AN INVESTIGATION OF COTTONSEED GLOBULINS.

XXI. THE 11S GLOBULINS OF TWO VARIETIES OF THE COTTON PLANT

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The 11S globulins of two varieties of the cotton plant — 108-F and Tashkent-1 — have been studied. It has been shown that the 11S globulins do not differ in quaternary structure and each consists of three subunits A, B, and C. It has been found that the subunits of these varieties differ in their amino acid compositions and the peptide maps of tryptic and chymotryptic hydrolysates.

The susceptibility of industrial crops to various diseases leads to enormous losses in agriculture. An important method of solving this problem is the creation of new varieties of plants. Wilt — the main disease of the cotton plant — is distributed throughout the world. In Uzbekistan the wilt-resistant variety of cotton Tashkent-1 has been developed and has come to replace the variety 108-F. These varieties differ not only in wilt resistance but also in a whole series of other characteristics. In the present communication we consider the structural differences of the main protein of the seeds of these plants — the 11S globulin. It consists of three types of subunits [1]. The investigations have shown that the 11S globulin of the Tashkent-1 variety also consists of three types of subunits. The main one is subunit C. The complete primary structure of this subunit from variety 108-F has been determined previously [2]. The presence of methionine, tyrosine, and different amounts of arginine shows a substantial difference between subunit C from the Tashkent-1 variety and the same subunit from the 108-F variety (Table 1). The amounts of tryptophan are identical. Both subunits are glycoproteins.

The different amounts of basic and aromatic amino acids lead to changes in the peptide maps (Fig. 1). The amount of glutamic acid in subunit A of the Tashkent-1 variety has increased greatly in comparison with the variety 108-F. The amounts of basic amino acids are approximately the same, but an analysis of tryptic hydrolysates of these subunits showed their fundamental difference (Fig. 2).

It must be mentioned that the digestion of subunit A of variety Tashkent-1 took place fairly rapidly without any treatment. However, the successful digestion of the same subunit from the variety 108-F required its thermal treatment. In the subunits B, as in the subunits C, a marked difference in arginine contents was observed. In subunit B from variety Tashkent-1, there was twice as much arginine as in the same subunit of variety 108-F, which is necessarily shown on the peptide maps (Fig. 3).

Analysis of the peptide maps of the chymotryptic hydrolysate of subunits B of variety Tashkent-1 showed the presence of seven tryptophan-containing peptides while in the case of variety 108-F there were only two. In all three subunits of variety Tashkent-1 there were greater amounts of proline than in the subunits from variety 108-F. Subunits A and B of variety Tashkent-1 contained approximately twice as much glutamic acid as the same subunits of variety 108-F. However, in the case of subunit C the opposite pattern was observed. Thus, an analysis of peptide maps of the various subunits of the 11S globulins of the varieties of cotton plant investigated has shown substantial differences in their primary structures. In view of the quantitative ratios of the subunits of the 11S globulins [1] of cotton seeds, it would be fairly difficult to detect appreciable differences directly in these proteins of varieties Tashkent-1 and 108-F [3], since a decrease in the amounts of some amino acids in one subunit is compensated by an increase in the amount of the same amino acid in another.

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TABLE 1. Amino Acid Compositions of Subunits A, B, and C of the 11S Globulins of the 108-F and Tashkent-1 Varieties

Amino acid	108-F			Tashkent-1		
	moles/mole of protein					
	A	B	C*	A	B	C
Asp	26	26	18	22	24	11
Thr	10	11	5	10	10	5
Ser	15	10	7	15	12	6
Glu	32	22	37	61	41	26
Pro	8	6	3	13	10	6
Gly	22	16	9	19	13	11
Ala	17	16	7	16	11	9
Val	17	10	7	18	12	6
Met	2	3	—	3	3	1
Ile	14	6	6	9	8	3
Leu	19	13	6	16	12	5
Tyr	3	4	—	5	5	2
Phe	9	13	7	12	10	3
His	5	5	6	6	6	2
Lys	6	7	2	6	5	2
Arg	20	8	14	22	16	7
Trp†	+	2	1	+	7	1

*Accurate composition determined from the primary structure.
†Tryptophan was determined from the number of tryptophan-containing spots on peptide maps of chymotryptic hydrolisates.

