dure proposed by Lutsik et al. [5]. The hemagglutinating activity was expressed by the titer. As the titer we took the maximum dilution of an extract at which agglutination of erythrocytes was still observed visually.

SUMMARY

151 species of plants of the Central Asian flora have been investigated. The presence of lectins has been established in 23 of them. The immunochemical specificities of the extracts and, for some species of plant, carbohydrate-binding specificities, as well, have been determined.

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PRIMARY STRUCTURE OF SUBUNIT B OF THE 11S GLOBULIN OF SEEDS OF COTTON PLANT VARIETY 108-F.

I. ACID-SOLUBLE PEPTIDES FROM COMPLETE TRYPTIC HYDROLYSIS OF SUBUNIT B

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The 11S globulin of cotton seeds consists of three types of subunits: A, B, and C. The complete tryptic hydrolysis of subunit B has given acid-soluble and acid-insoluble peptides. The amino acid compositions and amino acid sequences of the acid-soluble peptides have been determined.

The $His_{2\alpha}$ globulin (11S globulin) is the main component of the reserve proteins of cotton seeds. It possesses a complex quaternary structure and consists of three types of subunits, A, B, and C [1]. Subunit C has been studied [2].

We have continued an investigation of the $His_{2\alpha}$ globulin. Subunit B consists of 180-190 amino acid residues. We have used classical approaches to determine its primary structure and investigate its characteristics. Several types of cleavage have been performed with the aim of obtaining overlapping peptides: complete tryptic and limited tryptic hydrolyses at argine and lysine residues.

In the present communication we make an analysis of the acid-soluble peptides obtained in the complete tryptic hydrolysis of subunit B. The dependence of the degree of digestion of the protein by trypsin on the time of digestion has been studied. The degree of cleavage was checked by TLC and by the peptide-map method. It is interesting to note that the degree of cleavage of the protein by trypsin was practically independent of the time in the interval from 0.5 to 16 h (Fig. 1), i.e., the number and positions of the main spots did not change. Preparative hydrolysis was carried out for 4 h. On the basis of the nature of the peptide maps, it was assumed that trypsin cleavage took place with strict specificity and to completion. However, as will be shown below, in addition to low-molecular-weight peptides, high-molecular-weight peptides including both arginine and lysine residues were also obtained.

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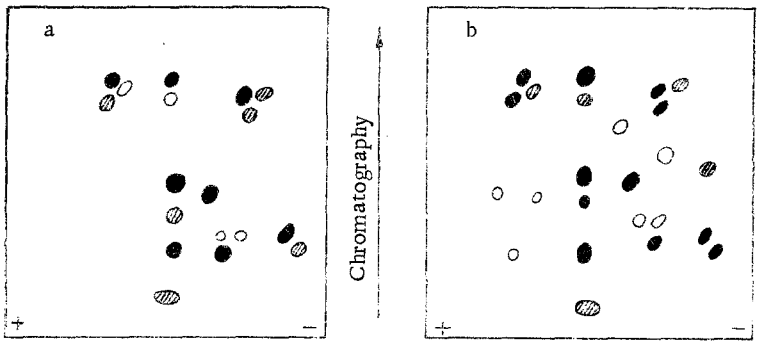

Fig. 1. Peptide maps of a complete tryptic hydrolysis of subunit B after hydrolysis times of 1 h (a) and 16 h (b).

The complete tryptic hydrolysis of the subunit was carried out in N-ethylmorpholine-acetate buffer for 4 h. The subunit did not dissolve under these conditions. After hydrolysis for 4 hours, the bulk of protein had passed into solution. Only 2% of the mass of the initial proteins constituted a residue which represented the fraction of uncleaved protein. To prove this, its amino acid composition was determined, and it was found to be identical with that of the initial protein.

The amino acid composition of subunit B (without taking tryptophan residues into account) is:

Asp	15.5	Met	1.1
Thr	5.2	Ile	6.2
Ser	14.4	Leu	12.5
Glu	38.6	Tyr	4.0
Gly	14.4	Phe	11.9
Pro	7.6	His	5.0
Ala	13.5	Lys	6.5
Val	12.1	Arg	15.0

On the basis of the amino acid composition of subunit B, it was expected to obtain 20-22 quantitatively important tryptic peptides. However, on a peptide map of the hydrolysate it was possible to distinguish only 12-14 main spots, and it is possible that the cleavage had not gone to completion in spite of the fact that an increase in the time of hydrolysis led to no appreciable changes.

