Protein–Ubiquinone Interaction: Synthesis and Biological Properties of Ethoxy Ubiquinone Derivatives†

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ABSTRACT: For investigation of the protein–ubiquinone interaction in the succinate-cytochrome *c* reductase region of the bovine heart mitochondrial electron-transport chain, ethoxy-substituted ubiquinone derivatives, 2-ethoxy-3-methoxy- or 3-ethoxy-2-methoxy-5-methyl-6-decyl-1,4-benzoquinone ($EtOQ_0C_{10}$) and 2,3-diethoxy-5-methyl-6-decyl-1,4-benzoquinone [$(EtO)_2Q_0C_{10}$], were synthesized and characterized. These compounds were synthesized from 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone (Q_0C_{10}) by reaction with sodium ethoxide/ethanol in hexane under anaerobic conditions. The products, $EtOQ_0C_{10}$ and $(ETO)_2Q_0C_{10}$, were separated by thin-layer chromatography using hexane/ether (3.5:1) as the developing solvent. The R_f values for diethoxy and monoethoxy derivatives are 0.7 and 0.6, respectively. The spectral and redox properties of $EtOQ_0C_{10}$ and $(ETO)_2Q_0C_{10}$ are very similar to those of Q_0C_{10}. The reducibility of these derivatives by succinate was measured with succinate-Q reductase (SQR), and their oxidizability was measured by ubiquinol-cytochrome *c* reductase (QCR). Ethoxy ubiquinone derivatives exhibit concentration-dependent inhibition of SQR activity, with $(ETO)_2Q_0C_{10}$ being the more potent inhibitor. These derivatives do not inhibit QCR and are reduced by succinate-cytochrome *c* reductase in an antimycin-insensitive manner. When used as substrate for QCR, $EtOQ_0C_{10}H_2$ has about 55%, and $(ETO)_2Q_0C_{10}H_2$ about 15%, of the activity of $Q_0C_{10}H_2$, but with lower apparent K_m values. The low efficiency of these compounds as electron donors is apparently not due to their weak binding to QCR. These results indicate that the binding environment of the benzoquinone ring in succinate-Q reductase is very specific and differs from that in ubiquinol-cytochrome *c* reductase.

The participation of ubiquinone (Q)[1] in mitochondrial electron transfer is well established (Green, 1960; Ernster et al., 1969; Crane, 1977; Yu et al., 1978). However, the interaction between Q and protein and the reaction mechanism of Q-mediated electron transfer are not yet fully understood. The relative abundance of Q in comparison to other redox components in the inner mitochondrial membrane and the strong lipophilic nature of Q have led investigators to propose that Q functions as a homogeneous mobile carrier (Green, 1960) which shuttles electrons between complexes. Several dynamic and kinetic studies support the idea of a pool function for ubiquinone in the mitochondrial electron-transport system (Kroger & Klingenberg, 1973; Gupte et al., 1984; Ragan & Cottingham, 1985; Fato et al., 1986; Hackenbrock et al., 1986; Lenaz & Fato, 1986). On the other hand, some studies indicate heterogeneity in the Q population of the inner mitochondrial membrane (Gutman, 1985; Van Hoek et al., 1987). The identification of Q-binding proteins in mitochondrial NADH-ubiquinone reductase (Suzuki & King, 1982; Suzuki & Ozawa, 1986), succinate-ubiquinone reductase (SQR) (Yu & Yu, 1980a; Yu et al., 1987), and ubiquinol-cytochrome *c* reductase (QCR) (Ohnishi & Trumpower, 1980; Yu & Yu, 1981, 1985b) supports the idea that a Q–protein complex is the active species during electron transfer. The proposed role of ubiquinone as a mobile pool and the idea that Q binds to protein as a prosthetic group are compatible, if bound quinone is capable of equilibrating with free quinone by either association–dissociation or Q–Q interaction. Both schools of thought regarding the reaction mechanism of Q acknowledge the presence of specific Q-binding sites in electron-transfer complexes.

