

Syntheses of Biologically Active Ubiquinone Derivatives[†]

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ABSTRACT: Various 6-alkylubiquinone or 6-(ω -haloalkyl)-ubiquinone derivatives were synthesized through a radical coupling reaction between alkanoyl or ω -haloalkanoyl peroxides and ubiquinone 0. The latter was synthesized from 2-methoxy-4-methylphenol via nitration, methylation, reduction, and oxidation by modifications of the reported methods. 6-(ω -Haloalkyl)ubiquinones were converted to 6-(ω -hydroxyalkyl)ubiquinones by a mercuric-assisted solvolysis technique. The 6-(ω -hydroxyalkyl)ubiquinones were then esterified with carboxylic acid anhydrides or carboxylic acid bearing reporting groups, such as a photoaffinity label, *N*-(4-azido-2-nitrophenyl)- β -alanine, or a spin-label, 3-carboxy-2,2,5,5-tetramethyl-3-pyrrolinyl-1-oxy. The esterification was catalyzed by dicyclohexylcarbodiimide and pyridine, and the esters were purified by preparative silica gel thin-layer chromatography, developed by 3% ethanol in benzene. The spectral properties

and biological functions of the synthesized ubiquinone derivatives were studied. The biological function of the synthesized compounds was followed by the ability to serve as an electron acceptor, donor, or mediator in the isolated mitochondrial electron transfer complexes of succinate-Q reductase, ubiquinol-cytochrome *c* reductase, and succinate-cytochrome *c* reductase, respectively. The concentration effect of these ubiquinone derivatives on the electron transfer reaction was compared with that of ubiquinone 10. The study of the inhibitory effect of synthesized arylazidoubiquinone on succinate-cytochrome *c* reductase after photolysis confirmed the existence of specific Q-binding proteins in this segment of the respiratory chain. The specific interaction between ubiquinone and protein has also gained support from the immobilization of the spin-label of a synthesized spin-labeled ubiquinone derivative.

Participation of ubiquinone (Q)¹ in biological energy conserving systems from mitochondria to bacteria has been well established (Crane, 1977) through studies of organic solvent (Ernster et al., 1969) or other biochemical (Yu, L., et al., 1978) extraction of Q followed by incorporation. Introduction of the protonmotive Q cycle (Mitchell, 1976) hypothesis and the observation of Q-binding proteins in the mitochondrial (Yu, C. A., et al., 1978) and photosynthetic (Marinetti et al., 1979) electron transport systems have stimulated a new tide of mechanistic studies of Q function in bioenergetic reactions (Blankenship & Parson, 1978; Gutman, 1980; Trumpower, 1981). One of the main problems encountered in the study of Q is the low solubility of Q in aqueous systems, coupled with the facts that the interaction between Q and protein has been shown to be essential to Q function and that the protein cannot tolerate organic solvent treatment.

The low molecular weight homologues of Q such as Q₁ or Q₂ are useful as electron acceptors but are usually not commercially available, and their role in phosphorylation remains to be established (Lenaz et al., 1981a). It has been reported that low molecular weight homologues of Q have an uncoupling effect although they are functionally active in electron transfer (Lenaz et al., 1981b). The synthesis of low molecular weight homologues of Q and their derivatives with defined properties, especially those with detectable reporting groups, has become an important task in the studies of bioenergetic reaction mechanisms because the active form of Q in the electron transfer reaction has been shown to be the Q-protein complexes (Yu & Yu, 1981a).

Although synthetic procedures for 2,3-dimethoxy-5-methylbenzoquinone (Q₀) (Oberlin, 1925) and straight-chain Q derivatives (Shunk et al., 1958; Wan et al., 1975) have been available in the literature, syntheses of Q derivatives with chemically or physically detectable reporting groups have not

been available. In this report we have adapted and modified the synthetic procedure to synthesize Q₀ from the currently commercially available compound, creosol. The procedure, in our opinion, can be carried out in the biochemical laboratory. We have also introduced a procedure to synthesize a Q derivative with a functional group to which a reporting group, such as a photoaffinity label (Yu & Yu, 1980a), spin-label (Yu & Yu, 1981b), or fluorescent group, can be easily attached. The procedures for synthesis of Q derivatives with reporting groups are described, and the enzymatic activity of these Q derivatives is reported as the electron donor of ubiquinol-cytochrome *c* reductase or as the electron acceptor of succinate-Q reductase and as mediators of the succinate-cytochrome *c* reductase system of beef heart mitochondria.

Experimental Procedures

Materials. Creosol (2-methoxy-4-methylphenol) was obtained from Eastman Chemical Co.; fuming nitric acid was a product of Mallinckrodt; high-concentration hydrogen peroxide (60%) was a gift from FMC Corp.; dimethyl sulfate, 3-carboxy-2,2,5,5-tetramethyl-3-pyrrolinyl-1-oxy (TMPOC), and other organic chemicals were purchased from Aldrich. Florisil, sodium cholate, cytochrome *c*, type III, and deoxycholic acid were obtained from Sigma. *N*-(4-Azido-2-nitrophenyl)- β -alanine (NAPA) was synthesized according to the method described by Jeng & Guillory (1975). Q₁ and Q₂ were synthesized in our laboratory according to the reported method (Shunk et al., 1958). The preparative thin-layer plate was a product of Supelco; asolectin was from Associate Concentrate; [³H]dimethyl sulfate was obtained from New England Nuclear. Other chemicals were obtained commercially at the highest purity available.

