

# Ubiquinone-1 Induces External Deamino-NADH Oxidation in Potato Tuber Mitochondria<sup>1</sup>

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The addition of ubiquinone-1 (UQ-1) induced  $\text{Ca}^{2+}$ -independent oxidation of deamino-NADH and NADH by intact potato (*Solanum tuberosum* L. cv Bintje) tuber mitochondria. The induced oxidation was coupled to the generation of a membrane potential. Measurements of  $\text{NAD}^+$ -malate dehydrogenase activity indicated that the permeability of the inner mitochondrial membrane to NADH and deamino-NADH was not altered by the addition of UQ-1. We conclude that UQ-1-induced external deamino-NADH oxidation is due to a change in specificity of the external rotenone-insensitive NADH dehydrogenase. The addition of UQ-1 also induced rotenone-insensitive oxidation of deamino-NADH by inside-out submitochondrial particles, but whether this was due to a change in the specificity of the internal rotenone-insensitive NAD(P)H dehydrogenase or to a bypass in complex I could not be determined.

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All plant mitochondria except those from fresh red beetroots oxidize exogenous NADH and NADPH in a  $\text{Ca}^{2+}$ -dependent manner. This oxidation is rotenone insensitive and is caused by at least two separate enzymes (Møller and Lin, 1986; Møller et al., 1993; Roberts et al., 1995). On the inner surface of the inner mitochondrial membrane NAD(P)H is oxidized by the rotenone-sensitive complex I, as well as by separate rotenone-insensitive NAD(P)H dehydrogenase(s) (Møller and Palmer, 1982; Møller and Lin, 1986; Møller et al., 1993).

The NAD(P)H analog deamino-NAD(P)H is oxidized very slowly by intact potato (*Solanum tuberosum* L.) tuber mitochondria and by inside-out SMP in the presence of rotenone with  $\text{O}_2$  as the electron acceptor. This indicates that only complex I and none of the rotenone-insensitive NAD(P)H dehydrogenases can use deamino-NAD(P)H as electron donors (Rasmusson and Møller, 1991a). In apparent conflict with this observation, a purified rotenone-insensitive NAD(P)H dehydrogenase thought to derive from the inner surface of the inner mitochondrial membrane (Rasmusson et al., 1993) oxidized deamino-NADH at almost the same rate as NADH when duroquinone, a low-molecular-weight UQ analog, was used as the electron acceptor.

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Menz et al. (1992) found that the addition of UQ-1, another low-molecular-weight UQ analog, induced rotenone-insensitive deamino-NADH oxidation in red beetroot SMP. This was interpreted to be due to the presence of a second quinone-binding site within complex I, where short-chained quinones could accept electrons and make them bypass the site of rotenone inhibition. However, considering our own observations of the internal rotenone-insensitive NAD(P)H dehydrogenase mentioned above, we thought that it might be possible that UQ-1 instead changes the specificity of the internal rotenone-insensitive NAD(P)H dehydrogenase to allow it to accept electrons from deamino-NADH. In the present study we have tested this hypothesis by investigating the effect of UQ-1 on the oxidation of deamino-NAD(P)H by inside-out SMP. We have also investigated the effect of UQ-1 on external deamino-NAD(P)H oxidation using intact mitochondria.

## MATERIALS AND METHODS

Mitochondria from tubers of potato (*Solanum tuberosum* L. cv Bintje) were prepared according to the method of Struglics et al. (1993), and inside-out SMP were prepared according to the method of Rasmusson and Møller (1991a). All concentrations given are final concentrations. The reaction medium used for all measurements ( $\text{O}_2$  consumption rates, MDH activities, and membrane potentials) contained 0.3 M Suc, 5 mM potassium phosphate, 5 mM Mops, and 2.5 mM  $\text{MgCl}_2$ , pH 7.2, and had a volume of 1.0 mL.

