Interactions of Mitochondria-targeted and Untargeted Ubiquinones with the Mitochondrial Respiratory Chain and Reactive Oxygen Species

IMPLICATIONS FOR THE USE OF EXOGENOUS UBQINONES AS THERAPIES AND EXPERIMENTAL TOOLS

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Antioxidants, such as ubiquinones, are widely used in mitochondrial studies as both potential therapies and useful research tools. However, the effects of exogenous ubiquinones can be difficult to interpret because they can also be pro-oxidants or electron carriers that facilitate respiration. Recently we developed a mitochondria-targeted ubiquinone (MitoQ10) that accumulates within mitochondria. MitoQ10 has been used to prevent mitochondrial oxidative damage and to infer the involvement of mitochondrial reactive oxygen species in signaling pathways. However, uncertainties remain about the mitochondrial reduction of MitoQ10 by the respiratory chain, and its pro-oxidant potential. Therefore, we compared MitoQ analogs of varying alkyl chain lengths (MitoQn, n = 3–15) with untargeted exogenous ubiquinones. We found that MitoQ10 could not restore respiration in ubiquinone-deficient mitochondria because oxidation of MitoQ analogs by complex III was minimal. Complex II and glycerol 3-phosphate dehydrogenase reduced MitoQ analogs, and the rate depended on chain length. Because of its rapid reduction and negligible oxidation, MitoQ10 is a more effective antioxidant against lipid peroxidation, peroxynitrite and superoxide. Paradoxically, exogenous ubiquinols also autoxidize to generate superoxide, but this requires their deprotonation in the aqueous phase. Consequently, in the presence of phospholipid bilayers, the rate of autoxidation is proportional to ubiquinol hydrophilicity. Superoxide production by MitoQ10 was insufficient to damage aconitase but did lead to hydrogen peroxide production and nitric oxide consumption, both of which may affect cell signaling pathways. Our results comprehensively describe the interaction of exogenous ubiquinones with mitochondria and have implications for their rational design and use as therapies and as research tools to probe mitochondrial function.

MITOCHONDRIA are the major site of reactive oxygen species generation (ROS) within cells (1, 2). When ROS production exceeds the capacity of detoxification and repair pathways, oxidative damage to protein, DNA, and phospholipid occurs, disrupting mitochondrial oxidative phosphorylation and leading to cell damage and death. This contributes to a number of human pathologies including Parkinson disease, Alzheimer disease, Friedreich ataxia, ischemia-reperfusion injury, diabetes, and aging (2, 3). In addition to this pathological role, ROS can also act as redox signaling molecules (4, 5). Hence, mitochondrial ROS production and oxidative damage are attractive targets for pharmacological intervention for both therapeutic and investigative purposes (6–10).

Antioxidants have the potential to block oxidative damage and redox signaling, and exogenous ubiquinones have been widely used for this purpose in mitochondrial studies. These molecules are based on the predominant human form of endogenous ubiquinone, coenzyme Q10 (CoQ10; Fig. 1A), which is synthesized in the mitochondrial inner membrane and comprises a ubiquinone head group attached to a tail of 10 five-carbon isoprenoid units (11). The ubiquinone moiety is redox-active, accepting two electrons and two protons in its reduction to a ubiquinol, while the extremely hydrophobic tail ensures that within the cell it is almost exclusively associated with phospholipid bilayers (Fig. 1B). The redox activity of the ubiquinone moiety enables it to act as a mobile electron carrier in the mitochondrial inner membrane where it is reduced to a ubiquinol by several membrane bound dehydrogenases and oxidized back to a ubiquinone by complex III. Furthermore, the reduced ubiquinol form of CoQ10 has an important protective function as a chain breaking antioxidant, terminating lipid peroxidation.

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The abbreviations used are: ROS, reactive oxygen species; ΔCOQ2, coenzyme Q-deficient yeast strain; cyt c, cytochrome c; cyt csto, acetylated cytochrome c; BHM, bovine heart mitochondrial membranes; CoQn, 2,3-dimethoxy-5-methyl-1,4-benzoquinone; CoQ1–10, coenzyme Q with a tail of 1–10 isoprenoid units (2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone); CsA, cyclosporin A; DETA-NONOate, 3,3′-bis(aminomethyl)-1-hydroxy-2-oxo-1-triazene; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; G3P, glycerol 3-phosphate; G3PDH, glycerol-3-phosphate dehydrogenase; MitoQ, ubiquinone linked to a triphenylphosphonium cation by an alkyl chain of unspecified length; MitoQ3–15, ubiquinone linked to a triphenylphosphonium cation by an alkyl chain of 3–15 carbons; PA, cis-pinaric acid; PBS, phosphate-buffered saline; PTP, mitochondrial permeability transition pore; SOD, superoxide dismutase; tBHP, tert-butyl-hydroperoxide; TPP, triphenylphosphonium cation; WT, wild-type yeast strain; TPMP, methytrithiophenylphosphonium; BSA, bovine serum albumin; HPLC, high performance liquid chromatography.
peroxidation in phospholipid bilayers (12, 13). Therefore, ubiquinone supplementation is an attractive therapeutic strategy in human pathology, as it could both stimulate oxidative phosphorylation by complementing any defects in respiration (14) and protect against oxidative damage (15). However, this duality complicates experimental interpretation, as any effects of CoQ_{10} can result from its interaction with oxidative phosphorylation, oxidative damage, or redox signaling pathways.

