INHIBITORY ACTIONS OF MERCURY COMPOUNDS AGAINST GLUCOSE-6- PHOSPHATE DEHYDROGENASE FROM YEAST

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Abstract.....Kinetic studies on the inhibition of the activity of glucose-6-phosphate dehydrogenase with mercuric chloride (MC) and methylmercuric chloride (MMC) have revealed that MC inhibited the enzyme non-competitively, while MMC inhibited it competitively. The K_m value was 5.26×10^{-5} M for glucose-6-phosphate and K_i value of MC was 2.17×10^{-5} M, while that of MMC was 4.35×10^{-5} M.

The strong complex formation of nicotinamide adenine dinucleotide phosphate (NADP) or amino acids (cysteine, cystine, histidine, tryptophan or tyrosine) with MC was demonstrated in the presence of phosphate buffer as compared with that of MMC in the same buffer.

Key words: glucose-6-phosphate dehydrogenase, nicotinamide adenine dinucleotide phosphate, amino acids, mercuric chloride, methylmercuric chloride.

INTRODUCTION

It is well known that inorganic and organic mercury compounds were found to be bound with SH groups of enzymes (Tsuzuki *et al.*, 1978; Hara and Nakao, 1976; Segawa *et al.*, 1978) and proteins (hemoglobin, metallothionein, albumin, and β -lactoglobulin) (Hughes, 1957; Takeda *et al.*, 1968; Giblin and Massaro, 1975; Piotrowski *et al.*, 1974; Fernandez-Diez *et al.*, 1964; Fand and Fallin, 1976). Recently, Okuda *et al.* (1978) estimated the activities of enzymes in the brains of rats with acute intoxication of methylmercuric chloride (MMC). It was concluded that the activities of monoamine oxidase, choline acetyltransferase, and acetylcholinesterase (SH enzymes) in the brains of these rats were remarkably inhibited by the MMC intoxication. In addition, the inhibitory actions of mercury compounds against many enzymes have been

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investigated. Ishikura and Sugimitsu (1968) reported that both phenylmercuric acetate and ethylmercuric phosphate inhibited the cholinesterase of rat sera competitively. In 1971, Ishikura and Yonaha described the inhibition of cholinesterase of rat erythrocytes by phenylmercuric acetate competitively, and by ethylmercuric phosphate non-competitively. Furuno *et al.* (1975) showed that mercuric chloride (MC), phenylmercuric acetate, and ethylmercuric chloride inhibited alcohol dehydrogenase from yeast competitively. Recently, Ohmiya and Nakai reported that MMC inhibited alcohol dehydrogenase obtained from yeast non-competitively. However, no report of binding sites other than the SH groups of enzymes with mercury compounds has appeared.

Recently, the authors studied the inhibition mechanisms of mercury compounds against glucose-6-phosphate dehydrogenase (G-6-PD) from yeast in the Tris-HCl and phosphate buffers in order to elucidate the mechanism of toxicity of mercury compounds. The results are briefly described in this paper.

MATERIALS AND METHODS

Chemicals and abbreviations used.

Methylmercuric chloride (MMC, CH_3HgCl) purchased from Wakō Chemicals was recrystallized in pure ethyl alcohol (mp 173°C) (Marvel and Gould, 1922). Mercuric chloride (MC, HgCl_2) and glucose-6-phosphate (G-6-P) (Nakarai Chemicals, Ltd.), glucose-6-phosphate dehydrogenase from yeast (G-6-PD) (Sigma Chemicals. Co.), nicotinamide adenine dinucleotide phosphate (NADP) (Sigma and Kohdin Chemicals, Ltd.), and amino acids (Kyowa Hakko Co. Ltd.) were obtained commercially and used in the experiment.

Determination of activity of G-6-PD (EC 1.1.1.49).

