NADH- and NADPH-dependent lipid peroxidation in bovine heart submitochondrial particles

Dependence on the rate of electron flow in the respiratory chain and an antioxidant role of ubiquinol

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Malondialdehyde formation by bovine heart submitochondrial particles supported by NADH or NADPH in the presence of ADP and $FeCl_3$ was studied. The NADH-dependent reaction was maximal at very low rate of electron input from NADH to the respiratory chain and it decreased when the rate became high. The reaction was stimulated by rotenone and inhibited by antimycin A when the input was fast, whereas it was not affected by the inhibitors when the input was slow. The input rate of the electrons from NADPH was also so low that the reaction supported by NADPH was not affected by the inhibitors. Most of the endogenous ubiquinone in the particles treated with antimycin A was reduced by NADH even in the presence of ADP–Fe^{3+} chelate, but ubiquinone was not reduced by NADPH when ADP–Fe^{3+} was present. Succinate strongly inhibited both NADH- and NADPH-dependent lipid peroxidation. The inhibition was abolished when ubiquinone was removed from the particles, and it appeared again when ubiquinone was reincorporated into the particles. Reduced ubiquinone-2 also inhibited the peroxidation, but duroquinol, which reduces cytochrome *b* without reducing endogenous ubiquinone, did not. Thus the malondialdehyde formation appeared to be inversely related to the extent of the reduction of endogenous ubiquinone. These observations suggest that both NADH- and NADPH-dependent lipid-peroxidation reactions are closely related to the respiratory chain and that the peroxidation is controlled by the concentration of reduced ubiquinone.

Non-enzymic lipid-peroxidation reactions of the mitochondrial membrane supported by reducing agents such as ascorbate or glutathione (Hunter *et al.*, 1964*a,b,c*; McKnight & Hunter, 1966) or those induced by chaotropic agents (Hatefi & Hanstein, 1970) have been intensively studied, and Pfeifer & McCay (1972) have shown the existence of an NADPH-dependent lipid-peroxidation reaction in rat liver mitochondria that is suggested by them to be a part of the non-energy-linked transhydrogenase reaction. We reported previously that bovine heart mitochondria and the submitochondrial particles prepared from them formed malondialdehyde with NADPH as an electron donor (Takeshige & Minakami, 1975). The properties of the reaction were quite different from those of rat liver mitochondria and microsomal fractions. Bovine heart mitochondria are a preferable material for the study of reduced-coenzyme-linked lipid-peroxidation reac-

tions, because the mitochondrial fragments, such as submitochondrial particles and NADH–ubiquinone reductase preparation (Complex I), have been obtained, and the oxidative reactions of reduced coenzymes including the O_2^--forming reactions (Takeshige & Minakami, 1979) have been characterized.

The present paper and the following one (Takeshige *et al.*, 1980) deal with the lipid-peroxidation reactions catalysed by bovine heart submitochondrial particles or by the NADH–ubiquinone reductase preparation with NADH or NADPH as an electron donor and in the presence of ADP and Fe^{3+}. We show in the present paper that not only NADPH but also NADH can support the peroxidation in heart mitochondria, electrons being supplied to the lipid-peroxidation reactions from a component between the substrate site and the rotenone-sensitive site of the NADH dehydrogenase

of the respiratory chain. We also show that the peroxidation of mitochondrial membrane is controlled by a potent antioxidant, ubiquinol.

Experimental

Preparation of submitochondrial particles containing various amounts of ubiquinone

Bovine heart mitochondria were prepared by the method of Blair (1967), and the submitochondrial particles were prepared from them as EDTA particles (Lee & Ernster, 1967). The particles with various contents of ubiquinone-10 were prepared as described by Norling et al. (1974), and the enzymically reducible ubiquinone was measured by the change in $A_{275-300}$ with succinate, an absorption coefficient of $12.2 \times 10^3 \, M^{-1} \cdot cm^{-1}$ being used.

