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Inactivation of Bakers' Yeast Glucose-6-phosphate Dehydrogenase by Aluminum†

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ABSTRACT: Preincubation of yeast glucose-6-phosphate dehydrogenase (G6PD) with Al(III) produced an inactive enzyme containing 1 mol of Al(III)/mol of enzyme subunit. None of the enzyme-bound Al(III) was dissociated by dialysis against 10 mM Tris-HCl, pH 7.0, containing 0.2 mM EDTA at 4 °C for 24 h. Citrate, $NADP^+$, EDTA, or NaF protected the enzyme against the Al(III) inactivation. The Al(III)-inactivated enzyme, however, was completely reactivated only by citrate and NaF. The dissociation constant for the enzyme–aluminum complex was calculated to be 4×10^{-6} M with NaF, a known reversible chelator for aluminum. Modification of histidine and lysine residues of the enzyme with diethyl pyrocarbonate and acetylsalicylic acid, respectively, inactivated the enzyme. However, the modified enzyme still bound 1 mol of Al(III)/mol of enzyme subunit. Circular dichroism studies showed that the binding of Al(III) to the enzyme induced a decrease in α-helix and β-sheet and an increase in random coil. Therefore, it is suggested that inactivation of G6PD by Al(III) is due to the conformational change induced by Al(III) binding.

Environmental stress such as acid rain has aroused interest in aluminum toxicity (Godbold & Huttermann, 1988). Although a unified hypothesis for aluminum toxicity remains to be established, Al(III) affects diverse cellular functions. For example, it inhibits (i) synaptosomal uptake systems with some selectivity toward the uptake of choline, glutamate, and γ-aminobutyric acid (Lai et al., 1980, 1982; Wong et al., 1981), (ii) in vitro assembly of tubulin into microtubules (Macdonald et al., 1987), and (iii) mitosis in murine cells in tissue culture (Jones et al., 1986). Al(III) affects the brain microsomal protein synthesis in immature rats (Magour & Maser, 1981) and the activity of the regulatory component of adenylate cyclase by fluoride (Northup et al., 1983). Al(III) binds to iron storage proteins such as transferrin (Cochran et al., 1984) and ferritin (Fleming & Joshi, 1987) and induces structural and functional changes in calmodulin (Siegel & Haug, 1983). Al(III) inhibits several enzymes such as acetylcholinesterase (Marquis & Lerric, 1982), Na^+,K^+-ATPase (Lai et al., 1980), ferroxidase (Huber & Frieden, 1970), dihydropteridine reductase (Altmann et al., 1987), and hexokinase from brain and yeast (Womack & Colowick, 1979).

We report here that Al(III) also inactivates yeast glucose-6-phosphate dehydrogenase, the first enzyme in the pentose phosphate pathway. Inactivation of G6PD[1] by specific reagents has shown that lysine and histidine residues are involved in the catalytic activity (Kuby & Roy, 1976; Jeffery et al., 1985; Domashke et al., 1969). We used a similar approach to determine the role of these amino acid residues in the binding of Al(III). The enzyme from yeast was chosen

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[1] Abbreviations: DEPC, diethyl pyrocarbonate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; G6P, glucose 6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; ASA, acetylsalicylic acid; CD, circular dichroism.

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for these studies because it has been well studied.

Experimental Procedures

Materials. Glucose-6-phosphate dehydrogenase from bakers' yeast (type XV), $NADP^+$, glucose 6-phosphate, EDTA, malate, sodium fluoride, acetylsalicylic acid, diethyl pyrocarbonate, and citrate were purchased from Sigma. $AlCl_3 \cdot 6H_2O$ was purchased from Fisher Scientific. Aluminum standard solution was purchased from Spex Industries, Inc.

Measurement of Aluminum. Concentration of Al(III) was measured with an Instrumentation Laboratory atomic absorption spectrophotometer 551 equipped with a graphite furnace atomizer 655. Pyrolytically coated graphite tubes and pyrolytical platforms were used with 10-μL aliquots. Before use, the tubes and platforms were conditioned by being heated twice at 2800 °C. The 309.2-nm Al(III) wavelength, a 160-μm slit width, and a lamp current of 8 mA were used. Background correction was performed throughout.

Enzyme Assay. Standard reaction mixture contained 2 μmol of G6P, 1 μmol of $NADP^+$, and 25 μmol of HEPES, pH 7.0. The reaction was started by the addition of enzyme. Various other reactants were added as required. The final volume of the reaction mixture was 1.0 mL. The enzyme activity was measured by following the initial rate of the formation of NADPH monitored at 340 nm at 25 °C. No Mg^{2+} was added unless otherwise indicated. Protein was monitored at 220 or 280 nm spectrophotometrically or assayed by the method of Larson et al. (1986).

