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

III. PEPTIDES FROM TRYPTIC HYDROLYSIS AT ARGININE RESIDUES

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To obtain peptides overlapping the lysine peptides obtained by complete tryptic hydrolysis, cleavage of maleylated subunit B with trypsin at arginine residues has been performed. Large peptides containing lysine have been obtained and characterized.

As a rule, the strategy of the determination of primary structures of proteins is determined by the features of their molecules. For subunit B limited tryptic hydrolysis has been widely used since chymotryptic hydrolysis did not give the necessary information because of the the production of a large number of short peptides on cleavage.

We give the results of a study of the tryptic hydrolysate of subunit B at arginine residues performed with the aim of obtaining lysine-containing peptides overlapping the lysine peptides from complete tryptic hydrolysis. Figure 1 gives the peptide map of the limited tryptic hydrolysis of the protein under study (for deposition on the plate, the maleyl protection was removed). The map corresponds to 5-hour hydrolysis. With an increase in the time of hydrolysis to 24 h several additional minor components appeared on the map, but the positions and number of the main spots did not change. The number of quantitatively major peptides obtained did not correspond to what was expected, since the protein molecules contains 14-15 arginine residues. The protein that was subjected to digestion contained no disulfide bridges, while the substrate was subjected to chemical modification, which, in general, should increase the accessibility of the various arginine bonds to the action of trypsin. In spite of this, a definite resistance of subunit B to the action trypsin was found. We shall discuss the possible reasons for this below.

To separate the hydrolysate we used a column of Sephadex G-25 equilibrated with 30% formic acid. The results of chromatography are given in Fig. 2.

Fraction 1 consisted of the practically uncleaved protein or fragments close in composition to the initial protein. Below we give information on the main peptides isolated from the various fractions (the percentage yields are given in parentheses):

Fraction 9

T_{Arg} 9,2 Arg (14)

T_{Arg} 9,3 Glu Arg (6,0)

Fraction 8

T_{Arg} 8,1 GlxAsxAlaArg (3,8)

T_{Arg} 8,4 GlyIleAsxGlxPhe (6,4)

Fraction 7

T_{Arg} 7,5 ValLeuGlxArg (4,8)

Fraction 6

T_{Arg} 6,1 GlyGlnSerGlnGlnArg (3)

T_{Arg} 6,6, 1 GlyGluIleAlaAspGluArg (3,2)

Fraction 5

T_{Arg} 5,3 PheArgGlxGlxGlxArg (4)

T_{Arg} 5,2 LeuGlxGlxGlxProGlxArg (2,4)

T_{Arg} 5,1, 4 LysAlaSerGluGluGluArg (0,64).

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and also a peptide identical with the peptide $T_{Arg}6,1$.

The amino acid compositions of the peptides of fractions 3 and 4 were as follows (the tryptophan-containing peptide $T_{Arg}4,6,1$ was determined by the Ehrlich reagent):

	T_{Arg} 4, 5, 1	T_{Arg} 4, 6, 1	T_{Arg} 4, 6, 2	T_{Arg} 4, 5, 2	T_{Arg} 3, 6, 1
Asp	3,0	2,7	1,9	2,8	4,8
Thr	1,1	0,5	0,6	1,4	2,0
Ser	1,9	2,1	0,8	1,6	2,8
Glu	4,1	3,7	1,3	3,7	4,6
Pro	0,9	1,1	0,6	1,7	0,9
Gly	2,2	2,2	1,8	2,2	3,5
Ala	2,5	3,0	1,9	3,0	2,9
Val	2,2	1,9	1,7	2,3	3,5
Ile	1,4	1,3	1,2	1,5	1,4
Leu	2,1	2,3	1,5	2,3	2,7
Tyr	1,2	1,3	—	1,1	0,9
Phe	2,0	1,9	1,4	1,7	2,4
His	0,6	0,5	0,6	0,6	0,7
Lys	1,0	1,0	1,0	1,0	1,0
Arg	1,8	2,2	1,4	2,2	2,0
N-terminal amino acid residue	Glv	Gly	Leu	Ile	Leu
Yield, %	1,2	2,4	2,4	1,3	0,8

