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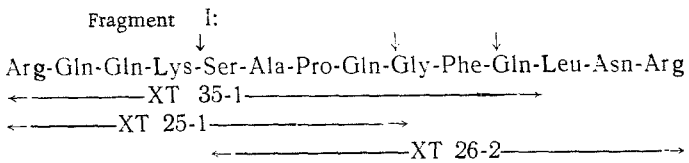
The chymotryptic hydrolysis of the carboxymethylated 7S globulin of cotton seeds and the separation, purification, and amino-acid compositions of the peptides obtained have been described previously [1]. In the present paper we consider the results of a study of the amino-acid sequences of the chymotryptic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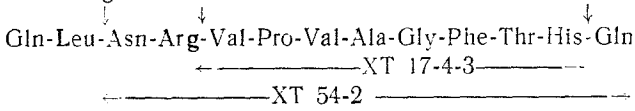
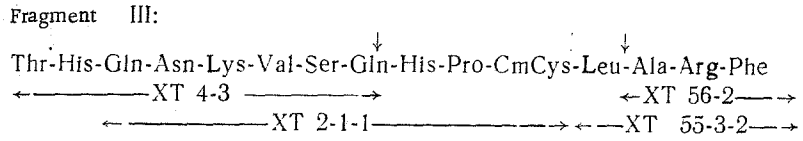
Fragment II:



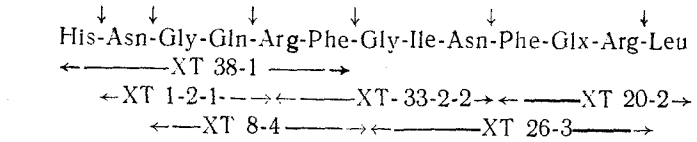
The amino-acid sequence of peptide XT 54-2 was established on the basis of its amino-acid composition, its N-terminal sequence, and the results of determinations of the sequences of peptides XT 17-4-3 and T 8-5-1, and T 17-1-2.

Fragment III:



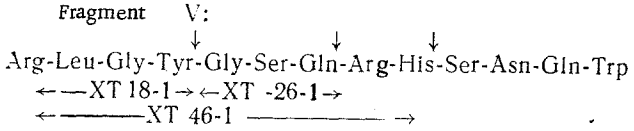
The amino-acid sequence of peptide XT 2-1-1 was determined from its amino-acid composition and N-terminal sequence and also on the basis of the sequences of amino acids in peptides XT 4-3 and T 20-1-2.

Fragment IV:



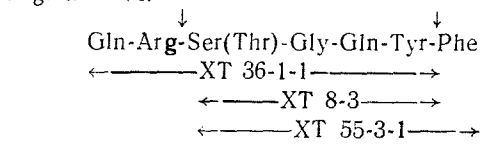
The amino-acid sequence of fragment (IV) was reconstructed on the basis of information on the primary structures of the peptides composing it and a comparison with the sequences of T 17-2-1 and T 13-1-2.

Fragment V:



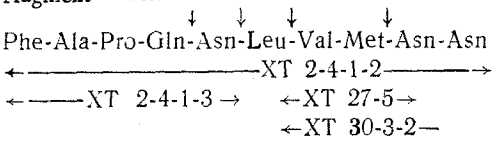
The amino-acid sequence of XT 46-1 is given on the basis of the results of the amino-acid analysis, the determination of the N-terminal sequence, and a comparison with the structures of peptides XT 18-1, XT 26-1, and T 8-1-1. The amino-acid sequence of fragment V was established from the structures of the peptides mentioned and of peptide T-9-4.

Fragment VI:



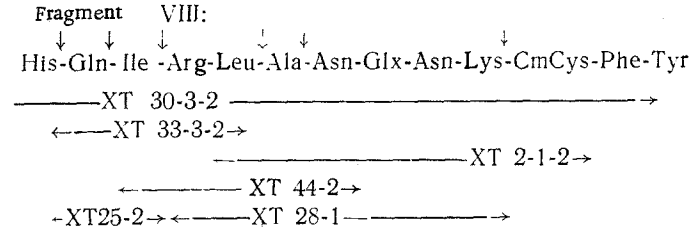
The amino-acid sequence of fragment VI is given on the basis of a comparison of the sequences of the amino-acid residues in the peptides of which it is composed.

