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Effect of substituents of the benzoquinone ring on electron-transfer activities of ubiquinone derivatives

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The effect of substituents on the 1,4-benzoquinone ring of ubiquinone on its electron-transfer activity in the bovine heart mitochondrial succinate-cytochrome *c* reductase region is studied by using synthetic ubiquinone derivatives that have a decyl (or geranyl) side-chain at the 6-position and various arrangements of methyl, methoxy and hydrogen in the 2, 3 and 5 positions of the benzoquinone ring. The reduction of quinone derivatives by succinate is measured with succinate-ubiquinone reductase and with succinate-cytochrome *c* reductase. Oxidation of quinol derivatives is measured with ubiquinol-cytochrome *c* reductase. The electron-transfer efficacy of quinone derivatives is compared to that of 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone. When quinone derivatives are used as the electron acceptor for succinate-ubiquinone reductase, the methyl group at the 5-position is less important than are the methoxy groups at the 2- and 3-positions. Replacing the 5-methyl group with hydrogen causes a slight increase in activity. However, replacing one or both of 2- and 3-methoxy groups with a methyl completely abolishes electron-acceptor activity. Replacing the 3-methoxy group with hydrogen results in a complete loss of electron-acceptor activity, while replacing the 2-methoxy with hydrogen results in an activity decrease by 70%, suggesting that the methoxy group at the 3-position is more specific than that at the 2-position. The structural requirements for quinol derivatives to be oxidized by ubiquinol-cytochrome *c* reductase are less strict. All 1,4-benzoquinol derivatives examined show partial activity when used as electron donors for ubiquinol-cytochrome *c* reductase. Derivatives that possess one unsubstituted position at 2, 3 or 5, with a decyl group at the 6-position, show substrate inhibition at high concentrations. Such substrate inhibition is not observed when fully substituted derivatives are used. The structural requirements for quinone derivatives to be reduced by succinate-cytochrome *c* reductase are less specific than those for succinate-ubiquinone reductase. Replacing one or both of the 2- and 3-methoxy groups with a methyl and keeping the 5-position unsubstituted (plastoquinone derivatives) yields derivatives with no acceptor activity for succinate-Q reductase. However, these derivatives are reducible by succinate in the presence of succinate-cytochrome *c* reductase. This reduction is antimycin-sensitive and requires endogenous ubiquinone, suggesting that these (plastoquinone) derivatives can only accept electrons from the ubisemiquinone radical at the Q_i site of ubiquinol-cytochrome *c* reductase, and cannot accept electrons from the QPs of succinate-ubiquinone reductase.

Introduction

The essential role of ubiquinone (Q) in mitochondrial [1–3] electron transfer is well established. The direct involvement of Q in proton translocation has been suggested [4,5]. However, the interaction between Q and protein and the reaction mechanism of Q-mediated electron transfer are not yet fully understood. There are two schools of thought regarding the reaction mechanism of Q: one places Q as a free mobile electron carrier shuttling electrons between electron-transfer complexes [6–10]; the other regards the Q-protein complex as the active species, even though the majority of Q in the inner mitochondrial membrane diffuses freely and equilibrates with protein-bound Q [11–13]. Both schools acknowledge the presence of specific Q-binding sites in the electron-transfer complexes.

Abbreviations: Q, quinone; QH_2, quinol; Q_2, 2,3-dimethoxy-5-methyl-6-geranyl-1,4-benzoquinone; PQ_2, 2,3-dimethyl-6-geranyl-1,4-benzoquinone; DMG, decanoyl-*N*-methylglucamide; DCIP, 2,6-dichlorophenol-indophenol.

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The existence of the Q-protein complex (Q-binding protein) in mitochondrial electron-transfer complexes is supported by the detection of the ubisemiquinone radical in NADH-Q [11,14], succinate-cytochrome *c* [12], and ubiquinol-cytochrome *c* reductases [15], and by the isolation of QPs * [16–19] from succinate-Q reductase. Recently, Q-binding proteins in cytochrome *b*-c_1 complexes from various sources were identified using arylazido- and azido-Q derivatives [20–22].

Study of the structural requirements for Q-mediated electron transfer is essential for the elucidation of the Q-binding site and its reaction mechanism. Both the benzoquinone ring and the alkyl side-chain of the Q molecule are reported to be essential for binding and electron transfer [1]. The effect of the variation at the alkyl side-chain of Q on the electron-transfer activity in mitochondrial succinate-cytochrome *c* reductase has been studied [23]. When Q is used as an electron acceptor for succinate-Q reductase, an alkyl side-chain of six or more carbons gives maximum activity. However, when quinol (QH_2) serves as an electron donor for ubiquinol-cytochrome *c* reductase, an alkyl side-chain of 10 carbons gives maximum activity. In addition to the chain length, a flexible portion of the alkyl side-chain immediately adjacent to the benzoquinone ring is needed in order for Q to exert maximum electron-transfer activity. Although the benzoquinone ring of the Q molecule was thought to be structurally more important than the alkyl side-chain for the electron transfer activity of Q in succinate-cytochrome *c* reductase [1], systematic studies on the effect of benzoquinone ring substituents on the electron-transfer activity of Q have not been reported.

In addition to the Q-reduction site (QPs) in succinate-Q reductase, according to the 'Q-cycle' mechanism, oxidation of ubiquinol in the cytochrome *b*-c_1 region involves two specific Q-binding sites: one site (Q_o) where quinol donates its first electron to Rieske's iron-sulfur protein and its second electron to cytochrome *b*-565; and another site (Q_i) where quinone receives an electron from cytochrome *b*-562 [4,24–27]. The fact that the Q_i site is capable of stabilizing the antimycin-sensitive ubisemiquinone radical [12,15], and that the Q_o site can form only a transient, antimycin-insensitive ubisemiquinone radical [28,29], suggests that the structural requirements for Q in these two binding sites may be different.

Succinate-Q and succinate-cytochrome *c* reductases can catalyze TTFA-sensitive electron transfer from succinate to 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone (Q_0C_{10}), but only succinate-cytochrome *c* reductase can catalyze antimycin-sensitive electron transfer from succinate to 2,3-dimethyl-6-decyl-1,4-benzoquinone (PQ_0C_{10}) [30,31]. This supports the existence of three different Q-binding sites in the succinate-cytochrome *c* reductase region: QPs of succinate-Q reductase and Q_o and Q_i sites of ubiquinol cytochrome *c* reductase. Q_0C_{10} can bind to the QPs of succinate-Q reductase and $Q_0C_{10}H_2$ to the Q_o and Q_i sites of ubiquinol-cytochrome *c* reductase. $PQ_0C_{10}H_2$, on the other hand, can only serve as an electron donor for the Q_o site of ubiquinol-cytochrome *c* reductase [31,32]. PQ_0C_{10} does not bind to QPs and thus cannot accept electrons from succinate-Q reductase. However, it can receive electrons from the Q_i site of ubiquinol-cytochrome *c* reductase.

