OXIDATION OF DUROHYDROQUINONE VIA THE CYANIDE-INSENSITIVE RESPIRATORY PATHWAY IN HIGHER PLANT MITOCHONDRIA

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1. Introduction

Duroquinone (2,3,5,6-tetramethyl-1,4-benzoquinone) has been used in studies of electron transfer reactions in mitochondria and submitochondrial particles from mammals [1–3], microorganisms [4] and chloroplasts [5]. Oxidized duroquinone has been shown to function as an electron acceptor in the NADH dehydrogenase portion of the respiratory chain [1], while the reduced duroquinone (durohydroquinone) is thought to donate electrons at a point between the natural quinone and cytochrome *b* [2] or directly to cytochrome *b* [3]. Lawford and Garland [6] have shown the oxidation of durohydroquinone by ox heart mitochondria to have app. K_M 28 μM and V_{max} 260 nmol/min/mg protein. Von Jagow and Bohrer [4] have shown that mitochondria isolated from *Neurospora crassa* grown in the presence of chloramphenicol possess a cyanide-resistant respiratory pathway which can be specifically inhibited by benzhydroxamic acids such as salicylhydroxamic acid (SHAM) [7]. In these mitochondria durohydroquinone can be oxidized in the presence of cyanide by a SHAM-sensitive pathway. However, in the *Neurospora* mitochondria [4] it was found that the oxidation of durohydroquinone was inhibited by antimycin A although NADH oxidation was not. They therefore postulated the site of electron donation from durohydroquinone to be cytochrome *b* and that antimycin A had dual sites of inhibition in the *Neurospora* mitochondria, one being between the natural quinone and cytochrome *b* and the other between cytochromes *b* and *c*. Thus in the presence of cyanide alone durohydroquinone can reduce the natural quinone via cytochrome *b* and hence donate to the alternate, cyanide-resistant pathway but cannot do so in the presence of antimycin A as cytochrome *b* is no longer able to reduce the natural quinone.

We have found that mitochondria from higher plant tissues, containing the cyanide-resistant respiratory pathway, such as cassava (*Manihot esculenta*), sweet potato (*Ipomea batatas*) and *Arum maculatum* are able to oxidize durohydroquinone via the cyanide-resistant pathway in the presence of both cyanide and antimycin A. The oxidation via the alternative pathway in *Arum maculatum* of durohydroquinone and NADH have been compared. The results indicate that durohydroquinone is as good a donor to the cyanide resistant pathway as is NADH and that it does not require the presence of the natural quinone in order to donate electrons to the SHAM-sensitive, cyanide-resistant pathway.

Abbreviations: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; SHAM, salicylhydroxamic acid

2. Materials and methods

Cassava from Kenya was provided by the Tropical Products Institute London, sweet potato was obtained from the local markets and *Arum maculatum* was collected from the wild. Mitochondria were isolated from these tissues as described in [8].

Oxygen uptake was measured polarographically using a Clark-type oxygen electrode. The reaction medium was 0.3 M sucrose, 10 mM *N*-[(trishydroxymethyl)methyl]-2-aminoethanesulphonic acid, 5 mM KH_2PO_4 and 5 mM $MgCl_2$, at pH 7.2.

Protein was assayed by the Lowry method [9] after solubilization with 0.2 ml (10% w/v) deoxycholate in final vol. 5.9 ml.

Salicylhydroxamic acid (SHAM) was obtained from Aldrich Chemical Co. Ltd, antimycin A from Calbiochem Ltd, duroquinone and durohydroquinone from K and K Chemicals Inc. Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) was a generous gift from Dr P. G. Heyter.

3. Results

Previous work with mitochondria and durohydroquinone has mainly been carried out with commercially obtained duroquinone which was reduced using crystalline borohydride in alcoholic solutions. Such solutions of durohydroquinone are fairly easily auto-oxidized if not kept under anaerobic conditions. In this study we have used commercially obtained durohydorquinone which was dissolved in dimethyl sulphoxide to produce a 100 mM solution. This preparation was found to remain stable under aerobic conditions at room temperature for considerably long periods of time. Comparative experiments showed that mitochondria oxidized both types of durohydroquinone in an identical manner and that they had identical absorption spectra in the ultraviolet region.

Figure 1 shows the oxidation of durohydroquinone to be resistant to cyanide and antimycin A but sensitive to SHAM in *Arum*, cassava and sweet potato mitochondria.

