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Interaction of aluminum ion with ATP. Mechanism of the aluminum inhibition of glycerol kinase and its reversal by spermine

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Aluminum ion inhibited yeast glycerol kinase competitively with respect to the substrate MgATP. The K_i value of the enzyme for aluminum ion was about 3 μM. Spermine at physiological concentrations prevented glycerol kinase from the inhibition by aluminum ion. Nuclear magnetic resonance spectroscopy showed the specific elimination by spermine of aluminum from the metal–ATP complex, but no dissociation of MgATP complex by spermine. Inhibition by aluminum ion of glycerol kinase as well as hexokinase can reduce the utilization of energy fuel in yeast. Change in polyamine concentration may control energy production *in vivo*, and is responsible for the development of age-related aluminum toxicity.

Keywords: aluminum ion, glycerol kinase, NMR, spermine, yeast

Introduction

Aluminum is the most abundant metal in the earth's crust, but is present in small amounts in living organisms because of its low solubility at neutral pH (Martin 1986, Macdonald & Martin 1988). Recent reports show aluminum toxicity on various biochemical reactions (Martin 1986, Macdonald & Martin 1988, Ganrot 1986). Ionic radius for Al^{3+} most closely resembles those of Mg^{2+} and Fe^{3+}, and thus, Al^{3+} substitution for Mg^{2+} at critical target sites causes the inhibition of several Mg^{2+}-dependent enzymes by aluminum (Martin 1986, Macdonald & Martin 1988). Potent inhibition by aluminum ion of hexokinase (EC 2.7.1.1) (Lai & Blass 1984, Womack & Colowick 1979, Yoshino *et al*. 1990) and NADP-linked isocitrate dehydrogenase (EC 1.1.1.42) (Yoshino *et al*. 1990, Yoshino & Murakami 1992, Yoshino *et al*. 1992) may contribute to the aluminum toxicity in cells.

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In this paper we report the inhibitory effect of aluminum ion on the activity of glycerol kinase (EC 2.7.1.30), the first enzyme of the glycerol-utilizing pathway in yeast. The inhibition of glycerol kinase by aluminum was effectively reversed by spermine the principal polyamine in eukaryotes. Nuclear magnetic resonance (NMR) studies revealed that spermine specifically ejected aluminum ion from the metal–ATP complex, but that spermine did not affect the binding of Mg^{2+} to ATP. Inhibition of glycerol kinase by aluminum ion and its reversal by spermine may be related to the appearance of aluminum toxicity in cells.

Materials and methods

Materials

NADP, purified glycerol kinase (specific activity of 160 μmol min^{-1} per mg protein) from yeast (*Candida mycoderma*) and rabbit muscle glycerol 3-phosphate dehydrogenase (EC 1.1.1.8, specific activity of 320 μmol min^{-1} per mg protein) were obtained from Boehringer-Mannheim-Yamanouchi (Tokyo, Japan). 4-Morpholino-propanesulfonic acid (Mops) was obtained from Dojindo Co. (Kumamoto, Japan).

Kinetic studies

Glycerol kinase activity was determined by following the change in absorbance at 340 nm. The assay medium of 0.5 ml contained 50 mM Mops–KOH buffer (pH 6.85), various concentrations of $MgCl_2$ and ATP, 4 mM glycerol and the enzyme in the absence and presence of $AlCl_3$. The reaction was started by adding the enzyme. After incubation at 37 °C for 5 min, the reaction was stopped by addition of 60 μl of 100 mM EDTA and 0.98 ml of 0.2 M glycine–KOH buffer (pH 9.8) containing 200 mg ml^{-1} hydrazine hydrate. NAD and purified glycerol 3-phosphate dehydrogenase were added to final concentrations of 0.1 mM and 10 units ml^{-1}, respectively. The mixture was incubated at 37 °C for 10 min, and the absorbance at 340 nm was determined.

Measurement of ^{27}Al- and ^{25}Mg-NMR spectra

^{27}Al-NMR and ^{25}Mg-NMR spectra were recorded at 104.26 MHz and 24.48 MHz, respectively, with a Bruker AM400 spectrometer. An equipped transmitter provided 90-degree pulse width of 46 μs for ^{25}Mg and 17 μs for ^{27}Al, respectively. Typical ^{27}Al spectra consists of collecting 320 to 960 transients using 16K data points over 10 000 Hz spectral width. Measurements of ^{25}Mg spectra were performed with the same condition of ^{27}Al using 960 to 56 000 transients. The signal to noise ratio was improved by exponential multiplication which introduced 1 to 10 Hz line broadening.