From fraction 2 were isolated the large lysine-containing peptides $T_{Arg}2,2$ and $T_{Arg}2,3,1$, and also the peptide $T_{Arg}2,1,1$, Glx-Glx-Glx-Arg (12.8%). Peptides $T_{Arg}2,2$ and $T_{Arg}2,3,1$ were subjected to additional cleavage the results of which will be given in subsequent publications. It must be mentioned that in the purification of the peptides of the fractions described above, a large number of minor peptides were isolated, and also identical peptides from different fractions, which shows the comparatively poor separation of the peptides on the column because of the formation of aggregates of peptides under the conditions of chromatography. The presence of free arginine ($T_{Arg}9,2$) in a limited tryptic hydrolysate indicates the presence in subunit B of an Arg-Arg bond, and one which is relatively accessive to the action of trypsin, since the arginine was obtained with good yield. There is a Arg-Lys bond in the protein, which is shown by the peptide $T_{Arg}5,1,4$. Furthermore, lysine has previously been isolated from the peptides of a complete tryptic hydrolysate. The remaining lysine-containing peptides were large ones mainly because of the presence in them of uncleaved bonds at arginine residues. In addition to lysine-containing peptides an uncleaved arginine bond was detected in peptide $T_{Arg}5,3$. Of the large peptides we must mention in the first place those of fractions 1 and 11 which remained relatively stable even in the case of the prolonged digestion of subunit B by trypsin. However, in the homogeneous state the peptides from these fractions were cleaved by trypsin almost completely in 2-4 h. The stability of the bonds can be explained by a number of factors; for example, the pronounced tendency of the peptides of partially hydrolyzed subunit B to undergo aggregation, and also the influence of certain accompanying substances (of nonprotein nature) on the resistance of arginine peptide bonds to the action of tryptin. A definitive consideration of this question will be possible only after a study of the peptides of complete tryptic hydrolysis and also of tryptic hydrolysis at the lysine residues of subunit B.

EXPERIMENTAL

The maleylation of subunit B was carried out in 0.1 M borate-alkali buffer, pH 8.85 at a 50-fold molar excess of anhydride per mole of lysine. The pH of the reaction mixture was maintained by the addition of a 5% solution of NaOH, and the temperature of the reaction vessel was kept at 10-15°C for 1 h. Then the mixture was stirred at 25°C for another 1 h. The excess of salts was eliminated by dialysis against distilled water with pH 8-9. The protein solution was then lyophilized. The completeness of modification was confirmed by dansylation.

The tryptic hydolysate of the protein was obtained in 0.2 M N-ethylmorpholine acetate buffer, pH 8.6, at an enzyme-substrate ratio of 1:100. The reaction was performed at 37°C for 5 h. Worthington trypsin was used.

The protection was eliminated in a 10% solution of acetic acid at 37°C for 40 h. The stability of the protein under the conditions of eliminating the protection was checked by disc phoresis, and also by dansylation.

The gel chromatography of the hydrolysate was carried out on a column (2 × 215 cm) of Sephadex G-25 (fine) equilibrated with 30% formic acid. The rate of elution was 10 ml/h.

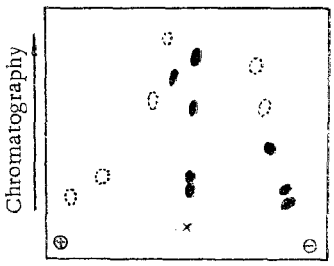

Fig. 1

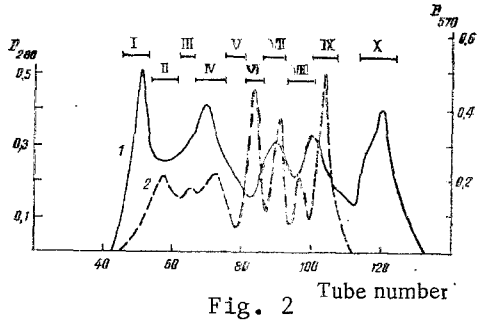

Fig. 2

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

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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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