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Effect of Zn^{2+} and Mg^{2+} on Alkaline Phosphatase from Human Placenta and Intestine[1]

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Alkaline phosphatases (orthophosphoric monoester phosphohydrolase, E.C. 3.1.3.1) from human placenta and intestine are activated by Mg^{2+}, but inhibited by Zn^{2+}. In these respects, the enzymes are different from the human biliary, bone, liver and kidney enzymes, which are much activated by Mg^{2+} and Zn^{2+}. At pH 10.5, the K_m of enzymes and enzymes inhibited by inorganic phosphate are not modified by Mg^{2+}. These results suggest that the activation by Mg^{2+} proceeds through a binding of Mg^{2+} with other part of the active site of alkaline phosphatase. It was also found that the activity of alkaline phosphatases was remarkably influenced by binding metals, and Zn^{2+} and Mg^{2+} contents in both enzymes were 4 g-atoms/mole and 3-5 g-atoms/mole, respectively.

Keywords——alkaline phosphatase; placenta; intestine; Mg^{2+}; Zn^{2+}; metal content

Alkaline phosphatase (E.C. 3.1.3.1) is a metal-containing enzyme. It requires metal ions both for preservation of its structure and for its enzyme activity.

Escherichia coli alkaline phosphatase contains 4 zinc atom/mole, two of which are very firmly bound and probably have structure-preserving properties, whereas the others are of importance for the catalytic process.[3] Although Zn^{2+} and Mg^{2+} play an important role in alkaline phosphatase activity, there is little conclusive information about the mechanism of the action of these ions on the enzyme.

Certain physiological and pathological states involving alkaline phosphatase production may be evaluated by the determination of its activity in the serum.[4] Clinically, the quantitative determination of serum alkaline phosphatase has yielded an aid to a diagnosis.[5] Since the amount of enzyme is determined by means of its activity in daily clinical test, it is required that the determination of alkaline phosphatase in the serum must be carried out in a constant condition under which alkaline phosphatase shows full activity.

This paper describes the examination of the effect of Zn^{2+} and Mg^{2+} on alkaline phosphatase activity by using human placental and intestinal alkaline phosphatases purified as previously described.[6]

Materials and Methods

Purification of Enzymes——Purification of human placental and intestinal alkaline phosphatase was performed as previously described.[6]

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3) J.A. Reynold, and M.J. Schlesinger, *Biochemistry*, **8**, 4278 (1969); C. Lazdunski, and M. Lazdunski, *Eur. J. Biochem.*, **7**, 294 (1969).
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Enzyme Assay——*p*-Nitrophenyl phosphate was used as a substrate. One ml of 10 mM substrate solution and 3 ml of 0.1M glycine–KCl–KOH buffer (pH 10.5) were preincubated at 37°. One ml of the enzyme solution was added and the enzyme reaction was carried out at 37° for 30 min. The reaction was stopped by addition of 2 ml of 0.1 N NaOH and the absorbancy was determined at 430 nm. Effect of Zn^{2+}, Mg^{2+}, and PO_4^{3-} on alkaline phosphatase activity was also determined in the same manner.

Metal Analysis——Sample I was first dialyzed for 24 hr against 10 mM Tris-HCl buffer (pH 7.4) containing 1 mM $ZnCl_2$. The excess Zn^{2+} was removed by dialysis against distilled water for 72 hr. Sample II was first treated in the same manner as sample I, and further dialyzed for 24 hr against 10 mM Tris-HCl buffer (pH 7.4) containing 1 mM $MgCl_2$. The excess Mg^{2+} was removed by dialysis against distilled water. These enzyme samples were lyophilized, weighed, and dissolved in 10 ml of 1 N HCl. Zn^{2+} and Mg^{2+} were quantitatively determined with a Hitachi atomic absorption spectrometer Model 303.

Results

Effect of Zn^{2+} and Mg^{2+} on Activity of Alkaline Phosphatase

Effect of Zn^{2+} and Mg^{2+} on alkaline phosphatase activity was examined in the standard assay system containing various concentrations of Zn^{2+} and Mg^{2+}. As shown in Fig. 1, the activity of human intestinal alkaline phosphatase varied in proportion to the concentration of Mg^{2+}, but that of human placental alkaline phosphatase was not considerably affected by Mg^{2+}. Both alkaline phosphatases were inhibited by Zn^{2+}.

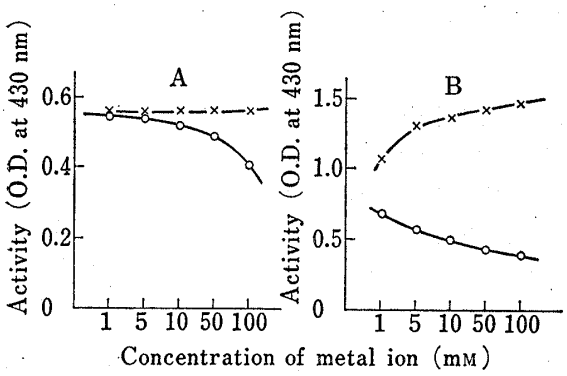

Fig. 1. Effect of Zn^{2+} and Mg^{2+} on the Activity of Alkaline Phosphatase

A: human placental alkaline phosphatase
B: human intestinal alkaline phosphatase
—○—: Zn^{2+}, —×—: Mg^{2+}