Study of the structural requirements for Q in Q-mediated electron transfer is essential for elucidation of the Q-binding site and the reaction mechanism. Both the benzoquinone ring and the alkyl side chain of Q are reported to be essential for binding and thus for electron transfer (Crane, 1977; Yu et al., 1985a; Gu et al., 1990). The effects of side-chain variations (Yu et al., 1985a) and substituent arrangement on the benzoquinone ring (Gu et al., 1990) on the electron-transfer activity in mitochondrial succinate-Q, ubiquinol-cytochrome *c*, and succinate-cytochrome *c* reductases have been studied. The structural requirements for Q to serve as an electron donor differ significantly from the requirements for its role as electron acceptor. When Q is used as an electron acceptor for succinate-Q reductase, an alkyl side chain of six or more carbons gives maximum activity (Yu et al., 1985a); whereas an alkyl side-chain of 10 or more carbons gives maximum activity when Q is an electron donor for ubiquinol-cytochrome *c* reductase (Yu et al., 1985a). When Q is used as an electron acceptor for succinate-Q reductase, the methyl group at the 5-position is less important than the methoxy groups at the 2- and the 3-position (Gu et al., 1990). Comparison of the electron-accepting activities of Q derivatives with only one methoxy group shows that Q with a methoxy at position 3 is more active than Q with a methoxy at position 2. The structural requirements for substituents on the benzoquinone

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[1] Abbreviations: Q, ubiquinone; DCPIP, 2,6-dichlorophenolindophenol; Q_0, 2,3-dimethoxy-5-methyl-1,4-benzoquinone; Q_{10}, 2,3-dimethoxy-5-methyl-1,4-benzoquinone with an alkyl group of 10 isoprenoid units at the 6-position; Q_0C_{10}, 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone; $Q_0C_{10}H_2$, 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinol; monoethoxy-Q_0C_{10}, a mixture of 3-ethoxy-2-methoxy-5-methyl-6-decyl-1,4-benzoquinone and 2-ethoxy-3-methoxy-5-methyl-6-decyl-1,4-benzoquinone; diethoxy-Q_0C_{10}, 2,3-diethoxy-5-methyl-6-decyl-1,4-benzoquinone; PL, phospholipid.

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ring are less stringent when Q is used as the electron donor for ubiquinol-cytochrome *c* reductase. These results suggest that the oxidation and reduction sites of Q in the succinate-cytochrome *c* reductase are structurally different. Herein we report the synthesis and electron-transfer properties of monoethoxy and diethoxy Q derivatives.

EXPERIMENTAL PROCEDURES

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 were from Analtech; 2,3-dimethoxy-5-methyl-6-geranyl-1,4-benzoquinone (Q_2), 2,3-dimethyl-6-geranyl-1,4-benzoquinone (PQ_2) and 2,3-dimethoxy-5-methyl-6-decyl-1,4-benzoquinone (Q_0C_{10}) and their reduced forms (Q_2H_2, PQ_2H_2, and $Q_0C_{10}H_2$) were synthesized as previously reported (Yu & Yu, 1982a). Azolectin was a product of Associate Concentrate. Other chemicals were of the highest purity commercially available.

Enzyme Preparations. Succinate-ubiquinone reductase (Yu & Yu, 1982b), succinate-cytochrome *c* reductase (Yu & Yu, 1982b), and ubiquinol-cytochrome *c* reductase (Yu & Yu, 1980b) were prepared and assayed as reported previously. The final preparation of succinate-ubiquinone reductase complex was dispersed in 50 mM phosphate buffer, pH 8.0, containing 0.2% sodium cholate, and ubiquinol-cytochrome *c* reductase was in 50 mM Tris-HCl buffer, pH 8.0, containing 0.66 M sucrose. Q- and phospholipid-depleted (PL-depleted) ubiquinol-cytochrome *c* reductase was prepared according to the method developed in this laboratory (Yu & Yu, 1980c). The preparation procedure involved five cycles of repeated ammonium sulfate precipitation in the presence of 0.5% sodium cholate at a protein concentration of 10 mg/mL. The final preparation was dispersed in 50 mM phosphate buffer, pH 7.4, containing 0.5% sodium cholate and 20% glycerol. The depleted preparation contained less than 10% of the endogenous Q (<0.08 mol/mol of protein) and phospholipid (<5 mol/mol of protein). The enzymatic activity of the depleted preparation could be fully restored upon reconstitution with phospholipids (azolectin) and Q. Freshly prepared, depleted preparation was diluted with phosphate buffer to a protein concentration of 1 mg/mL, mixed with 0.4 mg of azolectin per milligram of protein and various amounts of Q derivatives, and incubated at 0 °C for 1 h before the activity was measured. The stock solution of azolectin (10 mg/mL) was made in 1% sodium cholate.