¹ Abbreviations: CDI, carbodiimidazole; DCCD, dicyclohexylcarbodiimide; DCIP, 2,6-dichlorophenolindophenol; NAPA, *N*-(4-azido-2-nitrophenyl)- β -alanine; Q, ubiquinone; Q₀, 2,3-dimethoxy-5-methyl-1,4-benzoquinone; Q₀(CH₂)₁₀H, 6-decylubiquinone; Q₀(CH₂)₁₀Br, 6-(10-bromodecyl)ubiquinone; Q₀(CH₂)₁₀OH, 6-(10-hydroxydecyl)ubiquinone; Q₀(CH₂)₁₀NAPA, 2,3-dimethoxy-5-methyl-6-[10-[(4-azido-2-nitroanilino)propionyloxy]decyl]-1,4-benzoquinone; Q₀(CH₂)₁₀TMPOC, 2,3-dimethoxy-5-methyl-6-[10-[3-(1-oxy-3-carboxy-2,2,5,5-tetramethyl-3-pyrrolinyl)]decyl]-1,4-benzoquinone.

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Synthesis of 2,3-Dimethoxy-5-methyl-1,4-benzoquinone (Q_0). Creosol, which is the closest commercially available compound to Q_0 , was used as the starting material to synthesize Q_0 via steps of nitration (Oberlin, 1925), methylation, reduction, and oxidation (Anslow et al., 1938), with modification. The overall yield of the synthesis was about 10%. The main losses occurred in the initial nitration step and the final dichromate oxidation step. The nitration method of Oberlin (1925), which uses only fuming nitric acid as a nitrating reagent, was unsatisfactory, as it depended heavily on the concentration of fuming nitric acid. A nitrating reagent, prepared from 1.5 mL of concentrated H_2SO_4 and 4 mL of fuming HNO_3 (90%) in 50 mL of dry ether, was used instead. So that overnitration could be avoided, the nitrating reagent was added slowly, and the temperature of the ethereal solution of creosol was kept at 4 °C.

Reduction of 5-nitrohomoveratrole to 5-aminohomoveratrole was carried out by catalytic hydrogenation at a hydrogen pressure of 40 lb/in.² in a Parr shaker. Catalytic hydrogenation has the advantages of better recovery and cleaner operation over Sn-HCl reduction.

Although the recovery of Q_0 from dichromate oxidation of 5-aminohomoveratrole was poor with a typical yield of about 30%, there is no better method available for this particular reaction. Replacement of dichromate with Fremy's salt as the oxidizing agent did not increase the yield of product.

Synthesis of 6-(10-Bromodecyl)ubiquinone [$Q_0(CH_2)_{10}Br$]. The method reported for the synthesis of 6-alkylubiquinone by Wan et al. (1975) was adapted and modified to synthesis of 6-(ω -haloalkyl)ubiquinone derivatives.

11-Bromoundecanoic acid (18 g, 68 mmol) was converted to bromoundecanoyl chloride by treatment with $SOCl_2$ (7 mL) at 80–90 °C. After removal of excess $SOCl_2$, the resulting acyl chloride was dissolved in 120 mL of ether at 0 °C in an ice bath and 4 mL of 60% H_2O_2 was added. The mixture was stirred at 0 °C while 6 mL of pyridine was added dropwise over a 2-h period (Silbert & Swern, 1959). The ice bath was then removed and the mixture stirred at room temperature for an additional hour before it was diluted with 700 mL of ether, washed twice with 300 mL of H_2O , twice with 0.8 M HCl, once with H_2O , twice with 0.5 M $NaHCO_3$, and once with H_2O , and then dried over Na_2SO_4 . Approximately 16 g (30 mmol) of crude bromoundecanoyl peroxide (BUP) was obtained upon the removal of solvent.

A mixture of 0.445 g of Q_0 (3.0 mmol) and 1.2 g of BUP in 20 mL of glacial acetic acid was heated at 95 °C for 14 h. The mixture was then diluted with 200 mL of ether and the organic layer washed 2 times each with 200 mL of the following solvents, in order: H_2O , 1 N HCl, 0.5 M $NaHCO_3$, and H_2O . The washed ether solution was dried with magnesium sulfate and evaporated to dryness. The residue was dissolved in hexane and applied to a Florisil column (2 × 10 cm), which was equilibrated and developed with hexane. After removal of the front-running yellowish band, 6-(10-bromodecyl)ubiquinone was eluted with 5% ether in hexane. The material, 6-(10-bromodecyl)ubiquinone [$Q_0(CH_2)_{10}Br$], appeared as a golden band on the column, which, in the second solvent, overtook and eluted before a small brown band. Approximately 0.5 g (1.2 mmol) of $Q_0(CH_2)_{10}Br$ was obtained upon removal of the solvent. The purity of $Q_0(CH_2)_{10}Br$ was tested by thin-layer chromatography with different solvents. The R_f values were 0.15 and 0.24 on silica gel plates developed with benzene and 1,2-dichloroethane, respectively. Proton NMR spectra confirm that the compound obtained is indeed $Q_0(CH_2)_{10}Br$. The NMR spectra in DCl_3 showed the chemical

Table I: Solvolysis of (ω -Haloalkyl)ubiquinones

compounds	temp (°C)	time (h)	products	yield (%)
$Q_0(CH_2)_{10}Br$	60	2	$Q_0(CH_2)_{10}OH$	85
$H(CH_2)_{10}Br$	60	2	$H(CH_2)_{10}OH$	95
$Q_0(CH_2)_8Br$	60	2	$Q_0(CH_2)_8OH$	47
$H(CH_2)_8Br$	60	2	$H(CH_2)_8OH$	60
$Q_0(CH_2)_4Cl$	60	20	$Q_0(CH_2)_4OH$	5
$H(CH_2)_4Cl$	60	20	$H(CH_2)_4OH$	20

shifts and the numbers of hydrogen atoms as follows; 3.98 (s, 6), 3.00 (t, 2), 2.48 (br t, 2), 2.02 (s, 3), and 1.3 (br m, 16). These correspond to hydrogen atoms in CH_3OAr , $ArCH_2-$, $-CH_2Br$, $ArCH_3$, and $-(CH_2)-$, respectively. The largest m/e in mass spectra was found to be 392, which is the molecular weight of $Q_0(CH_2)_{10}Br$.