Fragment VII:



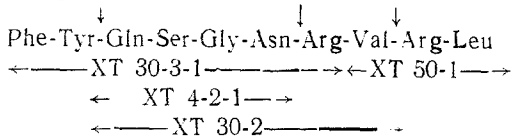
The amino-acid sequence of XT 2-4-1-2 was established on the basis of the results of the amino-acid analysis and the determination of the N-terminal sequence and also of the amino-acid sequences of the peptides obtained by the cleavage of the amide bonds of dicarboxylic acids:

Fragment VIII:



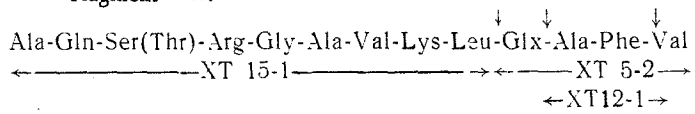
The amino-acid sequence of XT 30-3-2 was reconstructed on the basis of the results of the amino acid analysis, the determination of the N-terminal sequence, and a comparison of the sequences of the peptides composing it.

Fragment IX:



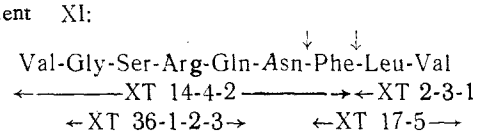
The amino-acid sequence of this fragment was established on the basis of the results of the amino-acid analysis and the determination of the N-terminal sequence and from the sequences of XT 50-1, XT 30-2, and T 15-1.

Fragment X:



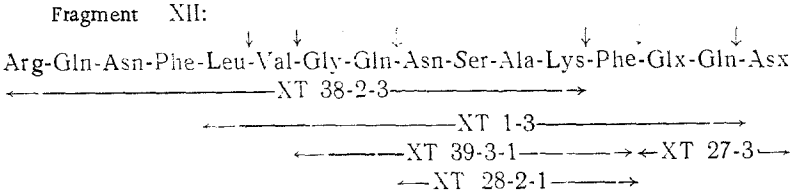
The amino-acid sequence of XT 15-1 was established on the basis of the amino-acid composition, the N-terminal sequence, and a comparison with the sequences of peptides T 5-2 and T-10-3-2.

Fragment XI:



The amino-acid sequence of XT 14-4-2 was reconstructed on the basis of the amino-acid analysis, the determination of the N-terminal sequence, and a comparison with the sequences of XT 36-1-2-3, XT 17-5, XT 2-3-1, and T 14-3-3.

Fragment XII:



Peptide XT 38-2-3 is the link between T 14-3-3 and T-6-1. Its amino-acid sequence was determined on the basis of the amino-acid composition the N-terminal sequence, and comparison with the N-terminal sequence of peptide XT 1-3. In this way we have determined the amino-acid sequences of 15 chymotryptic peptides.

The amino-acid compositions of peptides XT 2-1-2 and XT 21-2 and of XT-5-2 and XT 54-1-2, are the same, but their electrophoretic behaviors differ: 2-1-2 and 5-2 are anodic and XT 21-2 and XT 54-1-2 cathodic. On determining the amino-acid sequences of these peptides by the Edman method with identification of the amino acids in the form of their DNS and PTH derivatives it was established that the dicarboxylic amino acids of peptides 2-1-2 and 5-2 and of 21-2 and 54-1-2 were present in the forms of the acids and of the corresponding amides, respectively. Among the tryptic peptides we isolated pairs of peptides corresponding to them: T 4-1-2 and T 5-1-1, and T-13-1-2 and T-16-3.

In the determination of the amino-acid sequences of the peptides obtained on chymotryptic hydrolysis we also found two peptides, XT 21-2 and XT 26-2, which were products of the nonspecific cleavage of bonds at basic amino acids. This anomalous hydrolysis can be explained by the capacity intrinsic to chymotrypsin of cleaving certain bonds of basic amino acids. It must be mentioned that, in addition to the predominant cleavage of peptide bonds of amino acids, leucine, and methionine, hydrolysis was suffered by the bonds of other amino acids: His, Gln, Asn, Ile, Val, Ala, and Cys. A similar broad specificity of the action of chymotrypsin is particularly characteristic of its prolonged action i.e., under the conditions of the preparative cleavage performed.

