Antioxidant effect of caeruloplasmin on microsomal lipid peroxidation

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Microsomal NADPH-dependent lipid peroxidation catalyzed by ADP-Fe^{3+} was inhibited by the addition of caeruloplasmin. The antioxidant effect of caeruloplasmin was independent of the superoxide anion (O_2^-) scavenging activity. Since caeruloplasmin enhanced the function of ADP-Fe^{3+} acting as electron acceptor for microsomal electron transport system, the antioxidant effect of caeruloplasmin is considered to depend on the ferroxidase activity.

Lipid peroxidation *Antioxidant effect* *Caeruloplasmin* *Microsome*

1. INTRODUCTION

Superoxide dismutase and glutathione peroxidase are considered to be antioxidant enzymes against the lipid peroxidation in vivo. However, superoxide dismutase has little antioxidant effect on the lipid peroxidation of microsomes which catalyze the production of superoxide anion (O_2^-) in the presence of NADPH [1,2]. Thus, O_2^- may not be involved in microsomal lipid peroxidation or superoxide dismutase may be impermeable to the inner membrane which is subject to the lipid peroxidation catalyzed by O_2^-. Glutathione peroxidase catalyzes the reduction of peroxidized free fatty acids rather than peroxidized microsomal lipids, and has little antioxidant effect on microsomal lipid peroxidation [3].

Microsomal lipid peroxidation proceeds slowly after the addition of NADPH, and rapidly after the addition of NADPH and ADP-Fe^{3+} [4]. ADP-Fe^{2+} promotes the lipid peroxidation faster than ADP-Fe^{3+}, and ADP-Fe^{2+} formation via the reduction of ADP-Fe^{3+} by NADPH-cytochrome P450 reductase is considered to be involved in the initiation of microsomal lipid peroxidation [5]. Thus, the inhibition of the formation of ADP-Fe^{2+} is expected to reflect the inhibition of NADPH-dependent lipid peroxidation of microsomes catalyzed by ADP-Fe^{3+}.

This paper examines the possibility that microsomal NADPH-dependent lipid peroxidation catalyzed by ADP-Fe^{3+} is inhibited by the addition of caeruloplasmin having ferroxidase activity [6].

2. MATERIALS AND METHODS

Horse heart cytochrome *c*, Type III, was purchased from Sigma. Superoxide dismutase (EC 1.15.1.1), human caeruloplasmin and other reagents were obtained from Nakarai Chemicals. Rat liver microsomes were isolated from 100–150 g male Sprague–Dawley rats as in [7].

Lipid peroxidation occurred under the following conditions: 150 mM KCl, 50 mM Tris–HCl (pH 7.5), 15 μM $FeCl_3$, 1 mM ADP, 0.45 mM NADPH and 1 mg microsomal protein/ml. Total volume of reaction mixture was 1 ml, and the reaction mixture was mechanically shaken at 37°C for 10 min. The lipid peroxidation was measured by the formation of the thiobarbituric acid-reactive material, malondialdehyde [8].

The oxidation of NADPH was measured by following the decrease in the absorbance at 340 nm [8]. The O_2^- scavenging activity of caeruloplasmin was measured by the inhibition of cytochrome *c* reduction in the O_2^- generating system [9]. The reduction of cytochrome *c* was measured by following the increase in the absorbance at 550 nm [8],

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and was performed in a reaction mixture containing 30 μM cytochrome *c*, 40 μg xanthine oxidase/ml, 50 mM acetaldehyde and 50 mM phosphate buffer (pH 7.5) at 25°C.

Protein concentrations were determined by the Coomassie brilliant blue G-250 dye-binding method of Bradford using Bio-Rad dye reagent [10].

3. RESULTS

Fig.1 shows that caeruloplasmin has a larger antioxidant effect than superoxide dismutase in the microsomal NADPH-dependent lipid peroxidation catalyzed by ADP-Fe^{3+}. The antioxidant effect of caeruloplasmin decreased at high concentrations of ADP-Fe^{3+}.

Fig.2 shows that caeruloplasmin inhibits the reduction of cytochrome *c* by O_2^- generated by the acetaldehyde–xanthine oxidase system, and that the O_2^- scavenging activity of caeruloplasmin is smaller than the activity of superoxide dismutase. From fig.1 and 2, it is considered that the antioxidant effect of caeruloplasmin is independent of the

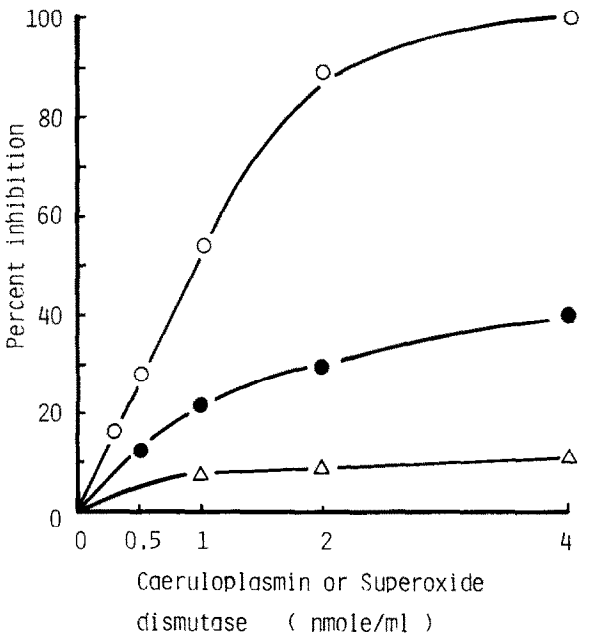

Fig.1. Antioxidant effects of caeruloplasmin and superoxide dismutase on microsomal lipid peroxidation catalyzed by ADP-Fe^{3+}: (○) caeruloplasmin at 15 μM $FeCl_3$; (●) caeruloplasmin at 30 μM $FeCl_3$; (△) superoxide dismutase at 15 μM $FeCl_3$.

