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LIPID PEROXIDATION BY BOVINE HEART SUBMITOCHONDRIAL PARTICLES STIMULATED BY 1,1′-DIMETHYL-4,4′-BIPYRIDYLIUM DICHLORIDE (PARAQUAT)

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Abstract—Paraquat enhanced the NADH-dependent lipid peroxidation of bovine heart submitochondrial particles in the presence of ADP-Fe^{3+} chelate. The enhancement at physiological pH was about 3-fold. The pH optimum of the lipid peroxidation was shifted from pH 5.5 to pH 6.5 by paraquat. The submitochondrial particles catalyzed the reduction of paraquat when incubated anaerobically with NADH, whereas they did not reduce paraquat with succinate. The reduction was inhibited by *p*-hydroxymercuribenzoate or amytal, but it was not inhibited by rotenone, antimycin A or cyanide. The respiratory-chain inhibitors similarly affected the NADH-dependent O_2 consumption stimulated by paraquat, indicating that the NADH-dehydrogenase is involved in the reduction of paraquat at a region between the mercurial-sensitive site and the rotenone-sensitive site. The NADH-dependent reduction of ADP-Fe^{3+} chelate, a key step in lipid peroxidation, was stimulated by paraquat about 5-fold at physiological pH. The stimulation could mainly be ascribed to the direct electron transfer from a paraquat radical to the chelate and partially to the electron transfer from O_2^- produced by the reoxidation of the paraquat radical. ADP-Fe^{2+} produced lipid hydroperoxide in liposomes and decomposed cumene hydroperoxide. These reactions, the initiation reaction and the propagation reaction of peroxidation, were stimulated by paraquat. These results suggest that paraquat enhanced lipid peroxidation by stimulating (1) the reduction of ADP-Fe^{3+} chelate, and (2) the ADP-Fe^{2+}-dependent initiation and propagation reactions of the peroxidation.

Paraquat‡ (methyl viologen; 1,1′-dimethyl-4,4′-bipyridylium dichloride) has been used as a herbicide. The compound is toxic not only to plants but also to animals including humans [1, 2], though the mechanism of its toxicity is controversial. Bus *et al.* [3] reported that paraquat caused lung damage by inducing the peroxidation of microsomal lipids which was mediated by superoxide anions generated from paraquat radicals and by singlet oxygen derived from the anions. Other investigators, however, proposed the depletion of NADPH or a decrease in fatty acid synthesis as a mechanism of paraquat toxicity [4, 5].

Bovine heart submitochondrial particles are a prefered material for the study of effects of paraquat on reduced coenzyme-linked lipid peroxidation reactions, because oxidative reactions of reduced coenzyme including O_2^--forming activities [6, 7] have been well characterized. We have reported previously that bovine heart submitochondrial particles can catalyze NADPH-dependent [8] and NADH-dependent [9] peroxidation in the presence of ADP-Fe^{3+} chelate. In this report, we show that paraquat stimulates the NADH-dependent lipid peroxidation of bovine heart submitochondrial particles and suggest the following mechanisms of the stimulation: (1) that paraquat stimulates the reduction of the ADP-Fe^{3+} chelate by the respiratory chain with NADH as an electron donor, and (2) that paraquat enhances both the initiation (formation of lipid hydroperoxide) and the propagation (breakdown of lipid hydroperoxide) reactions of lipid peroxidation. These results support the supposition that lipid peroxidation is one of the mechanisms of paraquat toxicity.

EXPERIMENTAL

Preparation of submitochondrial particles. Bovine heart mitochondria were prepared from slaughterhouse material by the method of Blair [10], and the submitochondrial particles were prepared from them as EDTA-particles [11]. Mitochondrial lipids were extracted under N_2 gas by the method of Folch *et al.* [12]. Liposomes were prepared by sonicating the lipids in 50 mM HEPES buffer, pH 7.5, under N_2 gas with a Tomy sonicator (UR-150P) at an output of 4 A for 3 min at 4°. The amount of lipids was estimated as lipid phosphorous by the method of Bartlett [13].