While positive therapeutic effects have been observed with CoQ_{10} supplementation in humans (16–19), its oral bioavail-

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**Fig. 1. Ubiquinones used in this study.** A, structures. B, octanol/PBS partition coefficients: a, calculated using Advanced Chemistry Development (ACD) Software Solaris v4.67 as described in Smith et al. (31); b, determined experimentally in this study; c, determined experimentally in Kelso et al. (29); d, determined experimentally in Asin-Cayuela et al. (30). C, a schematic of MitoQ_{10} in a phospholipid bilayer composed of C18 saturated fatty acids. The TPP cation and the alkyl chain favor the polar surface and hydrophobic core, respectively. The preferred position of the uncharged but moderately hydrophilic ubiquinone moiety is less clear.
ability is poor due to its extreme hydrophobicity (Fig. 1B). Consequently, only a small fraction of orally administered CoQ<sub>10</sub> reaches the circulatory system, and augmentation of mitochondrial CoQ<sub>10</sub> content is lower still (20–22). Therefore the beneficial effects of exogenous CoQ<sub>10</sub> require high doses and long term administration, and only subjects whose CoQ<sub>10</sub> levels have been depleted by defective synthesis, age, or disease are responsive (18, 20–24). The negligible water solubility of CoQ<sub>10</sub> and its poor diffusion to mitochondria in cultured cells also hinder its usefulness as a tool to study mitochondrial oxidative damage and redox signaling in vitro. As a result there is considerable interest in developing artificial ubiquinones with better bioavailability and pharmacokinetic properties. Idebenone is one such compound and it comprises a ubiquinone head group attached to a ten carbon alkyl tail with a terminal hydroxyl (Fig. 1A) (25). Clinical trials with idebenone have shown it can ameliorate cardiomyopathy in Friedreich ataxia patients (26–28).

Although decreasing hydrophobicity improves overall bioavailability, it would also be of benefit to target ubiquinones specifically to mitochondria, as they are the main site of ubiquinone utilization but represent only a small fraction of the cell volume. Lipophilic cations, such as methyltriphenylphosphonium (TPMP; Fig. 1A), are accumulated several hundred-fold by the large membrane potential (negative inside) generated by mitochondria during oxidative phosphorylation (6, 9). We have exploited this property by covalently attaching a ubiquinone moiety to the lipophilic triphenylphosphonium (TPP) cation generating a mitochondria-targeted ubiquinone (MitoQ<sub>10</sub>; Fig. 1A), which is selectively accumulated within isolated mitochondria, and within mitochondria in cells and in vivo (9, 29–31).

The interaction of amphipathic alkyltriphenylphosphonium cations with phospholipid bilayers occurs as follows: the TPP lipophilic cation is bound as a monolayer in a potential well at about the level of the phospholipid fatty acid carbonyls, while the hydrophobic alkyl chain is inserted into the hydrophobic core of the membrane (31–34). This is illustrated for MitoQ<sub>10</sub> in Fig. 1C. Although the large ionic radius and hydrophobicity of the TPP cation allows molecules such as MitoQ<sub>10</sub> to permeate phospholipid bilayers readily, their steady-state concentration within the hydrophobic core of the membrane is low. Furthermore, within energized mitochondria the membrane potential causes most MitoQ<sub>10</sub> to be adsorbed to the matrix surface of the inner membrane. MitoQ<sub>10</sub> is a particularly effective antioxidant against lipid peroxidation (29, 30) and has been used in a range of studies of mitochondrial dysfunction and oxidative stress where its mitochondrial localization has enabled the site of intracellular redox signaling to be inferred (35–42). However, the details of the mitochondrial processes affected by MitoQ<sub>10</sub> remain unclear because it could act as an antioxidant (thereby blocking oxidative damage and redox signaling) or as an electron carrier in the respiratory chain (thereby stimulating oxidative phosphorylation). A further consideration is that, an electron carrier in the respiratory chain (thereby blocking oxidative damage and redox signaling) or as a number of related short-chain exogenous ubiquinones interact with both the mitochondrial respiratory chain and ROS. For this we used a range of related short-chain exogenous ubiquinones that differ in the number of carbons linking the ubiquinone to the TPP moiety (MitoQ<sub>n</sub>; n = 3, 5, 10, or 15 CH<sub>2</sub> groups) (29, 30) and compared their interactions with those of the untargeted short-chain ubiquinone analogs, CoQ<sub>2</sub>, decylQ, and idebenone (Fig. 1A). This work has led to a better understanding of how MitoQ and other exogenous ubiquinones interact with the respiratory chain and ROS and has considerable implications for their rational design and use as therapies and as tools to probe mitochondrial oxidative damage and redox signaling.