Inactivation of G6PD by Al(III) and Quantitation of Al(III) Binding to G6PD. To determine the amount of Al(III) bound to the enzyme in a typical experiment, 3 nmol of enzyme was incubated at 25 °C for 20 min with 300 nmol of aluminum chloride in 100 mM Tris-HCl, pH 7.0, in a final volume of 200 μL. The completely inactivated enzyme was applied to a Sephadex G-25 column (1 × 30 cm), equilibrated with the same buffer. The protein was eluted with the same buffer and monitored spectrophotometrically at 220 nm. The fractions containing the protein were collected and assayed for protein and Al(III). An aliquot of the protein was also dialyzed twice against 2000 volumes of 10 mM Tris-HCl, pH 7.0, at 4 °C for 24 h with or without 0.2 mM EDTA and assayed for Al(III).

Fluoride (Brosset & Orring, 1943) was used to remove Al(III) from the enzyme–metal ion complex (E–Al). E–Al was prepared as described above and incubated with varying amounts of NaF. The Al–F complex was separated from the E–Al by centrifugation for 10 min at 2900 rpm in Centrifree filters (Amicon). The Al(III) concentration in the filtrate was used to measure the amount of Al(III) removed from E–Al by NaF.

Effect of Modification of Histidine on Al(III) Binding. Modification of histidine in G6PD with diethyl pyrocarbonate (DEPC) was performed by the method of Ovadi et al. (1967). A total of 1 mg of enzyme was incubated at 25 °C with 10 mM DEPC in 25 mM HEPES, pH 6.0, in a final volume of 1 mL. DEPC was dissolved in 95% ethanol, and the final concentration of ethanol was kept below 10% during the incubation of the enzyme with the modifying agent. DEPC concentration was diluted at least 10-fold during the assay for enzyme activity. The formation of *N*-carbethoxyhistidyl was monitored at 240 nm with the extinction coefficient of 3200 M^{-1} cm^{-1} at 240 nm (Ovadi et al., 1967). After the absorbance at 240 nm ceased to increase, the modified enzyme was incubated with a 100-fold molar excess of aluminum in 25 mM HEPES, pH 7.0, for 20 min. Free aluminum was removed by dialysis against 10 mM HEPES, pH 7.0, at 4 °C for 24 h, and the dialyzed enzyme was assayed for aluminum and enzyme activity.

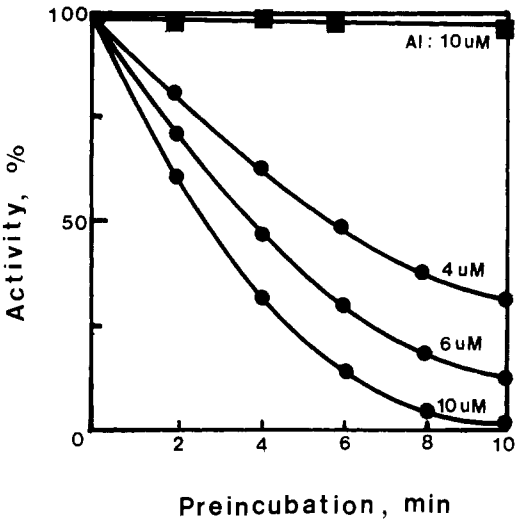

FIGURE 1: Time- and pH-dependent inactivation of G6PD by $AlCl_3$. $AlCl_3$ was added to the enzyme solution in 25 mM HEPES buffer, pH 8.0 (■) and pH 7.0 (●), at 25 °C. At indicated times, the remaining activity was assayed by the addition of the reaction mixture. This diluted the concentration of aluminum 10-fold during the assay.

Effect of Modification of Lysine on Aluminum Binding. Modification of lysine residues was achieved by the method of Jeffery et al. (1985). A total of 1 mg of enzyme was incubated at 25 °C with 1.5 mM acetylsalicylic acid in 10 mM Tris-HCl, pH 8.5, in a final volume of 1 mL. After 30 min, further acetylsalicylic acid was added to a final concentration of 3 mM, and aliquots withdrawn at different times were assayed for enzyme activity. When 95% of the enzyme activity was abolished, an equal volume of 150 mM ethanolamine acetate at pH 6.0 was added to stop the reaction. Reagents were removed by dialysis against 10 mM Tris-HCl, pH 7.0. The modified enzyme was treated with aluminum as described above (see histidine modification) and assayed for aluminum.

