Reduction of mercuric ion to metallic mercury *in vitro* by some enzymes

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ABSTRACT: The reduction rate of mercuric ion to metallic mercury by superoxide anion produced by xanthine-xanthine oxidase system increased with increased concentrration of xanthine oxidase in the presence of enough xanthine. The reduction rate of mercuric ion by superoxide anion in the presence of nitroblue tetrazolium (NBT) was proportional to the concentration of NBT. The results suggest that NBT is reduced to diformazan by superoxide anion produced by xanthine-xanthine oxidase system and mercuric ion will be reduced to metallic mercury by diformazan.

The reduction rate of mercuric ion by cytochrome C-β-nicotinamide-adenine dinucleotide (NADH) system in the presence of cytochrome C reductase was higher than that of cytochrome C-NADH system in the absence of cytochrome C reductase. On the other hand, the reduction rate of mercuric ion by superoxide anion produced by NADH-phenazine methosulfate (PMS) system increased with increased concentration of PMS.

INTRODUCTION

The vapor of metallic mercury is taken up *in vivo* by inhalation of the vapor and oxidized by catalase metallic mercury to mercuric ion. Kudsk[1] demonstrated that the uptake of mercury in the blood is inhibited by ethyl alcohol. Dunn *et al.*[2] also demonstrated that the mercury exhaled increased after the administration of ethyl alcohol to mice injected with a single dose of mercuric chloride. Ogata *et al.*[3] demonstrated that the oxidation of metallic mercury by catalase-hydrogen peroxide system with ethyl alcohol decreased as the concentration of ethyl alcohol was increased. The competitive nature of the inhibition of metallic mercury oxidation by ethyl alcohol illustrated by Lineweaver-Burk plots, with two straight lines having different slopes with or without ethyl alcohol. Ogata *et al*[4]. also demonstrated the

mechanism of metallic mercury oxidation *in vitro* by lactoperoxidase or horseradish peroxidase with L-dopa or pyrogallol may have served as a substrate and/or a reducing agent for lactoperoxidase or horseradish peroxidase. However, the result of metallic mercury exhaled from mice injected intraperitoneally with mercuric ion suggest that the reduction mechanism of mercuric ion to metallic mercury exists in the living-body. Ogata *et al.*[5] reported that superoxide anion produced from xanthine-xanthine oxidase system was reduced mercuric ion to metallic mercury in the living-body. The data of *in vitro* study show that some enzymes in tissues play an important role in reducing mercuric ion to metallic mercury. Clarkson *et al.*[6] reported the exhalation of small amounts of mercury from anesthetized rats injected with mercuric chloride and suggested that mercuric ion was reduced to metallic mercury in the blood. Ogata *et al.*[7] demonstrated that the total amount of mercury exhaled from acatalasemic mice in ten minutes after injection of metallic mercury was higher than that of normal mice. The phenomenon is explained by assuming that the reoxidation of mercury by catalase in actalasemic mice is less than that in normal mice.

The report concerns the reduction of mercuric ion to metallic mercury *in vitro* by some enzymes.

MATERIALS AND METHODS

Materials: Xanthine oxidase, Grade IV, 40 units/mg protein from milk (Sigma Chemical Co.); nitroblue tetrazolium, crystalline (Sigma Chemical Co.); cytochrome C from horse heart (Sigma Chemical Co.); NADH (Sigma Chemical Co.) ; riboflabin and tetramethyl ethylenediamine (Wako Chemical Co.), were obtained from respective sources.