Results

Addition of Al^{3+} inhibited the yeast glycerol kinase with a decrease in the affinity for the substrate MgATP (Figure 1(A)). Double reciprocal plot revealed the inhibition to be of a competitive type with respect to MgATP (Figure 1(B)). Figure 2 shows the effect of increasing concentrations of Al^{3+} on the glycerol kinase activity. Dixon plot showed that Al^{3+} acted as a competitive inhibitor of the enzyme with a K_i value of 3 μM (Figure 2(B)).

Spermine effectively protects hexokinase from the inhibition by aluminum ion (Yoshino *et al.* 1990). Thus, we examined the effect of spermine on the aluminum-induced inhibition of glycerol kinase. Apparent K_i values of the enzyme for aluminum ion increased with the increase in spermine concentration: addition of spermine at physiological concentration reduced the affinity of the enzyme for aluminum to half (Figure 3).

Reversal of aluminum-inhibition by spermine suggests that spermine inhibits the formation of the aluminum–ATP complex. We analyzed the action of spermine on the complex formation between aluminum and ATP using NMR spectroscopy.

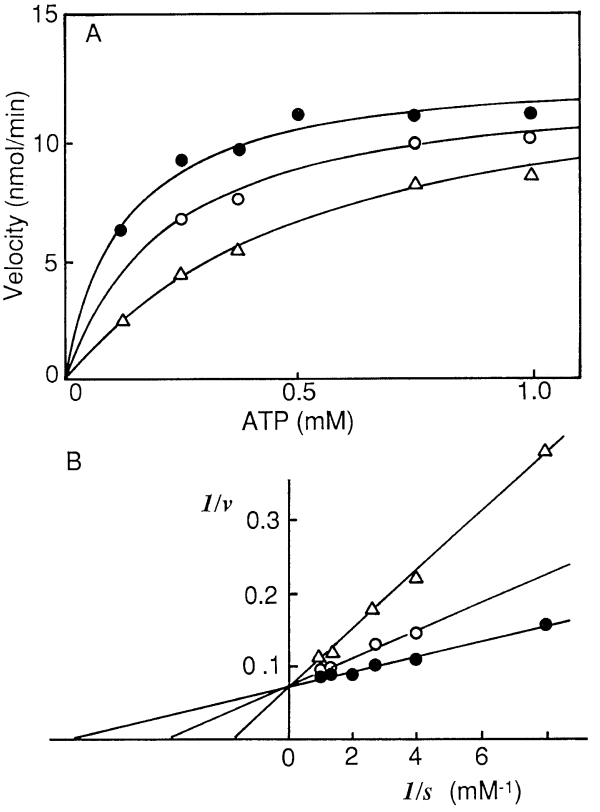

Figure 1. Effect of the concentrations of threo-Ds-isocitrate on the activity of yeast glycerol kinase in the absence and presence of aluminum ion. The reaction mixture of 0.5 ml contained 50 mM Mops–KOH buffer (pH 6.85), various concentrations of $MgCl_2$ and ATP, 4 mM glycerol and the enzyme in the absence and presence of $AlCl_3$. $MgCl_2$ was added to obtain the indicated concentrations of MgATP. The reaction was started by adding the enzyme. After incubation at 37 °C for 5 min, glycerol 3-phosphate formed was determined enzymatically as described in Materials and methods. (**A**) MgATP saturation curves in the absence (●) and presence of 5 μM (○) or 20 μM (△) aluminum ion. (**B**) Double reciprocal plot. Points represent experimental data, and lines are theoretically drawn according to the non-linear regression analysis (Duggleby 1981).

