

A STUDY OF THE GLOBULINS OF THE SEEDS  
OF THE COTTON PLANT OF VARIETY 108-F  
AND RADIOMUTANTS

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We have previously partially characterized the globulins of the seeds of cotton plants of radiomutants of the 1965-1966 crop, studying their N-terminal groups and amino acid composition [1], a change in the ratio of the globulin components in the radiomutants being found.

This paper gives the results of a study of the globulins of cotton seeds of radiomutants of the 1964-1966 crop and of the variety 108-F by a quantitative chromatographic fractionation of the N- and C-terminal amino acids and a comparative study of the amino acid composition of two peptide fragments from the 7S globulin.

The chromatography of a salt extract (10% solution of NaCl) of defatted cotton-seed flour (after the elimination of the water-soluble proteins) on a DEAE-cellulose column containing 8 M urea gave three fractions both in the case of variety 108-F and in the case of the radiomutants (Fig. 1). The  $E_{280}/E_{260}$  ratio showed that the first fraction was free from nucleic acids. After being chromatographed twice, this fraction proved to be homogeneous on disc electrophoresis on polyacrylamide gel and with respect to the N-terminal group [2]. The second fraction was heterogeneous and contained four or five electrophoretic components, including the 7S globulin the isolation and some characteristics of which have been described previously [3]. The third fraction consisted mainly of nucleic acids.

It can be seen from Table 1 that the contents of the first and second fractions of the globulins in the seeds of the varieties studied were different. In the radiomutants of the 1964-1965 crop the content of the first fraction was higher than in variety 108-F and the radiomutant of the 1966 crop. The same results were obtained in a study of the ratio of the N-terminal amino acids of the globulins isolated by diluting a salt extract of the seed flour with water (Table 2).

The decrease in the amount of N-terminal histidine shows a fall in the relative content of the 7S globulin in the cotton seeds of the radiomutants of the 1964-1965 crop.

The figures given in Tables 1 and 2 show that the content of globulin components is restored in the radiomutant of the 1966 crop. However, the results of a study of the C-terminal amino acids of the globulins of the variants mentioned by the carboxypeptidase method of cleavage show that the radiomutant of the 1966 crop also differs from the initial variety 108-F (Table 3). In the radiomutant of all the generations the amount of C-terminal aspartic acid is twice as great as in variety 108-F. The content of C-terminal threonine and glutamic acid shows a considerable restoration of the ratio of the globulin components in the radiomutant of the 1966 crop.

It has been observed [4] that the 7S-globulin from cotton seeds irradiated in the first generation with  $\gamma$  rays at a dose of 6 kr is dimerized. On investigating the sedimentation and diffusion constants in the ultracentrifuge, it can be seen that these properties of the 7S-globulin from the radiomutants of the 1966 crop have not changed: in view of the very small amount of the preparations of the globulins, the 7S-globulin from the radiomutants of the 1964-1965 crop was not studied. The N-terminal amino acid and the C-

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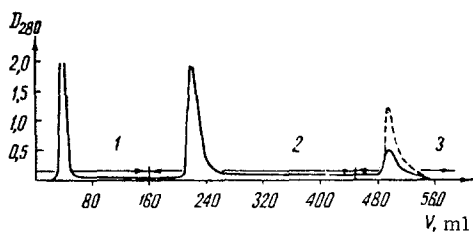


Fig. 1. Separation of a salt extract of cotton-seed flour on DEAE-cellulose: 1) 0.005 M tris-HCl, pH 8.6, in 8 M urea; 2) 0.01 M tris-HCl, pH 8.6, in 8 M urea and 2% NaCl; 3) 0.05 M tris-HCl, pH 7.5, in 8 M urea and 5% NaCl.

TABLE 3. Ratio of the C-Terminal Amino Acids in the Total Globulins of the Seeds of Cotton of Variety 108-F and Radiomutants, mole-%

Amino acid	Variety 108-F	Radiomutant of the crop (year)		
		1964	1965	1966
Aspartic acid	5,16	11,65	11,45	11,60
Threonine	24,50	32,90	31,60	21,75
Serine	9,65	10,05	9,27	10,75
Glutamic acid	9,20	3,10	2,50	10,40
Glycine	13,15	13,60	10,20	13,52
Alanine	16,90	15,00	13,50	18,35
Valine	4,78	2,84	6,08	4,40
Isoleucine	3,78	1,14	2,15	1,66
Leucine	5,48	4,07	8,80	3,79
Tyrosine	3,50	1,93	2,00	Traces
Phenylalanine	3,90	3,65	2,45	3,76