Assay of lipid peroxidation. Malondialdehyde for-

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‡ Abbreviations: paraquat, 1,1′-dimethyl-4,4′-bipyridylium dichloride; MES, 2-(*N*-morpholino)-ethanesulfonic acid; and HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid.

mation was quantitated colorimetrically by means of the thiobarbituric reaction according to the method of Bernheim *et al.* [14]. A reaction mixture containing 0.2 to 0.5 mg of the particles, 2 mM ADP, 0.2 mM $FeCl_3$ and 1 mM paraquat, in 1 ml of 50 mM buffer, was shaken in air at 37° for 8 min. The buffers used were: MES buffer for pH 5.25 to 7.5, HEPES buffer for pH 6.5 to 8.3, and glycine NaOH buffer for pH 8.25 to 10.5. The reaction was started by the addition of NADH (0.5 mM) and terminated by the addition of 10 μM 3,5-di-*tert*-butyl-4-hydroxytoluene. The rate of malondialdehyde formation supported by NADH was obtained by subtracting the rate of the reaction without NADH. Protein was determined by the method of Lowry *et al.* [15] with bovine serum albumin as standard.

Reduction of paraquat under anaerobic conditions. The reduced form of paraquat (the free radical) is oxidized so rapidly by molecular oxygen, producing O_2^-, that the radical does not accumulate under aerobic conditions [16]. The reduction of paraquat, therefore, was measured anaerobically in a Thunberg cuvette (1 cm path length) under N_2. The final composition of the reduction system was 0.06 mg of the particles, 1 mM paraquat, and a respiratory-chain inhibitor in 2 ml of 50 mM glycine–NaOH buffer, pH 9.0. After preincubation for 5 min at 37°, the reaction was started by the addition of NADH (0.5 mM) or succinate (10 mM) from the side bulb of the cuvette. Changes in the absorbance of the paraquat radical were measured at 600 nm with a Hitachi 556 spectrophotometer. The absorption coefficient of the radical used for the determination was 13.5×10^3 liters M^{-1} cm^{-1} [17].

Oxygen consumption. Oxygen consumption was assayed with a Clark-type polarographic electrode (Gilson 5/6 oxygraph). The reaction mixture consisted of 0.1 to 0.2 mg of the particles, 1.0 mM paraquat, 20 μg catalase, 10 μg superoxide dismutase (2500 units/mg), and a respiratory-chain inhibitor in 1.7 ml of 50 mM glycine–NaOH buffer, pH 9.0. We assayed oxygen consumption at pH 9.0 because the paraquat reductase activity was highest at this pH. The reaction mixture was preincubated for 5 min at 37°, and the reaction was started by the addition of NADH (0.5 mM).

Reductase activity toward ADP-Fe^{3+}. The reduction of ADP-Fe^{3+} chelate was determined colorimetrically with 4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) which forms a red complex with ferrous iron [18]. Although the agent is useful for determining the relative rate of ferric iron reductase activity, it does not represent the true rate of the reduction because the system contains another iron chelating agent, ADP. Therefore, the activities were expressed as the absorbance change per minute as an arbitrary unit. The reaction mixture consisted of the particles (0.07 mg), 2 μM antimycin A, 2 mM ADP, and 0.2 mM bathophenanthroline in 1 ml of 50 mM HEPES buffer, pH 7.5. After preincubation for 5 min at 37°, the reaction was started by the addition of NADH (0.5 mM), and the increase in absorbance of the Fe^{2+}–bathophenanthroline complex was measured at 535 nm.

Formation of O_2^- *by the submitochondrial particles.* Formation of O_2^- was determined by measuring the reduction of acetylated cytochrome *c* [19, 20]. The reaction mixture consisted of the particles (0.7 mg), 2 μM antimycin A, and 50 μM acetylated cytochrome *c* in 1 ml of 50 mM glycine–NaOH buffer, pH 9.0. After preincubation for 5 min at 37°, the reaction was started by the addition of 1 mM NADH or 10 mM succinate, and the absorbance changes were followed in dual-wave mode ($A_{540-550}$). The absorption coefficient used was 19.1×10^3 M^{-1} cm^{-1}.