Figure 4(A) shows the ^{27}Al-NMR spectra with a sharp singlet resonance of aluminum ion at pH 2. Addition of ATP at twice the concentration of aluminum ion decreased the sharp resonance of Al^{3+} markedly, and a broad signal appeared at 4 p.p.m. higher magnetic field than free aluminum ion (Figure 4(B)). We assigned this resonance to the aluminum–ATP complex (Karlik *et al.* 1982). The broadening of the resonance results from a decrease in the symmetry of the ligand field as a result of the substitution of P for H_2O in the Al^{3+} coordination

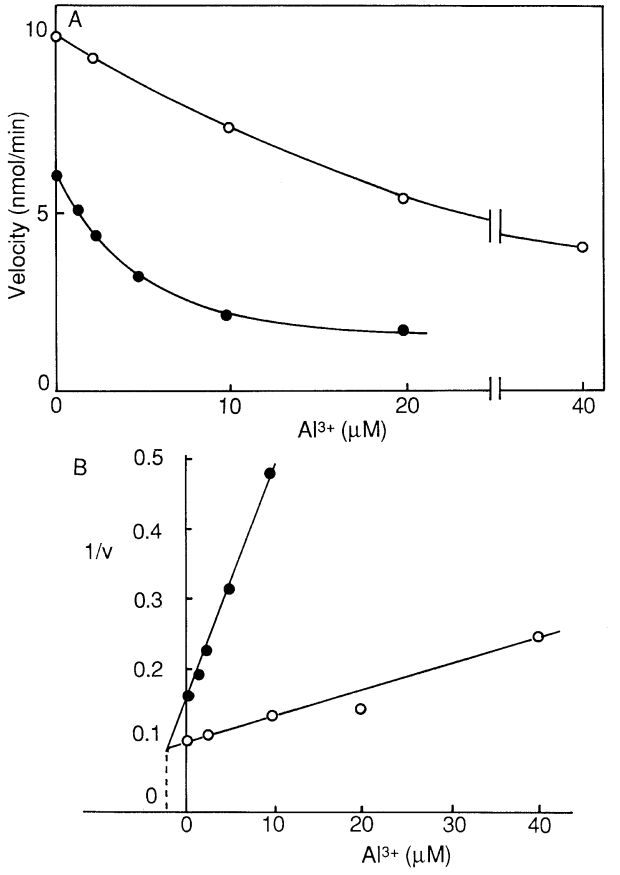

Figure 2. Effect of aluminum ion on the activity of yeast glycerol kinase in the presence of different concentrations of MgATP. Reaction mixture was similar to that in legend to Figure 1, except that $AlCl_3$ concentrations were varied. (**A**) Inhibition curves. (**B**) Dixon plot. (●) 0.125 mM MgATP. (○) 0.5 mM MgATP.

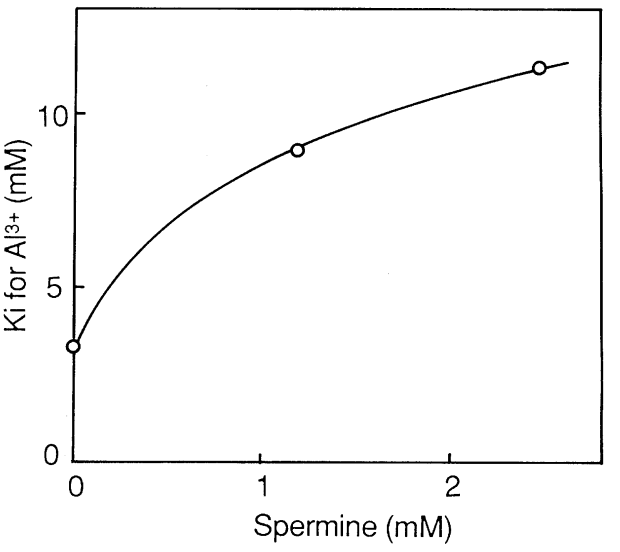

Figure 3. Effect of spermine on the K_i values of glycerol kinase for aluminum ion. Reaction mixture was similar to that in Figure 2, but spermine was included as indicated.

sphere. Further addition of excess spermine to the aluminum–ATP complex increased the intensity of the sharp resonance of free aluminum ion (Figure 4(C)). Increase in spermine concentration caused a further increase in the free aluminum ion signal with concomitant reduction of broad resonance of ATP-aluminum complex (Figure 4(D)). These results indicate that spermine can eject Al^{3+} from the aluminum–ATP complex.