Lipid peroxidation

The formation of malondialdehyde due to the peroxidation was measured by the thiobarbituric acid method of Bernheim et al. (1948). The particles (0.2–0.5 mg of protein) in 1.0 ml of a reaction mixture consisting of 2 mM-ADP, 0.2 mM-$FeCl_3$ and 50 mM-Tris/maleate buffer, pH 6.75 at 37°C, or Tris/acetate buffer, pH 7.4 at 37°C, were incubated at 37°C with shaking. The reaction was started by the addition of 0.5 mM-NADH or -NADPH and stopped by the addition of 0.05 ml of 100% (w/v) trichloroacetic acid (w/v) and 0.01 ml of 10 mM-2,6-di-t-butyl-p-cresol. In some experiments, reduced-coenzyme-generating systems were used. The malondialdehyde formation was usually linear with time for more than 5 min in the NADH-dependent reaction and for more than 10 min in the NADPH-dependent reaction. The linear portion of the time course was used for the determination of the rate. The rate of the peroxidation reaction supported by reduced coenzymes was obtained by subtracting the rate of the reaction without coenzymes.

Other analytical procedures

The redox state of ubiquinone was determined by extraction with methanol/light petroleum (b.p. 30–60°C) mixture as described by Kröger & Klinenberg (1966), except that pyrogallol (0.5 mg/ml) was added to the mixture used for the first extraction (Pumphrey & Redfearn, 1960) to prevent the oxidation of the reduced ubiquinone by ADP–Fe^{3+}. Although pyrogallol caused the underestimation of ubiquinone (Szarkowska & Klingenberg, 1963), it was only about 6% [e.g. in the same sample with and without pyrogallol, 4.08 ± 0.09 and 3.85 ± 0.09 nmol/mg of protein respectively (means $\pm$ S.E.M., $n = 7$)]. The NAD(P)$^+$ transhydrogenase, succinate–cytochrome c reductase and NADH–cytochrome c reductase activities were

assayed by the method of Kaplan (1967), King (1967) and Hatefi & Rieske (1967) respectively. The activities of NADH– and NADPH–ferricyanide reductase were measured by the decrease in A_{420}. Protein was determined by the method of Lowry et al. (1951), with bovine serum albumin as standard. Other analytical conditions are described in the legends to the Tables and Figures.

Reagents

Duroquinol (reduced tetramethyl-p-benzoquinone) and ubiquinol-2 were prepared by the reduction of oxidized forms in pure ethanol by $NaBH_4$ (Nelson & Gellerfors, 1978). Ubiquinones were generously supplied from Eisai Co., Tokyo, Japan. Other reagents used were of analytical grade.

Results

Lipid peroxidation in bovine heart mitochondria

Bovine heart submitochondrial particles produce malondialdehyde when they are incubated with NADPH, ADP and ferric ions (Takeshige & Minakami, 1975).

A reaction supported by NADH was also observed in the particles, as shown in Table 1. ADP–Fe^{3+} chelate was essential to both NADPH- and NADH-dependent reactions, because the rate of malondialdehyde formation in the absence of the chelate was negligibly low, for example 0.006 nmol/min per mg of protein without ADP–Fe^{3+} and 0.070 nmol/min per mg without NADH or NADPH. Both NADH- and NADPH-dependent lipid-peroxidation reactions required acidic pH; the pH optimum of the former was pH 5.5 and that of

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Table 1. *Malondialdehyde formation of the submitochondrial particles supported by reduced nicotinamide coenzymes*

The submitochondrial particles were incubated at 4°C for 200 min with or without trypsin (0.1 mg/mg of protein) in 50 mM-Tris/HCl buffer, pH 7.0 at 4°C, and then soya-bean trypsin inhibitor (0.4 mg/mg of protein) was added to stop the digestion. Malondialdehyde formation was determined as described in the Experimental section, except that 50 mM-Tris/acetate buffer, pH 7.4 at 37°C, and 0.8 mM-coenzymes were used. The values are given as means $\pm$ S.E.M. of four determinations.

| | Malondialdehyde formation (nmol/min per mg of protein) | |
Coenzyme(s)	Non-digested	Trypsin-digested
NADPH	4.55 ± 0.12	4.58 ± 0.07
NADH	1.82 ± 0.07	1.87 ± 0.08
NADPH + NADH	1.64 ± 0.08	1.87 ± 0.09
NADPH + NAD$^+$	2.87 ± 0.21	4.21 ± 0.11

Table 2. *Effects of respiratory chain inhibitors on the NADH- and NADPH-dependent malondialdehyde formation*

The submitochondrial particles (0.5 mg/ml) were incubated for 10 min at 37°C with the inhibitors before the addition of the reduced coenzymes. The assay conditions were as described in the Experimental section and the pH of the medium was 6.75. Thunberg tubes were used in Expt. 2. O_2 in the gas phase was 15% (v/v) and the remaining part was either N_2 or CO in Expt. 2. The values in Expt. 1 are given as means ± S.E.M. of four determinations. The values in parentheses are percentages of the control values.

