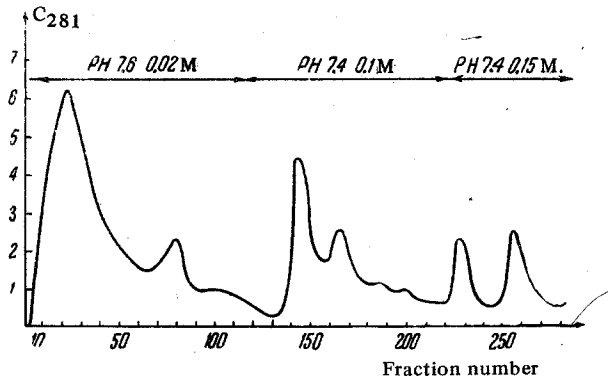

Separation of the albumin fraction on DEAE-cellulose.

dialyzed, and freeze-dried. The dried fractions were studied electrophoretically in acrylamide gel, using a micro method [4].

The presence of the following zones was established: two in the 1-st fraction, three in the 2-nd fraction, one in the 3-rd fraction, two in the 4-th fraction, two in the 5-th fraction, and one in the 6-th fraction.

The fraction corresponding to the 6-th peak possessed alanine-oxoglutarate aminotransferase activity.

REFERENCES

1. P. Kh. Yuldashev, N. K. Osmolovskaya, M. A. Kuchenkova, and N. R. Dzhanbaeva, KhPS [Chemistry of Natural Compounds], **4**, 64, 1968.

2. B. Mondovi, P. Fasella, C. Turano, A. P. Wolf, A. Scioscia-Santoro, P. Turini, F. Bossa, and D. Cavallini, G. di Biochim, **13**, 428, 1964.

3. C. Turano, F. Bossa, P. Fasella, and A. Rossi-Fanelli, Enzymologia, **30**, 185–197, 1966.

4. H. U. Bergmeyer, Methods of Enzymatic Analysis, 842, 1965.

5. Yu. Ya. Gofman, Biokhim., **32**, no. 4, 690, 1967.

7 July 1968

Institute of the Chemistry of Plant Substances, AS UzSSR

UDC 547.962

GLOBULINS OF SEEDS OF THE COTTON PLANT

Kh. Mirzarakhmatov and P. Kh. Yuldashev

Khimiya Prirodnykh Soedinenii, Vol. 4, No. 6, pp. 397–398, 1968

We have investigated the total globulin fraction obtained from the seeds of the cotton plant G. hirsutum variety 108-F. From the defatted flour, the protein was extracted with 2 M sodium chloride solution (pH 7), the globulins were precipitated by dialysis against distilled water, and the precipitate was repeatedly extracted with water to eliminate water-soluble proteins.

From the globulin fraction so obtained, by microelectrophoresis in acrylamide gel [1], we isolated three anodic components with R_f 0.34, 0.52, and 0.65 (Fig. 1, a and b).

By chromatography on columns of DEAE-cellulose, the total globulin fraction was separated into several chromatographic fractions differing by the ionic strength of their elution (Fig. 2). Columns of DEAE-cellulose (of the firm of Serva, German Federal Republic) with a capacity of 0.62 meq/g (1.35 × 37) were used. Elution was carried out with phosphate buffer (pH 7.3) with a stepwise increase in the ionic strength from 0.2 to 1 by the addition of sodium chloride. The electrophoretic composition of fraction 1 (ionic strength of elution 0.2) consisted of components with R_f 0.52 and 0.65 (see Figs. 1 and 2). In fractions 2–5 (ionic strength of elution 0.3, 0.35, 0.40, and 0.45, respectively), a single electrophoretic component with R_f on the gel 0.34 was found (see Figs. 1 and 2).

To study its composition further, the total globulin was fractionated by using its solubility in phosphate buffer (pH 7.3) and by increasing the ionic strength of the solution from 0.3 to 0.5 and 2. By cooling the extracts we obtained precipitates which were studied by gel electrophoresis, by ultracentrifugation, by their nitrogen content, and by their

amino acid composition. In all, six electrophoretically homogeneous globulin fractions with R_f 0.34 were obtained.

