

Communication

Modulatory effect of divalent metal cations on the phosphotyrosine activity of the frog liver acid phosphatase[⊙]

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Frog liver acid phosphatase hydrolyzes phosphotyrosine at acidic pH optimum. Mn^{2+} , Ca^{2+} and Mg^{2+} (but not Zn^{2+}) ions modulate this activity by shifting its pH optimum to physiological pH. This effect is not observed when *p*-nitrophenylphosphate is used as a substrate. Phosphoserine and phosphothreonine are not hydrolyzed under the same conditions.

Acid phosphatases (AcPases; EC 3.1.3.2) comprise a group of diverse enzymes considered as non-specific phosphomonohydrolases of acidic pH optimum. Although they hydrolyze a variety of substrates *in vitro*, they also show some substrate preferences. As the physiological role of the majority of acid phosphatases is still unknown, continuous attempts to find their natural substrates are being made. This resulted in establishing that the AcPases of molecular weight near 20000 function as protein tyrosine phosphatases

(PTPases, EC 3.1.3.48). In addition, there are growing data indicating that the tartrate-resistant purple acid phosphatases (PAPs) may display function of protein phosphatases [1-4] or that Zn^{2+} -dependent acid phosphatases may hydrolyze inositol-1-phosphate [5, 6].

The frog liver lower molecular weight acid phosphatase (LMW AcPase, M_r 35000) is a monomeric, heterogeneous metalloenzyme. Similarly to PAPs, it is not inhibited by L(+)-tartrate and its activity depends on divalent transition metal cation, but it essentially

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Abbreviations: AcPase, acid phosphatase; LMW AcPase, acid phosphatase of lower relative molecular weight; PAP, purple acid phosphatase; pNPP, *p*-nitrophenylphosphate; P-Ser, phosphoserine; P-Thr, phosphothreonine; PTPase, protein tyrosine phosphatase; P-Tyr, phosphotyrosine.

differs from these enzymes considering the kind of ion(s) involved in forming its active site [7]. The LMW AcPase hydrolyzes many synthetic and natural substrates, preferably phosphoric esters of aryl residues [8]. It is active also towards phosphotyrosine [9], but to date no natural substrate has been found to be hydrolyzed more efficiently than pNPP.

In this study we investigated the phosphotyrosine phosphatase function of the frog liver LMW AcPase and the effect exerted by some metal cations on this activity.

MATERIALS AND METHODS

Enzyme purification. The frog (*Rana esculenta*) livers were removed immediately after the death of animal, rinsed with isotonic solution (0.65% NaCl) and frozen at -20°C . LMW AcPase was isolated by extraction with 0.1 M Na-acetate buffer, pH 5.0, containing 0.1 mM phenylmethylsulfonyl fluoride and 1 mM dithiothreitol, ammonium sulfate fractionation, Con A-affinity chromatography and Bio-Gel P-200 filtration as described elsewhere [9, 10]. The enzyme sample used for further experiments was purified 300-fold as compared with the activity of the initial extract. This enzyme preparation had a specific activity of 20 U/mg protein when estimated with pNPP in 0.1 M Na-acetate buffer, pH 5.0.

Enzyme assays. Phosphatase activity with *p*-nitrophenylphosphate (pNPP) as a substrate was measured by following the release of *p*-nitrophenol from 3.5 mM substrate solutions in the appropriate buffers as described earlier [9].

Phosphatase activity towards phosphotyrosine, phosphoserine and phosphothreonine was determined by measuring the amount of inorganic phosphate released from 4.5 mM solutions of the above substrates. Quantitative colorimetric determination of phosphomolybdic acid was performed as described by Olczak *et al.* [11] according to the method of Saini & Van Etten [12] with the omission of

bovine serum albumin in the reaction mixtures.

In both methods one unit of enzyme activity corresponds to the hydrolysis of 1 μmol of substrate per min under the assay conditions.

pH Dependence of substrate hydrolysis was estimated in 4.5 mM substrate solutions in 50 mM sodium diethylbarbiturate/sodium acetate buffers at a range of pH 3.0–9.0 containing 0.1 M NaCl. The effect of metal cations on the reaction was examined in the same conditions with the addition of 5 mM Ca^{2+} , Mg^{2+} , Mn^{2+} and Zn^{2+} ions (in chloride forms). Metal ions at the same final concentration were also added to the control samples prepared separately for each tested pH.