Circular Dichroism Study. A total of 0.4 mg of enzyme was incubated with or without 0.5 mM $AlCl_3$ in 25 mM HEPES, pH 7.0, at 25 °C for 30 min and dialyzed against the same buffer. The spectrum of the inactive enzyme–aluminum complex was compared to that of the control enzyme. CD measurements were made in a Jasco J-500 C spectrophotometer using 0.02-cm pathway cell (Hellma Scientific Suprasil quartz) at room temperature. CD results were reported in terms of $[\theta]$ (deg cm^2 $dmol^{-1}$) with a computer program modulating the spectrophotometer. CD analyses were performed by the method of Yang et al. (1986) with reading from a "smoothed" curve through five successive point window average assigned to middle to determine the secondary conformation.

Results

Inactivation of Yeast Glucose-6-phosphate Dehydrogenase by Aluminum. Preincubation of yeast G6PD with $AlCl_3$ in 25 mM HEPES buffer, pH 7.0, at 25 °C produced an inactive enzyme. The loss of enzyme activity was proportional to the preincubation time and to the concentration of aluminum (Figure 1). The inactivation was pH dependent. A marked increase in the sensitivity to Al(III) was observed as the pH decreased. Above pH 8.0, aluminum did not affect the rate of the enzymatic reaction (Figure 1). The studies described here were performed at pH 7.0.

To quantitate the Al(III) bound to the enzyme, an inactive E–Al complex was prepared, excess Al(III) was removed by

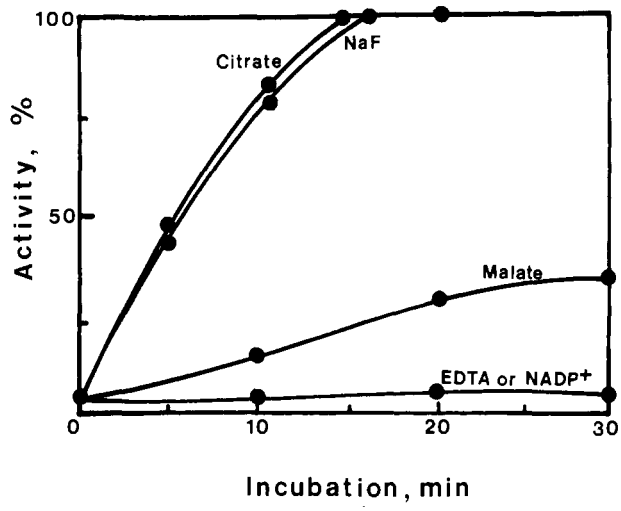

FIGURE 2: Reactivation of aluminum-inactivated G6PD by several chelators. Reaction mixtures containing the enzyme and aluminum chloride in 25 mM HEPES buffer, pH 7.0, were preincubated at 25 °C for 20 min. Chelators were added to the reaction mixture in a final volume of 1.0 mL. Final concentrations of aluminum chloride and the chelators were 50 μM and 2 mM, respectively. At different times, aliquots were withdrawn and assayed for activity.

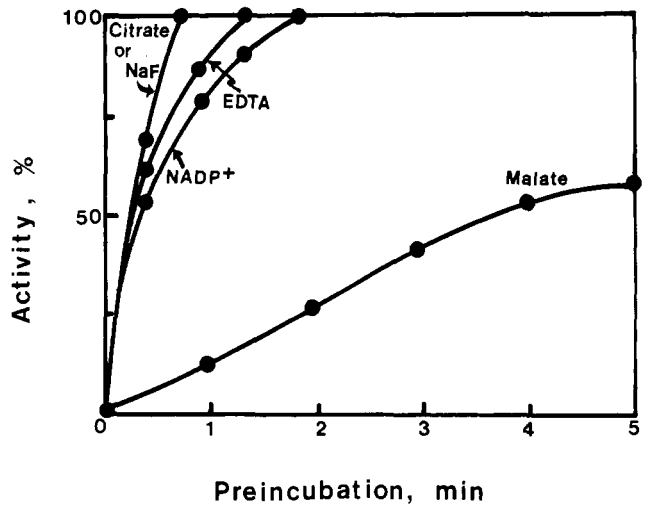

FIGURE 3: Effect of preincubation of Al(III) with the chelators on G6PD. Preincubation mixture contained 25 μmol of HEPES buffer, pH 7.0, 50 nmol of aluminum chloride, and 2 μmol of the indicated effectors in a final volume of 0.9 mL and was kept at 25 °C for various times. The reaction was started by the addition of enzyme and assay mixture with a final concentration of 2 mM G6P and 1 mM $NADP^+$ in 25 mM HEPES buffer, pH 7.0.