ADP-Fe^{2+}*-dependent lipid peroxidation in liposomes.* Lipid peroxidation was measured by the formation of lipid hydroperoxide and malondialdehyde. A reaction mixture contained liposomes (1.5 μmoles lipid phosphorous) and 2 mM ADP, with or without 1.0 mM paraquat, in 1 ml or 50 mM HEPES buffer, pH 7.5. The reaction mixture was preincubated for 10 min at 37° with shaking in air, and the reaction was started by the addition of either $FeCl_2$ or $FeCl_3$ (0.2 mM) and stopped by the addition of 1 μM 3,5-di-*tert*-butyl-hydroxytoluene for the assay of malondialdehyde and 5.0 ml of an ice-cold chloroform–methanol (2:1, v/v) mixture for the determination of lipid hydroperoxide. Lipid hydroperoxide was determined by the iodometric method as described by Buege and Aust [21].

ADP-Fe^{2+}*-dependent decomposition of cumene hydroperoxide.* A reaction mixture contained 200 μM cumene hydroperoxide and 2 mM ADP with or without 2 mM paraquat in 1 ml of 50 mM HEPES buffer, pH 7.5. After preincubation for 5 min at 37°, the reaction was started by the addition of either $FeCl_2$ or $FeCl_3$ (0.2 mM) and stopped by the addition of a chloroform–methanol (2:1, v/v) mixture. The content of cumene hydroperoxide was determined by the iodometric method as described by Buege and Aust [21].

Reagents. Superoxide dismutase was purified from ox blood by the method of McCord and Fridovich [22]. Catalase was purchased from Boehringer und Sohne, Mannheim, Germany, paraquat and antimycin A from the Sigma Chemical Co., St. Louis, MO, U.S.A., and cumene hydroperoxide from Nakarai Chemicals, Kyoto, Japan, respectively. Other reagents were of analytical grade.

RESULTS

Stimulation of lipid peroxidation by paraquat. When bovine heart submitochondrial particles were incubated with ADP-Fe^{3+} and NADH, the formation of malondialdehyde was observed. Figure 1 represents the pH profiles of the malondialdehyde formation. In the absence of paraquat, the pH profiles exhibited a progressive decrease from pH 6.0 to pH 8.0, and the pH optimum was at pH 5.5, consistent with that of the previous report [9]. In the presence of paraquat, however, a plateau was observed between pH 6.0 and pH 7.0 with the optimum pH shifted to pH 6.5. Malondialdehyde formation was stimulated by paraquat about 3-fold at physiological pH. No significant change in the apparent K_m value for NADH was observed by the addition of paraquat, whereas that for $FeCl_3$ decreased significantly by paraquat from 50 to 9 μM. The formation of malondialdehyde was negligibly low, even in the presence

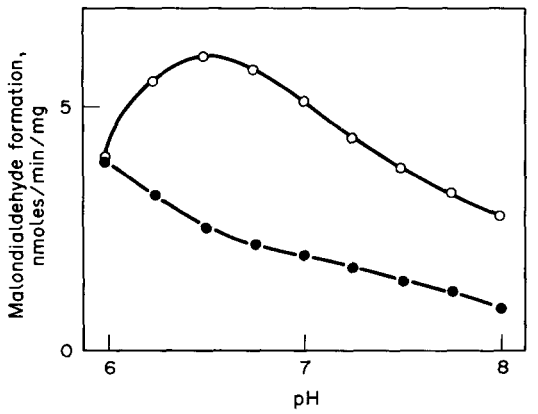

Fig. 1. Effect of pH on NADH-dependent malondialdehyde formation by submitochondria particles. A reaction mixture containing 0.2 to 0.5 mg of the particles, 2 mM ADP, 0.2 mM $FeCl_3$ and 0.5 mM NADH, with or without 1.0 mM paraquat, in 1 ml of 50 mM buffer was shaken in air at 37° for 8 min. The buffers used were MES for pH 5.25 to 7.5, HEPES for pH 6.5 to 8.3 and glycine–NaOH for pH 8.25 to 10.5. The samples that were incubated without NADH were taken as controls. Key: (○) with paraquat, and (●) without paraquat.

of paraquat, when the submitochondrial particles were incubated without $FeCl_3$ or NADH.

