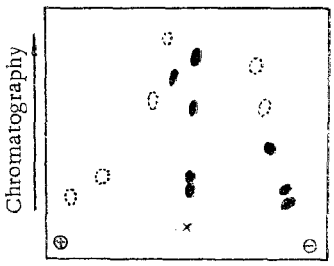

Fig. 1

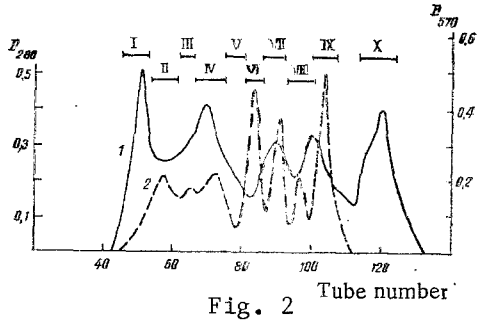

Fig. 2

Fig. 1. Peptide map of a tryptic hydrolysate of subunit B at arginine residues.

Fig. 2. Gel chromatography of a limited tryptic hydrolysate of subunit B: 1) D_{280}; 2) D_{570}.

Fractions with a volume of 10 ml each were collected. The eluate was analyzed from its absorption at 280 nm, and also by means of the ninhydrin reaction [1]. The conditions of the further separation and characterization of the peptides have been described previously.

SUMMARY

High-molecular-mass lysine-containing peptides necessary for the reconstruction of the polypeptide chain have been obtained from a tryptic hydrolysate of subunit B at arginine residues.

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PRIMARY STRUCTURE OF SUBUNIT B OF THE 11S GLOBULIN OF COTTON SEEDS OF VARIETY 108-F.

IV. HIGH-MOLECULAR-WEIGHT PEPTIDES FROM THE COMPLETE TRYPTIC HYDROLYSIS AND FROM TRYPTIC HYDROLYSIS AT THE ARGININE RESIDUES OF SUBUNIT B

A. L. Li and T. S. Yunusov

UDC 547.962.5

The high-molecular-weight peptides from the complete tryptic hydrolysis and tryptic hydrolysis at arginine residues of subunit B, which contained uncleaved bonds of the basic amino acids, have been studied. In the investigation of these peptides, use was made of additional cleavage by trypsin at lysine residues and of cyanogen bromide cleavage.

In the study of the primary structure of subunit B, several types of cleavages have been used with the aim of obtaining overlapping peptides. Complete tryptic hydrolysis and tryptic hydrolysis at arginine residues have been used. The feature of these types of cleavages is the production of high-molecular-weight peptides including arginine and lysine residues. We shall consider only the high-molecular-weight peptides of the complete tryptic cleavage and of tryptic cleavage at arginine residues. As has been shown previously, a tryptic hydrolysate of subunit B consisted of a mixture of acid-soluble (low-molecular-weight) and acid-insoluble (high-molecular-weight) peptides.

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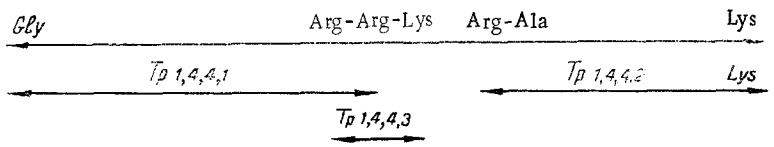
To separate the high-molecular-weight peptides we used the PC method. Three quantitatively predominating peptides were obtained which were purified additionally by high-voltage electrophoresis. The homogeneity of the peptides obtained was confirmed by TLC methods and by a determination of the N-terminal amino acids. In this way, the peptides $T_p1,4,4$; $T_p1,3,3$; and $T_p1,1,2$ with the N-terminal amino acid residues Gly, Gly, and His, respectively, were obtained. Their amino acid compositions were determined (Table 1). It is interesting to note that all the high-molecular-weight peptides contained residues of basic amino acids. This means that the cleavage of subunit B by trypsin did not take place completely although an increase in the time of hydrolysis did not lead to appreciable changes. On tryptic hydrolysis at arginine residues, high-molecular-weight peptides containing uncleaved arginine bonds were obtained. Consequently, some bonds of the basic amino acids in the protein molecule were resistant to the action of tryptin. This is possibly due either to conformational features of subunit B or to the blockage of the main acids by low-molecular-weight compounds.