In continuing our efforts to elucidate the reaction mechanism of the quinone-mediated electron transfer, we have synthesized a series of Q derivatives having various substituents (methyl, methoxy and hydrogen) at the 2, 3, 5 positions of the 1,4-benzoquinone ring but with the same alkyl side-chain at the 6-position, and have compared their electron transfer activity. The necessity of endogenous Q_{10} for the reduction of PQ derivatives by mitochondrial succinate-cytochrome *c* reductase is also demonstrated.

Experimental procedures

Materials

Creosol (2-methoxy-4-methylphenol) was from Eastman Chemical Co.; fuming nitric acid from Mallinckrodt. High-concentration hydrogen peroxide (60%) was a gift from the FMC Corp. Dimethylsulfate, geraniol and nerol were from Aldrich. Florisil, sodium cholate, cytochrome *c*, type III, 2,6-dichlorophenol-indophenol (DCIP) and deoxycholic acid were from Sigma. Preparative thin-layer plates were from Supelco; asolectin from Associate Concentrate. 2,3-Dimethoxy-5-methyl-6-geranyl-1,4-benzoquinone (Q_2) and 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone (Q_0C_{10}) were synthesized according to reported procedures [33]. *Cis*-Q_2 was synthesized by the method similar to that for Q_2 [33], except that nerol was used instead of geraniol. The reduced form of quinol derivatives was prepared by bubbling hydrogen gas into the quinone derivatives which was in 95% ethanol containing 1 mM HCl and few grains of palladium on active carbon (Pd-C) until the solution becomes colorless. The reaction takes only few seconds. Other chemicals were the highest purity available commercially.

Synthesis of Q derivatives

Q derivatives with a geranyl substituent at the 6-position were synthesized by acid-catalyzed alkylation of 1,4-benzoquinone. Q derivatives with a decyl sub-

* QPs refers to the membrane anchoring protein of mitochondrial succinate-ubiquinone reductase which converts succinate dehydrogenase to succinate-ubiquinone reductase. This protein fraction is also known as cytochrome *b*-560 [17], $CII_{3,4}$ [16] and PF [19].

stituent at the 6-position were synthesized through the radical coupling reaction between 1,4-benzoquinone and undecanoyl peroxide under conditions described previously [34]. The Q derivatives were purified by thin-layer chromatography and HPLC, and identified by the spectral characteristics in NMR, ultraviolet and mass spec-

TABLE I

Structure and spectral properties of quinone derivatives

Derivative	Substituent				Starting compound	Yield%	Spectral properties		
	R_2	R_3	R_5	R_6			^{1}H-NMR ($CDCl_3$), δ,	UV, λ_{max}	Mass (*m/e*)
Q_2	MeO	MeO	Me	geranyl (G)	2,3-dimethoxy-5-methyl-1,4-benzoquinone	14	5.00 (t, 2), 4.00 (s, 6) 3.20 (d, 2), 2.03 (m, 7) 1.66 (t, 9)	oxid. 276 red. 289	318.1824
2-H-Q_2	H	MeO	Me	G	3-methyl-2-nitroanisole	8	5.86 (s, 1), 4.95 (t, 2), 3.79 (s, 3), 3.22 (d, 2), 2.04 (m, 7), 1.66 (t, 9),	oxid. 273 red. 293	288.1764
3-H-Q_2	MeO	H	Me	G	4-methyl-3-nitroanisole	18	5.87 (s, 1), 4.96 (t, 2), 3.78 (s, 3), 3.20 (d, 2), 2.02 (m, 7), 1.66 (t, 9)	oxid. 272 red. 293	288.1742
3-H-5-MeO-PQ_2	Me	H	MeO	G	4-methyl-3-nitroanisole	15	6.42 (s, 1), 5.06 (t, 2), 4.01 (s, 3), 3.18 (d, 2), 2.02 (m, 7), 1.66 (t, 9)	oxid. 263 red. 288	288.1695
5-H-Q_2	MeO	MeO	H	G	2,3-dimethoxyphenol	6	6.34 (s, 1), 5.14 (t, 2), 4.02 (s, 3), 4.00 (s, 3) 3.12 (d, 2), 2.08 (m, 4), 1.66 (t, 9)	oxid. 267 red. 290	304.1609
PQ_2	Me	Me	H	G	2,3-dimethylaniline	11	6.50 (s, 1), 5.14 (t, 2), 3.14 (d, 2), 2.10 (m, 10), 1.66 (t, 9)	oxid. 261, 256 red. 289	272.1777
Q_0C_{10}	MeO	MeO	Me	decyl (D)	2,3-dimethoxy-5-methyl-1,4-benzoquinone	30	3.98 (s, 6), 2.35 (t, 2), 2.01 (s, 3), 1.26 (m, 16), 0.88 (t, 3)	oxid. 278 red. 286	322.2146
5-H-Q_0C_{10}	MeO	MeO	H	D	2,3-dimethoxyphenol	16	6.35 (s, 1), 4.00 (s, 3), 3.98 (s, 3), 2.39 (t, 2), 1.26 (m, 16), 0.88 (t, 3)	oxid. 268 red. 292	308.1980
2-H-Q_0C_{10}	H	MeO	Me	D	3-methyl-2-nitroanisole	6	5.86 (s, 1), 3.81 (s, 3), 2.45 (t, 2), 2.04 (s, 3), 1.26 (m, 16), 0.88 (t, 3)	oxid. 273 red. 292	292.2030
3-H-Q_0C_{10}	MeO	H	Me	D	4-methyl-3-nitroanisole	10	5.86 (s, 1), 3.79 (s, 3), 2.44 (t, 2), 2.04 (s, 3), 1.26 (m, 16), 0.88 (t, 3)	oxid. 273 red. 292	292.2019
3-H-5-Me-PQ_0C_{10}	Me	H	Me	D	2,5-dimethyl-1,4-benzoquinone	15	6.58 (s, 1), 2.48 (t, 2), 2.04 (d, 6), 1.26 (m, 16), 0.88 (t, 3)	oxid. 262, 256 red. 288	276.2045
2-Me-Q_0C_{10}	Me	MeO	Me	D	2,5-dimethyl-1,4-benzoquinone	0.5	3.98 (s, 3), 2.46 (t, 2), 2.06 (d, 6), 1.26 (m, 16), 0.88 (t, 3)	oxid. 272 red. 286	
2-H-5-Me-Pq_0C_{10}	H	Me	Me	D	2,6-dimethyl-1,4-benzoquinone	12	6.57 (s, 1), 2.46 (t, 2), 2.03 (d, 6), 1.26 (m, 16) 0.88 (t, 3)	oxid. 262, 256 red. 288	276.2156
3-Me-Q_0C_{10}	MeO	Me	Me	D	2,6-dimethyl-1,4-benzoquinone	0.5	3.97 (s, 3), 2.44 (t, 2), 2.06 (d, 6), 1.26 (m, 16), 0.88 (t, 3)	oxid. 272 red. 286	
5-Me-PQ_0C_{10}	Me	Me	Me	D	2,3,6-trimethylphenol	7	2.46 (t, 2), 2.02 (t, 9), 1.26 (m, 16), 0.88 (t, 3)	oxid. 267, 260 red. 288	290.2229

