AN INVESTIGATION OF THE GLOBULINS OF COTTON SEEDS

III. ISOLATION OF AN 11S GLOBULIN

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By gel chromatography in a thin layer of Sephadex G-150 (superfine) we have isolated two components of high molecular weight. In order to determine their molecular weights we used protein markers with known molecular weights: bovine serum albumin, glucose oxidase, and catalase. The molecular weight of the high-molecular-weight globulin present in greatest amount, according to gel chromatography, was 312,000, and that of the second component, the amount of which did not exceed 2% of the total weight of protein, was about 500,000.

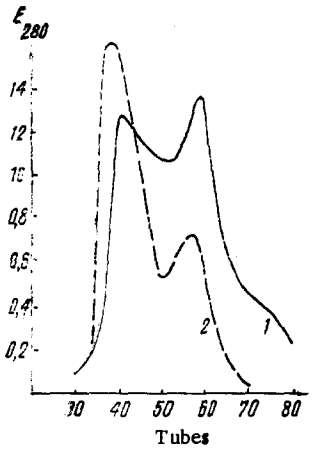

Fig. 1. Separation on a column of Sephadex G-200: 1) total globulin; 2) protein fraction after the elimination of the bulk of the 7S globulin.

In the total globulin, by sedimentation on ultracentrifugation we detected proteins with sedimentation coefficients of 7S, 11S, and 15S (10 mg of protein was dissolved in 1 ml of 10% sodium chloride, pH 7.4, or phosphate buffer, $\mu = 0.5$, pH 7.4; MOM-120 centrifuge, speed 50,000 rpm, time 40 min). According to the results of sedimentation, the molecular weight of the 7S component is 130,000, that of the 11S component 280,000, and that of the 15S component 560,000, which agrees well with the results of gel chromatography.

The N-terminal amino acids in the 11S and 15S globular components were determined by the dansylation method: histidine and traces of alanine and phenylalanine. For the preparative isolation of the 11S globulin we used gel filtration on a column of Sephadex G-200 [1] of the total globulin and protein fraction obtained after the elimination from the total protein of the bulk of the 7S globulin by a method described previously (Fig. 1).

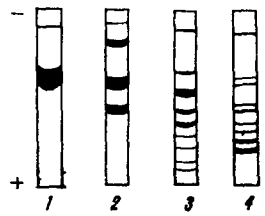

Fig. 2. Electrophoretograms obtained by disc electrophoresis in polyacrylamide gel: 1) 11S globulin in tris-buffer, pH 8.6; 2) in tris-buffer, pH 8.6, with the addition of 20% of sodium chloride; 3) in 8 M urea; 4) in 0.1% sodium dodecylsulfate.

Separation was monitored by determining the N-terminal amino acids by the dansyl method and a rough molecular weight by means of Determann's formula [2]. Further purification made use of ion-exchange chromatography on a column of DEAE-cellulose equilibrated with phosphate buffer, pH 7.4, μ 0.3 (column size 20 × 30 cm, rate of elution 12 ml/h). The protein was eluted by the superposition of an ionic strength gradient from 0.3 to 1 at a value of μ of 0.45-0.55. After dialysis and freeze drying, the protein had completely lost its capacity for dissolving in buffers and salt solutions, and, therefore, to determine the N-terminal amino acid it was dissolved in 8 M urea. Then the N-terminal amino acid was found, just as in the case of the 11S globulin obtained with a thin layer of Sephadex, to be histidine

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with trace amounts of alanine and phenylalanine. Figure 2 gives electrophoretograms [1] of the 11S component in tris-buffer (pH 8.6) and in tris-buffer with sodium chloride, and also of the 11S component previously incubated in 8 M urea and 1% dodecylsulfate at 50°C for 45 min. The results of electrophoresis show the presence of a quaternary structure in the 11S globulin of cotton seeds.

The amino-acid composition was determined after hydrolysis in 5.7 N hydrochloric acid at 105°C for 24, 48, and 72 h. The hydrolyzate was analyzed in a type AAA-881 amino-acid analyzer. The amino-acid composition was as follows (%): lysine 2.7, histidine 2.1, arginine 9.75, aspartic acid 8.57, threonine 2.8, serine 3.67, glutamic acid 15.42, proline 4.32, glycine 2.9, alanine 2.75, valine 4.12, methionine 0.625, isoleucine 2.2, leucine 5.2, tyrosine 3.27, and phenylalanine 6.12.

LITERATURE CITED

1. I. Koshiyama, Agr. Biol. Chem., 29, 885 (1965).
2. H. Determann, Gel Chromatography, Springer, New York (1968).