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EFFECTS OF Q METABOLITES AND RELATED COMPOUNDS ON MITOCHONDRIAL SUCCINATE AND NADH OXIDASE SYSTEMS

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The effects of Q metabolites (Q acid-I, Q acid-II) and related compounds (dihydro Q acid-I, dehydro Q acid-II, QS-*n*, and their esters) on mitochondrial succinate and NADH oxidase systems were investigated. The activity restoring succinate oxidation in acetone-treated beef heart mitochondria was found to decrease with descending order of carbon number (*n*) of the side chain of the Q metabolites; activity was restored with Q acid-I (*n* = 7) to one-third as much as that with Q-7 and Q-10, but Q acid-II (*n* = 5) did not restore any activity. Of the related compounds with a carboxyalkyl group (QS-*n*), QS-16–QS-18 (*n* = 16–18) were found to be most active, and their activities were also correlated with *n*. The relationship between the restoration of activity and the partition coefficient was considered. NADH oxidation in pentane-treated beef heart submitochondrial particles could be restored with esters of low molecular weight quinones to the same extent as with Q-10, but not with the metabolites.

Introduction

Q homologs are found mainly in the mitochondria of animals, higher plants and micro-organisms except the gram-positive organisms, and function as an electron carrier [1]. Animals obtain Q homologs from their diet as well as biosynthetically. Q homologs obtained exogenously are known to be metabolized into the low molecular weight quinones, Q acid-I and Q acid-II, probably by ω -oxidation of their terminal *cis*-methyl groups followed by β -oxidation [2,3].

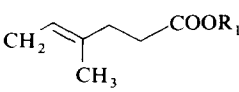
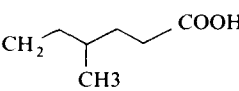
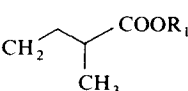
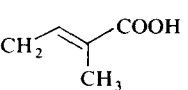
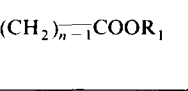
In this report, effects of Q metabolites and related compounds (Table I) on succinate and NADH oxidase systems of Q-depleted beef heart mitochondrial preparations are reported.

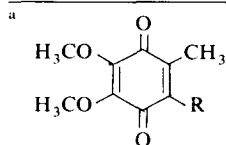
Materials and Methods

Q-10 and Q-7 were isolated from whale heart muscle [7] and cells of *Candida utilis* [8], respectively. Q-2 [9], Q metabolites and related compounds [4–6] were synthesized in our laboratories. Acetone, *n*-pentane (Wako Pure Chemical Industries, special grade), HCO-60, OP-10 (Nikko Chemicals), Tween 60, Tween 80 (Kao-Atlas Chemicals), cytochrome *c*, NADH (Sigma) and antimycin A (ICN Pharmaceuticals) were purchased. Rotenone was kindly supplied by Professor H. Fukami (Kyoto University). Beef hearts were obtained within 1 h after slaughter. Beef heart mitochondria [10] and submitochondrial particles [11] were treated with acetone [12] or pentane [13] as described previously. These preparations were stored at -20°C , and thawed or suspended in 0.25 M sucrose just before use. Oxygen consumption rates were measured with a Clark oxygen

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TABLE I
Q METABOLITES AND RELATED COMPOUNDS

Abbreviation	Structure ^a R	Reference
Q acid-I		4
Q acid-I Me		4
Q acid-I Et		5
Q acid-I Bz		5
Dihydro Q acid-I		6
Q acid-II		4
Q acid-II Et		5
Q acid-II Bz		5
Dehydro Q acid-II		5
QS-n		5
QS-n Me		
QS-n Bz		



electrode (Gilson oxygraph, Type K-IC) and the mean value of two or more measurements was determined. The succinate oxidase system (succinate:oxygen oxidoreductase) [14] and NADH oxidase system (NADH:oxygen oxidoreductase) [15] were assayed as described previously. These activities are given in terms of ngatom oxygen/min per mg protein unless otherwise noted. To compare the activities of various related compounds, a Q homolog (Q-2 or Q-10) was assayed in every three to five assays, and the activity (%) relative to the homolog was determined. Protein was determined by the Folin-Ciocalteu reagent [16]. Q homologs, Q metabolites and related compounds were added to the assay medium as an aqueous solution (1, 10, 20 mM) containing 5–20-times (w/w) as much detergent as compared to the compound, or as an ethanolic solution (1, 10, 20 mM).

