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Original Article

Interaction of 6-bromo- and 6-chloro-ubiquinone derivatives with the mitochondrial electron transfer system

Chang-An Yu*, Xiao-long Li, Lian-Quan Gu and Linda Yu

Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK 74078, United States of America.

ABSTRACT

To understand the reaction mechanism of quinone-mediated electron transfer, a series of ubiquinone (Q)-derivatives with a bromine or chlorine atom at the 6-position and a different alkyl side chains at the 5-position of the benzoquinone ring were synthesized and characterized. The chemical properties and electron transfer activities were compared with the native ubiquinone. The redox midpoint potential of 6-bromo- and 6-chloro-Q derivatives is 142 mV and 148 mV, respectively. These 6-halo-Q derivatives are reducible by ascorbate alone or by succinate in the presence of succinate-ubiquinone reductase. The reduced 6-halo-Q (6-halo-QH_2) can be oxidized by cytochrome *c* alone or *via* ubiquinol-cytochrome *c* reductase. In the presence of cytochrome *c* oxidase and cytochrome *c*, 6-halo-QH_2 is oxidizable by oxygen. 6-Halo-Q derivatives are reducible by succinate *via* succinate-ubiquinone reductase. They function as an electron-mediator connecting succinate-Q reductase and cytochrome c oxidase and bypasses ubiquinol-cytochrome *c* reductase in the mitochondrial electron-transfer chain. This bypassing results in a decrease of energy coupling efficiency and lower P/O ratio. These compounds might have therapeutic value in treating diseases that are caused by the oversupply of energy.

KEYWORDS: ubiquinone derivatives, cytochrome bc_1 complex, mitochondrial respiratory chain.

*Corresponding author: cayuq@okstate.edu

INTRODUCTION

The participation of ubiquinone (Q) in mitochondrial electron-transfer chain has been well established [1-3]. However, the interaction between Q and protein and the reaction mechanism of Q-mediated electron transfer is not yet fully understood. The relative abundance of Q compared to other redox components in the inner mitochondrial membrane, along with its strong lipophilic nature, have led investigators to propose that Q functions as a homogeneous mobile carrier that shuttles electrons between complexes [4]. Several dynamic and kinetic studies support the idea that ubiquinone has a pool function in the mitochondrial electron transport system [5]. On the other hand, some studies indicate heterogeneity in the Q population of the inner mitochondrial membrane [6]. The identification of the Q-binding proteins (site) in mitochondrial NADH-ubiquinone reductase (NQR) [7], succinate-ubiquinone reductase (SQR) and ubiquinol-cytochrome *c* reductase (QCR) supports the idea that a Q-protein complex is the active specie during electron transfer [8]. With regard to the reaction mechanism of Q, both schools of thought acknowledge the presence of specific Q-binding sites in electron transfer complexes. Q binding sites are clearly established by X-ray crystallographic studies in QCR [9-12], SQR [13] and quinol-fumarate reductase [14-16]. The presence of quinone binding sites in SQR and QCR has also been confirmed by the photo-affinity labeling [17] and molecular genetic [18] studies. Although the atomic structure of NQR

only became available recently [19, 20], the Q binding in the complex has been studied by photoaffinity labeling and inhibitor binding studies [21, 22].

One of the approaches to studying Q-protein interactions and Q-binding sites of the Q-mediated electron transfer complexes is to elucidate the structural requirements for Q. This is accomplished by using various synthetic Q-derivatives with different substituents on the benzoquinone ring [23] and various alkyl side chains [24]. The structural requirements for Q to serve as an electron donor or acceptor differ significantly. When Q is used as an electron acceptor for SQR, an alkyl side chain of six or more carbons gives maximum activity; when Q is used as an electron donor for QCR, an alkyl side chain of ten carbons gives maximum activity [25]. When Q is used as an electron acceptor for SQR, the methyl group at the 6-position is less important than the methoxy group at the 2- or 3-positions [23]. When the electron accepting activities of Q-derivatives with only one methoxy group are compared, the one with methoxy at the 3-position is more active than the one with methoxy at the 2-position [23]. The structural requirements for substituents on the benzoquinone ring are less strict when Q serves as an electron donor for QCR.

The use of chemically modified Q to study the interaction between Q and electron transfer protein complex has been well entertained since 1980s when many modified Q become available [25]. A modified Q known as $Q_0C_{10}OH$ was synthesized for the preparation of fluorescence- and photoaffinity-label Q in 1982. $Q_0C_{10}OH$ was later developed into a successful cardiac drug named idebenone [26]. In addition to the function in the electron transport, Q is also known as an active free radical scavenger and plays an important role in obesity and aging. Using synthetic Q, research on this aspect has been active in recent years, however, due to the extremely low solubility of Q in aqueous solution, the results are relatively difficult to evaluate [27].