Incubation and measurement of mercury: Incubation of reaction mixture was conducted at 37°C for 90 min, while shaking at 80 cycles/min. After incubation, reaction mixture is bubbled with nitrogen gas for 5 min. The nitrogen gas containing metallic mercury was washed with 0.5% L-cysteine solution and the mercury was trapped with 1% $KMnO_4$-1N H_2SO_4 mixture solution. The amount of mercury was determined by an elemental mercury analyzer (Hitachi Co., Model 207) with circulating air, which contained mercury vapor, as described in previous report.[8]

RESULTS AND DISCUSSION

Xanthine-Xanthine oxidase-NBT system

Reduction rate of mercuric ion to metallic mercury by superoxide anion produced by xanthine-xanthine oxidase system was investigated. The result is shown in Fig. 1. Xanthine oxidase catalysis the reaction between xanthine and oxygen, resulting the product of superoxide anion. The effect of xanthine oxidase on the reduction rate of mercuric ion to metallic mercury is given in Fig. 1, which shows

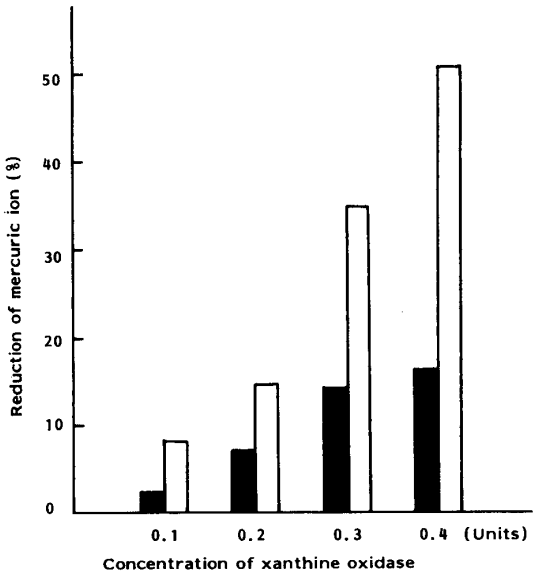

Fig. 1. Xanthine-Xanthine oxidase-NBT system.
■: Xanthine-Xanthine oxidase system.
□: Xanthine-Xanthine oxidase-NBT system.

that the increasing concentration of xanthine oxidase increased the level of reduction of mercuric ion to metallic mercury. The data indicate that the concentration of superoxide anion is proportional to the concentration of xanthine oxidase in the presence of enough xanthine. On the other hand, the reduction rate of mercuric ion by superoxide anion in the presence of nitroblue tetrazolium (NBT) was higher than that of xanthine-xanthine oxidase system in the absence of NBT. The result suggests that NBT is reduced to diformazan by superoxide anion is generated by xanthine-xanthine oxidase system, and mercuric ion is reduced to metallic mercury by diformazan. These results indicate that mercuric ion will be reduced by superoxide anion.

In order to study the reduction mechanism of mercuric ion to metallic mercury by superoxide anion, an experiment of riboflabin-tetramethylethylenediamine (TEMED)[9] system was conducted as follows. The result is shown in Fig. 2.

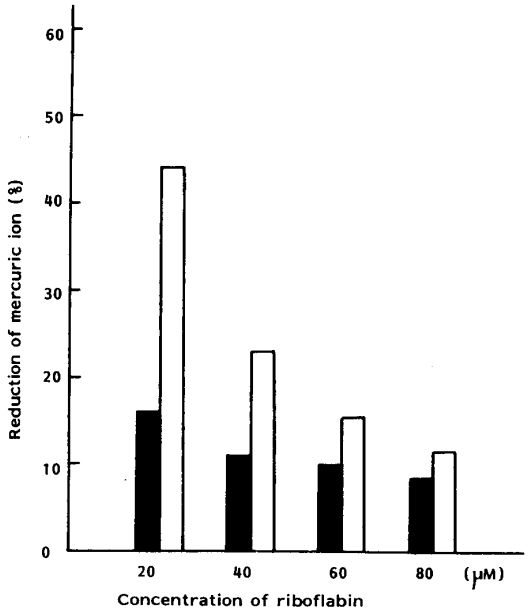

Fig. 2. Riboflabin-TEMED-NBT system.
■: Riboflabin-TEMED system.
□: Riboflabin-TEMED-NBT system.

Riboflabin produced to superoxide anion when fluorescent-light was irradiated to the system of riboflabin-TEMED. The reduction rate of mercuric ion to metallic mercury by superoxide anion was decreased with the increased concentration of riboflabin. The reduction rate of mercuric ion by superoxide anion in the presence of nitroblue tetrazolium (NBT) was significantly higher than that of the system in the absence of NBT. The data indicate that NBT was reduced to diformazan by superoxide anion produced by the riboflabin-TEMED-fluorescent light system, and mercuric ion was reduced to metallic mercury by diformazan similar to that of xanthine-xanthine oxidase-NBT system.

