REVERSAL BY POLYAMINE OF THE ALUMINUM-INDUCED INHIBITION OF HEXOKINASE FROM HUMAN BRAIN

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ABSTRACT

Hexokinase (EC 2.7.1.1) purified from human brain was inhibited by aluminum ion competitively with respect to the substrate MgATP. The K_i value was $1.84 \mu\text{M}$. Spermine and spermidine at physiological concentrations effectively prevented hexokinase from the inhibition by aluminum, whereas diamines hardly canceled the aluminum-induced inhibition of the enzyme. Decrease in polyamine may contribute to the age-related aluminum encephalopathy through the decreased energy production in brain.

Aluminum, one of the most abundant elements in the earth crust, is recognized as a causative agent in human encephalopathy (4), and increased aluminum levels in brain have been reported in Alzheimer disease, Down syndrome (2), and Parkinsonism-amyotrophic lateral sclerosis-dementia complex (10, 11, 14, 15). Recent studies have shown some toxic effects of aluminum on several enzymes and metabolic pathways (6-8, 13). A strong inhibition of brain hexokinase (EC 2.7.1.1) by aluminum ion has been considered to be related to a decrease in brain energy metabolism in dialysis dementia and other neurological diseases (13). In this communication, we report effective reversal by polyamine of the aluminum-inhibition of hexokinase from human brain, and the physiological role of polyamine is discussed in relation to the aluminum toxicity in cells, and to pathogenesis of some age-related encephalopathy.

MATERIALS AND METHODS

Chemicals

The sources of materials used in this study

were as follows: ATP and NADP from Boehringer-Mannheim-Yamanouchi (Tokyo), yeast glucose 6-phosphate dehydrogenase (292 U/mg protein) from Oriental Yeast (Osaka), 3-(*N*-morpholino)propane-sulfonic acid (MOPS) from Dojindo Laboratories (Kumamoto).

Methods

Assay The activity of hexokinase was determined in a mixture of 1 ml containing 0.1 M MOPS-KOH, pH 6.85, 5 mM D-glucose, various concentrations of ATP and MgCl_2 , 0.1 mM NADP, 1 U glucose 6-phosphate dehydrogenase in the absence or presence of different concentrations of AlCl_3 and polyamine. These components were pre-mixed at 37°C , and the reaction was started by the addition of hexokinase. Increase in absorbance at 340 nm was measured for 1-5 min.

Preparation of human brain hexokinase Hexokinase was partially purified from autopsied human brain by DEAE-cellulose chromatography according to Grossbard and Schimke (5) and the peak fractions were

Fig. 1 Effect of the MgATP concentrations on the activity of human brain hexokinase in the absence or presence of different concentrations of aluminum ion. Reaction mixture was prepared at pH 6.85. MgCl_2 was added to obtain the indicated concentration of MgATP. AlCl_3 was pre-mixed with ATP and the reaction was started by the addition of hexokinase. A: MgATP saturation curves in the absence ($\bullet$) and presence of $3 \mu\text{M}$ ($\circ$) or $5 \mu\text{M}$ ($\square$) aluminum ion. B: Double reciprocal plot. Points represent experimental data; lines in A are theoretically drawn according to the non-linear regression analysis (3).

pooled. The concentrated fractions were dialyzed against 0.1 M MOPS-KOH (pH 6.85).

RESULTS

Inhibition of Hexokinase by Aluminum Ion

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

Fig. 2 Effect of aluminum ion on the activity of human brain hexokinase in the presence of different concentrations of ATP. Reaction mixture was similar to that in legend to Fig. 1, except that AlCl_3 concentrations were varied. A: Inhibition curves. B: Dixon plot. $\circ$, 0.75 mM ATP; $\triangle$, 0.5 mM ATP; $\square$, 0.3 mM ATP

K_i value of about $1.8 \mu\text{M}$.

Reversal by Polyamine of the Al^{3+} -Inhibition of Hexokinase

Addition of spermine markedly prevented the hexokinase from the inhibition by Al^{3+} . In the presence of 2 mM spermine, only 25% of the hexokinase activity was inhibited by $10 \mu\text{M}$ Al^{3+} at physiological ATP concentrations (Fig. 3).