Continuing our efforts to understand the reaction mechanism of quinone-mediated electron transfer, a series of Q-derivative with a bromine or chlorine atom at the 6-position and a different alkyl side chain at the 5-position of a benzoquinone ring [6-bromo-2,3-dimethoxy-5-alkyl-1,4-benzoquinone and 6-chloro-2,3-dimethoxy-6-alkyl-1,4-benzoquinone] were synthesized and their chemical properties and electron transfer activities were compared to Q_0C_{10}. The non-enzymatic interaction of these derivatives with cytochrome *c* and their effect on energy coupling efficiency of mitochondria were also investigated.

MATERIALS AND METHODS

Materials

Cytochrome *c*, Type III, and 2, 3-dimethoxy-5-methyl-1, 4-benzoquinone (Q_0) were purchased from Sigma; silica gel G thin layer plates from Analtech; 2, 3-dimethoxy-5-methyl-6-decyl-1, 4-benzoquinone (Q_0C_{10}) were synthesized as previously reported [25]. Azolectin (crude soybean phospholipids) was a product of Associate Concentrate and it was partially purified according to Sone *et al.* [28]. Other chemicals were of the highest purity that were commercially available.

Enzyme preparations

Succinate-ubiquinone reductase (SQR) [29], succinate-cytochrome *c* reductase (SCR) [29], QCR [30], and cytochrome *c* oxidase (C*c*O) [31] were prepared from beef heart submitochondrial particles (SMP) and assayed according to the reported methods. The preparation of SQR was dispersed in 50 mM phosphate buffer, pH 8.0, containing 0.2% sodium cholate. The preparation of QCR was dispersed in 50 mM Tris-HCl buffer, pH 8.0, containing 0.66 M sucrose and 0.01% dodecyl maltoside. The preparation of the C*c*O was dispersed in 50 mM phosphate buffer, pH 7.4, containing 1.5% sodium cholate. Intact mitochondria were prepared from fresh rat liver and assayed by reported methods [32]. The consummation of oxygen for mitochondria was measured by YSI Model 53 Biological Oxygen Monitor at room temperature while stirring. The concentrations of cytochromes *b* and c_1 were determined spectrophotometrically, using difference millimolar coefficients of 28.5 and 17.5 for reduced minus oxidized absorbance at 562 nm minus 575 nm and at 552 nm minus 540 nm for

cytochromes *b* [32] and c_1 [33] respectively. Sodium dithionite and ascorbate were used as the reductants for cytochromes *b* and c_1, respectively. Protein assays were performed according to Lowry method in the presence of 1% SDS, with crystalline bovine serum albumin as standard [34].

Spectral measurements

Absorption spectra were measured in a Shimadzu spectrophotometer model 2101PC. ^{1}H NMR spectra were measured in a Varian XL-400 NMR spectrometer. The molecular weights of the Q derivatives were determined with a V6 ZAB-2SE high-resolution mass spectrometer.

Proton pumping assay for QCR vesicles

QCR was incorporated into azolectin phospholipid vesicles (liposomes) by cholate dialysis method [35]. The bulk-phase proton gradient generated by protein-PL vesicles was determined with a pH electrode (for outward proton ejection). For pH measurements, a Beckman electrode model 39849 and an Accument pH meter model 10 (Fisher Scientific) were used.

Redox potentials and relative hydrophobicity

The redox potentials of Q derivatives were determined from the ratio of reduced and oxidized forms of Q_0 and Q_0H_2 after a short exposure to pH 12. They were then neutralized with acid under anaerobic conditions. Reduced Q_0 was prepared by the treatment of $NaBH_4$ followed by hexane extraction. The buffer system used was 50% ethanol in 50 mM phosphate pH 7.0. A midpoint potential of 110 mV was used for Q_0 in the calculations. The relative hydrophobicity of Q derivatives was determined by their R_fs in the thin layer chromatography (TLC), using hexane/ether (3:1) mixture as developing solvent system.

Synthesis of 6-bromo-2, 3-dimethoxy-5-alkyl-1, 4-benzoquinones and 6-chloro-2, 3-dimethoxy-5-alkyl-1, 4-benzoquinones

Figure 1 summarizes the method used in the synthesis of those 6-bromo- and 6-chloro-Q derivatives. 6-Bromo-2, 3-dimethoxy-5-methyl-1, 4-benzoquinone (6-Br-Q_0): One hundred and eighty mg of Q_0 (2,3-dimethoxy-5-methyl-1,4-benzoquinone ,1 mmol) in 5 mL of acetic acid was mixed with 5 ml of 48% hydrobromic acid. The mixture was stirred at room temperature for 30 minutes, then diluted with 40 mL of water and extracted with ether. The ether extract was washed with H_2O and dried over anhydrous Na_2SO_4. One gram of Ag_2O was added to the dried ether solution and the mixture was stirred for one hour at room temperature. The solid phase was removed by filtration, and the filtrate solution was dried in a rotary evaporator. The crude product was purified by TLC (solvent system: ether/hexane mixtures), yield 92%. 6-Br-Q_0 is a red crystalline, m. p. 58-60 °C. H NMR ($CDCl_3$): 2.20 (s, 3H), 4.02 (s, 3H), 4.06 (s, 3H) ppm. UV^{EtOH}: oxid, 302 nm; red, 293 nm. MS (m/z), 260 (M), 262 (M+2); HRMS, 259.9681 (cal. 259.9684).