$\text{O}_2$  consumption was measured using an  $\text{O}_2$  electrode (Rank Brothers, Cambridge, UK). Carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone was added at 0.4  $\mu\text{M}$  to the reaction medium. The final concentration of each of the nicotinamide dinucleotides was 0.5 or 1.0 mM. Membrane potential measurements were made using a DW-2 UV/vis spectrophotometer (Aminco, Silver Spring, MD). Safranin (16  $\mu\text{M}$ ) was used as the marker for the generation of a membrane potential, which was measured as the difference between  $A_{533}$  and  $A_{511}$  (Åkerman and Wikström, 1976). For further details see the figure legends.

MDH activity was measured using the DW-2 UV/vis spectrophotometer or a UV-160A UV-visible recording spectrophotometer (Shimadzu, Kyoto, Japan). The sub-

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Abbreviations: deamino-NADH, nicotinamide hypoxanthine dinucleotide, reduced form; MDH,  $\text{NAD}^+$ -dependent malate dehydrogenase (EC 1.1.1.37); SMP, submitochondrial particles; UQ, ubiquinone.

strate was 2 mM oxaloacetate, and antimycin A (0.36  $\mu\text{M}$ ) was present. The concentration of each of the nicotinamide dinucleotides was 0.2 mM.

Protein determinations were made according to the method of Lowry et al. (1951) using BSA as the standard. The concentration of UQ-1 in the stock solution was determined by the method of Lawford and Garland (1972). The final concentration of UQ-1 was 60  $\mu\text{M}$  in all experiments.

## RESULTS AND DISCUSSION

### UQ-1 Induces Deamino-NADH Oxidation by Intact Mitochondria

Intact potato tuber mitochondria did not oxidize deamino-NADH (Table I), confirming that the external NADH dehydrogenase does not accept this substrate under normal conditions (Rasmusson and Møller, 1991a). However, the addition of 60  $\mu\text{M}$  UQ-1 induced deamino-NADH oxidation (Table I), the rate of which was about 20% of the maximum rate of NADH oxidation observed in the presence of  $\text{Ca}^{2+}$ . In contrast to normal external NAD(P)H oxidation (Table I; Arron and Edwards, 1979; Møller and Palmer, 1981; Møller et al., 1981), the UQ-1-induced deamino-NADH oxidation by intact mitochondria was not sensitive to  $\text{Ca}^{2+}$  addition (Table I). The oxidation of NADH by intact mitochondria was also stimulated by the presence of UQ-1 and in this state became less dependent on  $\text{Ca}^{2+}$  (Table I).

The UQ-1-induced deamino-NADH oxidation was completely sensitive to antimycin A (Table I), as was the membrane potential generated by this oxidation (see below). This indicates that the enzyme involved is part of the electron transport chain. It should be noted that in the presence of UQ-1 and absence of  $\text{Ca}^{2+}$  (1 mM EGTA present) the effect of antimycin A is slower (about 1 min rather than 20 s) and more antimycin A is required to give full inhibition (we used 0.9 instead of 0.36  $\mu\text{M}$ ).

**Table I.** The effect of UQ-1 on deamino-NADH and NADH oxidation by potato tuber mitochondria and inside-out SMPs

Rotenone was used at a final concentration of 20  $\mu\text{M}$  and antimycin was used at 0.9  $\mu\text{M}$ . Similar results were observed on four independent preparations and representative data are presented.

Substrate + Additions <sup>a</sup>	Oxygen Consumption		SMP
	Mitochondria		
	+ 1 mM EGTA	+ 1 mM $\text{Ca}^{2+}$	
	nmol min <sup>-1</sup> mg <sup>-1</sup> protein		
Deamino-NADH	10	14	339
+ Rotenone	5	14	<10
+ UQ-1	61	63	62
+ NADH	91	304	278
+ Antimycin A	3	1	ND <sup>b</sup>
NADH	32	252	373
+ Rotenone	40	252	129
+ UQ-1	126	313	258
+ Antimycin A	3	2	ND

<sup>a</sup> Measurements were made by consecutive additions of the solutions. <sup>b</sup> ND, Not determined.

