Short Communications

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Energy requirements and reducing power for cytochrome-linked pyridine nucleotide reduction in heart mitochondria

The requirement of biological reduction in aerobic systems for an energy source and reducing power is being studied in mammalian heart mitochondria using a terminal phosphorylating shunt of the respiratory chain. Interest in this problem originated from certain experiments with ascorbate–TMPD, which catalyzes a cytochrome oxidase shunt[1] coupled to a single phosphorylation (1.01 ± 0.20) and manifesting respiratory control (1.64 ± 0.45) with ADP provided the mitochondria are freed of detectable endogenous substrates by repeated washing[2]. Oxygen consumption, respiratory control, and phosphorylation are not inhibited by levels of antimycin A up to about 1 μg/ml but the pyridine nucleotide reduction which accompanies the oxidation of TMPD is antimycin sensitive[3–6] (Table I). Succinate oxidation leads to the same level of pyridine nucleotide reduction as with ascorbate–TMPD.

Although it is clear that ascorbate–TMPD is incapable of reducing pyridine nucleotide in the presence of antimycin A, it was nevertheless possible to effect a pyridine nucleotide reduction in this antimycin-inhibited system if succinate was present. After succinate is added to cardiac mitochondria, a steady-state pyridine nucleotide reduction occurs as in Table I; however, in the presence of antimycin,

TABLE I

PROPERTIES OF THE ASCORBATE–TMPD SHUNT

The basic reaction system contained: 0.07 M sucrose, 0.02 M phosphate (pH 7.5), 0.02 M KCl, and rabbit cardiac mitochondria (1 mg protein/ml). Other additions as indicated are: 0.5 mM ADP; antimycin A (1 μg/ml); 5 μM pentachlorophenol or Dicumarol; 5 mM ascorbate, glutamate, or succinate, and 200 μM TMPD. Oxygen utilization was followed with a polarograph simultaneously with fluorescence-intensity measurements[3]. Pyridine nucleotide fluorescence was excited at 365 mμ and monitored at 450 mμ using a photomultiplier positioned at 90° to the exciting light in a Brice-Phoenix light-scattering photometer modified for recording.

Condition	*O_2 utilization*	*Pyridine nucleotide reduction*
	Relative	*Relative fluorescence intensity*
Ascorbate–TMPD		
No addition	1.0	22–24
ADP	1.7–1.8	0
Antimycin A	1.0	0
Dicumarol or pentachlorophenol	1.7–1.8	0
Glutamate		
No addition	0.1–0.2	4–5
Succinate		
No addition	0.6–0.7	22–24

Abbreviation: TMPD, tetramethyl-*p*-phenylenediamine.

pyridine nucleotide reduction by succinate is inhibited[7]. This inhibition could result from failure to provide reducing power or energy to catalyze the reduction as a result of an antimycin block of succinate oxidation. In this case, it could be proved that the defect in pyridine nucleotide reduction results from the absence of phosphorylation associated with succinate oxidation in the more terminal regions of the chain. Ascorbate–TMPD in the presence of antimycin A as shown above continues to phosphorylate unabated. However when the experiment is done in the presence of both succinate and ascorbate–TMPD, a reduction of pyridine nucleotide in the steady state is catalyzed in the antimycin-blocked system, thus proving that ascorbate–TMPD provides the energy to drive a succinate-linked pyridine nucleotide reduction[4]. Using this same approach LÖW AND VALLIN[6] reduced externally added DPN^+ in submitochondrial particles with ascorbate–TMPD; TAGER, HOWLAND AND SLATER[5] have catalyzed the reduction of α-ketoglutarate and ammonia to glutamate and PENEFSKY[8] the reduction of ubiquinone in an antimycin A-blocked system in intact mitochondria by the "back door" approach. Cytochrome-linked pyridine nucleotide reduction has also been found in fragmented mitochondria by CHANCE AND FUGMANN[9] who used ATP directly.

In the presence of varying succinate to fumarate ratios, pyridine nucleotide reduction by succinate alone or in the complex antimycin-blocked system is suppressed as the succinate/fumarate ratio decreases, thus strengthening the argument that the role of succinate is that of providing the reducing power under these conditions (Table II). It is also found that the rate of pyridine nucleotide reduction in the antimycin-treated system is considerably lower than in the unblocked controls.