Results

Because of the insolubility of Q homologs in water, they were dissolved in ethanol or water containing detergents, and the effects of these solvents on the above-mentioned activities were investigated. Ethanol did not affect either oxidase activity in lyophilized beef heart mitochondria and submitochondrial particles, but the detergents inhibited NADH oxidation in both preparations (Table II). Following the finding that succinate oxidation in acetone-treated mitochondria could be restored specifically with Q homologs [17], pentane-treated mitochondria and submitochondrial particles were studied for their restoration of succinate and NADH oxidation with Q homologs [15,16,18–20]. From our comparison of restoration activities among these preparations, it was found

TABLE II

EFFECTS OF DETERGENTS (OP-10, TWEEN 60, TWEEN 80 AND HCO-60) AND ETHANOL ON SUCCINATE AND NADH OXIDASE SYSTEMS IN BEEF HEART MITOCHONDRIA AND BEEF HEART SUBMITOCHONDRIAL PARTICLES

Rates of oxygen consumption were recorded by means of a Clark oxygen electrode at 25°C as described in Materials and Methods. The final concentration of the reagents in the flask was as follows: 167 mM sucrose, 50 mM Tris-HCl (pH 7.5), cytochrome *c* 100 µg and 0.1–0.3 mg protein of lyophilized particles. An aqueous solution of a detergent or ethanol was added to the mixture in advance of the addition of 2.5 mM potassium succinate or 0.5 mM NADH. Results are expressed as activity (%) relative to control (100%). Values in parentheses are oxygen-consumption rates (ngatom oxygen/min per mg protein).

Addition	Amount	Lyophilized beef heart mitochondria: Oxidase activities		Lyophilized beef heart submitochondrial particles: Oxidase activities	
		Succinate	NADH	Succinate	NADH
None		100 (186.3 ± 32.3)	100 (268 ± 19.4)	100 (351)	100 (381.6 ± 31.1)
Ethanol	5 µl	100	97.5	97.3 ± 2.7	96.4
OP-10	50 µg	100	9.5	100	12.5
Tween 60	50 µg	100	27		
Tween 80	50 µg	100	2.5		
HCO-60	50 µg	100	27		

that succinate oxidation in acetone-treated beef heart mitochondria was clearly restored with an aqueous solution of Q homologs containing the detergent (OP-10) and NADH oxidation in pentane-treated beef heart submitochondrial particles was restored in a dose-dependent manner with an ethanolic solution of Q-10 (Table III).

Effect on succinate oxidation in acetone-treated beef heart mitochondria

The homologs, Q-2 and Q-10 (each 15 µM, 15.4 nmol/mg protein), restored succinate oxidation in acetone-treated beef heart mitochondria to 100 and 70%, respectively, of that of the nonextracted preparation. Since their restoration activities were fairly dose dependent at concentrations below 10 µM, the comparison of Q metabolites and related compounds was carried out at 5 µM (Fig. 1). The restoration activity of one of the Q metabolites, Q acid-I, was one-third that of Q-7 and Q-10, while the activity of another metabolite (Q acid-II) was nil. Dihydro Q acid-I showed less activity than Q acid-I, and dehydro Q acid-II seemed to be a little more active than Q acid-II. These results suggested a correlation between the restoration activity and

carbon number of the alkyl side chain. To confirm this suggestion, related carboxylic acids (QS-*n*, *n* = 3–22) and their esters were investigated. Of the carboxylic acids, QS-16 and QS-18 showed the most prominent activities. Esters of quinonyl acids (QS-*n*, Q acid-I and Q acid-II) had greater activities than those of the corresponding free acids, especially when the carbon numbers of the side chains were less than 7. Differences in the ester group (methyl, ethyl and benzyl) had little effect on the activity. The restoration activities of these low molecular weight quinones were almost completely inhibited with antimycin A (1 µg/mg protein).

