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ROLE OF MAGNESIUM AND PHOSPHATE IONS IN INDUCING COTTON PYROPHOSPHATASE ACTIVITY

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Magnesium ions activate pyrophosphatase from cotton seeds [1]. The Mg-depleted enzyme has no catalytic activity. However, pyrophosphatase is effectively inhibited in the presence of Mg^{2+}. A study of the kinetics of pyrophosphate hydrolysis by pyrophosphatase has shown that this involves an unusual and rarely encountered mechanism in which not only substrate (inhibition by an excess of substrate) but also orthophosphate formed in the reaction inhibit the enzyme. A detailed analysis of the experimental data explained the nature of this inhibition [2].

The effectiveness of metal binding at the active site of pyrophosphatase can also depend on the ion concentrations and their ratio. Furthermore, previously obtained data for the optimum pH of the enzyme changed significantly and were unexplicable [3].

Figure 1a shows the pyrophosphate hydrolysis rate catalyzed by cotton-seed pyrophosphatase as a function of Mg^{2+} concentration over the studied pH range. An interesting feature is that the optimal Mg^{2+} concentration is greater at the optimum pH (pH 7.4) than in the more acidic region (pH 6.4 and 5.8). Metal-ion concentrations in excess of the optimal ones inhibit pyrophsophatase.

It is known that Mg^{2+} is bound rather weakly to the active site of pyrophosphatase. For example, Ca^{2+} can easily displace it. Thus, Ca^{2+} acts as an enzyme inhibitor. The dissociation constant of Mg^{2+} is in the range 10^{-4}-10^{-3} M.

If we use the well-known expression

$$K_{diss} = \frac{[E][Mg^{2+}]}{[E\cdot Mg^{2+}]} = \frac{[E]_0[E\cdot Mg^{2+}]\cdot[Mg^{2+}]}{[E\cdot Mg^{2+}]} = \frac{V_0 - V_{Mg}[Mg^{2+}]}{V_{Mg}},$$

where V_0 is the hydrolysis rate of pyrophosphatase for the optimal concentration at a given pH value, V_{Mg} is the hydrolysis rate at a given $[Mg^{2+}]$, and $[Mg^{2+}] = [Mg^{2+}]_{tot}$ since $[Mg^{2+}]_{tot} >> [E]_0$ in the molar expression, then the following data are obtained: $K_{diss} = 10^{-7}$ (pH 7.4), $K_{diss} = 3\cdot10^{-4}$ (pH 6.2), and $K_{diss} = 5\cdot10^{-4}$ (pH 5.0).

Therefore, the strongest complexes are formed at pH 7.0-7.4. Shifting the pH to more acidic values increases the dissociation constant, i.e., the complexes become less stable. The enzyme activity can be increased or decreased depending on the pyrophosphate:orthophosphate ratio.

Figure 1a contains data obtained without orthophosphate present. However, adding various concentrations of orthophosphate substantially affects the shape of the curves and significantly changes the range of $[Mg^{2+}]$ necessary for optimal activity. Furthermore, inhibition of pyrophosphatase by Mg^{2+} in excess of the optimal amount becomes more noticeable. This can be seen in Fig. 1b, which was produced with 50% orthophosphate.

Thus, the catalytic activity of pyrophosphatase as a function of $[Mg^{2+}]$ can change depending on the composition of the medium in which the enzyme functions.

It is possible that the surface charge of the protein globule plays an important role in these changes. The availability of the enzyme catalytic site to Mg^{2+} changes because orthophosphate carries a large negative charge.

If this is so, then the pH profile of the activity should change with the optimal and additional amounts of Mg^{2+}. The experimental results confirm this.

Based on the results and considering the activation kinetics of pyrophosphatase [2], the functional features of this interesting enzyme can be explained.

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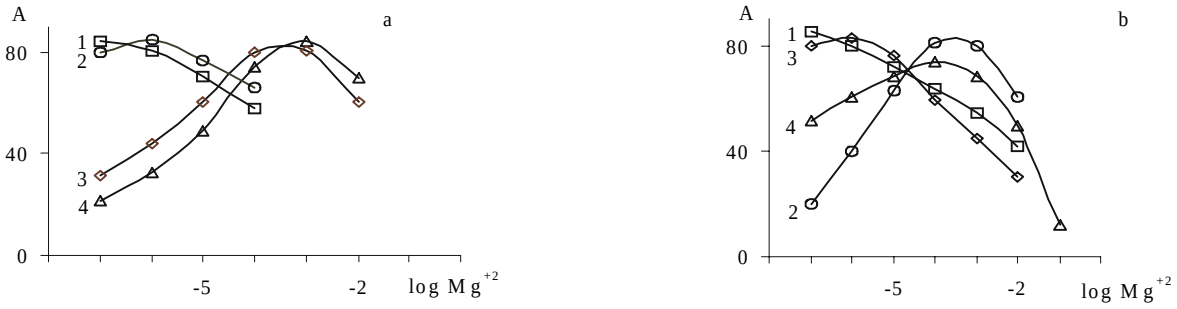

Fig. 1. Effect of Mg^{2+} (a) and presence of 50% orthophosphate (b) on pyrophosphatase activity at various pH values. pH: 7.4 (1), 7.0 (2), 6.2 (3), 5.8 (4).

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