FOUR QUINONE REDUCTION SITES IN THE NADH DEHYDROGENASE COMPLEX

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Summary

Quinones can be used as electron acceptors and donors in a new approach to the study and resolution of the respiratory chain-linked NADH dehydrogenase system of beef heart mitochondria. Juglone (5-hydroxynapthoquinone), ubiquinone, duroquinone (tetramethylbenzoquinone), and ferricyanide are found to react at four separate sites in the complex. The sites can be defined by chemical and enzymatic inhibition or by fractionation of the system. The reduced form of one quinone, duroquinol, has the unique property of being an electron donor for the energy-linked reduction of NAD^+.

We find that certain quinones show remarkable specificity in their ability to react at different sites with the NADH dehydrogenase complex of beef heart mitochondria. This specificity can be defined by selective inhibitor effects on the intact system or by a study of fractions derived from the NADH dehydrogenase complex.

The selective quinones which we have used as acceptors are: Juglone (5-hydroxy 1,4 naphthoquinone), ubiquinone 1 or 2 (Q_1 or Q_2) and duroquinone (DQ) as well as the generally used ferricyanide. 1,2 naphthoquinone can be used in place of juglone and 2,3 dicyano 5,6 dichloro 1,4 benzoquinone can be used in place of ferricyanide as site specific acceptors.

Methods and Materials

Quinones were obtained from commercial sources with the exception of the ubiquinones which were kindly provided by Dr. A. F. Wagner of Merck Sharp and Dohme. Assays of quinone reductase were carried out in 3 ml

at 30° by following the oxidation of NADH at 340 mμ after addition of quinone. The assay system contained: 30 μmoles Tris-Cl pH 7.4, 0.5 μmole NADH, 0.3 μmole quinone, 0.5 μg antimycin A, 3 μmole cyanide or 0.12 mμ mole piericidin A when applicable. NADH ferricyanide reductase assays were carried out at fixed ferricyanide concentration (1.7 mM) under conditions described by Minakami et al. (1). Electron transport particles (ETP) were prepared by sonic disruption of beef heart mitochondria. For parahydroxymercuribenzoate (PHMB) inhibition ETP was incubated with 1 mM PHMB in 0.05 M Tris-Cl pH 7.4 for one hour at 20°. Phospholipase a was purified from Naja naja venom according to Cremona and Kearney (2) and incubated at 0.1 μg/mg ETP protein at 30° for one hour. Phospholipase c (Sigma Chemical Co.) was incubated at 0.05 mg/mg ETP protein at 30° for one hour. Energy linked reduction of NAD^+ was assayed according to Ernster and Lee (3). 0.6 μmole of reduced quinone was substituted for

TABLE I

EFFECT OF CHEMICAL AND ENZYMATIC TREATMENTS

ON NADH-QUINONE REDUCTASE ACTIVITIES

	Reductase Activity μmoles NADH/min./mg. protein			
	ETP	ETP + PHMB	ETP + Piericidin A	ETP + Phospholipase c
Juglone	0.43	0.72*	0.35*	0.39*
Ferricyanide	13.1	9.7	13.0	12.7
Ubiquinone 1	0.49	0.06	0.03	0.27
Duroquinone	0.23	0.03	0.01	0.06

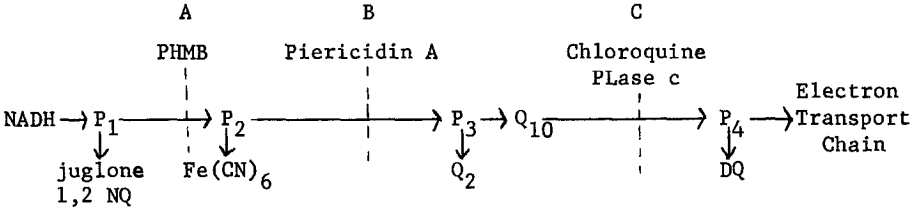
*Juglone activity assayed in the presence of piericidin A.

All activities not inhibited by antimycin A.

succinate. Inhibitor concentrations were 0.12 mμ moles piericidin A, 0.05 μg antimycin A, 1.2 μg oligomycin or 3.0 mμ moles FCCP.

