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—— BRIEF NOTE ——

METHEMOGLOBIN FORMATION BY PARAQUAT

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Abstract. Paraquat is a broad spectrum herbicide known to be highly lethal to man and animals. Its toxicity is characterized by acute lung injury. Paraquat probuces such toxic effects through the generation of the superoxide anion according to one proposed mechanism. The present experiment, methemoglobin formation was demonstrated after incubation of oxyhemoglobin with paraquat. The generation of the superoxide anion through the interaction of oxyhemoglobin with paraquat was suggested by chemiluminescence of luminol. Superoxide dismutase (SOD) and catalare inhibited methemoglobin formation. The generation of the superoxide anion is discussed in regard to methemoglobin formation by paraquat.

Key words : paraquat, superoxide anion, methemoglobin, superoxide dismutase, methemoglobin formation by paraquat.

Gramoxone (marketed by Plant Protection Co.) is a widely used weed killer. The effective component of Gramoxone is the herbicide paraquat (methyl viologen, 1,1'-dimethyl-4,4'-bipyridylium dichloride) (1). The herbicidal action of paraquat is a consequence of the intracellular reduction of molecular oxygen by reduced flavoprotein followed by oxidation of the paraquat radical by molecular oxygen. The hydrogen peroxide thereby evolved could account for the lipid peroxidation and membranolysis that has been observed (2).

Paraquat has been responsible for at least a hundred deaths after accidental of intentional ingestion. The primary cause of death is respiratory failure due to lung injury. One of the proposed mechanisms for the toxic effect of paraquat is the generation of the superoxide anion which causes lipid peroxidation in the lung tissue (3), (4).

However, the biochemical events underlying the toxicity of this compound are not well understood. The formation of the superoxide anion has been demonstrated in a completely reconstructed microsomal system consisting of isolated cytochrome C reductase, microsomal lipids and the NADPH generating system (5).

In this experiment, methemoglobin formation was demonstrated after incubation of oxyhemoglobin with paraquat. The generation of the superoxide anion was suggested by luminol chemiluminescence induced by the interaction

of oxyhemoglobin with paraquat. It already has been reported that the superoxide anion was related to methemoglobin formation induced by phenyl hydrazine (6) or nitrite (7), (8).

The generation of the superoxide anion by the interaction of oxyhemoglobin with paraquat is discussed in relation to methemoglobin formation.

Materials and methods. Venous blood was obtained from a healthy young man and hemolysed by addition of cold distilled water. The hemolysate was centrifuged at 25,000 r.p.m. for 2 h. The supernatant hemoglobin solution was dialysed against distilled water in a cold room for 24 h. Sephadex DE-52 moistened with 1 mM phosphate buffer at pH 6.9 was added to the hemoglobin solution and stirred slowly for one hour. Sephadex absorbed catalase was discarded by centrifugation. Catalase free hemoglobin solution was thus obtained (9).

0.7 mM of paraquat was added to the hemoglobin solution (0.6 mg/ml) in 0.1 M phosphate buffer at pH 7.0 and incubated for 2, 4 and 6 h at 37℃. After incubation, the absorbtion spectrum of hemoglobin was observed by an autorecording spectrophotometer (Hitachi Co. 100-50).

A hundred units/ml of SOD and a hundred units/ml of catalase were added to the paraquat-hemoglobin mixture and incubated for 4 h. Effects of SOD and catalase on methemoglobin formation by paraquat were studied.

In order to detect the generation of the superoxide anion and hydrogen peroxide, 10 mM of paraquat was added to a mixture of hemoglobin (0.3 mg/ml) and 0.25 mM luminol in 0.05 M bicarbonate buffer (pH 10.2) at 37℃.

Chemiluminescence of luminol was measured by a Luminescence Reader (Aloka Co. 102). Thirty units of SOD and thirty units of catalase were added through a needle with syringe. Changes in the intensity of chemiluminescence were recorded. SOD (3,000 units/mg protein) and catalase (300,000 units/mg protein) made by Sigma Co., U.S.A. were used in this experiment. The Paraquat standard was purchased from Wakojunyaku Co., Japan.

Results and Discussion. An increase at 630 nm. and decrease in the peaks at 577 nm. and 540 nm. were observed in the absorbtion spectrums of hemoglobin after incubation with paraquat (Fig. 1). These changes are specific to methemoglobin formation. Methemoglobin was formed after incubation with paraquat at 2, 4 and 6 h in increasing order.

A decrease at 630 nm. in the absorbtion spectrums was observed after the incubation of paraquat and hemoglobin with a hundred units of SOD or catalase or both of them together for 4 h, as shown in Fig. 2. SOD, catalase and a mixture of the two inhibited methemoglobin formation by paraquat in increasing order.

