Determination of Adlumine Sulfate as Such. An analytical sample of about 0.0200 g (accurately weighed) of the material was dissolved in a 500 ml measuring flask in 300 ml of water that had been heated to 30-35°C. After the solution had cooled to room temperature, its volume was made up to the mark with water and it was carefully mixed. The optical density of the solution obtained was measured on a spectrophotometer at a wavelength of 328 nm in a cell with a layer thickness of 10 mm. Water was used as the comparison solution.

In parallel, the optical density of a solution of a standard sample of adlumine sulfate was measured under the same conditions.

By using the average value of the optical density of the solution of the standard sample the percentage purity of the adlumine sulfate was calculated.

CONCLUSION

A spectrophotometric method for determining adlumine as such with an accuracy of ±0.79% and a chromato-spectrophotometric method for determining free adlumine in plant raw material with an accuracy of ±3.62% has been developed.

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THE CHANGE IN THE GLOBULINS DURING THE DEVELOPMENT OF COTTON SEEDS

A. L. Li, R. A. Rafikov,
Z. S. Yunusova, I. A. Bolotina,
T. S. Yunusov, and G. P. Moiseeva

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The biosynthesis of the 11S and 7S globulins — the main reserve proteins of cotton seeds — has been investigated. The periods at which the globulins appear in cotton seeds have been established. The changes in the amino acid composition and in the secondary structure of the 11S globulin during the ripening of cotton seeds have been studied.

The globulins of cotton seeds consist of two components — 11S (histidine) and 7S (arginine) proteins. The quantitatively predominating 11S globulin makes up ∿70% of the total globulin fraction [1]. We are performing investigations of the 11S globulin with the following aims:

1) to elucidate features of the quaternary structure of the protein and its link with the primary structures of the individual subunits in order to establish the roles of these structures in the appearance of the basic function of the reserve protein; and

2) to study the properties of the 11S globulin and their interrelationships with its structure for regulating the functional properties necessary in the production of edible cottonseed protein.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow.

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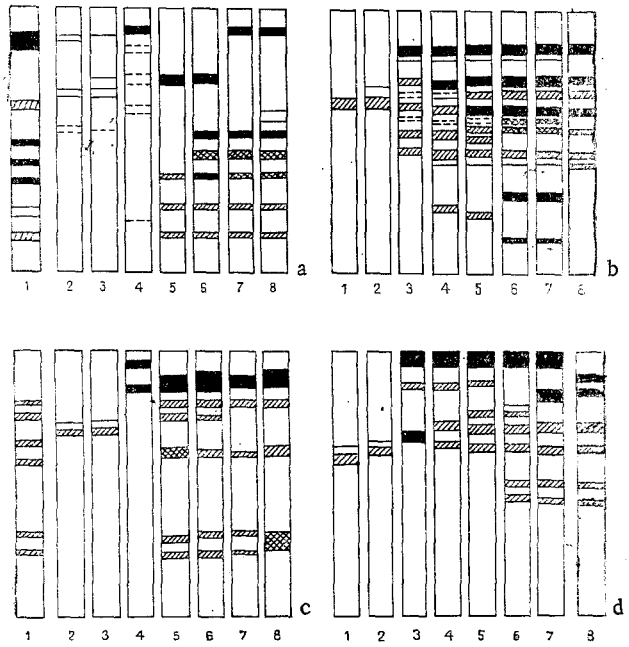

Fig. 1. Disc electrophoresis of the globulin fractions of the seeds: a) 70% gel, 8 M urea; 1) 11S globulin; 2-8) 10 days to > 60 days, respectively; b) 15% gel, 8 M urea; 8) 11S globulin; 1-7) 10 days to > 60 days, respectively; c) 7% gel, 0.1% DDSNa; 1) 7S globulin (arginine globulin); 2-8) 10 days to > 60 days, respectively; d) 15% gel, 0.1% DDSNa; 8) 7S globulin (arginine globulin); 1-7) 10 days to > 60 days, respectively.