gel filtration or by dialysis with or without EDTA (see Experimental Procedures), and the protein-bound Al(III) was quantified. The results showed that for a subunit molecular weight of 50 000 (Kawaguchi & Bloch, 1974) the Sephadex-treated enzyme contained 0.84 mol of Al per subunit, that dialyzed against buffer contained 0.8 mol of Al per subunit, and that dialyzed against EDTA contained 0.76 mol per subunit.

On the basis of a gel filtration study (Sephadex G-150) the molecular weight of the native enzyme was similar to that of the enzyme inactivated by Al(III) (data not shown). Therefore, Al(III) did not dissociate the active enzyme. These results indicate that there is a rather specific locus for the Al(III) binding on the enzyme.

Protection and Reactivation of G6PD by Chelators against Al(III) Inactivation. Figure 2 shows that the inhibitory effect of Al(III) is abolished by some chelators. A sample of the inactivated G6PD–aluminum complex, prepared as above, was treated with the known chelators for Al(III) (Womack & Colowick, 1979; Karlik et al., 1983) such as citrate, malate, EDTA, NaF, and $NADP^+$ at final concentrations of 2 mM. At different times, aliquots were withdrawn and assayed for the activity in the standard reaction mixture. The aluminum inhibition could be fully reversed only by citrate or NaF. Full activity was restored in 15 min. EDTA, even at 2 mM concentration did not reactivate the aluminum-inactivated enzyme. In a related experiment (Figure 3), Al(III) was preincubated with the chelators for various times and then tested as a source of aluminum available to inhibit the enzyme. The results showed that the rate of chelation of aluminum by malate was the slowest because a significant amount of free Al(III) was retained in a solution to produce 50% inhibition even after 5 min. Alternatively, it is likely that Al(III)–malate complex was also inhibitory to G6PD. This possibility was not explored, however. By contrast, only very small amounts of free Al(III) seemed to have remained in solution in the presence of citrate, EDTA, NaF, or $NADP^+$, as evidenced by the full recovery of the enzyme activity after 2 min of incubation with these chelators.

Measurement of Dissociation Constant for the Enzyme–Aluminum Complex. Dissociation constant of aluminum for the E–Al complex was calculated to be 4×10^{-6} M by an indirect method using NaF, a known reversible ligand for aluminum which forms AlF_3 (Brosset & Orring, 1943). Accordingly

$$\text{E–Al} \rightleftharpoons \text{Al} + \text{E} \qquad {}^{\text{Al}}K_d(\text{E–Al}) = \frac{[\text{Al}][\text{E}]}{[\text{E–Al}]} \quad (1)$$

$$\text{F} + \text{Al} \rightleftharpoons \text{Al–F} \qquad {}^{\text{Al}}K_a(\text{F}) = \frac{[\text{Al–F}]}{[\text{Al}][\text{F}]} \quad (2)$$

$$\text{sum: E–Al} + \text{F} \rightleftharpoons \text{E} + \text{Al–F} \qquad K_{eq} = \frac{[\text{E}][\text{Al–F}]}{[\text{E–Al}][\text{F}]} \quad (3)$$

Here [E], [Al], and [F] are unliganded forms. Thus the equilibrium expression (eq 3) of the reaction carried out is the sum of two individual elementary reactions (eq 1 and 2). From these three reactions

$$K_{eq} = \frac{[\text{E}][\text{Al–F}]}{[\text{E–Al}][\text{F}]} = {}^{\text{Al}}K_d(\text{E–Al})\,{}^{\text{Al}}K_a(\text{F}) \quad (4)$$

When half of the total Al(III) is removed from the enzyme and assuming that no significant E–F is present, the concentrations of enzyme and E–Al are equal. Therefore, eq 4 simplifies to

$$\frac{[\text{Al–F}]}{[\text{F}]_{\text{Total}} - [\text{Al–F}]} = {}^{\text{Al}}K_d(\text{E–Al})\,{}^{\text{Al}}K_a(\text{F}) \quad (5)$$

Half-maximal removal of aluminum (20 μM) was achieved at 25 μM NaF (Figure 4). We then substitute the value of ${}^{\text{Al}}K_a(\text{F})$ [from Brosset and Orring (1943)] and of $[\text{Al–F}]/([\text{F}]_{\text{Total}} - [\text{Al–F}])$ and calculated the dissociation for E–Al:

$$\frac{20 \times 10^{-6}}{(25 \times 10^{-6}) - (20 \times 10^{-6})} = {}^{\text{Al}}K_d(\text{E–Al})(1 \times 10^{6})$$

Therefore, ${}^{\text{Al}}K_d(\text{E–Al}) = 4 \times 10^{-6}$ M.