In this assay system, Q-10 had less restoration activity than Q-2. It is said that high molecular weight Q homologs rarely occupy the active site because of their higher lipophilicity [21]. Therefore, the relationship between the lipophilicity of these quinone compounds and their restoration activities of succinate oxidase system was investigated. The R_M value has been reported to correlate with the partition coefficient [22], and this value was calculated from the R_F value on reversed-phase thin-layer chromatography [23].

TABLE III

RESTORATION OF SUCCINATE AND NADH OXIDASE SYSTEMS IN ACETONE-TREATED BEEF HEART MITOCHONDRIA, PENTANE-TREATED BEEF HEART MITOCHONDRIA AND PENTANE-TREATED BEEF HEART SUBMITOCHONDRIAL PARTICLES WITH Q HOMOLOGS

Assay details as in Table II and legend to Fig. 1.

Mitochondrial preparation ^a	Acetone-treated beef heart mitochondria			Pentane-treated beef heart mitochondria			Pentane-treated beef heart submitochondrial particles		
Substrate	Succinate			Succinate			Succinate		
Vehicle of test compound	H ₂ O containing OP-10	EtOH		H ₂ O containing OP-10	EtOH		H ₂ O containing OP-10	EtOH	
Incubation temperature (°C)	25	25		30	30		25	30	30
Addition									
None	19 ^b	22		103	105	52	277	149	94
Q-10	(7.7) ^c (15.4)	79 101	(10) 36	(10) 130	(50) 158	(25) 123 (125) 134 (250) 211 (500) 277	(50) 532	(70) 316	(18) 233 (35) 280 (70) 396 (140) 512
Q-2	(7.7) (15.4)	140 161	(10) 91	(10) 175	(50) 229	(125) 101 (500) 101	(70) 469	(18) 94 (35) 94 (70) 141 (140) 188 (350) 188	

^a Succinate and NADH oxidase activities in nonextracted, lyophilized preparations (see Table II).

^b ngatom oxygen/min per mg protein.

^c nmol/mg protein.