It was demonstrated in an earlier study[3] that in the absence of antimycin, the cytochrome-linked pyridine nucleotide reduction brought about by ascorbate–TMPD could be reversed by ADP. It seemed therefore worth examining whether it was also possible to oxidize the pyridine nucleotide reduced in the steady state in the complex succinate ascorbate–TMPD–antimycin A-blocked system. The results are shown by time recordings of fluorescence and oxygen utilization as in Fig. 1. The experiment shows respiratory control and the expected rapid oxidation of pyridine nucleotide on adding ADP in the absence of antimycin A in the left chart. In the blocked system (shown on the right), ADP again led to reoxidation of pyridine nucleotide, although

TABLE II

EFFECT OF SUCCINATE–FUMARATE RATIO ON PYRIDINE NUCLEOTIDE REDUCTION DURING THE OXIDATION OF ASCORBATE–TMPD AND SUCCINATE

Conditions as in Table I. Pyridine nucleotide reduction rates and extent were calculated from the fluorescence changes[3].

	O_2 utilization (μmoles/sec/mg protein)	*Pyridine nucleotide reduction*	
		Rate (per cent/min)	*Total (%)*
No addition			
Succinate (2 mM)	0.21	270	100
Succinate (2 mM) + fumarate (30 mM)	0.12	90	57
Ascorbate–TMPD + antimycin A			
Succinate (2 mM)	—	72	100
Succinate (2 mM) + fumarate (30 mM)	—	38	60

at a rate calculated to be approx. 7 times slower than that found in the unblocked system. A full interpretation of the significance of this observation is not yet available for the nature of the pathway through which ADP reoxidizes reduced pyridine nucleotide in the antimycin-blocked system is unknown. It is possible that an alternative bypass system may exist or that the antimycin block leaks.

The action of oligomycin deserves some comment with regard to the above experiments. The oxidation of pyridine nucleotide by ADP is completely blocked by oligomycin (10 μg/ml) both in the ascorbate–TMPD system and in the complex antimycin-inhibited case where succinate is incubated together with ascorbate–TMPD. This inhibition is relieved by uncoupling reagents as pentachlorophenol and dinitrophenol.

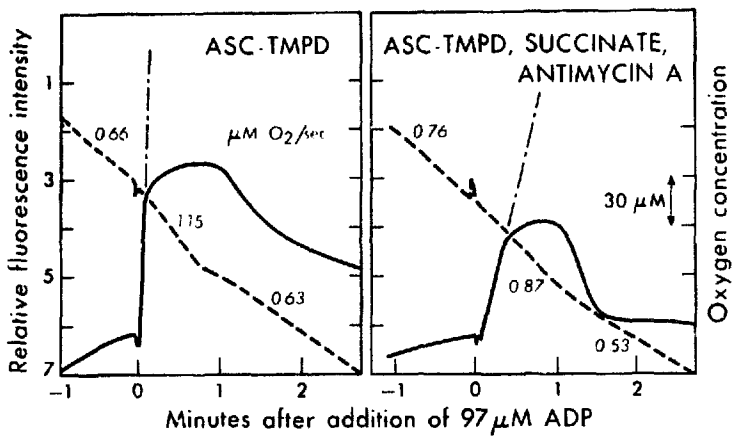

Fig. 1. Oxidation of mitochondrial pyridine nucleotide by ADP during the operation of the ascorbate–TMPD shunt.

The reversed electron-transport reaction however, in agreement with TAGER *et al.*[5] and PENEFSKY[8], is not oligomycin sensitive. These results are indicative of an action of oligomycin on the terminal phosphorylation reaction of the respiratory chain that is analogous to its action at earlier phosphorylation sites.

These results distinguish between energy requirements and reducing power for cytochrome-linked pyridine nucleotide reduction in intact mitochondria and illustrate the characteristics of the aerobic mechanism for the reduction of pyridine nucleotide from a terminal region of the respiratory chain.

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