A STUDY OF THE GLOBULINS OF COTTON SEEDS

VI. PEPTIDES OF A TRYTPIC HYDROLYZATE OF SUBUNIT I OF THE 7S GLOBULIN

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We have isolated and studied tryptic peptides from subunit I of the 7S globulin of cotton seeds. Reduction, carboxymethylation, and tryptic hydrolysis were performed under the conditions described previously [1]. Subunit I (200 mg) was hydrolyzed with TPCK-trypsin ("Serva," GFR) for 8 h. The hydrolyzate was dissolved in 2.5 ml of 0.1 M ammonium bicarbonate and deposited on a column of Sephadex G-25 (superfine, 1.5 × 180 cm) equilibrated with the same buffer. Fractions of 4 ml were collected at a rate of elution of 4 ml/h, and the absorption at 280 and 230 nm were measured (Fig. 1).

The peptides were separated by paper chromatography in the butan-1-ol–pyridine–acetic acid–water (15 : 10 : 3 : 12) system using "Filtrak" FN 17 paper (GDR) in strips of 7-10 cm, with 0.1 -0.2 μmole/cm, at room temperature for 18 h. The narrow bands of the chromatograms were reveated with 0.3% ninhydrin in acetone after drying in the air.

Preparative electrophoresis on plates with a thin layer of cellulose was performed under the conditions described previously for peptide maps [1]. The paper strips or the cut layers of cellulose were eluted with 10% acetic acid, and the eluates were concentrated in a rotary evaporator at room temperature. In some cases, rechromatography was used. The purity of the peptides was checked by means of the DNS derivatives as described by Vinogradova et al. [2]. This gave 17 homogeneous peptides the compositions and N-terminal amino acids of which are given below (the peptides were hydrolyzed with 6 N HCl at 110°C for 24 h, and their compositions were determined on a LKP 4101 amino-acid analyzer):

T-I-2	Glu (Asp 2, Thr, Ser, Ala, Val2, Ile, Leu)
T-I-4	Ser (Asp, Glu, Pro, Gly, Ala, Phe, Arg)
T-I-6	Gly (Asp, Glu, Ile, Phe, Arg)
T-II-1	Leu (Asp2, Glu, Ala, Lys)
T-II-3-1	Gly (Glu, Ser, Arg)
T-II-4-1	Leu (Thr, Ser, Glu, Ala, Arg)
T-III-1-1-1	His (Asp, Ser, Glu2, Arg)
T-IV-1-2	Ala, Arg

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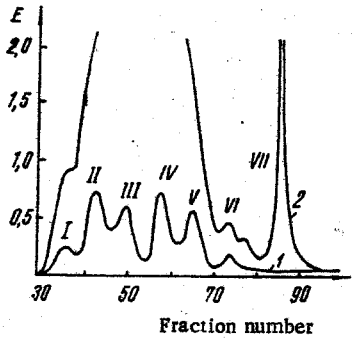

Fig. 1. Gel filtration of a tryptic hydrolyzate of subunit I of the 7S globulin on Sephadex G-25: 1) extinction at 280 nm; 2) at 230 nm.

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T-IV-3-2	Lys
T-IV-7-2	Leu, Arg
T-V-1-1	Arg
T-V-1-2	Val (Asp, Ser, Glu, Ala, Lys)
T-V-2-2	Phe (Glu, Arg)
T-V-3-1-2	Val (Asp, Thr, Glu, GLy, Ala, Phe, His, Lys)
T-V-3-2	Val (Ser, Gly, Arg)
T-VI-3-2	Thr (Asp, Glu, His, Lys)
T-VI-5-1	Phe (Asp, Glu, Gly, His, Arg)

LITERATURE CITED

1. E. F. Redina, M. A. Kuchenkova, and P. Kh. Yuldashev, Khim. Prirodn. Soedin., 229 (1976).
2. E. I. Vinogradova et al., Biokhimiya, 38, 3 (1973).

SPECIFICITY OF THE TRIACETINASE OF COTTON SEEDS

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The isolation of triacetinase – an enzyme which has been classified as a lipase – has been reported previously [1].

As Desnuelle and Sarda [2] have shown, the main difference between lipases and esterases is shown in their relationship to the physical state of the substrate – the lipases, unlike esterases, do not hydrolyze water-soluble substrates. This is shown best using the reaction with triacetin as example. This compound is readily soluble in water at low concentrations, and at higher concentrations it gives stable emulsions. In the case of lipases, hydrolysis takes place at an appreciable rate only when the concentration of triacetin is sufficiently high for the formation of micelles. In the case of esterases, hydrolysis begins at very low concentrations of triacetatin.

The results of our experiments are shown in Fig. 1. It is obvious from this graph that the triacetinase of cotton seeds is an esterase, K_m for this reaction being $1.6 \cdot 10^{-4}$.

When the triacetinase was incubated with diisopropyl phosphorofluoridate in a concentration of 10^{-3} M, the enzyme was 100% inactivated.

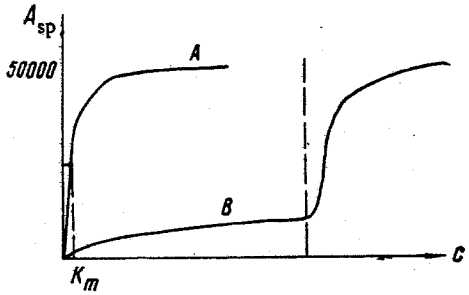

Fig. 1. Curves of the dependence of the rate of hydrolysis by triacetinase on the concentration of triacetin (A) and a typical curve of the hydrolysis of triacetin by lipases (B). The broken line denotes the limit of solubility of triacetin.

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