TABLE I (continued)

Derivative	Substituent				Starting compound	Yield%	Spectral properties		
	R_2	R_3	R_5	R_6			^{1}H-NMR ($CDCl_3$), δ,	UV, λ_{max}	Mass (m/e)
2-Me-5-He-Q_0C_{10}	Me	MeO	H	D	3-amino-*o*-cresol	1	6.47 (s, 1), 3.98 (s, 3), 2.41 (t, 2), 1.94 (s, 3), 1.26 (m, 16), 0.88 (t, 3)	oxid. 267 red. 288	292.2045
3-Me-5-H-Q_0C_{10}	MeO	Me	H	D	3-amino-*o*-cresol	5	6.37 (s, 1), 4.01 (s, 3), 2.42 (t, 2), 1.95 (s, 3) 1.26 (m, 16), 0.88 (t, 3)	oxid. 267 red. 288	292.2026
PQ_0C_{10}	Me	Me	H	D	2,3-dimethylaniline	7	6.54 (s, 1), 2.42 (t, 2), 2.01 (d, 6), 1.26 (m, 16), 0.88 (t, 3)	oxid. 262, 257 red. 288	276.2105
5-MeO-PQ_0C_{10}	Me	Me	MeO	D	2,3-dimethylaniline	2	3.96 (s, 3), 2.42 (t, 2), 0-.22 (s, 6), 1.26 (m, 16), 0.88 (t, 3)	oxid. 272 red. 286	306.2188
2,5-Di-H-Q_0C_{10}	H	MeO	H	D	2-methoxy-1,4-phenyl-enediamine	1	6.50 (s, 1), 5.86 (s, 1), 3.81 (s, 3), 2.25 (t, 2), 1.26 (m, 16), 0.88 (t, 2)	oxid. 267 red. 288	278.1884
3,5-Di-H-Q_0C_{10}	MeO	H	H	D	2-methoxy-1,4-phenyl-enediamine	2	6.50 (d, 1), 5.83 (d, 1), 3.81 (s, 3), 2.25 (t, 2), 1.26 (m, 16), 0.88 (t, 3)	oxid. 267 red. 288	278.1880

trometry. Table I summarizes the chemical structures, starting compounds and spectral properties of Q-derivatives.

A typical acid-catalyzed synthesis procedure for 6-geranyl Q-derivatives is exemplified by the synthesis of 3-methoxy-5-methyl-6-geranyl-1,4-benzoquinone (2-H-Q_2):

6 g of 3-methyl-2-nitroanisole were mixed with 1 g of 10% Pd-C in 95 ml of methanol. After addition of 5 ml of MeOH-HCl (2.47 M) the reaction was carried out for 40 min at 2 atm hydrogen pressure in a Parr shaker. The MeOH solvent was evaporated in vacuo and the deep greenish oil was dissolved in 600 ml cold 0.5 M sulfuric acid. Then 250 ml of a cold solution containing 27 g sodium dichromate and 20 ml concentrated sulfuric acid was added slowly with stirring, the temperature being maintained at about 5 °C. After stirring for 4 h at room temperature the mixture was extracted with 200 ml $CHCl_3$ four times. The combined deep brown extract was stirred with increasing amounts of florisil until the color of the solution became yellowish; florisil was removed by filtration. Upon removal of $CHCl_3$, 0.5 g of yellow crystals of 2-methoxy-6-methyl-1,4-benzoquinone were obtained. ^{1}H-NMR ($CDCl_3$): 6.55 (m,1), 5.88 (d,1), 3.82 (s,1), 2.09 (d,3). $UV_{max}^{95\%EtOH}$: oxi., 261 nm; red., 290 nm.

60 mg of 2-methoxy-6-methyl-1,4-benzoquinone were dissolved in 20 ml of diethyl ether and shaken with 0.5 g sodium dithionite in 4 ml water until the solution became colorless. The mixture was extracted with diethyl ether. The reduced benzoquinone, obtained as colorless crystals upon removal of solvent, was dissolved in 4 ml of dioxane, mixed with 80 μl of geraniol, and treated with 100 μl of $BF_3 \cdot O \cdot (C_2H_5)_2$ over 30 min with stirring. This mixture was stirred for 3 h at room temperature, treated with 12 ml of water, and extracted with diethyl ether. The ether extract was dried over Na_2SO_4 and oxidized with 0.1 g Ag_2O for 1 h with stirring. The solution was filtered and the filtrate was evaporated to yield a deep brown oil residue which was purified by silica-gel G plates developed with hexane/diethyl ether (3.5 : 1). 3-Methoxy-5-methyl-6-geranyl-1,4-benzoquinone has an R_F value of 0.4. A by-product, 3-methyl-5-methoxy-6-geranyl-1,4-benzoquinone, with an R_F value of 0.81, was also detected in the thin-layer plate. 3-Methoxy-5-methyl-6-geranyl-1,4-benzoquinone was eluted with ether and upon removal of solvent, 9 mg of orange oil were obtained. ^{1}H-NMR ($CDCl_3$): 5.86 (s, 1); 4.95 (t, 2); 3.79 (s, 3); 3.22 (d, 2); 2.045 (m, 7); 1.65 (t, 9). UV_{max}^{EtOH}: oxi, 2.73 nm; red., 293 nm. High-resolution mass spectra, m/e, 288.1764.

By using the same procedure, 2-methoxy-5-methyl- and 2-methyl-5-methoxy-6-geranyl-1,4-benzoquinones were synthesized from 4-methyl-3-nitroanisole. 2-Methyl-5-methoxy-6-geranyl-1,4-benzoquinone has a higher R_F value (0.67) than that of the 2-methoxy-5-methyl-derivative ($R_F = 0.22$) in the thin-layer plate developed with hexane/ether (3.5 : 1).

For preparation of 3-methoxy-5-methyl-6-(3,7-dimethyloctyl)-1,4-benzoquinone, 5 mg of 3-methoxy-5-methyl-6-geranyl-1,4-benzoquinone was dissolved in 3 ml of absolute ethanol, mixed with 2 mg of 10% Pd-C,

and hydrogenated at 1 atm of hydrogen for 30 min. The solution was filtered and the filtrate was oxidized with Ag_2O. The yield was over 90%. ^{1}H-NMR ($CDCl_3$): 5.87 (s, 1), 3.8 (s, 3), 2.41 (t, 2), 2.01 (s, 3), 1.22 (m, 10), 0.9 (t, 9). UV_{max}^{EtOH}: oxi., 274 nm; red., 292 nm. The same procedure was also used to convert other 6-geranyl-1,4-benzoquinone derivatives into 6-(3,7-dimethyloctyl)-1,4-benzoquinones.