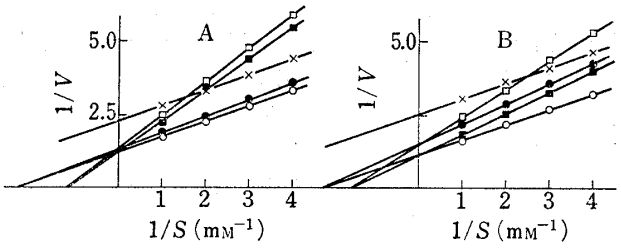

Fig. 2. Lineweaver-burk Plot of Alkaline Phosphatase Activity in the Presence of Zn^{2+} or Mg^{2+}

A: human placental alkaline phosphatase
B: human intestinal alkaline phosphatase
—●—: native, —×—: Zn^{2+} 1 mM, —○—: Mg^{2+} 1 mM,
—□—: PO_4^{3-} 1 mM, —■—: PO_4^{3-} 1 mM+Mg^{2+} 1 mM

Linewaver-burk Plot of Alkaline Phosphatase in the Presence of Zn^{2+} or Mg^{2+}

Effects of Zn^{2+}, Mg^{2+}, and PO_4^{3-} on the activity of alkaline phosphatase were examined. As shown in Fig. 2, Mg^{2+} had no effect on the K_m values of native enzyme and enzyme inhibited by PO_4^{3-}, and V_{max} increased by addition of Mg^{2+}, while Zn^{2+} had an effect on K_m and V_{max} of alkaline phosphatase.

Effects of Zn^{2+} and Mg^{2+} on the Optimum pH of Alkaline Phosphatase

Effects of Zn^{2+} and Mg^{2+} on the optimum pH were investigated in the standard assay system in the presence of 0.1 mM of Zn^{2+} and Mg^{2+}, and it was found that the activity of alkaline phosphatase was affected by Zn^{2+} and Mg^{2+}, but its optimum pH was not shifted by metal ions, as shown in Fig. 3.

Zn^{2+} and Mg^{2+} Binding Site of the Enzyme

Binding site of metal ion on the enzyme was examined by the use of Lineweaver-burk plot in the presence of various concentrations of Mg^{2+} against Zn^{2+}. The results are shown in Fig. 4. Lineweaver-burk plots show competitive type inhibition for both human placental and intesti-

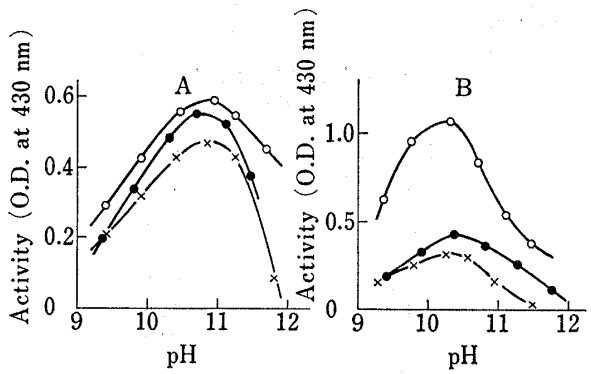

Fig. 3. Effects of Zn^{2+} and Mg^{2+} on the Optimum pH of Alkaline Phosphatase

A: human placental alkaline phosphatase
B: human intestinal alkaline phosphatase
—●—: none, —×—: Zn^{2+} 10 mM, —○—: Mg^{2+} 10 mM

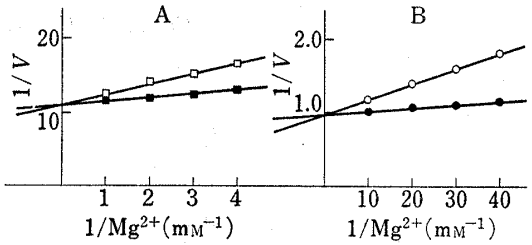

Fig. 4. Lineweaver-burk Plot of Human Alkaline Phosphatase

A: human placental alkaline phosphatase
B: human intestinal alkaline phosphatase
—■—, —●—: native, —□—, —○—: Zn^{2+} 1 mM

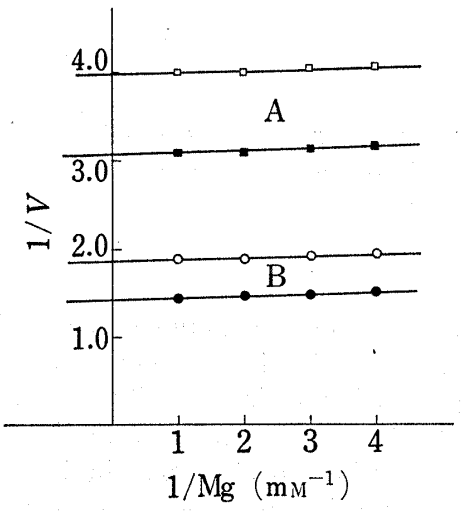

Fig. 5. Effects of Mg^{2+} and PO_4^{3-} on Alkaline Phosphatase

A: human placental alkaline phosphatase
B: human intestinal alkaline phosphatase
—■—, —●—: nafive, —□—: PO_3^{3-} 0.5 mM, —○—: PO_4^{3-} 5 mM

nal alkaline phosphatases, and it seems that Zn^{2+} and Mg^{2+} compete in the same site of alkaline phosphatase, and have an effect on alkaline phosphatase activity.

