BioMetals 1992, **5**, 217–221

Aluminum: a pH-dependent inhibitor of NADP-isocitrate dehydrogenase from porcine heart

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Recieved 13 July 1992; accepted for publication 17 July 1992

Aluminum showed a pH-dependent inhibitory effect on NADP-isocitrate dehydrogenase from porcine heart. Aluminum ions (Al^{3+}) acted as a partial competitive inhibitor of the enzyme with respect to the substrate *threo*-Ds-isocitrate and inhibited the enzyme non-competitively with respect to NADP at pH 6.85. Fractional velocity plot analysis showed the K_i of the enzyme for aluminum ions to be 0.88 μM. When pH was elevated to 8.0, aluminum ions, which occur as a form of the $Al(OH)_4^-$ anion, acted as partial uncompetitive and non-competitive inhibitors of the enzyme with respect to the substrates isocitrate and NADP, respectively. The K_i' of the enzyme was determined to be 5.64 μM at pH 8.0 by fractional velocity plot analysis. The inhibition of NADP-isocitrate dehydrogenase by two forms of aluminum ions may explain aluminum toxicity in various tissues and organs.

Keywords: aluminum, inhibition, NADP-isocitrate dehydrogenase

Introduction

Aluminum is abundant in the environment, but surface water concentrations of aluminum ions have until recently remained minimal due to the insolubility of aluminum hydroxide complexes at neutral pH (Martin 1986, Macdonald & Martin 1988). Therefore, aluminum is present in very small amounts in living organisms, but has been proposed to be the etiology of a variety of neurological and skeletal disorders (Parkinson *et al.* 1979, Willis & Savory 1983, Ganrot 1986, Martin 1986, Macdonald & Martin 1988). Experimental studies have shown that aluminum can induce some types of epilepsy (Ward 1972). Recently, toxic effects of aluminum on several enzymes and metabolic pathways have been reported (Ganrot 1986, Martin 1986, Macdonald & Martin 1988). A potent inhibition of hexokinase (EC 2.7.1.1) by aluminum ions may be one of the mechanisms by which aluminum can act as a neurotoxin (Womack & Colowick 1979, Lai & Blass 1984).

Aluminum is present as an Al^{3+} form and an $Al(OH)_4^-$ form at acidic and alkaline pH ranges, respectively (Martin 1986). Aluminum expresses its biological toxicity at acidic pH range, indicating that the toxic form of aluminum is Al^{3+}; however, the $Al(OH)_4^-$ anion has not been reported to show any toxic effects in biological systems.

In this paper we describe the inhibitory effects of aluminum on NADP-dependent isocitrate dehydrogenase (EC 1.1.1.42) from pig heart: aluminum showed differential inhibitory action on the enzyme at conditions below pH 7 and in the alkaline range. Aluminum may express its biological toxicity on energy metabolism as the Al^{3+} and $Al(OH)_4^-$ forms over a broad pH range.

Materials and methods

Threo-Ds-isocitrate, NADP and purified pig heart NADP-isocitrate dehydrogenase (specific activity 2 μmol min^{-1} mg $protein^{-1}$) were purchased from Boehringer-Mannheim-Yamanouchi (Tokyo, Japan). 4-Morpholinopropanesulfonic acid (MOPS) was obtained from Dojindo

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Co. (Kumamoto, Japan). 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 6.85 or 8.0), 0.5 mM $MgCl_2$, the enzyme, and various concentrations of NADP and *threo*-Ds-isocitrate in the absence and presence of $AlCl_3$. The reaction was initiated by the addition of the enzyme.