Estimation of K_m constants. The K_m constants for pNPP and P-Tyr were determined on the basis of the initial reaction velocity measured at the substrate concentration range 0.3–10 mM in 0.1 M Na-acetate buffer, pH 5.0, at 37°C . K_m were estimated by the Michaelis-Menten formula using Slide Write Plus 2.0 computer program (Advanced Graphics Software Inc., U.S.A.).

RESULTS AND DISCUSSION

Optimum pH of P-Tyr hydrolysis by the LMW AcPase

Figure 1 illustrates the effect of pH on the rate of P-Tyr and pNPP hydrolysis, referred to the maximal activity displayed towards pNPP at pH 4.0, taken as 100. Both substrates show similar pH dependence, being hydrolyzed most efficiently at acidic pH (pNPP at pH 4.0, and P-Tyr at 5.0). However, the LMW activity towards P-Tyr is much lower than towards pNPP (about 35–40% at pH optimum). None of the two substrates is hydrolyzed by the LMW AcPase at physiological pH.

Hydrolysis of both pNPP and P-Tyr follow the Michaelis-Menten kinetics. The K_m constants estimated at pH 5.0 in 0.1 M Na-acetate

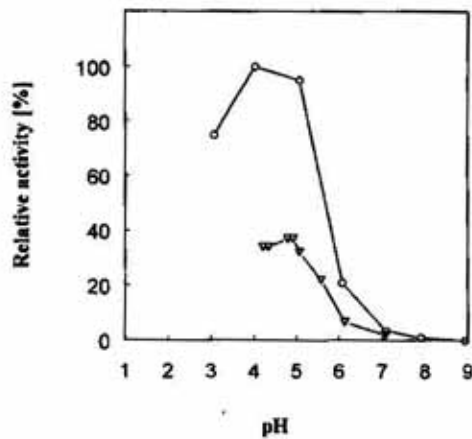


Figure 1. pH optimum of the lower molecular weight acid phosphatase towards phosphotyrosine (▽) and *p*-nitrophenylphosphate (○).

The activity of the enzyme was measured in the substrate solutions in 50 mM sodium diethylbarbiturate/sodium acetate buffers. Relative activity expresses the percentage of the maximal activity towards *p*-nitrophenylphosphate at pH 4.0 taken as 100.

buffer are: $1.38 \pm 0.15 \times 10^{-4}$ M for P-Tyr and $1.39 \pm 0.07 \times 10^{-4}$ M for pNPP. Thus, the comparable values of K_m constants indicate a similar apparent affinity of both substrates for the LMW AcPase under these conditions.

Effect of metal cations on P-Tyr hydrolysis

Except for Zn^{2+} , the addition of Mg^{2+} , Ca^{2+} and Mn^{2+} ions to the reaction mixture results in broadening the pH range of P-Tyr hydrolysis and in shifting its pH optimum to more physiological pH (Fig. 2). The most significant increase of enzyme activity is exerted by Mn^{2+} ions, which shifted the optimum pH to 7.4. At this pH the enzyme hydrolyzes P-Tyr more efficiently than pNPP at any pH (about 125% compared to the maximal enzyme activity towards pNPP at pH 4.0). Although the specific activity towards P-Tyr is still not very

