PROTEASES OF THE SEEDS OF THE COTTON PLANT. I.

M. A. Kuchenkova, N. L. Ovchinnikova and P. Kh. Yuldashev

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Proteolytic enzymes have been found previously in dormant seeds of the cotton plant [1, 2]. To isolate them we used a 0.1 M phosphate buffer, pH 7.4, and fractionation with ammonium sulfate, and to determine their activity we used casein and hemoglobin as substrates. Unit activity of the enzyme was taken to be that amount of it which in 1 min at 30° forms proteolysis products incapable of being precipitated by trichloroacetic acid containing one microequivalent of tyrosine [3].

The amount of protein in the solutions investigated was determined by the biuret reaction and by the Warburg–Christiani method [4].

To determine the optimum limit of the concentration of ammonium sulfate at which the proteases are precipitated, after centrifuging (18,000 rpm, 30 min) a solution of the first extract was brought successively to saturations from 20 to 100% in 10% steps. The amount of ammonium sulfate was calculated from a nomogram [5]. The maximum activity was obtained in the range from 0 to 60% saturation and the highest specific activity at an optimum saturation of 0 to 20%. The fraction obtained by precipitation in the range from 0 to 60% saturation was used for studying the action of the proteolytic enzymes of the seeds of the cotton plant on the reserve protein of the seeds and protein from factory extracted meal.

To find the optimum working conditions with the fraction mentioned, we determined the dependence of the proteolytic activity on the pH, time, and temperature of incubation, and also on the amount of enzyme added. The results of a comparison of the activity of the original total extract, determined on various substrates (casein and hemoglobin) showed that the dormant seeds of the cotton plant contain proteases the action of which is shown over a wide pH range with three maxima (at pH 2.8-3, 4.5-5, and 9-9.3), while the active fraction obtained by precipitation with ammonium sulfate at 0-60% saturation has two maxima (at pH 2.8-3 and 4.5-5) and corresponds to 75% of the activity of the original extract.

A preparation of the enzyme is activated by cysteine and by sodium sulfide. The rate of the reaction increases fourfold when the optimum amount of cysteine (0.005 M) is added, and twofold with the addition of sodium sulfide (0.01 M).

In a study of the dependence of the activity on the temperature of incubation, it was found that the protease possesses two temperature maxima: at 50-55°C and at 30-35°C.

The activity of the preparation investigated also changes as a function of the concentration of the enzyme added to the incubation mixture, increasing gradually with an increase in concentration. We consider a concentration of 1-3 mg/ml to be the most suitable. The addition of the salts NaCl and NH_4Cl to the incubation mixture in concentrations of from 0.03 to 1.5 M does not change the activity.

Thus, it can be seen that in the dormant seeds of the cotton plant there is a considerable amount of proteolytic enzymes: 75% of the activity is due to acid proteases.

The conditions for performing enzymatic hydrolysis have been determined.

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