**MATERIALS AND METHODS**

**Yeast Incubations**—The Saccharomyces cerevisiae strains used were W303-1A (ura3Δ trp1Δ ade2Δ leu2Δ trp1Δ::coq2 mating type MATa) and CEN.PK2–1C (CEN.PK2–1C coq2::URA3 His3), kindly supplied by Prof. Catherine Clarke, UCLA (47). CoQ<sub>2</sub> codes for the enzyme para-hydroxynicotinamide adenine dinucleotide transferase, which catalyzes the transfer of the polyisoprenoid chain to 4-hydroxybenzoic acid in CoQ biosynthesis. ΔCOQ2 is auxotrophic for CoQ and fails to grow on non-fermentable carbon sources, such as glycerol. Yeast were cultured in 10 ml of YPG (1% w/v yeast extract, 2% (w/v) peptone, 3% (w/v) glycerol). The initial cell density was adjusted to A<sub>600</sub> = 0.1. To achieve a reproducible transition to a respiratory phenotype, the medium was supplemented with 0.05% (w/v) glucose. This allowed fermentative growth of ΔCOQ2 up to A<sub>600</sub> = 0.4 after which growth rapidly ceased unless ubiquinone supplementation restored oxidative phosphorylation. Yeast were incubated in the dark at 30 °C with mechanical shaking at 250 rpm. Growth was monitored spectrophotometrically at 260 nm over 120 h. For in vivo experiments, ubiquinones and other hydrophobic compounds were added in Me<sub>2</sub>SO to 1% (w/v) of the total culture volume, which did not affect the growth of the WT and ΔCOQ2 strains on glucose (data not shown).

ΔCOQ2 yeast cultures for mitochondrial isolation were grown aerobically at 30 °C to mid-logarithmic phase (A<sub>600</sub> = 1) in lactate medium (2% (w/v) lactate, 0.3% yeast extract, 0.2% glucose, 0.05% CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.05% NaCl, 0.6% MgCl·6H<sub>2</sub>O, 0.1% KH·PO<sub>4</sub>, 0.1% KH·PO<sub>4</sub>, 0.1% NH·Cl (all w/v) (pH 5.5, NaOH). ΔCOQ2 yeast can use n-lactate as a respiratory substrate because it donates electrons to oxidative phosphorylation at complex IV via the reduction of cytochrome c (cyt c). For WT yeast, the level of glucose was kept at 0.05% (w/v). Mitochondria were isolated according to published protocols (48, 49). The protein concentration was measured by the bicinchoninic acid (BCA) assay as a standard (50). Aliquots of the mitochondrial preparation were mixed with 10 mg ml<sup>−1</sup> fatty acid-free BSA as a cryoprotectant, snap-frozen on dry ice, and stored at −80 °C. Upon thawing the mitochondria retained a membrane potential that was indistinguishable from that of freshly isolated yeast mitochondria as confirmed by the uncoupler-sensitive uptake of [14C]TPMP (data not shown) (29).

Oxygen consumption was measured with a Clark-type oxygen electrode (Rank Brothers, Bottisham, Cambridge, UK) in a 1-ml stirred chamber at 30 °C. Aliquots of frozen ΔCOQ2 yeast mitochondria were thawed rapidly, washed, and resuspended in mannitol buffer (0.6 mM mannitol, 10 mM Tris maleate, 5 mM K<sub>(O)</sub>, 0.5 mM EDTA (pH 6.8, KOH)) at 0.2 mg protein ml<sup>−1</sup>. The mitochondria were energized with 5 mM glycerol 3-phosphate (G3P) and uncoupled with 1 μM carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP). Ubiquinones (1–20 μM in Me<sub>2</sub>SO) were titrated in successively, followed by 1 μM myxothiazol to determine non-mitochondrial oxygen consumption. For some experiments mitochondria were sonicated (3 × 5 s, setting 4; Misonix XL-2020 with microtip) in an ice bath prior to the measurement of respiration and addition of ubiquinone. Uptake of MitoQ analogs by yeast mitochondria was measured using an electrode selective for TPP cations (39). For these experiments WT yeast mitochondria (0.4 mg protein ml<sup>−1</sup>) were incubated in 2.5 ml of mannitol buffer in the presence of 2 μM MitoQ analog at 30 °C, and the uptake of MitoQ in response to energization with 5 mM ethanol was measured.

**Mammalian Mitochondrial Preparations**—Bovine heart mitochondrial membranes (BHM) were prepared from isolated bovine heart mitochondria as described previously (51, 52). Rat liver mitochondria

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The text above is a continuation from the previous page, discussing the effects of MitoQ and related ubiquinones on cellular and mitochondrial processes, focusing on their bioavailability, oxidative stress protection, and mechanisms of action. It highlights the use of MitoQ and related compounds in vitro and in vivo, along with their potential therapeutic applications.
were prepared by homogenization followed by differential centrifugation as described previously (53). Rat heart mitochondria were prepared by tissue disruption using an Ultra-Turrax (5 s), followed by differential centrifugation (53). Protein concentration was determined using the biuret assay with BSA as a standard (54).

**Ubiquinone Reduction and Oxidation by Respiratory Complexes—** Assays were based on previously described methods for measuring respiratory complex activity (55). However, for all assays except complex III the spectrophotometric decrease in A275 (at this wavelength ubiquinone absorbs more strongly than ubiquinol) was monitored instead. Assays were performed in KPi buffer (50 mM KPi-KOH, 100 mM EDTA, 100 μM diethylenetriaminopentaacetic acid (pH 7.8) unless stated otherwise) at 37 °C. For complex I, the buffer was supplemented with 100 μg protein/ml -1 BHLM, 100 μM NADH, and 2 mM KCN, and the reaction was started by addition of 50 μM ubiquinone. For complex II, the buffer was supplemented with 100 μg protein/ml -1 BHLM, 5 mM succinate, 5 mM FAD, 1 mM rotenone, and 2 mM KCN, and the reaction was started by addition of 50 μM ubiquinone. For glycerol-3-phosphate dehydrogenase (G3PDH), EDTA and diethylenetriaminopentaacetic acid (DTPA) were omitted as Ca2+ may be required for activity (56), and the buffer was supplemented with 200 μg protein/ml -1 BHLM, 2 mM KCN, and 50 μM ubiquinone. The reaction was started with the addition of 10 mM G3P. Rotenone (8 μg/ml) and malonate (20 mM) did not affect the rate of ubiquinone reduction in the absence of succinate. In KPi buffer (pH 7.8) complex III, the buffer was supplemented with 50 μg protein/ml -1 BHLM, 50 μM bovine cyt c, 8 μM protein/ml -1 rotenone, and 2 mM KCN, plus or minus 400 mM myxothiazol. The reaction was started with the addition of 50 μM ubiquinol, and cyt c reduction was measured by an increase at A550 (55). The myxothiazol-insensitive rate of cyt c reduction was measured in parallel and subtracted as there is a significant non-enzymatic rate of cyt c reduction by ubiquinol

