THE GLOBULINS OF COTTON SEEDS

XII. THE STRUCTURE OF THE 7S-GLOBULIN

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We have previously reported that the 7S globulin of cotton seeds has a complex quaternary structure. After a study of various products of the dissociation of the protein in 8 M urea and 1% dodecyl sulfate it was established that the 7S globulin consists of 8 polypeptide chains of two types having molecular weights of 16,000 and 18,000 and differing from one another by their contents of carbohydrates and of amide groups [1, 2].

We have performed the preparative separation of the products of the dissociation of the protein in 8 M urea, consisting of three monotypical polypeptide chains, and have called them provisionally subunits I and II. A comparison of the peptide maps of the protein itself, of its subunits, and of the glycopeptide fraction has shown that the region of glycopeptides is the distinguishing part of the peptide maps of the subunits [2, 3]. No other appreciable differences were detected in the amino-acid compositions and the N- and C-terminal sequences of these subunits. Each peptide chain contains one disulfide bridge, one methionine residue and one tryptophan residue.

The study of the primary structure was begun with the trypsin cleavage of the reduced and carboxymethylated subunit I, containing no carbohydrates. For cleavage we used TPCK-trypsin (Serva, GFR). The product of tryptic hydrolysis was separated on a column of Sephadex G-25 equilibrated with 0.1 M ammonium bicarbonate. This yielded 7 fractions, each of which consisted on a fairly complex mixture of peptides.

The peptides isolated were then purified by paper chromatography and by electrophoresis in a thin layer of cellulose [4]. A total of seventeen peptides was obtained, and their N-terminal sequences and amino-acid compositions were established.

To determine the amino-acid sequences of the peptides we cleaved the carboxymethylated 7S globulin with trypsin (Spofa, Czechoslovakia) previously purified by recrystallization from ammonium sulfate and incubated with 0.001 N hydrochloric acid to suppress chymotryptic activity. The hydrolyzate was separated on Dowex 50WX4 cation-exchange resin. The peptides were purified by preparative paper chromatography and electrophoresis on paper and in a thin layer of cellulose [3]. The total number of tryptic peptides isolated and purified was 40, which considerably exceeds the number theoretically possible under the conditions of specific hydrolysis.

The amino-acid sequences of the tryptic peptides were determined by the Edman method with identification both of the phenylthiohydantoin derivatives of the amino acids and also of the amino-acid residues in the form of their dansyl derivatives [5]. For combining all the tryptic peptides into the polypeptide chain, the carboxymethylated 7S globulin was cleaved with chymotrypsin previously purified by recrystallization from ammonium sulfate. The chymotryptic peptides were isolated and purified in the same way as the tryptic peptides [6]. A total of 65 peptides was obtained, 38 of which included lysine and arginine. The amino-acid sequences were determined by the same methods as in the case of the tryptic peptides, taking the sequences of the latter [5] into account.

A comparison of the amino-acid compositions and N-terminal amino acids of the peptides of the tryptic hydrolyzate of subunit I containing no carbohydrates and of the tryptic peptides of the protein itself showed their identity, with the exception of the C-terminal peptide T 1-1, containing the carbohydrate moiety. This was the only peptide which, according to gas–liquid chromatography, contained 4-5 mannose residues and according to the results obtained on an amino-acid analyzer and by the Elson–Morgan reaction contained 3-4 glucosamine residues. In addition, we isolated two pairs of tryptic peptides (T 4-1-2 and T 5-1-1, and T 13-1-2 and T-16-3) and two pairs of chymotryptic peptides (XT 2-1-2 and XT 21-2, and XT 5-2 and XT 54-1-2)

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having the same amino-acid sequences but with aspartic and glutamic acids in the first peptides of each pair and the corresponding amides in the second peptides. This confirmed our hypothesis that the subunits of the 7S gobulin differ from one another by the inclusion or otherwise of the carbohydrate moiety and by the presence of amide groups. On the basis of the facts given, we considered that the two polypeptide chains have identical amino-acid sequences. Since the glycopeptide T-1-1 contained only mannose and glucosamine, it may be assumed that the oligosaccharide is attached to the amide group of asparagine through the N-acetyl group of N-acetylglucosamine [7]. However, peptide T-1-1 contained two aspartic acid residues. After two stages of the Edman splitting off of amino-acid residues, the reaction products were separated by electrophoresis in a thin layer of cellulose, and the presence of carbohydrates was found in each of them. Carbohydrates were also detected in the shortened peptide, which showed the attachment of the oligosaccharide to the second aspartic acid residue in the form of its amide corresponding to Asn 129 of the total amino-acid sequence of the polypeptide chain.