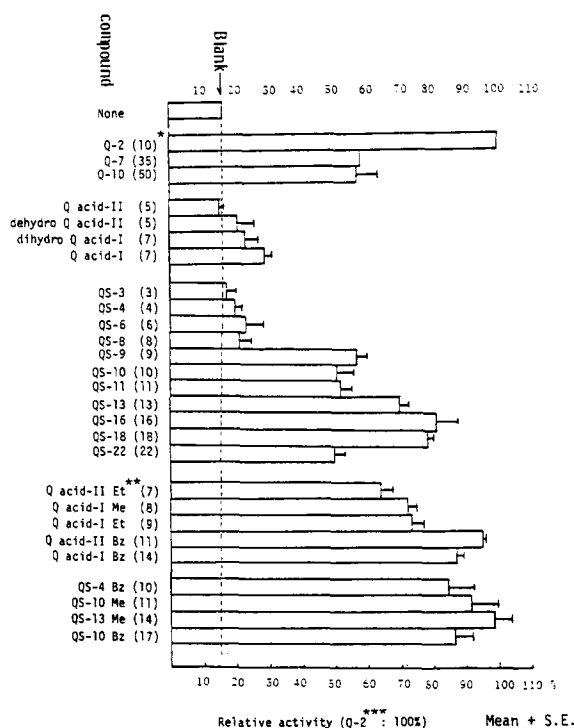


Fig. 1. Restoration of the succinate oxidase system in acetone-treated beef heart mitochondria with Q homologs and related compounds. Rates of oxygen consumption were measured by a Clark oxygen electrode as described in Materials and Methods. The reaction mixture consisted of 200 mM sucrose, 10 mM Tris-HCl (pH 7.4), 20 mM KCl, 3 mM $MgCl_2 \cdot 6H_2O$, 50 μM Na_2EDTA , 100 μg cytochrome *c*, 2.5 mM potassium succinate, 1.0 mg protein of acetone-treated beef heart mitochondria and a solution of a test compound (each at 5 μM) containing OP-10, or a solution of OP-10 (50 μg) as a control. Final volume, 2 ml; temperature, 25–27°C. * Carbon number of side chain. ** Et, Me and Bz, ethyl, methyl and benzyl ester, respectively (see Table I). *** 110.38 ± 5.20 ngatoms oxygen/min per mg protein.

A quadratic relationship exhibiting a maximum at $R_M = 0.5$ was observed between the R_M values and the restoration activities (Fig. 2), suggesting an intimate correlation between the restoration activity and the partition coefficient.

Effect on NADH oxidation in pentane-treated beef heart submitochondrial particles and pentane-treated beef heart mitochondria

Q-10 (25 μM , 350 nmol/mg protein) in an ethanolic solution restored NADH oxidation in pentane-treated beef heart submitochondrial par-

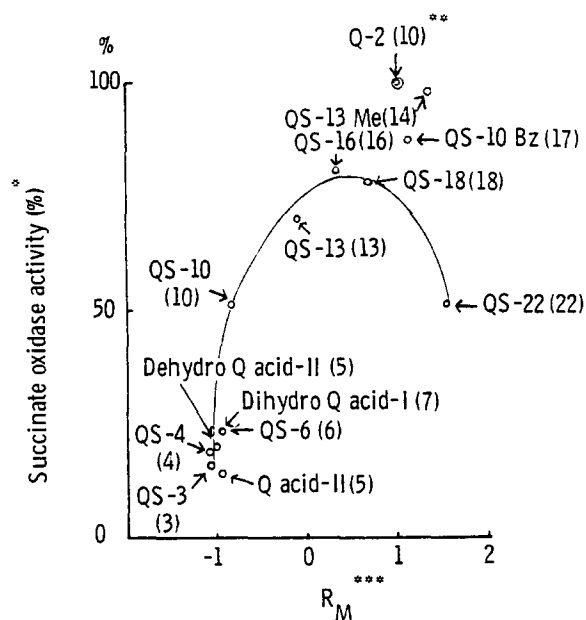


Fig. 2. The relationship between succinate oxidase activities of Q metabolites and related compounds and their R_M values. * Activity relative to Q-2 (Q-2: 100%). ** Carbon number of side chain. *** $R_M = \log\left(\frac{1}{R_F} - 1\right)$. R_F values were determined on paraffin-treated thin-layer chromatography developing with acetone-water (1:1, v/v).

ticles to the same level as that of lyophilized beef heart submitochondrial particles, but Q-2 attained only a 30% restoration. A rather large amount (35–150 nmol/mg protein) of Q-10 restored the NADH oxidase system in a dose-dependent manner without adding phospholipid. The restoration activities were determined mainly at 350 nmol/mg protein with regard to the metabolites and related compounds (Fig. 3), since their maximal activities were observed at this concentration. The ester derivatives of Q metabolites and related compounds showed restoration activity. QS-10 benzyl ester (QS-10 Bz) was about twice as active as Q-10 at low concentrations. These restoration activities were inhibited by rotenone and antimycin A (1–2 μg /mg protein) as observed on the same system of intact mitochondria.

