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Role of metal cations in the regulation of NADP-linked isocitrate dehydrogenase from porcine heart

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The regulatory role of divalent metal cations in the NADP-linked isocitrate dehydrogenase (EC 1.1.1.42) from porcine heart was analysed. Saturation curves with respect to the substrate *threo*-Ds-isocitrate complexed with the metals including manganous, cadmium, cobaltous and zinc ions showed sigmoid relationships characteristic of allosteric enzymes. The Hill's interaction coefficients were 1.90, 1.75, 1.28 and 1.12, respectively. Saturation kinetics of the substrate–metal complexes including magnesium, ferrous and nickel ions exhibited normal hyperbolic curves with Hill's coefficients of 1. The ionic radii of metal cations were closely correlated with the maximal velocity, the enzyme affinity and the Hill's n_H values for the substrate–metal complexes. Cooperative interactions of metal–substrate complexes with NADP–isocitrate dehydrogenase are discussed in relation to the sites of the enzyme for the binding of the metal–substrate complex.

Keywords: cooperativity, ionic radii, metal–isocitrate, NADP–isocitrate dehydrogenase

Introduction

NADP-linked isocitrate dehydrogenase (EC 1.1.1.42) catalyses oxidative decarboxylation of isocitrate to 2-oxoglutarate. The two isozymes of this enzyme, localized in mitochondria and cytosol, are under different genetic control (Huh *et al.* 1993, Jennings *et al.* 1994). In heart, the majority of the enzyme activity is located in mitochondria (Plaut *et al.* 1983), and the enzyme has been implicated to be responsible for the control of the tricarboxylic acid cycle (Plaut 1970). On the other hand, in ovary, mammary gland and liver (Plaut *et al.* 1983), most activity of the NADP–isocitrate dehydrogenase is located in cytosol, and the cytosolic enzyme is thought to participate in the supply of NADPH for biosynthetic reactions (Zirulnik & Giménez 1995). A metal requirement has long been established for the reaction catalysed by NADP–isocitrate dehydrogenase from porcine heart. This requirement may be satisfied by several divalent metals, including manganous, magnesium, zinc, cobaltous or cadmium ions (Colman 1972). Here we show that the porcine heart NADP–isocitrate dehydrogenase is regulated by metal cations in an allosteric manner: saturation kinetics with respect to Mn^{2+}–, Cd^{2+}–, Co^{2+}– and Zn^{2+}–isocitrate complexes showed sigmoid relationships. Allosteric properties closely related to the ionic radii of metal cations are discussed in relation to the interaction of the enzyme with metal–isocitrate complexes.

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Materials and methods

Threo-Ds-isocitrate was a product of Sigma (Tokyo, Japan); NADP and purified pig heart mitochondrial NADP–isocitrate dehydrogenase (specific activity 4 μmol min^{-1} mg $protein^{-1}$) were purchased from Boehringer-Mannheim (Tokyo, Japan). 4-Morpholinopropanesulfonic acid (Mops) was obtained from Dojindo Co., Kumamoto, Japan. The enzyme preparation is essentially homogeneous as judged by SDS–polyacrylamide gel electrophoresis. The activity of NADP–isocitrate dehydrogenase was measured by following the change in

absorbance at 340 nm. The assay medium of 1.0 ml contained 100 mM Mops–KOH buffer (pH 7.1), 0.5 mM $MgCl_2$ and *threo*-Ds-isocitrate in the presence of various metals as chloride salts. The reaction was initiated by addition of the enzyme.

The concentrations of metal–isocitrate complexes were calculated from the known total concentrations of metals and tribasic *threo*-Ds-isocitrate using the following stability constants (log stability constant): Mg, 3.6; Mn, 3.7; Fe, 4.4; Zn, 5.0; Co, 5.0; Cd, 5.0; Ni, 5.4.

Results

Kinetics with respect to the concentration of metal–isocitrate as substrate varied with the species of metal. When velocities were determined in the presence of Mg^{2+}, Fe^{2+} or Ni^{2+}, saturation curves with respect to the metal–isocitrate complexes gave hyperbolic relationships (Figure 1). The maximum velocities and the $S_{0.5}$ values, the concentration of metal–isocitrate required for half-maximal velocity, were largely dependent on the metal added.