By using the same method, a series of 6-bromo- and 6-chloro-Q derivatives were synthesized. Starting materials, such as 2,3-dimethoxy-1,4-benzoquinone, 2,3-dimethoxy-5-propy-1,4-benzoquinone, 2,3-dimethoxy-5-pentyl-1,4-benzoquinone, and 2,3-dimethoxy-5-decyl-1,4-benzoquinone, were prepared according to the methods described previously.

6-Bromo-2, 3-dimethoxy-5-propyl-1, 4-benzoquinone (6-Br-Q_0C_3): Red oil. ^{1}H NMR ($CDCl_3$): 1.02 (t, 3H), 1.75 (m, 2H), 2.20 (t, 2H), 4.02 (s, 3H), 4.06 (s, 3H) ppm. UV^{EtOH}: oxid, 302 nm; red, 293 nm. MS (m/z): 288 (M); 290 (M+2).

6-Bromo-2, 3-dimethoxy-5-pentyl-1, 4-benzoquinone (6-Br-Q_0C_5): Red oil. ^{1}H NMR ($CDCl_3$): 0.89 (t, 3H), 1.28-1.75 (m, 6H), 2.20 (t, 2H), 4.02 (s, 3H), 4.06 (s, 3H) ppm. UV^{EtOH}: oxid, 302 nm; red, 293 nm. MS (m/z): 316 (M); 318 (M+2).

6-Bromo-2, 3-dimethoxy-5-decyl-1, 4-benzoquinone (6-Br-Q_0C_{10}): Red oil. ^{1}H NMR ($CDCl_3$): 0.89 (t, 3H), 1.23-1.6 (m, 16H) 2.20 (t, 2H), 4.02 (s, 3H), 4.06 (s, 3H) ppm. UV^{EtOH}: oxid, 302 nm; red, 293 nm. MS (m/z): 386 (M); 388 (M+2); HRMS, 386.1098 (cal. 386.1093).

6-Chloro-2, 3-dimethoxy-5-methyl-1,4-benzoquinone (6-Cl-Q_0): Red crystalline. ^{1}H NMR ($CDCl_3$): 2.18 (s, 3H), 4.01 (s, 3H), 4.05 (s, 3H) ppm. UV^{EtOH}: oxide, 287 nm; red, 290 nm. MS (m/z), 216 (M), 218 (M+2); HRMS, 216.0185 (cal. 216.0190).

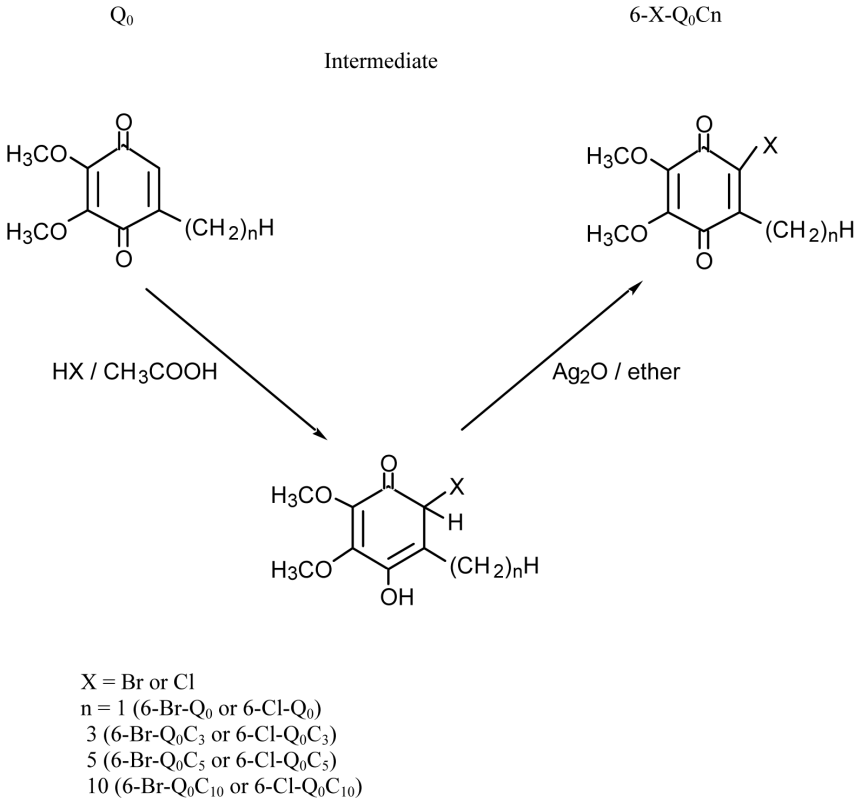

Figure 1. Synthetic pathway of 6-bromo- and 6-chloro-Q derivatives.

