Journal of Sciences, Islamic Republic of Iran 15(4): 315-320 (2004)
University of Tehran, ISSN 1016-1104

Inhibition of Human Erythrocyte Glucose 6-Phosphate Dehydrogenase by Cadmium, Nickel and Aluminium

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Abstract

The inhibition of human erythrocyte glucose 6-phosphate dehydrogenase (G6PD) by Cd (II), Al (III) and N_i (II) was studied. The enzyme was partially purified having specific activity of 1.4 U/mg protein. Cadmium inhibited G6PD activity progressively up to 1.5 mM concentration where about 65% of the enzyme activity was lost. The inhibition was uncompetitive and noncompetitive with respect to glucose 6-phosphate and $NADP^+$, respectively. Cd (II) also increased maximum emission spectrum of the intrinsic protein fluorescence. Sulfhydryl compounds such as glutathione (1.2 mM), β-mercaptoethanol (1.2 mM) or dithiothreitol (1.25 mM) protected the enzyme activity against Cd (II) inhibition and restored the native protein fluorescence. The data suggest that sulfhydryl groups are involved in Cd (II) inhibition. The inhibition patterns for Al (III) were mixed type and competitive when $NADP^+$ and glucose 6-phosphate were the variable substrates, respectively. The enzyme inhibition by Al (III) was increased as the pH of the incubation mixture decreased indicating that mainly the ionized form of Al (III) abolishes the enzyme activity. The types of the enzyme inhibition by N_i (II) were competitive with respect to $NADP^+$ and mixed type when glucose 6-phosphate varied. K_i values of 1.5 mM, 0.039 mM 0.05 mM for Cd (II), Al (III) and N_i (II) were calculated from the slope replots, respectively. The data suggest that direct interaction of these metal ions with human erythrocyte G6PD produces a reversible inhibition of the enzyme and that their toxicity, at least in part, may be due to the inhibition of this enzyme.

Keywords: Glucose 6-Phosphate dehydrogenase; Cadmium; Aluminium; Nickel

Introduction

Glucose 6-phosphate dehydrogenase (G6PD), E (1.1.1.46), the first key enzyme in hexose mono-phosphate shunt, is important in generating NADPH necessary for anabolic pathways. G6PD is an important enzyme in glutathione redox cycle essential for protecting the integrity of erythrocytes [1]. Deficiency or inhibition of G6PD by toxic compounds, may lead to the membrane damage and consequently anemia.

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Among toxic compounds, trace elements affect the cell metabolism and activities of several enzymes. Cadmium, Cd (II), at low concentrations causes transient increase of DNA synthesis [2] but at higher concentrations its toxic effect appears as inhibition of DNA, RNA and protein synthesis [3], lipid peroxidation [4] and impairing the DNA repair system [5]. Cadmium also inhibits the activities of several enzymes such as catalase, superoxide dismutase, glutathione peroxidase [6] and G6PD's from various sources [4,6-8]. Other trace elements such as aluminium, Al (III), and nickel, Ni (II), also affect enzyme activities. Al (III) inhibits the activities of several enzymes including acetylcholine esterase [9], Na^+, K^+-ATPase [10], feroxidase [11], dihydropteridin reductase in hemodialysis patients [12] and hexokinases [13]. Al (III) also affects G6PD activities of human and pig brain [14,15], and yeast [16]. The inhibitory effects of Ni (II) on several enzymes such as malate dehydrogenase [17], α-amylase [18] and G6PD's from rat liver [19] and human fibroblasts [20] have been reported. The mechanisms and the types of human G6DP inhibition by these metals have not been investigated. Kinetic studies of the enzyme inhibition lead to a better understanding of the mechanisms by which trace elements inhibit G6PD.

In the present study the kinetics of human erythrocycle G6PD inhibition by Al (III), Cd (II) and N_i (II) was investigated and the involvement of sulfhydryl groups in Cd (II) inhibition was elucidated.