### Deamino-NADH Can Generate a Membrane Potential Only in the Presence of UQ-1

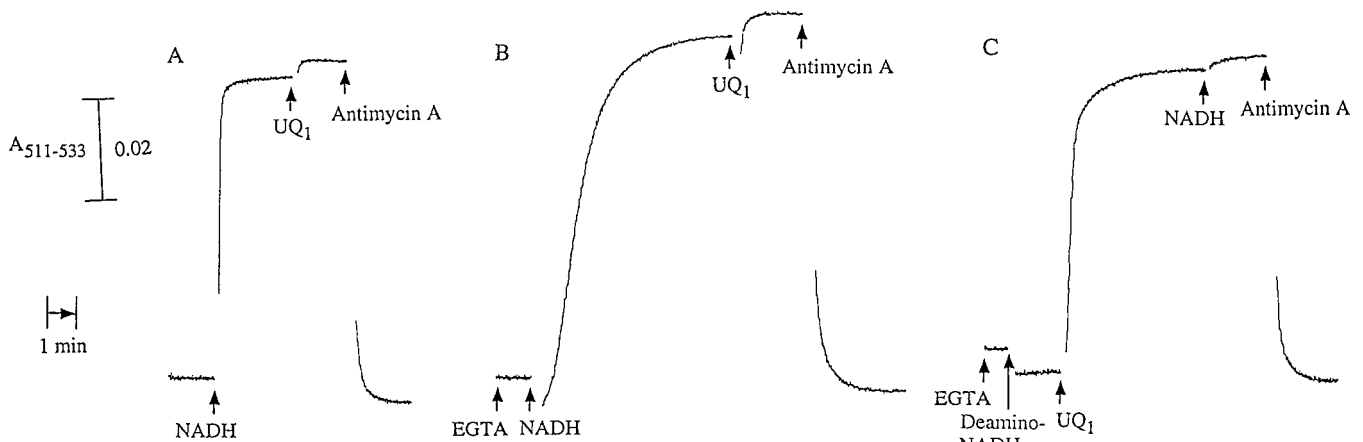
The effect of UQ-1 on the membrane potential of intact mitochondria with NADH and deamino-NADH as substrates was measured using safranin as a probe. NADH caused the rapid formation of a membrane potential, which increased only slightly in magnitude by the subsequent addition of UQ-1 (Fig. 1A). This experiment was performed without added  $\text{Ca}^{2+}$ , but there is sufficient  $\text{Ca}^{2+}$  in the standard reaction medium to allow close to maximum activity (Rasmusson and Møller, 1991b). Clearly, a rate of approximately 250 nmol  $\text{O}_2$  min<sup>-1</sup> mg<sup>-1</sup> (Table I) is sufficient to give a maximum membrane potential. In the presence of EGTA, the oxidation of NADH could also generate a full membrane potential, but this took much longer than with  $\text{Ca}^{2+}$  (Fig. 1B). This result is consistent with the much lower rate of oxidation with EGTA than with  $\text{Ca}^{2+}$  (Table I). The relationship between rate of oxidation and size of the membrane potential as monitored with safranin is shown in Figure 2 for intact potato tuber mitochondria. A rate greater than 50 nmol  $\text{O}_2$  min<sup>-1</sup> mg<sup>-1</sup> is required to give a full membrane potential under the conditions used, but very little decrease is seen unless the rate is less than 30 nmol  $\text{O}_2$  min<sup>-1</sup> mg<sup>-1</sup>.

The addition of deamino-NADH to intact mitochondria did not give rise to a membrane potential. However, the subsequent addition of UQ-1 led to the rapid formation of a membrane potential, the size of which could not be substantially increased by the addition of NADH (Fig. 1C). This is consistent with the observation that a rate of 60 nmol  $\text{O}_2$  min<sup>-1</sup> mg<sup>-1</sup> (Table I) is sufficient to give a full membrane potential (Fig. 2). Thus, UQ-1-induced,  $\text{Ca}^{2+}$ -independent (Fig. 1C) and  $\text{Ca}^{2+}$ -dependent (not shown) oxidation of deamino-NADH was coupled to the generation of a membrane potential. This membrane potential was completely collapsed by the addition of antimycin A (Fig. 1C) or carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (not shown), showing that proton pumping by complexes III and IV was involved in its generation. We conclude that UQ-1 induces oxidation of deamino-NADH through the electron transport chain, giving rise to a membrane potential.