	Malondialdehyde formation (nmol/min per mg of protein)	
	With NADH	With NADPH
Expt. 1		
Control	2.54 ± 0.09 (100)	5.10 ± 0.13 (100)
+ p-Hydroxymercuribenzoate (10 μM)	6.96 ± 0.18 (274)	5.20 ± 0.17 (102)
+ Rotenone (4 nmol/mg of protein)	7.11 ± 0.09 (280)	5.30 ± 0.16 (104)
+ Antimycin A (0.2 μg/mg of protein)	0.25 ± 0.01 (10)	4.54 ± 0.06 (89)
+ KCN (0.25 mM)	0.38 ± 0.02 (15)	5.30 ± 0.16 (104)
+ Rotenone + antimycin A	6.12 ± 0.13 (241)	4.44 ± 0.07 (87)
+ Rotenone + KCN	6.86 ± 0.26 (270)	5.00 ± 0.19 (98)
Expt. 2		
N_2/O_2 (control)	2.17 (100)	3.99 (100)
N_2/O_2 + rotenone (4 nmol/mg of protein)	4.23 (195)	4.04 (101)
CO/O_2	0.10 (5)	3.84 (96)
CO/O_2 + rotenone (4 nmol/mg of protein)	4.34 (200)	4.14 (104)

the latter was about pH 6.2 (results not shown). Most of the experiments were, however, done at physiological pH (7.4).

A possible contribution of the NAD(P)$^+$ transhydrogenase of the mitochondria, which was proposed by Pfeifer & McCay (1972), was ruled out because the peroxidation activities were resistant to the treatment of the particles with trypsin (Table 1) or with thiol-specific inhibitors such as p-hydroxymercuribenzoate (Table 2), to which the transhydrogenase is highly sensitive.

Simultaneous additions of NADH and NADPH did not give synergistic results, but the peroxidation rate was essentially the same as that with NADH only (Table 1). This suggests that NADH and NADPH give reducing equivalents through a common enzyme, probably through the NADH dehydrogenase of the respiratory chain, for which NADPH has a lower affinity than NADH. When NADH (0.5 mM) was used as an electron donor the peroxidation was slow, whereas when NADPH (0.5 mM) was used, the peroxidation was fast. This is an apparently contradictory phenomenon, because the electron input from NADH to the respiratory chain is much faster than that from NADPH.

Experiments with reduced-coenzyme-generating systems

The observation that the peroxidation with NADH was slower than that with NADPH, urged us to study the reactions at extremely low electron-input rate (Fig. 1). The electron input was controlled by changing the concentrations of NAD$^+$

and NADP$^+$ or the dehydrogenase in the regenerating systems, and the rate of the input was determined by the reduction of ferricyanide (Ragan, 1976). As shown in Fig. 1(a), the rate of the NADH-dependent peroxidation was maximal at a low concentration of NAD$^+$ (about 2 μM) with an apparent K_m value of 0.9 ± 0.2 μM (mean ± S.E.M., $n = 3$). The rate decreased sharply as the NAD$^+$ concentration increased above 2 μM, whereas the rate of the NADPH-dependent peroxidation increased continuously as the NADP$^+$ concentration increased. The apparent K_m value for NADP$^+$ was 96 ± 8 μM (mean ± S.E.M., $n = 3$). The relationship between the rate of electron input and the lipid peroxidation is shown in Fig. 1(b). The lipid-peroxidation reaction reached a maximum at an input rate of about 300 nmol of electrons · min^{-1} · mg of protein^{-1} and then decreased. The input rate was about 6500 nmol · min^{-1} · mg^{-1} under standard assay conditions with 0.5 mM-NADH and the rate from NADPH did not exceed 140 nmol · min^{-1} · mg^{-1}.

