PARTIAL RESOLUTION AND RECONSTITUTION OF THE ADENOSINE TRIPHOSPHATE-DEPENDENT REDUCTION OF DIPHOSPHOPYRIDINE NUCLEOTIDE BY SUCCINATE

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An ATP-dependent reduction of DPN by succinate (Reaction 1) has been demonstrated in submitochondrial particles from beef heart by Löw,

$$DPN^+ + \text{succinate} \xrightarrow{ATP} DPNH + H^+ + \text{fumarate} \qquad (1)$$

Krueger and Ziegler (1). Similar particles made in our laboratory have been found to catalyze the above reaction and also the thermodynamically favorable oxidation of DPNH in the presence of fumarate (Reaction 2) (2).

$$DPNH + H^+ + \text{fumarate} \longrightarrow DPN^+ + \text{succinate} \qquad (2)$$

In this communication, we wish to report a comparison of the response of the two reactions to various inhibitors and a partial resolution of the system into two components both of which are required for activity in Reaction 1.

The submitochondrial particles catalyze Reaction 1 at an initial rate of 30 to 100 mμmoles DPN reduced per min. per mg. protein under the conditions shown in Table I. In reaction 2, 20 to 30 mμmoles DPNH are oxidized per min. per mg., an activity which is 10 to 15 times greater than that observed by Slater (2) in heart muscle electron transport particles. Both activities were inhibited by Amytal and Antimycin A (Table I) as well as by malonate and 4, 4, 4-trifluro-1-(2-thienyl)-1, 3-butanedione. These compounds are known to inhibit different segments of the mitochondrial respiratory chain. The uncoupling agents, 2, 4-dinitrophenol (1), Dicumarol

TABLE I

Effect of electron transport inhibitors and uncoupling agents

Particle preparation	Compound	Conc.	ΔA/8 min. Reaction 1	Reaction 2
60	---		0.248	0.170
	Amytal	0.67 mM	0.017	0.051
	Amytal	1.0 mM	---	0.024
69	---		0.312	0.137
	Antimycin A	1.0 μg/ml	0.081	0.092
	Antimycin A	2.0 μg/ml	0.011	0.054
	Dinitrophenol	0.06 mM	0.024	0.154
	Dinitrophenol	0.12 mM	---	0.143
69*	---		0.244	0.154
	Dicumarol	6.0 μM	0.013	0.154
77	---		0.545	0.212
	carbonyl cyanide, p-chlorophenyl-hydrazone	0.04 μM	0.085	0.236
		0.05 μM	0.035	0.232

The reaction medium for both assays contained 0.05 M tris(hydroxymethyl)-aminomethane at pH 7.5, 3.3 mM $MgCl_2$, 1 mM ethylenediaminetetracetate, 1 mM KCN and 0.75 mg. protein (submitochondrial particles). In addition, the assay system for Reaction 1 contained 6.7 mM succinate, 1 mM DPN and 2 mM ATP, and that for Reaction 2 contained 0.19 mM DPNH and 3.3 mM fumarate. The reactions, carried out at 30° in 3 ml. volume, were started by the addition of either ATP or fumarate following a 3-min. incubation at 30°. The absorbancy change for Reaction 2 is corrected for a control run concurrently in the absence of fumarate. The fumarate independent oxidation of DPNH proceeded generally at 20 to 30% of the rate with fumarate present.

*Preparation 69 after storage for 2 days at -10°.

and the p-chlorophenylhydrazone of carbonylcyanide (3) inhibit the ATP-dependent reduction of DPN by succinate but have no effect on the oxidation of DPNH by fumarate. The previously reported (4) variable inhibition of the latter reaction by dinitrophenol has proved to be an artifact. Oligomycin

which is an inhibitor of an energy-coupling reaction in oxidative phosphorylation inhibits Reaction 1 (1) but has no effect on Reaction 2. Furthermore, the oxidation of DPNH by fumarate is not affected by the addition of Mg^{++}, ATP or ADP and phosphate. It would thus appear that the exergonic oxidation of DPNH by fumarate provides an independent assay for the electron transport sequence functioning in Reaction 1 independent of the phosphate esterification steps.

Repeated washing of the submitochondrial particles yields a particulate fraction which retains activity in the oxidation of DPNH by fumarate but has only feeble activity in Reaction 1 (Table II). Addition of an inactive 100,000 x g supernatant from sonically disrupted mitochondria to the washed particles results in several fold stimulation of the activity in Reaction 1.

TABLE II

Reconstitution of the ATP-dependent reduction of DPN by succinate

Particle	Soluble protein mg.	ΔA/8 min. Reaction 1	Reaction 2
Original, 0.75 mg.	----	0.780	0.232
Washed, 0.75 mg.	----	0.059	0.199
Washed, 0.75 mg.	1.21	0.331	0.198
Washed, 0.75 mg.	1.82	0.438	0.198
----	1.82	0.028	

The assays were performed as described in Table I. The soluble protein refers to the supernatant from the 100,000 x g spin of the sonically disrupted beef heart mitochondria.

The energy-dependent reduction of DPN by succinate or α-glycerophosphate has been considered to be a reversal of oxidative phosphorylation (5, 6). Since the activity of the particles in Reaction 2 is unaltered either by repeated washing or by addition of the heat-labile soluble supernatant, it appears likely that the reactivating fraction is mainly concerned with the

energy coupling events in oxidative phosphorylation. The site of energy coupling is probably in the region between DPNH and cytochrome b. The relationship of our coupling system to the previously reported coupling factors (7-10) remains to be clarified.

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