Reduction of paraquat under anaerobic conditions. Paraquat was reduced by bovine heart submitochondrial particles when they were incubated anaerobically with NADH. Succinate did not reduce paraquat even after prolonged incubation for 1 hr. The pH optimum of the reductase activity toward paraquat was found at pH 9.0. Then, we studied effects of respiratory-chain inhibitors on reductase activity toward paraquat. As shown in Table 1, *p*-hydroxymercuribenzoate inhibited the activity by 56% and amytal by 25%, whereas rotenone, antimycin A and cyanide did not affect the activity. These observations indicate that paraquat receives an electron [17] from an electron carrier between the mercurial-sensitive site and the rotenone-sensitive site.

Estimation, from O_2 consumption, of the rate of paraquat reduction. Paraquat stimulated the NADH-dependent oxygen consumption of the submitochondrial particles. This can be explained by the following reaction sequences:

$$4\,PQ + 4\,e^- \longrightarrow 4\,PQ\cdot \quad (1)$$

$$4\,PQ\cdot + 4\,O_2 \longrightarrow 4\,PQ + 4\,O_2^- \quad (2)$$

$$2\,O_2^- + 2\,O_2^- + 4\,H^+ \xrightarrow{SOD} 2\,H_2O_2 + 2\,O_2 \quad (3)$$

$$2\,H_2O_2 \xrightarrow{Catalase} 2\,H_2O + O_2 \quad (4)$$

$$4\,PQ\cdot + O_2 + 4\,H^+ \longrightarrow 2\,H_2O + 4\,PQ \quad (5)$$
$$[(2) + (3) + (4)]$$

(PQ, paraquat; PQ· paraquat radical)

Paraquat receives one electron from the respiratory chain, forming paraquat radical (reaction 1) which is reoxidized by molecular oxygen, forming O_2^- (reaction 2); O_2^- decomposes finally to H_2O_2 and O_2 (reactions 3 and 4). Thus, 1/4 mole of O_2 would be

Table 1. Effects of respiratory-chain inhibitors on paraquat reduction and O_2 consumpton, of submitochondrial particles, stimulated by paraquat*

Inhibitor	Paraquat reduction rate (μmoles/min per mg)	O_2 consumption rate (μmoles/min per mg)		Calculated rate of PQ reduction (μmoles/min per mg)
		Without PQ	With PQ	
Control	1.23 ± 0.05	0.20 ± 0.02	0.52 ± 0.07	1.28 ± 0.04
Rotenone (2 μM)	1.21 ± 0.04	0.01 ± 0.003	0.31 ± 0.02	1.21 ± 0.06
Amytal (2 mM)	0.93 ± 0.07	0.18 ± 0.06	0.39 ± 0.06	0.85 ± 0.06
Antimycin A (2 μM)	1.22 ± 0.07	0.03 ± 0.004	0.33 ± 0.04	1.20 ± 0.04
p-Hydroxymercuribenzoate (0.5 mM)	0.54 ± 0.08	0.02 ± 0.01	0.19 ± 0.01	0.67 ± 0.01
KCN (1 mM)	1.23 ± 0.04			

* The reduction of paraquat was assayed anaerobically in a Thunberg cuvette (1 cm path length). A reaction mixture, containing 0.06 mg of the particles, 1.0 mM paraquat and a respiratory-chain inhibitor, in 2 ml of 50 mM glycine–NaOH buffer, pH 9.0, was preincubated for 5 min at 37°, the reaction was started by the addition of NADH (0.5 mM), and the absorbance changes were measured at 600 nm. The O_2 consumption was assayed with a Clark-type oxygen electrode. The reaction mixture consisted of 0.1 to 0.2 mg of the particles, 1.0 mM paraquat, 20 μg catalase, 10 μg SOD (2500 units/mg) and a respiratory-chain inhibitor in 1.7 ml of glycine–NaOH buffer, pH 9.0; after preincubation for 5 min at 37°, the reaction was started by the addition of NADH (0.5 mM). The values are given as means ± S.D. of five experiments.

