MODIFICATION OF THE RESERVE PROTEINS DURING THE GERMINATION OF COTTON SEEDS

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The hydrolysis of the 11S globulin begins in the early stages of the germination of cotton seeds and is complete during the first 4-5 days. During the first 3 days, only limited hydrolysis of the reserve protein takes place, and only on the 4th day does it undergo far-reaching hydrolysis, after which the protein cannot be detected either by immunochemical methods or by electrophoresis and analytical ultracentrifugation.

We have established previously by TLC that protease A acts on the native reserve proteins, the 7S and 11S globulins (in vitro), as a result of which limited proteolysis of the globulins takes place, and only after the corresponding modification do the latter undergo complete degradation by other enzymes. In other words, protease A is an enzyme initiating hydrolysis.

Continuing these investigations using the methods of immunochemical analysis, electrophoresis, and ultracentrifugation, we have obtained additional confirmation of the results obtained. Two pathways for the proteolysis of reserve proteins are known from the literature [2]. In the first case, proteolysis begins a few days after the start of germination with the simultaneous synthesis of a proteinase which is absent from dormant seeds. In the second case, the limited proteolysis of the main reserve proteins begins almost simultaneously with the start of germination and is carried out by proteinases already present in the dormant seeds.

The results of immunochemical analysis showing that the hydrolysis of the proteins has the nature of limited proteolysis (modification) of the reserve proteins and begins simultaneously with the start of germination, may be convincing proof of the participation of protease A in the hydrolysis of the 7S and 11S globulins. To perform immunochemical analysis using a precipitation reaction, we obtained serum to one of the reserve proteins — the 11S globulin — and also isolated the 11S globulin from seedlings from the 1st to the 15th day of germination by the method described in [3] (Fig. 1).

As can be seen from Fig. 1, the 11S globulin underwent a change as early as the 1st day of germination, since the proteins under investigation from the dormant seeds and those that had germinated for 1 day no longer possessed antigenic identity. After the 3rd day of germination, the precipitation reaction changed, i.e., the 11S globulin underwent the action of other proteases, which led to more far-reaching hydrolysis of the reserve protein. In connection with the fact that the cleavage of the 11S globulin was detected during the first 24 h of growth, it is logical to assume the participation in this process of proteolytic enzymes from the dormant cotton seeds. The action of other proteases (B and C) of dormant cotton seeds on their own reserve proteins has been studied previously, and it was established that proteases B and C hydrolyze only modified reserve proteins, while protease A acts on the native 7S and 11S reserve proteins of cotton seeds [4]. When the amount of 11S globulin in growing cotton seeds was determined by Lowry's method [5], a fall in it was found even during the first 24 hours of growth (Fig. 2). The gradual fall in the amount of the 11S globulin continued up to the 5th day of germination. Thus, the results of immunochemical analysis have shown that the limited proteolysis of one of the main reserve proteins begins almost simultaneously with the start of germination and is effected by the protease A that is present in the dormant seeds.

In order to obtain additional information, we performed electrophoretic investigations of preparations of the 11S globulin in PAAG in the presence of sodium dodecyl sulfate. We investigated the 11S globulin from the 1st to the 13th days of germination (Fig. 3), and also the 11S globulin treated with protease A. It is interesting to note that on the electrophoresis

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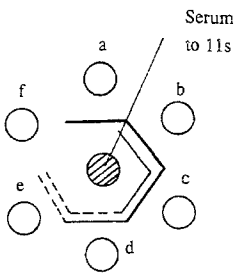

Fig. 1

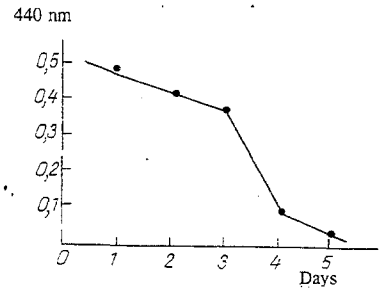

Fig.2

Fig. 1. Immunodiffusion of extracts obtained from dormant cotton seeds (a) and seeds that had grown for 1 (b), 3 (c), 5 (d), and 7 (e) days.