Materials and Methods

Materials

Human packed erythrocyte cells were obtained from Isfahan Blood Transfusion Service. Glucose 6-phosphate (Sodium salt),$NADP^+$, DEAE-cellulose, phosphor-cellulose, β-mercaptoethanol, EDTA, dithiothreitol and glutathione were obtained from Sigma Co (U.S.A). All other chemicals were reagent grade.

Enzyme Purification

Human erythrocyte G6PD was partially purified by chromatography on DEAE-cellulose and Phospho-cellulose as described by Defora *et al*. [21].

Enzyme Assay

G6PD activity was measured in 0.67 M Tris-HCl buffer pH 9 (unless otherwise specified in the Figure legends) containing 1 mM glucose 6-phosphate and 0.1 mM $NADP^+$ [6]. The increase in the absorbance of NADPH at 340 nm was measured to calculate the enzyme activity.

Enzyme Inhibition

The inhibition of G6PD by Cd (II), N_i (II) and Al (III) was done in the assay mixture containing different concentrations of each cation at 25°C (see Fig. Legends). The incubation times for Al (III) and Cd (II) were 10 and 5 min, respectively. N_i (II) exhibited its effect immediately after incubation. Double reciprocal plots and the corresponding replots were drawn using linear regression analysis. The effects of sulfhydryl compounds on the enzyme inhibition by Cd (II) were investigated by incubating the enzyme solution (11 μg/ml) in the presence of Cd (II) (0.6 mM) and either glutathione (1.2 mM), β-mercaptoethanol (1.2 mM) or dithiothreitol (1.25 mM) for 5 min at 25°C followed by measurement of the enzyme activity. Protein concentration was determined by the method of Lowry *et al*. [22].

Results

Enzyme Purification

G6PD from 2400 ml human erythrocyte packed cell was purified (1273-folld) using the procedure described in the methods and had a specific activity of 1.4 U/mg protein.

Cadmium Effect

Cd (II) inhibited G6PD activity progressively up to 1.5 mM concentration where 65% inhibition was achieved after 5 min (Fig. 1). The presence of glutathione, β-mercaptoethanol or dithiothreitol calculated from the decreased Cd (II)-induced enzyme inhibition (Fig. 1). The inhibition was noncompetitive and uncompetitive with respect to $NADP^+$ and glucose 6-phosphate, respectively (Fig. 2). A K_i of 0.74 mM was slope replot. Cadmium increased maximum emission of protein fluorescence by 123% (Fig. 3). The presence of glutathione, β-mercaptoethanol or dithiothreitol decreased the fluorescence change induced by Cd (II) (Fig. 3).

Aluminium Effect

G6PD activity was progressively inhibited by Al (III) concentration up to 100 μM where 60% of the enzyme activity was lost (Fig. 4). The inhibition was time and pH dependent; concerning pH, maximum inhibition

occurred at pH 7.4 but at alkaline conditions (pH 9) no inhibition was observed after 10 min incubation (Fig. 4). The inhibition was competitive with respect to glucose 6-phosphate but a mixed type pattern was obtained when $NADP^+$ was the variable substrate (Fig. 5). A K_i of 39 µM was calculated from the slope replot. Mixed type inhibition was also confirmed by determining the K_{mapp} values which their differences were significant.

Nickel Effect

N_i (II) also inhibited G6PD activity by 70% at 0.8 mM concentration immediately after incubation (Fig. 6). The inhibition patterns were competitive and mixed type when $NADP^+$ and glucose 6-phosphate varied, respectively (Fig. 7). An inhibition constant (K_i) of 50 µM was calculated from the results.

Discussion

The inhibition of human erythrocyte G6PD by Al (III), N_i (II) and Cd (II) may be relevant to their toxicity particularly for the first two cations with low K_i values. Obviously several mechanisms may be involved in metal toxicity, but G6PD inhibition may also be involved in these mechanisms. The data also demonstrated that direct interaction of these elements with G6PD produces a reversible inhibition of the enzyme activity.