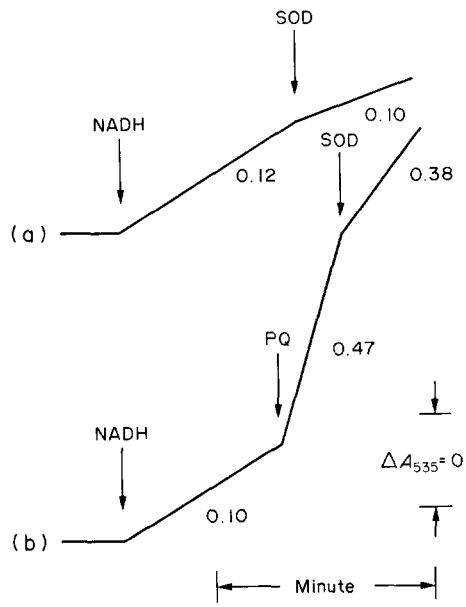

Fig. 2. Stimulation by paraquat of reductase activity of submitochondrial particles toward ADP-Fe^{3+}. An incubation mixture containing 0.07 mg of the particles, 2 mM ADP, 0.2 mM $FeCl_3$, 2 μM antimycin A, and 0.2 mM bathophenanthroline in 1 ml of 50 mM HEPES buffer, pH 7.5, was preincubated for 5 min at 37°. the reaction was started by the addition of NADH (0.5 mM). Paraquat (PQ) (10 μM) and SOD (10 μg; 2500 units/mg) were added as indicated. Values given on the traces indicate the initial velocities expressed as ΔA_{535}/min.

consumed when 1 mole of paraquat is reduced (reaction 5). We added superoxide dismutase (SOD) and catalase to the reaction mixture to facilitate reactions 3 and 4. Accordingly, the rate of paraquat reduction could be calculated from the O_2 consumption as follows:

Calculated rate of the PQ reduction = 4
× (O_2 consumption with PQ
− O_2 consumption without PQ).

As shown in Table 1 (the first line), the calculated rate of paraquat reduction agreed well with that of the reduction rate of paraquat under anaerobic conditions.

Effects of respiratory-chain inhibitors on the O_2 consumption stimulated by paraquat. The site where paraquat receives an electron from the respiratory chain was further investigated by measuring the effects of respiratory-chain inhibitors on the O_2 consumption by paraquat. As shown in Table 1 (calculated rate of PQ reduction), the effects of respiratory-chain inhibitors on the calculated rate of paraquat reduction were similar to those on the rates of paraquat reduction under anaerobic conditions. The results also confirm an aforementioned supposition that paraquat receives an electron from a region between the mercurial-sensitive site and the rotenone-sensitive site of the respiratory chain.

NADH-*dependent reductase activity toward* ADP-Fe^{3+}. We investigated the effect of paraquat on the reduction of ADP-Fe^{3+} chelate, because the reduction is a key step in lipid peroxidation [23]. The reaction was studied by the reduction of bathophenanthroline–iron complex, a qualitative assay procedure which does not give quantitative results. As shown in Fig. 2a, the addition of NADH caused the reduction, which could be inhibited by SOD only slightly when paraquat was absent, in agreement with little formation of O_2^- by the submitochondrial particles in this condition [6, 7]. In the presence of the herbicide, however, both reduction rate and extent of inhibition by SOD were pronounced (Fig. 2b), indicating that the iron was reduced by O_2^- formed mostly by the reoxidation of paraquat radicals. Thus, the reduction may happen by three different mechanisms in the presence of paraquat: (1) direct electron transfer from the respiratory chain, (2) reduction by paraquat radicals, and (3) reduction by O_2^-.