Effect of Modification of G6PD with Acetylsalicylic Acid or DEPC on Al(III) Binding. Consistent with the earlier observation (Jeffery et al., 1985), modification of one lysine residue of yeast G6PD with acetylsalicylic acid inactivated the enzyme. However, the modification did not affect the stoichiometry of the aluminum binding to the protein (Table I). One mole of aluminum still bound per mole of the modified

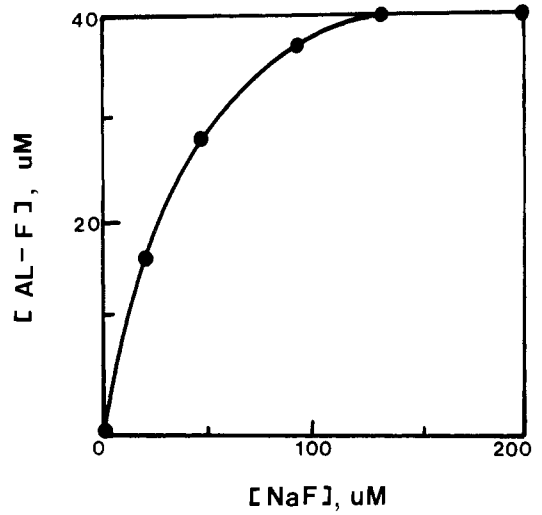

FIGURE 4: Removal of aluminum from enzyme–aluminum complex by NaF. The completely inactivated enzyme (protein concentration 20 μM containing 40 μM aluminum) was prepared as described under Experimental Procedures and incubated with increasing levels of NaF (final concentration 200 μM) in 20 mM Tris-HCl, pH 6.5, at 25 °C for 2 h in a 1-mL final volume. Aluminum released from the enzyme–aluminum complex was assayed as described under Experimental Procedures.

Table I: Effects of Treatments of G6PD with ASA or DEPC on the Enzyme Activity and Aluminum Binding to the Enzyme[a]

treatments	enzyme activity (% of control)	[Al]/[subunit][b]
enzyme (control)	100	0.07 (0)
enzyme + aluminum	0	0.80 (1)
ASA-modified enzyme	0	0.07 (0)
ASA-modified enzyme + aluminum	0	0.80 (1)
DEPC-modified enzyme	0	0.07 (0)
DEPC-modified enzyme + aluminum	0	0.80 (1)
DEPC-modified enzyme in the presence of G6P (2 mM) and Mg^{2+} (5 mM)	96	0.08 (0)
DEPC-modified enzyme in the presence of G6P (2 mM) and Mg^{2+} (5 mM) + aluminum	0	0.80 (1)

[a] The DEPC- or ASA-modified enzymes were prepared as described under Experimental Procedures, and the enzyme activities and aluminum contents were measured. The modified enzymes (protein concentration 5 μM) were separately incubated with 50 μM $AlCl_3$ and dialyzed. The enzyme activities and the contents of aluminum in the dialyzed samples were measured. [b] The numbers in parentheses are the nearest integer values. Removal of the traces of Al(III) from the control by dialysis against citrate (0.1 mM, pH 6.5) followed by removal of citrate against HEPES (25 mM, pH 6.0) did not increase the activity of the enzyme.

enzyme subunit. Therefore, it appears that the reactive lysine residue is not directly involved in the aluminum binding site.

Preliminary experiments showed that the Al(III) binding to the enzyme-induced spectral changes around 220 nm. In order to determine whether this was due to formation of a histidine–aluminum complex, diethyl pyrocarbonate (DEPC) was used as a group-specific reagent. DEPC specifically reacts with histidine residues in proteins at pH 6.0 to yield an ethoxycarbonyl derivative with a characteristic absorption maximum at 240 nm (Pradel & Kassab, 1968). The result showed the loss of the enzyme activity paralleled the binding of DEPC until 7 mol of DEPC was bound to the enzyme (Figure 5A). Quantitation from the spectral changes at 240 nm showed that the native enzyme and that denatured in 6 M urea contained 7 and 14 histidine residues, respectively (Figure 5B). Domschke et al. (1969) showed that G6P and Mg^{2+} protected

