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Enzymatic Reduction of Mercurous Ions in *Escherichia coli* Bearing R Factor

KAZUO IZAKI

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai, Japan

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Mercurous ion (Hg^+)-dependent reduced nicotinamide adenine dinucleotide phosphate oxidation was demonstrated in an extract from cells of *Escherichia coli* W2252 that bear R factor.

Mercury-resistant strains of *Escherichia coli* bearing R factor have the ability to reduce mercuric ion (Hg^{2+}) to metallic mercury (Hg^0) (2-7), and purification and properties of an Hg^{2+}-reducing enzyme have been reported (1, 8). I now report that a multiple-drug-resistant strain of *E. coli* W2252 bearing R factor (*E. coli* W2252 R) is not only resistant to Hg^{2+} but also to mercurous ion (Hg^+), and the same enzyme seems to catalyze reduction of both Hg^+ and Hg^{2+}.

Organisms used and methods for their cultivation, estimation of enzyme activity for Hg^+ or Hg^{2+} reduction, and enzyme purification were similar to procedures reported previously (1). It has not yet been demonstrated that an Hg^{2+}-resistant strain of *E. coli* W2252 R is also resistant to Hg^+. My recent experiments showed that the resistant strain could grow in a nutrient broth containing 0.01 mM CH_3COOHg; whereas the sensitive strain lacking R factor could not grow in the same medium (unpublished data).

Crude extract was prepared from the cells of *E. coli* W2252 R grown in the nutrient broth with or without 0.01 mM $HgCl_2$. Hg^+- or Hg^{2+}-dependent reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidation activity of the crude extract was measured. The crude extract from cells grown with $HgCl_2$ had both Hg^+- and Hg^{2+}-dependent NADPH oxidation (Fig. 1). The crude extract from cells grown in the nutrient broth without $HgCl_2$ had neither Hg^+- nor Hg^{2+}-dependent NADPH oxidation activity (data not shown). Both activities were induced to the same degree by adding either CH_3COOHg (for Hg^+) or $(CH_3COO)_2Hg$ (for Hg^{2+}) to the growth medium.

To clarify whether the same enzyme catalyzes Hg^+- and Hg^{2+}-reducing activities, both were measured at each step of purification, i.e., $(NH_4)_2SO_4$ fractionation, diethylaminoethyl-cellulose column chromatography, Sephadex G 200 gel filtration, or hydroxyapatite column chromatography. Both activities were not separated from each other at each step of purification. Elution profiles of both activities from the hydroxyapatite column showed that both activities were purified to the same degree (approximately 100-fold) at the final stage (Fig. 2). To confirm that the same enzyme catalyzes both

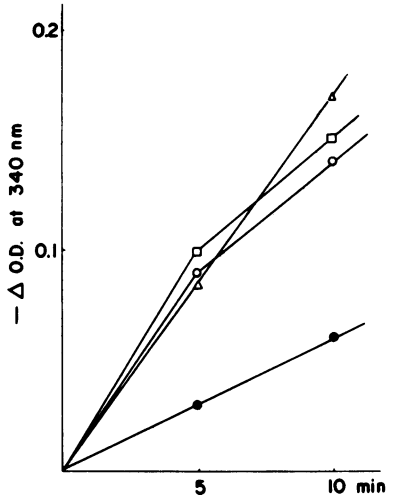

FIG. 1. *NADPH oxidation dependent on mercurous and mercuric ions by the crude extract of E. coli W2252 R. Crude extract was prepared from the cells of E. coli W2252 R grown in the nutrient broth containing 0.01 mM $HgCl_2$. NADPH oxidation activity of the crude extract dependent on Hg^+ or Hg^{2+} was measured. Reaction mixture contained the crude extract (2.6 mg of protein), 0.02 M potassium-phosphate buffer (pH 7.0), 0.04 mM mercury compound, 0.01 mM NADPH, and 2 mM mercaptoethanol in a total volume of 3 ml. Reaction mixture containing phosphate buffer, mercury compound, mercaptoethanol, and NADPH was preincubated for 5 min at 20°C, and the reaction was started by adding the enzyme to it. The following compounds were added to the reaction mixture: (-●-) no addition, (-□-) 0.04 mM $HgCl_2$, (-△-) 0.04 mM CH_3COOHg, (-○-) 0.04 mM $(CH_3CQO)_2Hg$.*