The same procedure was used for the syntheses of 6-(5-bromopentyl)ubiquinone and 6-(4-chlorobutyl)ubiquinone from 6-bromohexanoic and 5-chloropentanoic acid with a final yield of 33 and 38%, respectively.

Synthesis of 6-(10-Hydroxydecyl)ubiquinone [$Q_0(CH_2)_{10}OH$]. $Q_0(CH_2)_{10}Br$ was converted to $Q_0(CH_2)_{10}OH$ by mercuric-assisted solvolysis (McKillop & Ford, 1974). To a suspension of 224 mg of mercuric oxide in 2.6 mL of dimethoxyethane was added 0.176 mL of 70% perchloric acid. The mixture was warmed at 60 °C until all the mercuric oxide dissolved and 0.2 mL of water was added. The clear solution was then added to 82 mg (0.2 mmol) of $Q_0(CH_2)_{10}Br$, and the resulting mixture was heated at 60 °C for 2 h. The solution was mixed with 5 mL of water and extracted with 20 mL of ether. The ether layer was washed twice each with 0.5 M sodium bicarbonate and water, dried with sodium sulfate, and concentrated. The crude $Q_0(CH_2)_{10}OH$ was then purified on four preparative thin-layer silica gel plates (20 × 20 cm) developed with 3% ethanol in benzene. The R_f values of $Q_0(CH_2)_{10}OH$ and unreacted $Q_0(CH_2)_{10}Br$ were 0.15 and 0.58, respectively. The bands containing $Q_0(CH_2)_{10}OH$ were removed, combined, and eluted with acetone. Upon removal of the solvent, 56 mg (0.17 mmol) of $Q_0(CH_2)_{10}OH$ was obtained. The purity of $Q_0(CH_2)_{10}OH$ was confirmed by both silica gel chromatography and Al_2O_3 thin-layer chromatography with different solvent systems. Proton NMR (DCl_3) showed chemical shifts and hydrogen atoms as follows; 3.98 (s, 6), 3.63 (t, 2), 2.8 (br s, 1), 2.4 (br m, 2), 2.02 (s, 3), and 1.32 (br m, 16). These correspond to hydrogen atoms in CH_3Ar , $ArCH_2-$, $-CH_2OH$, $-CH_2O-$, $ArCH_3$, and $-(CH_2)_8-$. The largest m/e in mass spectra, as expected, is 329. The absorption spectra of $Q_0(CH_2)_{10}OH$ and its reduced form are similar to those of $Q_0(CH_2)_{10}Br$. Other (ω -haloalkyl)ubiquinones can be converted to their corresponding hydroxy derivatives. The yield for the shorter chain derivatives, however, was much lower than that for the longer chain derivatives, even when the reaction time was increased to 20 h. Table I compares the solvolysis of (ω -haloalkyl)ubiquinones.

Esterification of 6-(10-Hydroxydecyl)ubiquinone with Alkanoyl Anhydride or Alkanoyl Chlorides. Quantitative esterification of 6-(10-hydroxydecyl)ubiquinone with alkanoyl anhydrides or alkanoyl chlorides was obtained simply by mixing the alcohol and a 2-fold molar excess of anhydride or chloride at room temperature. The reaction proceeded immediately and smoothly. The excess reagent was removed either by vacuum distillation or by flushing with a stream of nitrogen, depending upon the boiling point of the reagents. For the higher boiling point alkanoyl chloride, the products were further purified by silica gel column or preparative thin-layer chromatography using 5% ethanol in benzene as the solvent

system. Acetyl, hexanyl, and decanyl esters have been made by this method. The yields were over 95% in all cases.

Synthesis of Photoaffinity- and Spin-Labeled Ubiquinone Derivatives. The following procedures were carried out in the dark. A total of 2.5 mg of NAPA was added to 0.2 mL of dry CH_2Cl_2 containing 20 mg of dicyclohexylcarbodiimide (DCCD) and incubated for 10 min. To this mixture $\text{Q}_0\text{-(CH}_2\text{)}_{10}\text{OH}$, 3.6 mg (10 μmol) in 0.15 mL of dry CH_2Cl_2 , was added, followed by the addition of 30 μL of pyridine. The mixture was then incubated at room temperature for 10 h with constant shaking. The salt formed during incubation was removed by filtration and the residue washed with a small amount of CH_2Cl_2 . The filtrate and CH_2Cl_2 wash were combined, concentrated, and applied to a preparative silica gel thin-layer plate (20 \times 20 cm). The plate was developed with 3% ethanol in benzene. The product, 2,3-dimethoxy-5-methyl-6-[10-[(4-azido-2-nitroanilino)propionyloxy]decyl]-1,4-benzoquinone [$\text{Q}_0\text{-(CH}_2\text{)}_{10}\text{NAPA}$], appeared as the second fastest moving band with an R_f of 0.66. The silica gel containing the $\text{Q}_0\text{-(CH}_2\text{)}_{10}\text{NAPA}$ band was collected and eluted with ether. Upon removal of the solvent, 3 μmol of product was obtained. The unreacted $\text{Q}_0\text{-(CH}_2\text{)}_{10}\text{OH}$ was recovered from the thin-layer plate, at an R_f value of 0.35. The pure $\text{Q}_0\text{-(CH}_2\text{)}_{10}\text{NAPA}$ showed the following chemical shifts in the proton NMR spectra in DCl_3 : 8.14 (br q, 1), 7.85 (d, 1), 7.15 (q, 1), 6.90 (d, 1), 4.14 (t, 2), 3.96 (s, 6), 3.68 (q, 2), 2.70 (t, 2), 2.44 (br t, 2), 2.00 (s, 3), and 1.28 (br m, 16). These correspond to hydrogen atoms in CNHAr , Ar-3'H, Ar-5'H, Ar-6'H, $-\text{CH}_2\text{OCO}-$, CH_3OAr , CCH_2N , $\text{OC}-\text{CH}_2\text{C}$, $\text{ArC}-\text{H}_2-$, ArCH_3 , and $-(\text{CH}_2)_8-$, respectively.