Effects of respiratory inhibitors

Table 2 shows the effects of respiratory inhibitors on the lipid-peroxidation reactions. The NADH-dependent reaction was greatly influenced by inhibitors; Amytal (2 mM) and N-ethylmaleimide (1 mM) (results not shown), as well as rotenone and p-hydroxymercuribenzoate, enhanced the reaction more than 2-fold. Antimycin A, KCN and CO strongly inhibited the reaction, but they no longer inhibited the reaction when the particles were pretreated with rotenone. In contrast, the NADPH-
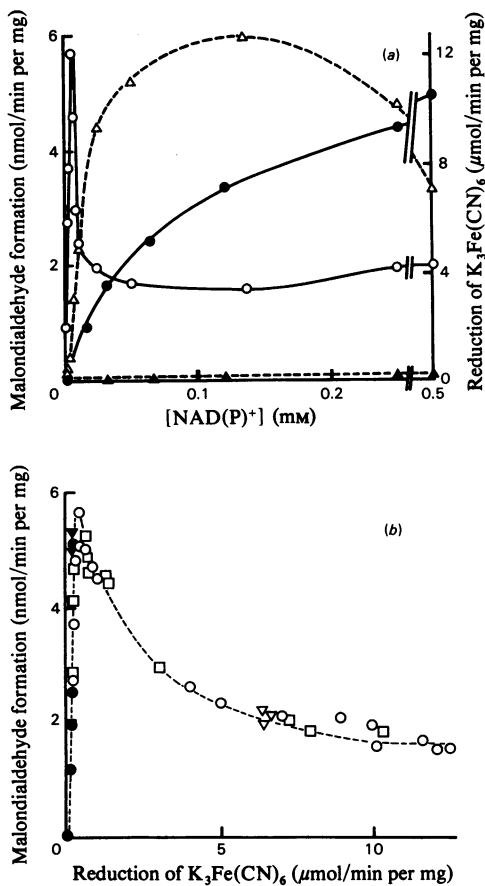
Fig. 1. *Rates of electron input (ferricyanide reductase activity) and malondialdehyde formation: (a) effects of the concentration of NAD(P)⁺ in regenerating systems; (b) relationship between the rates of ferricyanide reduction and malondialdehyde formation*

(a) The rates of both ferricyanide reduction and malondialdehyde formation were measured at pH 7.4 with an NADH-generating system (80 mM-ethanol, 17 units of alcohol dehydrogenase/ml and various concentrations of NAD⁺) or an NADPH-generating system (10 mM-glucose 6-phosphate, 4.5 units of glucose 6-phosphate dehydrogenase/ml and various concentrations of NADP⁺). Malondialdehyde formation without the addition of NAD⁺ was not zero because of the presence of endogenous NAD⁺. ○, NADH-dependent malondialdehyde formation; ●, NADPH-dependent malondialdehyde formation; △, NADH–ferricyanide reductase activity; ▲, NADPH–ferricyanide reductase activity. (b) The electron input was regulated by limiting NAD⁺ (○), alcohol dehydrogenase (□) and NADP⁺ (●). The results obtained under standard assay conditions without the generating systems are also given: ▽, with 0.5 mM-NADH; ▼, with 0.5 mM-NADPH.

dependent reaction and the reaction supported by the generating system with low concentrations of NAD⁺ (results not shown) were not affected by the inhibitors, except antimycin A. The antibiotic, which has a weak antioxidant property (Hunter *et al.*, 1964b), slightly inhibited the reactions. These observations suggest that the reduction of a component that lies between the rotenone- and antimycin A-sensitive sites inhibits the peroxidation reaction and that the component is presumed to remain in an oxidized state when the rate of electron input is so slow, as in the NADPH-dependent reaction.

Reduction level of ubiquinone in the presence of ADP–Fe³⁺

We studied the relationship between the malondialdehyde formation and the reduction level of endogenous ubiquinone in the presence and absence of ADP–Fe³⁺ chelate and at various incubation conditions (Table 3), because ubiquinone seemed to be one of the candidates for the key component that lies between rotenone- and antimycin A-sensitive sites of the respiratory chain and inhibits the peroxidation. When NADH was used as an electron donor, the ubiquinone was negligibly reduced in the presence of rotenone and it was highly reduced in the presence of antimycin A. Addition of ADP–Fe³⁺ did not influence the reduction levels. When NADPH was used, the reduction level of the ubiquinone was negligibly low, except in the presence of antimycin A. In the presence of the antibiotic, NADPH reduced 60% of the ubiquinone, but the addition of ADP–Fe³⁺ caused a complete oxidation of the ubiquinone, probably owing to a bypass of electrons to ADP–Fe³⁺ and a slow electron input from NADPH. The rate of the malondialdehyde formation under various incubation conditions was inversely related with the reduction level of endogenous ubiquinone. Similar results were obtained by the use of the particles treated with 1 mM-KCN.

