the solution was deposited on FN-17 chromatographic paper (Filtrak). Chromatography was carried out in system 2. The fractions obtained were eluted with 0.005% ammonia, the eluates were evaporated in a rotary apparatus, and the N-terminal amino acids and compositions were determined.

The glycopeptide was methylated by Hakomori's method [3]. The completeness of methylation was checked by TLC, for which purpose aliquots were taken and were analyzed after acid hydrolysis (3 N HCl, 100°C, 5 h) on plates (6 × 9 cm) with a fixed layer of type KSK silica gel in system 3. The revealing agent used was a 3% solution of anisidine in n-butanol and benzidine–sodium metaperiodate [7].

Periodate Oxidation of the Glycopeptide. A suspension of 2 mg of the substance in 2 ml of 0.005 M KIO_4 solution was left overnight at 4°C. The mixture was freeze-dried and the sugars were determined as described above.

SUMMARY

1. The oligosaccharide of the glycopeptide (I) from subunit C of the 11S globulin of cotton seeds consists of one molecule of glucosamine and three molecules of mannose.

2. The oligosaccharide is attached to the protein through the glucosamine and has a branched structure.

LITERATURE CITED

1. S. I. Asatov, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 291 (1977).
2. Methods of Carbohydrate Chemistry [in Russian], Moscow (1967), p. 32.
3. S. Hakomori, J. Biochem. (Tokyo), 55, 205 (1964).
4. R. G. Spiro, in: Methods of Carbohydrate Chemistry, Academic Press, New York, Vol. VII (ed. by R. L. Whistler and J. N. BeMiller) (1976), p. 185.
5. C. Cessi and F. Rillega, Biochem. J., 77, 508 (1960).
6. T. T. Gorovits, Khim. Prirodn. Soedin., 49 (1969).
7. Thin-Layer Chromatography [in Russian] (1965), Moscow, p. 478.

THE GLOBULINS OF COTTON SEEDS

X. STRUCTURES OF TRYPTIC PEPTIDES OF THE 7S GLOBULIN

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In preceding communications we have considered the isolation, purification, and characteristics of the amino-acid compositions of peptides from the tryptic hydrolysis of the 7S globulin [1, 2]. In the present paper we described the determination of the amino-acid sequences of the tryptic peptides.

Judging from the amount of basic amino acids in the polypeptide chain of the protein (12 arginine and five lysine residues) and from peptide maps [3], tryptic hydrolysis should give about 20 peptides. We have obtained such results by performing the hydrolysis of subunit I and the 7S globulin with TPCK-trypsin [1]. For preparative purposes we used unmodified trypsin (Spofa, Czechoslovakia) [2].

In all the peptides obtained, the sequences of the amino acids were determined by the method of Edman degradation with direct identification of the PTH derivatives of the amino acids, and also of the DNS derivatives. The structures of the long peptides were determined by comparing their N-terminal sequences while taking into account the structures of their fragments and the structures of overlapping chymotryptic peptides.

The amino-acid sequences of the tryptic peptides are as follows (x in the case of subunit I denotes an amide and in the case of subunit II an acid; the numbering of the peptides is given in the order of their sequence in the chain from the N-end):

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Peptide	Sequence
T1	Arg
T2	Gln-Gln-Lys
T3	Ser-Ala-Pro-Gln-Gly-Phe-Gln-Leu-Asn-Arg
T4	Val-Pro-Val-Ala-Gly-Phe-Thr-His-Gln-Asn-Lys
T5	Val-Ser-Gln-His-Pro-CmCys-Leu-Ala-Arg
T6	Phe-His-Asn-Gly-Gln-Arg
T7	Gly-Ile-Asn-Phe-Glx-Arg
T8	Leu-Gly-Tyr-Gly-Ser-Gln-Arg
T9	His-Ser-Asn-Gln-Trp-Gln-Arg
T10	Asn-Asn-His-Gln-Ile-Arg
T11	Leu-Ala-Asn-Glx-Asn-Lys (CmCys)
T12	Phe-Tyr-Gln-Ser-Gly-Asn-Arg
T13	Val-Arg
T14	Leu-Ala-Gln-Thr-Arg
T15	Gly-Ala-Val-Lys
T16	Leu-Glu-Ala-Phe-Val-Gly-Ser-Arg
T17	Val-Gly-Gln-Asn-Ser-Ala-Lys
T18	Glx-Gln-Asx-Val-Asn-Ile-Thr-Ser-Ala-Leu-Val

Below we give the facts permitting the elucidation of the structures of the peptides isolated and reconstructed on the basis of the structures of the chymotryptic peptides [4].

