

SUCCINATE OXIDASE ACTIVITY IN THE ABSENCE OF UBIQUINONE

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Received 3 February 1971

Szarkowska [1] showed that extraction of mitochondria with *n*-pentane abolishes their ability to catalyse the oxidation of succinate or NADH by oxygen, and that the activity is restored by re-incorporation of Q-10 into the particles. Lenaz et al. [2] found that Q-2 is as effective as Q-10 in restoring succinate oxidation, but that NADH oxidation has a marked preference for Q-10.

This paper describes the restoration of succinate oxidation by re-incorporation into pentane-extracted particles of a Q-10-free fraction of the pentane extract. The main finding was briefly reported in a recent symposium [3].

Keilin and Hartree heart-muscle preparation [4], suspended in water to a concentration of 20–30 mg protein/ml, was extracted 4–6 times with the methanol-petroleum ether mixture used by Kröger and Klingenberg [5] for the quantitative estimation of Q in particles. The petroleum ether layers containing the Q-10 were discarded. The methanol was removed from the aqueous phase (containing a floating protein layer) by evaporation under reduced pressure on a rotatory evaporator, and the residue was then lyophilized. The dry material was extracted 3 times with *n*-pentane. The combined pentane solutions contained the Q-free pentane extract (abbreviated P) of the heart-muscle preparation.

Reconstitution was carried out by suspending Q-free heart-muscle preparation (obtained by extracting the dry preparation 4 times with *n*-pentane) in an amount of the Q-free pentane extract corresponding to 6 times the amount of heart-muscle preparation, and stirring for 5 min at 20°. The pentane was then removed on a rotary evaporator under reduced pressure and the powder suspended in 0.25 M

sucrose–50 mM tris-HCl (pH 8.0). In a control experiment, Q was re-incorporated into pentane-extracted heart-muscle particles by suspending the latter in a pentane solution of Q-10 (Sigma Chemical Co.) containing 4 nmoles Q-10 per mg protein, followed by the same treatment as above.

Table 1 shows that P is almost as effective as Q-10 in restoring succinate oxidation, but that NADH oxidation is restored only by Q-10. The P-restored succinate oxidation is sensitive to antimycin and 2-thienyltrifluoroacetone. The addition of cytochrome *c* had no effect on the oxidase activities of the pentane-extracted preparations.

Fig. 1 shows the effects of different amounts of Q-10 (fig. 1A) and P (fig. 1B). Half-maximal restoration of the NADH and succinate oxidase activities was obtained with 1.2 and 0.75 nmole Q-10/mg protein, respectively. Half-maximal restoration of the succinate oxidase with no restoration of the NADH

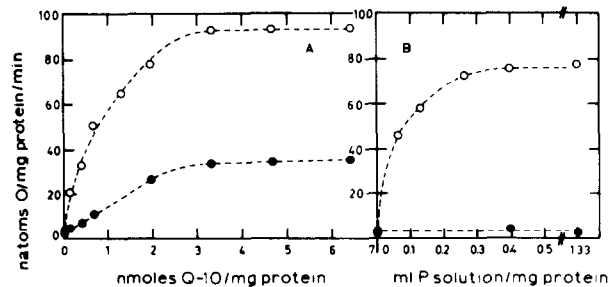


Fig. 1. Restoration of succinate and NADH oxidase activities of pentane-extracted heart-muscle preparation by Q-10 or P. Dried heart-muscle preparation was extracted with *n*-pentane and treated with Q-10 (A) or P (B) as described in the text. 1 ml of P solution was the pentane extract of 15 mg heart-muscle preparation. The activities were measured as in table 1. ○—○, succinate oxidase; ●—●, NADH oxidase.

Table 1

NADH and succinate oxidase activities of pentane-extracted heart-muscle preparation, and of pentane-extracted heart-muscle preparation with added Q-10 or P.

Substrate	Inhibitor	Oxidase activity (natoms oxygen/min per mg protein)		
		Pentane-extracted	Pentane-extracted + Q-10	Pentane-extracted + P
NADH	—	6	54	9
NADH	Rotenone (25 μ M)	6	8	8
Succinate	—	4	136	129
Succinate	Antimycin (10 μ M)	—	—	3
Succinate	TTFA* (0.25 mM)	—	18	14

Dried heart-muscle preparation was extracted with *n*-pentane and treated with Q-10 as described in text. Oxygen uptake was measured at 25° with a Clark electrode with heart-muscle preparation (4–6 mg protein/ml) suspended in 0.25 M sucrose-50 mM tris-HCl (pH 8.0), with 0.6 mM NADH or 2.5 mM succinate as substrate.

* 2-Thencyltrifluoroacetone

oxidase activity was obtained with an amount of P that, according to the assay of Kröger and Klingenberg [5], contained an amount of Q-10 corresponding maximally to 0.01 nmole/mg protein. It is clear, then, that the activity of P in restoring the succinate oxidase activity of pentane-extracted heart-muscle preparation, but not the NADH oxidase activity, is not due to any traces of Q-10 that it might contain.

The experiments described show unequivocally that succinate oxidase activity is possible in the absence of ubiquinone, provided that a substance present in the pentane extract of heart-muscle preparation is restored to the preparation. The nature of this substance (P) is under investigation. It is also possible to restore the succinate oxidase activity of pentane-extracted heart-muscle preparation, which is presumably deficient in, if not lacking, P, by addition of Q-10 or lower analogues of ubiquinone [2].

This work was carried out with support of the Life Insurance Medical Research Fund and The Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from The Netherlands Organization for the Advancement of Pure Research (Z.W.O.)

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