Results

Aluminum acted as an effective competitive inhibitor of pig heart NADP-isocitrate dehydrogenase with respect to the substrate isocitrate (Figure 1),

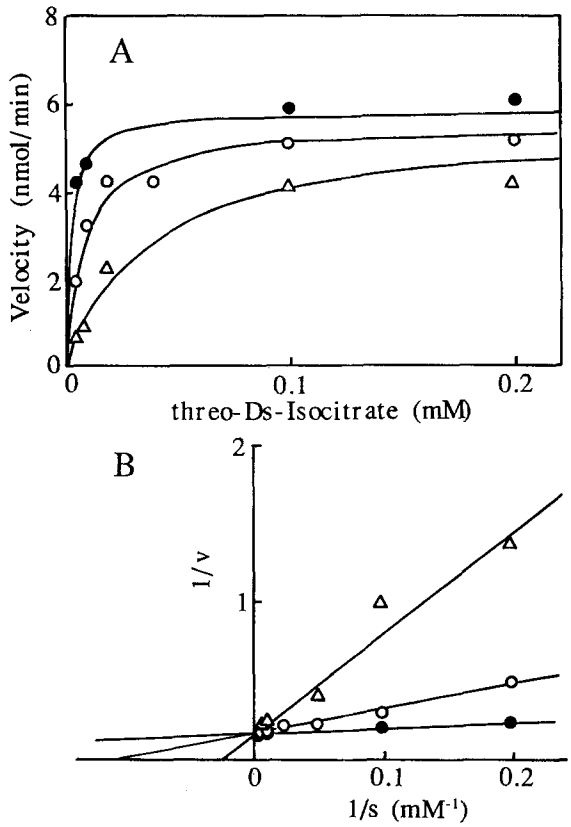

Figure 1. Effect of the concentrations of *threo*-Ds-isocitrate on the activity of pig heart NADP-isocitrate dehydrogenase in the absense and presence of aluminum ions at pH 6.85. The reaction mixture of 1 ml contained 100 mM MOPS–KOH buffer (pH 6.85), 0.5 mM $MgCl_2$, 0.1 M NADP, various concentrations of *threo*-Ds-isocitrate, and the enzyme in the absence and presence of 10 and 20 μM $AlCl_3$. (**A**) *Threo*-Ds-Isocitrate saturation curves in the absence (●) and presence of 10 (○) and 20 (△) μM $AlCl_3$. (**B**) Double reciprocal plot. Points are experimental data and lines in (**A**) are theoretically drawn with the partial competitive mechanism using the following kinetic parameters obtained by non-linear regression analysis (Duggleby 1981): $K_m = 2.0$ μM, $K_i = 0.88$ μM, $K_i' = K_s' = \infty$, $k' = k$, $V_{max} = 5.74$ μmol min^{-1}.

while it inhibited the enzyme non-competitively with respect to NADP at pH 6.85 (Figure 2). Figure 3 shows the effect of increasing concentrations of aluminum on the enzyme at pH 6.85. We analyzed the inhibition curves of the enzyme using fractional velocity plot analysis (Yoshino 1987). All the inhibition curves in Figure 3 were converted into straight lines converging on the abscissa at a point away from the origin (Figure 4A), suggesting that aluminum acts as a partial inhibitor (Yoshino 1987). The replot of the slope versus the substrate isocitrate concentration showed a straight line relationship (Figure 4B), confirming the partial competitive inhibition of aluminum. The K_i determined from the slope and abscissa intercept of the fractional velocity plot was 0.88 μM.

When the effect of aluminum on the activity of NADP-isocitrate dehydrogenase from pig heart was analyzed at pH 8.0, aluminum acted as an inhibitor of the enzyme, but an unusual double reciprocal plot suggested the inhibition to be uncompetitive toward