6-Chloro-2, 3-dimethoxy-5-propyl-1,4-benzoquinone (6-Cl-Q_0C_3): Red oil. ^{1}H NMR ($CDCl_3$): 0.95 (t, 3H), 1.62 (m, 2H) 2.16 (t, 2H), 4.01 (s, 3H), 4.04 (s, 3H) ppm. UV^{EtOH}: oxid, 287 nm; red, 290 nm. MS (m/z): 244 (M); 246 (M+2).

6-Chloro-2, 3-dimethoxy-5-pentyl-1,4-benzoquinone (6-Cl-Q_0C_5): Red oil. ^{1}H NMR ($CDCl_3$): 0.89 (t, 3H), 1.20-1.62 (m, 6H), 2.15 (t, 2H), 4.01 (s, 3H), 4.04 (s, 3H) ppm. UV^{EtOH}: oxid, 287 nm; red, 290 nm. MS (m/z): 272 (M); 274 (M+2).

6-Chloro-2, 3-dimethoxy-5-decyl-1, 4-benzoquinone (6-Cl-Q_0C_{10}): Red oil. ^{1}H NMR ($CDCl_3$): 0.89 (t, 3H), 1.18-1.60 (m, 16H), 2.14 (t, 2H), 4.01 (s, 3H), 4.04 (s, 3H) ppm. UV^{EtOH}: oxid, 287 nm; red, 290 nm. MS (m/z): 342 (M); 344 (M+2).

RESULTS AND DISCUSSION

Structures and properties of 6-bromo- and 6-chloro-Q derivatives

Figure 1 shows the chemical structures of 6-bromo- and 6-chloro-Q derivatives. The absorption spectral properties of 6-bromo- and 6-chloro-Q are similar to those of 2,3-dimethoxy-5-decyl-6-methyl-1,4-benzoquinone (Q_0C_{10}), but differ with 16 nm and 8 nm red shifts in the oxidized forms, and with 5 nm and 3 nm red shift in the reduced forms, respectively.

The midpoint potential (Em) of 6-bromo- and 6-chloro-Q derivatives were determined to be 142 mV and 148 mV, respectively. Introduction of an electron withdrawing group such as bromine or chlorine atom to the benzoquinone ring results in stabilization of the reduced form of the derivatives, thus increasing their redox midpoint potentials.

6-Bromo- and 6-chloro-Q derivatives as electron acceptors for succinate-ubiquinone reductase (SQR)

Table 1 summarizes the maximum electron acceptor activities of 6-bromo- and 6-chloro-Q derivatives as compared to that of Q_0C_{10} from succinate catalyzed by SQR.

Table 1. Comparison of electron-acceptor activity of 6-halo-Q derivatives.

Q-derivatives	Concentration used (μm)	Relative activity %
Q_0C_{10}	30	100
6-Br-Q_0	30	22
6-Br-Q_0C_3	30	34
6-Br-Q_0C_5	30	39
6-Br-Q_0C_{10}	30	54
6-Cl-Q_0	30	26
6-Cl-Q_0C_3	30	42
6-Cl-Q_0C_5	30	54
6-Cl-Q_0C_{10}	30	73

The electron-acceptor activity of Q-derivatives was measured by SQR using succinate as the substrate. The reaction mixture, with a final volume of 1.0 mL, contained 50 μmol of Na/K phosphate buffer, pH 7.4, 20 μmol succinate, 500 nmol EDTA, 0.5% sodium cholate, and 30 nmol of Q-derivative. The reduction of Q-derivative was followed spectrophotometrically by measuring the decrease in the absorption at 278 nm for Q_0C_{10}, 284 nm for 5-chloro-Q, and 293 nm for 5-bromo-Q, after the addition of SQR (1.2 μg). The maximum activity of Q_0C_{10} was considered to be 100%.

All the halo-Q derivatives show partial electron acceptor activities regardless of the presence or absence of alkyl side chain at the 5-position of the benzoquinone ring. The electron acceptor activity increased slightly as the size of the 5-alkyl group increased.

Interaction of reduced 6-bromo and 6-chloro-Q derivatives with cytochrome *c*

Unlike other ubiquinone homologs which show little interaction with cytochrome *c*, 6-halo-Q derivatives with an alkyl side chain of less than five carbons interact with cytochrome *c* freely in the absence of an electron transfer protein complex. The equilibration is dependent on the redox states and concentrations. Since the midpoint redox potentials of 6-halo-Q derivatives are lower than that of cytochrome *c*, the following reaction is favored on the reduction of cytochrome *c*.

$$6\text{-X-}QH_2 + \text{cyt. } c^{3+} \rightarrow 6\text{-X-Q} + \text{cyt. } c^{2+} + 2H^+$$
(X = Br or Cl)

As expected, the reaction is pH dependent. At pH lower than 7, very little 6-halo-QH_2 is oxidized by cytochrome *c*. This is because the midpoint potential of the 6-halo-Q derivatives increases as the pH decreases.