Cytochrome C-Cytochrome C reductase system

Mercuric ion was reduced to metallic mercury in the presence of superoxide anion, as shown in Fig. 1 and Fig. 2. The reduction rate of mercuric ion to metallic mercury in the presence of both superoxide anion and nitroblue tetrazolium was higher than that in the presence of superoxide anion alone produced by the xanthine-xanthine oxidase system or riboflabin-TEMED-fluorescent light system. The fact suggest that oxidative-form of cytochrome C is reduced to reductive-form of cytochrome C and nitroblue tetrazolium is reduced to nitroblue diformazan by superoxide anion is generated by the xanthine-xanthine oxidase system or riboflabin-TEMED-fluorescent light system.

In order to study the reduction mechanism of mercuric ion to metallic mercury *in vitro*, an experiment was conducted as follows. The reduction rate of mercuric ion by cytochrome C decreased with increased concentration of cytochrome C. On the other hand, the reduction of mercuric ion by cytochrome C in the presence of cytochrome C reductase was higher than that of cytochrome C in the absence of cytochrome C reductase (Fig. 3). Furthermore, the reduction of mercuric ion by

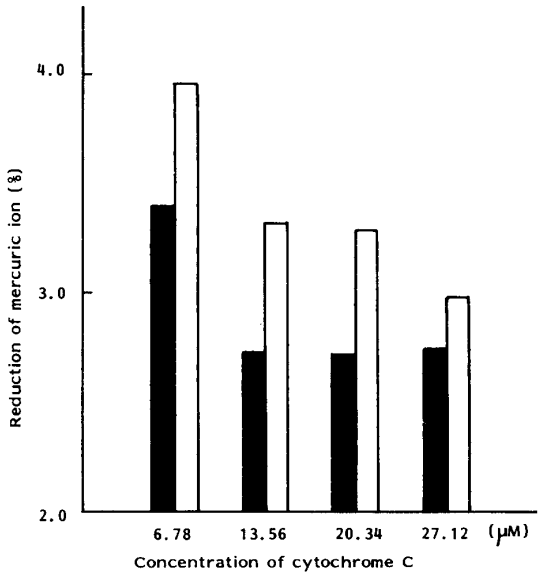

Fig. 3. Cytochrome C-Cytochrome C reductase system.
■: Cytochrome C system.
□: Cytochrome C-Cytochrome C reductase system.

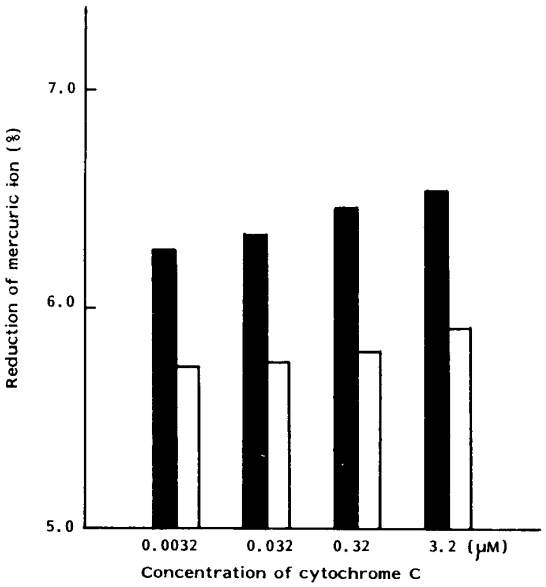

Fig. 4. Cytochrome C-Cytochrome C reductase-NADH system.
□: Cytochrome C-NADH system.
■: Cytochrome C-Cytochrome C reductase-NADH system.

cytochrome C-cytochrome C reductase-NADH (β-nicotinamido-adenine dinucleotide) system, occurring as a reduction-substrate in the living-body, was significantly higher than that of cytochrome C-NADH system (Fig. 4).