### UQ-1 Does Not Affect the Permeability of the Inner Mitochondrial Membrane

To confirm that the data in Table I reflect a change in specificity of the external NADH dehydrogenase upon addition of UQ-1, it was necessary to ensure that the permeability of the inner mitochondrial membrane, primarily to deamino-NADH, was not affected by the addition of UQ-1.

Measurements of the latency of the matrix enzyme, NAD<sup>+</sup>-dependent MDH, were used to determine the permeability of the inner mitochondrial membrane to NADH and deamino-NADH (Rasmusson and Møller, 1991b). The addition of UQ-1 slightly but reproducibly increased the MDH activity with NADH (Table II). UQ-1 had no significant effect on the equivalent rate with deamino-NADH. The MDH activity in the presence of Triton X-100 (Sigma)



**Figure 1.** The effect of UQ-1 on the membrane potential of intact potato tuber mitochondria oxidizing NADH or deamino-NADH. The membrane potential was monitored by safranin absorbance as described in "Materials and Methods." Starting conditions: reaction medium (see "Materials and Methods") plus 20  $\mu\text{g}/\text{mL}$  BSA and 0.2 mg/mL mitochondrial protein. In B and C, 1 mM EGTA was also present. Additions: 0.5 mM NADH and deamino-NADH, 60  $\mu\text{M}$  UQ-1, and 0.36  $\mu\text{M}$  antimycin A. Similar results were observed on at least five other preparations of mitochondria.

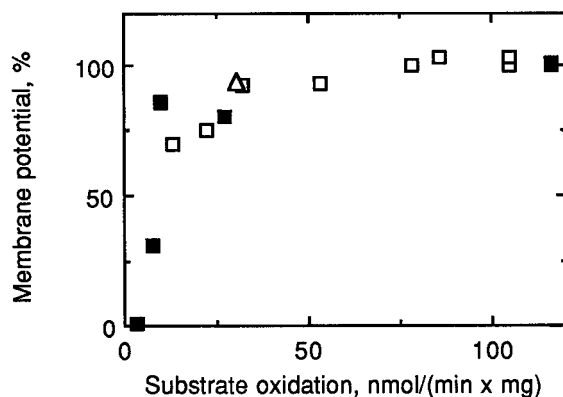
was not significantly affected by the addition of UQ-1 with either NADH or deamino-NADH. The latency of MDH was therefore unaffected by UQ-1 irrespective of the coenzyme used (Table II). We conclude that the permeability of the inner mitochondrial membrane to deamino-NADH and NADH was not significantly altered by the addition of UQ-1.

#### UQ-1 Induces Rotenone-Insensitive Deamino-NADH Oxidation by Inside-Out SMP

The NADH analog deamino-NADH is oxidized by complex I but not by the rotenone-insensitive dehydrogenases assayed in plant mitochondria or inside-out SMP (Rasmus-

son and Møller, 1991a). UQ-1 has been reported to induce rotenone-insensitive deamino-NADH oxidation by SMP from red beetroot mitochondria (Menz et al., 1992), an observation we confirmed for inside-out SMP from potato tubers. The induced rate of rotenone-insensitive deamino-NADH oxidation was 24% of the corresponding rate with NADH (Table I), and Menz et al. (1992) found a value of 66% using 150  $\mu\text{M}$  UQ-1 and beetroot SMP. Menz et al. (1992) suggested from their study that UQ-1 can interact with a second quinone-binding site on complex I, allowing deamino-NADH oxidation that is rotenone-insensitive without involving internal, rotenone-insensitive NAD(P)H dehydrogenase(s). An alternative interpretation is that UQ-1 changes the specificity of internal, rotenone-insensitive NAD(P)H dehydrogenase(s). To allow one of these two interpretations to be excluded requires either that complex I be specifically inhibited at the flavin or that the internal, rotenone-insensitive NAD(P)H dehydrogenase(s) be specifically inhibited—before the addition of deamino-NADH and UQ-1 in both cases.