TABLE 1. Ratio of the Protein Fractions in Salt Extracts of Cotton-Seed Flour

Fraction number	Variety 108-F	Radiomutant of the crop year		
		1964	1965	1966
1	38,5	48,7	43,9	36,4
2	54,0	44,9	47,3	55,3
3	7,5	6,4	8,8	8,3

TABLE 2. Ratio of the N-Terminal Histidine and Arginine in the Total Globulin of Cotton Seeds, mole-%

N-Terminal amino acids	Variety 108-F	Radiomutant of the crop year		
		1964	1965	1966
Histidine	58,5	20,0	23,2	62,2
Arginine	41,5	80,0	76,8	37,8

TABLE 4. Ratio of the Amino Acids in the Peptide Fragments of a Tryptic Hydrolysate of the DNP-7S-Globulins, %

Amino acids	Fragment I		Fragment II	
	variety 108-F	radiomutant	variety 108-F	radiomutant
Aspartic acid	13,05 ± 0,52	12,50 ± 0,45	9,80 ± 0,39	10,00 ± 0,46
Threonine	5,97 ± 0,23	5,56 ± 0,19	7,20 ± 0,23	6,90 ± 0,15
Serine	7,18 ± 0,35	9,30 ± 0,45	18,40 ± 0,95	15,95 ± 0,73
Glutamic acid	29,00 ± 1,60	28,50 ± 1,30	23,60 ± 1,20	24,64 ± 1,12
Proline	6,38 ± 0,60	6,40 ± 0,31	5,30 ± 0,14	4,90 ± 0,11
Glycine	5,42 ± 0,40	5,70 ± 0,20	11,80 ± 0,62	12,00 ± 0,38
Alanine	5,47 ± 0,20	5,56 ± 0,16	6,30 ± 0,35	8,62 ± 0,42
Valine	2,63 ± 0,30	2,61 ± 0,25	3,60 ± 0,17	3,81 ± 0,15
Isoleucine	4,03 ± 0,16	4,01 ± 0,10	3,25 ± 0,21	2,76 ± 0,18
Leucine	7,96 ± 0,25	5,82 ± 0,32	4,22 ± 0,19	4,50 ± 0,21
Tyrosine	2,04 ± 0,13	3,03 ± 0,11	1,95 ± 0,10	2,00 ± 0,10
Phenylalanine	10,95 ± 0,36	11,02 ± 0,46	4,60 ± 0,26	3,92 ± 0,33

terminal amino-acid sequence of four amino acid residues are similar to those of the 7S-globulin of variety 108-F [3].

Table 4 gives the amino acid composition of two peptide fragments from the 7S-globulin of the seeds of cotton of variety 108-F and of the radiomutant from the segments accessible to the action of trypsin.

The change in the amino acid composition of identical peptide fragments of the 7S-globulin of the radiomutants with no change in the N- and C-terminal groups apparently shows an intramolecular rearrangement in the primary structure of the protein. Further progress in the study of the proteins of cotton seeds will make it possible to characterize these changes in more concrete fashion.

## EXPERIMENTAL

Cotton seeds of the radiomutant of the 1964 crop were kindly given to us by P. Paiziev (Institute of Experimental Plant Biology, Academy of Sciences of the Uzbek SSR) and those of the 1965-1966 crop by the Institute of Experimental Plant Biology, Academy of Sciences of the Uzbek SSR, and the "Ak-Kurgan" collective farm.

The isolation of the globulins and the methods for their study have been described previously [1, 3] and so has the chromatographic separation of a salt extract of the flour in the presence of 8 M urea [2].

The C-terminal amino acids were determined by the carboxypeptidase method of cleavage [5] with subsequent analysis on an amino acid analyzer. The analysis was carried out on 100 mg of the total globulin isolated by the precipitation of a salt extract of cotton-seed flour on dilution using 3 mg of twice recrystallized carboxypeptidase ("Reanal," Hungary). Incubation was carried out at 37°C for 2 h. The splitting off of amino acids was stopped by bringing the incubation mixture to pH 2-3 by the addition of 2 N HCl.

The DNP-7S-globulin was hydrolyzed with trypsin as described previously [6].

The amino-acid composition of the peptide fragments was determined after acid hydrolysis (105°C, 24 h, closed tube) on an amino acid analyzer.

#### SUMMARY

1. A difference in the contents of the globulin components in radiomutants of the cotton plant in three generations has been established.

2. The ratio of the globulin components in the seeds of the radiomutant of the 1966 crop was restored, but there was a change in the primary structure of the 7S-globulin isolated from this plant.

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