Spectral Measurements. Absorption spectra were measured in a Shimadzu spectrophotometer, Model 2101-PC. ^{1}H-NMR spectra were measured in a Varian XL-400 NMR spectrometer. The molecular masses of the ethoxy-Q derivatives were determined with a V6 ZAB-2SE mass spectrometer.

Redox Potentials. The redox potentials of ethoxy-Q derivatives were determined from the ratio of reduced and oxidized forms of PQ_2 and an ethoxy-Q derivative after a short exposure to pH 12 and neutralization under anaerobic conditions (Yu et al., 1985a). The buffer system used was 50% ethanol in 50 mM phosphate buffer, pH 7.0. A midpoint potential of 85 mV for PQ_2 was used in the calculations.

Synthesis of 2-Ethoxy-, 3-Ethoxy-, and 2,3-Diethoxy-5-methyl-6-decyl-1,4-benzoquinone. Ethoxy-Q derivatives were synthesized from Q_0C_{10} by replacing its methoxy groups with ethoxy groups under anaerobic alkaline conditions. Since ubiquinone is very sensitive to oxygen in alkaline solution, strict anaerobic conditions are needed during the replacement process. The reaction was carried out in a Thunberg tube with a two-arm stopper. One milliliter of hexane solution

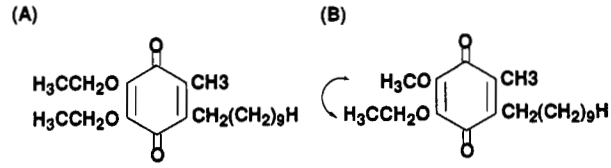

FIGURE 1: Chemical structures of diethoxy-Q_0C_{10} (A) and monoethoxy-Q_0C_{10} (B).

containing 20 μL of ethanol and 1 mg of sodium ethoxide was placed in the bottom of the tube. Six milligrams of Q_0C_{10} in 60 μL of hexane was placed in one arm of the stopper. Twenty microliters of 10 N acetic acid was placed in the other arm of the stopper. The assembly was then subjected to evacuation and argon flushing three times. The Q_0C_{10} solution was then carefully tipped into the ethanol/ethoxide solution. This mixture was incubated at room temperature in the dark for 20 min with constant shaking and then was acidified by tipping in the acetic acid. The acidified mixture was concentrated under vacuum, redissolved in 0.3 mL of hexane, and subjected to thin-layer chromatography (TLC) separation. The TLC plate was developed with hexane/ether (3.5:1). Under these developing conditions, 2,3-diethoxy-Q_0C_{10} has a higher mobility (R_f = 0.7) than 2- or 3-ethoxy-Q_0C_{10} (R_f = 0.6). Unreacted Q_0C_{10} moves slightly more slowly than monoethoxy-Q_0C_{10}, and any degradation products remain at the origin. The diethoxy- and monoethoxy-Q_0C_{10} bands were collected separately and eluted with ether. Upon removal of solvent, 2.8 and 1.2 mg of diethoxy- and monoethoxy-Q_0C_{10}, respectively, were obtained. The yield of 2,3-diethoxy-Q_0C_{10} was 37%: ^{1}H-NMR ($CDCl_3$) δ 4.24 (q, 4), 2.44 (t, 2), 2.02 (s, 3), 1.37 (t, 6), 1.26 (s, 16), 0.88 (t, 3); UV^{EtOH} oxidized, 277 nm, reduced, 290 nm; MS (*m/e*) 350.2447. The yield for monoethoxy-Q_0C_{10} was 17%: ^{1}H NMR ($CDCl_3$) δ 4.22 (q, 2), 4.00 (s, 3), 2.44 (t, 2), 2.02 (s, 3), 1.37 (t. 3), 1.26 (s, 16), 0.88 (t, 3); UV^{EtOH} oxidized, 278 nm, reduced, 290 nm; MS (*m/e*) 336.2303.

