C-TERMINAL SEQUENCE OF THE 8.2 S GLOBULIN OF COTTON SEEDS

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The C-terminal amino acid in the 8.2 S globulin that we had isolated [1, 2] was cleaved with the aid of hydrazine and carboxypeptidase [3, 4].

The protein (53.4 mg) was heated with 1 ml of anhydrous hydrazine in the thermostat at 100-105°C for 10 h. The excess of hydrazine was eliminated in a vacuum desiccator over sulfuric acid. The residue was dissolved in 2 ml of water and shaken with 0.2 ml of benzaldehyde for 2 h. The benzaldehyde layer with the condensation products of the hydrazides of the amino acids was separated from the aqueous part by centrifuging at 3000-4000 rpm. The aqueous layer was evaporated to dryness at 60-70°C in a rotary evaporator.

In the determination of the amino acids, the dry residue was chromatographed in a thin layer of silica gel [5]. It can be seen from Fig. 1 that the C-terminal amino acid of the 8.2 S globulin is threonine. To confirm the result obtained, the amount of the C-terminal amino acid of the 8.2 S globulinwas determined by carboxypeptidase cleavage. Carboxypeptidase A ("Reanal," Hungary) was recrystallized three times [6] and activated by a published method [4] before incubation.

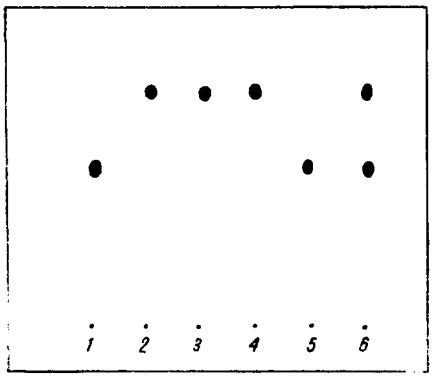

Fig. 1

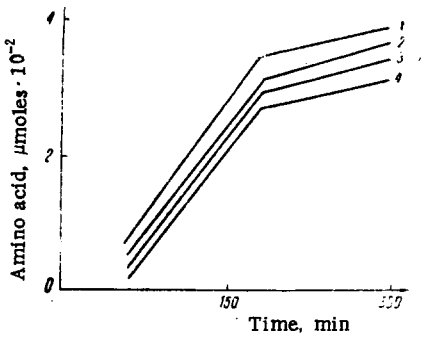

Fig. 2

Fig. 1. Thin-layer chromatogram in silica gel (10×10) in the butan-1-ol – conc. acetic acid – water (4:1:1) system (one-dimensional chromatography to a height of 9 cm three times): 1) threonine; 2) isoleucine; 3) phenylalanine; 4) leucine; 5) product of the hydrazinolysis of the 8.2 S globulin; 6) mixture of amino acids (1-4).

Fig. 2. Amino acids split off from the 8.2 S globulin on incubation with carboxypeptidase A: 1) threonine; 2) valine; 3) alanine; 4) glutamic acid.

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The 8.2 S globulin (34.6 mg) was dissolved in 9 ml of phosphate buffer with pH 8.0 and ionic strength 0.5. Then 1 ml of activated carboxypeptidase A was added to the solution and incubation was performed at 27°C.

Samples were taken after 30, 60, 120, 180, 240, and 300 min. The reaction was stopped in the test tubes taken by the addition of 1 N hydrochloric acid to pH 1-2, and the resulting precipitate of protein was separated off by centrifuging at 3000-4000 rpm. For the complete extraction of the amino acids split off, the precipitate was washed with water three times and was centrifuged. The aqueous solutions were evaporated in a rotary evaporator.

The amino-acid content of the dry residue was determined on a 4SAN (type 6020A) amino-acid analyzer. It can be seen from Fig. 2 that in the 8.2 S globulin the carboxypeptidase A first splits off threonine, then valine, alanine, and glutamic acid.

On the basis of these results, the sequence for the C-terminal section of the 8.2 S globulin can be given as glutamylvalylalanylthreonine.

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