Peptide T3 was not isolated from the products of tryptic hydrolysis and its structure was established on the basis of peptide T-8-3, containing no arginine in the C-terminal position because of the incomplete cleavage of the Arg-Val bond, and that of the overlapping peptide T-8-5-1 – the product of the partial cleavage of this bond:

T-8-3 Ser-Ala-Pro-Gln-Gly-Phe-Gln-Leu-Asn-Arg-Val-Pro-Val
T-8-5-1 Val-Pro-Val-Ala-Gly-Phe-Thr-His

Peptide T4 was determined from the fragments T-8-5-1 and T-17-1-2:

T-8-5-1 Val-Pro-Val-Ala-Gly-Phe-Thr-His
T-17-1-2 Thr-His-Gln-Asn-Lys

Peptide T8, likewise, was not isolated from the hydrolyzate, and its structure was established as the sum of fragments T-9-6-1 and T-8-1-1-1:

T-9-6-1 Leu-Gly-Tyr
T-8-1-1-1 Gly-Ser-Gln-Arg

Peptide T10 was probably split at a methionine residue, since only the fragments T-12-1 and T-14-2-1 were isolated; the complete structure of the tryptic peptide was restored from the methionine-containing chymotryptic peptides:

T10 Ser-Gly-Gln-Tyr-Phe-Ala-Pro-Gln-Asn-Leu-Val-
Met-Asn-Asn-His-Gln-Ile-Arg
T-14-2-1 Ser-Gly-Gln
T-12-1 Asn-Asn-His-Gln-Ile-Arg

In addition to the specific tryptic peptide T11 (T-5-1-1) we isolated a peptide T-4-1-2 differing by only one carboxymethylcysteine residue in the C-terminal position of the peptide, and also the fragment Lys-CmCys (T-7-1-3), formed as the result of the hydrolysis of the Asn-Lys bond of peptide T-5-1-1. In addition to this, in peptide T-4-1-2, the glutamic acid residue exists in the amide form in contrast to the peptide T-5-1-1. The circumstance that some peptides similar in sequence differ only by the fact that the dicarboxylic acids in them may also exist in the form of amides was observed by us previously on comparing peptide maps of subunits of the protein [3]. On investigating the tryptic peptides of the 7S globulin we isolated two pairs of such peptides:

T-5-1-1 Leu-Ala-Asn-Glu-Asn-Lys-CmCys
T-4-1-2 Leu-Ala-Asn-Gln-Asn-Lys
T-13-1-2 Gly-Ile-Asn-Phe-Glu-Arg
T-16-3 Gly-Ile-Asn-Phe-Gln-Arg

Peptide T12 is composed of fragments of T-16-4 and T-15-1:

T-16-4 Phe-Tyr
T-9-1-1 Tyr-Ser-Asn-Gly-Gln-Arg

Peptide T16 is the sum of fragments T-3-3-1 and T-14-3-3:

T-3-3-1 Leu-Glu-Ala-Phe
T-14-3-3 Val-Gly-Ser-Arg

Peptide T17 was completed on the basis of chymotryptic peptides, since the fragment Gln-Asn-Phe-Leu was not found among the tryptic peptides, and only the peptide T-6-1

Val-Gly-Gln-Asn-Ser-Ala-Lys

was isolated.

Peptide T18. Previously, in the determination of the C-terminal amino acid of the protein by the carboxypeptidase method it was possible to detect, in addition to the C-terminal amino acid valine, the appearance of leucine and alanine. This made it possible for us to establish that peptide T-1-1 is C-terminal and this conclusion was supported by the absence of Phe.

In an investigation of the tryptic peptides of subunit I of the 7S globulin we isolated the peptide T-1-1 having the composition Glu (Asp2, Thr, Ser, Ala, Val, Ile, Leu). The preparative hydrolysis of the protein gave the corresponding fragments Glu-Gln and Asp-Val-Asn-Ile-Thr-Ser-Ala-Leu-Val.

It was reported previously that the use of trypsin not treated with a chymotrypsin inhibitor gave a considerably larger number of peptides [2]. Analysis of the facts presented above and also of the structure of the residual fragments not given in the Table shows a nonspecific cleavage of the bonds predominantly at aromatic amino acids and amides, which can be explained by the existence of chymotrypsin activity in the trypsin preparation [5].

In sum (without taking the fragments into account) the peptides isolated correspond to approximately 85% of the amino-acid composition of the polypeptide chain of the protein.

EXPERIMENTAL

The amino-acid sequences of the peptides were determined by the Edman method with identification of the amino acids in the form of the DNS and PTS derivatives as described by Vinogradova et al [8].

To 0.1-0.2 μmole of peptide was added 0.3 ml of 50% aqueous pyridine and 0.1 ml of 5% phehylisothiocyanate (PITC) in pyridine. With liquid-nitrogen cooling, the air was pumped out from the test-tubes (vacuum of 0.1 mm) and was replaced by nitrogen (this operation was repeated three times). Then the tubes were placed in the thermostat at 45°C for 1 h. The solvent was distilled off in vacuum, the residue was carefully dried (55°C, 1 h), and 0.3 ml of trifluoroacetic acid (TFA) was added. The mixture was cooled, purged with nitrogen as described above, and left at 45°C for 30 min. The TFA was distilled off and the residue was dried in vacuum (55°C, 30 min). The dry residue was distilled in 0.3 ml of water and the solution was extracted with 1 ml of ethyl acetate. From the aqueous phase 10-15 nmole was taken for dansylation, and the remainder was dried in vacuum for the next stage of degradation.