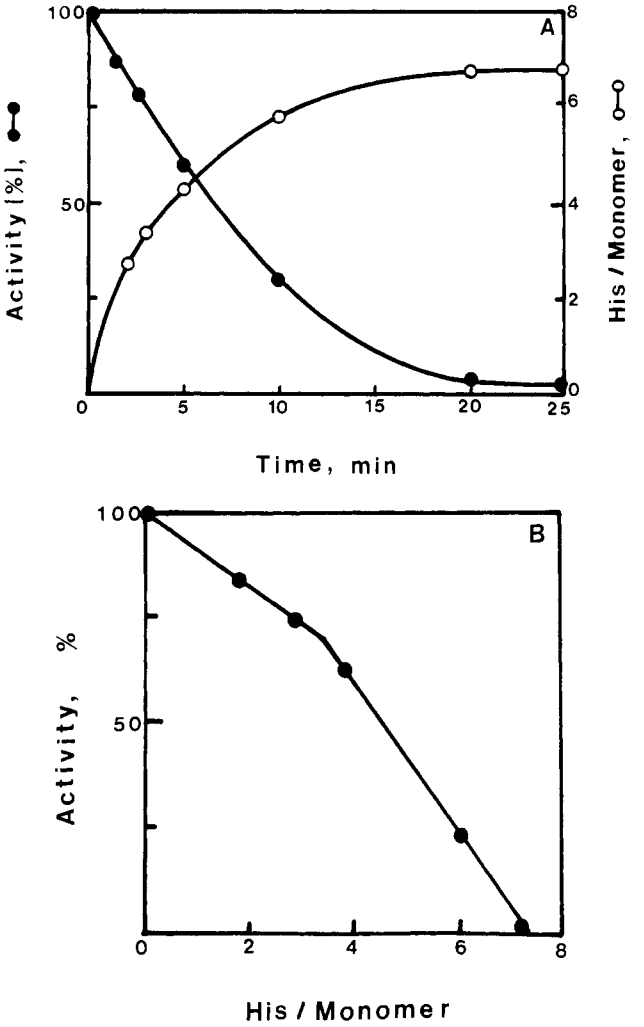

FIGURE 5: (A) Time course of DEPC incorporation into G6PD and inactivation of the enzyme. Enzyme (10 nmol) was incubated at 25 °C with 7 mM DEPC in 25 mM HEPES, pH 6.0. At indicated times, aliquots were withdrawn and diluted with an assay mixture for the enzyme activity or with the same buffer for the DEPC-induced spectral changes as described under Experimental Procedures. (B) Stoichiometry of DEPC incorporation and G6PD inactivation showing the number of histidine residues modified per monomer. The inactivation of G6PD is plotted as a function of moles of DEPC incorporated per mole of enzyme subunit. This is a replot of the data shown in (A).

the histidine residue of G6PD from *C. utilis* against photooxidation. As shown in Figure 6, DEPC did not inactivate the yeast G6PD in the presence of 2 mM G6P and 5 mM Mg^{2+}. Also, in the presence of these two reagents six rather than seven histidine residues were modified by DEPC. Thus, G6P and Mg^{2+} protected the catalytically essential histidine residue against DEPC-induced inactivation, but either the presence or the absence of these reagents did not protect the enzyme against Al(III) inactivation (Table I). In a separate experiment, the enzyme was preincubated with 5 mM dithiothreitol (DTT) in 25 mM HEPES, pH 7.0, at 25 °C for 15 min with or without 50 μM Al(III), and the resulting activities were compared with the corresponding controls. The results showed that DTT did not affect the activity of the enzyme or the inactivation by Al(III). Taken together, these data confirm the presence of lysine and histidine residues at the catalytic site of G6PD (Jeffery et al., 1985; Domschke et al., 1969), but exclude them, as well as cysteine residues, from being directly involved in the Al(III)-induced inactivation. These data suggest that the Al(III) binding site is distinct from the catalytic site, and the inactivation of G6PD by Al(III)