A spectrophotometric assay (total volume: 3.0 ml) was performed using 190 mM Tris-HCl buffer (phosphate buffer), pH 8.0, containing 2 mM MgCl_2 , 2.5 mM G-6-P, 0.1 mM NADP, 10^{-7} – 10^{-8} M mercury compounds, and limiting amounts (80 units/mg) of enzyme. The reaction mixture was incubated at 30°C for 10 min and each assay was made in duplicate by following the increase of absorbance at 340 nm in a double beam spectrophotometer (Model 124, Perkin-Elmer) (Grunwald and Hill, 1976), and the kinetics of the immobilized G-6-PD were carried out by the method of Gould and Goheer (1976). K_m values were obtained by a least squares fit of the Lineweaver-Burk Plots.

Complex formation of NADP with mercury compounds.

The NADP and mercury compounds dissolved in distilled water. The order of addition of reactants was altered to: 1.0 ml of 0.19 M Tris-HCl or phosphate buffer (pH 8.0), 0.5 ml of NADP (final concentration: 6.7×10^{-6} M) and 0.2 ml of mercury compounds (final concentration: 5×10^{-4} M). The reaction mixture was adjusted to 3.0 ml with distilled water. The spectral characteristics of the NADP alone and after the complex formation of NADP with mercury compounds (MC, MMC) were determined at 25°C for 5 min using a double beam spectrophotometer (Model 124, Hitachi Perkin-

Elmer) (Hamdy and Noyes, 1977).

Complex formations of amino acids with mercury compounds.

Various amino acids (all at 0.05 M) were added to mercury compounds (all at 0.01 M) in phosphate buffer and 5 or 10 μ l of each sample was spotted in duplicate and carried out in n-butanol: acetic acid: water (4:1:1, by volume) until the solvent front had traveled 11 cm from the origin. The detection of amino acid and mercury compounds of the plate was accomplished through visualization by spraying with ninhydrin (2% in actone) and dithizone (0.5% in benzene), and with heating at 105°C for 5 min, by thin layer chromatography, the amounts of amino acids in G-6-PD being measured by an amino acid analyzer (KLA-5 type) (Olson and Massaro, 1978).

RESULTS

Influence of mercury compounds on the activity of G-6-PD.

The comparative effects of MC or MMC on G-6-PD from yeast in Tris-HCl and phosphate buffers (63 mM, pH 8.0) were studied spectrophotometrically following the oxidation of NADPH by 6-phosphogluconate. The activity of G-6-PD in Tris-HCl buffer was determined in the presence of various concentrations of mercury compounds by *in vitro* experiments. The results are shown in Fig. 1. When a concentration (5×10^{-4} M) of MMC was present in the 3.0 ml reaction mixture, the activity of G-6-PD slightly decreased to 97% of that of the control, while in the presence of a concentration (10^{-3} M) of MMC, the enzyme was inhibited by 50%. The inhibition (50%) of G-6-PD by MMC (10^{-3} M) was quite similar to that of the enzyme by concentration (5×10^{-4} M) of MC. In the presence of 10^{-3} M of MC, the enzyme was remarkably

Fig. 1. Influences of various concentrations of mercury compounds (MC) on activity of glucose-6-phosphate dehydrogenase in Tris-HCl buffer (63 mM, pH 8.0)

—●— : CH₃HgCl; - - -○- - - : HgCl₂

Fig. 2. Influences of various concentrations of mercury compounds (MC) on activity of glucose-6-phosphate dehydrogenase in phosphate buffer (63 mM, pH 8.0)

—●— : CH₃HgCl; - - -○- - : HgCl₂

Fig. 3. Lineweaver-Burk plots of the inhibition of mercury compounds on activity of glucose-6-phosphate dehydrogenase

—●— : without HgCl₂ or CH₃HgCl
 - - -○- - : with CH₃HgCl
 ····●···· : with HgCl₂

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inhibited (97%). A strong inhibition of G-6-PD by various concentrations of MC was demonstrated as compared with those of MMC. The data of influences of various concentrations of mercury compounds on the activity of G-6-PD in phosphate buffer are shown in Fig. 2. In the presence of 5×10^{-5} M MMC, the enzyme slightly decreased and the enzyme was inhibited to 65% by a concentration (10^{-3} M) of MMC. On the other hand, in the case of a concentration (5×10^{-5} M) of MC, the enzyme was inhibited to 50%, and there was complex enzyme inhibition by a concentration (10^{-3} M) of MC.