A typical radical coupling synthesis procedure for 6-decyl-Q derivatives is exemplified by the synthesis of 2,3,5-trimethyl-6-decyl-1,4-benzoquinone (5-Me-PQ_0C_{10}): 1.25 g of 2,3,6-trimethylphenol was dissolved in 2 ml of 15 M sulfuric acid and cooled to 5 °C. Sodium dichromate (3.1 g) in 2 ml H_2O was added slowly over 30 min with stirring. This mixture was heated, maintained at 40 °C for 20 min, and repeatedly extracted with diethyl ether after cooling to room temperature. Upon removal of ether, crude 2,3,5-trimethyl-1,4-benzoquinone appeared as a brown oil which was purified with silica-gel G plates developed with hexane diethyl ether (3.5 : 1). Pure 2,3,5-trimethyl-1,4-benzoquinone was eluted from the plate with diethyl ether; removal of solvent yielded 50 mg of yellow crystals. ^{1}H-NMR ($CDCl_3$) 6.56 (s, 1), 2.04 (t, 9). UV_{max}^{EtOH}: oxi., 262, 253 nm; red., 228 nm.

50 mg of 2,3,5-trimethyl-1,4-benzoquinone and 0.2 g of undecanoyl peroxide prepared by reacting undecanoyl chloride with concentrated H_2O_2, were dissolved in 3 ml of benzene. The mixture was incubated at 85 °C for 4 h, cooled to room temperature, and purified on silica-gel G plates developed with hexane/diethyl ether (95 : 5). Pure 2,3,5-trimethyl-6-decyl-1,4-benzoquinone was eluted with diethyl ether, yield 8 mg of yellow crystal. ^{1}H-NMR ($CDCl_3$): 2.46 (t, 2), 2.02 (t, 9), 1.26 (m, 16), 0.88 (t, 3). UV_{max}^{EtOH}: oxi., 267, 260 nm; red., 288 nm. High-resolution mass spectra, *m/e*, 290.2229.

By using the same procedure, other 6-decyl-1,4-benzoquinones, those listed in Table I, were synthesized from commercially available compounds. 2-Methoxy-5-methyl- and 2-methyl-5-methoxy-6-decyl-1,4-benzoquinones were synthesized from 4-methyl-3-nitroanisole and separated by thin-layer plate in the final step. The R_F values of these two compounds are similar to those given for their corresponding 6-geranyl derivatives. 2-Methyl-3-methoxy- and 2-methoxy-3-methyl-6-decyl-1,4-benzoquinones were synthesized from 3-amino-*o*-creosol. Separation of these two isomers was achieved by HPLC using a C-18 column (4.6 × 25 cm) with 85% methanol as the mobile phase. The retention times were 15.1 and 16.5 min for 2-methyl-3-methoxy and 2-methoxy-3-methyl-6-decyl-1,4-benzoquinones, respectively, at a flow rate of 0.8 ml/min [39].

Since the absorption spectra of these synthesized Q derivatives are very similar to that of Q_0C_{10} or Q_2, a millimolar extinction coefficient of 12.25 for the ultraviolet absorption peak of the oxidized-minus-reduced form was used in the calculation of the concentrations of all the synthesized Q derivatives in 95% ethanol.

Enzyme preparations

Succinate-cytochrome *c* reductase [35] and its Q- and phospholipid (PL)-depleted preparation [36], succinate-Q reductase [35], and ubiquinol-cytochrome *c* reductase [37] were prepared and assayed as previously reported. Replenishment of Q_2 and PL to the Q- and PL-depleted enzyme was accomplished by addition of an ethanolic solution of Q_2, or its derivatives, followed by the addition of micelle asolectin [36]. When Q_{10} was used in reactivation of the Q- and PL-depleted reductases, it was added as a Q_{10}-PL mixture, prepared by mixing Q_{10} and asolectin in an alcoholic solution which was dried and sonified with 0.5% decanoyl-*N*-methylglucamide (DMG) and 0.1% sodium cholate. Protein was estimated by the biuret method in the presence of hydrogen peroxide [38] using crystalline bovine serum albumin as a standard.

Absorption spectra measurements and spectrophotometric assays of enzymic activity were done in a Cary spectrophotometer, Model 219, at room temperature. NMR spectra were measured in a Varian XL-300 NMR spectrometer. Mass spectra were measured with a high resolution CEC 21-110B spectrometer with a Nora data acquisition system.

The redox potentials of quinone derivatives were determined from the ratio of reduced and oxidized forms of Q_0C_{10} (or PQ_2) and a given derivative in the system containing the given derivative and $Q_0C_{10}H_2$ (or PQ_2H_2) after a short exposure to pH 12 and then neutralized to neutral pH 'alkaline shock' under anaerobic conditions [20]. The buffer system used was 50% EtOH in 50 mM phosphate buffer (pH 7.0). Midpoint redox potentials of 100 and 85 mV were used for Q_0C_{10} and PQ_2, respectively, in the calculation. Whether $Q_0C_{10}H_2$ or PQ_2H_2 was used depended upon the spectral characteristics of the Q-derivatives. The relative hydrophobicity of quinone derivatives were determined by partition chromatography by HPLC using a C_{18} column. 80% methanol was used as mobile phase. The retention time of each derivatives was used to indicate its hydrophobicity. A direct correlation between the retention time and partition coefficient has been observed [39].

Results and Discussion

Effect of configuration of the alkyl side-chain on the electron-transfer activity of ubiquinone derivatives

We have reported a systematic comparison of Q derivatives, possessing alkyl side-chains of 1–10 carbons and of different flexibilities, as electron acceptors, donors and mediators for individual complexes [23]. When used as electron acceptors for succinate-Q re-

ductase, six or more carbons in the side-chain gave maximal activity. However, when used as electron donors for ubiquinol-cytochrome *c* reductase, ten or more carbons in the side-chain gave full activity. The incorporation of two or more conjugated double bonds to the alkyl side-chain decreased, relative to the saturated side-chain, electron acceptor activity more than electron donor activity. The effect of the configuration of an isolated double bond in the alkyl side-chain on electron-transfer activity has not been studied.

The *cis*-(2,3-dimethoxy-5-methyl-6-neonyl-1,4-benzoquinone) and *trans*-(2,3-dimethoxy-5-methyl-6-geranyl-1,4-benzoquinone) isomers of Q_2 are equally effective when used as electron acceptors for succinate-Q reductase. However, *cis*-Q_2H_2 (2,3-dimethoxy-5-methyl-6-neonyl-1,4-benzoquinol) is only 70% as efficient as an electron donor for ubiquinol-cytochrome *c* reductase as the *trans* isomer (2,3-dimethoxy-4-methyl-6-geranyl-1,4-benzoquinol). This suggests that configurational variation in the alkyl side-chain affects donor activity more than acceptor activity. These results, together with our previously reported observations [23], lead us to conclude that the structural requirements of the alkyl side-chain for Q to serve as an electron acceptor are less strict than those needed for QH_2 to function as an electron donor.