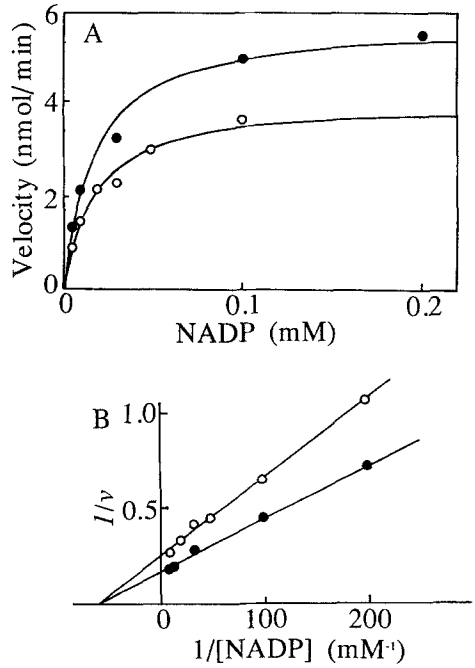

Figure 2. Effect of the concentrations of NADP on the activity of pig heart NADP-isocitrate dehydrogenase in the absence and presence of aluminum ions at pH 6.85. The reaction mixture was similar to that described in the legend to Figure 1 except that NADP concentrations were varied in the presence of 0.2 mM *threo*-Ds-isocitrate and 0.5 mM $MgCl_2$. (**A**) NADP saturation curves in the absence (●) and presence (○) of 10 μM $AlCl_3$. (**B**) Double reciprocal plot. Points are experimental data and lines in (**A**) are theoretically drawn with the non-competitive inhibition mechanism using $K_m = 17$ μM for NADP.

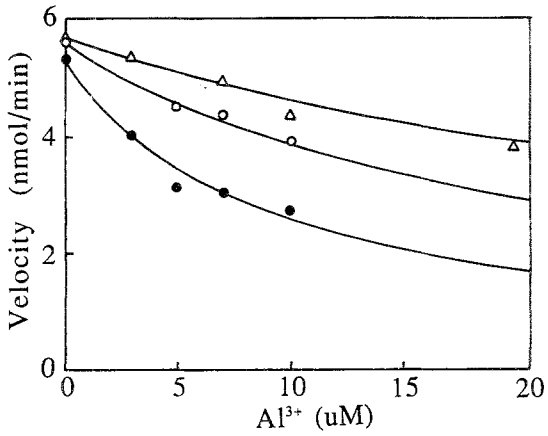

Figure 3. Effect of aluminum ions on the activity of pig heart NADP-isocitrate dehydrogenase in the presence of different concentrations of *threo*-Ds-Isocitrate at pH 6.85. The reaction mixture was similar to that described in the legend to Figure 1 except that $AlCl_3$ concentrations were varied in the presence of 0.1 mM NADP and different concentrations of *threo*-Ds-isocitrate. *Threo*-Ds-isocitrate concentration: ●, 20 μM; ○, 50 μM; △, 100 μM. Points are experimental data and lines are theoretically drawn according to the kinetic parameters indicated in the legend to Figure 1.

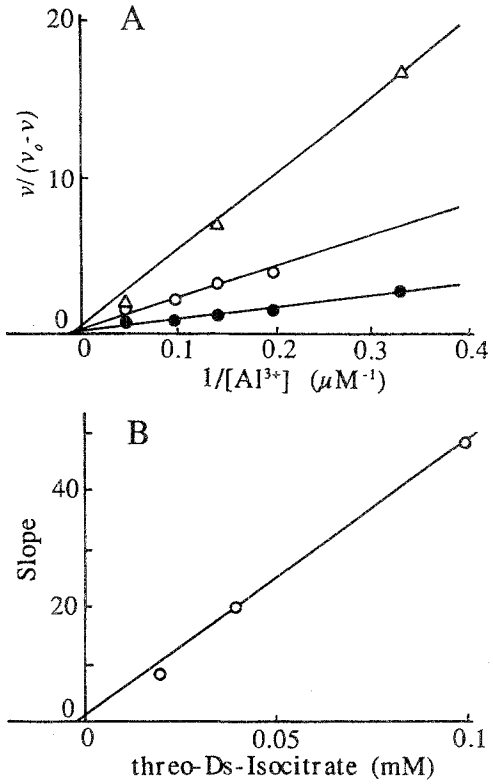

Figure 4. Fractional velocity plot analysis of the inhibition by Al^{3+} of pig heart NADP-isocitrate dehydrogenase. (**A**) Fractional velocity plot. All the inhibition curves in Figure 3 were converted to the straight lines according to the equation $v/(v_0 - v) = (1/[I] + 1/K_i') \cdot (1 + [S]/K_m)/(1/K_i - 1/K_i')$ (see Yoshino 1987). ●, 20 μM *threo*-Ds-isocitrate; ○, 40 μM; △, 100 μM. (**B**) Replot of the slope of the fractional velocity plot versus the concentration of *threo*-Ds-isocitrate.