A similar procedure was used successfully in the synthesis of a spin-labeled ubiquinone derivative, 2,3-dimethoxy-5-methyl-6-[10-[3-(1-oxy-3-carboxy-2,2,5,5-tetramethyl-3-pyrrolinyl)]decyl]-1,4-benzoquinone [$\text{Q}_0\text{-(CH}_2\text{)}_{10}\text{TMPOC}$], from $\text{Q}_0\text{-(CH}_2\text{)}_{10}\text{OH}$ and TMPOC (Yu & Yu, 1981b).

Enzyme Preparation. Succinate-cytochrome *c* reductase (Yu & Yu, 1980b) and its Q- and phospholipid-depleted preparation (Yu, L., et al., 1978), succinate-Q reductase or complex II (Ziegler & Doeg, 1962), and ubiquinol-cytochrome *c* reductase (Yu & Yu, 1980b) were prepared and assayed by the reported methods. Replenishment of Q and its derivatives to the Q- and phospholipid-depleted enzyme was carried out by first adding an ethanolic solution of Q or its derivatives, followed by the addition of phospholipids, which were prepared in micelle form (Yu & Yu, 1980c). Protein was estimated by the biuret method in the presence of hydrogen peroxide (Yonetani, 1961) using crystalline bovine serum albumin as a standard.

Spectral Measurement. Absorption spectral measurements and spectrophotometric assays of enzymic activity were done in a Cary spectrophotometer, Model 219, at room temperature. EPR measurements were made in a Varian E-4 EPR spectrometer. The detailed instrument settings are listed in the legends of the figures. NMR spectra were measured in either a Hitachi Perkin-Elmer Model R24B or a 100-MHz Varian XL-100 NMR spectrometer.

Photoinactivation of succinate-cytochrome *c* reductase in the presence of $\text{Q}_0\text{-(CH}_2\text{)}_{10}\text{NAPA}$ was done at 3–5 $^\circ\text{C}$ in a glass windowed Dewar. Illumination was carried out with a long-wavelength UV lamp, Model UVSL-25, at a distance of 5 cm.

Results and Discussion

Synthesis and Properties of 6-(ω -Haloalkyl)ubiquinone. Although the syntheses of 6-(4-chlorobutyl)- and 6-(5-bromopentyl)ubiquinones are achieved by the same procedure,

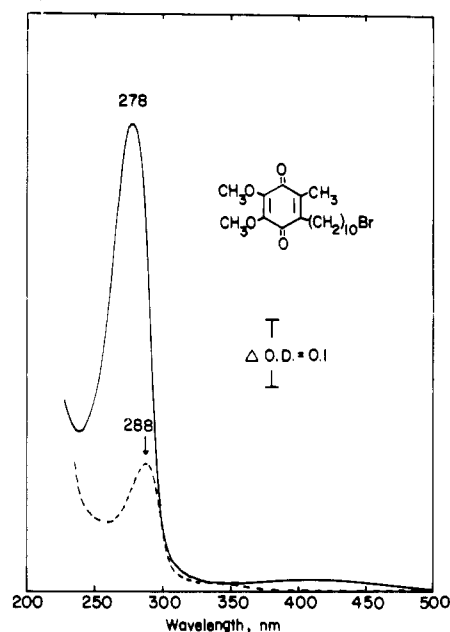


FIGURE 1: Absorption spectra of $\text{Q}_0\text{-(CH}_2\text{)}_{10}\text{Br}$ in 95% ethanol. The dashed curve is the NaBH_4 -reduced form.

the radical coupling reactants, chloropentanoyl peroxides and bromohexanoyl peroxides, must be freshly prepared, because they are liquids that decompose upon storage. Bromoundecanoyl peroxide and undecanoyl peroxide are solids that can be stored for extended periods. A coupling reaction between Q_0 and chloropropanoyl peroxide gave very poor yield, and attempts to synthesize haloacetyl Q derivatives were not successful.

Purified 6-(ω -haloalkyl)ubiquinone is an orange liquid. The absorption spectra are given in Figure 1. The oxidized form has an absorption maximum at 278 nm in 95% ethanol. Upon reduction with NaBH_4 , the absorption maximum shifted to 288 nm with a decrease in molar absorption of about 73%. These absorption spectral properties are very similar to those of the corresponding 6-alkylubiquinone.

Synthesis and Properties of 6-(ω -Hydroxyalkyl)ubiquinone. Since the methoxy groups of Q_0 are rather labile in alkaline conditions, conversion of the halo group to a hydroxy group by conventional methods, which generally involve the use of alkaline conditions, was not successful. The method introduced by McKillop & Ford (1974) is suitable because it uses mercury-assisted solvolysis under acidic conditions, in which the benzoquinone ring is more stable. The procedure described under Experimental Procedures gave a yield of over 85% in the conversion of 6-(10-bromodecyl)ubiquinone to 6-(10-hydroxydecyl)ubiquinone in 2 h of incubation at 60 $^\circ\text{C}$. The yield of the conversion, however, decreased as the alkyl groups became smaller, and the incubation time required to reach the maximal conversion increased, as indicated in Table I. The yield of solvolysis of the corresponding alkyl halides is included for comparison.