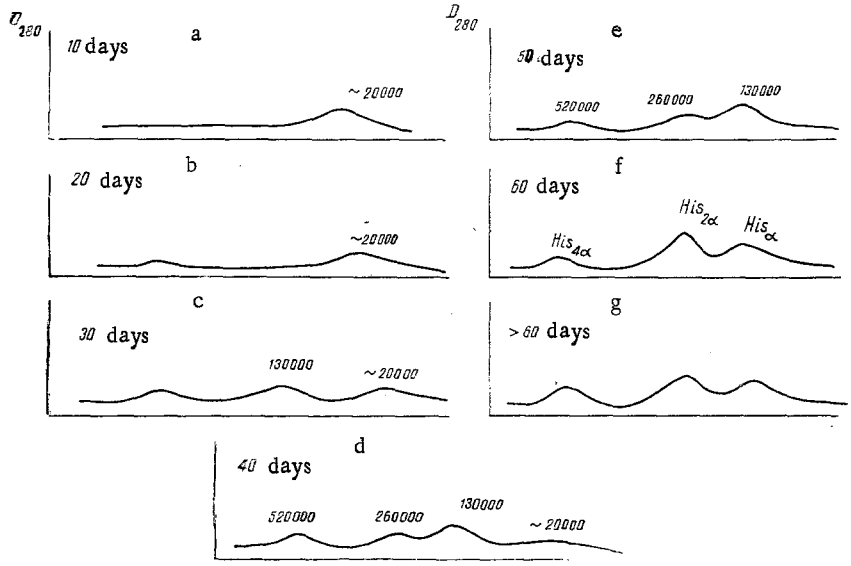

Fig. 2. Gel chromatography of the globulin fractions in the process of development of seeds on Ultrogel AcA-34.

The 11S globulin of cotton seeds possesses a low solubility in comparison with the analogous proteins from other sources, is thermolabile, is irreversibly denatured in 8 M urea solution [2], does not dissociate into subunits in 0.1% DDSNa solution [3], is present in three multiplet forms in 10% NaCl solution [4], and has a relatively low mean hydrophobicity according to Bigelow [5], amounting to about 900 cal/res. In spite of this, hydrophobic intersubunit interactions are definitely manifested in the 11S globulin molecule [3]. On gel filtration in an 8 M urea solution, no definite products of the dissociation of the pro-

TABLE 1. Amino Acid Compositions of the Total Protein Fractions of 10- and 20-Day Cotton Seeds and of the Histidine Globulins of the 40-, 50-, and 60-Day Cotton Seeds

Amino acid	Molar percentage				
	10 days	20 days	40 days	50 days	60 days
Asp	10,9	7,9	8,5	8,5	8,7
Thr	6,7	6,1	3,9	3,9	3,9
Ser	8,3	7,7	7,7	6,5	6,7
Glu	13,6	9,9	19,8	22,9	22,7
Gly	8,1	9,7	7,9	6,6	6,9
Ala	13,6	11,4	6,0	7,2	6,0
Val	6,3	7,7	6,7	7,3	6,5
Met	1,8	3,3	1,3	+	0,9
Ile	4,7	4,2	3,2	3,5	3,4
Leu	6,9	4,6	6,7	6,6	7,2
Tyr	3,4	3,3	3,9	2,2	2,8
Phe	4,9	4,3	6,9	7,4	6,0
His	2,1	3,6	3,4	3,9	3,6
Lys	4,3	8,2	3,5	3,3	3,2
Arg	4,7	8,1	9,0	10,6	11,5

tein whatever are detected, and on disc phoresis under the same conditions a large number of conformers is produced.

The structure of the main globulin of cotton seeds likewise has a number of differences from the legumins of legume seeds [6]. It has the subunit composition $A_4B_2C_6$, which corresponds to a comparatively low molecular weight — 260,000. The protein with a molecular mass of 260,000 consists of two half-molecules (α-subunits). In addition, the subunits with a large molecular mass, A and B, are basic and those with a smaller molecular mass are acidic. The formation of the 11S globulin of cotton seeds, like the legumins of other plants, apparently takes place through the appearance of ion pairs of acidic and basic subunits, which is common for all plants. However, the mechanism of the combination of these pairs into the final product must undoubtedly have its specific features in different plants. The aim of the present investigation was to determine the laws of the formation of the quaternary structure of the main reserve protein in the process of the development of the seeds.

Cotton seeds take about 60 days to reach full ripeness. The starting point for this reckoning was the day of appearance of the flower, and then an analysis of the protein fractions was carried out every ten days. The fractions insoluble in distilled water were considered to be the globulins. There were no water-insoluble proteins in the 10- and 20-day seeds, and therefore the total protein fraction was studied. The extract from the 30-day seeds now separated into globulins and albumins, which made it possible, from this period onwards, to investigate the total globulin fraction. The albumin fractions of the 10- and 20-day seeds was represented by fairly simple mixture consisting mainly of a single component (Figs. 1 and 2) with a molecular mass of about 20,000. The amino acid composition of the albumin fraction differed from that of the globulin (Table 1).