**Measurement of the Ubiquinone Redox State—** Spectrophotometric measurements were made at 275 nm. The ubiquinone redox state was measured at 37 °C in KPi buffer supplemented with 100 μg protein/ml -1 BHLM, 8 μM protein/ml -1 rotenone, and 10 μM ubiquinone. BHLM were also supplemented with 5 μM bovine cyt c, as this can be lost during membrane isolation. For succinate (5 mM) oxidation, fumarase (5 units) was added as an additional source of NADH in the absence of ubiquinone. For succinate oxidation, the buffer was supplemented with 50 μg protein/ml -1 BHLM, 50 μM bovine cyt c, 8 μM protein/ml -1 rotenone, and 2 mM KCN, plus or minus 400 mM myxothiazol. The reaction was started with the addition of 50 μM ubiquinol, and cyt c reduction was measured by an increase at A550 (55). The myxothiazol-insensitive rate of cyt c reduction was measured in parallel and subtracted as there is a significant non-enzymatic rate of cyt c reduction by ubiquinol

**Exogenous Ubiquinones as Therapies and Tools**—Ubiquinone redox changes were measured at 275 nm. ONOOH has a strong absorbance maximum at 302 nm (ε302 = 1.67 mM -1 cm -1 (57)) leading to a transient spike in A275 until it has decayed. The decay back to baseline absorbance took ~8 s in KP buffer. For cis-parinaric acid (PA; Molecular Probes, Eugene, OR) oxidation, the incubation was performed using a Shimadzu RF-5301PC fluorimeter in a stirred 3-ml cuvette thermostatted at 37 °C; PA was excited at 324 nm, and its fluorescence was monitored at 413 nm. The assay was in KP buffer supplemented with 100 μg protein/ml -1 BHLM, 8 μg/ml -1 rotenone, 5 μM cyt c, and 10 μM ubiquinone. BHLM were supplied with 5 mM succinate, then after 1 min 2 mM PA was added, followed by 20 μM ONOO - and each minute thereafter. ONOO - was prepared as described previously (58).

**Measurement of Reactive Oxygen Species and Autoxidation—** Reduced MitoQ10 (50 μM) was prepared as described previously (29). Ubiquinol oxidation by O2- was measured spectrophotometrically at 275 nm in a stirred 3-ml cuvette. Oxidation of reduced MitoQ10 (50 μM) by O2- generated from 0.015 unit/ml -1 xanthine oxidase and 5 mM ascorbate was measured at 37 °C in KP buffer. After 10 min 100 units/ml -1 SOD was added. Oxidation of reduced MitoQ10 (50 μM) by O2- generated from LPO was measured at 37 °C in PBS-solution with 1 μM KP buffer (pH 7.3). A saturated solution of KO2 (10 mM) was prepared by dissolving 1.4 mg solid KO2 in 2 ml of 10 mM 18-crown-6 ether in Me2SO. A solution where KO2 had degraded to H2O2 was prepared by mixing ~10 mM KO2 in 10 mM 18-crown-6 ether with 1 volume of H2O followed by incubation for 2 min. Autoxidation of reduced MitoQ10 (50 μM) was measured spectrophotometrically in KP buffer, (pH 6.8, 7.8, 8.8, or 9.8) with 5 units of apocytochrome c (cyt c) as the electron acceptor. The rate measured from reduced MitoQ10 (50 μM) was measured using acetylated cytochrome c (cyt cacet) reduction, which was measured spectrophotometrically at 550 nm and 37 °C. KP buffer was supplemented with 100 μg protein/ml -1 BHLM, 8 μg/ml -1 rotenone, 50 μM cyt c, and 100 μM ubiquinone, and 5 mM succinate. The reaction was repeated in the presence of 100 units/ml -1 SOD to determine H2O2 production. After using an Apollo-4000 H2O2 electrode (World Precision Instruments) in an open stirred chamber at 37 °C, KP buffer was supplemented with 200 μg protein/ml -1 BHLM, 8 μg/ml -1 rotenone, 5 μM cyt c, and 100 units/ml -1 SOD. To this, 400 mM myxothiazol, 50 μM CoQ9, and 5 mM succinate were added as indicated. The electrode was calibrated with known amounts of H2O2. NO- was measured using an ISO-NO NO electrode (World Precision Instruments). NO- was added open stirred KP buffer, and 20 μg protein/ml -1 BHLM, 8 μg/ml -1 rotenone, 5 μM cyt c, and 10 μM CoQ9, 250 μM 3,3-bis(aminomethyl)-1-hydroxy-2-oxo-1-triazene (DETA-NONOate; 100 mM in 10 mM KOH), 5 mM succinate, 400 mM myxothiazol, and 100 units/ml -1 SOD were added as indicated. The electrode was calibrated with known amounts of S-nitroso-N-acetyl penicillamine (SNAP) to saturated CuCl. NO- production in H2O2-sensitive mitochondria was measured spectrophotometrically by a coupled enzyme assay linking isocitrate production by aconitase to NADP+ reduction by isocitrate dehydrogenase (ε340 NADPH = 6.22 mM -1 cm -1 (59, 60). Aliquots of frozen WT (EGI03 MATa his3 leu2 trpl ura3 yeast mitochondria (61) were thawed rapidly, then washed in 0.6 M mannitol buffer and resuspended in this buffer. The phase transitions were measured by estimating the change in absorbance at 240 nm (1.000 g x 1000 ml -1 x 1000 g) at 37 °C. The mitochondrial protein was determined using the biuret assay with BSA as a standard (54).