When Mn^{2+} or Cd^{2+} were used, kinetics with respect to the metal–isocitrate complex gave sigmoid saturation curves characteristic of allosteric properties (Figure 2). Hill's interaction coefficients indicating the cooperative binding of the substrate were calculated as 1.90 and 1.75 for the substrates Mn– and Cd–isocitrate, respectively. Theoretical saturation curves were computed in the presence of these metals using V_{max}, apparent K_m, and n_H values according to the equation shown below, and the best-fit curves were obtained (Figure 2).

$$v = \frac{V_{max}\,[S]^n}{[S]^n + K_m{}^n}$$

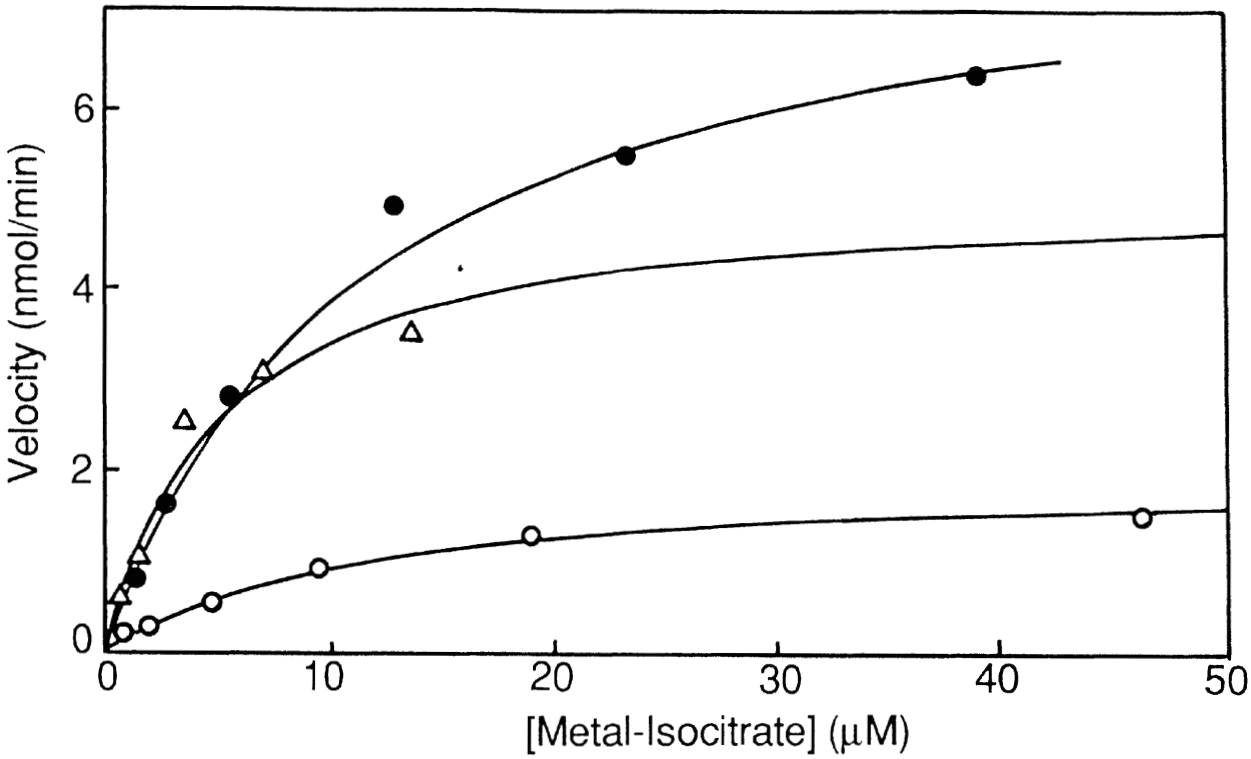

Figure 1. Rate of the reaction catalysed by NADP-linked isocitrate dehydrogenase as a function of the substrate Mg–, Fe– and Ni–isocitrate concentration. The reaction mixture of 1 ml contained 50 mM Mops–KOH buffer (pH 7.1), 0.1 mM NADP, various concentrations of $MgCl_2$, $FeCl_2$ or $NiCl_2$, *threo*-Ds-isocitrate and the enzyme. The reaction was initiated by addition of the enzyme, and was carried out at 37°C. Points are experimental data, and lines are theoretically drawn by using the text equation, with the obtained values for the following kinetic parameters essentially based on non-linear regression analysis (Duggleby 1981). ●, Mg: K_m = 12 μM, V_{max} = 8.34 nmol min^{-1} and n_H = 0.98. △, Fe: K_m = 5.5 μM, V_{max} = 5.11 nmol min^{-1} and n_H = 0.98. ○, Ni: K_m = 13 μM, V_{max} = 1.58 and n_H = 1.0.

