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A REQUIREMENT FOR UBIQUINONE IN ATPase ACTIVITY AND OXIDATIVE PHOSPHORYLATION

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Summary. Ubiquinone-3 uncouples succinate-driven oxidative phosphorylation in heart mitochondria; the uncoupling is reversed by ubiquinone-7, with normalization of both respiratory control and P:O ratios. Moreover ubiquinone-3 induces a decrease of oligomycin sensitivity of ATPase in submitochondrial particles; sensitivity is restored by ubiquinone-7. Also pentane extraction of lyophilised mitochondria induces decrease of oligomycin sensitivity of ATPase: only long-chain ubiquinones can restore oligomycin sensitivity. The results suggest an involvement of endogenous ubiquinone in the coupling process.

We have previously shown that ubiquinones (Q) having short isoprenoid side chains inhibit NADH oxidation competitively with long-chain ubiquinones, whereas the rate of succinate oxidation is not affected (1, 2). During the course of these investigations we have observed that Q-3 stimulates the rate of state-4 succinate oxidation in coupled mitochondria.

This study reports our preliminary observations on the uncoupling effect of Q-3.

METHODS

Rat heart mitochondria (RHM) were prepared according to Sordahl et al. (3) and beef heart mitochondria (BHM) by a large-scale procedure (4).

Submitochondrial particles ETP have been prepared by sonication of BHM (5). Pentane extraction of lyophilised mitochondria in order to remove the endogenous Q was accomplished according to Szarkowska (6) modified as appearing in the legend of Fig. 4.

ATPase activity was assayed in a medium containing 6 mM-ATP, 3 mM $MgCl_2$ and 50 mM Tris-acetate, pH 8.5. The phosphate liber-

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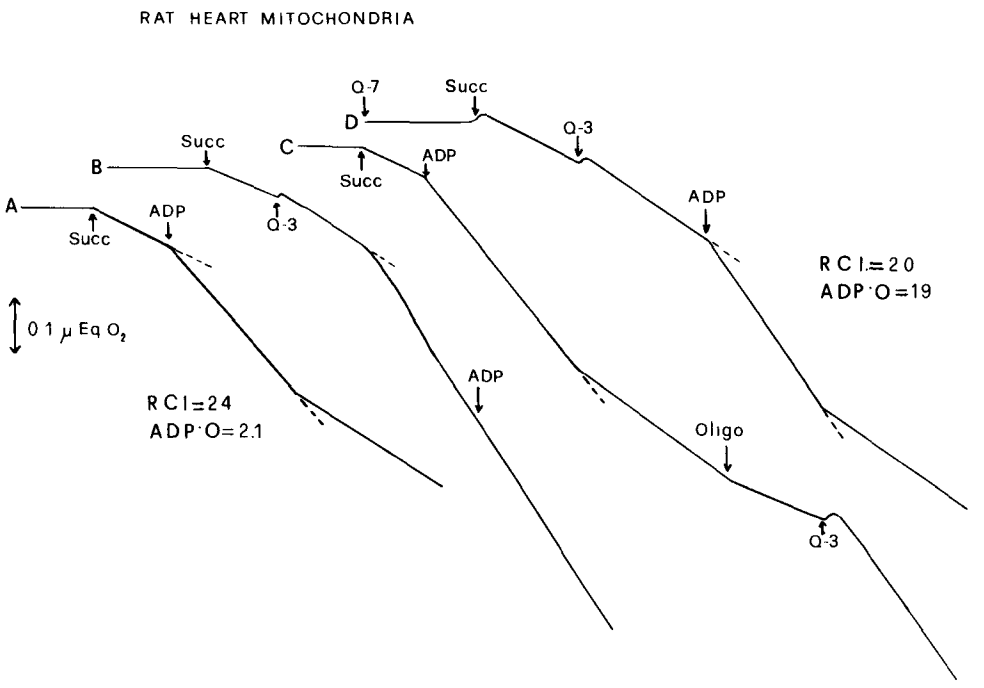

Fig. 1. Uncoupling effect of Q-3 and its reversal by Q-7 in rat heart mitochondria. The assay medium contained in a total volum of 3 ml: 180 mM KCl; 0.5% BSA; 3 mM K phosphate, pH 7.4; 0.5 mM EDTA; rotenone, 7.35 ng; ADP when added was 0.75 μmoles. Q-3 and Q-7 were 300 μmoles.

ated was assayed as described elsewhere (7). Respiratory control was determined with an oxygen electrode, and oxidative phosphorylation manometrically as described elsewhere (8).

