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A STUDY OF THE STABILIZATION OF PHOSPHOLIPASE D

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To determine the thermal stability of phospholipase D we have studied the kinetics of the inactivation of the enzyme at temperatures of from 40 to 70°C. The phospholipase D was isolated from tubers of the Central Asian radish (garden radish) *Raphanus sativus* by the method of [1], and the activity of the enzyme was determined on choline [2] and protein concentrations by Lowry's method [3]. It can be seen from Fig. 1 that at temperatures up to 55°C a slow smooth fall in the activity of the enzyme took place during incubation. At 60 and 70°C the enzyme was inactivated almost completely in the first few minutes of incubation. The times of half-inactivation were 6 and 4 minutes, respectively. When the enzyme was incubated at 40°C, its activity fell by 25% in 13 minutes and on futher heating the activity remained at the same level.

It is known that phospholipase D from radishes exhibits a high hydrolytic activity in an acid medium at pH 5.6 [4] and a synthetase activity at broader pH ranges [5,6]. The pH of the medium also exerts a considerable influence on the stability of the enzyme in the manifestation of both its hydrolase and its transferase functions. In view of this, we have studied the stability of phospholipase D at various pH values of the medium, and at 55°C using 0.1 M sodium acetate buffer in the pH interval of 4.0-7.0. It can be seen from Fig. 2 that the enzyme exhibited no activity at pH 4. With a rise in the pH of the

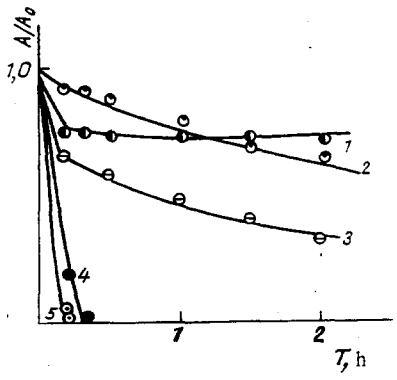

Fig. 1

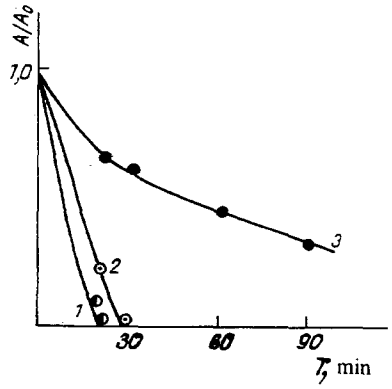

Fig. 2

Fig. 1. Thermal stability of phospholipase D from the roots of the Central Asian radish at various incubation temperatures. Enzyme concentration 1 mg/ml in 0.1 M Na acetate buffer,pH 5.6: 1) 40°C; 2) 50°C; 3) 55°C; 4) 60°C; 5) 70°C.

Fig. 2. Thermal stability of phospholipase D at various pH values of the medium. Enzyme concentration 1 mg/ml, incubation temperature 55°C: 1) pH 4.5; 2) pH 5.0; 3) pH 5.5.

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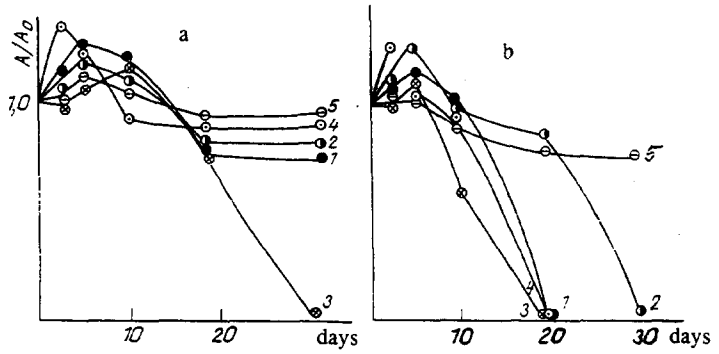

Fig. 3. Influence of various agents on the stability of phospholipase D. Concentration of phospholipase D 0.25 mg/ml in 0.1 M Na acetate buffer, pH 5.6. Initial specific activity 1.2 unit/mg: 1) control preparation; 2) 1.25 mM total phospholipids; 3) 1.25 mM lecithin; 4) 0.5 mM Na-DS; 5) 250 mM glycerol; a) incubation at +4°C; b) incubation at +22°C.

medium the stability of the enzyme became higher. While after incubation at pH 4.5 for 20 min only 15% of its initial activity was retained, at pH 5.0 the corresponding figure was 20%, and at pH 5 after the same time 70% was retained. The time for the half-inactivation of phospholipase D at pH 5.5 and an incubation temperature of 55°C was 45 min. An increase in the pH to 6.0 amd 7.0 did not lead to stabilization of the enzyme. Thus, the enzyme retained its greatest catalytic activity and stability in a medium with pH 5.5.

The influence of various agents on the stability of phospholipase D is shown in Fig. 3. After 30 days at 4°C, a control preparation containing 0.1 M acetate buffer, pH 5.6, had retained 75% of its initial activity, while the addition of 1.25 mM lecithin led to complete inactivation of the enzyme after the same time. The same pattern was observed for total phospholipids at 22 °C. In the control preparation, at 22°C complete inactivation of the enzyme was reached on the 20th day. Complete inactivation was also reached after this time with the use of lecithin and sodium dodecyl sulfate. Incubation of the enzyme at 4°C for 30 days with glycerol, sodium dodecyl sulfate, and egg-yolk phospholipids led to the retention of 100, 91, and 83% of the initial activity, respectively. The retention of the activity of phospholipase D at the same temperature in the presence of the total phospholipids can be explained by a stabilizng effect of the other phospholipd components of the total mixture. In the presence of glycerol at 22°C, 75% of the activity of the enzyme was retained after 30 days.

Thus, the influence of the components of the reaction medium on the stability of radish phospholipase D at various incubation temperatures has been studied. Glycerol was found to exert a stabilizing effect at 4 and 22°C. The results that we have obtained can be used in a study of the synthetase properties of this enzyme.

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