Mode of the inhibition by mercury compounds.

Fig. 3 shows the Lineweaver-Burk plots of the inhibitions of mercury compounds of the activity of G-6-PD. Inhibition of G-6-PD by MC was non-competitive, while MMC inhibited it competitively with respect to G-6-P. The K_m value was 5.26×10^{-6} M for G-6-P and K_i value of MC was 2.17×10^{-5} M, while K_i value of MMC was 4.35×10^{-3} M.

Complex formation of NADP with mercury compounds.

Fig. 4 shows the absorption spectra of the complexes of NADP with mercuric

Fig. 4. Absorption spectra of the complexes of nicotinamide adenine dinucleotide phosphate (NADP) with mercuric chloride (HgCl_2) in the presence of Tris-HCl or phosphate buffer (63 mM, pH 8.0)

— : NADP + Tris-HCl or phosphate buffer
 --- : NADP + HgCl_2 + Tris-HCl buffer
 - · - : NADP + HgCl_2 + phosphate buffer
 NADP : 6.7×10^{-6} M ; HgCl_2 : 5.0×10^{-4} M

chloride in the presence of Tris-HCl and phosphate buffers (63 mM, pH 8.0). As shown in Fig. 4, the normal absorption spectra of the final concentration (6.7×10^{-6} M) of NADP in Tris-HCl and phosphate buffers exhibited one maximum at 260 nm. Characteristic absorption and difference spectra in phosphate buffer were observed upon addition of MC. A strong complex formation of NADP with MC was demonstrated in phosphate buffer, while the complex formation in Tris-HCl buffer was rather weak. However, the complex formation of NADP with MMC was very weak as compared with that of MC.

Changes of amounts of amino acids in G-6-PD treated with mercury compounds.

Complex formations of amino acids with mercury compounds were confirmed by thin layer chromatography and the amounts of amino acids in G-6-PD treated with these compounds were measured by an amino acid analyzer. The results are shown in Table 1. MC strongly combined with cysteine, cystine, histidine, tryptophan, and tyrosine, respectively, while MMC was shown by thin layer chromatography to be bound only with cystine. By the determination of the amounts of amino acids in G-6-PD, the amounts of cysteine and histidine in G-6-PD treated with MC decreased to 66.8%, and 92.2% of those of control, respectively, while the amount of cysteine in G-6-PD treated with MMC decreased to 87.1% as compared with that of the control.

Table 1. Changes of amounts of amino acids in glucose-6-phosphate dehydrogenase treated with mercuric chloride (HgCl_2) or methylmercuric chloride (CH_3HgCl)

Amino acids	Control ($\mu\text{moles/ml}$)	HgCl_2 ($\mu\text{moles/ml}$)	CH_3HgCl ($\mu\text{moles/ml}$)
Cysteine	5.60	3.74 (+++)	4.88 (++)
Serine	3.84	3.82 (—)	3.82 (—)
Histidine	2.56	2.36 (+)	2.55 (—)
Isoleucine	2.34	2.33 (—)	2.34 (—)
Glycine	1.76	1.76 (—)	1.76 (—)
Cystine	1.60	1.61 (++)	1.60 (—)
Arginine	0.93	0.92 (—)	0.92 (—)
Alanine	0.59	0.58 (—)	0.58 (—)
Tryptophan	trace	trace (+)	trace (—)
Tyrosine	trace	trace (+)	trace (—)