NADH-*dependent* O_2^- *formation by submitochondrial particles.* Bus *et al.* [3] suggested that paraquat toxicity may be mediated by O_2^-. The submitochondrial particles produce O_2^- at the NADH-dehydrogenase [6] and at the ubiquinone-cytochrome *c* region [7]. Therefore, we investigated the effects of paraquat on the NADH-dependent O_2^--forming activity of the submitochondrial particles. The activity was measured at pH 9.0 because the O_2^--

Table 2. Effects of paraquat on the reduction of acetylated cytochrome *c**

	Reduction of acetylated cytochrome *c* (nmoles/min per mg)	(%)
NADH (1.0 mM)	5.03 ± 0.29	100
+ PQ (5 μM)	11.20 ± 1.05	223
+ PQ (5 μM) + SOD	2.49 ± 0.20	49
+ PQ (10 μM)	21.50 ± 1.25	427
+ PQ (10 μM) + SOD	5.09 ± 0.38	101
Succinate (10 mM)	6.25 ± 0.87	100
+ PQ (10 μM)	5.82 ± 0.58	93

* A reaction mixture containing 0.7 mg of submitochondrial particles, 2 μM antimycin A and 50 mM acetylated cytochrome *c* in 1 ml of 50 mM glycine–NaOH buffer, pH 9.0, was preincubated for 10 min at 37°; the reaction was started by the addition of NADH (1.0 mM) or succinate (10 mM). The reduction of acetylated cytochrome *c* was assayed in dual-wave mode ($A_{550-540}$). The values are given as means ± S.D. of four experiments.

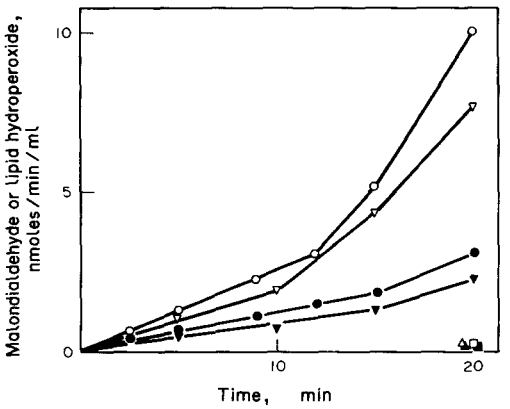

Fig. 3. ADP-Fe^{2+}-dependent lipid peroxidation in liposomes. Liposomes (1.5 μmoles of lipid phosphorous) were preincubated for 10 min at 37° with shaking in air in 1 ml of reaction mixture containing 2 mM ADP and 50 mM HEPES buffer, pH 7.5, with or without 1 mM paraquat. The reaction was started by the addition of either $FeCl_2$ or $FeCl_3$ (0.2 mM) and was terminated by the addition of 1 μM 3,5-di-*tert*-butyl-hydroxytoluene for the assay of malondialdehyde formation and 5.0 ml of an ice-cold chloroform–methanol (2:1) mixture for the determination of lipid hydroperoxide. Key: (▲) MDA without addition of paraquat and $FeCl_2$ or $FeCl_3$; (△) MDA with paraquat; (■) MDA with $FeCl_3$; (□) MDA with $FeCl_3$ and paraquat; (●) MDA with $FeCl_2$; (○) MDA with $FeCl_2$ and paraquat; (▼) lipid hydroperoxide with $FeCl_2$; and (▽) lipid hydroperoxide with $FeCl_2$ and paraquat.