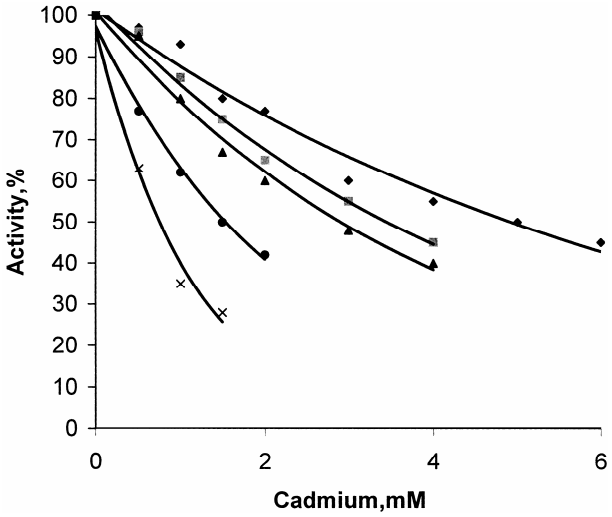

Figure 1. The effect of different concentrations of cadmium on G6PD activity in the absence (×) or presence of 25 mM dithiothreitol (♦), 25 mM β-mercaptoethanol (■), 2.3 mM glutathione (▲) and 25 mM glutathione (●). Cd (II) choride was added to the assay mixture at 25°C and after 5 min the enzyme activity measured as described in Methods. Each point represents the average of two independent experiments.

Cd (II) binds either to the enzyme (E) or to enzyme-substrate (ES) complexes producing binary and ternary complexes, respectively. This results in conformational changes leading to the enzyme inactivation. Protection of the inhibition by glutathione, β-mercaptoethanol or dithiothreitol demonstrated that sulfhydryl groups are involved in Cd (II) binding to the enzyme. The protection by glutathione was concentration dependent indicating that Cd (II) and these sulfhydryl compounds compete for binding to the enzyme. Previous reports have shown that certain toxic cations such as Hg (II) and Cd (II) have high affinity for binding to sulfhydryl groups [23]. The conformational change induced upon Cd (II) binding to G6PD was also prevented in the presence of sulfhydryl group compounds as judged by the change in emission spectrum of the enzyme.