RESULTS AND DISCUSSION

Synthesis, Structure, and Chemical Properties of Ethoxy Ubiquinone Derivatives. Figure 1 shows the chemical structures of 2,3-diethoxy-Q_0C_{10} (A) and 2- or 3-ethoxy-Q_0C_{10} (B). These structures were confirmed by ^{1}H-NMR, and their molecular masses were determined by mass spectrometry. As expected, these ethoxy-Q derivatives have absorption properties similar to those of Q_0C_{10}. An ethanolic solution of the oxidized form of monoethoxy-Q_0C_{10} has an absorption peak at 277 nm with a millimolar extinction coefficient of 15. Upon reduction, the absorption maximum shifts to 290 nm with a millimolar extinction coefficient of 4. The absorption maxima of the diethoxy-Q_0C_{10} are at 278 and 290 nm for the oxidized and reduced forms, respectively, and the millimolar extinction coefficients are the same as those of monoethoxy derivatives. Midpoint redox potentials of mono- and diethoxy-Q_0C_{10} are very close to those of Q_0C_{10} (100 mV). In 50% ethanol solution, the midpoint redox potentials of monoethoxy- and diethoxy-Q_0C_{10} are 103 and 105 mV, respectively. The hydrophobicity, as measured by the mobility of these compounds in HPLC using a C-18 column, increases as the number of ethoxy groups increases. The hydrophobicity of these Q derivatives follows the order Q_0C_{10} < monoethoxy-Q_0C_{10} < diethoxy-Q_0C_{10}. Since the chromatographic properties of 3-ethoxy-2-methoxy-5-methyl- and 2-ethoxy-3-methoxy-5-methyl-6-decyl-1,4-benzoquinone are very similar, separation of these two compounds is tedious; no effort was made to separate them in this

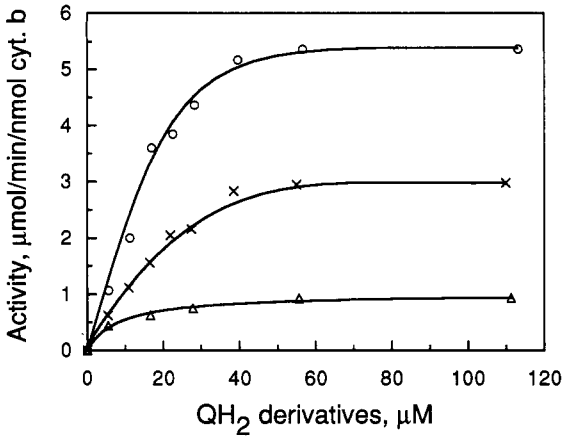

FIGURE 2: Effect of reduced ethoxy-Q_0C_{10} concentration on ubiquinol-cytochrome *c* reductase activity. The assay mixtures contained 100 mM sodium/potassium phosphate buffer, pH 7.0, 0.3 mM EDTA, 100 μM cytochrome *c*, and the indicated concentrations of $Q_0C_{10}H_2$ (○), monoethoxy-$Q_0C_{10}H_2$ (×), or diethoxy-$Q_0C_{10}H_2$ (△). The reduction of cytochrome *c* was followed at 550 nm using a Cary 219 spectrophotometer at room temperature. The reaction, in a total volume of 1 mL, was started by the addition of 5 μL of ubiquinol-cytochrome *c* reductase, 0.2 mg/mL, in 50 mM phosphate buffer, pH 8.0, containing 0.01% deoxycholate, after 20 s tracing for nonenzymatic reduction of cytochrome *c*.

investigation. The term monoethoxy-Q_0C_{10} used in this report refers to a mixture of both isomers.

Electron-Donating Activities of Reduced Ethoxy Ubiquinone Derivatives. Figure 2 shows the concentration-dependent electron-donating activity of the reduced ethoxy-Q_0C_{10} derivatives [$EtOQ_0C_{10}H_2$ and $(EtO)_2Q_0C_{10}H_2$] to cytochrome *c* catalyzed by ubiquinol-cytochrome *c* reductase. $Q_0C_{10}H_2$ is included for comparison. As indicated in Figure 2, both ethoxy-$Q_0C_{10}H_2$ derivatives are less active than $Q_0C_{10}H_2$, and the electron-donating activity is concentration dependent. Maximum electron-donating activities for diethoxy- and monoethoxy-$Q_0C_{10}H_2$ are 15 and 55% of the maximum activity of $Q_0C_{10}H_2$. One hundred percent activity represents 5.5 μmol of quinol oxidized per minute per nanomole of cytochrome *b* of ubiquinol-cytochrome *c* reductase. Monoethoxy-Q_{10} and diethoxy-Q_{10} have been shown to be able to restore a partial succinate oxidase activity of isooctane-extracted electron-transport particles from bovine heart mitochondria (Hendlin & Cook, 1960).

