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IMMUNOCHEMICAL CHARACTERIZATION OF A PROTEOLYTIC ENZYME — PROTEASE A FROM COTTON SEEDS

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It has been established by double immunodiffusion in agar that protease A (a proteolytic enzyme from dormant cotton seeds hydrolyzing the native reserve proteins) is present for the first 3-4 days during the germination of the seeds. An immunological affinity between trypsin and protease A has been revealed which indicates the presence of common structural elements in them.

In the germination of plant seeds, degradation of the reserve proteins takes place under the action of proteolytic enzymes. In the initial phase of this complex multistage process a modification of the reserve proteins takes place as a result of which they become accessible to the action of other proteases [1]. Which of the proteinases is the initiator of proteolysis (that which is present in the dormant seed or that which is synthesized anew in the process of growth) it has not yet been possible to determine [2, 3]. It is known that a criterion of the participation of a protease in the breakdown of a reserve protein is not only its capacity for hydrolyzing the latter but also its presence in the germinating seeds [4]. It is unlikely that a protease disappearing during germination can play a fundamental role in the hydrolysis of reserve proteins.

Proteases A, B, and C have been isolated from dormant cotton seeds in the homogeneous state and have been characterized completely. Protease A [5] cleaves native reserve proteins, while proteases B and C [6] act only on modified reserved proteins. To reveal protease A in extracts of cotton seeds in various stages of germination we used the method of immunochemical analysis — double diffusion in gel. The performance of this reaction presupposes the presence of antigens and antibodies. If in two organisms there are similar or identical antigenic determinants, precipitation bands are formed, while when there are no similar determinants the reaction does not take place.

Rabbit antiserum to protease A was obtained. Its specificity was determined by double immunodiffusion in a gel. The results of the immunodiffusion of the extracts obtained from dormant and swollen cotton seeds and those that had germinated for 1-3 days showed that protease A from the dormant seeds and those that had germinated for 3 days possessed antigenic identity, i.e., protease A is present during the first 3 days of germination (Fig. 1), and, as we had determined previously by thin-layer chromatography [5], hydrolyzes the native reserved proteins, modifying them for deeper hydrolysis by the other proteases. The maximum proteolytic activity in the shoots was observed on the 3rd-4th and also on the 8th-9th days of germination in the total fractions. The results of immunochemical analysis

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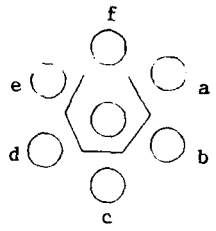

Fig. 1

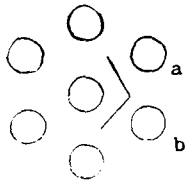

Fig. 2

Fig. 1. Double immunodiffusion of extracts obtained from dormant (a) and swollen (b) cotton seeds and those that had germinated for 1 (c), 2 (d), 3 (e), and 4 (f) days.

Fig. 2. Immunodiffusion between antiserum to protease A and the protease (a) and trypsin (b).

serve as an additional proof of the fact that the protease A detected in dormant seeds is capable of initiating proteolysis and modifying the reserve proteins in the first 3-4 days of germination.

With the aid of the antiserum to protease A we obtained additional information on the specificity of the enzyme. To detect common structural elements of protease A and trypsin we performed double immunodiffusion between trypsin and the antiserum to protease A. The formation of precipitation bands was observed (Fig. 2) when the central well was filled with the antiserum to protease A, which indicated the presence of common structural elements in protease A and trypsin and served as an additional proof of the fact that protease A is a trypsin-like enzyme.

EXPERIMENTAL

Seeds of a cotton plant of the T-1 variety were germinated in a thermostat in moist sand in the dark at 25°C for 15 days.

To obtain the antiserum, a rabbit was injected with 15 mg of the preparation three times in the course of a month with complete Freund's adjuvant. Blood was collected from an ear vein a week after the last immunization and was kept at room temperature for 1 h and then at 4°C for 12 h, during which time retraction of the fibrin clot took place. The clot was removed by centrifugation at 2500 rpm for 10 min, and the serum was collected and freeze-dried.

The specificity of the antiserum with respect to protease A was determined by the method of double immunodiffusion [8]. Ouchterlony double immunodiffusion was performed in 1% agar gel in the presence of Merthiolate [thimerosal] at room temperature. Then the gel was washed for three days with 0.14 M NaCl and for a day in distilled water and was dried. The washed gels were stained with a 0.1% solution of Coomassie Blue GL (Serva) in 7% acetic acid. The excess of dye was washed out with 7% acetic acid.

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