Results

Inhibition of quinone and ferricyanide reduction in the presence of the inhibitors PHMB and piericidin A and after phospholipase c treatment is shown in Table I. Juglone reduction is not inhibited by any of these agents and is strongly stimulated in presence of PHMB. Ferricyanide reduction is inhibited by PHMB but not by piericidin A or phospholipase c. Preincubation of ETP with NADH (4,5) causes 90% inhibition of ferricyanide reduction by PHMB and causes slight (20%) inhibition of the stimulated rate of juglone reduction. Q_1 or Q_2 reduction is inhibited by PHMB and piericidin A (5,7), but is only partially inhibited by phospholipase c. Rotenone will inhibit like piericidin A. Duroquinone reduction is inhibited by all three agents, PHMB, piericidin A and phospholipase c. Thus we can define three sites of electron transport inhibition in the NADH system and four acceptor sites as shown in the diagram. Note that all



of the inhibitors also inhibit NADH oxidase activity. The inhibitory effect of phospholipase c on DQ reductase activity is also seen with chloroquinone diphosphate (8). Extraction of ubiquinone 10 from the particle with pentane (9) also inhibits only DQ reduction which is restored by addition of Q_{10} but not by addition of Q_2. Lenaz et al. (10) have shown that restoration of NADH oxidation after pentane extraction requires ubiquinones higher than Q_4 and that Q_2 does not restore activity.

TABLE II

QUINONE REDUCTION BY NADH DEHYDROGENASE PREPARATIONS

Acceptor	ETP	Complex I	Complex I* flavo-protein	Complex I* Non-heme Fe protein	Complex I* insol prot.	Heat acid ethanol enzyme	Phospho-lipase a preparation
			μmoles NADH oxidized/min, mg protein				
Juglone	0.28	1.36	11.4	0.31	0.16	28	5.28
Ferricyanide	16.1	117	21.6	1.47	0.97	91.7	186
UQ_2	0.25	0.34	0	0	0	0**	0**
DQ	0.23	0.32	0	0	0	0**	0**
Cytochrome c	***0.05	0.06	3.55	0.16	0.06	20.9	

*Fractions derived from complex I by the procedure at Hatefi and Stempel (12).

**UQ_2 + DQ (piericidin sensitive activity) complex I flavoprotein, heat acid ethanol enzyme and phospholipase a enzyme show activity with UQ_2 and DQ which is not piericidin sensitive.

***Cytochrome c activity in presence of antimycin A.

Several preparations of purified NADH dehydrogenase from ETP have been described. In Table II we compare the activity of three of these preparations using the four acceptors. Complex I prepared according to Hatefi et al. (11) shows increased activity with all acceptors. When the complex is fragmented by urea treatment according to Hatifi et al. (12) all activity except Juglone reductase is decreased in the purified flavoprotein fraction and a small amount of antimycin insensitive cytochrome c reductase activity appears. From this result it appears that the naphthoquinones, juglone or 1,2 naphthoquinone are better acceptors for the primary dehydrogenase than is ferricyanide. In the enzyme prepared by heat, acid, ethanol treatment by Pharo et al. (13), and by phospholipase a treatment (2) the piericidin A sensitive Q_2 and DQ reductase activities are lost, although these enzymes do show some Q_2

TABLE III

DUROQUINONE AS SUBSTRATE FOR

ENERGY-LINKED REDUCTION OF NAD^+ WITH ETP_H

Electron Donor	NAD^+ Reduction μmoles NADH/min./mg. protein					
	No ATP	+ ATP	+ Piericidin A	+ Antimycin A	+ Oligomycin	+ FCCP
Succinate	0	0.170	0	0.166	0	0
Reduced Duroquinone	0	0.083	0	0.077	0	0
Reduced Ubiquinone 2	0.018	0.015	0.013	0.015		

Phosphorylating electron transport particles (ETP_H) were prepared by the method of Hansen and Smith (14).

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and DQ reductase activity which is not inhibited by piericidin. The relative ratio of juglone to ferricyanide reductase activity in these two enzymes shows significant differences which may relate to some change in the primary dehydrogenase as is suggested by the PHMB stimulation of juglone reductase in ETP.

Reduced duroquinone can also be used as an electron donor for the energy-linked reduction of NAD^+ using the phosphorylating electron transport particles. The activity is completely dependent on ATP and is inhibited by piericidin A, oligomycin or FCCP (carbonyl cyanide p-trifluoro methoxyphenylhydrazone). Table III. The reduction is not inhibited by antimycin A which acts between cytochromes b and c_1. Reduced ubiquinone 2 does not function as an electron donor for energy-linked reversed electron transport and when added together with reduced duroquinone inhibits reversed electron transport from the duroquinol.

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