INFLUENCE OF IONS ON COTTON PYROPHOSPHATASE

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The role of certain ions in the functioning of inorganic pyrophosphatase, one of the key enzymes of the phosphorus metabolism, has been little studied, particularly for the vegetable kingdom, including the cotton plant. The aim of the present work was to study the influence of certain ions on the hydrolytic activity of cotton pyrophosphatase [1].

Influence of Co^{2+} and Mn^{2+}. In an investigation of the action of Co^{2+} and Mn^{2+} on the hydrolysis of PP_i by cotton pyrophosphatase, activation of the enzyme was detected, as in the case of the ions Mg^{2+} [2] and Zn^{2+} [3]. It must be mentioned that the Co^{2+} and Mn^{2+} ions, unlike Mg^{2+} and Zn^{2+}, stimulate inorganic pyrophosphatase at comparatively high concentrations of PP_i, (8 mM) in the incubation medium, the optimum concentrations for $CoCl_2$ and $MnCl_2$ being 0.5 mM. A further rise in the concentration of Co^{2+} and Mn^{2+} in the incubation medium appreciably inhibited the pyrophosphatase reaction. However, inhibition by an excess of Co^{2+} was weaker than by an excess of Mn^{2+} in the medium. This phenomenon may apparently be connected with the complication of the formation of polymeric complexes in the Mn^{2+}–PP_i system, as has been established for yeast pyrophosphatase [4].

Influence of Ca^{2+}, Cu^{2+}, and Sr^{2+}. With respect to the enzyme under investigation, Ca^{2+} ions occupy a neutral position or they inhibit the hydrolytic capacity of cotton pyrophosphatase. Investigations of the action of Cu^{2+} and Sr^{2+} on the activity of the enzyme have shown that these ions are inhibitors of the pyrophosphatase reaction, while the inhibiting capacity of Cu^{2+} is almost an order of magnitude greater than that of Sr^{2+}. The inhibition constant (K_i) of the magnesium-activated reaction of the pyrophosphatase from cotton-plant shoots was determined from the dependence of the rate on the concentration of copper: it proved to be 90 μM.

Influence of F^-. The results of a study of the influence of fluoride ions on pyrophosphatase activity has shown that fluoride ions also inhibit the enzymatic activity, lowering K_i and V. It must be mentioned that this inhibition of cotton pyrophosphatase by F^- belongs to the noncompetitive type of inhibition. Hence, it may be assumed that fluorine is capable of adding only to the enzyme-substrate complex and not to the free pyrophosphatase enzyme, which is more characteristic for the noncompetitive inhibition of enzymatic reactions. As a result of this, the inhibitor strongly stabilizes the intermediate compound of the protein with a PP_i molecule, as has been shown in the case of yeast pyrophosphatase [4]. The value of K_i for F^- ions amounts to 420 μM.

Summarizing what has been said above, it may be observed that, in the first place, Mg^{2+}, Zn^{2+}, Co^{2+}, and Mn^{2+} ions participate in the formation of the catalytically active complex of the substrate and cotton pyrophosphatase, and the maximum rates of the pyrophosphatase reaction are shown with the Mg^{2+} and Zn^{2+} ions. In the second place, from the influence of Cu^{2+} and F^- ions on the enzymatic activity it has been found that they are effective inhibitors that can prevent the activation of cotton pyrophosphatase by bivalent metals.

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