The tryptic hydrolysate consisted of a mixture of acid-soluble and acid-insoluble peptides. Subsequent investigations showed that the acid-soluble peptides had low molecular weights and the acid-insoluble had high molecular weights.

A mixture of the acid-soluble peptides from the tryptic hydrolysate was separated by column ion-exchange chromatography in the presence of a linear-stepwise gradient of a buffer solution increasing molarity and pH. The combination of the fractions was based on the absorption at a wavelength of 570 nm of the ninhydrin derivatives of the peptides after alkaline hydrolysis. As a result 23 combined fractions were obtained (Fig. 2). From the distribution of the ninhydrin-positive spots on the peptide map of the tryptic hydrolysate of subunit B it was assumed that in this case no strongly acidic peptides were formed. The elution of the peptides was carried out by using four gradients. When gradient I was switched on, two fractions were obtained, with gradient II fractions 3 to 15, with gradient III fractions 3 to 20, and with gradient IV fractions 21 to 23. Then the column was washed with a 4 M solution of pyridine. Each combined fraction obtained from the ion-exchange column was analyzed by TLC, and its N-terminal amino acid was determined by the dansyl method. Fractions T6, T20, and T23 were homogeneous peptides and the others were subjected to additional purification by PC and high-voltage paper electrophoresis. The acid-soluble fraction of the tryptic hydrolysate yielded 20 individual peptides.

For the peptides obtained in low yield we determined amino acid compositions and N-terminal amino acids, and for the others complete or partial amino acid sequences (Table 1).

The amino acid sequences of the peptides obtained were studied by the Edman method with identification of the amino acids in the form of the dansyl derivatives and by the "direct" Edman method with identification of the phenylthiohydantoin derivatives of the amino acids:

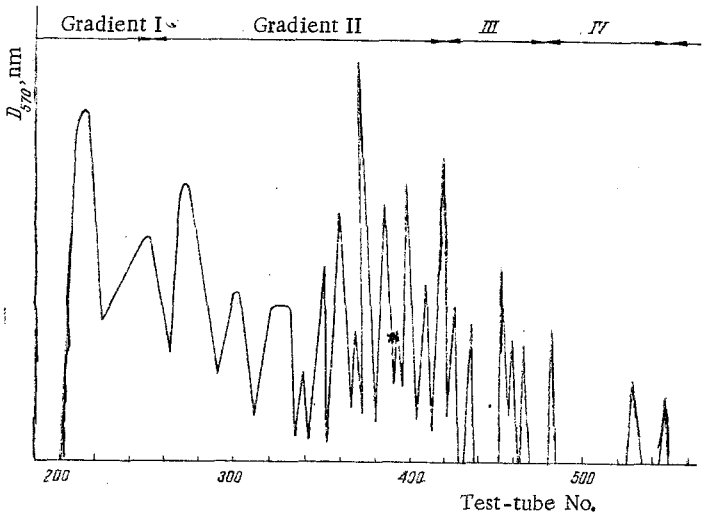

Fig. 2. Chromatography of a tryptic hydrolysate of subunit B.

1. T1.0.1.2 Glu-Asp-Glu-(Arg)
2. T1.0.2.2 Gly-Asp-Gly-X-Glu-Ser-(Arg)
3. T1.0.2.3 Ala-Gly-Asp-Glu-Gly(Ser)-Val(Gly, Ser, Arg)
4. T1.0.2.4 Val-Pro-Ile-Gly-Asn-Ser-Asp-(Val, Glu, Ala, Leu)-Phe-Gly-(Asp, Glu_2, Ala)-Arg
5. T2.0.1.1 Gly-Asp-Ser(Glu)-X-Asp-Glu-Glu-Leu-(Arg)
6. T4.3 Gly-Val-Ser-Glu-Asp-Leu-Glu-(Lys)
7. T5.1.1 Gly-Glu-Ser-Glu-Lys
8. T5.1.3 Gly-Glu-Ser-Glu_n-Arg
9. T8.1.3 Arg
10. T9.2 Glu-Glu-Gly-Asp-Phe-(Arg)
11. T10.2 Arg
12. T11.0.3 Arg
13. T5.1.2 Lys

In spite of the fact that the peptides had relatively low molecular weights, the determination of their structures was somewhat complicated by the presence of dicarboxylic amino acid residues. In the determination of the amino acid sequences of some of the peptides we used additional cleavage by an enzyme.

