

Ryan, R. J., Jiang, N. S. & Hanlon, S. (1971) *Biochemistry* **10**, 1321–1330

Saxena, B. B. & Rathnam, P. (1967) *J. Biol. Chem.* **242**, 3769–3775

Sherwood, O. D., Grimek, H. J. & McShan, W. H. (1970) *J. Biol. Chem.* **245**, 2328–2336

Shiu, R. P. C., Kelly, P. A. & Friesen, H. G. (1973) *Science* **180**, 968–971

Shome, B. & Parlow, A. F. (1974*a*) *J. Clin. Endocrinol. Metab.* **39**, 199–202

Shome, B. & Parlow, A. F. (1974*b*) *J. Endocrinol. Metab.* **39**, 203–205

Steelman, S. L. & Pohley, F. M. (1953) *Endocrinology* **53**, 604–616

Stockell-Hartree, A. (1975) *Methods Enzymol.* **37**, Part B, 380–389

Tsushima, T. & Friesen, H. G. (1973) *J. Clin. Endocrinol. Metab.* **37**, 334–337

Verrier, B., Fayet, G. & Lissitzky, S. (1974) *Eur. J. Biochem.* **42**, 355–365