The titration of ethoxy-$Q_0C_{10}H_2$ derivative concentrations versus activity exhibits saturation behavior similar to that of $Q_0C_{10}H_2$, but the double-reciprocal plots of these data show that the apparent K_m values differ from that of $Q_0C_{10}H_2$. The apparent K_m values for $Q_0C_{10}H_2$, $EtOQ_0C_{10}H_2$, and $(EtO)_2$-$Q_0C_{10}H_2$ are 7.1, 5.0, and 2.0, respectively. It should be mentioned that the apparent K_m values of these hydrophobic compounds vary significantly depending on the assay conditions. Especially important is the amount of detergent present. The lower activities of ethoxy-$Q_0C_{10}H_2$ derivatives compared to that of $Q_0C_{10}H_2$ cannot be explained by weaker binding to the reductase, as the K_m values for these derivatives are lower than that of $Q_0C_{10}H_2$. Although no actual binding study was carried out in this investigation, it was assumed that the low apparent K_m is an indication of high binding affinity. The lower apparent K_m values for diethoxy-Q_0C_{10} and monoethoxy-Q_0C_{10} compared to the K_m of Q_0C_{10} might be due in part to their higher partition coefficients (Fato et al., 1988). Since the midpoint potentials of ethoxy-Q_0C_{10} are similar to that of Q_0C_{10}, the lower activity cannot be attributed simply to the difference in redox properties.

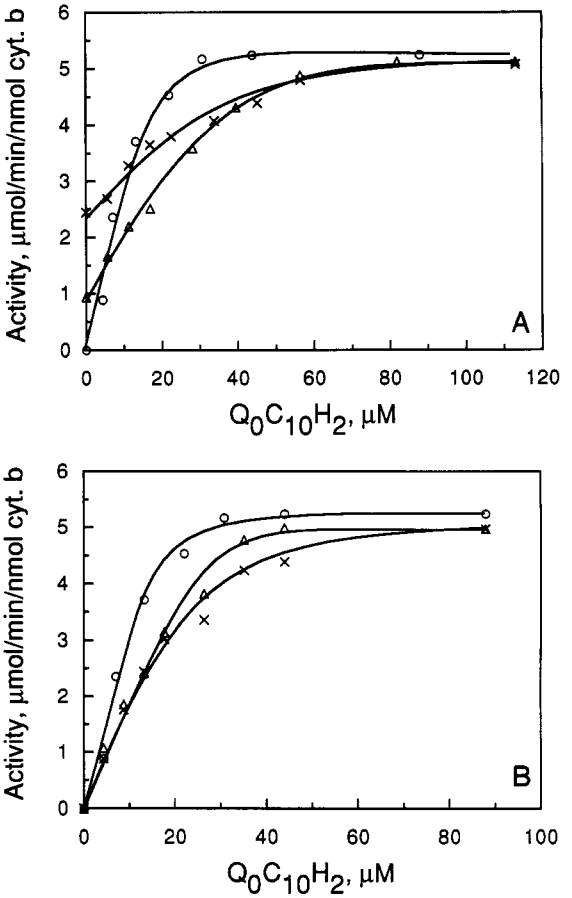

FIGURE 3: Titration of QCR activity with $Q_0C_{10}H_2$ in the presence of ethoxy-Q_0C_{10} derivatives. (A) In the presence of reduced ethoxy-Q_0C_{10} derivatives. The assay conditions were the same as those described for Figure 2 except that 28 μM monoethoxy-$Q_0C_{10}H_2$ (×) or diethoxy-$Q_0C_{10}H_2$ (△) was included in the assay mixture. The curve with circles (○) represents the titration with $Q_0C_{10}H_2$ alone. (B) In the presence of oxidized ethoxy-Q_0C_{10} derivatives. The assay conditions were the same as described in (A), except that 25 μM monoethoxy-Q_0C_{10} (×) or 22 μM diethoxy-Q_0C_{10} (△) was included.

