hydrochloric acid at 100° C for 16 hr. The N-terminal amino acids were isolated by thin-layer chromatography on silica gel [4]. Two spots were found with R_f 0.70 and 0.38 in the propanol—34% ammonia system, corresponding to histidine and arginine.

The C-terminal amino acids were determined by the hydrazine and carboxypeptidase methods. A mixture of 50 mg of protein, 0.125 g of hydrazine sulfate, and 0.5 ml of anhydrous hydrazine was sealed in a tube which was heated in a water bath at 60° C for 16 hr. The excess of hydrazine was eliminated in a vacuum desiccator over sulfuric acid. The hydrazides of the free amino acids were separated on B3 polyacrolein resin [5]. The latter was filtered off and the filtrate was investigated for its content of amino acids by thin-layer chromatography on silica gel and in a mixed layer of cellulose and silica gel [6]. In the ethanol—34% ammonia system on silica gel we identified valine with R_f 0.45 and aniline with R_f 0.55; in the mixed layer in the phenol—water system we identified the same amino acids with R_f 0.55 and 0.66. The revealing agent was 0.5% ninhydrin in ethanol.

In the carboxypeptidase method, 0.02 ml of a suspension of carboxypeptidase in water (corresponding to ≈1 mg of enzyme) was added to 10 ml of a 0.5% solution of globulin A in a 1% solution of sodium hydrogen carbonate (ph 7.8), and the mixture was incubated at 27° C for 4 hr. The enzyme was inactivated by boiling in the water bath for 5 min, and after the addition of 2 ml of 20% TCA the mixture was centrifuged. The supernatant liquid was separated off and evaporated to dryness in vacuum at 40° C. The residue was dissolved in 0.02 ml of water. The amino acids were determined by thin-layer chromatography on silica gel in the ethanol—34% ammonia system. The revealing agent was 0.5% ninhydrin in ethanol. The two spots with R_f 0.45 and 0.55 were identified as valine and alanine.

Thus, the C-terminal amino acids of globulin A are valine and alanine, and the N-terminal acids histidine and arginine.

REFERENCES

1. A. Rossi-Fanelli et al., Biochemical and Biophysical Research Communications, **15**, no. 2, 110–115, 1964.
2. P. Andreus, Biochem. J. **91**, 222, 1964.
3. J. M. Dellacha and V. F. Angelina, Exper., **21** (6), 351, 1965.
4. D. Walz, A. R. Fahmy, G. Pataki, A. Niederzieser, and M. Brenner, Exper., **19**, 217, 1963.
5. J. Kauffmann and F. P. Boettcher, Ann. Chem., **625**, 123, 1959.
6. N. A. Turner and R. J. Redwell, J. Chromatog., **21**, no. 1, 129, 1966.

7 July 1968

Institute of the Chemistry of Plant Substances, AS UzSSR

UDC 547.962

ALANINE-OXOGLUTARATE AMINOTRANSFERASE FROM THE SEEDS OF THE COTTON PLANT

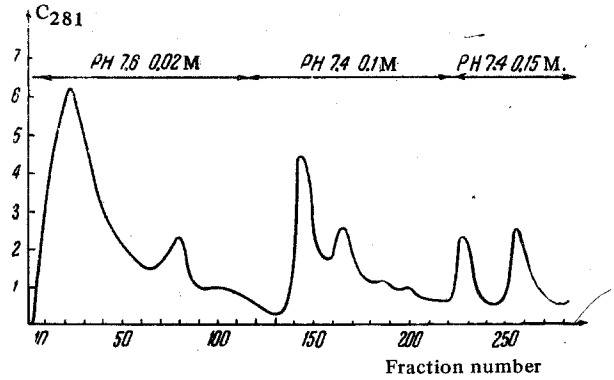
N. R. Dzhanbaeva, M. A. Kuchenkova, and P. Kh. Yuldashev

Khimiya Prirodnykh Soedinenii, Vol. 4, No. 6, pp. 396–397, 1968

Continuing an investigation of the albumin fraction of the proteins of cottonseed [1], we have isolated a fraction with alanine-oxoglutarate aminotransferase activity.

The acetone powder obtained by B. Mondovi's method [2] was suspended in five volumes of a 10^{-3} M solution of glutathione pyruvate, the pH of the mixture was brought to 6, and it was stirred at 4° C [3]. The suspension was centrifuged at 9000 × g for 15 min. The residue was discarded and the supernatant was centrifuged at 45 000 × g for 90 min. The liquid present in the intermediate layer was collected, filtered through glass wool, concentrated, and dialyzed in the cold against 3 *l* of 0.02 M phosphate (potassium) buffer (pH 7.6) for 12 hr. The contents of the dialysis bag were centrifuged at 100 000 × g for 30 min and transferred to a column of DEAE-cellulose (2.5 × 35 cm) equilibrated with the same buffer. The rate of elution was 24 ml/hr. The column was washed with 500 ml of the same buffer and eluted with 0.1 M phosphate (potassium) buffer (pH 7.4) until the density of the fraction at 280 mμ had become less than 0.3. Then, it was eluted with 0.15 M phosphate (potassium) buffer (pH 7.4) and elution was continued until the density at 280 mμ had fallen to 0.1.

The experimental results enabled a graph of the elution of the protein from the column to be plotted. All the fractions eluted were investigated for alanine-oxoglutarate aminotransferase activity [4]. The fractions were collected,



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