Peptide T1.0.2.4 was subjected to cleavage with chymotrypsin, since it contained a single phenylalanyl residue. As a result, the two peptides XT1.0.2.4.1 and XT1.0.2.4.2 were obtained with the N-terminal amino acids Val and Gly, respectively. The N-terminal sequence of the peptide XT1.2.0.4.1 coincided with that of the initial peptide and therefore the peptide XT1.0.2.4.2 was C-terminal and the initial peptide contained a -Phe-Gly- bond.

It is interesting to note that the peptides T8.1.3, T10.2, and T11.0.3 consisted of the free amino acid arginine. Since they were obtained from the complete hydrolysate, it was assumed that the molecule of subunit B of the $His_{2\alpha}$ globulin contains an -Arg-Arg- or a -Lys-Arg- bond. In the tryptic hydrolysis of subunit B at arginine residues, likewise, the free amino acid arginine was obtained.

We came to the conclusion that the protein molecule most probably contains an -Arg-Arg- bond, although the presence of an -Arg-Arg-Lys- bond is not excluded, since peptide T5.1.2 consisted of the free amino acid lysine. The production of free lysine is also possible in the case of a -Lys-Lys- bond, but we excluded this possibility: When subunit B underwent tryptic hydrolysis at lysine residues no free lysine was detected.

Thus, the molecule of subunit B of $His_{2\alpha}$ globulin contains -Arg-Arg- and -Arg-Arg-Lys- bonds. The presence of such bonds in the molecule is one of the features of the primary structure of subunit B. In a study of the primary structure of subunit C, an -Arg-Lys-bond was found in the 85-86 position [2]. The existence of such bonds is possibly a feature of all three types of subunits of the $His_{2\alpha}$ globulin.

TABLE 1. Amino Acid Compositions of Some Acid-Soluble Peptides from the Tryptic Hydrolysate

	T1.0.1.2	T1.0.2.2	T1.0.2.3	T1.0.2.4	T3.3	T5.1.1	T5.1.2	T5.1.3	T8.1.3	T10.2	T11.3	T6.5	T9.2
Asp	1.1 (1)	0.9 (1)	1.3 (1)	3.2 (3)	1.3 (1)	—	—	—	—	—	—	1.1 (1)	0.8 (1)
Thr	—	—	—	—	0.9 (1)	—	—	—	—	—	—	—	—
Ser	—	1.0	1.0	1.7 (2)	1.3 (1)	—	—	1.1 (1)	—	—	—	—	—
Glu	2.3 (2)	2.5 (3)	3.1 (3)	4.4 (4)	2.4 (2)	2.8 (3)	—	3–4	—	—	—	1.4 (1)	1.8 (2)
Gly	—	2.1 (2)	1.2 (1)	2.3 (2)	1.4 (1)	1.2 (1)	—	1–0	—	—	—	1.0	0.7 (1)
Ala	—	—	1.5 (2)	2.2 (2)	—	—	—	—	—	—	—	—	—
Val	—	—	0.7 (1)	2.4 (2)	1.1 (1)	—	—	—	—	—	—	0.7 (1)	—
Met	—	—	—	—	—	—	—	—	—	—	—	—	—
Ile	—	—	—	0.8 (1)	—	—	—	—	—	—	—	—	—
Leu	—	—	—	1.5 (2)	1.3 (1)	—	—	—	—	—	—	1.4 (1)	—
Tyr	—	—	—	—	—	—	—	—	—	—	—	—	—
Phe	—	—	—	1.2 (1)	1.0	—	—	—	—	—	—	0.7 (1)	0.6 (1)
His	—	—	—	—	—	—	—	—	—	—	—	—	0.5 (1)
Lys	—	—	—	—	—	0.9 (1)	1.0	—	—	—	—	—	0.4
Arg	1.0	1.1 (1)	1.2 (1)	1.0	0.4	—	—	1.0	1.0	1.0	1.0	0.4	1.0
Number of residues	4	8	10	18–19	9–10	5	1	4–6	1	1	1	5–7	6–7
N-terminal	Glu	Gly	Ala	Val	Gly	Gly	Lys	Gly	Arg	Arg	Arg	Glu	Gly

EXPERIMENTAL

Subunit B was isolated from the 11S globulin ($His_{2\alpha}$ globulin) by a method described previously [3].