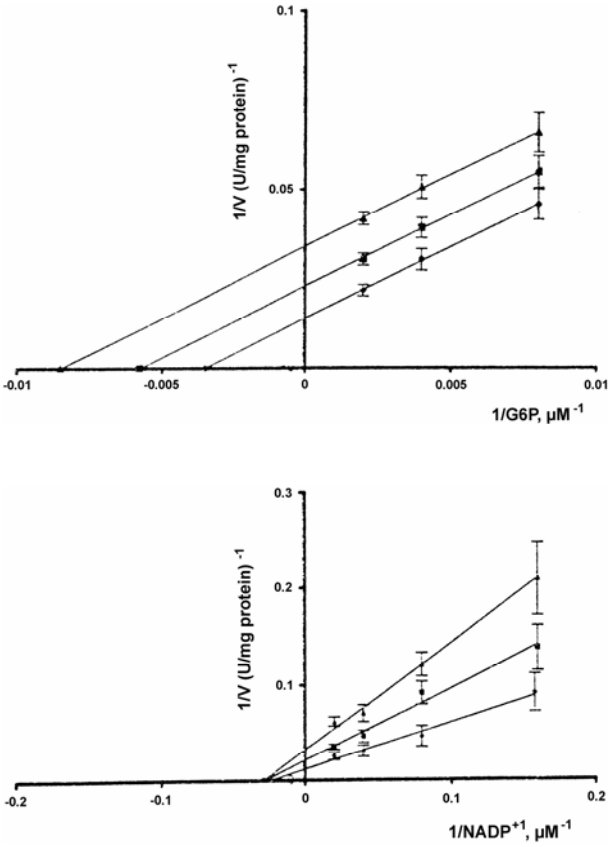

Figure 2. Lineweaver-Burk reciprocal plot of the G6PD in the presence of different fixed concentrations of Cd (II) as an inhibitor. Top: glucose 6-phosphate (G6P) is the varied substrate and $NADP^+$ is fixed at saturated concentration. Bottom: $NADP^+$ is the varied substrate and G6P is fixed at saturated concentration. Cd (II) Concentrations are zero (♦), 0.5 mM (■) and 1 mM (▲). Values represent Mean±S.D. of three independent experiments.

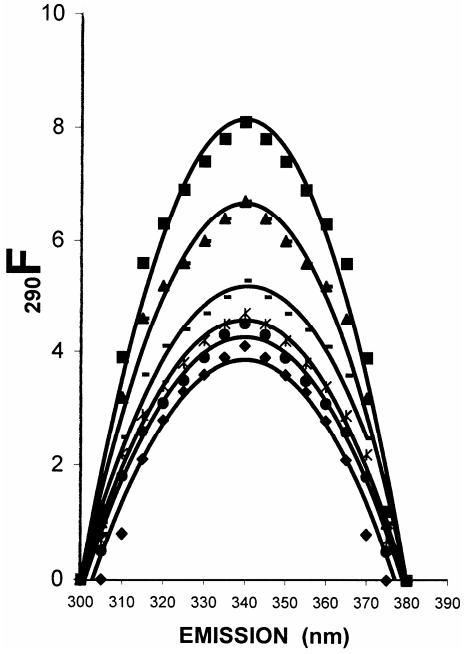

Figure 3. Emission spectra of G6PD in the absence (♦) and presence of Cd (II) (■) and Cd (II) plus 2.3 mM glutathione (▲), 25 mM gluththione (-), 25 mM β-mercaptoethanol (*) or 25 mM dithiothreitol (•) as specified. The enzyme (8 µg/ml) in 0.67 M Tris-HCl buffer pH 9 was incubated with each reagent for 5 min at 25°C and the emission spectra were recorded using excitation wave length of 290 nm. Each point represents the average of three independent experiments.

Cadmium toxicity via food consumption in humans have been reported [24,25]. Cadmium stimulates production of nitrate peroxide and H_2O_2 [26] which in turn causes oxidation of membrane lipids. Cd (II)-induced G6PD inhibition, as observed in this study, diminishes NADPH concentration and stimulates erythrocyte membrane damage. The liver [25] and kidney [27] toxicity by Cd (II) may also cause G6PD inhibition in these organs.

The results of Al (III) study clearly established the sensitivity of human erythrocyte G6PD to a low level of this metal. G6PD inhibition, therefore, may indicate Al (III) toxicity. The pure competitive inhibition observed by Al (III) with respect to glucose 6-phosphate seems surprising since these two molecules are not structurally analogous. It is, therefore, conceivable that metal-induced conformational changes prevent proper substrate binding. The finding that inactivation of G6PD by Al (III) was more pronounced at acidic pH suggests that the inhibition is probably resulted from Al^{+3}-enzyme interaction, probably due to $Al(OH)_4^-/Al^{+3}$ ratio declines following pH decrease [28]. The finding that Al (III) functions only at acidic pH [29], may be a general characteristic of the reactions involving this metal ion. The previous reports of Al (III)-induced anemia [30] and decreased heme synthesis caused by its toxicity [31] may be consistent with its inhibitory effect on G6PD presented in this study. The presence of natural chelators of Al (III) in blood and in erythrocytes such as $NADP^+$, citrate and ATP may prevent its binding to transferrin, ceruloplasmin and G6PD [14].