**Aconitase Activity—** Aconitase activity was assayed in a 96-well plate with a 10-μl sample added to 190 μl of assay buffer (50 mM Tris-HCl, 0.6 mM MnCl2, 5 mM sodium citrate, 0.2 mM NADP+, 0.1% w/v Trition X-100, and 0.4 unit/ml -1 isocitrate dehydrogenase (pH 7.4) at 30 °C. A450 readings were carried out at 15 s intervals over 7 min in an EL3110 Ultra Microplate Reader (Bio-Tek Instruments). Each time point was measured in quintuplicate, and plotting the natural logarithm of activity versus time linearized the time course of aconitase inactivation. The slope of the line corresponded to the pseudo-first order rate constant of aconitase inactivation. The background rate of NADPH formation was determined in the presence of fluorocitrate (100 μM), a competitive inhibitor of aconitase, and was always less than 10% of the initial rate.

**Efflux of H2O2 from Isolated Rat Heart Mitochondria—** Efflux of H2O2 from isolated rat heart mitochondria was measured using a Shimadzu RF-5301PC fluorimeter in a stirred 3-m1 cuvette thermostatted at 25 or 37 °C. Mitochondria (200 μg protein/ml -1 ) were incubated in 120 mM KC1, 3 mM HEPES-KOH, 1 mM EGTA, and 0.01% (w/v) fatty acid-free BSA (pH 7.2), containing 4 units/ml -1 horseradish peroxidase (HRP), 100 μM Amplex Red (Molecular Probes), and 100 units/ml -1 SOD. 5 mM succinate and 8 μg/ml -1 rotenone were added as indicated. Amplex Red was excited at 560 nm, and its fluorescence was monitored at 590 nm.

**Partition Coefficients—** Octan-1-ol/PBS partition coefficients (the concentration of ubiquinone in octan-1-ol relative to the concentration in PBS) were determined as described previously (30). Membrane/PBS partition coefficients (the concentration of ubiquinone in membranes...
relative to the concentration in PBS) were estimated by incubating 2 ml of PBS containing 250 µg protein·ml⁻¹ BHM and 50 µM ubiquione for 5 min at 37 °C. BHM were pelleted by centrifugation (30 min at 16,000 g), after which the supernatant was removed to a test tube and back-extracted with 1 volume of octan-1-ol. One extraction was sufficient for all ubiquiones except MitoQ and MitoQ₂, which were extracted with 2 ml of octan-1-ol two and three times, respectively. The pellet was fully aspirated to remove as much water as possible, and then all ubiquiones were extracted four times with 100 µl of octan-1-ol.

To estimate the partition coefficient, the inner membrane surface area per unit volume of rat heart mitochondria (61 µm²·µm⁻³) was used along with values for membrane thickness (6 nm) and mitochondrial volume (0.6 µl/mg protein) (62). This gave an estimated membrane volume of 0.22 µl/mg protein.

Thiol Oxidation—Thiol oxidation by H₂O₂ was measured at 37 °C in KP buffer containing 10 mM glucose, 500 µM GSH, 200 µM NADPH, 0.4 unit/ml glutathione reductase, and/or 100 µM decyl-TPP, but not with 10 µM NADPH oxidation. The rate of NADPH oxidation was low in the absence of glutathione reductase and glutathione reductase. Glutathione reductase activity (0.02 unit·ml⁻¹) was in excess of glucose oxidase activity, yet kept to a minimum to allow for reaction of H₂O₂ with MitoQ₁₀ and CoQ₁₀.

For opening of the mitochondrial permeability transition pore (PTP) by t-butylhydroperoxide (tBHP) in isolated rat liver mitochondria, the final centrifugation during mitochondrial isolation was in 250 mM sucrose, 5 mM Tris (pH 7.4, HCl). Measurements of 5'-[3H]butadienyl methionine under argon. Excess NaBH₄ was quenched with 10% (v/v) methanol and this was identical in the presence or absence of H₂O₂.

