

COTTONSEED GLOBULINS

II. N-TERMINAL AMINO ACIDS

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By various methods of isolation the cotton plant of variety 108F has yielded three globulin components: 8.2 S with mol. wt. 100,000 and N-terminal amino acid arginine [1]; 7.2 S with mol. wt. 140,000 and N-terminal amino acid histidine [2]; and globulin A with mol. wt. 170,000 and N-terminal amino acids arginine and histidine [3]. To determine the amounts of these globulins in the native state in the seeds, we extracted the defatted seed flour of cotton plants of the variety 108F with 10% sodium chloride solution. The extract obtained was analyzed by gel filtration in a thin layer of Sephadex G-150 Superfine equilibrated with the same sodium chloride solution in order to exclude possible changes in the structure of the proteins during their separation and purification (dilution, dialysis, ion-exchange chromatography). Five components were detected, two of which were present in major amount and had mol. wt. 130,000 and \approx 300,000.

The fractions were removed from the thin layer and the amounts of N-terminal amino acids were determined by Edman's dansyl method. In a hydrolyzate of the dansylated globulin with mol. wt. 130,000 we found only one DNS-amino acid - DNS-arginine - while in the high-molecular-weight globulin the N-terminal amino acid present in major amount is histidine, and arginine is absent.

On the basis of the results of the determination of the N-terminal amino acid and a comparison of some other properties, the identity of the globulin component with mol. wt. 130,000 and the 8.2 S globulin described previously [1] was established. Since the N-terminal amino acid histidine that we found in the fraction with mol. wt. 300,000 was absent from the fraction with mol. wt. 130,000, it must be assumed that the histidine component with mol. wt. 140,000 and a sedimentation coefficient of 7.2 S possibly forms a product of dissociation in the process of the isolation of the high-molecular-weight globulin component, while globulin A is probably a mixture of the 8.2 S globulin and the high-molecular-weight globulin or the product of its dissociation.

Using the dinitrophenylation method [4] we have determined the amount of N-terminal amino acid - arginine - in the globulin with mol. wt. 130,000; this is 6 mole of amino acid per mole of protein. By disk electrophoresis in polyacrylamide gel with electric focusing [5] we have found the isoelectric point for the globulin with mol. wt. 130,000. On electric focusing in a pH gradient (3-10) the value of the pH at the isoelectric point was 3.6, while when using a pH (4-6) gradient it was 4.3. The latter value is the more accurate in view of the smoother superposition of the gradient in this case.

Thus, the N-terminal amino acid of the globulin component with mol. wt. 130,000 is arginine in an amount of 6 mole per mole of protein the isoelectric point of which is pH 4.3.

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