We obtained thyroglobulin from human nontoxic goitrous thyroids [6] by Karlsson's method [7] with our modification of ultrafiltration through 1–3% perlodium. The lyophilized protein was electrophoretically homogeneous at pH 4.7–6.7 and 8.5. A sedimentation analysis showed that 90% of the protein had 19S, 5% had 27S, and 5% had 12S. The appearance of 27S and 12S is explained by the influence of the preliminary lyophilization of the protein. The protein contained 15.5% of nitrogen.

In order to avoid steric hindrance [5], the sialic acids were removed by Spiro's method [2]: 50 mg of a mixture of the sample and salt (about 5 mg of pure protein) was dissolved in 0.3 ml of water. The solution was treated with 1 N sulfuric acid to pH 1.5 and then 0.05 N sulfuric acid was added to give a 0.3% solution of the protein and this was heated in the water bath at 80° C for 1 hr. The protein was freed from sialic acid by dialysis (initially against 0.1 M NaCl and then against water), and the protein precipitate was collected in the centrifuge.

Edman degradation in Eriksson and Sjöquist's modification [8] was carried out with some of our own modifications. To the protein precipitate was added 1 ml of water and 2 ml of a mixture of pyridine, triethylamine, and phenyl isothiocyanate (100:3:1). The mixture was thermostated at 40° C for 1.5 hr, the excess of solvent was removed by extraction with benzene 7–8 times, and the mixture was dried over phosphorus pentoxide or sodium hydroxide and paraffin wax. Cyclization was carried out by our combination of the two methods of Sjöquist [8] and Light [9]. To the PTH-protein were added 1 ml of water and then 2 ml of a mixture of glacial acetic acid and concentrated hydrochloric acid (5:1) and the resulting mixture was thermostated at 40° C for 2 hr, after which it was dried over sodium hydroxide in vacuum. The dry residue was treated with 2 ml of water saturated with a mixture of ethyl acetate and methyl ethyl ketone. The PTH-derivatives were extracted with a mixture of ethyl acetate and methyl ethyl ketone (2:1) and dried by Belitser's method [10].

Hydrolysis of the PTH-amino acids was carried out with 11.86 N ammonia for 14–16 hr [11]. Descending one-dimensional chromatograms were carried out in aqueous phenol and in the butanol—acetic acid—water (4:1:5) system. On each of the two chromatograms of the material studied we obtained two spots. On comparing the spots obtained with reference samples it was found that the N-terminal amino acids of human thyroglobulin are alanine and glycine. Consequently, the molecule of thyroglobulin consists of two chains beginning with alanine and glycine.

REFERENCES

1. B. de Crombrugghe, R. Pitt-Rivers, and H. Edelhoch, J. Biol. Chem. 241, 2766, 1966.
2. R. G. Spiro and M. J. Spiro, J. Biol. Chem., 240, 997, 1965.
3. J. Roche, R. Michel, and J. Nunez, Bull. Soc. Chem. Biol., 37, 219, 1955.
4. J. Roche, R. Michel, and J. Nunez, Bull. Soc. Chem. Biol., 37, 229, 1955.
5. T. A. A. Dopheide and V. M. Trikojus, Nature, 201, 1128, 1964.
6. I. G. Pierce, A. B. Rawitch, D. M. Brown, and P. G. Stanley, Biochim. Biophys. Acta, 111, 247, 1965.
7. R. Karlsson, V. Kivikangas, and B. A. Lamberg, Acta Chem. Scand., 17, 2347, 1963.
8. S. Eriksson and J. Sjöquist, J. Biochim. Biophys. Acta, 45, 290, 1960.
9. A. Light and J. Greenberg, J. Biol. Chem., 240, 258, 1965.
10. V. A. Belitser and N. N. Orlovskaya, Biokhimiya, 29, 741, 1964.
11. G. Ya. Lamm, A. R. Rakhimov, K. G. Ioffe, and I. A. Asatov, KhPS [Chemistry of Natural Compounds], 62, 1968.

11 November 1967

Institute of Biochemistry,
AS UzSSR

UDC 547.962.3

A STUDY OF THE WATER-SOLUBLE FRACTION OF THE PROTEINS OF THE SEEDS OF THE COTTON PLANT

P. Kh. Yuldashev, N. K. Osmolovskaya, M. A. Kuchenkova, and N. Dzhanbaeva

Khimiya Prirodnykh Soedinenii, Vol. 4, No. 1, pp. 64–66, 1968

In the Laboratory of the Chemistry of Plant Proteins of the Institute of the Chemistry of Plant Substances, AS UzSSR, a study of the protein composition of the seeds of the cotton plant (variety 108-F) has been begun. The seeds freed from the pods and comminuted were defatted [1] (total nitrogen 8.72, protein nitrogen 7.65% of the weight of the absolutely dry defatted meal). To select the conditions for the maximum extraction of the water-soluble fraction of the proteins we used extraction with water at 0° C and extraction with 10% sodium chloride (total nitrogen in the extract 6.68; protein nitrogen 6.19%). The extracts obtained were dialyzed and the globulin components that deposited were separated off by

centrifuging. The extract obtained in this way (nitrogen of the albumin fraction 1.72%, globulin nitrogen 4.47%) was freeze-dried. The yield was 4% of the initial defatted meal in the cases both of the aqueous extract and the salt extract.