Further, the restoration activities of Q homologs and a related compound were investigated by using pentane-treated beef heart mitochondria (Table IV). Q-2 barely restored its NADH oxidase activity also in our work and its high concentra-

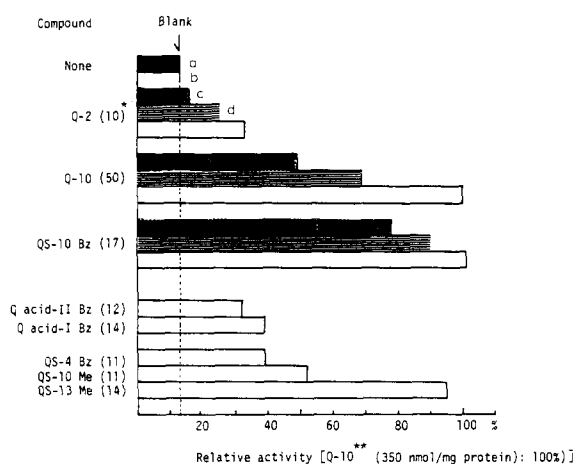


Fig. 3. Restoration of NADH oxidase system in pentane-treated beef heart submitochondrial particles with Q homologs and related compounds at 30°C. Assay details as in Table II. * Carbon number of side chain. ** 574.75 ± 36.60 ngatom oxygen/min per mg protein. Shading: ^a 0, ^b 35, ^c 70, ^d 350 nmol/mg protein.

tion inhibited the oxidase activity in beef heart mitochondria and submitochondrial particles as already reported [24], but QS-10 Bz showed rather high levels of the restoration activity and hardly inhibited its activity at a high concentration of 350 nmol/mg protein in both unextracted preparations.

TABLE IV
RESTORATION OF NADH OXIDASE SYSTEM WITH QS-10 BENZYL ESTER (QS-10 Bz) IN PENTANE-TREATED BEEF HEART MITOCHONDRIA

Assay details as in Table II. Results are expressed as ngatom oxygen/min per mg protein.

Addition	$\mu\text{mol/mg}$ protein	NADH oxidase activity ^a	
		Expt. 1 ^b	Expt. 2
None		34	70
QS-10 Bz	0.5	—	246
	1.0	168	—

^a NADH oxidase activities of lyophilized beef heart mitochondria were 285 (Expt. 1) and 334 (Expt. 2) ngatom oxygen/min per mg protein, respectively.

^b The restoration activities with Q-10 (1 μmol) and Q-2 (1 μmol) were 252 and 101, respectively.

Discussion

The restoration activity of succinate oxidation in acetone-treated beef heart mitochondria was more potent with Q-2 than with Q-10 as already described. This was considered to depend on decreased accessibility to the active site of the longer side chain quinones in comparison with the lower homologs [21]. Therefore, R_M values of the test compounds were measured and it was found that the most active compounds, QS-16, QS-18 and ester derivatives, showed similar R_M values to that of Q-2 (Fig. 2). This result suggested that the magnitude of restoration activity in succinate oxidation depended on a proper balance of lipophilicity and hydrophilicity in addition to the essential quinone structure.

On the other hand, the restoration of NADH oxidation with Q-2 was only 30% of that of Q-10 both in pentane-treated beef heart mitochondria (Table IV) and submitochondrial particles (Fig. 3), as already described [19,20]. The restoration activities of quinonyl acids (metabolites and QS-*n*) were very low also. However, some of ester derivatives (QS-10 Bz and QS-13 Me) have comparable activities to Q-10, and QS-10 Bz was more active than Q-10 at low concentrations (Fig. 3). QS-10 Bz hardly inhibited the NADH oxidation in unextracted beef heart mitochondria and submitochondrial particles, thus differing from low homologs of Q.

Q is an indispensable component of the electron-transfer chain [15,17], and functions as a mobile carrier between fixed lipoprotein Complex I or II to III [1]. Our studies show that QS-10 Bz can restore both succinate and NADH oxidation in Q-depleted preparations to the level of the original unextracted preparation. From this result, it is concluded that the positioning of exogenous Q and related compounds with respect to the mitochondrial electron-transfer chain depends on their respective physicochemical properties. In this sense, QS-10 Bz and related ester derivatives may contribute to investigation of the role of exogenous Q in mitochondria.

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