LIPOLYTIC ACTIVITIES OF ENZYMES IN COTTON SEEDS

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Plant seeds are characterized by a high content of one [1, 2] or more [3] enzymes that cleave triglycerides. The present paper gives the results of a study of the lipolytic enzymes (glycerol ester hydrolaze, EC 3.1.1.3) in extracts from an acetone powder of dormant cotton seeds [4].

The lipase activity was measured by titrating the fatty acid liberated on the hydrolysis of tributyrin with 0.01 M KOH at a constant pH. The change in pH was monitored by an LPM-60M pH-meter. The titrimetric method used enables the activities of enzymes to be determined without the use of buffer solutions at any pH. The method was checked by measuring the activity of pancreatic lipase. The medium for the determination of activity contained 60 mg of tributyrin emulsified in 0.1 M tris with 0.1% of Triton X-100, 180 mmole of $CaCl_2$, and 3.0-60 mg of enzyme: the total volume was 30 ml. The protein was measured by the biuret method. The errors in the determination of the lipase activity did not exceed ±2%.

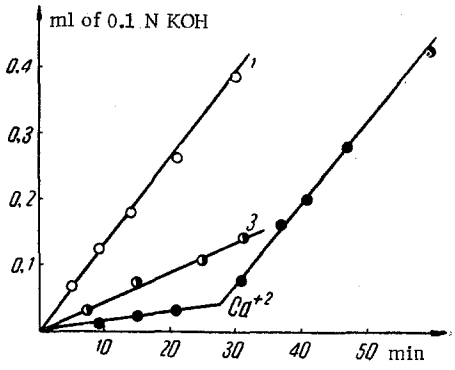

Fig. 1

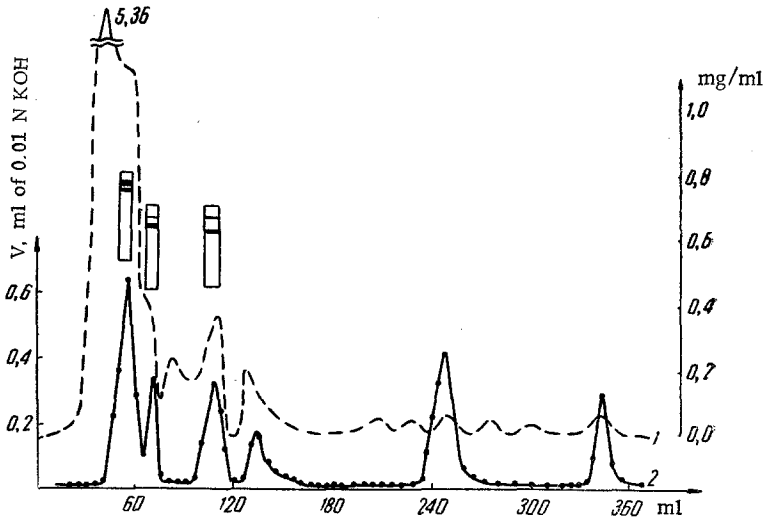

Fig. 2

Fig. 1. Influence of phosphate and Ca^{2+} on the lipase activity at pH 8.75 (tributyrin 2 mg/ml; enzyme 0.33 mg/ml): 1) activity in an aqueous extract; 2) in the presence of 2.32 μmole/ml of phosphate; 3) in the presence of 33.3 μmole/ml of $CaCl_2$. In experiment 2, 33.3 μmole/ml of $CaCl_2$ was added 28 min after the beginning of the reaction.

Fig. 2. Chromatographic separation of the lipases of cotton seeds on Sephadex G-100 (column dimensions 35 × 2.2, amount of protein deposited 100 mg, rate of elution 18 ml/h, fraction volume 4.5 ml). Electrophoresis was carried out on 5% agar gel in 0.1 M tris buffer, pH 8.8 [5]: 1) concentration of protein in the fractions; 2) lipase activity at pH 8.75.

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The extracts were obtained by triturating 10 g of the acetone powder with an equal amount of powdered glass in 10 ml of double-distilled water or buffer. The suspension was centrifuged for 30 min at 18,000 × g at 2°C, and the supernatant was diluted to a protein concentration of 10-30 mg/ml. Homogenization or prolonging the extraction was less effective.

Measurements of the lipase activity in the pH range 2.0-12.0 showed that cotton seeds contain at least two enzymes cleaving tributyrin: the first with the pH optimum at 4.75 and the second at pH 8.75, the amount of the first always being smaller. The yield of the lipase with the optimum pH of 4.75 increased if sucrose or glycerol was present in the extraction medium. Conversely, for the other lipase a good yield was obtained on extraction in water, in 0.1 M phosphate buffer at pH 7.4, and in 10% NaCl solution. It was stable in aqueous solution at 0°C but unstable at higher temperatures. The heat stability increased considerably in phosphate and tris buffers; 85% of the initial activity was retained after 15 min at 50°C. The lipase was inhibited by Ca^{2+} ions and by phosphate, but the inhibiting effect of the phosphate could be prevented by the addition of $CaCl_2$ (Fig. 1).

The preparative purification of the enzyme by fractionation with ammonium sulfate at saturations from 10-55% with subsequent chromatography on Sephadex G-100 with 0.1 M phosphate buffer (pH 7.4) showed the presence of six protein components possessing lipase activity at pH 8.75 (Fig. 2). Some of them were homogeneous (electrophoresis) [5].

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