We further explored the effect of spermine on the MgATP complex using ^{25}Mg-NMR spectroscopy. Magnesium ion gave a sharp singlet resonance (Figure 5(A)), but addition of ATP caused a marked broadening and reduction of the signal intensity (Figure 5(B)). ^{25}Mg nuclei has a large quadrupolar moment and the broad resonance is also due to the coordinated H_2O (Shimizu & Hatano 1982). Further addition of spermine to the MgATP complex could not recover the sharp resonance of Mg^{2+} (Figure 5(C)). Thus, spermine can specifically eject aluminum ion from the metal–ATP complex, but not magnesium ion from the complex.

Discussion

Aluminum, the most abundant metal in the environment, shows various toxic effects on biological and biochemical processes, but the molecular mechanism of aluminum toxicity has remained obscure. The toxic effect of aluminum may be the substitution for some essential metal ions at critical sites in the cells (Ganrot 1986). Binding of metal ion is primarily electrostatic and therefore, in addition to charge, ionic size is an important parameter. Ionic radius for Al^{3+} most closely resembles those of Mg^{2+} and Fe^{3+}, and Al^{3+} would be substituted for these ions under physiological conditions (Martin 1986, Macdonald & Martin 1988). Thus, aluminum ion can act as an inhibitor of several Mg^{2+}-dependent enzymes in cells (Ganrot 1986). Typical examples are the inhibition of hexokinase (Womack & Colowick 1979, Lai & Blass 1984, Yoshino *et al.* 1990) and NADP-isocitrate dehydrogenase (Yoshino *et al.* 1992, Yoshino & Murakami 1992), which results in the inhibition of the energy-yielding reaction in cells. Aluminum inhibition of these enzymes may be responsible for some neurological and skeletal disorders (Ganrot 1986). Furthermore, inhibitory action of aluminum ion on

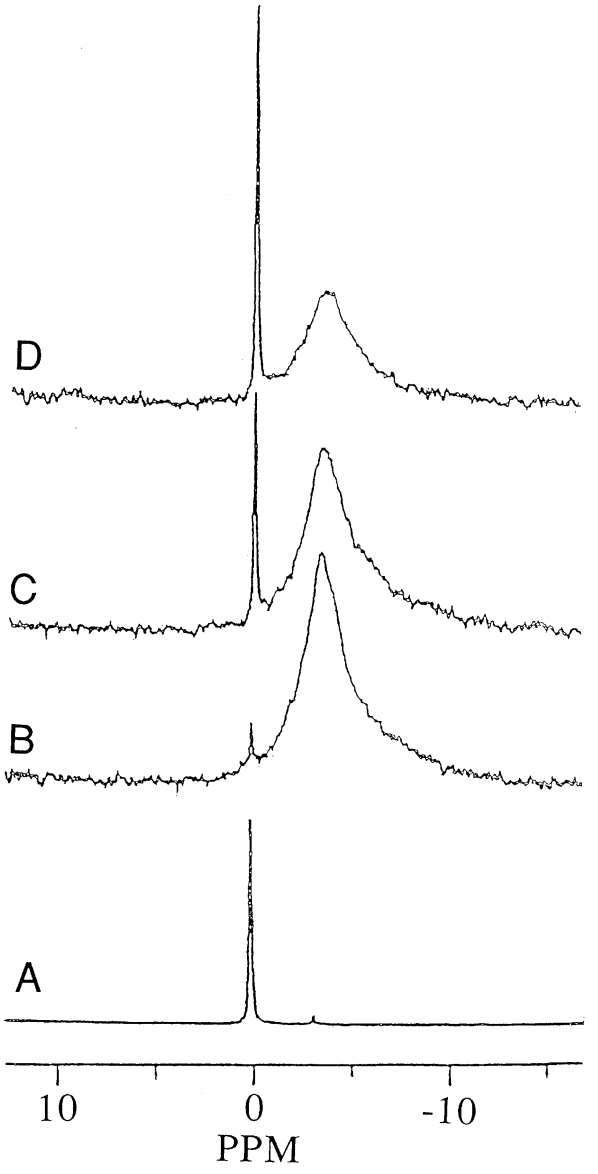

Figure 4. ^{27}Al–NMR spectra of aluminum ion and aluminum–ATP complex in the absence and presence of spermine. pH of the solution was kept at 2.0. (**A**) 1 mM $AlCl_3$. (**B**) 2 mM ATP plus 1 mM $AlCl_3$. (**C**) 20 mM spermine, 2 mM $AlCl_3$ and 1 mM ATP. (**D**) 140 mM spermine, 2 mM $AlCl_3$ and 1 mM ATP.