Figure 2 shows that the rate of oxidation of durohydroquinone via the SHAM-sensitive, cyanide-insensitive pathway in *Arum* mitochondria is directly proportional to the concentration of mitochondrial protein.

The percentage inhibition of oxygen uptake by increasing concentrations of SHAM in *Arum* mitochondria oxidizing NADH and durohydroquinone in the presence and absence of antimycin A are shown in fig.3. These indicate that the inhibition characteristics of SHAM on NADH oxidation via the cyanide-resistant pathway are essentially the same as with the oxidation of durohydroquinone.

The oxidation characteristics via the cytochrome pathway with respect to cyanide sensitivity are also very similar with *Arum* mitochondria oxidizing

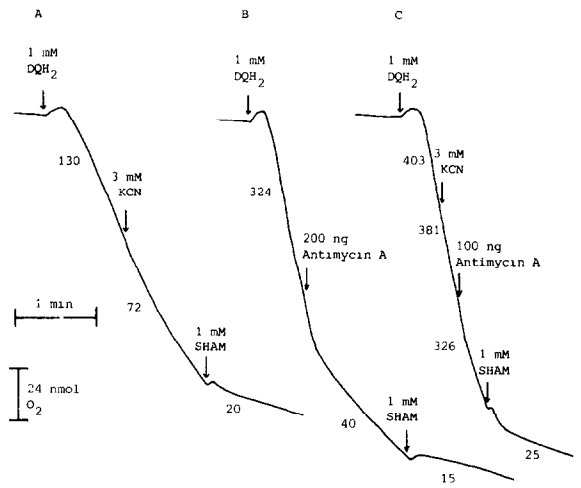

Fig.1. Inhibitor sensitivity of durohydroquinone oxidation by *Arum*, sweet potato and cassava mitochondria. Oxygen uptake was measured polarographically using 1 ml reaction mixture as described in section 2, with 2×10^{-7} M FCCP. Figures indicate rates of oxygen uptake in nmol oxygen/min/mg protein, using (A) 0.5 mg cassava mitochondrial protein/ml, (B) 0.6 mg sweet potato mitochondrial protein/ml and (C) 0.42 mg *Arum* mitochondrial protein/ml.

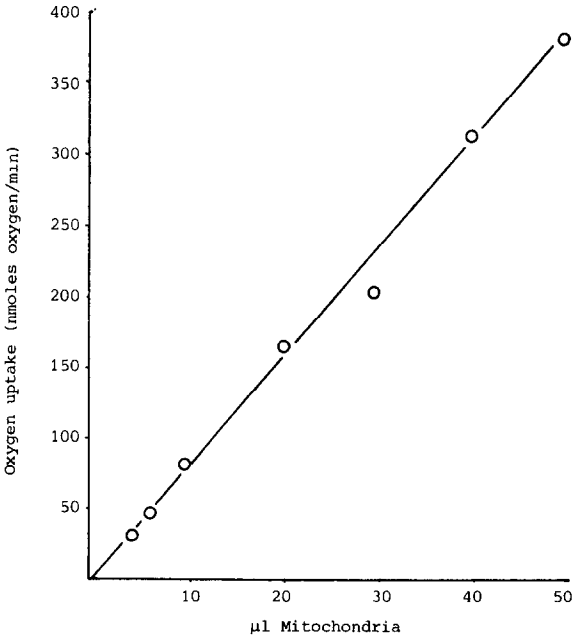

Fig.2. Effect of mitochondrial protein concentration on rate of durohydroquinone oxidation. Oxygen uptake was measured as described in fig.1, using *Arum* mitochondria (42 mg protein/ml).