Exogenous Ubiquinones as Therapies and Tools

The ubiquinol form of MitoQ₁₀ was an ineffective substrate for ubiquinone reductases (Fig. 3), since it fails to restore respiration even after it accumulates in mitochondria. We next focussed on determining why MitoQ₁₀ was ineffective at stimulating respiration in isolated mitochondria, even though it was rapidly accumulated by energized yeast mitochondria (data not shown). This confirms that MitoQ₁₀ is an ineffective electron carrier for respiration and explains its failure to restore yeast growth in non-fermentable media. Surprisingly, CoQ₁₀ was also ineffective at restoring respiration, despite being able to restore growth. However, this was due to its slow uptake by isolated mitochondria as respiration more than doubled (+110% versus intact mitochondria with CoQ₁₀) if mitochondria were sonicated before CoQ₁₀ additions were made (data not shown). Sonication did not increase respiration with CoQ₉ or MitoQ₁₀. Therefore, CoQ₁₀ is presumably too hydrophobic to diffuse rapidly through the mitochondrial outer membrane and stimulate respiration in these short term experiments. In summary we observe three classes of exogenous ubiquinone interaction: CoQ₁₀, CoQ₂, and CoQ₆, which all migrate to mitochondrial intermembrane space and can restore respiration in yeast cells; and MitoQ₁₀, which fails to restore respiration even after it accumulates in mitochondria. We next focussed on determining why MitoQ₁₀ was unable to complement the respiratory defect in the CoQ₁₀-deficient mitochondria.

MitoQ Analogs Are Not Oxidized by Complex III but Are Reduced by Complex II and Glycerol-3-Phosphate Dehydrogenase—The failure of MitoQ₁₀ to stimulate respiration in CoQ₁₀-deficient mitochondria was general to both yeast and mammals, as it did not complement respiration in CoQ₁₀-deficient human fibroblasts, which could be rescued by decyl-TPP. Therefore MitoQ₁₀ is either poorly oxidized by complex III or poorly reduced by mitochondrial ubiquinone reductases (Fig. 3A). To find out which we determined whether the reduced form of MitoQ₁₀ and other short-chain ubiquinols were oxidized by complex III in bovine heart mitochondrial membranes (BHM). The ubiquinol form of MitoQ₁₀ was an ineffective substrate for complex III, while those of CoQ₂, decyl-TPP, and idebenone were

2 P. Rustin, personal communication.
all rapidly oxidized (Fig. 3B). We then measured how effectively the oxidized forms of MitoQ10, CoQ2, decylQ, and idebenone were reduced by complex I, complex II, and G3PDH. MitoQ10 was an ineffective substrate for complex I (Fig. 3C) but was well reduced by complex II (Fig. 3D) and G3PDH (Fig. 3E). In contrast, CoQ2, decylQ, and idebenone were effective substrates for all three ubiquinone reductases (Fig. 3, C–E).

We next determined how the carbon chain length between the TPP and ubiquinone moieties of MitoQ analogs affected their interaction with the respiratory chain (Fig. 3, B–E). None of the MitoQ analogs reacted effectively with complexes I and III. In contrast, MitoQ analogs did react with complex II and G3PDH in a manner that was sensitive to alkyl chain length, with the reduction rate slowing as the chain length decreased from 10 to 3 carbons. Although increasing the carbon chain length to 15 led to an apparent decrease in reduction rate relative to MitoQ10, this may be an artifact due to the low solubility of MitoQ15, as reduction of MitoQ15 at a lower concentration (10 μM rather than 50 μM) by complex II is comparable with MitoQ10 (Fig. 4A).

Reduction of MitoQ10 by succinate is via complex II as it is completely inhibited by the competitive inhibitor malonate and other electron carriers, such as O2, do not mediate it as reduction occurred at a similar rate under anaerobic conditions and in the presence of SOD (data not shown). Direct electron transfer from the reduced endogenous CoQ pool is also unlikely to contribute to the reduction of MitoQ10 as electron transfer between a ubiquinol and a ubiquinone occurs by sequential deprotonation/electron transfer reactions that cannot occur within the phospholipid bilayer (66). Therefore we conclude that the predominant sources of electrons for MitoQ10 in mitochondria are the active sites of ubiquinone reductases. In summary, MitoQ analogs cannot complement defects in respiration because they are poorly oxidized by complex III. The longer chain MitoQ analogs are extensively reduced by complex II and G3PDH but not by complex I.

**MitoQ10 Remains Reduced under Conditions Where CoQ2, DecylQ, and Idebenone Are Oxidized**—In addition to transferring electrons in oxidative phosphorylation, the ubiquinol form of CoQ10 also acts as a chain breaking antioxidant in lipid peroxidation through donation of a hydrogen atom to a carbon or oxygen-centered radical (12). Therefore if an exogenously added ubiquinone is to be an effective antioxidant, its redox state is critical. The poor reactivity of MitoQ10 with complex III...
FIG. 3. Reactivity of MitoQ and other ubiquinone analogs with respiratory chain complexes. 

A, interaction of respiratory chain enzymes with the mitochondrial ubiquinone pool. DHAP, dihydroxyacetone phosphate; UQ, ubiquinone; UQH₂, ubiquinol; Cyt c₉ and Cyt c₉₀, oxidized and reduced cyt c, respectively. 

B, oxidation of ubiquinol analogs (50 μM) by complex III. 