As can be seen from Table 1, the amino acid composition of the 10- and 20-day protein fractions were nontypical for plant globulins, as is shown by the high lysine content and the relatively low content of dicarboxylic acids. The N-terminal amino acid in proteins of these fractions was glutamic acid (or its amide). Thus neither from its molecular mass nor from its amino composition is it possible to assign the basic protein component of the 10-day and 20-day seeds to a precursor of the reserve proteins of cotton seeds. Apparently, the process of formation of the precursors and then the reserve proteins from them in the plant is not so well-defined as in other plants [7, 8].

The first period of development of the seeds (the period of cell division) is completed by the 20th-25th day [9]. Then the second period — the accumulation of reserve substances — sets in, and it lasts to the 50th-55th day. By the 30th day of the development of the seeds, appreciable amounts of globulins have appeared which can be separated preparatively from the albumin fraction. However, the predominance of albumins in this period leads to a contamination of the globulins with them. This is observed on gel chromatography and disk phoresis. Furthermore, in this period the globulin fraction isolated contains substantial amounts of water-soluble polysaccharides which are partially separated from the proteins on gel filtra-

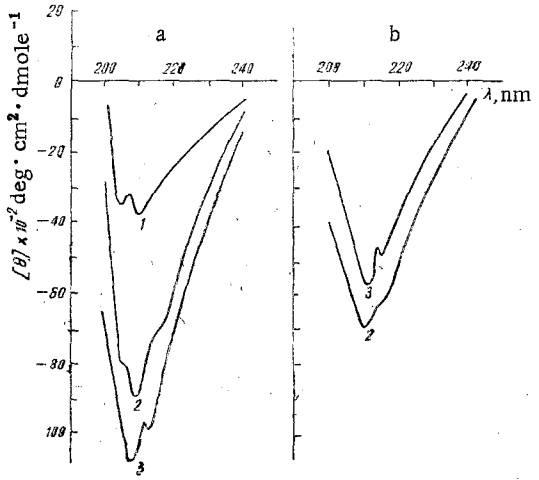

Fig. 3. CD spectra of the histidine globulins with molecular masses of 260,000 (a) and 130,000 (b): 1) histidine globulin from 40-day seeds; 2) from 50-day seeds; 3) from 60-day seeds.

tion. This is apparently connected with an intensive process of the formation of cellulose in the early stages in the development of the cotton bolls.

To study the change in the quaternary structure of the 11S globulin we must know the periods of the appearance of the different globulins. Cotton seeds contain two globulin components — 11S (legumin) and 7S (vicilin) globulins, which differ in their N-terminal amino acids, amino acid compositions, and properties. The N-terminal amino acid of the 11S globulin is histidine and that of the 7S globulin is arginine. The sequence of formation of the legumins and vicilins has been studied in a number of plants. Thus, in a number of leguminous seeds the vicilin is synthesized first and then the legumin [10, 11]. However, in soybean seeds these proteins are synthesized simultaneously [12], with the only difference that in the initial stage of development of the seeds one type of subunit is absent from each of the proteins. The process of forming the main components of the reserve proteins apparently depends on a number of factors even within a single family.

In the cotton seeds investigated, the globulins were synthesized in the early vegetation periods. Our conclusions on this question were based on three criteria:

1) marked differences in the solubilities of the 11S and 7S proteins;

2) the electrophoretic behaviors of the proteins in 8 M urea solution; and

3) analysis of the N-terminal amino acids.

It is difficult to assign the individual bands obtained on disc phoresis in the presence of urea and of DDSNa, as has been done, for example, for soybean proteins [12], but from the nature of the whole pattern it is possible to state that only the 11S globulin is present in the 30-day seeds. By the 40th day 15 bands assigned to the products of dissociation of the 7S globulin have appeared. Thus, the 11S globulin does not dissociate in 0.1% DDSNa, and the 7S globulin gives five or six dissociation products. The dissociation products that are fast-migrating in 8 M urea also belong to the 7S globulin. Furthermore, the amount of the readily soluble globulin fraction in the seeds has increased appreciably by the 50th day of ripening of the seeds. Analysis of the N-terminal amino acids definitively confirmed the facts stated. Thus, by the 30th day of the development of the seeds the biosynthesis of the globulins represented mainly by the 11S globulin had begun, and by the 40th day an appreciable accumulation of the 7S globulin had stopped. The times of appearance of these proteins in the seeds may apparently change according to the external conditions, as, in general, may the whole process of accumulation of reserve proteins in cotton seeds.