Fig. 2. Amount of 11S globulin in germinating cotton seeds.

of the 11S globulin from dormant cotton seeds that had been treated with protease A and of a preparation of the 11S globulin isolated from cotton seeds in the 1st and 3rd days of germination, similar patterns were obtained. These results may be considered as a proof of the hypothesis that protease A is the initiating enzyme in the germination of cotton seeds. The limited nature of proteolysis was also confirmed by the absence of changes in the electrophoretograms with an increase in the amount of added enzyme and a lengthening of the time of incubation.

Moreover, it can be seen from the results of electrophoresis that after the 5th day of germination, the high-molecular-mass fragments from the cleavage of the 11S globulin had disappeared and low-molecular-mass fragments had appeared. It was shown by ultracentrifugation that the molecular masses of samples of the 11S globulins isolated from 1-, 3-, and 5-day shoots fell from 67 to 63 and 35 kDa, respectively. A correspondence of the molecular masses of the 11S globulin seeds of the cotton plant that had been treated with protease A (67 kDa) and the 11S globulin from 1-day shoots [6] was also established.

The results of the immunochemical and electrophoretic analyses of the products of the cleavage of one of the reserve proteins, the 11S globulin, and also the results of ultracentrifugation permit the conclusion that the protease A under study effects the limited proteolysis of the protein concerned. The results obtained may give an idea of the process of degradation of the globulins under the action of protease A from dormant cotton seeds. It is obvious that this modification intensifies the hydrolysis of the reserve protein which leads, in its turn, to a more effective supply of the germ with nutrient substances.

The study of the substrate specificity of the enzyme isolated showed that it possesses no carboxypeptidase activity in relation to the synthetic peptides Z–Gly–Tyr, Z–Ala–Val, Z–Val–Leu and no amino peptide activity in relation to α-naphthylamidoleucine, which permits us to assign the enzyme to the endopeptidases.

The mobilization of reserve substances is an important process in the germination of seeds. In a study of the proteolysis of one of the main reserve proteins, the 11S globulin, it has been established that the process is completed on the 5th day of germination, and up to the 3rd day proteolysis has a limited nature.

We express our gratitude to colleagues from the Laboratory of Biopolymeric Systems for supplying us with the 11S globulin from dormant cotton seeds.

EXPERIMENT

The precipitation reaction was carried out in 1% agar–agar gel, which was prepared by mixing equal volumes of hot 2% agar and 1.7% NaCl solution [7]. To prevent the growth of microorganisms in the agar we added sodium methiolate (thiomerosal) to give a concentration of 1:10,000. The diluted agar (1%) was poured onto glass in a layer of from 1 to 3 mm. After setting, wells were made in the agar with a punch of definite shape and they were filled with antigen and antiserum. The plates with the filled wells were placed in a humid chamber and were kept at room temperature for 5-7 days with observation for the appearance of precipitation lines every day. Then the plates with the agar were washed for 5-7 days in physiological solution, which was changed 2-3 times per day. The washed gels were stained with a 0.1% solution of Coomassie Blue G1 in 7% acetic acid. The excess of dye was washed out with 7% acetic acid.

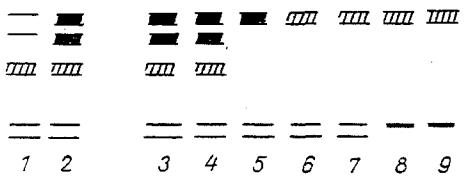

Fig. 3. Electrophoretograms of preparations of the 11S golbulin from dormant seeds (1) treated with protease A (2), and that had germinated for 1 (3), 3 (4), 5 (5), 7 (6), 9 (7), 11 (8), and 13 (9) days.

Electrophoresis was conducted in 7.5% PAAG in Tris-glycine buffer, pH 8.3, in the presence of sodium dodecyl sulfate for 3 h at U = 300 V, I = 50 mA per plate [8]. After separation, the plates were fixed in 10% TCA solution for 20-30 min and were stained with 0.2% Coomassie Bright Blue p-250 for 1 h. The excess of dye was washed out with a mixture of 7% acetic acid and 10% ethanol until the background of the plate had clarified.

The cotton seeds were germinated in moist sand for 15 days.

The action of protease A on the 11S globulin of cotton seeds was studied under the following conditions: 3 mg of the 11S globulin was suspended in 3 ml of phosphate buffer solution, pH 7.4, and protease A was added in a ratio of 1:30. Hydrolysis was conducted at 40°C, pH 7.4, for 18 h. After the end of the reaction, the hydrolysates were freeze-dried.

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