A SPLIT PATHWAY OF ELECTRON TRANSPORT IN MITOCHONDRIA

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Schemes for electron transport in mitochondria usually postulate sequential electron flow through a series of carriers. Only recently has there been some suggestion that a simple linear system of carriers may not account for observed redox changes. Jacobs et al. (1965) have described redox changes in cytochromes a and a_3 which indicate separate sources of electrons for each of these carriers. Baum and Rieske (1967) have shown the possibility that the redox state of cytochrome b may influence the ability for cytochrome c_1 to be reduced which opens up the possibility that sequential electron flow is only apparent. In other words one carrier must be reduced before electrons from a separate source are able to reduce the second carrier.

Studies of redox changes of cytochromes in mitochondria in presence of piericidin A or after pentane extraction provide additional evidence that the reduction of cytochrome b and c_1 may proceed by separate pathways. The inhibition of either pathway serves to prevent significant electron flow through both pathways.

Piericidin A inhibits NADH oxidase, NADH and succinate cytochrome c reductase (Hall et al. 1966) and NADH-coenzyme Q reductase activity. It does not inhibit cytochrome oxidase at the levels used. The amount required for complete inhibition is very small and equivalent to the amount of rotenone required. Since the site of action appears to be close to the site of rotenone inhibition (Ernster et al. 1963) it would appear that both of these reagents

inhibit at the same site. The pattern of cytochrome reduction observed with the two reagents is different. In the presence of rotenone no cytochromes are reduced when NADH is added as substrate. With piericidin present at levels which completely inhibit NADH oxidase cytochromes c_1 and a appear reduced but no cytochrome b appears as shown in Figure 1. Cytochrome b is reduced if succinate is subsequently added.

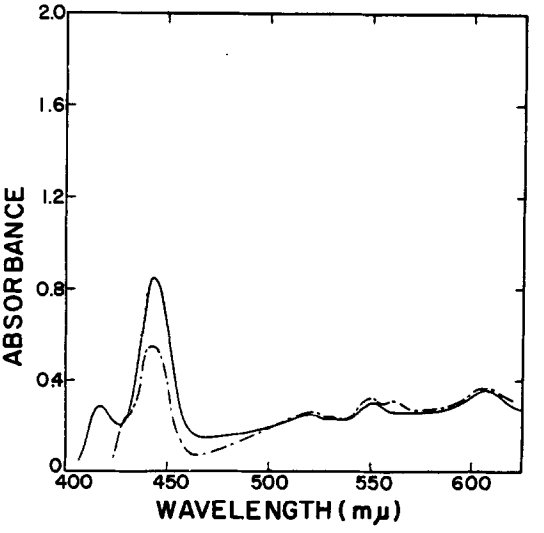

Figure 1. The difference spectrum of the mitochondria in the presence of piericidin A, NADH as substrate. The cell contains 14 mg of mitochondria, 0.5 ml of 0.1 M phosphate buffer pH 7.4, 0.4 ml of NADH (20 mg/ml). Total volume is 3 ml. 1.2×10^{-7} M of piericidin A is added into the reaction mixture. The dotted line represents the difference spectrum of the mitochondria after further addition of dithionite into the reaction mixture.

Antimycin A inhibits the reduction of cytochromes c_1 and a in mitochondria but does not interfere with reduction of cytochrome b. If both antimycin A and piericidin A are added with succinate as substrate then cytochrome c_1 is reduced and cytochrome b and cytochrome a are not reduced. With NADH as substrate no cytochromes are reduced when both inhibitors are present. (cf. Table I)

When piericidin is added to mitochondria which are incubated in the

TABLE I

INHIBITORS OF REDUCTION OF COENZYME Q AND CYTOCHROMES BY NADH AND SUCCINATE

Inhibitor Added	Conc.	Substrate: NADH			Substrate: Succinate		
		Cytochromes b	Cytochromes c_1	Coenzyme Q	Cytochromes b	Cytochromes c_1	Coenzyme Q
		% Reduced					
None	----	75	88	50	75	88	90
+dithionite		100	100	100	--	--	--
Piericidin A	0.12μM	0	70	0	--	--	--
	2 mM	--	--	--	60	0	0
Antimycin A	0.3mg/ml	75	0	--	75	0	--
P and A		--	--	--	0	50	--
Extraction with pentane		25	0	--	25	0	0
Rotenone	12μM	0	0	0	--	--	--
Thenoeyltrifloro acetone		--	--	--	0	0	--

presence of NADH or succinate all of the endogenous coenzyme Q is found in the oxidized form whereas 40-50% is found reduced when no inhibitor is added. Piericidin therefore inhibits electron transport between coenzyme Q and the primary dehydrogenase.