Marks in parentheses were the expressed extent of complex formations of amino acids with mercury compounds by thin layer chromatography; +++ : very strong complex; ++ : strong complex; + : weak complex; — : no complex

DISCUSSION

In order to find the biochemical significance of G-6-PD (SH enzyme), the authors studied the inhibition of G-6-PD by mercury compounds. As described above, MC

strongly inhibited G-6-PD in both Tris-HCl and phosphate buffers as compared with MMC (Figs. 1 and 2). The difference of inhibition of G-6-PD between these two mercury compounds could also be due to their configuration and molecular size as reported by Hamdy and Noyes (1977). Many reports on the inhibitory mechanisms of mercury compounds on many enzymes have been published hitherto. In 1977, Hamdy and Noyes reported that MC inhibited lactate dehydrogenase non-competitively and the K_i value of MC was 1.61×10^{-8} M. Ohmiya and Nakai (1977) showed that inhibition of alcohol dehydrogenase by MMC was non-competitive. Nakamura and Ogura (1968) indicated that MC inhibited glucose oxidase non-competitively. As shown in Fig. 3, kinetic studies on the inhibition of the activity of G-6-PD with mercury compounds (MC, MMC) demonstrated that MC inhibited the enzyme non-competitively and MMC inhibited it competitively with respect to G-6-P.

The K_m value was 5.26×10^{-5} M for G-6-P and K_i value of MC was 2.17×10^{-5} M, while the K_i value of MMC was 4.37×10^{-3} M. The authors studied the complexes of mercury compounds with NADP in the presence of Tris-HCl and phosphate buffers. A strong complex formation of NADP with MC was demonstrated in phosphate buffer, while the complex formation in Tris-HCl buffer was rather weak. The complex formation of NADP with MMC in Tris-HCl and phosphate buffers was very weak as compared with that of MC. Then, complex formations of amino acids with mercury compounds were carried out by thin layer chromatography as reported previously (Okuda and Tsuzuki, 1978). The amounts of amino acids in G-6-PD treated with mercury compounds were measured by an amino acid analyzer. As shown in Table 1, MC combined with cysteine, cystine, histidine, tryptophan, and tyrosine, while MMC bound only cysteine to form a complex with mercury compounds. Amounts of cysteine and histidine in G-6-PD treated with decreased to 66.8% and 92.2% of those of the control, respectively, while that of cysteine in G-6-PD treated with MMC decreased to 87.1% as compared with that of the control. Kahn *et al.* (1972) and Gözükar (1974) reported that one histidine residue and one SH group played a major role in the catalytic activity of G-6-PD. This datum is quite similar to ours.

From these results, it can be seen that MC inhibited G-6-PD more than did MMC, probably due to the monofunctional nature of MMC, which was found to bind only with cysteine, whereas MC was bifunctional and could react with the adenine of NADP or various amino acids (cysteine, cystine, histidine, tryptophan, tyrosine) in the presence of phosphate buffer.

SUMMARY

The influence of mercuric chloride (MC) and methylmercuric chloride (MMC) on the activity of glucose-6-phosphate dehydrogenase (G-6-PD) were studied in Tris-HCl or phosphate buffer (63 mM, pH 8.0). The results were as follows.

- (1) MC inhibited strongly G-6-PD in both buffers as compared with MMC.

(2) Kinetic studies on the inhibition of the activity of G-6-PD with MC and MMC revealed that MC inhibited G-6-PD non-competitively, while MMC inhibited it competitively with respect to G-6-P. The K_m value was 5.26×10^{-5} M for G-6-P and the K_i value of MC was 2.17×10^{-5} M, while that of MMC was 4.35×10^{-5} M.

(3) A strong complex formation of nicotinamide adenine dinucleotide phosphate (NADP) or amino acids (cysteine, cystine, histidine, tryptophan, and tyrosine) was demonstrated in phosphate buffer as compared with that of MMC in the same buffer, while the complex formation in Tris-HCl buffer was very weak.

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