6-(10-Hydroxyalkyl)ubiquinone have absorption spectral properties very similar to those of the halogen compound, except that a low absorption peak at 345 nm was observed in the reduced form of the hydroxyl compound.

Usefulness of 6-(ω -Hydroxyalkyl)ubiquinone Derivatives in the Synthesis of Q Derivatives with Reporting Groups. 6-(10-Hydroxydecyl)ubiquinone is easily esterified with the carboxylic acid anhydride or alkanoyl chloride with good recovery. However, for the synthesis of more complicated molecules with reporting groups such as NAPA, milder con-

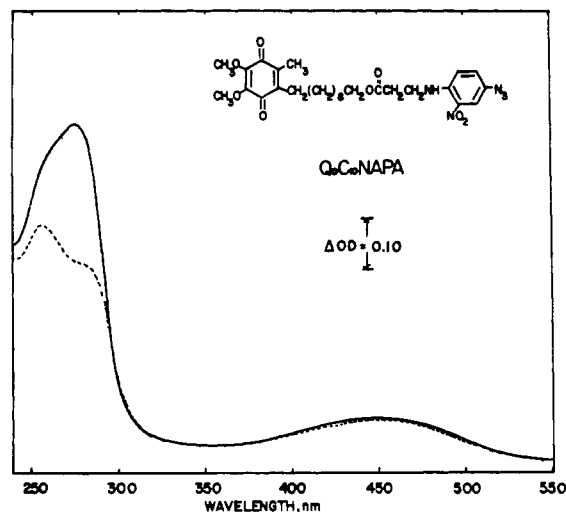


FIGURE 2: Absorption spectra of $Q_0(CH_2)_{10}NAPA$ in 95% ethanol. The dashed curve is the $NaBH_4$ -reduced form.

densing conditions are needed. Coupling between $Q_0-(CH_2)_{10}OH$ and NAPA by DCCD and pyridine gave a 30% yield, which is better than that of any other method tested. When carbodiimidazole (CDI), which was used in the esterification of NAPA with ATP (Jeng & Guillory, 1975), was used, a yield of only about 1% was obtained. In the DCCD-catalyzed esterification reaction, pyridine was found to be necessary; without pyridine only a low recovery of ester was obtained. No effort was made to optimize the condensing conditions.

The spin-labeled Q derivative, $Q_0(CH_2)_{10}TMPOC$, was synthesized by a similar method. In this case, however, no alcoholic solvent was used because the product hydrolyzed easily in alcoholic solvent. Crude $Q_0(CH_2)_{10}TMPOC$ was purified on preparative thin-layer chromatography and then eluted with acetone.

The purity of $Q_0(CH_2)_{10}NAPA$ obtained was established by several criteria: a single spot on silica gel and Al_2O_3 thin-layer chromatography in various solvent systems, H NMR spectra, and absorption spectral properties. Figure 2 shows the absorption spectra of both reduced and oxidized forms of $Q_0(CH_2)_{10}NAPA$. By use of the reported values of the molar extinction coefficients of $Q_0(CH_2)_{10}OH$ and NAPA to calculate the concentration of both moieties, it was found that $Q_0(CH_2)_{10}NAPA$ is indeed composed of 1 mol each of $Q_0-(CH_2)_{10}OH$ and NAPA. The concentration of Q_0 was determined by the decrease in absorption at 278 nm upon reduction by $NaBH_4$ and the concentration of NAPA was estimated by the absorption at 450 nm ($\epsilon_{mM}^{450} = 5$) (Jeng & Guillory, 1975).

The absorption spectra of $Q_0(CH_2)_{10}TMPOC$ shows little difference from that of $Q_0(CH_2)_{10}OH$ and other simple esters such as $Q_0(CH_2)_{10}OCOCH_3$, because very little of the absorption is contributed by the TMPOC moiety. However, the presence of TMPOC was verified by H NMR and EPR spectra.

Biological Activity of Synthetic Q Derivatives. The effectiveness of Q homologues and 6-alkylubiquinones in the electron transfer or the mitochondrial respiratory chain has been well established and has been reviewed recently (Crane, 1977; Wan et al., 1975). The effect of chain length of 6-alkyl groups on the enzyme activity differs significantly between the enzyme complexes. In many cases the reported data concern only overall effectiveness of Q analogues in the reaction of NADH or succinate oxidases. Very few studies have dealt

Table II: Relative Effectiveness of Q Analogues in the Electron Transfer Reaction of Succinate-Q and Succinate-Cytochrome *c* Reductases

analogues	succinate-Q reductase ^a		succinate-cytochrome <i>c</i> reductase ^b	
	act. (%)	concn (μM)	act. (%)	concn (nmol/mg)
Q_2	100	17	100	20
Q_0	0	30	0	120
Q_1	83	16	10	60
$Q_0(CH_2)_8Cl$	100	17	17	48
$Q_0(CH_2)_8H$	98	14	24	48
$Q_0(CH_2)_8Br$	99	13	26	48
$Q_0(CH_2)_{10}H$	111	17	125	20
$Q_0(CH_2)_{10}Br$	115	17	130	20
$Q_0(CH_2)_{10}OH$	80	20	20	24
$Q_0(CH_2)_{10}NAPA$	90	17	90	24
$Q_0(CH_2)_{10}TMPOC$	91	17	116	24
$Q_0(CH_2)_{10}OC(=O)CH_2CH_3$	87	28	89	32
$Q_0(CH_2)_{10}OC(=O)(CH_2)_4CH_3$	54	30	54	32
$Q_0(CH_2)_{10}OC(=O)(CH_2)_8CH_3$	35	33	33	32