The investigation of the protein fractions in phosphate buffer (pH 8.05, μ = 0.5) on a Spinco ultracentrifuge showed that only fraction I (μ of extraction 0.3) was homogeneous, having a sedimentation coefficient of 8.2S. In all

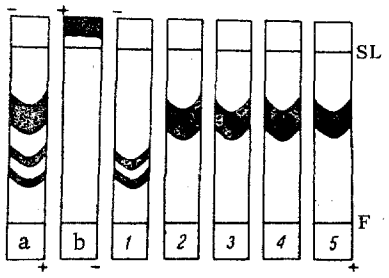

Fig. 1. Electrophoregrams of the total globulin (a, b) and of fractions 1–5. SL—starting line; F—electrophoresis front.

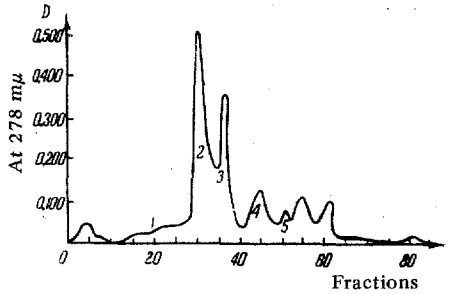

Fig. 2. Fractionating curves of the total globulin: fractions 1–5.

the other fractions, besides the 8.2S component there was a heavier component with a sedimentation coefficient of 11.8S, its content being higher in the later fractions. In fraction VI (μ of extraction 2), we found a third component, heavier than 11.8S. However, it was impossible to determine its sedimentation coefficient because of the diffuseness of the sedimentation peak.

The total nitrogen contents in the fractions were determined by the Kjeldahl and Conway method [2] (% on the absolutely dry weight): fraction I—16.05, II—13.27, III—16.04, IV—14.49, V—16.83, and fraction VI—14.76.

All six fractions differed considerably in amino acid composition, which was determined on an amino acid analyzer (% on the absolutely dry weight of the homogeneous 8.2S globulin component): alanine 3.6; arginine 10.7; aspartic acid 7.2; glutaric acid 12.5; glycine 3.6; histidine 3.3; isoleucine 3.2; leucine 6.1; lysine 3; phenylalanine 8.1; proline 4.7; serine 5.2; threonine 3.3; tyrosine 3.3; and valine 5.2.

These figures show the differing properties of the proteins making up electrophoretically homogeneous globulin fractions.

REFERENCES

1. Yu. Ya. Gofman, Biokhim., **32**, no. 4, 690, 1967.

2. A. N. Belozerskii and N. I. Proskuryakov, Practical Handbook on Plant Biochemistry [in Russian], Moscow, pp. 106, 115, 1951.

30 July 1968

Institute of the Chemistry of Plant Substances, AS UzSSR

UDC 612.015

SPECIFIC INHIBITION OF HUMAN PEPSIN AND GASTRICSIN

L. S. Lobareva, V. M. Stepanov, T. A. Solov'eva, and L. M. Ginodman

Khimiya Prirodnykh Soedinenii, Vol. 4, No. 6, pp. 398–399, 1968

It has been shown previously that N-diazoacetyl-N'-dinitrophenylethylenediamine (DDE) inhibits porcine and hen pepsins in the presence of Cu^{++} at pH 5.0 [1, 2], one inhibitor residue being added to the molecule of the enzyme which inhibits the carboxy group of aspartic acid [3]. We have established that the proteases of human gastric juice—pepsin and gastricsin [4]—are also inhibited by DDE.

The inhibition was performed by the following method: to 10 mg of the enzyme in 5 ml of 0.04 N acetate buffer with pH 5.0 was added 0.05 ml of a 0.1 M solution of copper acetate; the mixture was kept at room temperature for 10 min, and, with stirring, 1 mg of DDE in 0.1 ml of moist acetone was added. After 15 min, the proteolytic activities of human pepsin and gastricsin (with respect to the degradation of hemoglobin) had fallen to 0.25–0.5% of their initial values. The inhibited enzymes were separated from the low-molecular-weight components of the mixture by filtration through Sephadex G-25. The UV spectra of the inhibited enzymes showed the two absorption maxima at 280 and 360 mμ