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THE GLOBULINS OF COTTON SEEDS

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

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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

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THE GLOBULINS OF COTTON SEEDS

XVIII. THE ACID-LABILE BOND OF SUBUNIT C OF THE 11S GLOBULIN

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In determining the amino acid composition of subunit C [1], traces of methionine were detected. To determine methionine in the protein we cleaved it with cyanogen bromide. With stirring, 500 mg of cyanogen bromide was added to a solution of 10 mg of the protein in 70% HCOOH, and the mixture was kept at 20°C for 20 h. On a peptide map one additional ninhydrin-positive spot was observed (Fig. 1).

On separating the mixture on a column (2 × 70 cm) of Sephadex G-50 (fine) equilibrated with 50% acetic acid we obtained two fractions (Fig. 2). The high-molecular-weight fraction (1) had the N-terminal amino acid His, and fraction 2 had Pro. Fraction 2 was obtained with a yield of 7%. The low yield could be explained by nonspecific cleavage and by the absence of methionine. In actual fact, an attempt to carry out the cleavage of the polypeptide chain at the methionine with freshly prepared Raney nickel [2] proved unsuccessful. The cleavage of an Asp-Pro bond in proteins under the conditions of cyanogen bromide hydrolysis is known [3]. Subsequently, from the peptides of the chymotryptic hydrolysate we isolated a peptide (XT3) containing such a bond.

We determined the amino-acid composition and N-terminal sequences of both fractions: 1) His-Asn-Gln...; 2) Pro-Gln-Asn-Gln.... Fraction 1 gave a positive reaction for a sugar. The presence of such a bond in the protein and the fact of its cleavage undoubtedly facilitated the reconstruction of the whole polypeptide chain of subunit C. The amino acid composition was determined as described previously [1]. The reaction for sugar was also carried out as described previously [4].

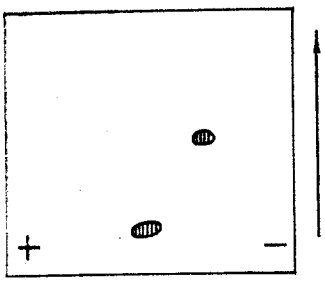

Fig. 1

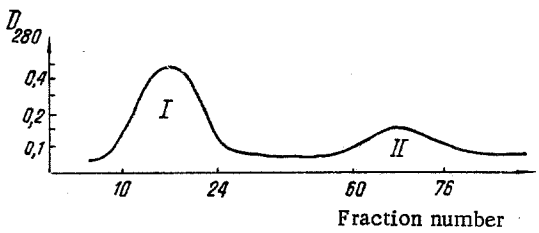

Fig. 2

Fig. 1. Peptide map of the cyanogen bromide hydrolysate of subunit C (conditions described in [1]).

Fig. 2. Chromatography of the cyanogen bromide hydrolysate of subunit C on a column of Sephadex G-50 (5-ml fractions, rate 60 ml/h).

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