C–E, reduction of ubiquinone analogs (50 μM) by complex I (C), complex II (D), and G3PDH (E). Activity was measured in BHM as described under “Materials and Methods.” The activities of the various ubiquinone analogs are given relative to that of CoQ₂. The values are the means ± S.E. of three independent experiments. DQ, decylQ; Ide, idebenone; MQ₃–15, MitoQ₃–15.
FIG. 4. Ubiquinone redox state on reduction by mitochondrial membranes. A, MitoQ analogs, unlike other short-chain ubiquinones, are predominantly in a reduced form in succinate-energized uninhibited BHM. BHM were supplemented with rotenone, cyt c, fumarase, and 10 μM CoQ2 (a), MitoQ3 (b), MitoQ5 (c), MitoQ10 (d), or MitoQ15 (e). Succinate was added where indicated. The background rate in the absence of ubiquinone was subtracted. B, BHM were supplemented with rotenone, cyt c, and either ethanol (a), 10 μM CoQ2 (b), succinate, myxothiazol, and 10 μM CoQ2 (c), or succinate and 10 μM CoQ2 (d), then incubated for 2 min, extracted, and run on an HPLC that measured A220. C, same as described for B but supplemented with 10 μM MitoQ10 instead of CoQ2. D, reduction state of CoQ2 or MitoQ10 on incubation with BHM. The ratios of the ubiquinol to ubiquinone peak areas for MitoQ10 (filled bars) and CoQ2 (open bars) were determined by HPLC as shown in B and C. Data are means ± S.E. of three independent experiments. E, MitoQ analogs and untargeted ubiquinones remain predominantly oxidized in the presence of an NADH regeneration system. BHM were supplemented with cyt c, NAD+, lactate, and either 10 μM CoQ2 (a), MitoQ3 (b), MitoQ5 (c), MitoQ10 (d), or MitoQ15 (e). Lactate dehydrogenase (LDH) and cyanide were added where indicated. A background rate in the absence of ubiquinone was subtracted. F, the redox state of exogenous ubiquinones in intact liver mitochondria. Mitochondria (200 μg protein·ml⁻¹) were supplemented with rotenone and a 5 μM concentration of either idebenone (a), CoQ2 (b), or MitoQ10 (c). Succinate, FCCP, and myxothiazol were added as indicated. A decrease in A275 indicates ubiquinone reduction, whereas an increase indicates ubiquinol oxidation.
implies that MitoQ10 may be persistently reduced and consequently a better antioxidant than CoQ2, decylQ, or idebenone. To investigate this, we measured the steady-state ubiquinone/ubiquinol ratio for exogenous ubiquinones in the presence of BHM respiring on succinate (Fig. 4A). Under these conditions CoQ2 remained largely oxidized (Fig. 4A, trace a), as did decylQ and idebenone (data not shown). In contrast, MitoQ10 and MitoQ15 (Fig. 4A, traces d and e) were rapidly reduced. MitoQ3 and MitoQ5 (Fig. 4A, traces b and c) were slowly reduced, consistent with their lower rate of reduction by complex II (Fig. 3D). For CoQ2 and MitoQ10, the relative amounts of ubiquinone and ubiquinol were determined by HPLC (Fig. 4, B and C), and the ratios of their peak areas are shown in Fig. 4D (open bars, CoQ2; filled bars, MitoQ10). CoQ2 and MitoQ10 were both largely in the oxidized form on incubation with BHM alone, but on addition of substrate, CoQ2 remained oxidized, while MitoQ10 was reduced to its ubiquinol form. The complex III-inhibitor myxothiazol caused CoQ2 to become reduced but had no effect on MitoQ10 as it was already in the ubiquinol form. The ratio of reduced to oxidized idebenone was qualitatively similar to CoQ2 (data not shown), but co-eluting peaks present in BHM alone prevented precise quantification at 220 nm by HPLC.

That CoQ2, decylQ, and idebenone were all largely oxidized on incubation with mitochondrial membranes was somewhat unexpected as endogenous CoQ10 is ~75–90% reduced in isolated mitochondria during State 4, dropping to 50–60% reduced in State 3 (67, 68). To determine whether this was specific to using succinate as an electron donor, we used NADH to drive reduction through complex I. Under these conditions, MitoQ3 and MitoQ5, as well as CoQ2, decylQ, and idebenone, remained predominantly in the oxidized form (Fig. 4E), while MitoQ10 and MitoQ15 were slightly reduced (Fig. 4E, traces d and e). Ubiquinone reduction by NADH was possible as the respiratory inhibitor cyanide led to the rapid reduction of CoQ2, decylQ, and idebenone and to the gradual reduction of the MitoQ analogs, consistent with their slow reduction by complex I. Therefore the relatively oxidized steady state of the exogenous untargeted ubiquinones is independent of the electron donor and is determined by the relative rates of electron entry to and efflux from the ubiquinone pool.

We next investigated whether the membrane potential in intact rat liver mitochondria largely prevented the oxidation of exogenous ubiquinones by complex III. In contrast to BHM, CoQ2, idebenone, and MitoQ10 were all largely reduced in mitochondria energized with succinate (Fig. 4F). When the membrane potential was collapsed with the uncoupler FCCP, CoQ2 and idebenone rapidly became oxidized but were re-reduced on addition of myxothiazol. DecylQ behaved in a manner similar to CoQ2 and idebenone (data not shown). Therefore the reduced state of exogenous ubiquinones in coupled mitochondria was due to the membrane potential slowing their oxidation by complex III. This behavior was in marked contrast to MitoQ10 as its negligible oxidation by complex III meant that it remained reduced even when the mitochondria were uncoupled. Interestingly, a partial decrease in the membrane potential upon addition of ADP (State 3) did not lead to large scale oxidation of CoQ2 (data not shown), thus ATP synthesis does not cause extensive oxidation of exogenous untargeted ubiquinones, but complete collapse of the membrane potential does.

