EFFECT OF METAL IONS ON THE ACTIVITY OF EXTRACELLULAR PHYTASE OF *Bacterium* SP.

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The effect of Ca²⁺, Mg²⁺, Cu²⁺, and Fe³⁺ on the activity of *Bacterium* sp. phytase is studied. The ions Ca²⁺ and Mg²⁺ at low concentrations act as activators whereas Cu²⁺ and Fe³⁺ are phytase inhibitors. Inhibition of phytase activity by Cu²⁺ is proposed to be competitive whereas Fe³⁺ noncompetitively inhibits sodium phytate.

Most enzymes that transfer phosphate require divalent metal ions to manifest activity or to be activated. There is usually an optimal concentration, above which inhibition is observed. The magnitude of this optimal concentration depends on the nature of the metal. Phosphatases also usually require cations, although exceptions are known, for example, nonspecific phosphatase.

We previously isolated and purified phytase (myoinositolhexaphosphatephosphohydrolase, KF 3.1.3.8) from the culture medium of *Bacterium* sp. [1]. Studies of certain physicochemical properties showed that it is similar to analogous phytases isolated from other samples [2-11]. We studied the effect of certain ions on the activity of this enzyme because metal ions play an important role in the manifestation of phytase activity.

Figure 1 shows that Ca²⁺ and Mg²⁺ at concentrations of 0.5-1.0 mM act as phytase activators. Phytase activation reaches ~60% for Ca²⁺ and 35% for Mg²⁺. Increasing the concentrations further causes phytase inhibition. This effect is explained in the literature by the precipitation of phytates as an insoluble metal salt [2, 4].

![Graph showing the effect of Ca²⁺ and Mg²⁺ ions on activity of *Bacterium* sp. phytase.](image)

**Fig. 1.** Effect of Ca²⁺ and Mg²⁺ ions on activity of *Bacterium* sp. phytase. Conditions: salts were dissolved at the appropriate concentrations (0.1, 0.5, 1.0, 1.5, 2.0) in acetate buffer at pH 5.4; incubation temperature 50°C; incubation time 30 min; substrate Na phytate 10 g/L.


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An excess of substrate and hydrolysis products inhibit certain phytases, including that studied by us. In particular, phytate concentrations above 2 mM inhibit phytase preparations from bean [3] and the fungus Asp. ficuum [4]. Only substrate levels above 0.3 M inhibit phytase from Aerobacter aerogenes [5]. Orthophosphate inhibits phytase from wheat and soy [6, 7]. The inhibiting concentrations, \( K_i \), are 0.3 and 0.018 mM, respectively. Our experiments on the effect of orthophosphate ions formed through hydrolysis on bacterial phytase activity found that increasing the phosphate concentration to 2 mM inhibits the enzyme. The phytase activity falls to 83% of the initial value (Fig. 2).

According to the literature, Ca\(^{2+}\) activates many phytases at certain concentrations [2-7]. Activation of this phytase, similar to Bacillus subtilis phytase [7], is seen at a CaCl\(_2\) concentration of 0.5 mM. Magnesium ions activate mung-bean phytase [2] but inhibit phytase from Bacillus subtilis, Pseudomonas sp., and Lilium longiflorium [8-10]. At low concentrations (10-50 \(\mu\)M) Ca\(^{2+}\) and Mg\(^{2+}\) weakly activate phytase from three-day cotton seedlings [11]. With increasing concentration of these cations in the incubation medium, slight inhibition is observed. Effective inhibitors of phytase from cotton seedlings are Fe\(^{3+}\), Zn\(^{2+}\), and Cu\(^{2+}\).

According to our studies (Fig. 2), Cu\(^{2+}\) effectively inhibits extracellular phytase from Bacterium sp. Thus, phytase activity is 8% of the initial value in the presence of 2 mM Cu\(^{2+}\); 29%, with Fe\(^{3+}\) at the same concentration.

Figure 3 shows \(1/V\) as a function of the Cu\(^{2+}\) and Fe\(^{3+}\) concentration in the incubation medium. The inverse rate of phytin hydrolysis by phytase depends linearly on the concentration of inhibitor ions in the incubation medium. On the basis of these diagrams we calculated the constants of phytase inhibition by these ions from the slopes of tangent angle: \( K_i = 0.040 \) (Fe) and 0.093 (Cu).

We also studied the effect of substrate concentration on inhibition by these ions. Figure 4a indicates that the substrate concentration (sodium phytate) depends linearly on the Cu\(^{2+}\) concentration in the incubation medium. Increasing the substrate concentration at constant Cu\(^{2+}\) concentration decreases phytase inhibition.

It should be noted that we obtained identical results from studies of inhibition by Fe\(^{3+}\) of phytase with varying substrate concentrations in the incubation medium (Fig. 4b).

Figure 4 shows that Cu\(^{2+}\) competes with substrate to inhibit phytase whereas Fe\(^{3+}\) acts noncompetitively. Therefore, it can be proposed that the observed competition appears owing to substitution by inhibiting ions of Ca\(^{2+}\), which enhances the interaction between the substrate and enzyme. On the other hand, it is highly likely that an excess of phytin binds Cu\(^{2+}\) and Fe\(^{3+}\), reducing their effective concentrations for inhibiting phytase.