Effect of various polyamines on the Al^{3+} -inhibition of hexokinase was examined. We added spermine, spermidine and putrescine to the hexokinase reaction mixture in the presence of $5 \mu\text{M}$ Al^{3+} . Spermine and spermidine effectively reversed the Al^{3+} -inhibition of the enzyme, but putrescine failed to remove the inhibition (Fig. 4). Other diamines including cadaverine and diaminodecane were ineffective. Furthermore, all polyamines showed no direct stimulating effect on the hexokinase reaction in the absence of Al^{3+} (data not

Fig. 3 Effect of spermine on the inhibition of hexokinase by aluminum ion. Reaction mixture was similar to that in Fig. 2, except that 1.8 mM or 1.3 mM ATP was used, and that 2 mM spermine was included. $\circ$, $\bullet$, 1.8 mM MgATP; $\triangle$, $\blacktriangle$, 1.3 mM MgATP. Open symbols, spermine not included; closed symbols, spermine included

Fig. 4 Effect of polyamine on the activity of hexokinase in the presence of 5 μ M aluminum chloride. Reaction mixture was similar to that in Fig. 3, but MgATP was fixed at 1.8 mM and spermine, spermidine, or putrescine was included as indicated. $\circ$, spermine; $\triangle$, spermidine; $\square$, putrescine

shown).

DISCUSSION

Aluminum is closely related to some types of encephalopathy, and action of aluminum on several enzymes was demonstrated and discussed in relation to the pathogenesis of dementia (4, 7, 8). A most striking effect of aluminum is its inhibition of hexokinase, which may be responsible for the impairment of brain energy metabolism in diseases with dementia (6, 13). Human brain hexokinase was also inhibited by aluminum ion competitively with respect to the substrate MgATP. The affinity of the human enzyme for aluminum ion was higher than that of the yeast and rat brain enzyme (6, 13). The mechanism of the aluminum-inhibition of the human brain enzyme appears to be somewhat different from that of the yeast enzyme, which shows uncompetitive and competitive inhibition at lower and higher MgATP concentrations, respectively (13). However, uncompetitive inhibition of the yeast enzyme by aluminum can be observed only at considerably lower MgATP concentrations (below 0.2 mM). Competitive nature of the aluminum-inhibition of the human enzyme is in good agree-

ment with the inhibition pattern of the yeast enzyme at physiological concentrations of intracellular MgATP (13). Inhibition of hexokinase and glucose utilization by Al^{3+} may be involved in Al^{3+} neurotoxicity; Al^{3+} at micromolar range did not inhibit the activity of glycolytic enzymes other than hexokinase (6, 13).

Aluminum-inhibition of hexokinases can be reversed by various compounds including citrate, 3-phosphoglycerate, phosphate, and malate (13); it appears that these substances exert their action by forming coordination complexes with aluminum. It should be noted that polyamine is the only non-chelating substance that reverses the aluminum-inhibition of hexokinase. Polyamine does not seem to directly activate hexokinase; the enzyme was not stimulated by polyamine in the absence of aluminum. In view that polyamine tightly binds to polyphosphate compounds (15), and that the reversing effect on aluminum-inhibition is decreased when polyamine exists in excess, polyamine-ATP complexes seem to compete with the aluminum-ATP at specific sites of the enzyme.

In contrast with a potent inhibitory effect of aluminum ion on isolated hexokinase, the ion inhibits only weakly the glycolytic flux in rat brain extract. The concentration of Al^{3+}

necessary for 50% inhibition of lactate production in cell-free extract of rat brain was 280 μ M (6). The value is markedly higher than the K_i value (1.8 μ M) for the human enzyme and $I_{0.5}$ value (4 μ M) for the rat brain enzyme. Increase in intracellular polyamines can participate in the reversal of the inhibition of hexokinase by aluminum, resulting in a decrease in the inhibition of lactate production both *in vivo* and *in situ*.

Polyamine accumulates with cell proliferation in animal tissues (1, 9, 12), and directly contributes to the stimulation of glycolysis: polyamine can stimulate phosphofructokinase (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.41) by an increase in ammonium ion produced through the activation of AMP deaminase (EC 3.5.4.6) (16, 17). In proliferative cells which contain higher levels of polyamines, glycolysis can be inhibited by aluminum ion only slightly, but can be stimulated by polyamines. Aging of cells causes a decrease in polyamines, which can augment the aluminum-induced inhibition of hexokinase. Aluminum may exert its toxic effect by inhibiting brain energy metabolism when polyamines are decreased with aging. Accumulation of aluminum and a decrease in polyamines may be involved in the pathogenesis of senile dementia.

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