Considering the above data, including those of Menz et al. (1992), we cannot draw a firm conclusion as to whether



**Figure 2.** Correlation between rate of respiration and the size of the membrane potential as monitored by safranin absorbance changes. Different rates of NADPH oxidation were obtained by adding EGTA (10–1000  $\mu\text{M}$ ; ■), different rates of succinate oxidation were obtained by adding malonate (0.2–10 mM; □), and a low rate of NADH oxidation was obtained by adding 1 mM EGTA (▲). The maximal membrane potential observed with NADH and  $\text{Ca}^{2+}$  (rate always more than 200  $\text{nmol min}^{-1} \text{mg}^{-1} \text{protein}$ ) was set at 100%. Similar results were observed with another independent preparation of mitochondria.

**Table II.** The effect of UQ-1 on the MDH activity of potato tuber mitochondria

Triton X-100 was used at a final concentration of 0.025% (w/v). The experiment was done on two independent preparations of mitochondria with similar results. The results presented are means  $\pm$  SD ( $n = 4$ ) from one preparation of mitochondria.

Additions	MDH Activity		Latency
	– Triton X-100	+ Triton X-100	
	$\mu\text{mol min}^{-1} \text{mg}^{-1} \text{protein}$		%
NADH	2.7 $\pm$ 0.1	26.9 $\pm$ 0.2	90 $\pm$ 0.5
NADH + UQ-1	4.0 $\pm$ 0.2	32.1 $\pm$ 1.0	88 $\pm$ 0.3
Deamino-NADH	1.2 $\pm$ 0.1	12.0 $\pm$ 0.9	90 $\pm$ 1.4
Deamino-NADH + UQ-1	1.0 $\pm$ 0.1	9.9 $\pm$ 0.6	90 $\pm$ 0.5

the effect of UQ-1 on deamino-NADH oxidation by inside-out SMP is due to a change in the specificity of internal, rotenone-insensitive NAD(P)H dehydrogenase(s) or to the induction of a rotenone-insensitive bypass in complex I. However, by analogy with the effect of UQ-1 on the external, rotenone-insensitive NADH dehydrogenase shown in the present paper, we believe that the former conclusion is more likely.

### CONCLUSIONS

The addition of UQ-1 to intact potato tuber mitochondria induces deamino-NADH oxidation via the electron transport chain (Table I; Fig. 1) without affecting the permeability of the inner membrane to deamino-NADH (Table II). We therefore conclude that UQ-1 induces a change in specificity of external, rotenone-insensitive NADH dehydrogenase(s) such that deamino-NADH is accepted as an electron donor. Since UQ-1 also induces rotenone-insensitive deamino-NADH oxidation by inside-out SMP, we suggest that internal, rotenone-insensitive NAD(P)H dehydrogenase(s) may be affected by UQ-1 in the same way as is external, rotenone-insensitive NADH dehydrogenase(s).

A change in specificity upon addition of UQ-1 (or other short-chained quinones) could be the reason that purified internal, rotenone-insensitive NAD(P)H dehydrogenase (assayed with duroquinone) from red beetroot can use deamino-NADH as a substrate (Rasmusson et al., 1993), whereas the enzyme in situ (assayed with O<sub>2</sub> in the absence of added quinones) cannot use deamino-NADH (Rasmusson and Møller, 1991a). On the other hand, the 32-kD NADH dehydrogenase purified by Knudten et al. (1994) from maize mitochondria also oxidized deamino-NADH as well as NADH, but the electron acceptor used was 2,6-dichlorophenolindophenol and no small quinone was present.

The present study also shows that it is not possible to utilize deamino-NADH (and probably deamino-NADPH as well) in the presence of UQ-1 (and likely other UQ analogs as well) to distinguish between the activities of the different dehydrogenases in the electron transport chain of plant mitochondria.

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