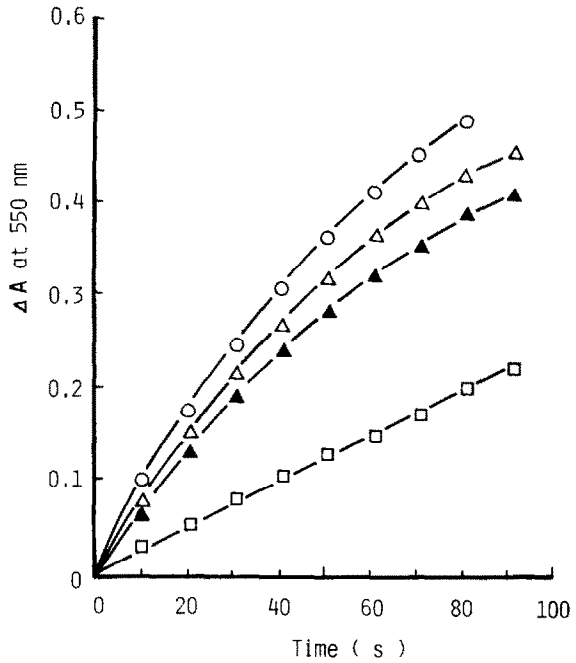

Fig.2. Effects of caeruloplasmin and superoxide dismutase on the reduction of cytochrome *c* by O_2^- generated in xanthine oxidase system: (○) complete system; (□) 0.2 μM superoxide dismutase; (△) 0.4 μM caeruloplasmin; (▲) 0.8 μM caeruloplasmin.

O_2^- scavenging activity, and that O_2^- liberated from microsomes is not significantly involved in microsomal NADPH-dependent lipid peroxidation catalyzed by ADP-Fe^{3+}.

Table 1 shows that NADPH oxidation is stimulated by the addition of ADP-Fe^{3+}, indicating electron transport from NADPH to ADP-Fe^{3+}. NADPH oxidation stimulated in the presence of ADP-Fe^{3+} was enhanced by the addition of caeruloplasmin. This result suggests that caeruloplasmin acting as ferroxidase catalyzes the reoxidation of ADP-Fe^{2+} to enhance the electron transport from NADPH to ADP-Fe^{3+}. As the antioxidant effect of caeruloplasmin decreased with increasing concentration of ADP-Fe^{3+}, it is considered that the antioxidant effect of caeruloplasmin depends on the ferroxidase activity/ADP-Fe^{3+} ratio.

4. DISCUSSION

Caeruloplasmin is synthesized in liver microsomes [11], and is present in normal human plasma at a concentration of about 300 μg/ml [12,13]. Re-

Table 1

Effects of caeruloplasmin on NADPH oxidation catalyzed by ADP-Fe^{3+} in microsomal electron transport system

Additions	ΔA at 340 nm/min	(%)[a]
None	−0.06	50
20 μM $FeCl_3$ and 1 mM ADP (ADP-Fe^{3+})	−0.12	100
ADP-Fe^{3+} and 1.3 μM caeruloplasmin	−0.15	125
ADP-Fe^{3+} and 2.6 μM caeruloplasmin	−0.17	142

[a] Percentage was calculated on the basis of the rate of NADPH oxidation stimulated by the addition of ADP-Fe^{3+}.

Reaction mixtures contained 1 mg microsomal protein/ml 0,25 mM NADPH, 150 mM KCL and 50 mM Tris–HCL (pH 7.5). Reaction mixtures were incubated at 25°C.

cently, it has been suggested that caeruloplasmin may be an antioxidant enzyme, because caeruloplasmin inhibits the autoxidation of lipids of ox-brain homogenate [14] and purified ox-brain-phospholipid liposomes [15,16] induced by inorganic iron or by ascorbic acid. However, the mechanism of the antioxidant effect of caeruloplasmin has not been clarified in complex systems such as in vivo, because the antioxidant effect of caeruloplasmin may depend on the ferroxidase activity [6], ascorbate oxidase activity [17] and the O_2^- scavenging activity [9]. In the model system such as lipid peroxidation of liposomes induced by Fe^{2+}, the antioxidant effect of caeruloplasmin depends on the ferroxidase activity [16].

Here, it was found that caeruloplasmin inhibited microsomal NADPH-dependent lipid peroxidation catalyzed by ADP-Fe^{3+}, and that the antioxidant effect of caeruloplasmin was independent of the O_2^- scavenging activity. As caeruloplasmin enhanced NADPH oxidation stimulated by ADP-Fe^{3+} acting as electron acceptor, caeruloplasmin is considered to catalyze the reoxidation of ADP-Fe^{2+}. Thus, the antioxidant effect of caeruloplasmin on microsomal NADPH-dependent lipid peroxidation catalyzed by ADP-Fe^{3+} is considered to depend on the ferroxidase activity rather than the O_2^- scavenging activity.

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