In this equation, [*S*] is the concentration of metal–isocitrate, and K_m the concentration required for half-maximal velocity. The Hill coefficient, *n* value, was estimated from the maximum slope in the Hill diagram.

Kinetics with respect to the concentration of Zn– and Co–isocitrate as substrates gave weak sigmoid relationships (Figure 3). Hill interaction coefficients were calculated to be 1.12 and 1.28 for the Zn– and Co–isocitrate complexes, respectively.

Relationships between the kinetic parameters including n_H, V_{max} and $S_{0.5}$ values, and ionic radii of the metals are demonstrated in Figure 4. The values of the Hill coefficients and the maximal velocities increased with the increase in ionic radii of the metals. The only exception was the Ni^{2+} ion, which showed a remarkably low V_{max} value. On the other hand, the $S_{0.5}$ values of the enzyme for metal–isocitrate complexes varied depending on the ionic radii of metal cations: metals with ionic radii of approximately 0.7 nm showed a maximum $S_{0.5}$ value. Mn^{2+} and Cd^{2+} ions, with larger ionic radii, showed higher values for the maximal velocity, the Hill interaction coefficient and the enzyme affinity. However, Mg^{2+}, Ni^{2+} and Fe^{2+} ions, with smaller ionic radii, showed normal Michaelis–Menten type kinetics, with the largest K_m values and the lowest maximal velocity.

Discussion

Most eukaryotic cells contain three types of isocitrate dehydrogenase: mitochondrial NAD-linked, mitochondrial NADP-linked and cytosolic NADP-dependent enzymes (Plaut 1970). The first enzyme is known to be allosterically regulated by AMP or ADP and can act as a key enzyme of the citric acid cycle (Chen & Plaut 1963, Gabriel & Plaut 1984).

