A STUDY OF THE STRUCTURE OF THE 11S GLOBULIN FROM COTTON SEEDS

XIV. TRYPTIC PEPTIDES OF SUBUNIT C

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UDC 547.962.5

We have reported previously that the 11S globulin has a complex quaternary structure including three types of subunit [1]. To study the primary structure of subunit C we have cleaved it with trypsin (Worthington). The reaction was performed with a suspension of 0.25 g of the reduced and carboxymethylated protein in 0.2 M ammonia–acetate buffer, pH 8.8 at an enzyme:substrate ratio of 1:50 at 37°C. To determine the degree of cleavage and the nature of the peptides, the hydrolyzate was deposited on a plate (20 × 20 cm) coated with cellulose in order to obtain peptide maps. Chromatography was performed in the butan-1-ol–pyridine–acetic acid–water (15:10:3:12) system and electrophoresis in pyridine–acetate buffer, pH 6.5, 800 V, 40 min (Fig. 1).

To separate the peptides of the tryptic hydrolyzate we used a method described by Ovchinnikov et al. [2]. When the dried mixture of peptides was dissolved in 0.2 M pyridine-

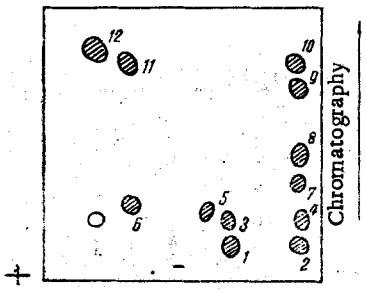

Fig. 1. Peptide map of a tryptic hydrolyzate of subunit C.

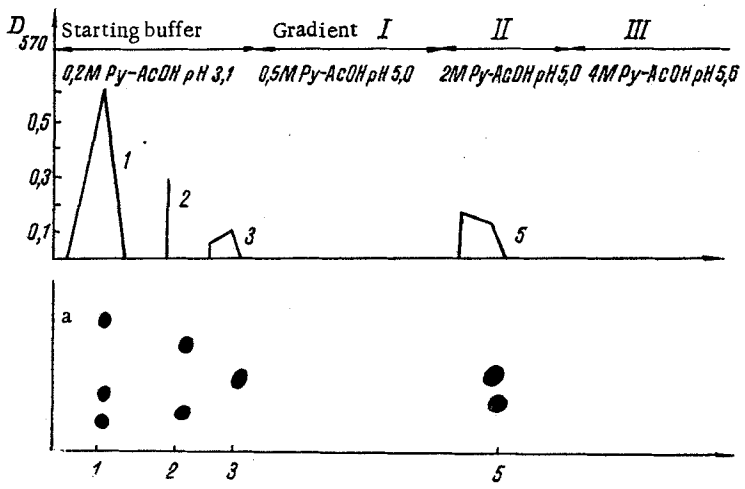

Fig. 2. Chromatography of a Tryptic hydrolyzate of subunit C on a column of Dowex 50 × 4; thin-layer chromatography of the combined fractions on cellulose (a).

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 272-273, March-April, 1978. Original article submitted November 17, 1977.

0009-3130/78/1402-0231$07.50

acetate buffer, pH 2.2, it was found that only 50% of the hydrolyzate dissolved. After centrifuging, the supernatant liquid was deposited on a column (1.2 × 145 cm) of Dowex 50 × 4 (400 mesh) using molar and pH-gradient elution for chromatography. The eluate was collected in 10-ml fractions, and after alkaline hydrolysis the ninhydrin reaction was carried out [3] (Fig. 2).

The purity of the combined fractions was chekced by thin-layer chromatography on plates (6 × 9 cm) of cellulose in the same system as for the peptide maps. According to the results of TLC and N-terminal amino-acid analysis, only the third fraction was homogeneous. The other fractions were separated by paper chromatography (FN 17, "Filtrak," GDR) and by preparative electrophoresis in a thin layer of cellulose under conditions similar to those used for the peptide maps.

In this way, for the soluble part of the tryptic hydrolyzate of subunit C we isolated nine peptides and determined their amino-acid compositions and N-terminal amino acids.

T1-1 Gly (Glu_3, Val, Asp, Ser_3, Gly, Ala, Lys)
T1-2 Gly (Ser, Asp, Val, Glu)
T1-3 Gly (Val, Asp, Glu)
T2-1 Asp (Ser, Glu_3)
T2-2 Glu (Val, Asp)
T3 Ser (Glu_2, Asp)
T5-1-1 Glu (Glu, His, Gly, Asp, Phe, Arg)
T5-2-1 Ser (Phe, Glu_2, Ser, His, Arg)
T5-3-1 Glu (Glu_2, Asp, Phe, Ser_2, His, Val, Ala, Arg)

The compositions of the peptides were determined after hydrolysis with 5.7 N HCl on a LKB 4101 analyzer.

LITERATURE CITED

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3. T. Déveni and Ya. Gergei, Amino Acids, Peptides, Proteins [in Russian], Moscow (1976), p. 199.