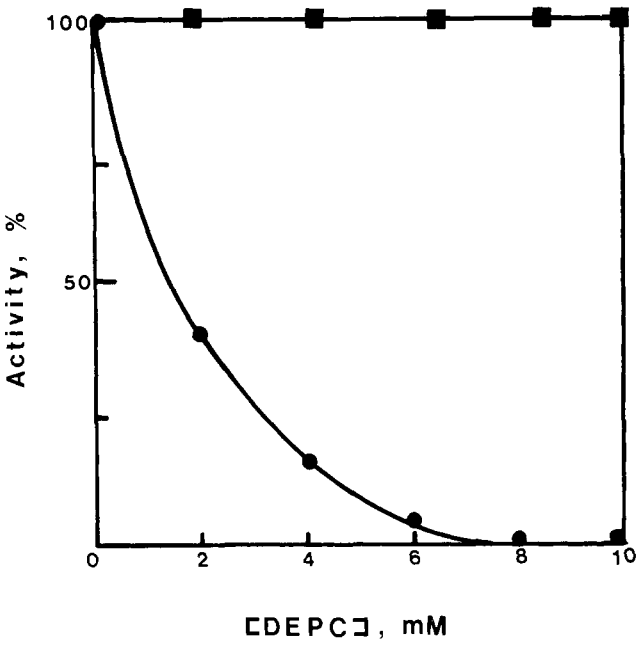

FIGURE 6: Protection of G6PD by G6P and Mg^{2+} against DEPC-induced inactivation. The enzyme (10 nmol) was treated with increasing concentrations of DEPC in the presence (■) and absence (●) of 2 mM G6P and 5 mM Mg^{2+} for 20 min at 25 °C in 25 mM HEPES, pH 6.0, and assayed for enzyme activity.

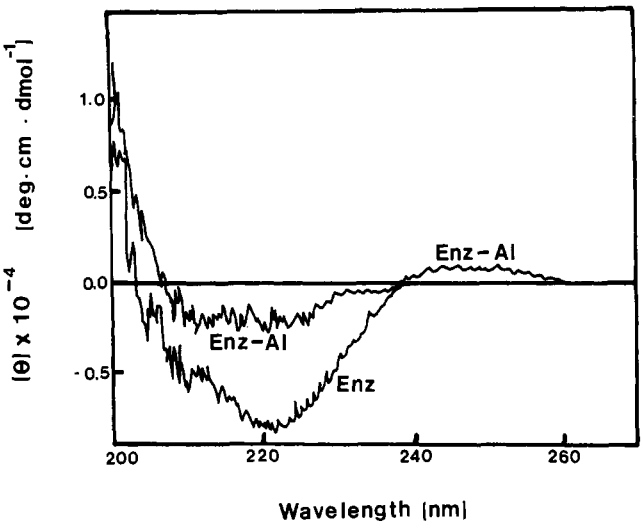

FIGURE 7: Circular dichroism spectra of G6PD and G6PD–aluminum complex at pH 7.0. See text for detail.

might be due to the conformational changes induced by the metal ion.

Circular Dichroism Study of Al(III)-Induced Structural Changes. The results of the circular dichroism studies on the G6PD–aluminum complex are shown in Figure 7. With the method of Yang et al. (1986), the data in Figure 7 were used to quantify the conformational change induced by the binding of Al(III) to the enzyme. As seen, the binding of Al(III) to the enzyme induced a reduction in the ordered configuration (α-helix and β-pleated sheet) and an increase in random coil. Spectral signals at 200–250-nm range showed that the conformation of the aluminum-free enzyme was 30% α-helix, 30% β-pleated sheet, and 40% random coil, whereas that of the aluminum–enzyme complex was 20% α-helix, 15% β-pleated sheet, and 65% random coil. Large negative peaks in the native enzyme were apparent at 208 and 222 nm, respectively. Since random and β-conformations approach to zero at 208 nm, the signal at 208 nm is due to α-helix conformation. Increase in the β-turn conformation may be due to the relaxed helical configuration since the signal for 3_{10} helix was similar to that of β-turn.

Discussion

The results presented here clearly establish the sensitivity of bakers' yeast G6PD to a low level of Al(III). The sensitivity of the yeast enzyme to Al(III) suggests that the inhibition of this enzyme may be relevant to Al(III) toxicity. The inhibition is more pronounced at acidic pH, and this is consistent with the profound solubility of Al(III) at low pH. The suggestion that Al(III) functions only at acidic pH (MacDonald & Martin, 1988), therefore, may be a general characteristics of the reactions involving this metal ion.