The mixed type inhibition shown by N_i (II) when glucose 6-phosphate was the variable substrate has a competitive character since the intersection of several straight lines of the reciprocal Lineweaver-Burk plots was in the positive 1/v axis region [32]. If N_i (II) was a cofactor of the erythrocyte enzyme, it might compete with the substrate but this is not the case. The effect of N_i (II) is, therefore, a rather indirect one in which the conformation of the enzyme is altered resulting in the

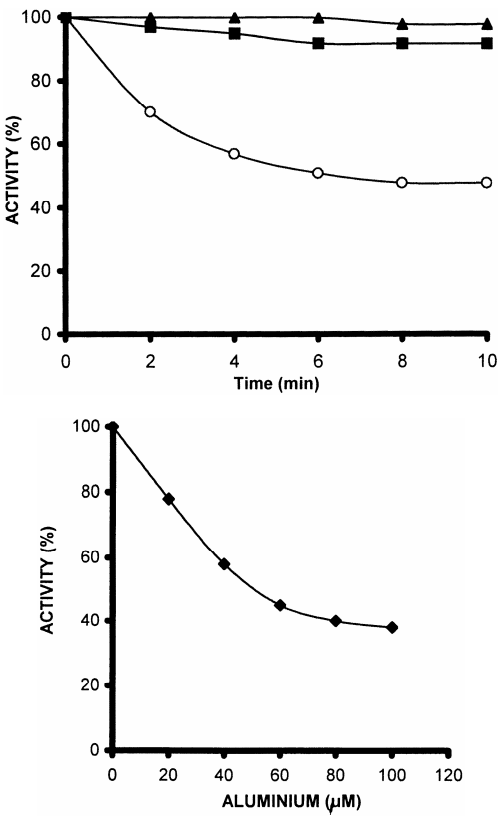

Figure 4. Inactivation of G6PD by aluminum. Top: time course and pH-dependent inactivation at pH values of 9 (▲), 8.4 (■) and 7.4 (○) Using Tris-HCl buffer. Aluminium concentration was 60 µM. Bottom: different concentrations of Al (III) were added to the assay mixture and the enzyme activity measured in pH 7.4 at 25°C. Each point represents the average of two independent experiments.

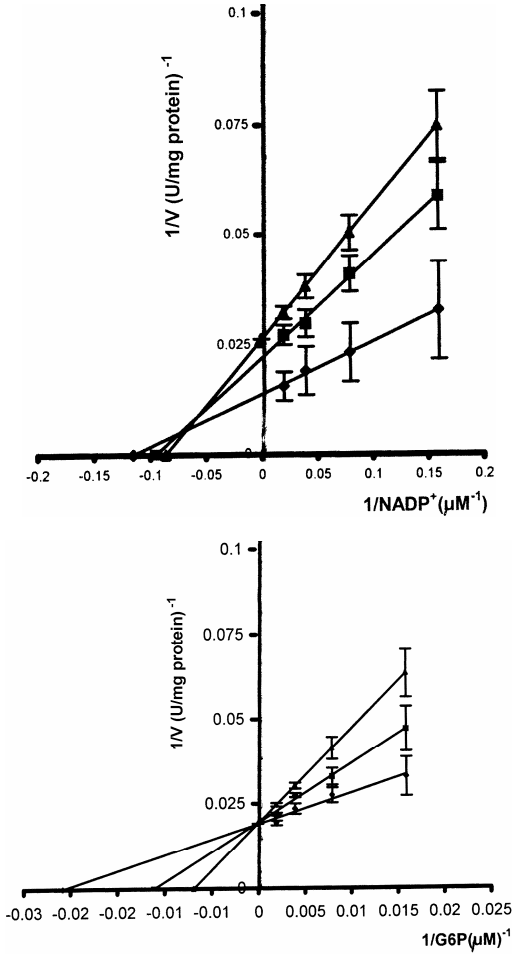

Figure 5. Lineweaver-Burk double reciprocal plot of G6PD in the presence of different concentrations of Al (III) as an inhibitor. Top: $NADP^+$ is the varied substrate and glucose 6-phosphate (G6P) is fixed at saturated concentration. Bottom: G6P is the varied substrate and $NADP^+$ is fixed at saturated concentration. Al (III) concentrations are zero (♦), 60 μM (■) and 80 μM (▲). Values are Mean±S.D. of three independent experiments.

