THE GLOBULINS OF COTTON SEEDS

XVI. THE STRUCTURE OF SOME CHYMOTRYPTIC PEPTIDES OF SUBUNIT C OF THE 11S GLOBULIN

É. G. Yadgarov, T. S. Yunusov, and P. Kh. Yuldashev

UDC 547.962.5

In determining the sequence of amino acids of the acid-soluble chymotryptic peptides of subunit C [1], we were unable to determine the complete structure of a number of peptides by the Edman method. To elucidate their structures the peptides were subjected to additional cleavage. In peptide XT2 with the composition Asp, Glu, Val, Leu we determined only the N-terminal amino acid — Asp. The peptide was subjected to partial acid hydrolysis. Hydrolysis could not be carried out under mild conditions with 75% formic acid or with dilute solutions of hydrochloric acid, and therefore it was performed with 5.7 N HCl at 100°C for various times. After hydrolysis for only 60 min we obtained the two fragments K1 and K2, which were separated by PC under the conditions described previously [1]. The composition of fragment K1 was Asp, Glu, and of K2 Val, Leu. The sequence of K1 was Asn-Gln and of K2 Val-Leu. Consequently, the structure of XT2 is Asn-Gln-Val-Leu.

In peptides XT10 and XT11 (the two peptides were identical in relation to their N-terminal sequences and compositions) it was possible to determine the sequence of four residues: Val-Gln-Gly-Asn-(Glu, Ileu, Leu, Arg). As a result of treating the peptide with trypsin at 37°C (pH 8.8) with an enzyme–substrate ratio of 1:50 for 16 h we obtained two fragments with N-terminal amino acids Val and Leu. In view of the fact that in the peptide XT10 itself the N-terminal sequence had been determined, we found the sequence of the peptides without their separation. After the second step Gln and Ileu were identified, and subsequently Gly-Asn-Gln-Arg. Consequently, we had a mixture of the two peptides Val-Gln-Gly-Asn-Gln-Arg and Leu-Ileu. Peptide XT10 has the structure Val-Gln-Gly-Asn-Gln-Arg-Leu-Ileu.

In peptide XT19 with the composition Val-Ala-Leu-Gly-Glu-(Glu_2, Asp, Arg, Ser) we determined the sequence up to the fifth amino acid, inclusive. On cleavage of the peptide with trypsin under the conditions described above we obtained two fragments the structure of which were determined without their previous separation. Fragment 1 was Val-Ala-Leu-Gly-Glu-Gln-Asp-Arg and fragment 2 was Ser-Gln. Thus, peptide XT19 has the structure Val-Ala-Leu-Gly-Glu-Gln-Asp-Arg-Ser-Gln.

In peptide XT29,3 with the composition Val-Thr-His-Lys-Asp-(Glu_3, Ser, Gly, Arg_2) we determined the sequence up to the fifth residue, inclusive. The composition includes lysine and two arginines and therefore the peptide was cleaved with trypsin under the conditions described above. Three peptides were isolated by the PC method and their structures were determined:

XT29.3.1 Val-Thr-His-Lys-Asp-Gln-Arg
XT29.3.2 Asp-Gln-Arg
XT29.3.3 Gly-Gln-Glu-Ser-Arg.

Using the results obtained we reconstructed peptide XT29,3: Val-Thr-His-Lys-Asp-Gln-Arg-Gly-Gln-Glu-Ser-Arg.

The amino-acid analyses and conditions of chromatography of the peptides are given in our previous paper [1]. The structures of the peptides were determined by the methods described by Vinogradova et al. [2].

Thus, we have determined the structures of peptides XT2, XT10, XT19, and XT29,3, which include 34 nonoverlapping amino acids. The structures of the remaining chymotryptic peptides will be given in subsequent papers.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 540-541, July-August, 1978. Original article submitted March 27, 1978.

0009-3130/78/1404-0469$07.50

LITERATURE CITED

1. É. G. Yadgarov, T. S. Yunusov, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 271 (1978).
2. E. I. Vinogradova et al., Biokhimiya, 38, 3 (1973).

THE GLOBULINS OF COTTON SEEDS

XVII. PRIMARY STRUCTURE OF THE CARBOHYDRATE-CONTAINING SUBUNIT C OF THE 11S GLOBULIN

S. I. Asatov, É. G. Yadgarov,
T. S. Yunusov, and P. Kh. Yuldashev

UDC 547.962.5

We have shown previously [1] that the 11S globulin of cotton seeds consists of three types of subunits. We have begun the determination of the sequence of amino acids and the structure of the carbohydrate moiety of subunit C [2-4]. In order to obtain overlapping fragments of the polypeptide chain the protein was cleaved with trypsin and chymotrypsin (both enzymes of Worthington brand).

Subunit C has a very low solubility under the conditions of enzymatic cleavage, which makes it necessary to increase the time of digestion of the protein: in the case of chymotrypsin to 16-20 h and for trypsin to 2-3 days. Only with such prolonged action was it possible to achieve appreciable cleavage of the chain. Of course, with an increase in the time of digestion the specificity of the enzymes fell. This led to a large number of fragments.

To isolate and purify the peptides we used the classical methods of ion-exchange chromatography on a column of Dowex 50 × 4, paper chromatography, preparative electrophoresis in a thin layer of cellulose, and also separation of the acid-insoluble peptides by gel filtration on a column of Sephadex. Under the conditions of cyanogen bromide cleavage the rupture of a Asp-Pro bond was observed which undoubtedly facilitated the assembly of the molecule.

Subunit C lacks methionine, tyrosine, and cysteine. The sequences of the peptides were determined by the direct Edman method [5] in combination with dansylation. The amino acid analysis of the peptides was carried out after hydrolysis with 5.7 N HCl for 24 h in an LKB 4101 analyzer (Sweden). The results obtained enabled us to determine the complete primary structure of subunit C (* — position of attachment of the carbohydrate):

10
His-Asn-Gln-Trp-Glu-Glu-His-Gly-Asn-Asn-Phe-Arg-Gly-Asp-Ala-Glu-
20* 30
Glu-Leu-Val-Ileu-Asx-Ser-Thr-Pro-Arg-Val-Gln-Gly-Asn-Gln-Arg-
40
Leu-Ileu-Ser-Phe-Val-Ala-Asx-Glx-Arg-Val-Thr-His-Lys-Asp-Gln-
50 60
Arg-Gly-Gln-Glu-Ser-Arg-Glx-Ileu-Asn-Gly-Phe-Leu-Glu-His-Glu-
70
Asn-Arg-Glx-Ala-Gly-Val-Thr-Glu-Ala-Asx-Gly-Leu-Glx-Glx-Thr-
80
Phe-Ser-Glx-Arg-Gln-Phe-His-Gln-Asn-Arg-Lys-Phe-Ileu-Glx-Glu-
100
Asn-Arg-Ileu-Pro-Gln-Ala-Ser-Ala-Arg-Gln-Asn-Pro-Gln-Asn-Gln-
110 120
Val-Leu-Gln-Arg-Gln-Thr-Phe-Gln-Ser-His-Gln-Asn-Arg-Gln-Glu-
130
Gly-Asp-Ileu-Val-Ala-Leu-Gly-Glu-Gln-Asp-Arg-Ser-Gln-Gln-Asn

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 541-542, July-August, 1978. Original article submitted March 27, 1978.

 0009-3130/78/1404-0470$07.50