the substrate *threo*-Ds-isocitrate (Figure 5). Furthermore, aluminum showed a non-competitive inhibition of the enzyme with respect to NADP (Figure 6). The mechanism of aluminum inhibition of the enzyme was analyzed under different concentrations of isocitrate at pH 8 (Figure 7A). We applied the fractional velocity plot to the inhibition curves in Figure 7(A). All of the inhibition curves in Figure 7(A) were converted into straight lines converging on the abscissa at a point away from the origin (Figure 7B), suggesting that aluminum acted as a partial inhibitor. The values of the x-intercept and the slope gave the K_i for the aluminum ions and k'/k to be 5.64 μM and 0.55, respectively. A decrease in the slope of the plot accompanied by the increase in the substrate concentration confirmed the partial uncompetitive nature of the inhibition (Yoshino 1987).

Discussion

Aluminum, the most abundant metal in the Earth's crust, exists as two principal soluble forms dependent on pH in aqueous solutions. In acidic solutions, aluminum exists as the octahedral hexahydrate, $Al(H_2O)_6^{3+}$, often abbreviated as Al^{3+}. Neutral solutions give an $Al(OH)_3$ precipitate that redissolves in basic solutions, owing to the formation of tetrahedral $Al(OH)_4^-$ (Martin 1986, Macdonald & Martin 1988). Recently, several studies showed that aluminum exerts biological toxicity: aluminum is an etiologic agent in some types of encephalopathy (Ward 1972, Parkinson *et al.* 1979, Willis & Savory 1983, Ganrot 1986) and acts as an inhibitor of several enzymatic reactions (Womack & Colowick, 1979, Lai & Blass 1984, Ganrot 1986, Martin 1986, Macdonald & Martin 1988). Experimental and theoretical considerations have led investigators to hypothesize that among all the possible aluminum species in solutions, the trivalent free aluminum ion, Al^{3+}, is the active form responsible for many of the observed aluminum effects (Martin 1986, Macdonald & Martin 1988).

Although the biological processes and the molecular mechanisms through which Al^{3+} may exert its toxicity are not well understood, the principal mechanism has been considered to be substitution

