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The mechanism of uncoupling of oxidative phosphorylation by 2-methyl-1,4-naphthoquinone

ERNSTER and collaborators[1] showed that rat-liver mitochondria contain an enzyme, now known as NAD(P)H dehydrogenase (NAD(P)H:acceptor oxidoreductase, EC 1.6.99.2), that accepts reducing equivalents from both NADH and NADPH. A number of compounds act as electron acceptors, *e.g.* 2-methyl-1,4-naphthoquinone. The reduced quinone is oxidized by the respiratory chain, probably *via* ubiquinone[2]. Thus, when 2-methyl-1,4-naphthoquinone is added to rat-liver mitochondria, NAD(P)H may be oxidized by an Amytal- or rotenone-insensitive pathway parallel to a portion of the respiratory chain (Fig. 1). Upon adding the quinone to rat-liver mitochondria oxidizing a NAD(P)-linked substrate, the P:O ratio should be lowered by about 1 unit (*cf.* ref. 3).

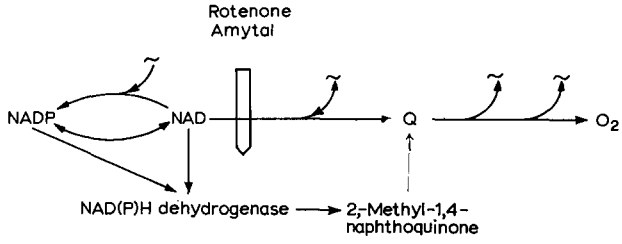

Fig. 1. Mechanism of uncoupling by 2-methyl-1,4-naphthoquinone.

However, 2-methyl-1,4-naphthoquinone has also an uncoupling effect in rat-liver mitochondria; it slightly decreases the P:O ratio with succinate as a substrate[3] and induces a small ATPase activity[4–6]. A mechanism for this uncoupling has been proposed by WU AND CHENG[6] who found that stimulation of the ATPase activity by 2-methyl-1,4-naphthoquinone is sensitive to both Amytal and low concentrations of dicoumarol. They suggested that in the presence of 2-methyl-1,4-naphthoquinone, a cyclic transfer of hydrogen occurs which consists of reversed electron transfer from Q to NAD^+ followed by the oxidation of NADH *via* the NAD(P)H dehydrogenase and 2-methyl-1,4-naphthoquinone by Q. Since reversed electron transport consumes energy which is not regained after completion of the cycle, the net result is an uncoupling of oxidative phosphorylation.

We have confirmed the finding of WU AND CHENG[6] that 2-methyl-1,4-naphthoquinone stimulates the ATPase activity of rat-liver mitochondria in the presence of Na_2S and that this stimulation is sensitive to rotenone. Furthermore, the effect of 2-methyl-1,4-naphthoquinone on succinate oxidation in the presence of oligomycin was studied. Table I shows that the quinone stimulates oxygen consumption and that this effect is also rotenone sensitive. In Table II is given the effect of 2-methyl-1,4-naphthoquinone on the P:O ratio with succinate as substrate. A suboptimal amount of hexokinase was added in Expts. 1 and 2 in order to make competition for ~ between oxidative phosphorylation and quinone uncoupling favorable for the latter (*cf.* ref. 7). 2-Methyl-1,4-naphthoquinone caused a 10–40% decrease in the P:O ratio, unless rotenone was present.

TABLE I

EFFECT OF 2-METHYL-1,4-NAPHTHOQUINONE ON SUCCINATE OXIDATION IN RAT-LIVER MITOCHONDRIA IN THE PRESENCE OF OLIGOMYCIN

1 ml reaction mixture contained 15 mM KCl, 5 mM $MgCl_2$, 2 mM EDTA, 50 mM Tris–HCl (pH 7.5), 60 mM succinate, 10 μg oligomycin, 3% ethanol and 3.8 mg mitochondrial protein. Oxygen uptake was measured manometrically. Temp., 25°. Reaction time, 20 min.

Additions	$-\Delta O$ (μatoms)
None	4.1
2-Methyl-1,4-naphthoquinone (10 μM)	6.8
Rotenone (2 μg)	3.8
2-Methyl-1,4-naphthoquinone + rotenone	3.9

TABLE II

EFFECT OF 2-METHYL-1,4-NAPHTHOQUINONE ON THE P:O RATIO WITH SUCCINATE AS SUBSTRATE IN RAT-LIVER MITOCHONDRIA

Reaction mixture contained 15 mM KCl, 5 mM $MgCl_2$, 2 mM EDTA, 50 mM Tris–HCl, 60 mM succinate, 0.5 mM ADP, 30 mM glucose, 30 mM P_i, 1–3 units (Expts. 1 and 2) or 5 units (Expt. 3) hexokinase (EC 2.7.1.1), 2% ethanol and 3.2 mg, 2.3 mg or 3.6 mg mitochondrial protein in Expts. 1, 2 and 3, respectively. Final pH 7.5. Oxygen uptake was measured manometrically. Esterified phosphate was determined by the method of SLATER[8].

Additions	*P:O ratio*		
	Expt. 1	*Expt. 2*	*Expt. 3*
None	0.38	1.08	1.60
2-Methyl-1,4-naphthoquinone (10 μM)	0.22	0.75	1.44
Rotenone (2 μg)	0.36	1.04	1.55
2-Methyl-1,4-naphthoquinone + rotenone	0.35	0.98	1.58

These experiments demonstrate that the uncoupling effect of 2-methyl-1,4-naphthoquinone is always rotenone-sensitive and corroborate the hypothesis of WU AND CHENG[6] that quinone completes a redox cycle through which reducing equivalents are transported at the expense of energy (Fig. 1). However, it is possible that not only NAD but also NADP is involved in the cyclic process. If both are involved, it follows that, when reducing equivalents complete one full cycle, two high-energy intermediates of oxidative phosphorylation are lost, one in the energy-linked reduction of NAD^+ by ubiquinone and the second in the energy-dependent transhydrogenation between NADH and $NADP^+$ (see Fig. 1 and ref. 9).

Lowering of the P:O ratio by 2-methyl-1,4-naphthoquinone in phosphorylating particles from *Alcaligenes faecalis*[10] or *Azotobacter vinelandii*[11] may partly be due to uncoupling by the quinone. If it can be demonstrated that this uncoupling occurs according to the mechanism of WU AND CHENG[6], this would be the first indication that reversal of the respiratory chain is possible in these micro-organisms.

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