6-Halo-Q derivatives with an alkyl side chain of more than five carbons show little interaction with cytochrome *c*, but the interaction with cytochrome *c* increases significantly in the presence of QCR. Table 2 shows the interactions between 6-halo-Q derivatives and cytochrome *c* in the absence or presence of QCR.

Reduced 6-bromo- and 6-chloro-Q derivatives as electron donors for ubiquinol-cytochrome *c* reductase

Table 2 summarizes oxidation of reduced 6-halo-Q derivatives by cytochrome *c* in the presence or absence of QCR. Reduced 6-halo-Q derivatives with short alky side chain or no side chain can be oxidized by cytochrome *c* non-enzymatically. Their oxidizability decreases as the sizes of the alkyl side chain increases. The reduced 6-halo-Q derivatives with alkyl side chain can function as electron donors for QCR. The enzymatic oxidizability increases as the sizes of the alky side chain

Table 2. Oxidation of reduced 6-halo-Q derivatives by cytochrome *c*.

Reduced Q derivatives	Concentration used	Non-enzymatic (absence of QCR)	Enzymatic (presence of QCR)*
	(μM)	μM/min	μM/min
$Q_0C_{10}H_2$	30	0.14	6.39
6-Br-Q_0H_2	30	4.10	0
6-Br-$Q_0C_3H_2$	30	3.42	2.57
6-Br-$Q_0C_5H_2$	30	2.43	4.38
6-Br-$Q_0C_{10}H_2$	30	0.36	8.02
6-Cl-Q_0H_2	30	15.32	0
6-Cl-$Q_0C_3H_2$	30	11.31	1.08
6-Cl-$Q_0C_5H_2$	30	9.10	4.50
6-Cl-$Q_0C_{10}H_2$	30	1.54	7.69

Non-enzymatic oxidation of reduced Q-derivatives was measured by cytochrome *c* reduction in the absence of a protein complex. The reaction mixture, at a final volume of 1.0 mL, contained 100 μmol of Na/K phosphate buffer, pH 7.0, 300 nmol EDTA, and 100 nmol cytochrome *c*. The interaction was measured by following the reduction of cytochrome *c* at 550 nm, after 30 nmol of reduced Q-derivative was added.

Enzymatic oxidation of reduced Q-derivatives by cytochrome *c* was measured by QCR. The assay mixture is the same as that for the non-enzymatic activity assay. The assay mixture was first mixed with QCR (1.2 μg) and then reduced Q-derivative was added to start the reaction.

*This activity is the total activity minus the non-enzymatic one.

increases. The maximal oxidizability is reached with the derivative having ten carbons in the alkyl side chain.

Figure 2 shows the concentration-dependent oxidation of reduced 6-halo-Q derivatives by cytochrome *c* catalyzed by QCR. The activity increased to a maximum with increasing concentrations of the 6-halo-Q derivatives and then decreased. At a high concentration of reduced derivatives very little oxidation was observed. This substrate inhibition is not observed in regular ubiquinol unless an extremely high concentration is used. The sharp decrease in oxidation of halo-Q derivatives is an interesting phenomenon that cannot be explained easily. One possibility is that with the high concentrations of reduced halo-Q derivatives they compete with the oxidized form Q derivatives for the quinone reduction (Qi or Q_N) site of QCR and thus inhibit the activity. According to the structural information of the Q_N site of QCR, ubiquinol is bound through two hydrogen bonds, one between the oxygen in 2-methoxy and the hydrogen of the hydroxyl of serine-205, and the other between 1-hydroxyl of ubiquinol and asparatate-228 bridged by a water molecule [36]. Near the methyl group of ubiquinol, there is lysine-227 [36], which may increase the binding of ubiquinol having its methyl group replaced by a bromine or chlorine atom. It should be mentioned that this enzymatic electron-transfer reaction is partially sensitive to antimycin A (Figure 2).

6-Bromo- and 6-chloro-Q derivatives function as an electron shunt between SQR and cytochrome *c* oxidase (C*c*O) in the electron transfer chain

Since the 6-halo-Q derivatives are reducible by succinate in the presence of succinate-Q reductase, and the resulting reduced derivatives are oxidized