The results of an electrophoretic investigation (see Fig. 1) showed that in the 30-day seeds of the cotton plant there are all three types of subunits of the 11S globulin since it is just in this case that the product of the association of these subunits appears which does not dissociate in 0.1% DDSNa solution. The absence of any of these three types of subunits gives a clear separation of the other two on disc phoresis. At this stage of development

(Fig. 2c), an 11S globulin (α form) arises and the 2α form with a molecular mass of 260,000 is absent. Subsequently (Figs. 2d, e, f, g), the 2α form of the globulin accumulates, which shows possible chemical changes in the protein during its synthesis.

The amino acid composition of the main globulin component of cotton seeds (after the elimination of the 7S — the arginine — globulin) is given in Table 1. The proteins of the 50- and 60-day seeds are close in amino acid composition and, at the same time, differ from the protein of the 40-day seeds. The differences are apparently due to the presence in the protein samples of accompanying carbohydrates which, on acid hydrolysis, may lead to a change in the amino acid composition.

We have made conformational investigations of the 11S globulin of the seeds during their development. Figure 3 shows circular dichroism (CD) spectra for the two forms of the 11S globulin with molecular masses of 130,000 and 260,000. Since the main final form of the His globulin is the form with a molecular mass of 260,000, we have calculated the elements of its secondary structure:

Sample	α-Helix, %	β-Structure, %	β-Bend, %	Irregular section, %
40 days	11	16	19	54
50 days	19	17	19	45
60 days	27	22	24	27

The calculation was carried out by the method described in [13-15, 19].

It has been shown [15] that the antiparallel and parallel β-structures are not separated very accurately, and we have therefore given the total value for the β-structures. It can be seen from the facts given that the secondary structure of the 2α form of the 11S globulin changes as the seeds ripen.

As can be seen from Fig. 3, the natures of the change in the orderedness of the molecule of the two forms during ripening are different. While the changes in the 260,000 molecular form (Fig. 3a) have a tendency towards an increase in their degree of orderedness in the process of development, the 130,000 form has the opposite tendency (Fig. 3b). Since the form with a molecular mass of 260,000 (2α form) is a product of the association of the form with a molecular mass of 130,000, the appearance of the 2α form apparently leads to definite changes in the secondary structures of the individual subunits. Thus, in the process of development of the seeds the following molecular-conformational equilibrium between the two forms exists:

$$2\alpha' \rightleftarrows \alpha + \alpha,$$

where α' and α are subunits with different secondary structures but each having a molecular mass of 130,000.

The increase in the amount of the form with a molecular mass of 260,000 by the end of the ripening of the seeds (Fig. 2f) is apparently connected with a change in the degree of amidation of the protein during ripening. The nitrogen contents of the globulin fractions of the reserve proteins were as follows, %:

40 days	10.8
50 days	13.6
60 days	16.1

It must be mentioned that this equilibrium between the forms is characteristic only for the intensive development of the seeds, since in conformational studies of the same forms in dormant seeds no appreciable differences in their secondary structures were observed.

EXPERIMENTAL

The globulins were isolated from graded seeds of the cotton plant of the Tashkent 1 variety (Tashkent province) by a method described previously [16].

The electrophoretic investigation of the samples was carried out by the method of Ornstein and Davis [17]. For electrophoresis we used the total globulin fraction. The gel chromatography of solutions of the 11S globulins was carried out as described in [4].

The CD spectra were taken on a JASCO J-20 spectropolarimeter at protein concentrations of 0.5-0.7 mg/ml in a cell 0.05 cm long with a sensitivity of the apparatus of 0.002° per

1 cm and a time constant of 4 sec. The concentrations of the solutions were determined by the Biuret method [18]. The results obtained were expressed in the form of molar ellipticities calculated to the mean amino acid composition [θ]. The mean molecular weight of a residue, calculated from the amino acid composition of the protein, was 129.

The secondary structures of the proteins were determined from their CD spectra by the method described in the following paper in this issue [19].

CONCLUSION

1. The biosynthesis of globulins in the cotton plant begins between the 20th and 30th days of development of the seeds. On the 30th day, these proteins are represented mainly by the 11S globulin.

2. The first intermediate molecular form of the 11S (His) globulin during the process of biosynthesis is the α form with a molecular mass of 130,000. As the seeds ripen, the amount of the 2α form with a molecular mass of 260,000 increases.

3. In the process of development of the seeds, a change takes place in the secondary structures of the α and 2α forms. For the 2α forms the degree of orderedness of the molecules rises, and for the α form it falls.

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