

INFLUENCE OF SOME IONS ON THE ACTIVITY OF COTTONPLANT PHYTASE

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The activity of the phytase from cottonplant shoots has been studied. It has been shown that Ca^{2+} and Mg^{2+} ions in low concentrations weakly stimulate the activity of the phytase, while Fe^{3+} , Zn^{2+} , and Cu^{2+} ions are inhibitors. It is suggested that the Fe^{3+} and Cu^{2+} ions accumulating in the aleurone grains may fulfill the function of phytase inhibitors during the dormancy of the seeds.

Continuing a study of the influence of ions on the phosphohydrolases of the cotton plant, we have investigated the effect of some ions on the activity of the phytase of three-day cottonplant shoots [1, 2]. The results obtained have shown that, at low concentrations (1×10^{-5} - 5×10^{-5} M), Ca^{2+} and Mg^{2+} ions weakly stimulate the activity of the phytase, but with an increase in their concentration a slight inhibition of the phytase activity is observed. Thus, at a concentration of Mg^{2+} and Ca^{2+} in the incubation medium of 5×10^{-3} M, the phytase activity was inhibited by 20 and 12%, respectively. A further increase in the concentration of these ions did not affect the kinetics of phytase inhibition.

A substantially different picture was observed in the case of Fe^{3+} , Zn^{2+} , and Cu^{2+} ions. They are all phytase inhibitors, and with a rise in their concentration in the incubation medium the phytase activity inhibiting effect increases. As can be seen from Fig. 1, a linear relationship exists between the concentration of ions in the incubation medium and the rate of hydrolysis of phytin by the phytase. From the tangents of the angles of slope of the curves in Fig. 1 we calculated the inhibition constants of these ions for phytase activity as 0.025 mM for Fe^{3+} and 0.018 mM for Cu^{2+} ; i.e., iron and copper ions scarcely differ with respect to the inhibition of phytase.

The study of the influence of the concentration of the enzyme preparation on the effect of inhibition by Fe^{3+} , Zn^{2+} , and Cu^{2+} ions showed the existence of a linear relationship between the concentration of iron ions in the incubation medium and the concentration of the phytase enzyme preparation. Thus, an increase in the concentration of enzyme preparation in the incubation medium with no change in the concentration of iron ions led to a decrease in the inhibition of phytase activity.

Analogous results were obtained in an investigation of the influence of copper and zinc ions on the phytase activity at various concentrations of the enzyme preparation in the incubation medium.

The results obtained on varying the concentrations of the phytase enzyme preparation and Fe^{3+} and Cu^{2+} ions permit the conclusion that not only the Fe^{3+} and Cu^{2+} ions but also phytase molecules participate in the inhibition of enzyme activity. Even though their concentration is very low, Fe^{3+} and Cu^{2+} cations accumulating in aleurone grains may fulfill the function of phytase inhibitors during the dormancy of the seeds.

EXPERIMENTAL

We used an enzyme preparation isolated from cotyledons of three-day shoots of a cotton plant of the 108-F variety. An enzyme extract was obtained by triturating the frozen shoot cotyledons with glass powder in a small volume of 0.1 M sodium acetate buffer, pH 5.2, followed by centrifugation at 10,000 g for 30 min with cooling (0-2°C). The deposit was removed, and the supernatant liquid was used for the subsequent investigations.

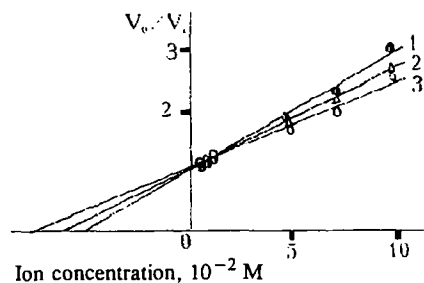


Fig. 1. Effect of some ions on phytase activity: 1) Fe^{3+} ; 2) Cu^{2+} ; 3) Zn^{2+}

Two volumes of cooled acetone were added to the solution, and the mixture was left for 2 h for the complete precipitation of proteins. The resulting precipitate was collected by centrifugation in the cold and was dissolved in a small volume of the initial buffer, and this solution was centrifuged at 10,000 g. The supernatant was dialyzed at 2-4°C for 16-18 h. The precipitate that formed during dialysis was also removed by centrifugation, and the supernatant liquid was used for measurement. This enzyme preparation contained 80-90% of the phytase activity in the cottonplant shoot homogenate.

Phytase activity was determined from the amount of orthophosphate formed in the incubation medium. The incubation mixture contained 0.2 ml of 0.1 M sodium acetate buffer, pH 5.2, 0.1 ml of 1 mM sodium phytate obtained as in [3, 4] and brought to pH 5.2 by the addition of 1 M HCl, and 0.1 ml of enzyme preparation. The mixture was incubated at 37°C and the reaction was stopped by the addition of 0.5 ml of cooled 20% trichloroacetic acid. In a control sample, the same amount of enzyme preparation was added after the trifluoroacetic acid. We used chlorides of the metals concerned as the sources of the ions. Orthophosphate was determined by the method of [5] and protein by that of [6].

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