Inhibition of lipid peroxidation by succinate

The inhibitory effect of reduced endogenous ubiquinone was further confirmed by the following observations. Succinate, which reduces ubiquinone and cytochrome *b* in the rotenone-treated particles, strongly inhibited the NADH- and NADPH-dependent peroxidation (Table 4). The inhibition by succinate was not observed in the presence of malonate or *p*-hydroxymercuribenzoate, and the inhibition was intensified by antimycin A and KCN. Malonate, *p*-hydroxymercuribenzoate and fumarate had no effect on the peroxidation reaction when succinate was not present. A possibility that the reduced cytochrome *b* inhibits the peroxidation was ruled out, because duroquinol, which reduces cytochrome *b* without reducing ubiquinone in the

Table 3. *Malondialdehyde formation and the reduction level of ubiquinone with or without ADP–Fe³⁺*

A reaction mixture (1.75 ml) containing the particles (1.26 mg/ml), NAD(P)H-generating sytems and 50 mM-Tris/acetate buffer, pH 7.4 at 37°C, was incubated in the presence and absence of 2 mM-ADP and 0.2 mM-FeCl₃ with shaking at 37°C for 10 min. The NAD(P)H-generating systems were as given in the legend for Fig. 1 (with 0.5 mM-NADH or 0.5 mM-NADPH). The assay procedures for malondialdehyde formation and ubiquinone are given in the Experimental section. Total content of ubiquinone was 3.86 ± 0.10 nmol/mg of protein. The values in the table are given as means $\pm$ s.e.m. for three experiments.

	Reduction level of ubiquinone (% of total)		Malondialdehyde formation (nmol/10 min per ml)	
	$-ADP-Fe^{3+}$	$+ADP-Fe^{3+}$	$-ADP-Fe^{3+}$	$+ADP-Fe^{3+}$
NADH	43 ± 2	43 ± 1	0.1 ± 0.0	6.0 ± 0.6
NADH + rotenone (2 μM)	1 ± 1	1 ± 1	0.1 ± 0.0	38.1 ± 0.9
NADH + antimycin A (0.2 μg/mg of protein)	80 ± 1	78 ± 2	0.0 ± 0.0	1.6 ± 0.6
NADPH	4 ± 2	1 ± 1	0.1 ± 0.0	29.3 ± 0.5
NADPH + rotenone	1 ± 1	0	0.1 ± 0.0	30.9 ± 1.3
NADPH + antimycin A	59 ± 2	0	0.1 ± 0.0	26.3 ± 1.4

Table 4. *Effects of electron-transport inhibitors and succinate on the NADH- and NADPH-dependent malondialdehyde formation by the rotenone-treated submitochondrial particles*

The control assay system was as described in the Experimental section, except for the use of 2 μM-rotenone and 50 mM-Tris/acetate buffer, pH 7.4 at 37°C.

	Malondialdehyde formation (nmol/min per mg of protein)	
	With NADH	With NADPH
Control	4.54	4.28
+ Succinate (20 mM)	0.81	0.73
+ Succinate + p-hydroxymercuribenzoate (0.1 mM)	3.95	4.15
+ Succinate + malonate (0.25 mM)	2.63	2.23
+ Succinate + malonate (10 mM)	4.43	4.24
+ Succinate + antimycin A (0.2 μg/mg of protein)	0.04	0.02
+ Succinate + KCN (1 mM)	0.09	0.05
+ Malonate (10 mM)	4.56	4.30
+ Fumarate (20 mM)	4.22	4.23
+ Duroquinol (0.4 mM) + antimycin A	4.18	3.92
+ Duroquinone (0.4 mM) + antimycin A	4.13	3.93
+ Ubiquinol-2 (0.05 mM) + antimycin A	0.11	0.04
+ Ubiquinone-2 (0.05 mM) + antimycin A	3.98	3.91

antimycin A-treated particles (Kröger & Klingenberg, 1973; Jagow & Bohrer, 1975), did not inhibit the peroxidation in the submitochondrial particles treated with rotenone plus antimycin A. A possibility that the component was ubiquinone was further demonstrated by the inhibition of the peroxidation by ubiquinol-2 but not by ubiquinone-2.