The amino-acid sequences of the peptides obtained in the chymotryptic hydrolysis of the carboxymethylated 7S globulin are as follows:

Peptide	Amino-Acid Sequence
XT 1-2-1 XT 31-2-1	Asn-Gly-Gln
XT 1-3	Leu-Val-Gly-Gln-Asn-Ser-Ala-Lys-Phe-Glx-Gln
XT 2 1-1	Gln-Asn-Lys-Val-Ser-Gln-His-Pro-CmCys-Leu
XT 2-2-2	Glu-Asn
XT 2-4-1-2 XT 4-1	Phe-Ala-Pro-Gln-Asn-Leu-Val-Met-Asn-Gln-Asn
XT 2-4-1-3	Phe-Ala-Pro-Gln-Asn
XT 4-2-1 XT 34-2	Gln-Ser-Gly-Asn
XT 4-3 XT 31-1	Thr-His-Gln-Asn-Lys-Val-Ser-Gln
XT 4-4-2	Asn-Gly-Gln-Phe
XT 5-2	Glx-Ala-Phe-Val
XT 5-3-2	Arg-Phe-Gly-Ile-Asn
XT 8-3	Ser-Gly-Gln-Tyr
XT 8-4	Gly-Gln-Arg-Phe
XT 12-1	Ala-Phe
XT 14-4-2	Val-Gly-Ser-Arg-Gln-Asn-Phe
XT 15-1	Ala-Gln-Thr-Arg-Gly-Ala-Val-Lys-Leu
XT 17-4-3	Val-Pro-Val-Ala-Gly-Phe-Thr-His
XT 17-5	Phe-Leu-Val
XT 18-1	Leu-Gly-Tyr
XT-20-2	Phe-Glx-Arg-Leu
XT 23-1	Leu-Val-Gly-Gln-Asn-Ser-Ala-Lys-Phe
XT 25-1-1	Arg-Gln-Gln-Lys-Ser-Ala-Pro-Gln
XT 25-2-2	Gln-Ile
XT 26-1	Gly-Ser-Gln
XT 26-3 XT 31-2-2 XT 37-2	Gly-Ile-Asn-Phe-Glx-Arg
XT 27-3	Gln-Gln-Asn
XT 27-5	Val-Met
XT 28-1	Arg-Leu-Ala-Asn-Glx-Asn-Lys
XT 28-2-1 XT 28-2-2	Asn(Thr)-Ser-Ala-Lys-Phe
XT 30-1	Asn-Gly-Gln-Arg
XT-30-2	Gln-Ser-Gly-Asn-Arg-Val
XT-30-3-1	Phe-Tyr-Gln-Ser-Gly-Ash-Arg
XT 30-3-2	Val-Met-Asn-Asn-His-Gln-Ile-Arg-Leu-Ala-Asn Glx-Asn-Lys-CmCys-Phe-Tyr
XT 32-1-1 XT 32-2-1	Thr-His-Gln-Asn-Lys
XT 33-2-2	Arg-Phe-Gly-Ile-Asn
XT 33-2-2 XT 36-1-2-2	Gln-Ile-Arg-Leu
XT 35-1-1	Arg-Gln-Gln-Lys-Ser-Ala-Pro-Gln-Gly-Phe
XT 36-1-1	Gln-Arg-Ser(Thr)-Gly-Gln-Tyr
XT 36-1-2-3	Gly-Ser-Arg-Gln
XT 38-1 XT 39-1	His-Asn-Gly-Gln-Arg-Phe
XT 38-2-3	Arg-Gln-Asn-Phe-Val-Leu-Gly-Gln-Asn-Ser-Ala-Lys
XT 39-3-1	Gly-Gln-Asn-Ser-Ala-Lys-Phe
XT 44-2 XT 51-2	Ile-Arg-Ala-Leu
XT 46-1	Leu-Gly-Tyr-Gly-Ser-Gln-Arg-His
XT 50-1	Val-Arg-Leu
XT 54-1-2	Gln-Ala-Phe-Val
XT 54-2	Asn-Arg-Val-Pro-Val-Ala-Gly-Phe-Thr-His-Gln
XT 55-3-1	Ser(Thr)-Gly-Gln-Tyr-Phe
XT 55-3-2	Ala-Arg-Phe
XT 56-2	Leu-Ala-Arg-Phe

EXPERIMENTAL

The isolation, purification, and amino-acid compositions of the chymotryptic peptides have been described previously [1]. The amino-acid sequences of the peptides of the chymotryptic hydrolyzate were determined by the Edman method and by the same method in Gray and Hartley's modification [2].

SUMMARY

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

LITERATURE CITED

1. N. L. Ovchinikova, M. A. Kuchenkova, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 560 (1977).
2. É. F. Redina, M. A. Kuchenkova, L. Petrosyan, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 679 (1977) [in this issue].