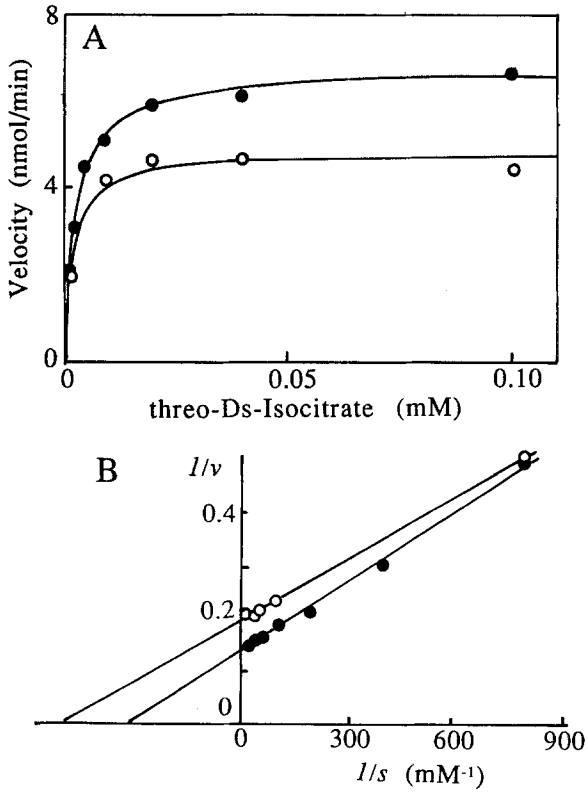

Figure 5. Effect of the concentrations of *threo*-Ds-isocitrate on the activity of pig heart NADP-isocitrate dehydrogenase in the absence and presence of 10 μM $AlCl_3$ at pH 8.0. The reaction mixture was similar to that described in the legend to Figure 1 except that the pH 6.85 buffer was replaced by the pH 8.0 buffer. (**A**) *Threo*-Ds-Isocitrate saturation curves in the absence (●) and presence (○) of 10 μM $AlCl_3$. (**B**) Double reciprocal plot. Points are experimental data and lines theoretically drawn with the partial uncompetitive mechanism using the following kinetic parameters obtained by non-linear regression analysis (Yoshino 1987): $K_m = 2.79$ μM; $K_i' = 5.64$ μM; $k'/k = 0.55$.

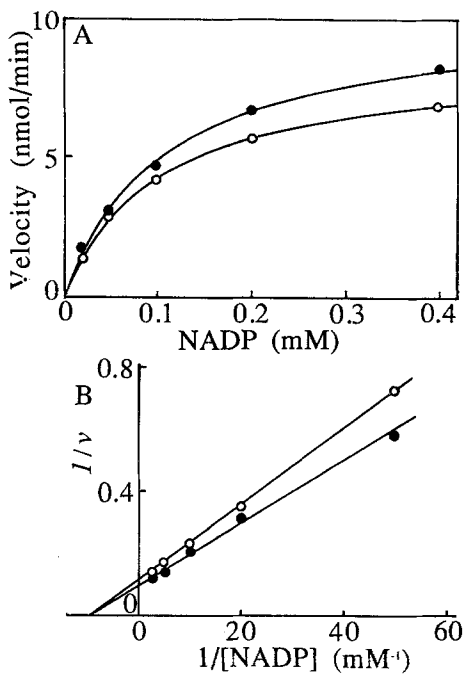

Figure 6. Effect of the concentration of NADP on the activity of pig heart NADP-isocitrate dehydrogenase in the absence and presence of of $AlCl_3$ at pH 8.0. The reaction mixture was similar to that described in the legend to Figure 2 except that the pH 6.85 buffer was replaced by the pH 8.0 buffer. (**A**) NADP saturation curves in the absence (●) and presence (○) of 10 μM $AlCl_3$. (**B**) Double reciprocal plot. Points are experimental data and lines in (**A**) are theoretically drawn with the noncompetitive inhibition mechanism using parameters: $K_m = 97$ μM, $V_{max} = 8.67$ μmol min^{-1}.

for Mg^{2+} at critical sites in the cell (Macdonald & Martin 1988). Binding of aluminum is primarily electrostatic and, therefore, in addition to charge, ionic size is an important parameter. Since the radius of Al^{3+} most resembles that of Fe^{3+}, the appearance of Al^{3+} in Fe^{3+} sites seems likely. The binding of Al^{3+} and Fe^{3+} to transferrin has been demonstrated (Cochran *et al.* 1984). Though Mg^{2+} is somewhat larger than Al^{3+}, displacement of the ubiquitous Mg^{2+} in biological systems by Al^{3+} appears likely (Macdonald & Martin 1988). Mg^{2+} is often associated with phosphate groups and acts as a essential cofactor of many enzyme reactions. Al^{3+} binds almost 10^7 times more strongly to ATP than does Mg^{2+}; thus, in these systems, very low amounts of Al^{3+} can compete with Mg^{2+} for the phosphate sites (Macdonald & Martin 1988). The inhibitory effects of aluminum ions on enzyme reactions at acidic pH can be explained by this Al^{3+}/Mg^{2+} substitution mechanism.