^a The activity was followed by the Q-mediated succinate-DCIP reductase activity of complex II. The concentration refers to the final concentrations of Q analogues in the assay mixture. ^b The activity refers to that restored from the Q- and phospholipid-depleted succinate-cytochrome *c* reductase upon addition of Q analogues and PL. The concentration represents nanomoles of Q analogues used per milligram of reductase protein in the reconstituted system.

with the separated enzyme segments such as succinate-Q, or ubiquinol-cytochrome *c* reductases. In the first case, Q is used as an electron acceptor and in the second as a donor. A given Q derivative could be very effective as an electron acceptor for succinate-Q reductase but not as an electron donor for ubiquinol-cytochrome *c* reductase. In Table II, the effectiveness of Q derivatives in electron transfer is reported in both activities, and the effectiveness in general is given under the described concentration. Only in some cases was the concentration dependence of effectiveness studied.

Introduction of a halogen at the end of the 6-alkyl group produces little change in the electron transfer ability, compared to that of the corresponding 6-alkyl group without substitution. Introduction of a hydroxy group to the end of the 6-alkyl group greatly decreased the electron transfer activity but only slightly decreased the effectiveness as an electron acceptor. Esterification of the hydroxy groups restored electron transfer activity. The number of carbons in the ester group exerted a significant effect on the electron transfer efficiency. The activity decreased as the carbon chain length of the alkanoyl group increased. A comparison of electron transfer activity of $Q_0(CH_2)_{10}OCO(CH_2)_nH$ ($n = 1, 2, 3, 4$, and 9) is given in Figure 3. The lower activity of the longer chain ester groups probably resulted from increased hydrophobicity rather than from the increase in size. $Q_0(CH_2)_{10}NAPA$ is as active as $Q_0(CH_2)_{10}OCOCH_3$, indicating that the bulky NAPA group does not decrease the activity. The high effectiveness of these Q derivatives with reporting groups in the electron transfer reaction gives researchers the needed confidence to use these Q derivatives in their structure-function studies of electron transfer complexes.

Effect of Concentration of Q Derivatives on the Electron Transfer Activity of Succinate-Cytochrome *c* Reductase. For confirmation of the different effectiveness of Q derivatives given in Table II, the concentration dependence of the effectiveness of each type of derivative was investigated. The Q- and phospholipid-depleted succinate-cytochrome *c* reductase was first reconstituted with Q derivatives, followed by addition

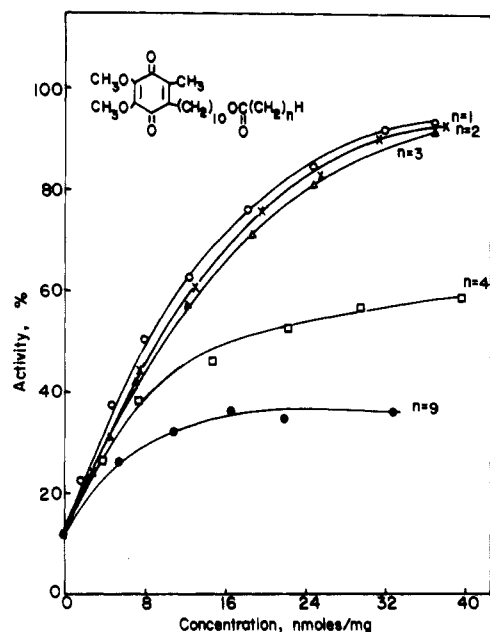


FIGURE 3: Effect of carbon chain length of the simple ester of ubiquinone derivatives on the restoration of succinate-cytochrome *c* reductase. 0.1-mL aliquots of Q- and phospholipid-depleted succinate-cytochrome *c* reductase (6 mg/mL, 24.3 μ M cytochrome *b*) in 50 mM phosphate buffer, pH 7.4, containing 10% glycerol were added to 5 μ L of cold acetone containing the indicated amount of Q derivatives. After a 2-min incubation, 30 μ L of asolectin micellar solution (10 mg/mL) was added, and the mixture was then diluted with 0.2 mL of 50 mM phosphate buffer, pH 7.4, and incubated for 2 h at 0 °C before the succinate-cytochrome *c* reductase was assayed. Q₂ was used as a control in the reconstituted system, and the activity of Q₂ (at 35 nmol/mg of protein) was used as 100%.

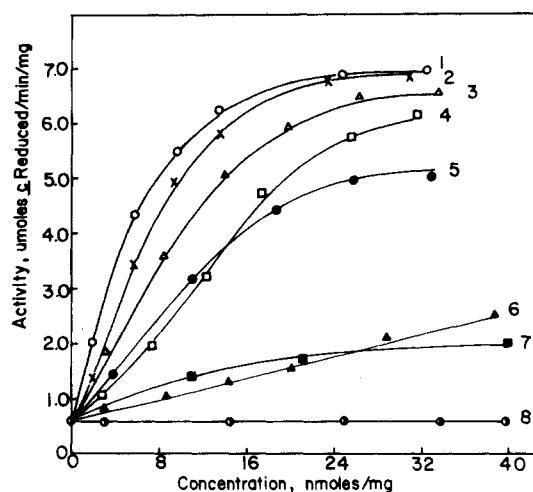


FIGURE 4: Restoration of enzymatic activity of the Q- and phospholipid-depleted succinate-cytochrome *c* reductase by Q derivatives. The conditions used were the same as those described in Figure 3 except the Q- and phospholipid-depleted reductase used was 6.67 mg/mL, containing 26.7 μ M cytochrome *b*. Q derivatives used were in 95% ethanolic solution except Q₀(CH₂)₁₀TMPOC and Q₀(CH₂)₁₀NAPA, which were in acetone. Curve 1, Q₀(CH₂)₁₀Br; curve 2, Q₀(CH₂)₁₀H; curve 3, Q₀(CH₂)₁₀TMPOC; curve 4, Q₂; curve 5, Q₀(CH₂)₁₀NAPA; curve 6, Q₀(CH₂)₁₀OH; curve 7, Q₀(CH₂)₅H; curve 8, Q₀.