Effect of the ring substituents on electron-acceptor activity of ubiquinone derivatives for succinate-Q reductase

Although it has been grossly demonstrated [40] that ring substituents are essential for the electron-transfer activity of Q, a systematic study of these effect has not been reported. For the simplicity of comparison we have synthesized a series of Q derivatives that have a decyl group at the 6-position and various arrangements of methyl, methoxy and hydrogen groups at the 2-, 3- and 5-positions. Maximum electron-transfer activity of these Q derivatives, compared to those of 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone (Q_0C_{10}), as electron acceptors for succinate-Q reductase is summarized in the left column of Table II.

Replacing one or both of the methoxy groups at the 2- and 3-positions of Q_0C_{10} with the methyl group completely abolishes electron-acceptor activity, indicating that these two methoxy groups are essential for acceptor activity. The 3-methoxy is more specific than the 2-methoxy, because replacing the 3-methoxy group with hydrogen (3-H-Q_0C_{10}) results in a complete loss of acceptor activity, but replacing the 2-methoxy group with hydrogen (2-H-Q_0C_{10}) causes only a 70% reduction of activity. However, the presence of a 3-methoxy group is not absolutely necessary for activity, because when replaced with a methyl group, partial activity (30%) is obtained if the 2-position is unsubstituted. But, if the 3-position is unsubstituted, replacing the 2-methoxy group with a methyl group (3-H-5-Me-PQ_0C_{10}) abrogates all activity. These results suggest that a complementary effect between substituents at the 2- and 3-positions of Q_0C_{10} exists.

In contrast to complete or partial loss of acceptor activity by replacing the 2- or 3-methoxy group with hydrogen, replacing the 5-methyl group of Q_0C_{10} with hydrogen (5-H-Q_0C_{10}) causes a slight increase in acceptor activity, indicating that this methyl is structurally

TABLE II

Comparison of electron-transfer activity of Q derivatives

SQR, succinate-Q reductase; QCR, ubiquinol-cytochrome *c* reductase; SCR, succinate-cytochrome *c* reductase. The activities given are the maximum activities of each compound obtained through the concentration titration. The maximum activity of Q_0C_{10} was used as 100% in calculation.

Q derivatives	R_2	R_3	R_5	Activities (%)		
				as acceptor for SQR	as donor for QCR	as acceptor for SCR
Q_0C_{10}	MeO	MeO	Me	100	100	100
2-Me-Q_0C_{10}	Me	MeO	Me	0	15	25
3-Me-Q_0C_{10}	MeO	Me	Me	0	14	33
5-Me-PQ_0C_{10}	Me	Me	Me	0	20	3
2-H-Q_0C_{10}	H	MeO	Me	29	54	53
3-H-Q_0C_{10}	MeO	H	Me	0	27	30
2-H-5-Me-PQ_0C_{10}	H	Me	Me	32	22	60
3-H-5-Me-PQ_0C_{10}	Me	H	Me	0	20	36
5-H-Q_0C_{10}	MeO	MeO	H	117	91	94
2-Me-5-H-Q_0C_{10}	Me	MeO	H	18	25	48
3-Me-5-H-Q_0C_{10}	MeO	Me	H	9	23	40
PQ_0C_{10}	Me	Me	H	0	27	24
3,5-Di-H-Q_0C_{10}	MeO	H	H	0	6	30
2,5-Di-H-Q_0C_{10}	H	MeO	H	30	11	48
5-MeO-PQ_0C_{10}	Me	Me	MeO	0	20	4

less important than the two methoxy groups when Q derivatives serve as electron acceptors for succinate-Q reductase. If the 5-position is unsubstituted, replacing the methoxy groups at the 2-position, the 3-position, or both, with methyls results in a drastic reduction of the activity. 2-Methyl-3-methoxy-6-decyl-1,4-benzoquinone (2-Me-5-H-Q_0C_{10}) or 2-methoxy-3-methyl-6-decyl-1,4-benzoquinone (3-Me-5-H-Q_0C_{10}) has only less than 20% of the activity of Q_0C_{10} and 2,3-dimethyl-6-decyl-1,4-benzoquinone (PQ_0C_{10}) has absolutely no acceptor activity. Also, when the 5-position is unsubstituted, subsequent replacement of the 2- or 3-methoxy group with hydrogen results in 70% or 100% loss of activity, respectively. This also suggests that the 3-methoxy is more essential than the 2-methoxy.

Although the effect of ring substituents on the electron-acceptor activity of Q derivatives is clearly demonstrated, it is not known whether the observed effect results from the difference in chemical properties of Q derivatives, such as hydrophobicity, binding affinity, or redox potential, or from the structural specificity of the electron-donating site of succinate-Q reductase. It is our hope that examining the relationship between the chemical parameter and electron-donor activity of Q derivatives may provide some insight into the nature of the effect. A set of Q derivatives, 2-H-Q_0C_{10}, 3-H-Q_0C_{10}, 5-H – Q_0C_{10} and Q_0C_{10}, were chosen for the chemical property and activity comparison study because they cover the full range of acceptor activity.

Fig. 1 shows the concentration-dependent electron-acceptor activity of 2-H-Q_0C_{10}, 3-H-Q_0C_{10}, 5-H-Q_0C_{10} and Q_0C_{10}. It should be mentioned that in this experiment the succinate-Q reductase activity is measured for its ability to catalyze the Q-mediated TTFA-sensitive

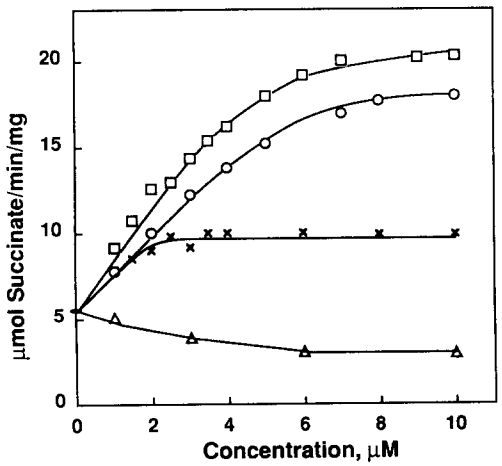

Fig. 1. Titration of succinate-Q reductase activity with Q derivatives. 1 ml assay mixture contains 50 μmol sodium/potassium phosphate buffer (pH 7.0), 0.015% Triton X-100, 20 μmol succinate, 1.1 μmol EDTA, 50 nmol DCIP and indicated amounts of Q_0C_{10} (○), 2-H-Q_0C_{10} (×), 3-H-Q_0C_{10} (△), and 5-H-Q_0C_{10} (□). The reaction was started with the addition of succinate-Q reductase.

