SUCCINATE OXIDASE ACTIVITY IN THE ABSENCE OF UBIQUINONE

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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 estimatation 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 oxidase, but that NADH oxidation is restored only by Q-10. The P-restored succinate oxidation is sensitive to antimycin and 2-thenoyltrifluoroacetone. 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

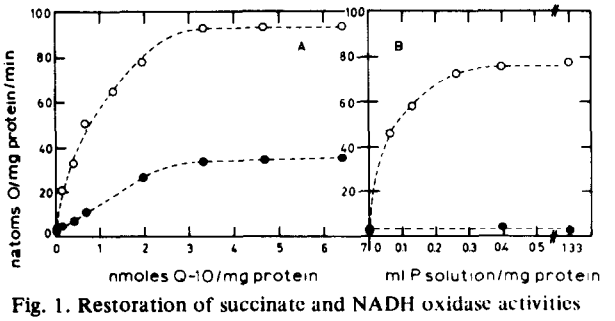

Fig. 1. Restoration of succinate and NADH oxidase activities of pentane-extracted heart-muscle preparation by Q-10 or P. Dried heart-muscle preparation was extracted with *n*-pentane and treated with Q-10 (A) or P (B) as described in the text. 1 ml of P solution was the pentane extract of 15 mg heart-muscle 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 (25μM)	6	8	8
Succinate	–	4	136	129
Succinate	Antimycin (10μM)	–	–	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].

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