Figure 3 shows the titration of QCR with $Q_0C_{10}H_2$ in the presence of reduced (Figure 3A) and oxidized (Figure 3B) forms of ethoxy-Q_0C_{10} derivatives. The titration patterns are quite similar to those observed in the absence of ethoxy-Q derivatives, except that higher concentrations of $Q_0C_{10}H_2$ are needed to obtain the same level of activity. This suggests that $Q_0C_{10}H_2$ competes with the ethoxy-Q derivatives for the same binding site in the QCR complex. As expected, at lower concentrations of $Q_0C_{10}H_2$, the presence of reduced ethoxy-Q derivatives increases the reduction rate of cytochrome *c*, obviously at the expense of reduced ethoxy-Q derivatives. Since in the presence of oxidized ethoxy-Q derivatives higher concentrations of $Q_0C_{10}H_2$ are required to reach the same level of activity as in their absence, $Q_0C_{10}H_2$ and ethoxy-Q derivatives compete for the same binding site (see Figure 3B). The competition is most likely due to the decyl side chain of the Q molecule.

It was reported previously that most quinol derivatives are oxidized by QCR when sufficient endogenous ubiquinone is present in the enzyme complex. Quinol derivatives without methoxy substituents, such as duroquinol or plastoquinol, are not oxidized by ubiquinone-depleted QCR. It is of interest to know whether or not the required methoxy group can be replaced by ethoxy groups. Ubiquinone-depleted QCR was prepared by reconstitution of Q- and PL-depleted QCR with PL. Figure 4 shows the titration of Q-depleted QCR with

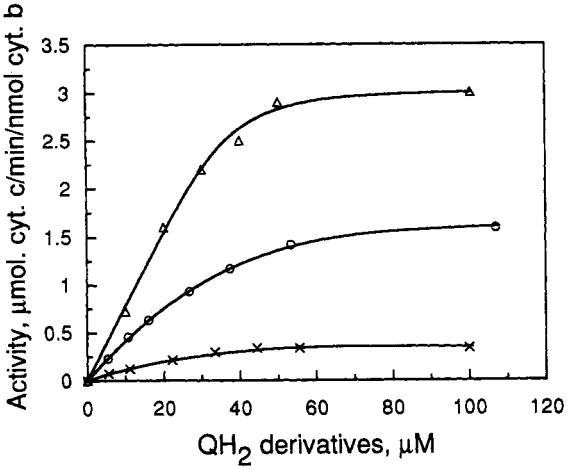

FIGURE 4: Effect of reduced ethoxy-Q_0C_{10} concentration on the activity of ubiquinol-cytochrome *c* reductase in delipidated succinate-cytochrome *c* reductase. The assay conditions were the same as those described in Figure 2, except that Q-depleted phospholipid-reconstituted succinate-cytochrome *c* reductase was used instead of ubiquinol-cytochrome *c* reductase.

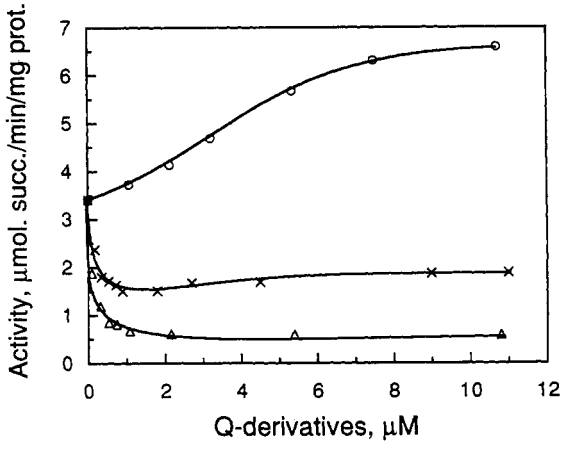

FIGURE 5: Concentration-dependent inhibition of succinate-ubiquinone reductase by ethoxy-Q_0C_{10} derivatives. The assay mixture contained 50 mM sodium/potassium phosphate buffer, pH 7.0, 0.01% Triton X-100, 20 mM succinate, 1.1 mM EDTA, 50 μM DCPIP, and the indicated amounts of Q_0C_{10} (○), monoethoxy-$Q_0C_{10}H_2$ (×), or diethoxy-Q_0C_{10} (△). The reaction, in a total volume of 1 mL, was started with the addition of 5 μL of succinate-ubiquinone reductase, 1 mg/mL, in 50 mM Tris–succinate buffer, pH 7.0, containing 0.01% deoxycholate, after 20 s tracing for nonenzymatic reduction of DCPIP.

