PREPARATION OF PEPTIDE FRAGMENTS ENRICHED IN CERTAIN AMINO ACIDS FROM A TRYPTIC HYDROLYSATE OF THE DINITROPHENYL DERIVATIVE OF THE 7S-GLOBULIN OF COTTONSEED

A. P. Ibragimov and Sh. Yunuskhanov

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For a comparative study of the amino acid composition of the globulins of seeds of the cotton plant of variety 108-F and its radiomutant we have developed a method for obtaining the 7S-globulin component from cottonseed using chromatography [1]. Continuing the investigation, the dinitrophenyl derivative of 7S- globulin from cottonseed was subjected to tryptic hydrolysis.

The tryptic hydrolysate of the DNP-7S-globulin was separated on Sephadex G-25. After 48 hr incubation with trypsin (Fig. 1a), two fractions were obtained which, after freeze-drying, were readily soluble in ethanol. The UV absorption spectra of both fractions (Fig. 2) had two absorption maxima, one at 255–260 mμ and the other at 350–355 mμ. The numerical ratio of the two maxima for the first fraction E_{350}/E_{255} = 0.995 and for the second fraction E_{350}/E_{255} = 1.345 shows that the amounts of aromatic and dinitrophenylated amino acids in these fractions differ.

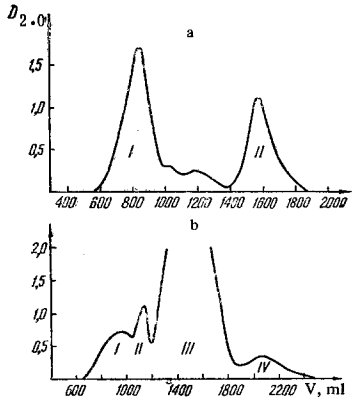

Fig. 1. Separation of a tryptic hydrolysate of DNP-7S-globulin after incubation for a) 48 hr and b) 96 hr. (I–IV represent fraction numbers).

Separation of a tryptic hydrolysate of DNP-7S-globulin after 96 hr incubation (Fig. 1b) gave four fractions, of which the third was colorless and its yield was greatest, The ratio of the maxima at 350 and 255 mμ for the first fraction was 0.777, for the second fraction 1.06, and for the fourth 1.370; the third fraction had only one maximum, at 255 mμ.

Consequently, with an increase in the amount of dinitrophenyl amino acids per unit of aromatic acids, the volume of eluent required for the elution of the fractions increased.

On comparing the fractions obtained from the tryptic hydrolysis of DNP-7S-globulin for 48 and 96 hr, we see that E_{350}/E_{255} of the first fraction of the first incubation is similar to the second fraction of the 96 hr, incubation, and the second fraction is equal to the fourth fraction of the second incubation. The first and third fractions in the 96-hr tryptic hydrolysate of the DNP-globulin were apparently formed as a result of a far-reaching hydrolysis of the protein or of the action of other enzymes, such as chymotrypsin, which is present in crystalline trypsin in trace amounts in the form of an impurity. Consequently, further study was carried out on the product of the 48-hr incubation. On disk electrophoresis on polyacrylamide gel, the residue remaining after trypic hydrolysis RTH (residue of the tryptic hydrolysis of CNP-7S-globulin) migrates as one yellow band. This takes up the stain when the gel is treated with the dye Coomassie Bright-Blue. The two fractions obtained on a column of Sephadex G-25 each gave

a single band on electrophoresis which was washed out in the staining process because of its good solubility in aqueous and ethanolic solutions.

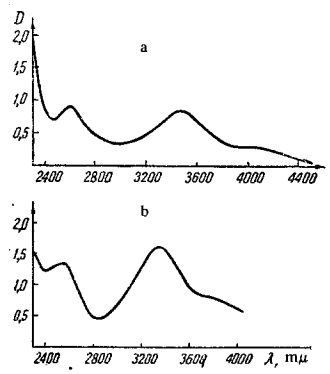

Fig. 2. UV absorption spectrum of a) the first, and b) the second fractions.