NADH-PMS-NBT system

Phenazine methosulfate (PMS) is reduce relative quickly by NADH under aerobic conditions followed by reduction of oxygen to yield the superoxide anion. Superoxide anion was capable of reducing NBT. The overal reaction should be inhibited not only by oxidants of NADH and $PMSH_2$, but by scavengers of O_2. The reaction is connsidered by Nishikimi *et al.*[9] to be as follows;

$$NADH + PMS \longrightarrow NAD^+ + PMSH_2$$

$$PMSH_2 + 2O_2 \longrightarrow 2O_2^- + 2H^+ + PMS$$

$$2NBT + 2Cl^- + 4O_2^- + 4H^+ \longrightarrow Diformazan + 4O_2 + 2HCl$$

In considering the mercury reduction, a similar mechanism may be suggested for *in vivo* conditions.

The reduction rate of mercuric ion by NADH-PMS system in the presence of NBT was conducted. The result is shown in Fig. 5. The reduction rate ot mercuric

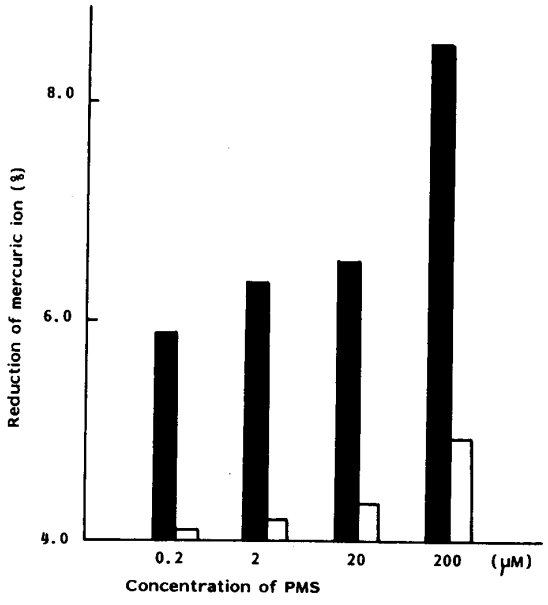

Fig. 5. NADH-PMS-NBT system.
■: NADH-PMS system.
□: NADH-PMS-NBT system.

ion to metallic mercury by NADH-PMS system tended to increase with increased concentration of PMS. However, the reduction rate of mercuric ion by NADH-PMS system in the presence of NBT was lower than that of NADH-PMS system in the

absence of NBT. The data suggest that the reduction of mercuric ion by NADH-PMS system was inhibited by the interposition of NBT. The result is now under investigation.

Effect of the concentration of NADH on the mercury reduction is shown in Fig. 6. The reduction rate of mercuric ion by NADH increased with increased

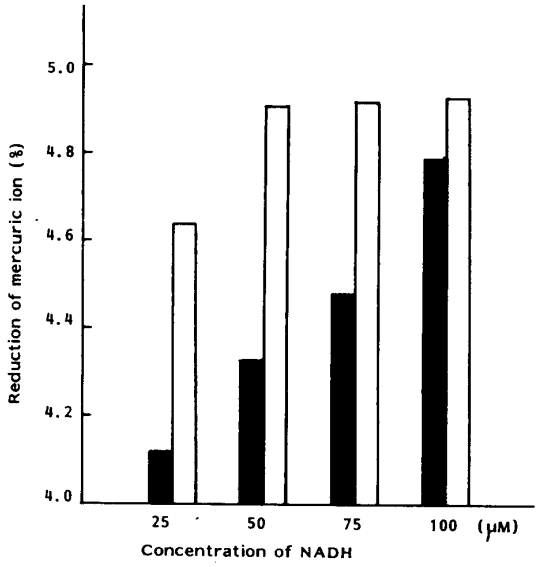

Fig. 6. NADH-Glucose-6-phosphate system.
■: NADH system.
□: NADH-Glucose-6-phosphate system.

concentration of NADH. The reduction rate of mercuric ion in the presence of both NADH and glucose-6-phosphate tended to increase compared with that of NADH alone.

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