It must be mentioned that the 7S globulin of cotton seeds, like other plant proteins, is characterized by a high content of glutamic and aspartic acids, which are present in the molecule in the form of the amides. The enzymes trypsin and chymotrypsin that we used were not distinguished by high specificity, because of which a large number of nonspecific peptides was obtained through the cleavage of the peptide bond at the amides of the acids mentioned. Thus, subunit II containing carbohydrates has the following primary structure:

10
Arg-Gln-Gln-Lys-Ser-Ala-Pro-Gln-Gly-Phe-Gln-Leu-Asn-Arg-Val-Pro-
20 30
Val-Ala-Gly-Phe-Thr-His-Gln-Asn-Lys-Val-Ser-Gln-His-Pro-CmCys-Leu-
40
Ala-Arg-Phe-His-Asn-Gly-Gln-Arg-Phe-Gly-Ile-Asn-Phe-Glx-Arg-Leu-
50 60
Gly-Tyr-Gly-Ser-Gln-Arg-His-Ser-Asn-Gln-Trp-Gln-Arg-Ser-Gly-Gln-
70 80
Tyr-Phe-Ala-Pro-Gln-Asn-Leu-Val-Met-Asn-Asn-His-Gln-Ile-Arg-Leu-
90
Ala-Asn-Glx-Asn-Lys-CmCys-Phe-Tyr-Gln-Ser-Gly-Asn-Arg-Val-Arg-
100 110
Leu-Ala-Gln-Thr-Arg-Gly-Ala-Val-Lys-Leu-Glx-Ala-Phe-Val-Gly-Ser-
120
Arg-Gln-Asn-Phe-Leu-Val-Gly-Gln-Asn-Ser-Ala-Lys-Phe-Glx-Gln-Asx-
130
Val-Asn-Ile-Thr-Ser-Ala-Leu-Val
|
$[(GlcNAc)_{3-4}(Man)_{4-5}]$

(X in the formula denotes an amide in the case of subunit I and an acid in the case of subunit II).

EXPERIMENTAL

The isolation and purification of the 7S globulin, the reduction of the disulfide bridges, and the carboxymethylation of the SH groups were performed by known methods [1].

The subunits were separated and purified, and the peptide maps were obtained by a method described previously [2].

The cleavage of the carboxymethylated 7S globulin by trypsin and chymotrypsin and the separation and purification of the peptides obtained were performed by methods described previously [3, 6].

The amino-acid analyses of the 7S globulin and of all the products of its cleavage were performed on a LKB 4101 amino-acid analyzer (Sweden).

The amino-acid sequences of the tryptic and chymotryptic peptides were determined by the Edman method and by the modified Edman method with identification of the amino-acid residues in the form of their DNS derivatives [5, 6]. The carbohydrates of the glycopeptides were determined as described previously after acid hydrolysis by the GLC method in the form of their TMS derivatives [8] and by the Elson–Morgan reaction. The glucosamine was determined on the amino-acid analyzer in the hydrolyzate obtained for determining the amino acids. In its retention time, standard glucosamine coincided with tyrosine (LKB 4101 amino-acid

analyzer, Sweden). The results of the amino-acid analysis of the hydrolyzate were also used to calculate the amount of carbohydrates in the glycopeptide.

Electrophoresis in a thin layer of cellulose was used to separate the products obtained after two stages of the Edman splitting off of amino-acid residues in the glycopeptide T-1-1. Electrophoresis was performed on a 20 × 6 cm plate under the conditions described previously for peptide maps [2]; the spots were detected in UV light and with ninhydrin. Two fractions were found, and each was eluted with 10% acetic acid and, after evaporation in vacuum, hydrolyzed with 3 N HCl at 100°C for 3 h. The hydrolyzate was dried, kept in a vacuum desiccator over caustic soda, dissolved in water, and chromatographed on a plate with a thin layer of silica gel (6 × 6 cm) in the N-propanol–ethyl acetate–water (70 : 20 : 10 system), using orcinol to reveal the spots. The amino sugars in the hydrolyzate were revealed by the Elson–Morgan reaction.

SUMMARY

1. The 7S globulin of cotton seeds consists of eight polypeptide chains of two types differing by their contents of carbohydrates and of amide groups.

2. On the basis of the results of a study of the amino-acid sequences of the peptides obtained by cleaving the 7S globulin with trypsin and chymotrypsin, its complete primary structure has been put forward.

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METABOLITES OF THE PATHOGENIC FUNGUS *Verticillium dahliae*

VII. THE PHYTOTOXIC PIGMENT PKZh-1 FROM THE CULTURE LIQUID

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Many workers have reported the great importance of phytotoxins in plant diseases caused by microorganisms [1]. Among the phytotoxins are known substances of various chemical structures: peptides, glycosides, terpenoids, etc.

We have previously reported the presence in the culture liquid of the fungus *Verticillium dahliae* Kleb. of the Yangiyul' population of phytotoxic pigments – verticillins – belonging to the siderochrome group [2]. For their isolation we have developed a method using ion-exchange chromatography on CM-Sephadex C-25 and on DEAE-Sephadex A-25 [3].

We give here the results of a study of the phytotoxic pigment (PKZh-1) from the culture liquid of *V. dahliae*. It has been established that in a concentration of 100-150 μg/ml PKZh-1 causes the formation of chlorotic zones, necrotic spots, and the withering of the leaves of the cotton plant when the plants in the three-

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