of the optimal amount of asolectin, and the restored activity was then plotted against the concentration of Q derivatives used. Figure 4 summarizes the concentration-dependent effectiveness of Q₀(CH₂)₁₀Br, Q₀(CH₂)₁₀H, Q₀(CH₂)₁₀TMPOC, Q₂, Q₀(CH₂)₁₀NAPA, Q₀(CH₂)₁₀OH, Q₀(CH₂)₅H, and Q₀. Q₀(CH₂)₁₀OH showed the least effectiveness when used as the electron mediator between the succinate-Q reductase and

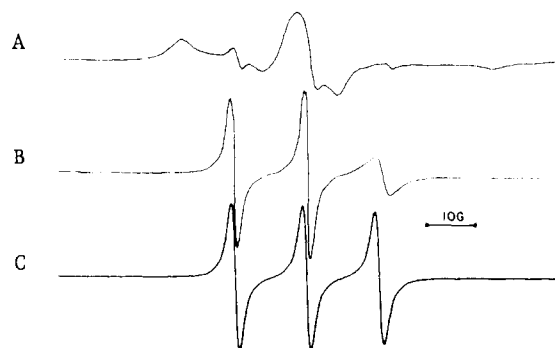


FIGURE 5: Immobilization effect of the Q- and phospholipid-depleted cytochrome *b*-*c*₁ III complex on Q₀(CH₂)₁₀TMPOC. EPR spectra of Q₀(CH₂)₁₀TMPOC in 50 mM phosphate buffer, pH 7.4, containing 20% glycerol in the presence (A) and absence (B) of the Q- and phospholipid-depleted cytochrome *b*-*c*₁ III complex (13 mg/mL). Spectrum C shows an EPR spectrum of Q₀(CH₂)₁₀TMPOC in 95% ethanol. The spectra were taken on a Varian E-4 EPR spectrophotometer at room temperature, with the instrument settings as follows: field modulation frequency, 100 kHz; microwave power, 10 mW; microwave frequency, 9.5 GHz; modulation amplitude, 1 G, time constant, 0.3 s; scan rate, 12.5 G/min. The concentrations of Q₀(CH₂)₁₀TMPOC used and the receiver gain settings were as follows: 140 μ M and 2×10^3 for spectrum A; 93 μ M and 8×10^2 for spectrum B; 87 μ M and 4×10^2 for spectrum C.

ubiquinol-cytochrome *c* reductase. The low effectiveness apparently resulted from the hydrophilic hydroxy group at the end of the side chain of this particular derivative.

Although most of the Q derivatives are very similar to Q₂ in effectiveness, the concentration dependencies are quite different. Q₂ shows a sigmoidal titration curve while other derivatives have hyperbolic titration curves.

Inhibitory Effect of Q₀(CH₂)₁₀NAPA on Succinate-Cytochrome *c* Reductase upon Illumination. When a Q- and phospholipid-depleted preparation of succinate-cytochrome *c* reductase was mixed with Q₀(CH₂)₁₀NAPA and incubated with phospholipids in the absence of light, the enzymatic activity was restored. Upon illumination with light, in either the presence or absence of phospholipids, the restored enzymatic activity was diminished because of the formation of a covalent linkage between the photoactivated nitrene and protein. The inactivation was not reversed by further addition of Q after photolysis. When Q₀(CH₂)₁₀NAPA is made in a radioactive form coupled with NaDodSO₄-polyacrylamide gel electrophoresis, identification of specific Q-binding protein(s) in the electron transfer chain can be made with ease. Preliminary results on the identification of Q-binding protein in the cytochrome *b*-*c*₁ complex have been reported (Yu & Yu, 1980a). An extension of this method would be expected to be instrumental in the identification of other Q-binding proteins of the mitochondrial or photosynthetic electron transfer chains.

As expected, if Q in succinate-cytochrome *c* reductase was not removed, addition of high concentrations of Q₀(CH₂)₁₀NAPA caused only partial inactivation after photolysis. These results support the existence of specific Q-protein binding in succinate-cytochrome *c* reductase.

Specific Interaction between Spin-Labeled Ubiquinone Derivative and Ubiquinol-Cytochrome *c* Reductase. Figure 5 shows EPR spectra of synthesized spin-labeled Q₀(CH₂)₁₀TMPOC in various solvents. Typical nitroxide spin-label EPR spectra were observed when Q₀(CH₂)₁₀TMPOC was dissolved in ethanol or in 50 mM phosphate buffer, pH 7.4, containing 20% glycerol. When Q₀(CH₂)₁₀TMPOC was incubated with the Q- and phospholipid-depleted cytochrome *b*-*c*₁ III complex (ubiquinol-cytochrome *c* reductase), an immobilization of the spin-label resulted from the interaction of