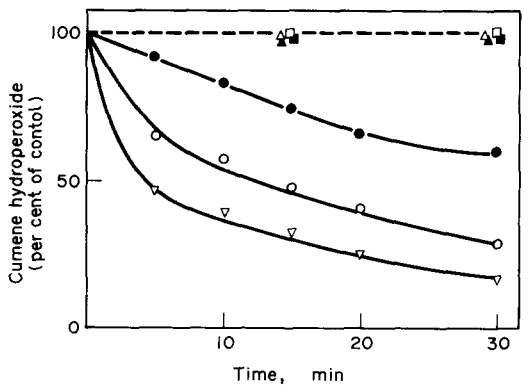

Fig. 4. ADP-Fe^{2+}-dependent decomposition of cumene hydroperoxide. A reaction mixture containing 200 μM cumene hydroperoxide (CHP), and 2 mM ADP, with or without 2 mM paraquat, in 1 ml of 50 mM HEPES buffer, pH 7.5, was preincubated for 5 min at 37°. The reaction was started by the addition of $FeCl_2$ or $FeCl_3$ (0.2 mM) and was terminated by the addition of a chloroform–methanol (2:1, v/v) mixture. The content of cumene hydroperoxide was measured iodometrically as described in the Experimental section. Key: (▲) CHP alone; (□) CHP + $FeCl_3$ + paraquat (2 mM); (○) CHP + $FeCl_2$ + paraquat (2 mM); (▽) CHP + $FeCl_2$ + paraquat (10 mM); (△) CHP + paraquat (2 mM); (■) CHP + $FeCl_3$; and (●) CHP + $FeCl_2$.

forming activity of the submitochondrial particles was highest at this pH. Acetylated cytochrome *c* was used as an electron acceptor, because the submitochondrial particles reduced the modified cytochrome *c* with NADH or succinate, and the reactions were completely inhibited by SOD. As shown in Table 2, the reduction with NADH was stimulated 4-fold by the addition of 10 μM paraquat, but SOD did not completely inhibit the reduction. The SOD-insensitive reduction may be attributed to a direct electron transfer from paraquat radical to acetylated cytochrome *c*, whereas the SOD-sensitive reduction, which was stimulated about 3-fold, is attributed to O_2^- generated by the reoxidation of paraquat radical and by the NADH-dependent O_2^--forming activity of the submitochondrial particles. The reduction with succinate, however, was not affected by paraquat, probably because succinate does not reduce paraquat.

ADP-Fe^{2+}-*dependent lipid peroxidation in liposomes*. Lipid peroxidation consists of two sequential reactions, the initiation reaction which is defined as the formation of lipid hydroperoxides and the propagation reaction which is ferrous iron-catalyzed breakdown of the lipid hydroperoxides formed by the initiation reaction [24].

The time-course of lipid peroxidation in liposomes is shown in Fig. 3. ADP-Fe^{2+} catalyzed the formation of both lipid hydroperoxides and malondialdehyde whereas ADP-Fe^{3+} did not. Paraquat stimulated the peroxidation in the presence of ADP-Fe^{2+}, but it did not stimulate the peroxidation in the absence of the ferrous iron chelate. Formation of both lipid hydroperoxides and malondialdehyde was linear for at least 20 min in the absence of paraquat, but the formation in the presence of the herbicide increased exponentially after the linear phase of about 10 min. After 20 min of incubation, lipid hydroperoxides (2.3 nmoles/ml) and malondialdehyde (2.7 nmoles/ml) were found in the samples incubated without paraquat whereas about three times that amount was found in the samples incubated with the herbicide (8.0 nmoles/ml and 10 nmoles/ml respectively). The finding that almost equimolar amounts of lipid hydroperoxides and malondialdehyde were present in both cases seems to disagree with the report of Tam and McCay [25] that a small percentage of lipid hydroperoxide is converted to malondialdehyde, but this may arise from the difference in the experimental systems; they used an NADPH-dependent microsomal system and we used a system with liposomes and a ferrous iron chelate. Furthermore, the amount of lipid hydroperoxide measured does not correspond to the formation of the hydroperoxides, but it depends on both the rates of formation and degradation. Thus, the parallel change of both intermediates and product suggests that paraquat stimulates both the initiation and the propagation reactions.