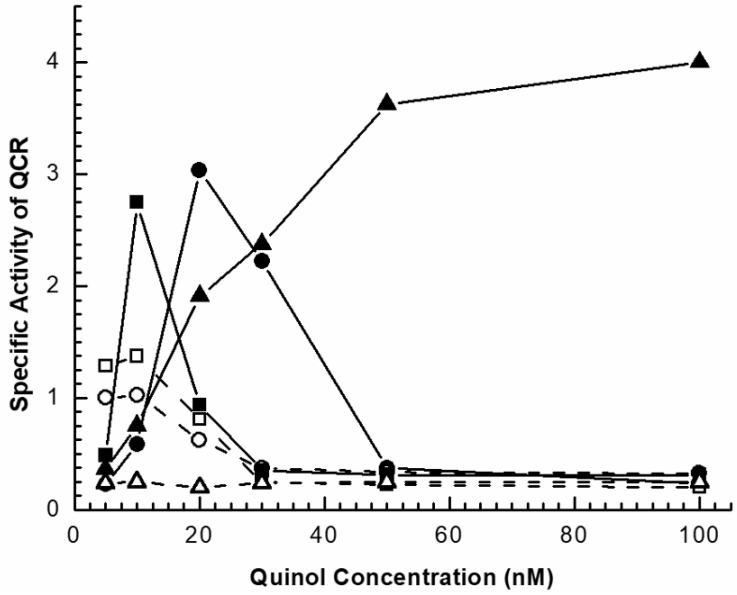

Figure 2. Effect of quinol concentration on QCR activity. One mL assay mixture contained 100 μmol Na/K phosphate buffer, pH 7.4, 100 nmol cytochrome *c*, and the indicated concentration of quinols. The reduction of cytochrome *c* was followed at 550 nm. The reaction was started by the additions of QCR (2 μg) in 0.01% sodium cholate. Curves with open and closed circles (o, ●) represent 6-Br-$Q_0C_{10}H_2$; squares (□, ■), 6-Cl-$Q_0C_{10}H_2$; triangles (△, ▲), $Q_0C_{10}BrH_2$. Open symbols (o, □, △) indicate the presence of antimycin.

by cytochrome *c* directly in the absence of QCR, these derivatives are expected to function as an electron shunt between SQR and C*c*O complexes. In the presence of SQR and C*c*O an electron is transferred from succinate to oxygen directly without involving QCR.

Succ. → SQR → 6-X-Q → Cyt. *c* → C*c*O → O_2

As expected, this electron transfer reaction is sensitive to C*c*O inhibitors, such as CO or KCN. When purified QCR is embedded in phospholipid vesicles, they pump protons out of the vesicles during the oxidation of ubiquinol ($Q_0C_{10}H_2$). The vesicles also transfer an electron to cytochrome *c* with an H^+/e^- ratio of about 2. When reduced 6-halo-Q derivatives were used as substrate for cytochrome bc_1 embedded vesicles, very little proton pumping activity was observed (see Table 3), even with the derivatives having a large alkyl side chain. The lack of proton pumping activity during the oxidation of reduced 6-halo-Q derivatives cannot be simply attributed to the non-enzymatic oxidation of derivatives by cytochrome *c*. Although derivatives with a large alkyl side chain have better enzymatic oxidation activity (80-95%) than the non-enzymatic one (5-20%), their proton pumping activities are still very low.

Table 4 summarizes the oxygen uptake rates in the electron transfer chain from SQR to C*c*O in the presence or absence of cytochrome *c*, using 6-halo-Q derivatives as an electron mediator. The bypass ability of 6-halo-Q derivatives to QCR in the electron transfer chain is dependent on the carbon chain length of the alkyl group of the derivatives. Derivatives with an alkyl side chain of less than five carbons show significant bypass function. The compounds with an alkyl side chain of ten carbons have much less bypass activity. The observation that the 6-Br-Q_0 and 6-Cl-Q_0 have a much higher oxygen uptake rate than other derivatives with no halo-substituent such as Q_0 is consistent with the results of the higher rates observed for the electron transfer from reduced Q to cytochrome *c* and the stronger interaction between reduced 6-halo-Q_0H_2 and oxygen (Table 4). We found that reduced form 6-halo-Q_0H_2 can be oxidized by oxygen in the absence or presence of C*c*O in the reaction mixture. As expected the oxidation in the absence of cytochrome c was not sensitive to cyanide. The interaction between

Table 3. Proton pumping activity (H^+/e^- ratio) of cytochrome bc_1 vesicles.

Reduced Q-derivatives	Proton pumping activity (H^+/e^-)
$Q_0C_{10}H_2$	2.01
6-Br-Q_0 H_2	1.03
6-Br-Q_0C_{10} H_2	1.07
6-Cl-Q_0 H_2	1.07
6-Cl-Q_0C_{10} H_2	1.19

For the determination of proton pumping activity of cytochrome bc_1 complex liposomes, the reaction mixture, at a final volume of 2.0 mL, contained 150 mmol KCl, 5 mmol $MgCl_2$, 10 μmol cytochrome *c*, 1 μg valinomycin, 12.5 μmol reduced QH_2, and 30 μL bc_1 vesicles (1 mg proteins/mL). 5 nmol of $K_3[Fe(CN)_6]$ was added to accept the electron reduced cytochrome *c*. 1 μmol HCl was added to the assay system and the pH change observed was used as reference to calculate the proton ejection induced by the addition of $K_3[Fe(CN)_6]$.

Table 4. The oxygen uptake rates in the electron-transfer from succinate-Q reductase to cytochrome *c* oxidase.