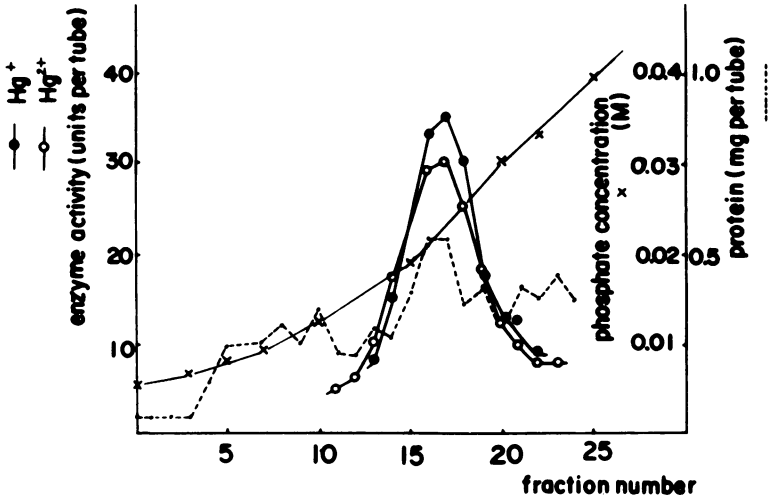

FIG. 2. *Purification of mercurous and mercuric ions-reducing enzyme by hydroxyapatite column chromatography. The active fraction from the Sephadex G 200 eluate (33 mg of protein) was applied to a column of hydroxyapatite (11 by 1.5 cm), washed with 0.01 M potassium-phosphate buffer (pH 7.0) containing 4 mM mercaptoethanol (PM buffer). Elution was carried out with a linear gradient of PM buffer from 0.01 to 0.1 M.*

TABLE 1. *Heat stabilities of mercurous and mercuric ions-reducing activities*

Treatment[a]	Inactivation of reducing activity	
	Hg^+ (%)	Hg^{2+} (%)
70°C, 5 min	0	0
75°C, 8 min	24	33
80°C, 5 min	53	60

[a] The reaction mixture contained 38 μg of heat-treated purified enzyme (hydroxyapatite eluate fraction; specific activity, ca. 60 U/mg), 0.02 M potassium-phosphate buffer (pH 7.0), 0.1 mM CH_3COOHg or $(CH_3COO)_2Hg$, 0.1 mM NADPH and 2 mM mercaptoethanol in a total volume of 3 ml. The reaction mixture containing phosphate buffer, mercury compound, mercaptoethanol, and NADPH was preincubated for 5 min at 20°C, and the reaction was started by adding the enzyme to it. Hg^+- or Hg^{2+}-dependent oxidation was measured.

activities of Hg^+ and Hg^{2+} reduction, effects of heat treatment and various inhibitors on both activities were examined; and both activities were similarly inactivated by various heat treatments (Table 1). Numerous heavy metal ions such as Ag^+, Cu^{2+}, and Co^{2+} were tested for their inhibitory action on both activities. Both activities had similar sensitivities to these heavy metal ions. Effects of various sulfhydryl inhibitors on both activities were also examined. Both Hg^+- and Hg^{2+}-reducing activities were severely inhibited by iodoacetamide but not by either *p*-chloromercuribenzoate or *N*-ethylmaleimide (Table 2). Although further complete purification is needed, to conclude, the results describing purification and properties of an Hg^+- and Hg^{2+}-reducing enzyme suggest that a single enzyme catalyzes both Hg^+ and Hg^{2+} reduction.

TABLE 2. *Effects of various sulfhydryl reagents on mercurous and mercuric ions-reducing activities*

Inhibitor[a]	Inhibition of reducing activity	
	Hg^+ (%)	Hg^{2+} (%)
p-Chloromercuribenzoate 10^{-4} M	0	0
N-Ethylmaleimide 10^{-3} M	0	0
Iodoacetamide		
10^{-4} M	71	64
10^{-3} M	91	93

[a] The purified enzyme (hydroxyapatite eluate fraction) was dialyzed against more than 1,000 volumes of 0.02 M potassium-phosphate buffer (pH 7.0). The reaction mixture containing 30 μg of the dialyzed enzyme, 0.3 or 3 μmol of each inhibitor, and 240 μmol of potassium-phosphate buffer (pH 7.0) in a total volume of 2.15 ml, was preincubated for 5 min at 20°C, and the reaction was started by adding 6 μmol of mercaptoethanol, 0.3 μmol of NADPH, and 0.3 μmol of CH_3COOHg or $(CH_3COO)_2Hg$ successively. Hg^+- or Hg^{2+}-dependent NADPH oxidation was measured. The final volume of the reaction mixture was 3 ml.

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