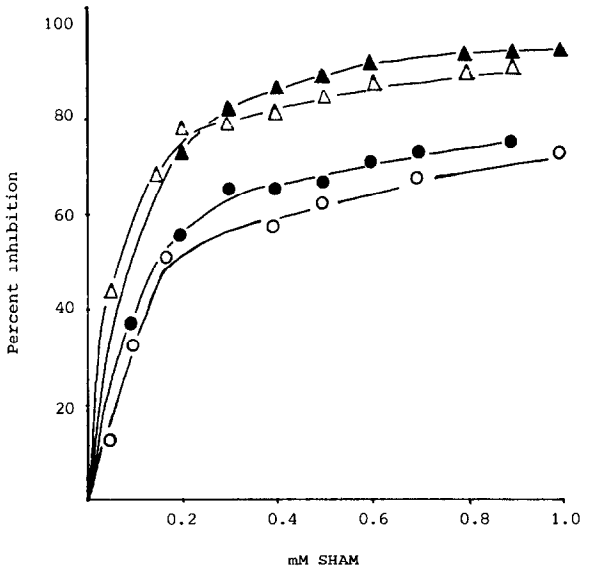

Fig.3. SHAM inhibition of NADH and durohydroquinone oxidation. Oxygen uptake was measured as described in fig.1 using 0.42 mg *Arum* mitochondrial protein oxidizing: (o–o) NADH (uninhibited rate 630 nmol O_2/min/mg protein); (•–•) durohydroquinone (uninhibited rate 593 nmol O_2/min/mg protein); (△–△) NADH + 100 ng antimycin A (uninhibited rate 423 nmol O_2/min/mg protein); and (▲–▲) durohydroquinone + 100 ng antimycin A (uninhibited rate 427 nmol O_2/min/mg protein).

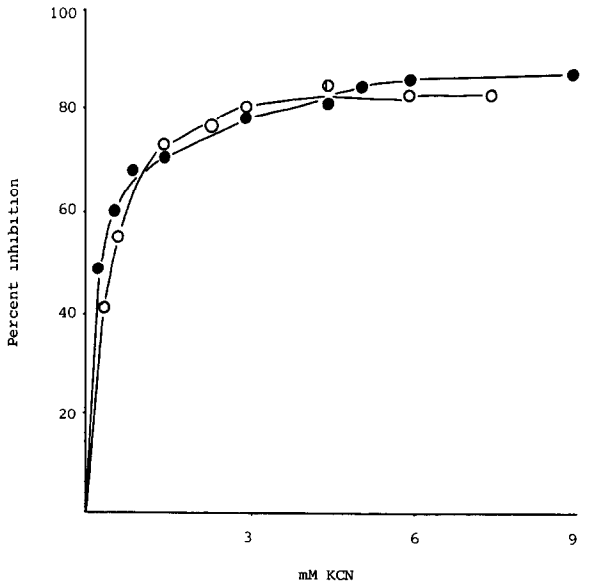

Fig.4. Effect of KCN on oxygen uptake by *Arum* mitochondria in the presence of SHAM. Oxygen uptake was measured as described in fig.1 using 0.42 mg *Arum* mitochondrial protein and 2 mM SHAM. (o–o) Oxidizing 0.5 mM NADH; (•–•) oxidizing 1.0 mM durohydroquinone.

NADH or durohydroquinone as shown in fig.4.

Mitochondria from *Arum* were treated with pentane after lyophilization to extract the natural quinone [10]. The quinone-depleted mitochondria were not able to oxidize NADH at all without the addition of duroquinone or the re-incorporation of its own extracted quinone or ubiquinone 50. However, the oxidation of durohydroquinone was unaffected by the extraction of the natural quinone (table 1).

Table 1
Oxidation of NADH and durohydroquinone by lyophilized and quinone-depleted *Arum* mitochondria (nmol oxygen/min/mg lyophilized mitochondria)

	Control			Quinone-depleted		
		+ 1 mM SHAM	+ 3 mM KCN		+ 1 mM SHAM	+ 3 mM KCN
NADH	112	18	4	10	10	1.4
Durohydroquinone	112	10	4	115	6	4

Oxygen uptake was measured as described in fig.1. Control mitochondria were lyophilized in 0.15 M KCl, washed and resuspended in reaction medium (described in section 2). Quinone depletion was done by pentane extraction from lyophilized mitochondria as described in [10]

4. Discussion

Durohydroquinone has been shown to be oxidized, in *Arum*, sweet potato and cassava mitochondria, via the cyanide-resistant, SHAM-sensitive respiratory pathway. The sensitivity to antimycin A, cyanide and SHAM of the respiratory electron transport chain has been shown to be essentially the same whether the mitochondria oxidized NADH or durohydroquinone and were thus different from *Neurospora crassa* as shown [4]. Mitochondria that had been depleted of their natural quinone such that NADH could no longer be oxidized were shown to be able to oxidize durohydroquinone via the cyanide-resistant pathway. The oxidation of durohydroquinone via the cyanide-resistant pathway in higher plant mitochondria therefore does not require the natural quinone to be present. As cytochromes have been shown not to be involved in electron transport via the cyanide-resistant pathway [11] we believe that the cyanide-resistant, SHAM-sensitive oxidation of durohydroquinone may prove a useful assay for the alternative oxidase as it does not require the presence of any of the known components of the electron transport chain and may donate close to or directly to the alternative oxidase itself.

Acknowledgements

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