As can be seen from Table 1, peptide $T_p1,4,4$ contained six residues of basic amino acids — two lysine residues and four arginine residues. To determine the positions of the lysine residues we carried out additional cleavage of the peptide that had first been modified with cyclohexanedione. On separating the hydrolysate we obtained two peptides as the main products — $T_p1,4,4,1$ and $T_p1,4,4,2$ — and one peptide in low yield — $T_p1,4,4,3$. Since the cleavage took place at lysine residues and this gave only two peptides in major yield, the second lysine residue must be the C-terminal amino acid residue of the initial peptide $T_p1,4,4$. However, the sum of the amino acid residues of the $T_p1,4,4,1$ and $T_p1,4,4,2$ peptides differed somewhat from the number of amino acids of the initial peptide $T_p1,4,4$. It was assumed that modification by cyclohexanedione took place partially and in the course of hydrolysis a peptide containing one arginine residue was obtained. But no such peptide was isolated. The amino acid sequence of the peptide $T_p1,4,4,3$ was Arg-Lys. It was assumed that the peptide Tp1,4,4 contained a -Arg-Arg-Lys- or a -Lys-Arg-Lys- bond. If a -Lys-Arg-Lys- bond had been present in the peptide, then on tryptic hydrolysis at lysine residues we should have obtained three peptides in high yield: a peptide containing one arginine residue, the peptide Arg-Lys, and a peptide containing one lysine and two arginine residues. However, only two peptides were isolated in major yield. Consequently, the existence of a -Arg-Arg-Lys- bond is the most likely. The formation of the peptide $T_p1,4,4,3$, Arg-Lys became possible on the incomplete modification of one of the two adjacent arginine residues, apparently because of a steric effect.



Peptide $T_p1,3,3$ contained two lysine and three arginine residues. Supplementary cleavage with trypsin of this peptide previously modified with cyclohexanedione gave two peptides — $T_p1,3,3,1$ and $T_p1,3,3,2$ with the N-terminal amino acids Gly and Ala (Val), respectively. Their homogeneity was confirmed by TLC and high-voltage electrophoresis and by a determination of their N-terminal acid residues. The formation of only two peptides indicates that one lysine residue was the C-terminal amino acid; otherwise three peptides would have been obtained.

Subunit B contains only one methionine residue, which is present in the fourth position and, consequently, all the peptides containing a methionine residue were N-terminal. On complete tryptic hydrolysis, peptide Tp1,1,2 containing a methionine residue was isolated. Like all the high-molecular-weight peptides, it contained uncleaved bonds of basic amino acids. This peptide was not studied, since on tryptic hydrolysis at arginine residues a similar N-terminal peptide was obtained that differed in composition by a few amino acid residues.

As can be seen from Table 1, all the acid-insoluble high-molecular-weight peptides of the complete tryptic hydrolysis of subunit B differed with respect to their amino compositions and their contents of basic amino acids and tyrosine and methionine residues. It has been suggested that they all form definite nonoverlapping sections of the polypeptide chain of subunit B.

The tryptic hydrolysis at arginine residues of subunit B also yielded high-molecular-weight peptides containing uncleaved arginine bonds: $T_{Arg}2,1,1$; $T_{Arg}2,2$; and $T_{Arg}2,3,1$.

TABLE 1. Amino Acid Compositions of the High-Molecular-Weight Peptides

Amino acid	T_p 1, 4, 4	T_p 1, 4, 4.1	T_p 1, 4, 4,2	T_p 1, 3, 3	T_{Arg} 2, 2	T_{Arg}2, 3, 1	T_p 1, 1, 2
Asp	7,1 (7)	3,6 (4)	2,9 (3)	3,6 (4)	7,4 (7)	3,8 (4)	8,5 (9)
Thr	3,1 (3)	1,4 (1)	1,5 (2)	1,4 (1)	4,4 (4)	1,7 (2)	2,9 (2)
Ser	3,9 (4)	1,8 (2)	2,2 (2)	2,4 (2)	4,9 (5)	1,8 (2)	4,5 (5)
Glu	11,0 (11)	4,2 (4)	2,8 (3)	7,1 (7)	12,9 (13)	5,5 (6)	11,0 (11)
Gly	5,4 (5)	2,1 (2)	2,8 (3)	3,6 (4)	6,4 (6)	2,5 (3)	5,9 (6)
Ala	4,6 (5)	2,2 (2)	1,5 (2)	2,4 (2)	5,1 (5)	2,5 (3)	4,6 (5)
Val	5,3 (5)	3,5 (3)	2,3 (2)	3,4 (3)	4,8 (5)	4,3 (4)	4,7 (5)
Met	–	–	–	–	1,0 (1)	–	1,0 (1)
Ile	2,2 (2)	1,1 (1)	1,5 (2)	1,3 (1)	2,3 (2)	1,1 (1)	2,1 (2)
Leu	4,1 (4)	1,8 (2)	2,1 (2)	6,3 (6)	5,7 (6)	2,2 (2)	4,6 (5)
Tyr	–	–	–	3,3 (3)	2,3 (2)	–	3,7 (4)
Phe	4,8 (5)	3,1 (3)	2,1 (2)	2,2 (2)	2,1 (2)	2,1 (2)	1,7 (2)
His	1,7 (2)	1,4 (1)	–	2,0 (2)	2,5 (3)	1,0 (1)	1,9 (2)
Lys	2,0 (2)	1,0 (1)	1,1 (1)	2,0 (2)	2,3 ( )	1,0 (1)	2,1 (1)
Arg	3,7 (4)	1,9 (2)	1,0 (1)	3,1 (3)	4,1 (4)	2,3 (2)	4,1 (4)
Number of residues	59–60	29–30	24–25	42–44	72–73	30–33	64–66
N-terminal amino acid	Gly	Gly	Ala	Gly	His	Gly	His
Yield, %	7	2,3	1,1	4	20	2	3 5

T_p – acid-insoluble peptides from complete tryptic hydrolysis; T_{Arg} – peptides obtained on tryptic hydrolysis at arginine.