The DNS-(amino acid)s were identified by two-dimensional chromatography on 6 × 6 cm plates with a fixed layer of type KSK silica gel [6, 7] in the systems used by Vinogradova [8].

The phenylthiohydantoin derivatives (PTHs) of the amino acids were used to determine the sequence of peptides containing tryptophan and amino dicarboxylic acids and their amides. To 0.2-0.4 μmole of peptide was added 0.2 ml of 50% aqueous pyridine and 25 μl of a 5% solution of PITC in pyridine. The mixture was cooled with nitrogen, the air was pumped out and replaced by nitrogen (the operation was repeated three times), and it was incubated in an atmosphere of nitrogen at 45°C for 1 h and was then dried, after which 0.1 ml of water was added and the solution was extracted with benzene (2 × 0.2 ml; the mixtures were centrifuged). The aqueous layer was dried, 0.1 ml of water and 0.2 ml of acetic acid were added and the mixture was saturated with gaseous HCl, thermostatted in a current of nitrogen at 45°C for 1 h, and dried in vacuum, and the residue was treated with 0.2 ml of water and the solution was extracted with ethyl acetate (2 × 0.2 ml). The PTHs of the amino acids in the ethyl acetate were dried in vacuum and were then dissolved in acetone and identified by one-dimensional chromatography on 6 × 6 cm plates in the presence of markers. LS_{254} silica gel with a luminescent indicator (5/40 μ, 13% of gypsum) (Chemapol, Czechoslovakia) was used. Chromatography was performed in the chloroform–ethanol (98 : 2) and chloroform–ethanol–methanol (88.2 : 1.8 : 10) systems. The PTHs of the amino acids were detected in UV light (260 nm).

SUMMARY

The amino-acid sequences of the tryptic peptides of the 7S globulin of cotton seeds have been determined.

An analysis of the peptides obtained has been performed and on the basis of the overlapping chymotryptic peptides the sequence of tryptic peptides in the chain of the 7S globulin has been established.

LITERATURE CITED

1. É. F. Redina, M. A. Kuchenkova, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 585 (1977).
2. É. F. Redina, M. A. Kuchenkova, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 556 (1977).
3. É. F. Redina, M. A. Kuchenkova, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 229 (1976).
4. N. L. Ovchinnikova, M. A. Kuchenkova, T. D. Kasymova, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 682 (1977) [in this issue].
5. J. Jentsch, J. Chromatogr., 57, 450 (1971).
6. B. G. Belen'kii et al., Dokl. Akad. Nauk SSSR, 172, 91 (1967).
7. B. G. Belen'kii, Molekul. Biol., 1, 184 (1967).
8. E. I. Vinogradova et al., Biokhimiya, 38, 3 (1973).

THE GLOBULINS OF COTTON SEEDS

XI. THE STRUCTURE OF THE CHYMOTRYPTIC PEPTIDES OF THE 7S GLOBULIN

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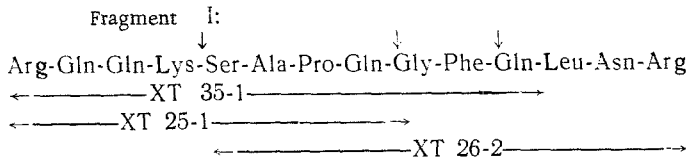
The chymotryptic hydrolysis of the carboxymethylated 7S globulin of cotton seeds and the separation, purification, and amino-acid compositions of the peptides obtained have been described previously [1]. In the present paper we consider the results of a study of the amino-acid sequences of the chymotryptic peptides.

In the process of chymotryptic hydrolysis, 65 peptides were isolated and characterized. The amino-acid sequences of the chymotryptic peptides were investigated by the Edman method with identification of the amino acids in the form of their 2-dimethylaminonaphthalene-5-sulfonyl (DNS) or phenylthiohydantoin (PTH) derivatives. The latter variant was used in determining the amino-acid sequences of peptides containing residues of dicarboxylic acids and of tryptophan.

In the case of chymotryptic peptides containing no basic amino acids and, evidently, being fragments of tryptic peptides, we limited ourselves to the determination of their partial structure. The complete amino-acid sequences of some chymotryptic peptides were established on the basis of the results of determinations of the primary structures of the tryptic peptides [2]. Below we give proofs of the structures of the chymotryptic peptides. For convenience of consideration, the whole peptide chain has been divided into fragments.



The amino-acid sequence of fragment I is given on the basis of the primary structures of the three overlapping peptides XT 35-1, XT 25-1, and XT 26-2, the latter having been formed as the result of the cleavage of bonds to basic amino acids.

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