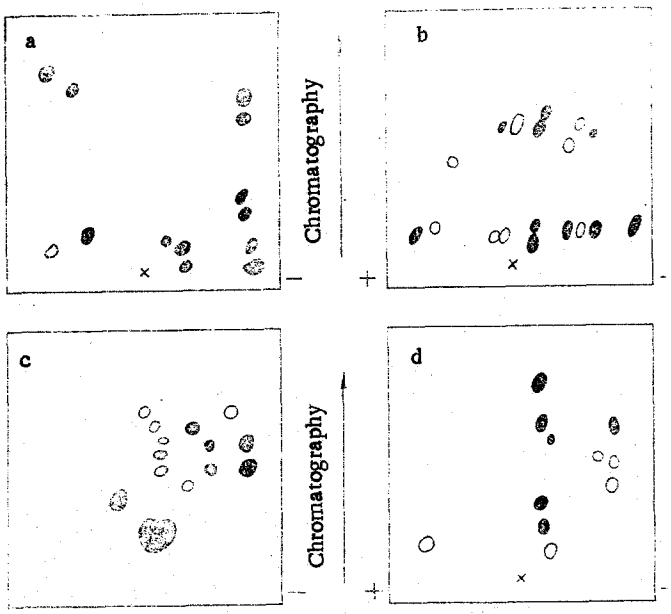

Fig. 1. Peptide maps of subunits C from cotton plants: a) tryptic hydrolysate (108-F); b) tryptic hydrolysate (Tashkent-1); c) chymotryptic hydrolysate (108-F); d) chymotryptic hydrolysate (Tashkent-1).

EXPERIMENTAL

The 11S globulins were isolated by a known method [1].

The subunits were isolated by a method described previously with some modification. Subunits A and B were separated on a column (4.5 × 16 cm) of DEAE-cellulose (DE 32, United Kingdom) equilibrated with a 8 M solution of urea, pH 8.0. Subunit A was eluted with the free volume. Subunit B was eluted with a 1 M solution of NaCl in 8 M urea.

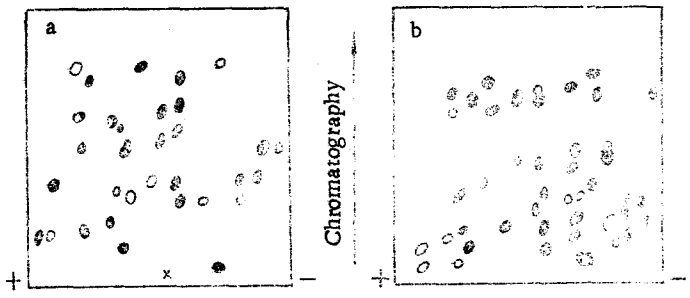

Fig. 2. Peptide maps of subunits A from cotton plants: a) tryptic hydrolysate (108-F); b) tryptic hydrolysate (Tashkent-1).

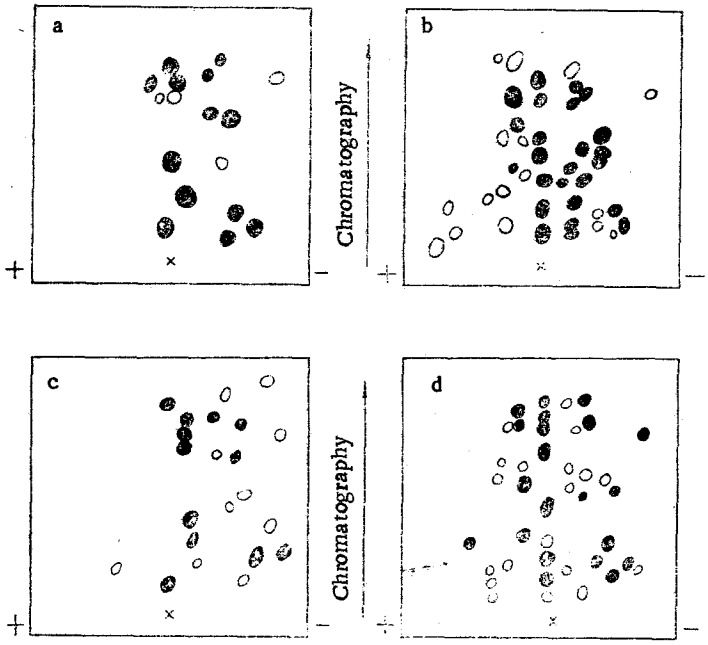

Fig. 3. Peptide maps of subunits B from cotton plants: a) tryptic hydrolysate (108-F); b) tryptic hydrolysate (Tashkent-1); c) chymotryptic hydrolysate (108-F); d) chymotryptic hydrolysate (Tashkent-1).