It is well known that inorganic phosphate competes with phosphomonoester as substrate at the active site of alkaline phosphatase and then the active site of alkaline phosphatase is phospholyrated by inorganic phosphate.[7] As shown in Fig. 5, the inhibition of alkaline phosphatase by inorganic phosphate was uncompetitive. These results suggest that the binding site of Mg^{2+} on the enzyme is different to its active site, *i.e.* catalytic site, of them.

Activation of Alkaline Phosphatase by the Ratio of Zn^{2+} and Mg^{2+}

Effect of the ratio of Zn^{2+} and Mg^{2+} on alkaline phosphatase activity was examined in the standard assay system. As shown in Table I, human intestinal alkaline phosphatase was more activated at the concentration of 1 mM Mg^{2+} and 0.01 mM Zn^{2+}, but human placental alkaline phosphatase was hardly affected by these concentrations. From these results, it was found that there was some suitable conditions of the ratio of Zn^{2+} and Mg^{2+} to give maximum alkaline phosphatase activity.

Zn^{2+} and Mg^{2+} Contents Alkaline Phosphatases

Metal contents of alkaline phosphatase from the human placenta and the intestine were determined by atomic absorption spectrometer, as shown in Table II, and it was found that both alkaline phosphatases contained 4 g-atoms of Zn/mole of enzyme. Both enzymes also contained 3 g-atoms of Mg/mole of enzyme and after dialysis in the presence of Mg^{2+}, they contained maximum 5 g-atoms of Mg/mole of enzyme. These results exhibited that 4 g-atoms of Zn/mole of enzyme and 3 g-atoms of Mg/mole of enzyme were essential for alkaline phosphatase and additional 2 g-atoms of Mg/mole of enzyme affected their activity. However, the

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Table I. Effect of Zn^{2+} and Mg^{2+} Ratio on Alkaline Phosphatase Activity

No.	Concentration (mM)		Alkaline Phosphatase activity (%)	
	Zn^{2+}	Mg^{2+}	Placenta	Intestine
1	0	0	100	100
2	0	1	103	258
3	0.01	0.5	129	318
4	0.05	0.1	136	301
5	0.1	0.05	133	170
6	0.5	0.01	112	118
7	1	0	91	68

Table II. Zn^{2+} and Mg^{2+} Contents of Alkaline Phosphatases

No.	Placental alkaline phosphatase (gatom/mole)		Intestinal alkaline phosphatase (gatom/mole)	
	Zn^{2+}	Mg^{2+}	Zn^{2+}	Mg^{2+}
I	4	3	4	3
II	4	5	4	5

gatom/mole is expressed as the nearest integer.

competition of Zn^{2+} and Mg^{2+} on alkaline phosphatase was not observed. It seems that this difference from the result of competition between Zn^{2+} and Mg^{2+} on the enzyme may be due to the additional Zn^{2+} which is easily removed in the course of dialysis.

Discussion

Human intestinal alkaline phosphatase was more activated by Mg^{2+} than human placental alkaline phosphatase, and both enzymes were inhibited by Zn^{2+}. In these reports, human placental and intestinal alkaline phosphatases were differentiated from human biliary, liver and kidney alkaline phosphatases.[8] The K_m values of both enzymes and enzymes inhibited by PO_4^{3-} were not modified by Mg^{2+}. It was found that Mg^{2+} had no effect on the binding of substrate *i.e.* phosphomonoester on the active site of enzyme, and it was suggested that the activation by Mg^{2+} proceeds through a binding Mg^{2+} with other part of the active site of alkaline phosphatase.

The optimum pH for the activation and inhibition of alkaline phosphatase by Mg^{2+} and Zn^{2+}, respectively, was not altered and exhibited the same optimum pH in the absence of metal ion.

From Lineweaver-burk plots of Zn^{2+} and Mg^{2+} against human placental and intestinal alkaline phosphatase, it was suggested that the binding sites of Zn^{2+} and Mg^{2+} on the enzyme were the same and also Mg^{2+} competed with Zn^{2+} on the enzyme. However, this fact was not observed in the results of metal analysis. Native enzymes contained 4 g-atoms of Zn/mole of enzyme like calf intestinal[9] and Escherichia coli alkaline phosphatase,[3] and 3—5 g-atom of Mg/mole of enzyme. It is well known that Mg^{2+} is required for the maximum activity of alkaline phosphatase. However, in this respect, it became evident that the maximum activity for organ specific alkaline phosphatase was obtained at a constant ratio of Mg^{2+} and Zn^{2+}, and clinically, the determination of alkaline phosphatase activity should be performed under an optimal condition.

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