Removal and reincorporation of ubiquinone-10

The succinate-induced inhibition of the NADH-dependent lipid peroxidation in rotenone-treated particles was dependent on the amount of ubiquinone in the preparation (Fig. 2). The inhibition was not observed in the particles from which the ubiquinone was exhaustively removed by n-pentane (about 95% of the content) (curve A), whereas the inhibition was essentially the same between the control freeze-dried particles (curve B) and the particles to which a physiological amount of ubiquinone was reincorporated (about 5 nmol/mg of protein) (curve C). The intermediate content of the ubiquinone caused an intermediate extent of inhibition (curve D). Similar results were obtained in the NADPH-dependent peroxidation. Thus the inhibition of the peroxidation by succinate is ascribed to the reduction of endogenous ubiquinone by the succinate dehydrogenase. When we extract the ubiquinone, the activities of succinate–cytochrome *c* reductase and NADH–cytochrome *c*
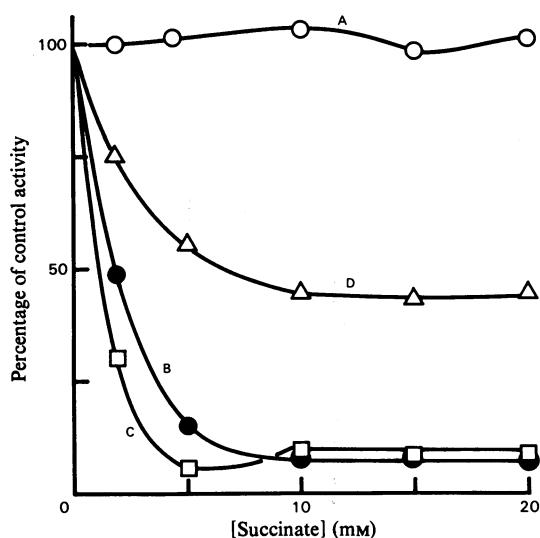
Fig. 2. *Effect of succinate on the NADH-dependent lipid peroxidation of the submitochondrial particles with various ubiquinone contents*

The lipid peroxidation was assayed with the particles treated with $2\,\mu$M-rotenone. The contents of enzymically reducible ubiquinone are given below in parentheses (nmol/mg of protein). Curves: A (O) ubiquinone extracted (below 0.1); B (●), freeze-dried particles (3.61); C (□), ubiquinone reincorporated (5.12); D (△), ubiquinone partially reincorporated (1.43). The control activities of malondialdehyde formation (nmol/min per mg of protein) were 3.89, 4.09, 3.83 and 3.92 for A, B, C and D respectively.
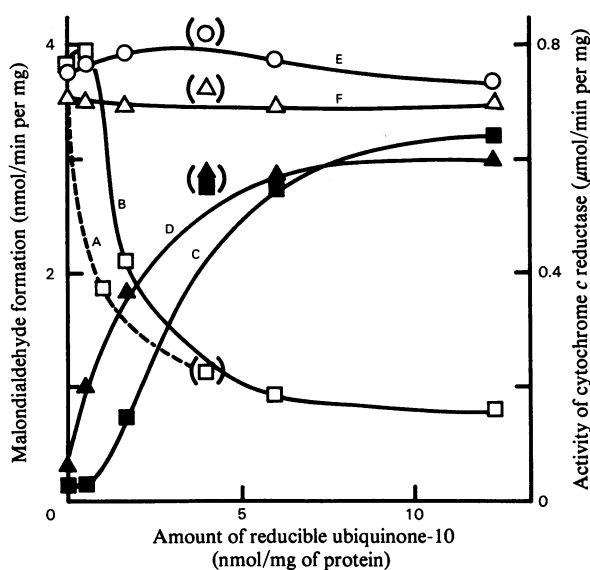

Fig. 3. *Effects of the extraction and reincorporation of ubiquinone on the NADH- and NADPH-dependent lipid peroxidation*

Extraction and reincorporation of ubiquinone were carried out as described in the Experimental section. The enzymically reducible ubiquinone of the reincorporated particles was 70–89% of the total ubiquinone. Curves: A (□) and B (□), lipid peroxidation with 0.5 mM-NADH in which the dotted line (A) represents the effect of the extraction and solid line (B) represents the effect of the reincorporation; C (■), NADH–cytochrome c reductase activity; D (▲), succinate–cytochrome c reductase activity; E (O), lipid peroxidation with 0.5 mM-NADH and $2\,\mu$M-rotenone; F (△), with 0.5 mM-NADPH. The values in the Figure with symbols in parentheses represent the activities of the freeze-dried particles.