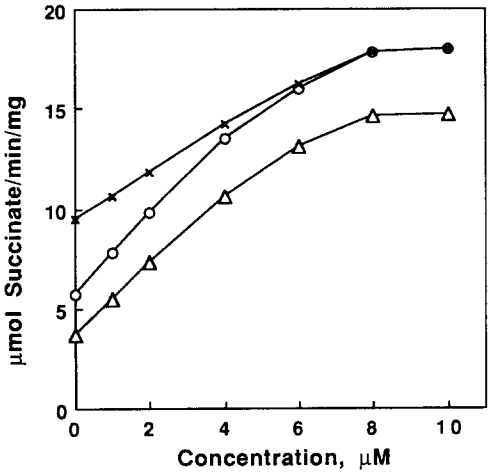

Fig. 2. Titration of succinate-Q reductase activity with Q_0C_{10} in the presence of 2-H-Q_0C_{10} or 3-H-Q_0C_{10}. The assay conditions were the same as those described for Fig. 1, except that 5 μM of 2-H-Q_0C_{10} (×) or 3-H-Q_0C_{10} (△) is present in the assay mixture. The curve with circles (○) represents the titration with Q_0C_{10} alone.

electron transfer from succinate to DCIP. Since isolated succinate-Q reductase contains endogenous Q, a partial activity is expected when assayed in the absence of Q derivative. When succinate-Q reductase is measured with increasing concentrations of these four derivatives, the activity increases as the concentration of 2-H-Q_0C_{10}, 5-H-Q_0C_{10}, or Q_0C_{10} in the system increases, whereas the endogenous Q-mediated activity (5 μmol succinate oxidized per min per mg protein) decreases as the concentration of 3-H-Q_0C_{10} increases. The acceptor activity of Q derivatives is defined as the ability of Q derivatives to mediate the DCIP reduction by succinate via succinate-Q reductase. Maximum electron-acceptor activities for 2-H-Q_0C_{10}, 5-H-Q_0C_{10} and Q_0C_{10} are 4.6, 15.3, and 13.1 μmol succinate oxidized per min per mg of succinate-Q reductase protein, respectively. If one takes the concentration that gives half of maximum activity as the apparent K_m, then the K_m values for 2-H-Q_0C_{10}, 5-H-Q_0C_{10} and Q_0C_{10} are 1.05, 2.54 and 2.94 μM, respectively. No K_m for 3-H-Q_0C_{10} can be obtained. Since 2-H-Q_0C_{10} has a K_m lower than those of 5-H-Q_0C_{10} and Q_0C_{10}, the lower acceptor activity of 2-H-Q_0C_{10} cannot be simply due to the insufficient concentration of 2-H-Q_0C_{10} in the succinate-Q reductase assay system; other factors must have contributed to the low electron-transfer efficiency of this Q derivative.

Since 2-H-Q_0C_{10} has a lower K_m than Q_0C_{10} and 3-H-Q_0C_{10} inhibits the endogenous Q-mediated electron transfer activity, it is of interest to examine the effect of these two Q derivatives on electron-acceptor activity of Q_0C_{10}. Fig. 2 shows the succinate-Q reductase activity assayed with increasing concentrations of Q_0C_{10} in the absence and presence of 5 μM 2-H-Q_0C_{10} or 3-H-Q_0C_{10}. When 5 μM of 2-H-Q_0C_{10} are

present, at low concentrations of Q_0C_{10} the competition between Q_0C_{10} and 2-H-Q_0C_{10} slightly favors 2-H-Q_0C_{10}. However, when the Q_0C_{10} concentration in the system increases, the competition is gradually in favor of Q_0C_{10}, and finally the same maximum activity as that of Q_0C_{10} alone is reached. This result suggests that 2-H-Q_0C_{10} and Q_0C_{10} are bound to the same site, and 2-H-Q_0C_{10} has a slightly higher binding affinity. This is consistent with the observation that succinate-Q reductase has a slightly lower K_m for 2-H-Q_0C_{10} than for Q_0C_{10}.

When 5 μM 3-H-Q_0C_{10}, which inhibits 35% of the endogenous Q-mediated electron-transfer activity, is present along with Q_0C_{10} in the assay mixture, the activity increases as the concentration of Q_0C_{10} increases. However, the maximum activity reached is only about 80% of that obtained with Q_0C_{10} alone. Since the inhibition is not overcome by Q_0C_{10}, either 3-H-Q_0C_{10} is not bound to the same site as Q_0C_{10}, or the binding is so tight that it cannot be reversed by Q_0C_{10}.

Table III compares the relative hydrophobicity, midpoint redox potential (E_m), K_{cat}/K_m, and electron-acceptor activity of Q_0C_{10} derivatives. The hydrophobicity is indicated by the retention time of Q derivatives from a C_{18} HPLC column eluted with 80% methanol. The electron-acceptor activity of Q derivatives does not correlate with the hydrophobicity, midpoint redox potential, or the K_{cat}/K_m.

Effect of the ring substituents on electron-donor activity of Q-derivatives for ubiquinol-cytochrome c reductase

Ubiquinol is the substrate for ubiquinol-cytochrome *c* reductase. This enzyme complex catalyzes the antimycin-sensitive electron transfer from ubiquinol to cytochrome *c*. Maximum electron donating activity of 1,4-benzoquinol derivatives to cytochrome *c* via ubiquinol-cytochrome *c* reductase is summarized in the middle column of Table II. Although the electron-donating activity of 1,4-benzoquinol derivatives is affected by the substituents on the benzoquinone ring, all derivatives examined show partial donor activity. This suggests that the structural requirements of the ring

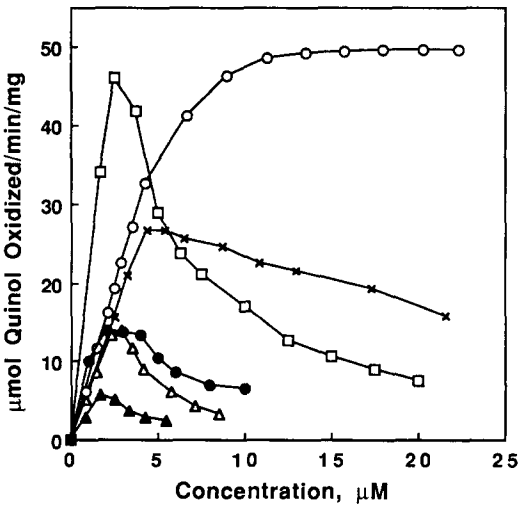

Fig. 3. The effect of quinol concentration on the ubiquinol-cytochrome *c* reductase activity. 1 ml assay mixture contains, 100 μmol sodium/potassium phosphate buffer (pH 7.0), 0.3 μmol EDTA, 100 nmol cytochrome *c* and indicated amounts of $Q_0C_{10}H_2$ (○), 2-H-$Q_1C_{10}H_2$ (×), 3-H-$Q_0C_{10}H_2$ (△), 5-H-$Q_0C_{10}H_2$ (□), $PQ_0C_{10}H_2$ (●), and 2,5-diH-$Q_0C_{10}H_2$ (▲). The reduction of cytochrome *c* was followed at 550 nm with a Cary 219 spectrophotometer at room temperature. The reaction was started by the addition of ubiquinol-cytochrome *c* reductase, 0.2 mg/ml, in 50 mM phosphate buffer (pH 7.8) containing 0.01% sodium deoxycholate, after 20 s tracing for non-enzymatic reduction of cytochrome *c*.

substituents for Q in electron-donating and accepting sites are different.