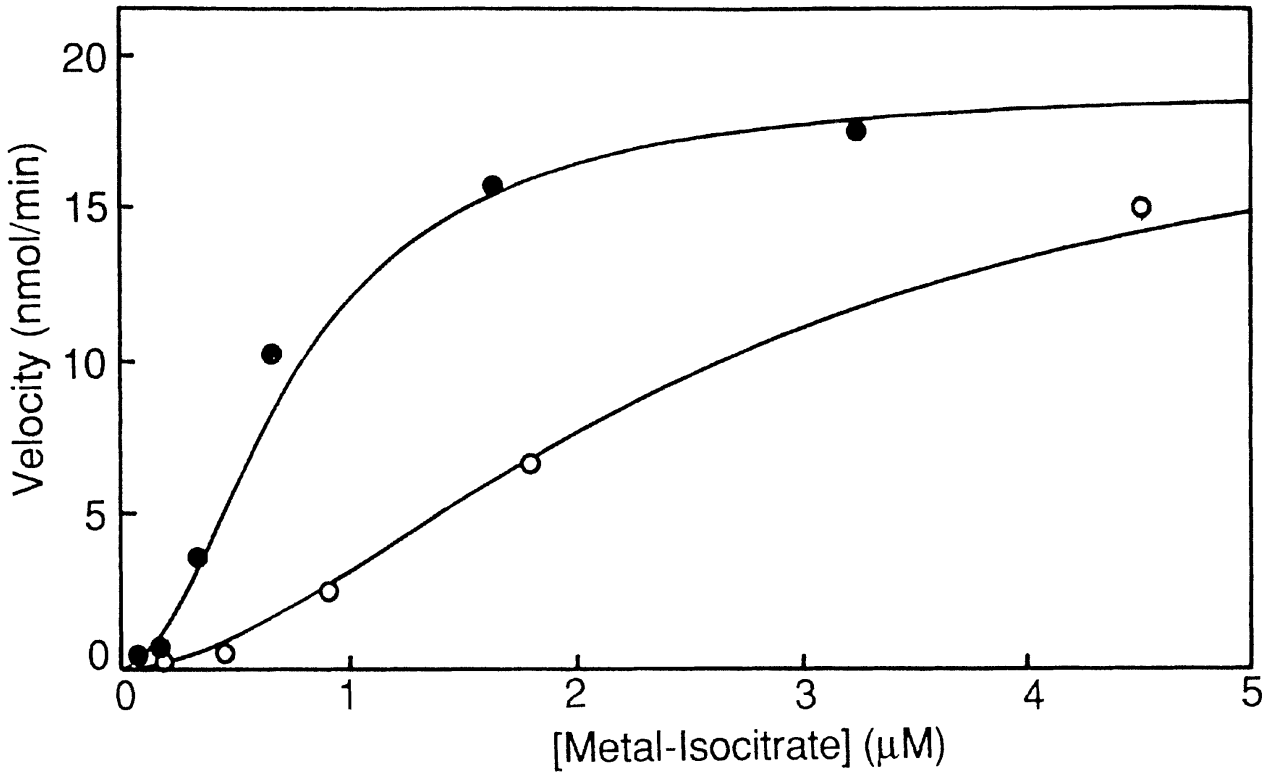

Figure 2. Rate of the reaction catalysed by NADP-linked isocitrate dehydrogenase as a function of the substrate Mn– and Cd–isocitrate concentration. The reaction mixture of 1 ml contained 50 mM Mops–KOH buffer (pH 7.1), 0.1 mM NADP, various concentrations of $MnCl_2$ or $CdCl_2$, *threo*-Ds-isocitrate and the enzyme. The reaction was initiated by addition of the enzyme, and was carried out at 37°C. Points are experimental data, and lines are theoretically drawn by using the text equation, with the obtained values for the following kinetic parameters essentially based on non-linear regression analysis (Duggleby 1981). ● Mn: K_m = 0.76 μM, V_{max} = 18.8 nmol min^{-1} and n_H = 1.9. ○, Cd: K_m =2.6 μM, V_{max} = 19.24 nmol min^{-1} and n_H = 1.75.