reductase decreased to 12 and 5% of the original activities respectively, and 20% of phospholipids were lost. The NADH-dependent lipid-peroxidation activity, on the contrary, was accelerated by the removal of ubiquinone (Fig. 3, curve A) and decreased again when ubiquinone-10 was reincorporated to the particles (curve B). The activities of the NADH–cytochrome c reductase and succinate–cytochrome c reductase appeared again as the ubiquinone was reincorporated (curves C and D). The effects were clearly observed at a physiological concentration of the ubiquinone. The NADH-dependent peroxidation of the rotenone-treated particles and the NADPH-dependent reaction were not affected by the removal and reincorporation of ubiquinone (curves E and F). These observations agreed well with the results in Tables 2 and 3.

Discussion

Lipid peroxidation was observed when bovine heart mitochondria were incubated in the presence of ADP and $FeCl_3$ and with NADH or NADPH. The

properties of the NADPH-dependent peroxidation reaction were apparently different from those of the NADH-dependent reaction; for example, the NADH-dependent reaction was stimulated by rotenone, amytal, thiol-specific inhibitors and the extraction of ubiquinone, and inhibited by antimycin A, KCN and CO, whereas the NADPH-dependent reaction was not affected by the inhibitors and the extraction of ubiquinone. The difference in properties, however, can be explained by the difference in the rate of the electron flow to ubiquinone and the antioxidant property of ubiquinol. As shown in Scheme 1, both NADH and NADPH reduce ADP–Fe^{3+} chelate through a site that is situated before the rotenone-sensitive site of the NADH dehydrogenase. ADP–Fe^{2+} is supposed to provide electrons for the lipid peroxidation, as is further discussed in the following paper (Takeshige *et al.*,
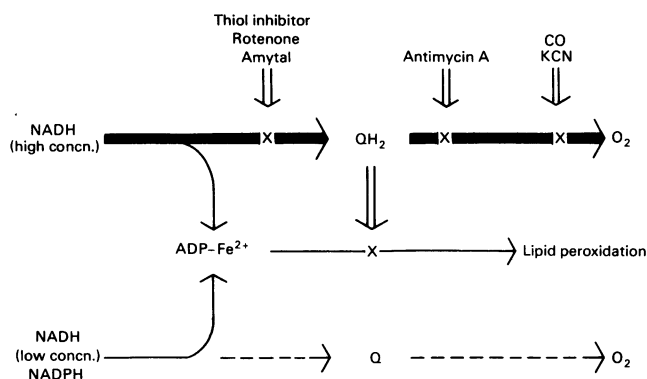
Scheme 1. *Schematic representation of NADH- and NADPH-dependent lipid peroxidation of bovine heart mitochondria*

1980). When the concentration of NADH is sufficiently high, electrons flow to ubiquinone so that ubiquinol is formed, whereas at low input rate of electrons, such as with very low concentrations of NADH or with NADPH, an inefficient electron donor, essentially all electrons are consumed for the reduction of ADP/Fe^{3+}, so that ubiquinone remains in the oxidized state, even in the presence of antimycin A or KCN (Table 3). Actually the NADPH consumption in the presence of ADP–Fe^{3+} was almost the same regardless of rotenone treatment of the submitochondrial particles (results not shown). The higher is the reduction level of ubiquinone, the slower is the lipid peroxidation. A possibility that ubiquinol affects the reduction activity of ADP–Fe^{3+} and inhibits the peroxidation can be neglected, because non-enzymic mitochondrial peroxidation (ascorbate-induced and without ADP–Fe^{3+}) was inhibited by succinate (results not shown), and because the reduction rate of ADP–Fe^{3+} by the NADH–ubiquinone reductase preparation (Complex I), which contains ubiquinone-10, was not affected by rotenone (Takeshige *et al.*, 1980).

When both NADH and NADPH were simultaneously used as electron donors, only the NADH-dependent reaction was observed. NADPH has been considered to be oxidized directly by the NADH dehydrogenase at the substrate site common with NADH (Rydström *et al.*, 1978; Ragan, 1976; Hatefi & Bearden, 1976). These observations, together with the results discussed in Scheme 1 support the conclusion that both NADH- and NADPH-dependent lipid-peroxidation reactions are catalysed by the same enzyme, the NADH dehydrogenase of the respiratory chain.