Q-derivatives	Concentration used (μm)	O_2 up-take [ΔO nmol/min]	
		(without cytochrome *c*)	(with cytochrome *c*)
Q_0	30	1.20	2.30
Q_0C_{10}	30	0.65	1.77
6-Br-Q_0	30	25.56	55.23
6-Br-Q_0C_3	30	10.23	20.32
6-Br-Q_0C_5	30	3.53	19.98
6-Br-Q_0C_{10}	30	1.53	8.59
6-Cl-Q_0	30	24.97	66.09
6-Cl-Q_0C_3	30	11.41	23.45
6-Cl-Q_0C_5	30	2.64	18.21
6-Cl-Q_0C_{10}	30	1.65	8.99

The oxygen uptake in the presence of Q-derivatives was measured by SQR and C*c*O, using succinate as the substrate. The reaction mixture, at a final volume of 1.3 mL, contained 50 μmol of Na/K phosphate buffer, pH 7.4, 10 μmol succinate, 500 nmol EDTA, 0.2% sodium cholate, 100 nmol cytochrome *c*, 30 nmol of Q-derivative, and 0.2 mg C*c*O. The consumption of oxygen was measured by YSI Model 53 Biological Oxygen Monitor at room temperature while stirring. The reaction was started by the addition of SQR (24 μg).

reduced 6-halo-Q_0 and oxygen is pH dependent. Figure 3 shows the effect of pH on O_2-uptake rate of SQR-reduced halo-Q-derivatives. These results indicate that the electron transfer from reduced 6-Cl-Q_0H_2 to oxygen in the presence of C*c*O in the neutral pH range 7.0 to 8.0 is a pH less-sensitive, enzymatic reaction. For the reaction in the absence of C*c*O, (a non-enzymatic reaction), the oxidation rate increases as the pH of the reaction mixture increases.

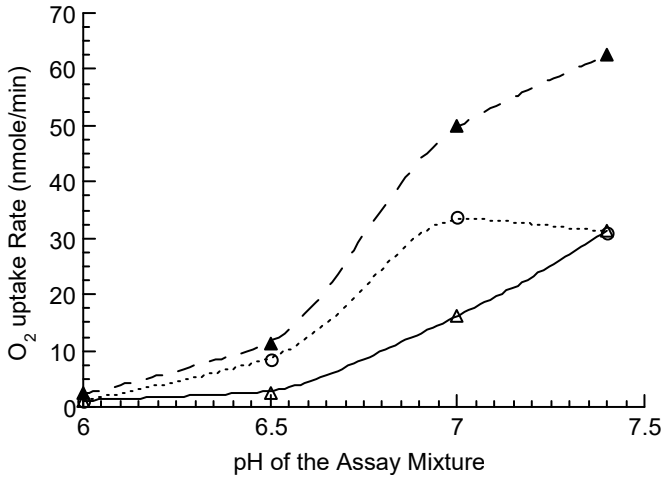

Figure 3. Effect of pH on the O_2-uptake rate of SQR in the presence of reduced 6-Cl-Q derivatives. The oxygen uptake in the presence of Q-derivatives was measured by SQR and C*c*O, using succinate as the substrate. The reaction mixture, at a final volume of 1.3 mL, contained 50 μmol of Na/K phosphate buffer, pH 7.4, 10 μmol succinate, 500 nmol EDTA, 0.2% sodium cholate, 100 nmol cytochrome *c*, 30 nmol of Q-derivative, and 0.2 mg cytochrome *c* oxidase (C*c*O). The consumption of oxygen was measured by YSI Model 53 Biological Oxygen Monitor at room temperature while stirring. The reaction was started by the addition of SQR (24 μg). The curve with solid triangles (▲) represents oxygen uptake in a reaction system containing SQR, cytochrome *c*, C*c*O, and 5-Cl-Q_0. The O_2-uptake includes both enzymatic and non-enzymatic reactions. The curve with open triangles (△) represents the reaction system containing SQR and 6-Cl-Q_0, but not cytochrome *c* and C*c*O. Only a non-enzymatic reaction between reduced 6-Cl-Q_0H_2 and oxygen was detected. The curve with open circles (O) represents the difference between the curve with ▲ and the curve with △. It indicates the pure enzymatic reaction between reduced 6-Cl-Q_0H_2 and oxygen through cytochrome *c* and C*c*O.

The electrons transfer from 6-halo-Q_0H_2 to oxygen may take two pathways. One is *via* normal C*c*O and the reaction is cyanide sensitive. Another is that reduced 6-halo-Q derivatives directly interact with molecular oxygen and the reaction is cyanide insensitive.

$$\text{6-X-}Q_0H_2 \rightarrow \begin{cases} \rightarrow \text{cyt. } c \rightarrow Cc\text{O} \rightarrow O_2 \text{ (cyanide sensitive pathway)} \\ \rightarrow O_2 \text{ (cyanide insensitive pathway)} \end{cases}$$

X = Br or Cl

Effects of 6-bromo-and 6-chloro-Q derivatives on state 3 respiration of mitochondria

Unlike other homologs of ubiquinone, 6-halo-Q derivatives showed an inhibitory effect on the state 3 of NADH or succinate-supported respiration when the concentration is higher than 20 μM [35]. This concentration dependent inhibition apparently results from the bypass of site 2 energy conservation. The electrons derived from NADH or succinate, can bypass the QCR region of the respiratory chain to reduce oxygen. As a result, less energy is conserved leading to a decrease in stimulation of respiration upon the addition of ADP in the mitochondria. The P/Q ratio [37] decreased as the concentration of 6-halo-Q derivatives used increased (Figure 4).