RESULTS

Figure 1 shows oxygen electrode recordings of succinate oxidation in RHM; Q-3 uncouples state 4 respiration (tracing B) and also oligomycin-inhibited respiration (tracing C); Q-7 incubated with RHM almost completely prevents the effects of Q-3 (tracing D). The uncoupling effect of Q-3 is not the consequence of a bypass of the respiratory chain through the quinone, since Q-3 stimulated respiration is still completely inhibited by antimycin A. Table I shows the inhibitory effect of Q-3 on succinate-driven phosphorylation and its reversal by Q-7.

Table I

Effect of Q-3 on oxidative phosphorylation of rat heart mitochondria.

Addition	Oxidative rate (μatoms/min.mg protein)		P:O Ratio	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
None	0.095	0.125	1.86	1.37
Q-3 67 μM	0.145	0.140	0.7	0.04
Q-7 67 μM	-	0.100	-	1.46
Q-3 + Q-7 (67 μM each)	0.087	0.120	0.72	0.51

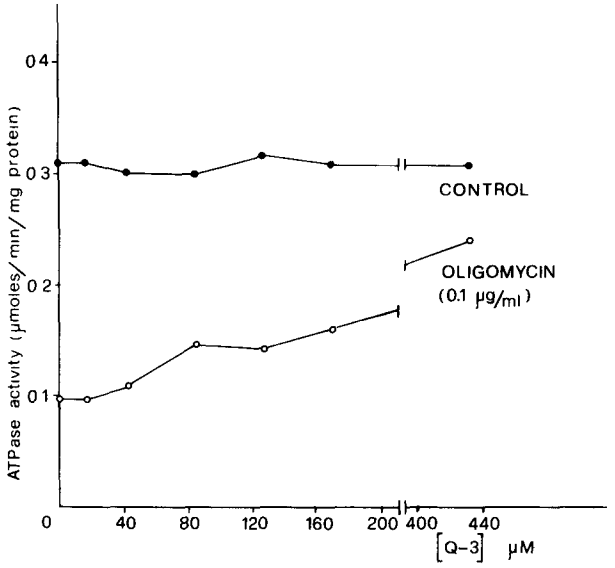

Fig. 2. Effect of Q-3 on oligomycin sensitivity of ATPase in ETP.

In order to attempt a localization of Q-3 uncoupling in the energy-transfer pathway we have also investigated the effect of Q-3 on ATPase activity; although no effect was shown on the kinetics of ATPase in submitochondrial particles, a striking loss of its oligomycin sensitivity was apparent (Fig. 2 and 3). Q-7 again prevented the loss of oligomycin sensitivity (Table II).

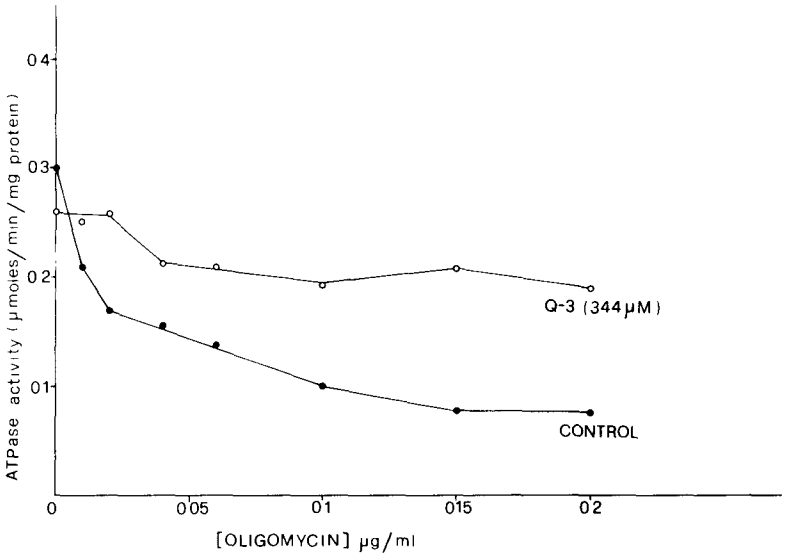

Fig. 3. Effect of oligomycin on ATPase activity of ETP in presence of Q-3.