The generation of the superoxide anion by paraquat and hemoglobin was suggested by chemiluminescence of luminol, which was inhibited by addition of SOD as shown in the left side of Fig. 3. The generation of hydrogen peroxide

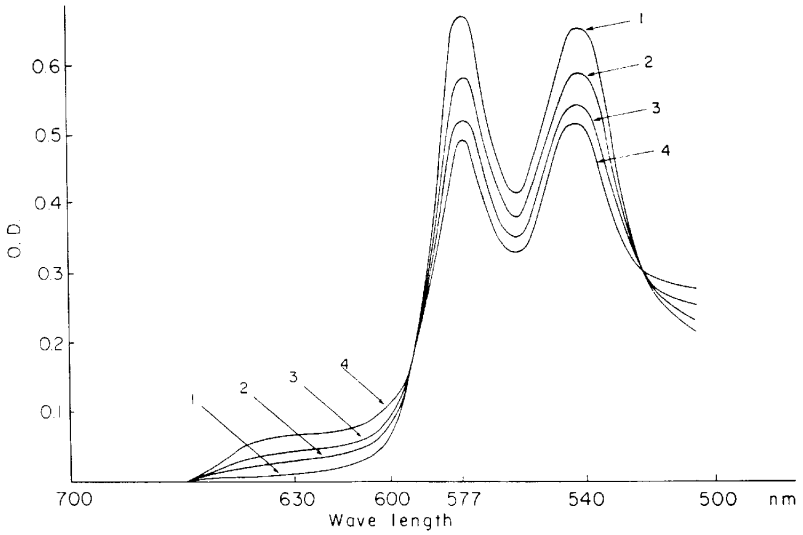

Fig. 1. Absorption spectrum of hemoglobin after incubation with paraquat. 0.7 mM of paraquat and 0.6 mg/ml of hemoglobin in 0.1 M phosphate buffer at pH 7.0 were incubated for 2, 4 and 6 h at 37℃.

1. control (hemoglobin incubated without paraquat); 2. incubated with paraquat for 2 h; 3. incubated with paraquat for 4 h; 4. incubated with paraquat for 6 h.

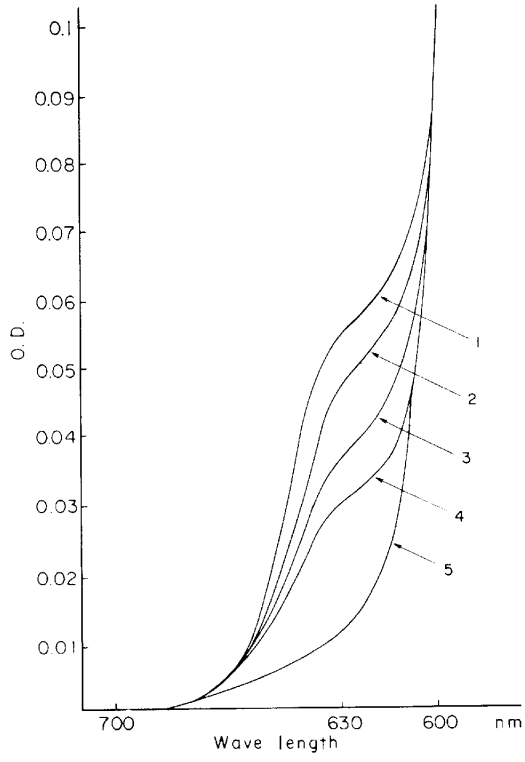

Fig. 2. Absorption spectrums of hemoglobin after incubation with paraquat, SOD and catalase. 0.6 mg/ml of hemoglobin in 0.1 M phosphate buffer at pH 7.0 were incubated with 0.7 mM paraquat, SOD and catalase for 4 h at 37℃.

1. hemoglobin with paraquat; 2. hemoglobin with paraquat and SOD; 3. hemoglobin with catalase and paraquat; 4. hemoglobin with SOD and catalase and paraquat; 5. control (hemoglobin incubated for 4 h).

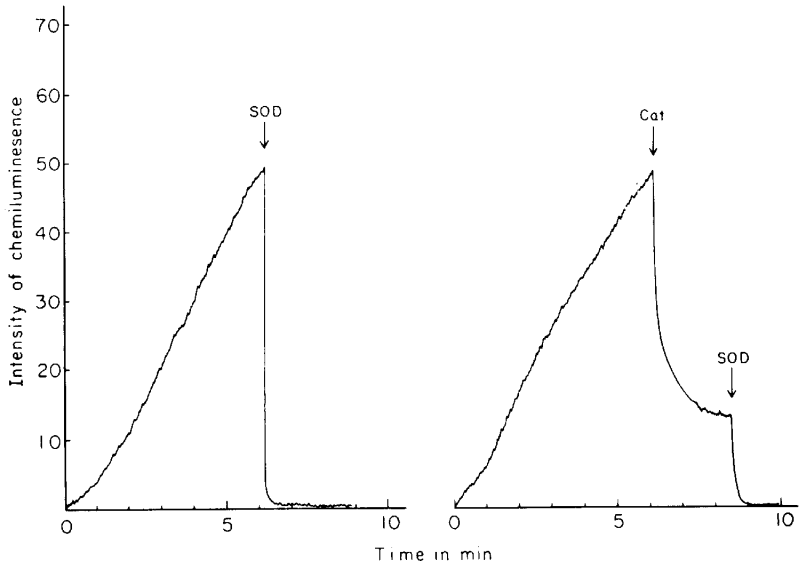

Fig. 3. Luminol chemiluminescence induced by hemoglobin and paraquat. 0.3 mg/ml of hemoglobin and 10 mM paraquat with 0.25 mM luminol in 0.05 M bicarbonate buffer at pH 10.2. After initiation of the reaction, 30 units of SOD was added as indicated in the figure (left). Thirty units of catalase was added first and afterwards 30 units of SOD was added as indicated in the figure (right).

was suggested by the chemiluminescence of luminol, which was inhibited 70 % by addition first of catalase and disappeared after addition of SOD as shown in the right half of Fig. 3. The superoxide anion was converted to hydrogen peroxide (10).

Consequently, it is suggested that the superoxide anion generated by the interaction of oxyhemoglobin with paraquat oxidized oxyhemoglobin to form methemoglobin and hydrogen peroxide.

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