CONCLUSION

It is generally known that more than 90% of the energy required to sustain life, growth, and the

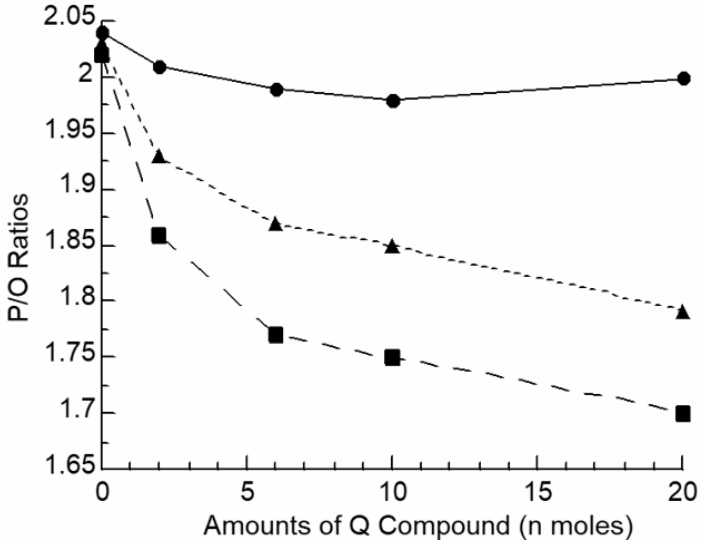

Figure 4. The effect of quinone concentration on the P/O ratios of mitochondria. Mitochondria were prepared from fresh rat liver, according to methods reported previously [31]. 1.3 mL assay mixture contained 225 µmol sucrose, 20 µmol Na/K phosphate buffer, pH 7.4, 20 µmol KCl, 5 µmol $MgCl_2$, 1 µmol EDTA, and 1.3 µg BSA. The oxygen consumption was measured by YSI Model 53 Biological Oxygen Monitor at room temperature while stirring. After three minutes incubation with ubiquinone derivatives at the indicated concentrations, 1 mg (100 µl, 10 mg/mL) protein of mitochondria was added for each assay. The state 4 respiration was started by the additions of 30 µmol succinate, pH 7.4, and the state 3 respiration was stimulated by the addition of 250 nmol ADP. The curve with solid cycle (●), triangles (▲) and solid squares (■) represents Q_0C_{10}, 6-Cl-Q_0C_{10} and 6-Br-Q_0C_1, respectively.

structural integrity of a living organism is derived from a process called oxidative phosphorylation. This process takes place in the mitochondrial inner membrane of eukaryotic cells or in the cytoplasmic membrane of prokaryotic organisms, *via* a coupling reaction of two multi-subunit membrane protein complexes: the electron transfer chain complexes I (NQR), II (SQR), III (QCR) and IV (C*c*O), and Complex V (ATP synthase complex). Coenzyme Q, which functions as an electron acceptor for NQR and SQ, and as an electron donor for QCR, is the only component whose concentration can be modified externally. The idea of using chemically synthesized Q to improve the bioenergetic function has been well entertained. The idea of using modified coenzyme Q to decrease the bioenergetic function, however, is relatively new. The results described in this report clearly indicate that 6-Br- or 6-Cl-Q_0C_{10} can reduce the energy production efficiency of oxidative phosphorylation. Taking advantage of the availability of 6-Br-Q_0C_{10}, we have recently investigated the effect of this compound on cell growth using commercially available cell lines and the body weight gain in rats. As expected, our preliminary results show that the 6-Br-Q_0C_{10} indeed reduces cell growth significantly depending on the type of cell-lines used. It decreases rat's body weight gain in a concentration-dependent manner. These suggest that 6-Br-Q_0C_{10} or 6-Cl-Q_0C_{10} has a great potential to reduce obesity and to slow the growth of cancer cells. A patent application based on these findings has been developed and submitted.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ABBREVIATIONS

C*c*O : cytochrome *c* oxidase
Q_0 : 2,3-dimethoxy-5-methyl- 1,4-benzoquinone
Q_0C_{10} : 2,3-dimethoxy-5-decyl-6-methyl-1,4-benzoquinone
QCR : ubiquinol-cytochrome *c* reductase
SCR : succinate-cytochrome *c* oxidoreductase
SQR : succinate-ubiquinone oxidoreductase
NADH : reduced form of nicotinamide adenine dinucleotide

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