An electrophoretic investigation using the acrylamide gel micromethod [2] showed the presence in the total aqueous extract of 14 zones: two zones in the cathode region and twelve zones in the anode region (Fig. 1). An electrophoregram of the freeze-dried albumin fraction showed eight zones migrating in the direction of the anode.

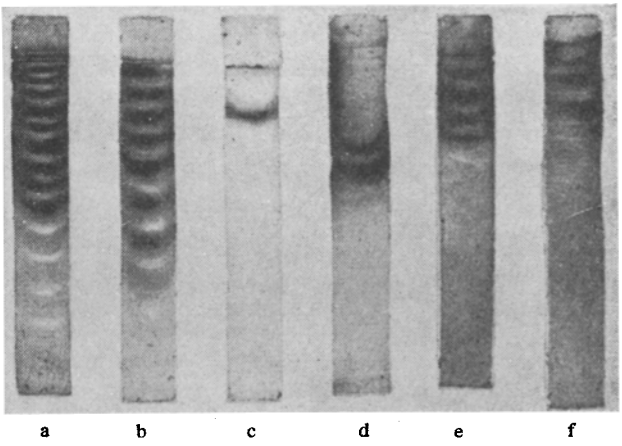

Fig. 1. Electrophoregrams in acrylamide gel. a) Total water-insoluble fraction; b) albumin fraction; c) second fraction from DEAE-cellulose; d) third; e) fourth; f) fifth fraction.

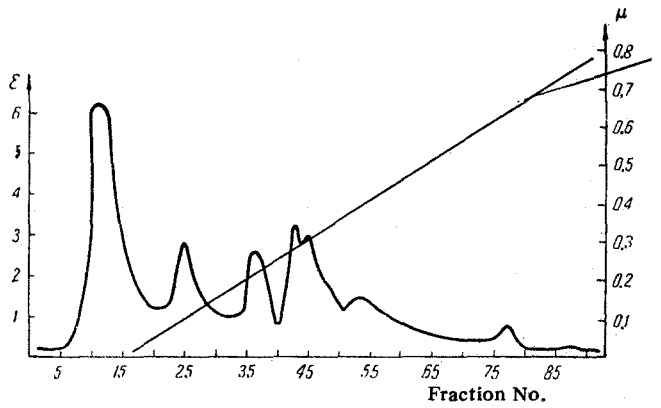

Fig. 2. Separation of the albumin fraction on DEAE-cellulose.

The separation of the total water-soluble fraction on DEAE-cellulose and on calcium phosphate gave similar results (column diameter 2.5 cm, rate of elution 20 ml/hr, 700 mg of protein; elution with phosphate buffer, pH 7.4, with NaCl gradient) (Fig. 2).

The fractions belonging to the individual peaks were combined, dialyzed, and freeze-dried. The dried fractions were subjected to electrophoresis in acrylamide gel. The fraction corresponding to the first peak proved to be nonprotein material. The second fraction (yield 14.1%) showed a single zone on the electrophoregram, the third (yield 17.8%) two zones, the fourth (yield 20.1%) four zones, and the fifth (yield 26%) two zones. Work on the isolation and further separation of the albumin fraction of the proteins is continuing.

REFERENCES

1. B. Mondovi, P. Fasella, C. Turano, A. M. Wolf, A. Scioscia-Santoro, P. Turini, F. Bossa, and D. Cavallini, Italian Journal of Biochemistry. V. XIII, 6, 429, 1964.

2. Yu. Ya. Gofman, Symposium on the Chemistry and Biochemistry of Plant Proteins [in Russian], p. 15, Kishinev, 1966.

26 September 1967

Institute of the Chemistry of Plant Substances, AS UzSSR

UDC 577.17

SOME DATA ON THE STUDY OF BOVINE LUTEINIZING HORMONE

F. Yu. Ryshka, N. K. Assonova, and A. S. Khokhlov

Khimiya Prirodnykh Soedinenii, Vol. 4, No. 1, p. 66, 1968

We have previously described a complex method for isolating and purifying the hormones of the hypophysis [1]. Luteinizing hormone has been obtained from the cakes remaining after the extraction of bovine hypophyses with acidified acetone. By subsequent purification by salt and alcohol fractionation, a sample has been obtained which is ten times more active than the NIH-LH-SI standard. Further purification of the sample by chromatography on carboxymethylcellulose and diethylaminoethylcellulose did not raise the luteinizing activity. The activity of the sample was determined by the superovulation method. The content of follicle-stimulating hormone determined from the increase in weight of the mouse uterus was not more than 0.04%. The testing was carried out by A. A. Molodyk in the Laboratory of Hormone Therapy (Director Professor N. I. Lazarev) in the Institute of Experimental and Clinical Oncology, AMS USSR.