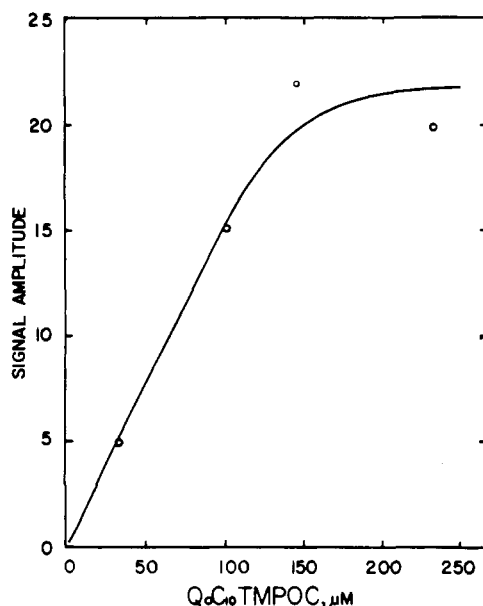


FIGURE 6: Titration of the Q- and phospholipid-depleted cytochrome *b-c*₁ III complex with Q₀(CH₂)₁₀TMPOC. Aliquots of 0.3 mL of the Q- and phospholipid-depleted cytochrome *b-c*₁ III complex, 13 mg/mL in 50 mM phosphate buffer, pH 7.4, containing 20% glycerol, were mixed with various amounts of Q₀(CH₂)₁₀TMPOC. The EPR signal was measured under the same conditions as those used for spectrum A in Figure 5.

Q and protein. Immobilization was indicated by the broadened spectrum and the appearance of low- and high-field signals. The broadening effect of the spectra and the magnitude of the low-field signal are dependent on the concentration of enzyme. Titration of a given enzyme concentration with Q₀(CH₂)₁₀TMPOC showed that the maximal binding of Q₀(CH₂)₁₀TMPOC to the cytochrome *b-c*₁ III complex was at a ratio of 1 mol/mol of cytochrome *b*, as the low-field signal showed saturation at that concentration (see Figure 6). These results confirm the existence of a specific Q-binding protein in the cytochrome *b-c*₁ III complex and demonstrate the usefulness of this type of Q derivative in future studies of protein-Q interaction. Since phospholipids are required in the cytochrome *b-c*₁ III complex for enzymatic activity, the study of protein-Q interaction may be complicated by the presence of phospholipids. A systematic study of Q-protein interaction in the presence of a given phospholipid system is needed in order to resolve the complicated interactions among all participating components.

Acknowledgments

We express our gratitude to Joy Steidl and Paul Haley for their technical assistance and to L.-Q. Gu for the measure-

ments of NMR and mass spectra.

References

- Anslo, W. K., Ashley, J. N., & Raistrick, H. (1938) *J. Chem. Soc.*, 439-442.
- Blankenship, R. E., & Parson, W. W. (1978) *Annu. Rev. Biochem.* 47, 635-654.
- Crane, F. L. (1977) *Annu. Rev. Biochem.* 46, 439-469.
- Ernster, L., Lee, I.-Y., Norling, B., & Person, B. (1969) *Eur. J. Biochem.* 9, 299-310.
- Gutman, M. (1980) *Biochim. Biophys. Acta* 594, 53-84.
- Jeng, S. J., & Guillory, R. J. (1975) *J. Supramol. Struct.* 3, 448-468.
- Lenaz, G., Esposti, M., Bertoli, E., Castelli, G. P., Mascarello, S., Pato, R., & Casali, G. (1981a) in *Function of Quinones in Energy Conserving Systems* (Trumpower, B. L., Ed.) Academic Press, New York (in press).
- Lenaz, G., Esposti, M. D., Pato, R., & Cabrini, L. (1981b) in *Biomedical Aspects of Coenzyme Q* (Folkers, L., Ed.) North-Holland Publishing Co., Amsterdam (in press).
- Marinetti, T. D., Okamura, M. Y., & Feher, G. (1979) *Biochemistry* 18, 3126-3133.
- McKillop, A., & Ford, M. E. (1974) *Tetrahedron* 30, 2467-2475.
- Mitchell, P. (1976) *J. Theor. Biol.* 62, 327-367.
- Oberlin, M. (1925) *Arch. Pharm. Ber. Dtsch. Pharm. Ges.* 263, 641-668.
- Shunk, C. H., Linn, B. O., Wong, E. L., Wittreich, P. E., Robinson, F. M., & Folkers, K. (1958) *J. Am. Chem. Soc.* 80, 4753-4756.
- Silbert, L. S., & Swern, D. (1959) *J. Am. Chem. Soc.* 81, 2364-2367.
- Trumpower, B. L. (1981) *J. Bioenerg. Biomembr.* 13, 1-29.
- Wan, Y. P., Williams, R. H., Folkers, K., Leung, K. H., & Racker, E. (1975) *Biochem. Biophys. Res. Commun.* 63, 11-15.
- Yonetani, T. (1961) *J. Biol. Chem.* 236, 1680-1688.
- Yu, C. A., & Yu, L. (1980a) *Biochem. Biophys. Res. Commun.* 96, 286-292.
- Yu, C. A., & Yu, L. (1980b) *Biochim. Biophys. Acta* 591, 409-420.
- Yu, C. A., & Yu, L. (1980c) *Biochemistry* 19, 5715-5720.
- Yu, C. A., & Yu, L. (1981a) *Biochim. Biophys. Acta* 639, 99-128.
- Yu, C. A., & Yu, L. (1981b) *Biochem. Biophys. Res. Commun.* 98, 1063-1069.
- Yu, C. A., Nagaoka, S., Yu, L., & King, T. E. (1978) *Biochem. Biophys. Res. Commun.* 84, 1070-1078.
- Yu, L., Yu, C. A., & King, T. E. (1978) *J. Biol. Chem.* 253, 2657-2663.
- Ziegler, D. M., & Doeg, K. A. (1962) *Arch. Biochem. Biophys.* 97, 41-50.