Reduction of Mercuric Ion by Superoxide Anion Radical *In Vitro*

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The reduction rate of mercuric ion by superoxide anion radical produced by the system of riboflabin-tetramethylethylenediamine (TEMED) or phenazine methosulfate-nicotinamide adenine dinucleotide (NADH), both with and without nitroblue tetrazolium (NBT) was investigated. The reduction rate of mercuric ion by superoxide anion radical produced by the system of riboflabin-TEMED with NBT decreased with the increased concentration of riboflabin. On the other hand, the reduction rate of mercuric ion by superoxide anion radical produced by the system of PMS-NADH with NBT tended to decrease in comparison with that in the presence of NBT. The two results indicated that superoxide anion radical produced by the two systems reduced mercuric ion to metallic mercury similar to that of xanthine-xanthine oxidase.

INTRODUCTION

It has been known that metallic mercury inhaled is oxidized by catalase in the presence of hydrogen peroxide generated by oxidase, indicating that the oxidation of metallic mercury occurs in the tissues[1~7], However, some of the oxidized mercury is restored to metallic mercury by some enzymes[8] and is exhaled in the expiration. Ogata et al.[9] reported that metallic mercury exhaled from acatalasemic mice after intraperitoneal injection of metallic mercury was significantly higher than that from normal mice. Sugata et. al.[10] also reported that mercuric ion may be reduced to metallic mercury in body tissues, because of metallic mercury exhaled from animals treated with mercuric chloride confirmed.

Ogata et al.[11] reported that superoxide anion radical produced by xanthine-xanthine oxidase system reduced mercuric ion to metallic mercury. To elucidate the reduction of mercuric ion by superoxide anion radical, the reduction of mercuric ion to metallic mercury using superoxide anion radical produced by the systems of chemical materials was investigated.

The present study concerns the reduction rate of mercuric ion to metallic mercury by superoxide anion radical produced by the system of riboflabin-TEMED or PMS-NADH with or without NBT.

MATERIALS AND METHODS

MATERIALS : Riboflabin, TEMED, NBT and PMS purchased from Wako Chemical Co., Tokyo, Japan. β-NADH (reduced form) purchased from Oriental Yeast Co., Tokyo, Japan. Other reagents used were of analytical grade.

METHODS : The reaction mixture containing riboflabin-TEMED or PMS-NADH with and without NBT were incubated at 37 °C for 90 minutes, while shaking at 80 cycles/min. After incubation, the reaction mixture was bubbled with nitrogen gas for 5 minutes. After the nitrogen gas containing metallic mercury was washed with a 0.5 % L-cysteine solution, mercury trapped with 6 % potassium permanganate-sulfuric acid. The amount of mercury was determined by a elemental mercury analyzer (Model 207, Hitachi, Tokyo, Japan) with circulating air, which contained mercury vapor, as described in a previous report[12].

RESULTS AND DISCUSSION

To study the reduction mechanism of mercuric ion to metallic mercury *in vitro*, an experiment was conducted as follows. Metallic mercury was oxidized to mercuric ion in the presence of catalase. Mercuric ion was reduced to metallic mercury in the presence of superoxide anion radical as described in the previous report[11].

The reduction rate of mercuric ion by superoxide anion radical produced by the system of riboflabin-TEMED or PMS-NADH, both with and without NBT, was investigated. The result is shown in Table 1. The reduction rate of mercuric ion to metallic mercury in the system of riboflabin-TEMED decreased with the increased concentration of riboflabin in the constant concentration of TEMED. The reduction rate increased in the presence of NBT when the riboflabin concentration was same. The results suggest that nitroblue tetrazolium is reduced to nitroblue diformazan by superoxide anion radical, which is produced by the system of riboflabin-TEMED. The data is similar to the result of the system of xanthine-xanthine oxidase[11]. Phenazine methosulfate (PMS) is reduce relative quickly by NADH under aerobic condition followed by reduction of oxygen to yield superoxide anion radical. The reaction is considered by Nishikimi et al.[13] to be as follows ;

Table 1 Reduction rate of mercuric ion by superoxide anion radical produced by the system of riboflabin-tetramethylethylenediamine (TEMED).