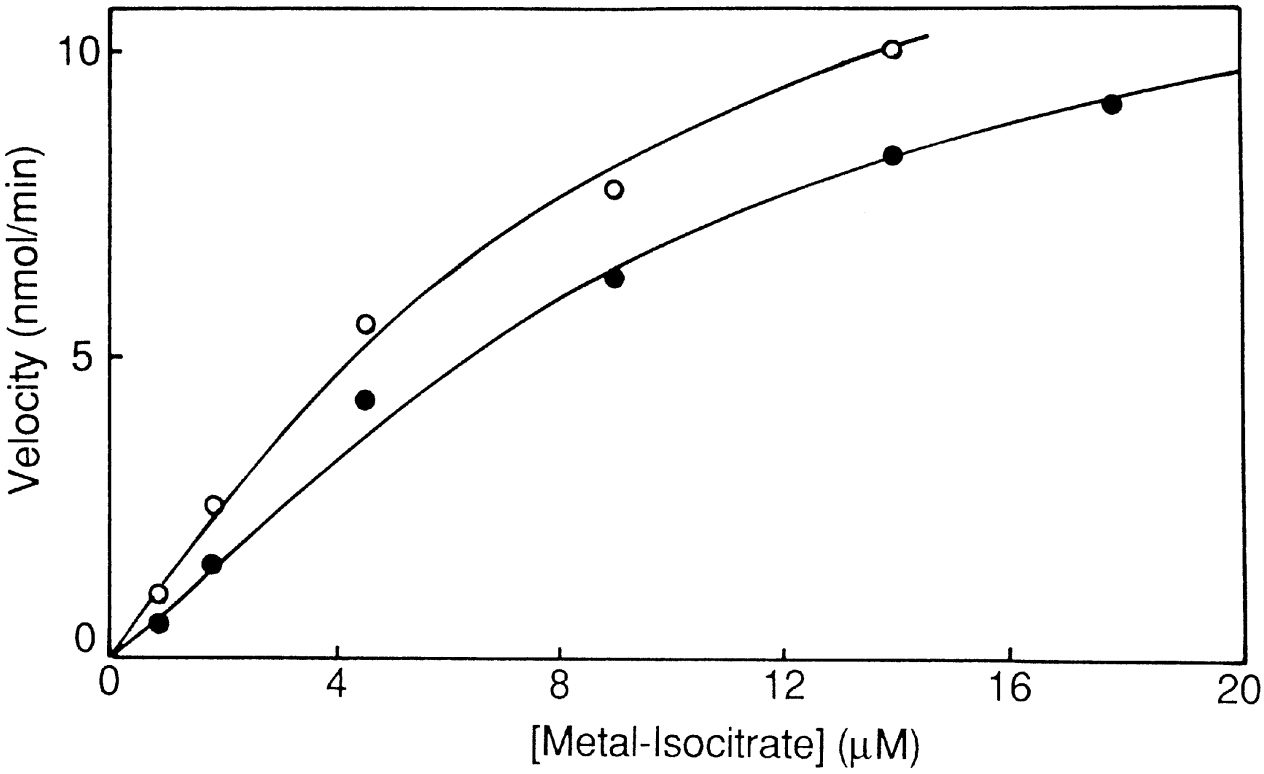

Figure 3. Rate of the reaction catalysed by NADP-linked isocitrate dehydrogenase as a function of the substrate Zn– and Co–isocitrate concentration. The reaction mixture of 1 ml contained 50 mM Mops–KOH buffer (pH 7.1), 0.1 mM NADP, various concentrations of $ZnCl_2$ or $CoCl_2$, *threo*-Ds-isocitrate and the enzyme. The reaction was initiated by addition of the enzyme, and was carried out at 37°C. Points are experimental data, and lines are theoretically drawn by using the text equation, with the obtained values for the following kinetic parameters essentially based on non-linear regression analysis (Duggleby 1981). ○, Zn: K_m = 9 μM, V_{max} = 15.94 nmol min^{-1} and n_H 1.12. ●, Co: K_m = 10 μM, V_{max} = 13.35 nmol min^{-1} and n_H = 1.28.

On the other hand, the two types of NADP–isocitrate dehydrogenase are thought to show no regulatory properties. The requirement for metal ions for the reactions catalysed by the mitochondrial NADP–isocitrate dehydrogenase from pig heart has been extensively investigated. The predominant substrate for the mitochondrial enzyme is the metal–isocitrate complex (Colman 1972), although some researchers have suggested that, under some conditions, isocitrate can be a substrate for the cytosolic NADP–isocitrate dehydrogenase from beef liver (Carlier & Pantaloni 1976a, 1976b). In this paper we have examined the regulatory effect of several metal cations on the activity of NADP–isocitrate dehydrogenase from pig heart. Mn^{2+}, Cd^{2+}, Zn^{2+} and Co^{2+} ions complexed with isocitrate showed typical sigmoid concentration–velocity relationships. On the other hand, Mg^{2+}, Fe^{2+} and Ni^{2+} showed normal hyperbolic saturation functions.

Binding of metal cations is primarily electrostatic and therefore, in addition to charge, ionic size is an important parameter. In the present work, kinetic parameters including Hill's interaction coefficient, $S_{0.5}$ and V_{max} values, were correlated with the ionic radii of the metal cations. Increases in ionic radii raised both the maximal velocity and the Hill's interaction coefficient, characteristic of allosteric properties. The relationship between ionic radii and the $S_{0.5}$ values for metals was complex: the metal cations with an ionic radius of approximately 0.7 nm showed a maximal value of $S_{0.5}$, but metals with smaller or larger ionic radii exhibited higher affinities for the enzyme. These results suggest that the binding of the substrate isocitrate to the active sites of the enzyme is highly dependent on the ionic size of the metal, forming a cyclic metal bridge structure in which isocitrate and metal bind to separate groups on the enzyme and to each other (Colman 1974).