The methoxy groups at the 2- and 3-positions are important for both acceptor and donor activity. However, they are more important for Q as an electron acceptor for succinate-Q reductase than as an electron donor for ubiquinol-cytochrome *c* reductase, since replacing one methoxy group of Q_0C_{10} with a methyl abolishes all electron-acceptor activity while retaining 15% of electron-donating activity. Of the $Q_0C_{10}H_2$ derivatives that have an unsubstituted 2, 3 or 5 position, 2-methoxy-5-methyl-6-decyl-1,4-benzoquinol (3-H-$Q_0C_{10}H_2$) has the lowest electron-donor activity (26%) and 2,3-dimethoxy-6-decyl-1,4-benzoquinol (5-H-$Q_0C_{10}H_2$) has the highest (90%), indicating that the 3-methoxy is structurally more important than the 2-

TABLE III

Comparison of the redox potential, apparent K_m, K_{cat}/K_m, relative hydrophobicity, and electron-acceptor activity of Q derivatives

Q derivatives	Acceptor activity (units [a])	E_m (mV)	K_m (μM)	K_{cat}/K_m	Retention time (min) [b]
Q_0C_{10}	2382	100	2.94	810	21.9
2-H-Q_0C_{10}	836	41	1.05	796	17.6
3-H-Q_0C_{10}	0	40	–	–	17.5
5-H-Q_0C_{10}	2727	67	2.54	1095	15.9

[a] One unit equals 1 mol of DCIP reduction mediated by Q derivative per mol succinate-Q reductase per min, at 23° C.

[b] The hydrophobicity increases as the retention time increases.

TABLE IV

Comparison of the redox potential, apparent K_m, K_{cat}/K_m, relative hydrophobicity and electron-donor activity of Q derivatives

Q derivative	Donor activity [a] (units)	E_m (mV)	K_m [b] (μM)	K_{cat}/K_m	retention time [c] (min)
Q_0C_{10}	10000	100	3.4	2941	21.9
2-H-Q_0C_{10}	5400	41	2.5	2160	17.6
3-H-Q_0C_{10}	2700	40	1.2	2250	17.5
5-H-Q_0C_{10}	9100	67	1.1	8272	15.9
PQ_0C_{10}	2700	85	1.3	2076	34.4
2-H-5-Me-PQ_0C_{10}	2200	41	0.8	2750	35.2
3-H-5-Me-PQ_0C_{10}	2000	68	1.9	1052	36.5

[a] Maximum activity obtained from substrate titration is used. One unit equals 1 mol of quinol oxidized per mol of ubiquinol-cytochrome *c* reductase (based on cytochrome c_1) per min, at 23° C.

[b] For quinols which show substrate inhibition the apparent K_m was obtained from the double-reciprocal plot using data points obtained at concentrations without inhibition.

[c] The hydrophobicity increases as the retention time increases.

methoxy group or the 5-methyl group. Of the $PQ_0C_{10}H_2$ derivatives, the 2,3-dimethyl-6-decyl-1,4-benzoquinol ($PQ_0C_{10}H_2$) has the highest activity. However, its activity is only 27% of that obtained with $Q_0C_{10}H_2$. Rearrangement of the two methyl groups of $PQ_0C_{10}H_2$ to become 2,5- or 3,5-dimethyl-6-decyl-1,4-benzoquinol further decreases electron-donor activity to about 20%.

During the titration of ubiquinol-cytochrome *c* reductase with increasing concentrations of quinol derivatives to obtain maximum activity, an interesting phenomenon is observed. All benzoquinol derivatives with one unsubstituted position at 2, 3 or 5 show substrate inhibition at high concentration (Fig. 3). This substrate inhibition is more apparent with Q derivatives unsubstituted at the 5- or 3-positions than at the 2-position. Substrate inhibition is not observed when a fully substituted ubiquinol derivative is used. Substrate inhibition is not affected by the presence of endogenous Q, as a similar effect is observed with PL-reconstituted, Q- and PL-depleted ubiquinol-cytochrome *c* reductase.

Table IV compares the electron-donor activity, K_m, K_{cat}/K_m, E_m and hydrophobicity of representative quinol derivatives. The electron-donor activity of Q derivatives does not correlate with the K_m values, K_{cat}/K_m or hydrophobicity of the derivatives, indicating that the difference in electron-donating activity of various Q derivatives for ubiquinol-cytochrome *c* reductase results more from the structural specificity of the Q-binding site than from the difference in chemical properties of Q derivatives.

It should be mentioned that the effect of ring substituents on electron-accepting and -donating activities of Q-derivatives with geranyl groups at the 6-position is similar to that with decyl groups at the same position.

Reduction of Q-derivatives catalyzed by succinate-cytochrome c reductase

It was reported by Von Jagow and Bohrer [30] in 1975 that mitochondria and succinate-cytochrome *c* reductase can catalyze the antimycin-sensitive electron transfer from succinate to plastoquinone. A similar result was observed with duroquinone [41]. However, this activity is not found in isolated, functionally active succinate-Q reductase, which is the first electron-transfer segment of succinate-oxidase. Succinate-Q reductase catalyzes a TTFA-sensitive electron transfer from succinate to Q. The antimycin-sensitive reduction of plastoquinone and duroquinone by mitochondria or succinate-cytochrome *c* reductase is thought to occur by electron transfer between the free form quinone molecule and a ubisemiquinone radical at the Q_i site of the ubiquinol-cytochrome *c* reductase moiety of succinate-cytochrome *c* reductase. It is not due to electron transfer from the QPs of succinate-Q reductase. Thus the structural requirement for the Q-binding site of succinate-Q reductase (QPs) and the Q_i site of ubiquinol-cytochrome *c* reductase is expected to be different.

When Q derivatives are examined for their ability to be reduced by succinate-Q and succinate-cytochrome *c* reductases using succinate as substrate, seven out of fifteen show no activity for succinate-Q reductase but all of them can be reduced by succinate-cytochrome *c* reductase (see Table II). This indicates that the structural requirement for Q derivatives to be reduced by succinate-cytochrome *c* reductase (via Q_i site) is less demanding than for reduction by succinate-Q reductase (va QPs). This may be explained by the electron transfer between Q derivatives and Q^- at the Q_i site of ubiquinol-cytochrome *c* reductase, which is a part of the succinate-cytochrome *c* reductase complex. When Q derivatives are used to accept electrons from Q^- formed at the Q_i site, a tight binding of Q to this site may not be required. That is, electron transfer may occur when Q derivatives are loosely bound near the Q_i site. If so, the structural specificity of Q for this reaction depends more on the Q^- formed at the Q_i site than the acceptor itself. The bound Q at the Q_i site which forms Q^- upon accepting electrons from its donor is structurally specific