It should be noted that Al(III) concentrations presented in this study are those of Al(III) added to the solution, and the calculated K_d value of 4×10^{-6} M is based on the total concentration of Al(III). The same is true for numerous studies reported by others (Crapper-McLachlan et al., 1983; Lange et al., 1986; Altmann et al., 1987; Womack & Colowick, 1979). Solubility of aluminum salts is very high at acidic pH and very low at physiological pH (Macdonald & Martin, 1988). At acid pH, this is due to hydrolysis [$Al(H_2O)_6^{3+}$] rather than increased solubility of any particular salt of aluminum. This is also true at higher pH [$Al(OH)_4^-$] as well. This would explain why Al(III) is a better inhibitor of G6PD at acidic pH. The free Al^{3+} concentration rather than the much greater $Al(OH)_4^-$ concentration will be therefore the relevant quantity in assessing ligation and in determining stability constants for such association (Macdonald & Martin, 1988).

Therefore, it is highly possible that the aluminum concentration, at which inhibitory effects on enzymatic reaction occur, will actually be much less than those reported in this study as well as in previous studies (Lange et al., 1986; Altmann et al., 1987; Womack & Colowick, 1979; Huber & Frieden, 1970; Cochran et al., 1984).

The importance of the order of addition of the reagents to produce the observed effects of Al(III) on G6PD and the reactivation of the Al(III)-inactivated enzyme by citrate or NaF, but not by EDTA, malate, or $NADP^+$ (Figures 2 and 3), are similar to those observed for hexokinase (Womack & Colowick, 1979).

The protection of G6PD by $NADP^+$ against Al(III) inactivation is of particular interest. A very similar protection was reported for the Al(III) binding to RNA polymerase (Crapper-McLanchlan, 1983) in which preincubation of the RNA polymerase with 0.5 mM Al(III) decreased the rate of incorporation of nucleotides by 50%, but when Al(III) was premixed with the nucleotides or with the template, there was no inhibition until the Al(III) concentration exceeded 2 mM. These protective effects are most likely a result of the phosphate of $NADP^+$ or nucleotides complexing with the metal ion (Karlik et al., 1983).

Modification of histidine of the G6PD from *C. utilis* by photooxidation (Domashke, 1969), of lysine of the enzyme from *L. mesenteroides* by pyridoxal 5-phosphate (Haghighi, 1982), or of lysine of the yeast enzyme by acetylsalicylic acid (Jeffery et al., 1985) produced inactive enzyme. We chose acetylsalicylic acid and DEPC to modify lysine and histidine residues, respectively, because of the specificity of DEPC (Ovadi et al., 1967) and the successful application of acetylsalicylic acid to the yeast enzyme (Jeffery et al., 1985). The results in this study confirm the role for histidine and lysine residues at the active site but exclude them, as well as the sulfhydryl groups, from being involved in Al(III) binding. The

possibility of the aluminum binding to the carboxyl groups was not explored. It appears likely that the inactivation may be due to conformational changes induced by Al(III) binding to the carboxyl or hydroxyl groups. The results of circular dichroism studies support this possibility. This result shown in Figure 7 is very similar to that observed for structural changes induced by Al(III) in calmodulin (Siegel et al., 1983), showing that the Al–calmodulin structure is a more random, open polypeptide than the structure of Ca–calmodulin on the basis of their EPR resonance studies. Also, Al(III)-induced structural changes in calmodulin decrease helical content, increase random coiling, and increase hydrophobic surface expression (Siegel & Haug, 1983).

The protection of the G6PD by citrate against Al(III) binding is also very similar to the previous observation (Suhayda & Haug, 1984), showing that at a molar ratio of 10:1 for [citrate]/[calmodulin] citrate can prevent Al(III) binding to calmodulin as determined by fluorescence and circular dichroism spectroscopy.

Environmental pollution by soluble Al(III) has elevated aluminum from a "harmless" to a "toxic" metal ion. Indeed, Al(III) is one of the major toxicants to plants and animals (Godbold et al., 1988; Havas & Likens, 1985). This paper shows that the concentration of Al(III) required to produce 50% inhibition of yeast G6PD is similar to that reported for inhibiting hexokinase from brain (Lai & Blass, 1984; Womack & Colowick, 1979). The resulting reduction in glucose utilization is unfavorable for normal growth of yeast. Such effects are likely to be more damaging to brain, a tissue known to accumulate aluminum in Alzheimer's disease and other neuronal disorders. Together with the previous reports on the inhibition of pig brain G6PD by Al(III) (Cho & Joshi, 1988) and Al(III)-induced reduction in glucose utilization in rat brain in vitro (Johnson & Jope, 1986), the results presented here increase the significance of Al(III) toxicity in the regulation of energy metabolism.

Registry No. G6PD, 9001-40-5; His, 71-00-1; Lys, 56-87-1; Al, 7429-90-5.

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