Table II

The effect of Q-3 on oligomycin sensitivity of ATPase in ETP.

Addition	ATPase activity - oligo.	ATPase activity + oligo. (0.05 μg/ml)	% inhibition
	μg Pi/min.mg protein		
None	0.34	0.17	50
Q-3 (300 μM)	0.30	0.25	17
Q-3 + Q-7 (300 μM each)	0.31	0.20	36

In order to test more directly the role of Q in the ATPase reaction, we have depleted ETP of their endogenous Q by means of pentane extraction after lyophilization.

We found that the treatment resulted in decrease of oligomycin sensitivity of ATPase (Fig. 4); addition of long-chain quinones (Q-9) but not of short-chain quinones (Q-3) could restore oligomycin sensitivity of ATPase.

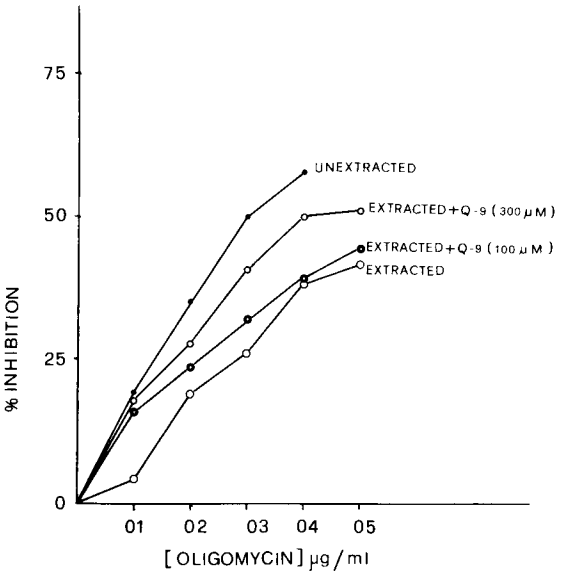

Fig. 4. Effect of Q extraction on the oligomycin sensitivity of lyophilized ETP. Pentane extraction was accomplished according to Szarkowska (6) except that ETP were suspended in 10 mM $MgCl_2$ prior to lyophilization, and pentane extraction was performed by shaking in the cold 3 times for a total of 3 hours.

Moreover the ATP-^{32}Pi exchange could be measured under the same experimental conditions: it was found that Q extraction results in a low exchange rate, which is enhanced by long-chain ubiquinones (E. Bertoli and M. Carver, unpublished).

DISCUSSION

We have shown that Q-3 reversibly uncouples succinate-driven oxidative phosphorylation. The uncoupling effect of Q-3 is not an unspecific effect due to high quinone concentrations, since it is not induced by long-chain quinones which are rather able to reverse uncoupling and to restore both respiratory control and normal P:O ratios. Such reversible uncoupling by Q-3 is accompanied by a decrease of oligomycin sensitivity of ATPase; long-chain quinones again restore the original sensitivity. These data suggest that Q-3 acts by displacing the endogenous Q from

a specific site. We have attempted to show a coupling effect of endogenous Q more directly by investigating the effects of Q extraction by pentane treatment of mitochondria; unfortunately such conditions lead to loss of oxidative phosphorylation, since Q is required as an electron carrier; no selective effects could be tried on phosphorylation versus oxidation since phosphorylation could not be recovered under our experimental conditions. However Q depletion induces a reversible loss of oligomycin sensitivity and a reversible decrease of ATP-^{32}Pi exchange reaction.

The results of this investigation point out a role of ubiquinone in the energy-transfer pathway.

It cannot be decided presently whether Q is directly required in some step of the coupling process or it is an allosteric modulator of the ATPase-ATP synthetase reaction.

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