Riboflabin (mM)	TEMED ($\times 10^{-2}$ M)	NBT (0.1 mM)	Reduction rate (%, mean ± SD)
0.02	+	+	44.05 ± 2.15
	+	−	16.03 ± 1.02
0.04	+	+	22.94 ± 1.12
	+	−	11.12 ± 0.82
0.06	+	+	15.46 ± 0.96
	+	−	10.15 ± 0.73
0.08	+	+	11.52 ± 0.52
	+	−	8.25 ± 0.70

$$NADH + H^+ + PMS \longrightarrow NAD^+ + PMSH_2$$
$$PMSH_2 + 2O_2 \longrightarrow 2O_2^{\cdot -} + 2H^+ + PMS$$
$$2NBT + 2Cl^- + 4O_2^{\cdot -} + 4H^+ \longrightarrow diformazan + 4O_2 + 2HCl$$

Rates of the reduction of mercuric ion to metallic mercury by superoxide anion radical produced by the systems of PMS-NADH with and without NBT were investigated. The result is shown in Table 2. The reduction rate of mercuric ion by superoxide anion produced by the system with NBT tended to decrease in comparison with that without NBT. The results suggest that the following reaction occurs readily and that oxygen strongly interferes with this reaction.

$$PMSH_2 + NBT \longrightarrow formazan + PMS$$

The reduction mechanism of mercuric ion by superoxide anion radical can be depicted as shown in Fig. 1. PMS is reduced to $PMSH_2$ by NADH. $PMSH_2$ reduced oxygen to superoxide anion radical. Superoxide anion radical reduced NBT to formazan, and formazan reduced mercuric ion to metallic mercury.

Finally, the finding of metallic mercury exhaled from mice injected intraperitoneally with mercuric ion suggest that the reduction of mercuric ion occurs in the tissues by some reducing substants. One of the reducing substants is considered to be superoxide anion radical produced by the system of xanthine-xanthine oxidase[11]. To elucidate the reduction of mercuric ion by superoxide anion radical, the reduction of mercuric ion by superoxide anion radical produced by the two systems, riboflabin-TEMED and PMS-NADH with or without NBT as the system of chemical materials was investigated. As

Table 2 Reduction rate of mercuric ion by superoxide anion radical produced by the system of nicotinamide adenine dinucleotide (NADH)-phenazine methosulphate (PMS).

PMS (μM)	NADH (70 μM)	NBT (80 μM)	Reduction rate (%, mean ± SD)
0.20	+	+	4.12 ± 0.09
	+	−	5.92 ± 0.24
2.00	+	+	4.23 ± 0.13
	+	−	6.36 ± 0.42
20.0	+	+	4.36 ± 0.15
	+	−	6.56 ± 0.74
200	+	+	4.92 ± 0.34
	+	−	8.56 ± 1.01

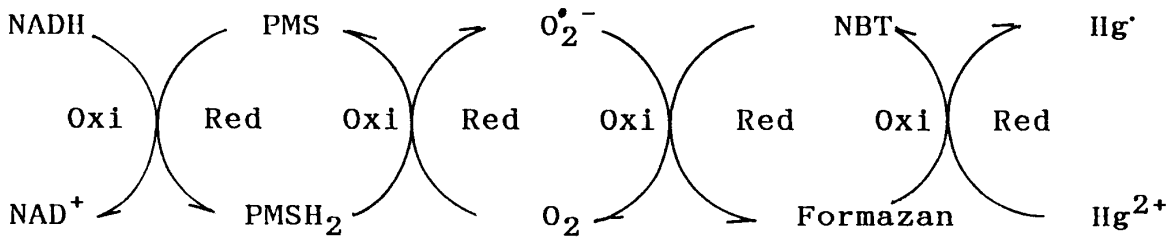

Fig. 1 Systematic pathway for reduction of mercuric ion to metallic mercury.

a result, author confirmed that superoxide anion radical produced by the two systems reduced mercuric ion to metallic mercury. In conclusion, metallic mercury exhaled from mice pretreated with mercuric ion is explained by assuming that superoxide anion radical reduced mercuric ion to metallic mercury in the tissues.

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