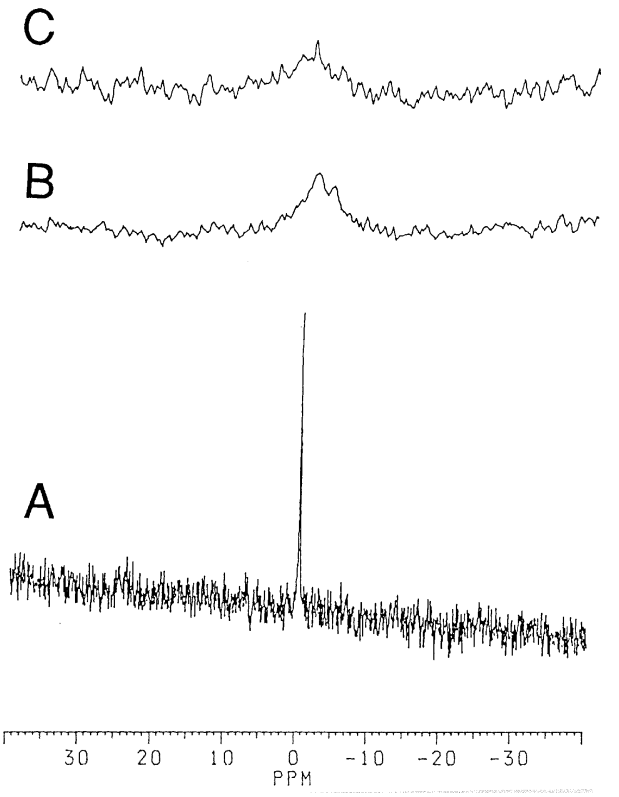

Figure 5. ^{25}Mg–NMR spectra of magnesium ion and MgATP complex in the absence and presence of spermine. (**A**) 10 mM $MgCl_2$ at pH 6.25. (**B**) 10 mM $MgCl_2$ plus 10 mM ATP at pH 7.20. (**C**) 10 mM $MgCl_2$ plus 10 mM ATP in the presence of 50 mM spermine.

these enzymes may participate in the inhibition of root elongation of plants causing forest decline (Godbold *et al.* 1988, Yoshino *et al.* 1992).

The present results show that aluminum ion inhibits glycerol kinase in a competitive manner with respect to the substrate MgATP. Effect of aluminum ion on the enzyme was similar to the inhibition of hexokinase (Womack & Colowick 1979, Lai & Blass 1984). Aluminum ion forms a chelate complex of aluminum–ATP, and can bind to the MgATP-sites of these enzymes. The K_i values of these two enzymes for aluminum ion were similar to each other: 3 and 1.8 μM for glycerol kinase and hexokinase, respectively. Furthermore, spermine, which is not a direct activator of these enzymes, reversed the inhibition by aluminum ion. NMR spectroscopy showed that spermine specifically eliminates aluminum ion from the aluminum–ATP complex, but cannot eject Mg^{2+} from the MgATP complex. Effect of spermine on the metal–ATP complex can explain the reversal by spermine of aluminum inhibition of Mg^{2+}-dependent enzymes.

Glycerol kinase and hexokinase utilize glycerol and glucose, respectively, as an energy source by phosphorylation and subsequent glycolysis or oxidation in yeast (Thorner & Paulus 1973). The inhibition by aluminum ion of these enzymes can suppress the energy production in yeast. Spermine concentration necessary for the reversal of the aluminum inhibition of glycerol kinase and hexokinase (Yoshino *et al.* 1990) is about 1 to 2 mM, within

physiological range (Abrahams & Pihl 1981). Spermine the principal polyamine responsible for the macromolecular synthesis accumulates with cell proliferation in cells, whereas aging of cell decreases polyamines (Abrahams & Pihl 1981). Decrease in polyamine can enhance the inhibition by aluminum ion of these enzymes, resulting in the reduction of the phosphorylation of glycerol and glucose. Aluminum toxicity may be explained by the inhibition of these enzymes, and further by the decreased reversal of the inhibition resulting from the decreased polyamine.

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