An antioxidant property of exogenously added ubiquinone-6 has already been observed by Mellors & Tappel (1966), who proposed that ubiquinol may quench lipid free radicals. An antioxidant role of endogenous ubiquinol is also confirmed by the present observations that: (*a*) succinate strongly inhibited both enzymic (NADH- and NADPH-dependent) and non-enzymic (ascorbate-dependent) lipid peroxidation of mitochondria; (*b*) the inhibition by succinate was dependent on the content of ubiquinone in the submitochondrial particles; and (*c*) duroquinol, which reduces cytochrome *b* without reducing ubiquinone, did not inhibit the peroxidation.

The results given in the present paper may help to elucidate some clinical observations, e.g. myocardial damage by an anticancer drug, adriamycin, which lowers mitochondrial ubiquinone content (Folkers *et al.*, 1977) and induces myocardial malondialdehyde formation (Myers *et al.*, 1977).

References

Bernheim, F., Bernheim, M. L. & Wilkur, K. M. (1948) *J. Biol. Chem.* **174**, 257–264

Blair, P. V. (1967) *Methods Enzymol.* **10**, 78–81

Folkers, K., Liu, M., Watanabe, T. & Porter, T. H. (1977) *Biochem. Biophys. Res. Commun.* **77**, 1536–1542

Hatefi, Y. & Bearden, A. J. (1976) *Biochem. Biophys. Res. Commun.* **69**, 1032–1038

Hatefi, Y. & Hanstein, W. G. (1970) *Arch. Biochem. Biophys.* **138**, 73–86

Hatefi, Y. & Rieske, J. S. (1967) *Methods Enzymol.* **10**, 225–231

Hunter, F. E., Jr., Scott, A., Hoffsten, P. E., Guerra, F., Weinstein, J., Schneider, A., Schultz, B., Fink, J., Ford, L. & Smith, E. (1964*a*) *J. Biol. Chem.* **239**, 604–613

Hunter, F. E., Jr., Scott, A., Hoffsten, P. E., Gebicki, J. M., Weinstein, J. & Schneider, A. (1964*b*) *J. Biol. Chem.* **239**, 614–621

Hunter, F. E., Jr., Scott, A., Weinstein, J. & Schneider, A. (1964*c*) *J. Biol. Chem.* **239**, 622–630

Jagow, G. V. & Bohrer, C. (1975) *Biochim. Biophys. Acta* **387**, 409–424

Kaplan, N. O. (1967) *Methods Enzymol.* **10**, 317–322

King, T. E. (1967) *Methods Enzymol.* **10**, 216–225

Kröger, A. & Klingenberg, M. (1966) *Biochem. Z.* **344**, 317–336

Kröger, A. & Klingenberg, M. (1973) *Eur. J. Biochem.* **39**, 313–323

Lee, C.-P. & Ernster, L. (1967) *Methods Enzymol.* **10**, 543–546

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

McKnight, R. C. & Hunter, F. E., Jr. (1966) *J. Biol. Chem.* **241**, 2757–2765

Mellors, A. & Tappel, A. L. (1966) *J. Biol. Chem.* **241**, 4353–4356

Myers, C. E., McGuire, W. P., Liss, R. H., Ifrim, I., Grotzinger, K. & Young, R. C. (1977) *Science* **197**, 165–167

Nelson, B. D. & Gellerfors, P. (1978) *Methods Enzymol.* **53**, 80–91

Norling, B., Glazek, E., Nelson, B. D. & Ernster, L. (1974) *Eur. J. Biochem.* **47**, 475–482

Pfeifer, P. M. & McCay, P. B. (1972) *J. Biol. Chem.* **247**, 6763–6769

Pumphrey, A. M. & Redfearn, E. R. (1960) *Biochem. J.* **76**, 61–64

Ragan, C. I. (1976) *Biochem. J.* **158**, 149–151

Rydström, J., Montelius, J., Backström, D. & Ernster, L. (1978) *Biochim. Biophys. Acta* **501**, 370–380

Szarkowska, L. & Klingenberg, M. (1963) *Biochem. Z.* **338**, 674–697

Takeshige, K. & Minakami, S. (1975) *J. Biochem. (Tokyo)* **77**, 1067–1073

Takeshige, K. & Minakami, S. (1979) *Biochem. J.* **180**, 129–135

Takeshige, K., Takayanagi, R. & Minakami, S. (1980) *Biochem. J.* **192**, 861–866