The tryptic hydrolysis of the subunit was carried out in a 0.2 M N-ethylmorpholine-acetate buffer, pH 8.3. A weighed sample of protein (300 mg) was suspended in 2 ml of buffer and the mixture was thermostated at 50°C for 1 h. Then the enzyme was added in an enzyme-substrate ratio of 1:100. Hydrolysis was carried out at 37°C for 4 h. The degree of hydrolysis was monitored by the TLC method. After the end of the reaction, the hydrolysate was neutralized and freeze-dried. The solid residue (6 mg) was analyzed separately.

The separation of the acid-soluble fraction of the tryptic hydrolysate was carried out on a column (1.2 × 60 cm) of Aminex Q 150SFOR equilibrated with 0.2 M pyridine-acetate buffer (PAB), pH 3.1. The tryptic hydrolysate was dissolved in 0.2 M PAB with pH 2.2. This gave acid-soluble and acid-insoluble mixtures of peptides. To separate the acid-soluble peptides on the column described we used a linear-stepwise gradient increasing with respect to the molarity of the pH of the solution. The elution of the peptides was begun with 0.2 M PAB, pH 3.1 — the starting buffer — with the column jacket having a temperature of 35°C. In the subsequent chromatographic process, the temperature of the column jacket was raised to 50°C and gradient I was created, using the starting buffer as buffer A and 0.5 M PAB, pH 5.0, as buffer B. To create gradient II, buffer B and buffer C — 2 M PAB, pH 5.0 — were used; for gradient III, buffers C and D — 4 M PAB, pH 5.6; and for gradient (IV), buffers D and E — 8 M PAB, pH 6.0. In the concluding stage, the column was washed with a 4 M aqueous solution of pyridine. The elution of the peptides was carried out at the rate of 30 ml/h. Fractions with a volume of 3 ml were collected, 0.2 ml being taken from each second test-tube for analysis of the ninhydrin reaction after alkaline hydrolysis [4].

The peptide maps were obtained on plates (20 × 20 cm) with a thin layer of cellulose (type FND). Chromatography was performed in the 1-butanol—acetic acid—pyridine—water (15:3:10:12) system, and electrophoresis in pyridine-acetate buffer, pH 6.4, 1000 V, 30 min. The peptide maps were sprayed with ninhydrin-collidine developing agent and were dried at 100-105°C for 3-5 min. To detect tryptophan, the plates were treated with Ehrlich's reagent (0.1 g of p-dimethylaminobenzaldehyde, 1 ml of concentrated HCl, and 9 ml of acetone).

The TLC of the combined fractions was carried out on plates, 6 × 9 cm, coated with cellulose (FND) in the same solvent system as for the peptide maps. The plates were sprayed with the same revealing agent as for the peptide maps.

Preparative PC was carried out on FN-17 paper (Filtrak) in the 1-butanol—acetic acid—pyridine—water (15:3:10:12) system in a hermetically sealed chamber at room temperature.

The chromatograms were dried and the peptides were detected by spraying narrow bands with a 0.5% solution of ninhydrin in acetone.

For electrophoresis we used chromatographic paper of the FN-17 type (Filtrak) and PABs with various pH values (6.4, 3.5, and 5.0). Electrophoresis was performed at 1000 V for 30, 45, and 60 min. The presence of peptides was detected as in the case of PC. The peptides were eluted with 50% pyridine solution and 30% acetic acid solution.

Chymotryptic hydrolysis was carried out in 0.2 M N-ethylmorpholine acetate buffer, pH 8.3. After the previous thermal denaturation of the protein at 35°C, the enzyme was added in an enzyme-substrate ratio of 1:100. Hydrolysis was carried out at 37°C for 2 h.

N-Terminal amino acids were determined as described by Gray [5].

Amino acid compositions were determined after acid hydrolysis in 5.7 N HCl at 110°C for 24 h. The hydrolysate was analyzed on a LKB 4101 amino acid analyzer (Sweden).

The structures of the peptides were determined by the Edman method [5].

Solvents were purified as described by Edman and Begg [6].

SUMMARY

1. From a complete tryptic hydrolysate of subunit B, 20 peptides of low molecular weight have been isolated.

2. The amino acid sequences of 13 of the peptides have been determined.

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