Amino acid compositions were determined after acid hydrolysis with 5.7 N HCl (24, 48, and 72 h (110°C), on a LKB-4101 analyzer (Sweden).

Subunits A and B were digested with trypsin and chymotrypsin (both from Worthington) for 16 h in 0.2 M N-ethylmorpholine—acetate buffer, pH 8.3, at 37°C. The enzyme:substrate ratio was 1:50. Subunit C was cleaved by the same enzymes in 0.2 M ammonia—acetate buffer, pH 8.8, at 37°C for 16 h at an enzyme:substrate ratio of 1:50.

The peptide maps were obtained in plates (20 × 20 cm) with a thin layer of cellulose (type FND, GDR). Chromatography was performed in the butanol—acetic acid—pyridine—water (15:3:10:12) system and electrophoresis in pyridine—acetate buffer, pH 6.4, at 800 V for 45 min. The peptide maps were first sprayed with ninhydrin-collidine chromogenic agent and dried at 100°C for 5 min, and were then treated with Ehrlich's reagent (0.1 g of dimethylaminobenzaldehyde, 1 ml of concentrated HCl, and 9 ml of acetone).

SUMMARY

It has been shown that the 11S globulins of the seeds of cotton plants of varieties Tashkent-1 and 108-F consist of three types of subunits.

The subunits of the 11S globulin of variety Tashkent-1 differ in their primary structures from the corresponding subunits of variety 108-F.

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PREPARATION OF HYDRAZIDES OF AMINO ACIDS AND PEPTIDES

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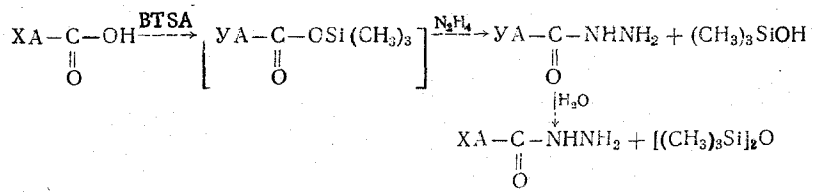
A new method of synthesizing hydrazides of amino acids and peptides is considered which involves the hydrazinolysis of the silyl esters of the corresponding compounds. A number of hydrazides of derivatives of amino acids and peptides has been obtained in high purity with yields close to quantitative. The physicochemical characteristics of the compounds synthesized are given.

The usual method of obtaining hydrazides of amino acids and peptides, which are used mainly for the synthesis of azides, is the hydrazinolysis of the methyl or ethyl esters of the corresponding compounds [1]. As a rule, the process is performed under mild conditions (at room temperature) in ethanol or dimethylformamide (DMFA). The hydrazine is used in the form of hydrazine hydrate or, more rarely, as anhydrous hydrazine, the hydrazine being taken in excess to prevent the formation of symmetrical bishydrazides. A disadvantage of this method is the necessity for the previous synthesis of the methyl or ethyl ester of the corresponding compound which, in a number of cases, is associated with certain difficulties. Thus, for example, esters of derivatives of N^{ε}-tert-butoxycarbonyl-L-lysine can be obtained only by using diazomethane [2].

We have developed a convenient method for obtaining hydrazides of amino acids or peptides which consists in the preliminary silylation of the corresponding amino acid or peptide followed by the hydrazinolysis of the trimethylsilyl ester with anhydrous hydrazine [3]. The reaction is performed at room temperature in DMFA or methylene chloride in accordance with the following scheme:



where X represents a protective group or a hydrogen atom; Y, a trimethylsilyl or protective group; A, an amino acid or peptide residue; and BTSA represents bis(trimethylsilyl)acetamide.

Bis(trimethylsilyl)acetamide is used as the silylating agent [4]. The hydrazides of the corresponding amino acids and peptides are obtained in yields of 80-98% and in high purity.

It is interesting that the process under consideration differs from known reactions of nucleophilic substitution at a silicon atom in acyloxysilanes [5]. In spite of the fact that

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