loss of the enzyme activity. This is also consistent with the noncompetitive inhibitory effect of N_i (II) with respect to $NADP^+$. The inhibition patterns of N_i (II) for human erythrocyte G6PD are in agreement with those reported for G6PD from *Saccharomyces cervisiae* [19].

Increased N_i (II) concentration in sera of the patients undergoing chronic hemodialysis and its related complications have been reported before [33]. It has been also found that in rat N_i (II) decreases thermostability and exhibits a deformability effect on erythrocytes [34]. These effects may be related to the inhibitory effect of N_i (II) on G6PD activity which plays a role in providing the reducing potential and protecting the integrity of erythrocytes.

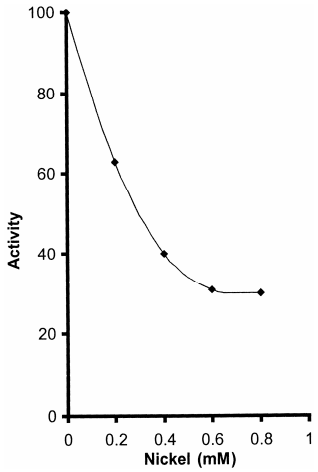

Figure 6. Concentration-dependent inactivation of G6PD by Ni (II). Indicated concentrations of Ni (II) were added to the assay mixture and the enzyme activity measured at 25°C. Each point is the average of two independent experiments.

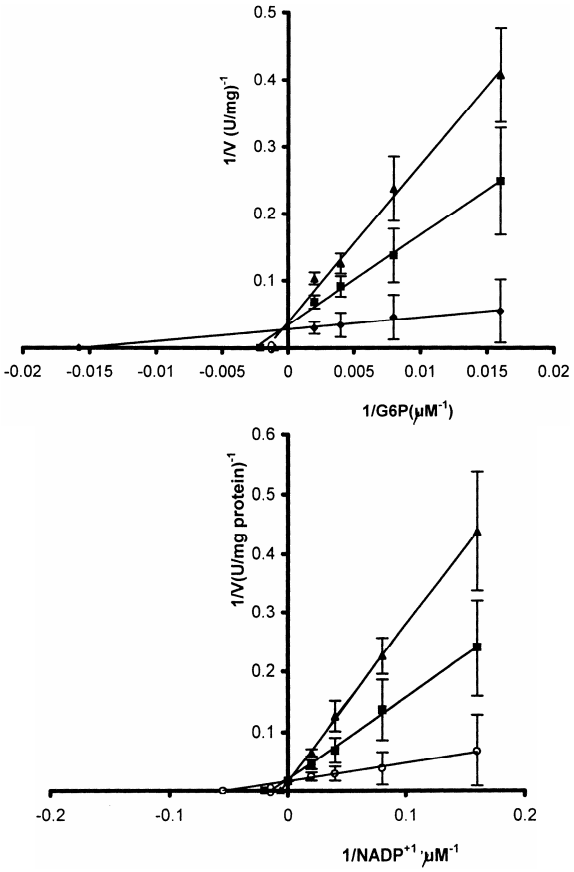

Figure 7. Lineweaver-Burk double reciprocal plot of G6PD in the presence of different concentrations of N_i (II) as an inhibitor. Top: glucose 6-phosphate (G6P) is the varied substrate and $NADP^+$ is fixed at saturated concentration. Bottom: $NADP^+$ is the varied substrate and G6P is fixed at saturated concentration. Ni (II) concentrations are zero (♦), 0.2 mM (■) and 0.4 mM (▲). Values represent Mean±S.D. of three independent experiments.

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