If coenzyme Q is extracted from mitochondria with pentane both NADH oxidase and succinic oxidase activity are lost. The activity can be restored to both systems by adding back coenzyme Q. After extraction of coenzyme Q, cytochrome b is reduced when NADH or succinate is added to the extracted particles. Normal reduction of cytochromes b and c_1 is restored when coenzyme Q is added.

Inhibition of succinoxidase by piericidin is not as complete as inhibition of NADH oxidase and larger amounts of piericidin are required to cause inhibition. When succinate is used as a substrate in presence of piericidin at high levels, cytochrome b is reduced first then cytochromes a and c_1. Since the inhibition is not so complete the effects of piericidin are not as dramatic as with NADH as substrate.

The conclusion can be drawn that cytochromes b and c_1 and coenzyme Q can be reduced independently by the NADH dehydrogenase which indicates the possibility of a three-pronged pathway for electron entry into the first stage of the electron transport chain. The conditions which control each pathway will be important for further study. In the succinate based system the pathway to cytochrome c_1 is not clearly evident but it is clear that cytochrome b can be reduced when coenzyme Q is fully oxidized.

When succinate is substrate, high concentrations of piericidin stop the reduction of coenzyme Q and slow down the reduction of cytochrome c_1 as if a slow pathway to cytochrome c_1 remained open. Cytochrome b reduction is slower than normal but certainly faster than c_1 reduction. At present we cannot explain why only cytochrome c_1 is reduced when both piericidin and antimycin a are added with succinate as substrate. One could postulate that piericidin acts to relieve antimycin inhibition of c_1 reduction and

that the two together can inhibit b reduction. (cf. Figure 2)

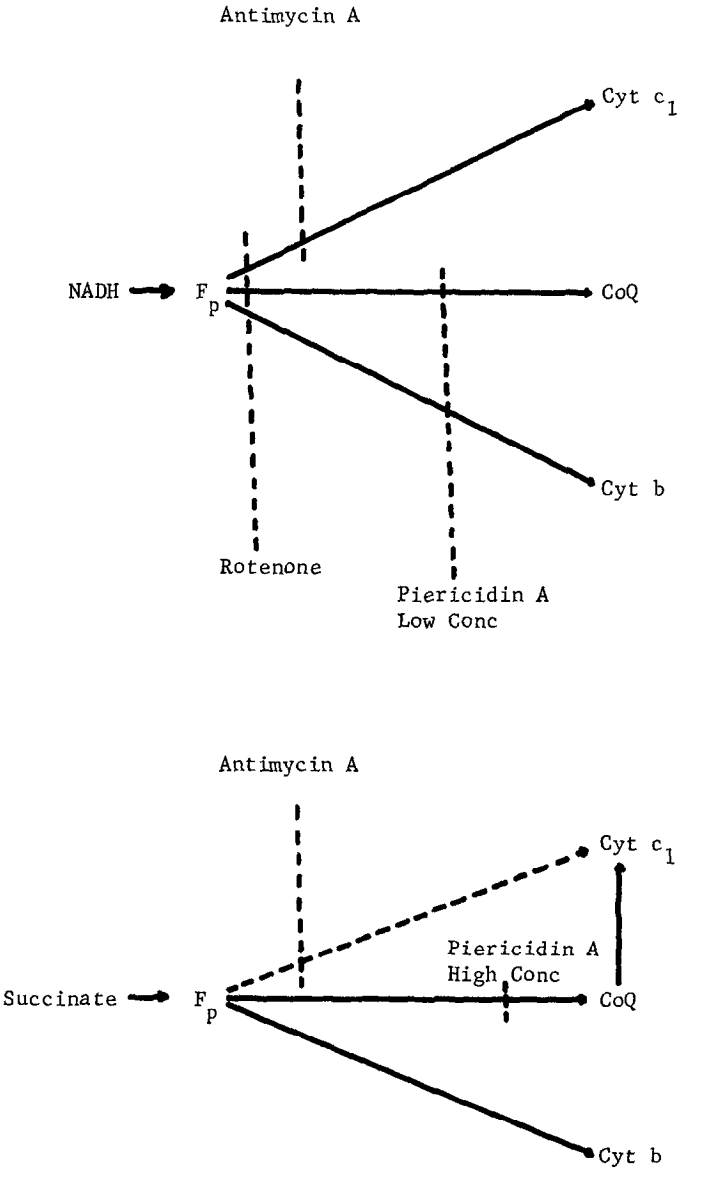

Figure 2. Proposed three-pronged pathway for electron transport. The dashed line indicates that the pathway from succinate to cytochrome c_1 is seen only under special conditions. Antimycin A can also inhibit between coenzyme Q and cytochrome c_1.

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