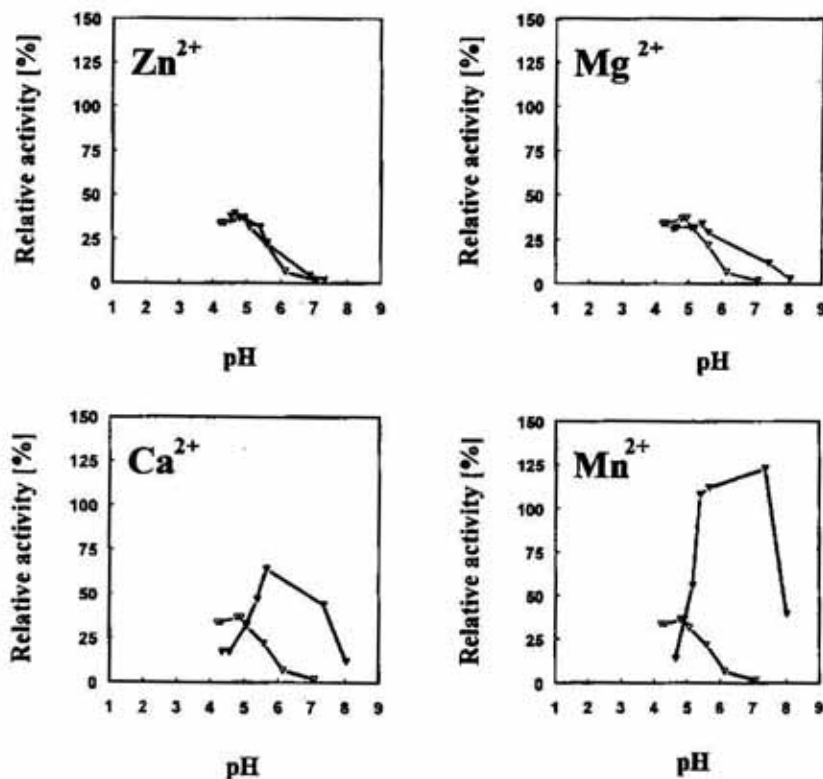


Figure 2. Hydrolysis of phosphotyrosine in the absence (▼) and in the presence (▽) of 5 mM metal cations as the function of pH.

The reaction was carried out in 4.5 mM substrate solutions in 50 mM sodium diethylbarbiturate/sodium acetate buffers. Relative activity expresses the percentage of the activity towards pNPP at pH 4.0 taken as 100. Details under Materials and Methods.

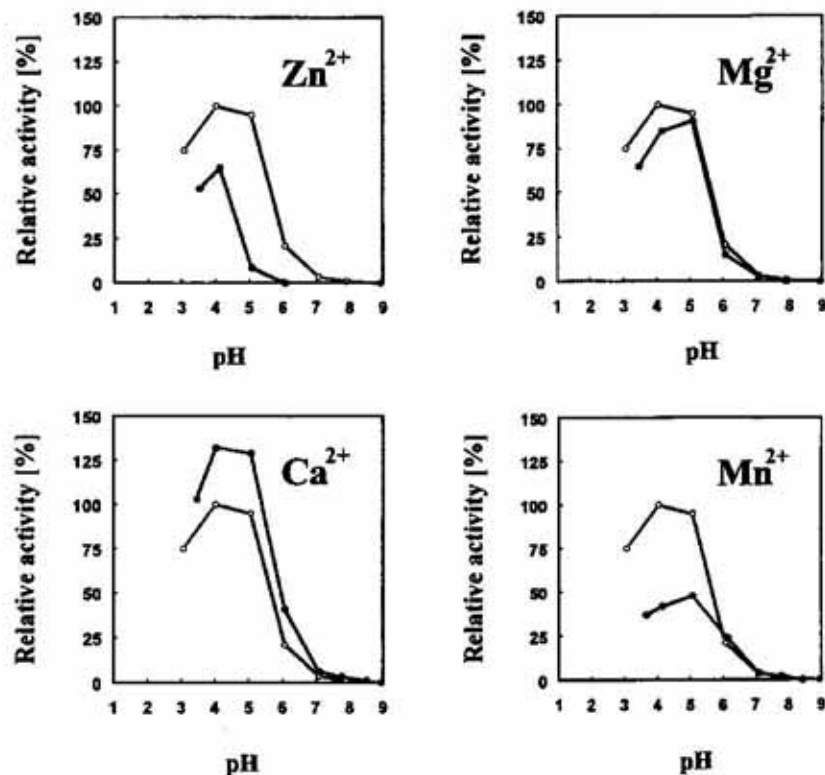


Figure 3. Hydrolysis of *p*-nitrophenylphosphate in the absence (○) and in the presence (●) of 5 mM metal cations as the function of pH.

The reaction was carried out in 3.5 mM substrate solutions in 50 mM sodium diethylbarbiturate/sodium acetate buffers. Relative activity expresses the percentage of the activity towards pNPP at pH 4.0 taken as 100. Details under Materials and Methods.

high (about 25 U/mg), it is for the first time when the natural substrate is processed better than the synthetic one.

The effect of metal cations was also tested in identical conditions with pNPP (Fig. 3). In this case, however, no shift of optimum pH was observed. The activity of acid phosphatase was only slightly higher in the presence of Ca²⁺ ions, it was almost unchanged by Mg²⁺ and diminished by Mn²⁺ and Zn²⁺ ions. This suggested that the effect observed for P-Tyr might be specific for this substrate.

Other phosphorylated amino acids, namely phosphoserine and phosphothreonine, are not hydrolyzed by LMW AcPase in the whole range of pH, either in the absence or presence of the tested metal ions.

Our results suggest that the frog liver LMW AcPase supplemented with metal ions can

function at physiological conditions as a protein phosphotyrosine phosphatase. This requires further investigation.

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