Peptide $T_{Arg}2,2$ was N-terminal, since it contained a methionine residue. This peptide was subjected to additional cleavage by cyanogen bromide. As a result, two peptides were obtained, $T_{Arg}2,2,0,1$ and $T_{Arg}2,2,0,3$, with the N-terminal amino acids valine and histidine. The amino acid sequence of the histidine peptide was His-Phe-Arg. When subunit B was cleaved with cyanogen bromide, the same peptide was isolated and the N-terminal sequence of the initial protein coincided with this sequence of the low-molecular-weight peptide obtained. Peptides $T_p1,1,2$ and $T_{Arg}2,2$ were N-terminal.

The amino acid compositions of the high-molecular-weight peptides obtained on tryptic hydrolysis at arginine residues were determined.

EXPERIMENTAL

Subunit B was isolated by a method described previously [1].

The modification of subunit B and the peptides by cyclohexanedione was performed in a 0.25 M Na borate buffer, pH 9.0. A weighed sample of protein or peptide was suspended in this buffer and the mixture was incubated at 45°C for 50 min. The cyclohexanedione was used in the reaction in 20-fold excess. The reaction was performed for 30 h at room temperature in the dark. The process was stopped by the addition of an equivalent amount of 30% acetic acid. The precipitate was separated from the solution and analyzed separately.

The arginine protection was removed in 0.5 M hydroxylamine hydrochloride at pH 7.0 and a temperature of 37°C for 8 h. The dione was detected by spraying the chromatogram with a 0.01 M solution of nickel chloride.

The cleavage of the peptide by cyanogen bromide was carried out in 70% formic acid solution. The reagent was added in 200-fold excess. The reaction was performed at room temperature for 16 h. Then the hydrolysate was evaporated and was washed with water several times.

The other methods for investigating the structures of the peptides have been described previously.

SUMMARY

1. From a complete tryptic hydrolysate of subunit B, three high-molecular-weight peptides comprising 165-170 amino acid residues of the polypeptide chain of subunit B have been obtained.

2. It has been established that the polypeptide chain of subunit B contains peptide bonds of basic amino acids that are resistant to the action of trypsin.

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PRIMARY STRUCTURE OF SUBUNIT B OF THE 11S GLOBULIN OF COTTON SEEDS OF VARIETY 108-F.

VI. RECONSTRUCTION OF THE POLYPEPTIDE CHAIN

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Subunit B of the 11S globulin of cotton seeds, the polypeptide chain of which contains about 190 amino acid residues, has been reconstructed on the basis of large fragments from three types of tryptic hydrolyses.

The study of proteins from thermophilic and mesophilic sources has attracted a broad group of research workers in recent years [1]. The elucidation of the interrelationship of these proteins with their properties is of both theoretical and practical interest. It is primarily of practical importance for the reserve proteins of plant seeds.

The production of the proteins of food isolates is usually connected with the treatment of the raw material under various conditions, including different temperatures. As a rule, for the reserve proteins, a correlation is made between composition and thermal stability [2]. Thus, Biglow [3] assigned proteins with a high value of the mean hydrophobicity to the thermophilic proteins. The mean hydrophobicity of the main globulin of cotton seeds (11S) is about 900 cal/res. At the same time, the protein has a comparatively low solubility in solvents of high ionic strength and exhibits a high thermolability [4].

It must be mentioned that the hydrophobicity of protein globules is undoubtedly connected with the total number of hydrophilic residues in the molecule. However, the thermophilicity of proteins is, in our view, determined also by the serial arrangement of the hydrophobic residues along the polypeptide chain and, in particular, by the distribution of the hydrophobic radicals between the hydrophobic core of the molecule and the hydrophobic clusters on the surface of the protein globule. For proteins with a complex quaternary structure, this factor is of fundamental importance. In the protein that we are studying there are no disulfide bonds, and in the intersubunit interactions, in addition to ionic forces, hydrophobic forces are definitely involved [5] — all this, and also the generally low mean hydrophobicity, imparts a highthermolability to the molecule.

In a study of the interconnection of the structure of a protein with its properties, in addition to chemical modification and enzymatic cleavage of the native molecule with various enzymes, we are determining the primary structures of the individual subunits in order to elucidate the features of each of them. The complete amino acid sequence of subunit C has been shown [6].

In the present paper we give results on the reconstruction of the polypeptide chain of subunit B. The strategy of the study included chymotryptic hydrolysis, complete tryptic hydrolysis, tryptic hydrolysis at arginine residues, and tryptic hydrolysis at lysine residues, and also the chemical cleavage of the chain at methionine and tryptophan residues. The study of all types of cleavage has been described in individual publications. Basic information on the polypeptide chain was given by a study of large fragments obtained with different types of cleavage of the protein by tyrpsin. Chymotryptic hydrolysis led to the formation of a large number of short peptides, part of which were lost on the ion-exchange column.

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