$Q_0C_{10}H_2$, $EtOQ_0C_{10}H_2$, and $(EtO)_2Q_0C_{10}H_2$. Except for the somewhat lower activity, the data in Figure 4 are comparable to those shown in Figure 1, suggesting that the oxidation of ethoxy-Q derivatives does not require the presence of endogenous Q in the enzyme complex. In other words, ethoxy-Q derivatives are capable of binding directly to the quinol oxidation site, perhaps with a high affinity, yet they are less rapidly oxidized.

Electron-Accepting Activities of Ethoxy Ubiquinone Derivatives. The electron acceptor activity of Q derivatives is defined as the ability to be reduced by SQR directly or to mediate (stimulate) the thenoyltrifluoroacetone (TTFA)-sensitive DCPIP reduction by succinate in the presence of succinate-Q reductase. Since isolated succinate-Q reductase contains endogenous Q, partial activity is expected in the absence of added Q. Figure 5 shows the results of the titration of succinate-Q reductase activity by ethoxy-Q_0C_{10} derivatives. Titration of Q_0C_{10} is included for comparison. When succinate-Q reductase was titrated with increasing concentrations of ethoxy-Q_0C_{10} derivatives, activity decreased as the

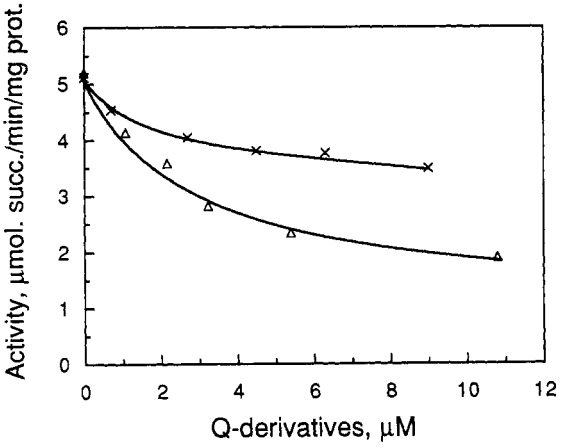

FIGURE 6: Titration of succinate-ubiquinone reductase activity with ethoxy-Q_0C_{10} derivatives in the presence of Q_0C_{10}. The reaction conditions were the same as those described in Figure 5 except a constant amount (5.4 μM) of Q_0C_{10} was included in the assay mixtures. The curve with crosses (×) represents monoethoxy-Q_0C_{10}, and the curve with triangles (△) represents diethoxy-Q_0C_{10}.

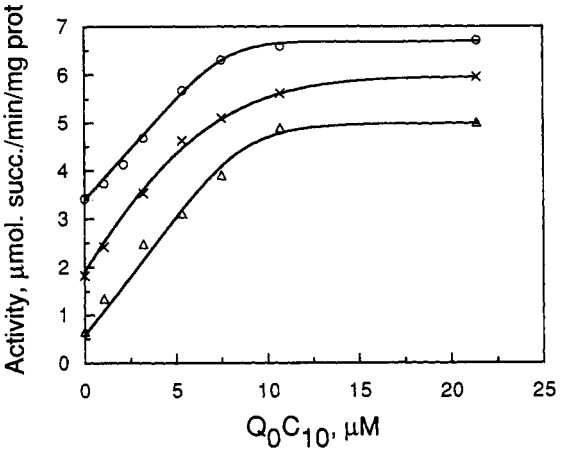

FIGURE 7: Titration of succinate-ubiquinone reductase activity with Q_0C_{10} in the presence of ethoxy-Q_0C_{10} derivatives. The reaction conditions were the same as those described in Figure 5 except a constant amount (2.7 μM) of monoethoxy-Q_0C_{10} (×) or (3.2 μM) diethoxy-Q_0C_{10} (△) was included in the assay mixtures. The curve with circles (○) represents the the titration with Q_0C_{10} alone.