On electrophoresis, RTH is highly mobile, while the DNP-7S-globulin is immobile. Table 1 gives the results of a quantitative analysis of the 12 amino acids contained in the fractions. The ratio of these acids in the DNP-7S-globulin is given for comparison. Compared with the second fraction, the first contains large amounts of leucine, phenylalanine, proline, and glutamic and aspartic acid. The second fraction has more than two and a half times as much as serine, and almost three times as much glycine as the first. The amino acid composition of the RTH likewise differs from that of the DNP-7S-globulin. In the fractions of the tryptic hydrolysate the amount of proline is greater than in the DNP-7S-globulin. The basic amino acids were not determined qualitatively.

Table 1. Ratio of the Twelve Amino Acids in the Fragments from the Tryptic Hydrolysis of DNP-7S-globulin from Cottonseed

Amino acid	Content, %			
	first fraction	second fraction	RTH	DNP-7S-globulin
Asparagine	12.70	9.30	17.44	14.30
Threonine	5.65	7.40	4.50	4.21
Serine	6.76	18.50	5.51	7.55
Glutamic acid	25.72	19.50	17.10	29.20
Proline	6.88	4.63	5.43	5.90
Glycine	4.11	11.60	4.94	5.94
Alanine	5.81	6.74	5.30	5.56
Valine	2.11	3.58	9.86	5.54
Isoleucine	3.44	3.25	5.04	3.85
Leucine	7.62	4.92	9.55	8.12
Tyrosine	1.95	1.95	1.35	0.306
Phenylalanine	10.42	4.60	8.40	9.50

In the dinitrophenylation of the globulin, the OH group of tyrosine and the ε-amino group of lysine are dinitrophenylated. This is seen in Table 2, which gives the results of a determination of the amino acid composition of the DNP-7S-globulin. Compared with the normal 7S-globulin, the amounts of lysine and tyrosine in the DNP-7S-globulin are very low. The amount of tyrosine in the fractions should probably be higher than is shown by the results obtained.

What has been said above allows us to assume that the amino acids are found with different frequencies in the molecule of the 7S-globulin of cottonseed. It is possible that there are sections where individual amino acids are concentrated. Thus, serine and glycine are localized in large amounts in the second fraction of the tryptic hydrolysate of DNP-7S-globulin isolated on a column of Sephadex G-25.

EXPERIMENTAL

The 7S-globulin was isolated from the seeds of the cotton plant of variety 108-F by the method described previously [1]. Dinitrophenylation was carried out by Sanger's method [2]. The reaction mixture was stirred in a dark place for 2 hr. The DNP-globulin that had deposited was centrifuged off, and the residue was treated with 1 N

HCl to a feebly acid reaction. Then it was centrifuged again and washed with distilled water until neutral. After this the DNP-globulin was freeze-dried.

Table 2. Amino Acid Composition of the 7S-globulin from Cottonseed and Its Dinitrophenyl Derivatives

Amino acid	Content, %	
	7S-globulin	DNP-7S-globulin
Lysine	2.62	0.206
Histidine	2.60	2.76
Arginine	12.70	12.54
Aspartic acid	11.50	12.06
Threonine	3.28	3.56
Serine	5.41	6.40
Glutamic acid	22.90	24.70
Proline	4.19	4.98
Glycine	5.15	5.01
Alanine	4.80	4.70
Valine	4.96	4.67
Isoleucine	3.91	3.26
Leucine	6.47	6.86
Tyrosine	1.86	0.258
Phenylalanine	6.92	8.01

The DNP-protein (200 mg) was dissolved in 10 ml of 8 M urea and denatured at 60° C for 2 hr [3]. The denatured protein was incubated with trypsin (from the firm Spofa, Czechoslovakia) at 37° C for 48 and 96 hr. The ratio of enzyme to substrate was 1 : 100 in the first, and 1 : 50 in the second. After incubation, the mixture was centrifuged. The residue was washed successively with acidified water, ethanol, and ether. The centrifugate was passed through a column of Sephadex G-25 (from the firm Pharmacia, Sweden) with dimensions of 4.5 × 90 cm. The fractions were subjected to spectrophotometry at 280 mμ in a SFD-2 spectrophotometer and were freeze-dried. Electrophoresis in a polyamide gel was carried out by Davis's method [4]. Amino acid analysis was carried out after acid hydrolysis on an amino acid analyzer from the firm Hitachi (Japan, Model KLA-3B).

CONCLUSIONS

The tryptic hydrolysis of DNP-7S-globulin from cottonseed gave peptide fragments, whose amino acid composition was studied.

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Institute of Biochemistry, AS UzSSR