This report demonstrated that aluminum showed potent inhibitory effect on pig heart NADP-isocitrate dehydrogenase. Aluminum occurs mainly as the Al^{3+} form at pH 6.85 and, thus, the inhibition of the enzyme by aluminum at pH 6.85 seems to be by the Al^{3+}/Mg^{2+} substitution mechanism. At pH 6.85, Al^{3+} forms a complex with the substrate *threo*-Ds-isocitrate and the complex can compete the magnesium-isocitrate competitively at the substrate sites of the enzyme. However, the uncompetitive inhibition of the enzyme by aluminum at pH 8.0 was clearly discriminated from the competitive inhibition at pH 6.85. Since all the soluble aluminum ions occur as $Al(OH)_4^-$ with a negligible Al^{3+} level at alkaline pH range (Macdonald & Martin 1988), the inhibition at pH 8.0 is due to $Al(OH)_4^-$; the

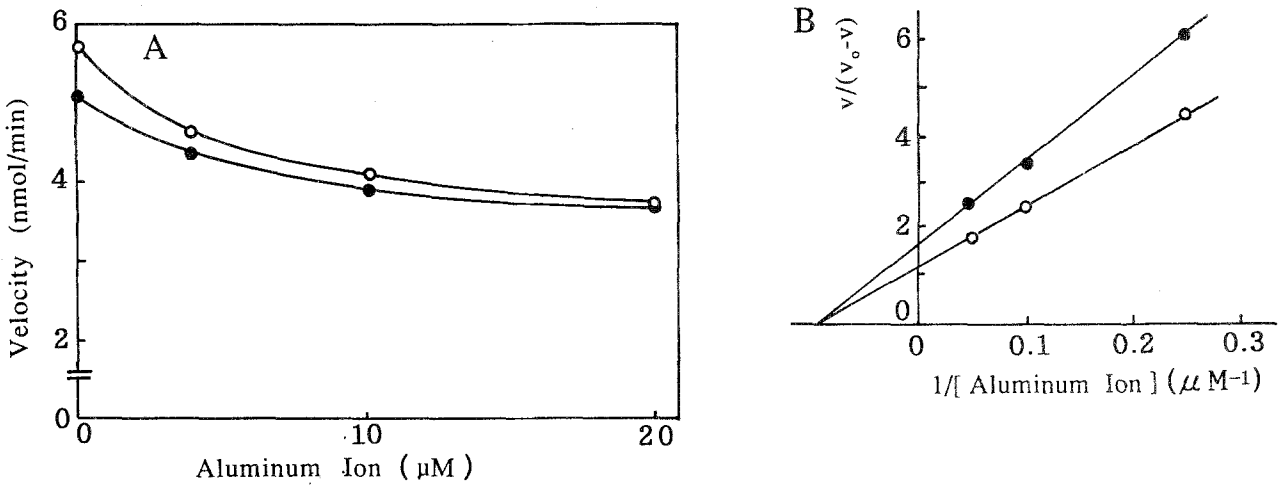

Figure 7. Effect of aluminum on the activity of pig heart NADP-isocitrate dehydrogenase in the presence of different concentrations of *threo*-Ds-Isocitrate at pH 8.0. (**A**) Inhibition curves. The reaction mixture was similar to that described in the legend to Figure 3 except that the pH of the buffer was changed to 8.0. *Threo*-Ds-Isocitrate concentration; ●, 20 μM; ○, 100 μM. (**B**) Fractional velocity plot. Points are experimental data and lines are theoretically drawn according to the kinetic parameters indicated in the legend to Figure 5.

$Al(OH)_4^-$ anion can bind to the enzyme–magnesium-isocitrate complex at the sites separate from the active sites, but cannot bind to the free enzyme.

The toxic effect of aluminum ions other than Al^{3+} has not yet been demonstrated. Recently, inhibition by aluminum of voltage-dependent closure of mitochondrial channels of *Neurospora* was shown to be the direct action of $Al(OH)_3$ at neutral pH (Zhang & Colombini 1984). Inhibition of NADP-isocitrate dehydrogenase by aluminum at pH 8.0 is the first example of the biological effect of aluminum in the $Al(OH)_4^-$ form. Toxicity of $Al(OH)_4^-$ and $Al(OH)_3$ on biological and enzyme systems may be a more general phenomenon. The combined effect of Al^{3+} and $Al(OH)_4^-$ on NADP-isocitrate dehydrogenase should be considered as an important candidate for aluminum-induced cellular effects.

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