concentration of ethoxy-Q_0C_{10} increased. The maximum decreases of the intrinsic activity of succinate-Q reductase were 57% and 84% for monoethoxy-Q_0C_{10} and diethoxy-Q_0C_{10}, respectively. Maximum inhibition was observed at a concentration of 1 μM for both derivatives. At higher concentrations of monoethoxy-Q_0C_{10}, the inhibition decreased slightly whereas the inhibition of diethoxy-Q_0C_{10} remained at the maximum level. Similar inhibitory effects were also observed in Q_0C_{10}-stimulated SQR activity. Figure 6 shows the titration of Q_0C_{10}-stimulated succinate-ubiquinone reductase activity with ethoxy-Q_0C_{10} derivatives. Activity decreased as the concentrations of both mono- and diethoxy-Q_0C_{10} increased. Maximum inhibitions were 43% and 62% for diethoxy- and monoethoxy-Q_0C_{10} at concentrations of 8 and 11 μM, respectively. As indicated in Figure 7, the degree of inhibition of SQR by monoethoxy-Q_0C_{10} and diethoxy-Q_0C_{10} was not Q_0C_{10} concentration dependent; the titration curves of Q_0C_{10} are parallel in the presence and absence of ethoxy-Q derivatives. They behave as typical noncompetitive inhibitors of SQR, in contrast to the behavior of ethoxy-$Q_0C_{10}H_2$ derivatives during their oxidation by QCR.

It has been reported that for Q derivatives to serve as electron acceptors for succinate-Q reductase, the arrangement of

Table 1: Summary of Redox Potential, Relative Hydrophobicity, Apparent K_m and K_i, and Electron Acceptor and Donor Activities of Ethoxy-Q_0C_{10} Derivatives

				QCR	
O derivatives	E_m (mV)	RT[a] (min)	inhibition (%)	activity (%)	K_m[b] (μM)
Q_0C_{10}	100	21.9	0	100	7.1
$EtOQ_0C_{10}$	103	28.2	40	55	5.0
$(EtO)_2Q_0C_{10}$	105	38.0	80	15	2.1

[a] RT (retention time) indicates relative hydrophobicity, which increases as the retention time increases. [b] K_m, apparent K_m.

substituent groups on the benzoquinone ring is very critical (Gu et al., 1990). Two methoxy groups at positions 2 and 3 are very important, and any modification at these positions results in a drastic reduction in electron acceptor activity. Therefore, the lack of electron acceptor activity of ethoxy-Q derivatives is expected. The noncompetitive inhibition of ethoxy-Q derivatives to succinate-Q reductase, however, is rather difficult to visualize. There are two possible explanations: the ethoxy-Q derivative may bind irreversibly to the quinone-binding site, or alternatively, the decyl side chain may bind to a hydrophobic domain of succinate-Q reductase other than the one which binds Q_0C_{10}.

CONCLUSIONS

Previous studies on the effect of substituents of the benzoquinone ring on electron-transfer activities of ubiquinone derivatives have shown that the structural requirements for quinone derivatives to be reduced by succinate-Q reductase are more strict than those for quinol derivatives to be oxidized by ubiquinol-cytochrome *c* reductase. The presence of two methoxy groups at positions 2 and 3 of the quinone ring is essential for reduction of a quinone derivative by succinate-Q reductase. Two ethoxy-Q derivatives were synthesized to examine the different structural requirements of quinone derivatives in enzymatic reduction and oxidation. The present work further indicates that the binding site for the methoxy groups on the benzoquinone ring in succinate-Q reductase must be very restricted, as it cannot even accommodate an ethoxy group, which is only slightly larger than a methoxy group. On the other hand, the quinol-oxidizing sites (Qo and Qi) in the ubiquinol-cytochrome *c* reductase are not very restricted; most quinol derivatives are partially oxidizable by the reductase, either directly or via bound ubiquinol. There is no absolute requirement for a specific arrangement of small alkyl or alkoxy substituents on the benzoquinone ring. Ethoxy-Q derivatives are oxidized directly by ubiquinol-cytochrome *c* reductase in the presence and absence of endogenous ubiquinone, suggesting that the quinol-oxidizing site in the reductase is spacious enough to accommodate a substituent larger than a methoxy group. These results support our previous conclusion that the quinone-binding sites in succinate-ubiquinone reductase and ubiquinol-cytochrome reductase are very different.

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