ADP-Fe^{2+}-*dependent breakdown of cumene hydroperoxide*. We investigated the effect of paraquat on the second step of lipid peroxidation (propagation reaction) by using cumene hydroperoxide as a model organic hydroperoxide. As shown in Fig. 4, ADP-Fe^{2+} catalyzed the breakdown but ADP-Fe^{3+} or paraquat, alone, did not. The addition of

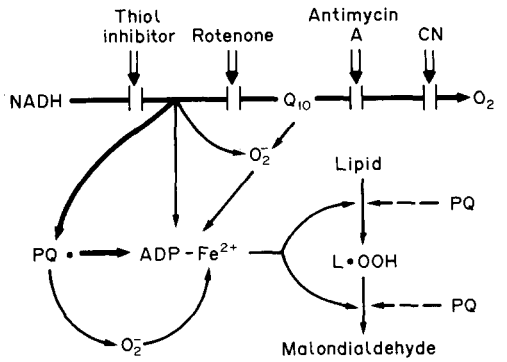

Fig. 5. Proposed scheme for the stimulation of lipid peroxidation by paraquat in bovine heart submitochondrial particles.

2 mM paraquat stimulated the ferrous iron-dependent breakdown about 2-fold (30-min incubation) which confirms a supposition that the herbicide stimulated the propagation reaction.

DISCUSSION

The results presented in this report indicate that paraquat stimulated an NADH-dependent lipid peroxidation in bovine heart submitochondrial particles, and the stimulation can be explained by a scheme (Fig. 5).

The respiratory chain transmitted a single electron from NADH to paraquat, forming a paraquat radical. The site where paraquat receives an electron could be identified as a region between the mercurial-sensitive site and the rotenone-sensitive site, because the reduction of paraquat under anaerobic conditions and the O_2 consumption stimulated by paraquat were not inhibited by rotenone, antimycin A or cyanide but were inhibited by *p*-hydroxymercuribenzoate or amytal. These effects of respiratory chain inhibitors on the reduction of paraquat were similar to those on the O_2^--forming activity of the bovine heart submitochondrial particles [6] and the reductase activity toward ADP-Fe^{3+} of the NADH-ubiquinone reductase preparation [23]. These observations suggest that a similar site of the NADH-dehydrogenase is responsible for the reduction of paraquat, the formation of O_2^- and the reduction of ADP-Fe^{3+}. The site which is sensitive to mercurial or amytal is not clear, but we suggested in the previous report [6] that a slowly reactive thiol group might be a candidate for the mercurial-sensitive site. It is conceivable that amytal inhibits the respiratory chain at the substrate side of the rotenone-sensitive site, because the reduction of paraquat under anaerobic conditions and the O_2 consumption stimulated by paraquat were not inhibited by rotenone but inhibited by amytal. Gage [26] has reported that the reduction of paraquat is inhibited by amytal.

The reduction of the ADP-Fe^{3+} chelate is a key step in lipid peroxidation [23], and the stimulation of the reduction by paraquat may partly be responsible for the stimulation of lipid peroxidation by the drug. The stimulation could mainly be ascribed to the electron transfer from a paraquat radical to the chelate, at higher concentrations of paraquat. As shown in Figs. 3 and 4, ADP-Fe^{2+} catalyzed both initiation and propagation reactions of lipid peroxidation, and the herbicide stimulated both reactions of lipid peroxidation. The mechanisms of the stimulation is not clear at present, but an interaction between the non-reduced form of the herbicide and the intermediate(s) of ADP-Fe^{2+}-dependent lipid peroxidation may be suggested because ADP-Fe^{2+} does not reduce paraquat (unpublished observation).

Paraquat intoxication results in an energy-dependent accumulation of the herbicide into the lung [27] with subsequent occurrence of pulmonary fibrosis [17]; microsomal lipid peroxidation has been proposed as a mechanism underlying the effects of the drug on the lung [3]. We have shown in this report that paraquat stimulated lipid peroxidation in heart mitochondria. Mitochondria of the lung may also be affected by paraquat, because paraquat accumulates in mitochondria of the lung [28]. Lipid peroxidation in mitochondria results in the inhibition of electron transport and oxidative phosphorylation [29]. Furthermore, Gage [26] reported that paraquat interacts with the mitochondrial respiratory chain, and Ogata and Hasegawa [30] reported that paraquat is an uncoupler of the mitochondrial energy transfer reaction. Thus, the survival of cells may be affected by peroxidation of mitochondrial lipids and by uncoupling of mitochondrial energy production in the presence of paraquat.

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