The present study cannot clarify the mechanism

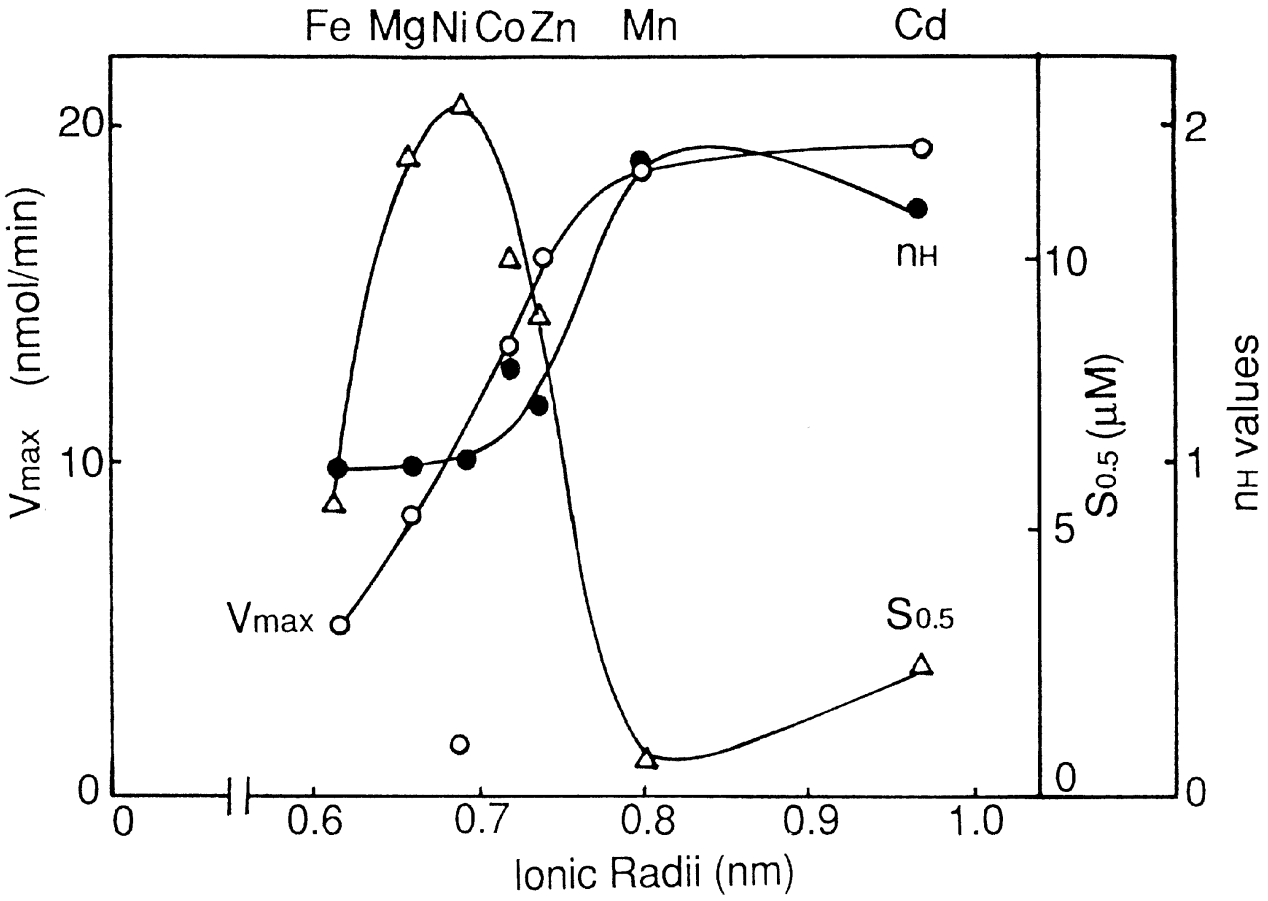

Figure 4. Relationship between ionic radii of metal cations and kinetic parameters for metal–isocitrate complexes. Kinetic parameters including $S_{0.5}$, V_{max} and Hill's interaction coefficient, n_H, determined in Figures 1 to 3 were plotted against ionic radii of metal cations. ●, n_H; ○, V_{max}; △, $S_{0.5}$.

for the metal cation-dependent allosteric properties of the NADP–isocitrate dehydrogenase. Several lines of evidence have accumulated concerning the structure and nature of the metal binding site. Earlier studies revealed that the pig heart enzyme is inactivated by chemical modification of lysine: the Mn^{2+} ion potentiates the inactivation by increasing the availability of lysyl residues, but Mg^{2+} cannot influence the modification reaction (Colman 1972). The lysyl residue, which may participate in the binding of Mn^{2+} in the active sites, is conserved at Lys230, Lys212 and Lys213 in the *E. coli*, yeast and pig heart enzymes, respectively (Hurley *et al.* 1991, Loftus *et al.* 1994). Interaction of the metal ion including Mn^{2+}, with the Lys213 residue of the enzyme may be responsible for the allosteric interactions of the substrate binding and other specific kinetic properties. By contrast, the Mg^{2+} ion, which cannot interact with Lys213, does not show any allosteric character.

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