(see next section). Antimycin prevents formation of the ubisemiquinone radical from the Q_i site, thus inhibiting the reduction of these derivatives. It should be noted that quinone derivatives, such as 2-H-Q_0C_{10}, 2-H-5-Me-PQ_0C_{10}, 2-Me-5-H-Q_0C_{10}, 3-Me-5-H-Q_0C_{10} and 2,5-di-H-Q_0C_{10}, which show acceptor activity for both succinate-Q and succinate-cytochrome *c* reductase with the latter higher than the former may accept electrons from both QPs of succinate-Q reductase and the bound Q in the Q_i site of ubiquinol-cytochrome *c* reductase moiety of succinate-cytochrome *c* reductase in the absence of antimycin. For example, 2-H-Q_0C_{10} has 30% of normal acceptor activity with succinate-Q reductase, but more than 50% with succinate-cytochrome *c* reductase, indicating that about 20% of the observed activity is from the Q_i site of ubiquinol-cytochrome *c* reductase. Q derivatives which have no acceptor activity for succinate-Q reductase but can be reduced by succinate-cytochrome *c* reductase interact only with the bound Q in the Q_i site. For example, all of the 30% acceptor activity observed for 3-H-Q_0C_{10} with succinate-cytochrome *c* reductase comes from the Q_i site of ubiquinol-cytochrome *c* reductase because the derivative has no acceptor activity with succinate-Q reductase.

The requirement of endogenous ubiquinone for the antimycin-sensitive reduction of plastoquinone derivatives by succinate-cytochrome c reductase

The requirement of endogenous Q for reduction of plastoquinone derivatives (PQ) by succinate-cytochrome *c* reductase has been reported [31,41] and is confirmed here in by using Q- and PL-depleted succinate-cytochrome *c* reductase reconstituted with PL alone, the Q_2-PL micelle and the Q_{10}-PL micelle (see Table V). Intact succinate-cytochrome *c* reductase contains about one mol of Q_{10} mol enzyme complex, based on cytochrome c_1, and about 0.2 mg phospholipid per mg protein. The fully active complex catalyzes electron transfer from succinate to cytochrome *c*, to Q_2 and to PQ_2 at the rate of 8, 4 and 1 μmol cytochrome *c*, Q_2 and PQ_2 reduced, per min per mg protein, respectively, at 23°C. This enzyme complex can also catalyze electron transfer from Q_2H_2 and from PQ_2H_2 to cytochrome *c* at the rate of 52 and 13 μmol quinol oxidized, respectively, per min per mg protein, at 23°C. When Q_{10} and phospholipid are removed (90%) by repeated ammonium sulfate precipitation in the presence of cholate [18], the resulting enzyme shows practically no succinate-cytochrome *c*, succinate-PQ_2, Q_2H_2-cytochrome *c*, and PQ_2H_2-cytochrome *c* reductase activities, but retains full succinate-Q_2 reductase activity. When micelle asolectin is added to the Q- and PL-depleted reductase, the Q_2H_2-cytochrome *c* and PQ_2H_2-cytochrome *c* reductase activities are completely restored, indicating that PQ_2H_2 and Q_2H_2 are able to bind to the quinol oxidation site in the absence of endogenous Q. However, this addition does not restore the succinate-cytochrome *c* and succinate-PQ_2 reductase activities, indicating that these activities require endogenous Q.

TABLE V

Effect of endogenous Q on the reduction and oxidation of PQ derivatives

Preparation [a]	Activity (%) [b]		
	succ. → cyt. *c*	succ. → PQ_2	PQ_2H_2 → cyt. *c*
Intact SCR	100	100	100
dSCR + PL	8	4	89
dSCR + Q_2 + PL	80	27	95
dSCR + Q_{10} + PL	80	54	95

[a] SCR represents succinate-cytochrome *c* reductase and dSCR is the Q- and phospholipid-depleted succinate-cytochrome *c* reductase. The concentration of the phospholipid (PL, asolectin) used was 0.2 mg per mg protein. The concentration of Q used was 3 mol per mol protein, based on cytochrome c_1. The PL and Q mixture was made in 0.5% DMG and 0.1% sodium cholate.

[b] 100% activity represents 6.4 μmol cytochrome *c* reduced, 1.04 μmol PQ_2 reduced, and 12 μmol PQ_2H_2 oxidized per min per mg protein, at 23°C, for succinate-cytochrome *c*, succinate-PQ_2, and PQ_2H_2-cytochrome *c* reductase activities, respectively, measured with intact succinate-cytochrome *c* reductase. The PQ_2H_2 concentration used in the assay system was 3 μM.

Addition of a Q_2-PL micelle solution to Q- and PL-depleted reductase restores the succinate-cytochrome *c* reductase activity (80% of the original activity) to the same extent as that obtained with a Q_{10}-PL micelle. However, the restored succinate-PQ_2 reductase activity in the Q_2-PL-reconstituted succinate-cytochrome *c* reductase is only about half of that in the Q_{10}-PL-reconstituted reductase, indicating that Q_2 is less effective than Q_{10} in mediating electron transfer from succinate to PQ_2 via succinate-Q reductase and the Q_i site of ubiquinol-cytochrome *c* reductase. This result correlates with the observation that Q_2 and PL-reconstituted succinate-cytochrome *c* reductase or ubiquinol-cytochrome *c* reductase shows less ubisemiquinone radical formation than that reconstituted with Q_{10} and PL. Apparently, the Q_2- radical at the Q_i site is less stable than the Q_{10}^- radical. The observation that the PQ reduction catalyzed by succinate-cytochrome *c* reductase is antimycin sensitive and that the reduction efficiency depends on the ability of the endogenous UQ to form UQ^- suggests that PQ receives the electron from UQ^- on the Q_i site and not directly from the Q_i site. This deduction does not contradict the fact that a UQ-less ubiquinol-cytochrome *c* reductase is capable of catalyzing the antimycin-sensitive oxidation of PQ or other Q-derivatives which cannot serve as acceptor for succinate-Q reductase. PQ may only loosely bind to the Q_i site to serve as the electron acceptor for cytochrome *b*-562 during the oxidation of PQH_2. The binding of PQ

at the Q_i site may not specific enough to acceptor electrons from succinate-Q reductase.

In the past, restoration of the enzymatic activity to a Q-depleted reductase with Q_{10} has been only 30% effective compared to that with lower Q homologs, such as Q_2. This is simply due to the low solubility of Q_{10}. In the course of this study, we have developed a new Q_{10}-PL micelles system that can effectively introduce Q_{10} into Q- and PL-depleted succinate-cytochrome *c* reductase to restore the full enzymatic activity. The Q_{10}-PL micelle is prepared by pre-mixing Q_{10} and PL (asolectin) in alcohol followed by sonication in the presence of 0.5% DMG and 0.1% sodium cholate after removal of organic solvent. Electron transfer from succinate to PQ_2 is directly proportional to the reconstituted succinate-cytochrome *c* reductase activity when Q_{10} is used. This reconstituted activity in turn proportional to the amount of Q_{10} incorporated. The maximum reconstituted activity is obtained when 2 mol Q_{10} per mol enzyme are used.

Acknowledgements

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