In summary, the equilibrium redox state of an exogenous ubiquinone is determined by its relative rates of reduction and oxidation (Fig. 3A). The oxidation of MitoQ10, by complex III is negligible, while it is rapidly reduced by complex II, hence MitoQ10 is fully reduced under most conditions. This is not the case for CoQ2, decylQ, and idebenone: although they are fully reduced in coupled mitochondria, they are rapidly oxidized by complex III when the membrane potential is low. This may be critical during pathological conditions where depolarization occurs such as during ischemic injury or following induction of the PTP.

The Ineffective Oxidation of MitoQ10 by Complex III Enhances Antioxidant Protection against Peroxynitrite—The antioxidant efficacy of MitoQ10 is due to its conversion to a ubiquinol, as the ubiquinone is inactive (29, 30). To see if the greater tendency of MitoQ10 to remain in the reduced form enhanced its antioxidant ability, we examined its interaction with the biologically significant oxidant peroxynitrite (ONOO\(^-\)), which is produced in vivo by the reaction of O\(_2^-\) with nitric oxide (NO\(^-\)) (69). Among the oxidizing reactions of ONOO\(^-\) is the one electron oxidation of ubiquinol to a ubisemiquinone radical which then dismutates (70) (Reactions 1 and 2).

\[
2\text{UQ}_2^+ + 2\text{ONOO}^- \rightarrow 2\text{UQ}^+ + 2\text{H}_2\text{O} + 2\text{NO}_2^-
\]

\[
2\text{UQ}^+ + 2\text{H}^- \rightarrow \text{UQ}_2^+ + \text{UQ}
\]

**Reactions 1 and 2**

An advantage of using ONOO\(^-\) as the ubiquinol oxidant is that at pH 7 it decays within a few seconds to unreactive end products (69), leading to a pulse of ubiquinol oxidation after which regeneration of its antioxidant function by re-reduction can be assessed. CoQ2 incubated with BHM respiring on succinate remained in its ubiquinone form (Fig. 5A, trace a), while MitoQ10 was reduced by the respiratory chain (Fig. 5A, trace b). MitoQ3 was also reduced but to a lesser extent due to its slower reaction with complex II (Fig. 5A, trace c). Addition of ONOO\(^-\) led to a sharp upward spike in A\(_{275}\) for all three ubiquinones. For CoQ2 (Fig. 5A, trace a) the transient increase in A\(_{275}\) was solely due to the absorbance of ONOO\(^-\) itself (\(\epsilon_{602} = 1.67 \text{ mm}^{-1} \text{cm}^{-1}\) (57)), which decayed away over ~8 s. For MitoQ3 (Fig. 5A, trace c) there was a transient spike in A\(_{275}\) due to both ONOO\(^-\) itself and the formation of oxidized MitoQ3. After the ONOO\(^-\) had decayed away, A\(_{275}\) decreased as the ubiquinone was slowly reduced back to the ubiquinol. For MitoQ10, there was also a dramatic spike in A\(_{275}\) due to both ONOO\(^-\) and ubiquinol oxidation, but in this case the ubiquinone was re-reduced back to the ubiquinol rapidly by complex II (Fig. 5A, trace b). This oxidation and re-reduction of MitoQ10 by ONOO\(^-\) could be repeated several times and two such reaction cycles are shown in Fig. 5A. The re-reduction of the ubiquinone was by complex II, as malonate prevented reduction of MitoQ3 and MitoQ10 after addition of ONOO\(^-\) (Fig. 5A).

The slower reduction of MitoQ3 by complex II enabled ONOO\(^-\) decay and ubiquinone reduction to be easily distinguished in Fig. 5A as a biphasic change in A\(_{275}\) after addition of ONOO\(^-\). The biphasic nature of MitoQ10 re-reduction after ONOO\(^-\) addition was not obvious so the traces from Fig. 5A were expanded to clearly show that the decay in ONOO\(^-\) differs from the re-reduction of MitoQ10 (Fig. 5B). The base lines of the traces have been aligned to emphasize the relative changes in A\(_{275}\). For CoQ2 ± malonate (traces c and d) and for MitoQ10 + malonate (trace b), the addition of ONOO\(^-\) leads to an increase in A\(_{275}\) that decays back to base line over ~8 s due to the breakdown of ONOO\(^-\). In contrast, addition of ONOO\(^-\) to MitoQ10 in the presence of uninhibited BHM (trace a) is biphasic with an initial decay in A\(_{275}\) due to ONOO\(^-\) that is followed by a slower decrease in A\(_{275}\) due to reduction of the ubiquinone formed by ONOO\(^-\) oxidation. Idebenone behaved in the same way as CoQ2 (data not shown).

To confirm that the ubiquinol forms of idebenone and CoQ2 could also react with ONOO\(^-\), we repeated these experiments in myxothiazol-inhibited BHM. Addition of succinate led to the
complete reduction of idebenone (Fig. 5C, trace a) and MitoQ10 (Fig. 5C, trace b). ONOO\(^-\) rapidly oxidized these ubiquinols, and this was reversed by the respiratory chain within ~20 s in a malonate-sensitive fashion. CoQ\(_2\) behaved similarly to idebenone (data not shown). Thus, the ubiquinol forms of all exogenous ubiquinones can be oxidized by ONOO\(^-\) and then recycled by the respiratory chain.

We next tested whether MitoQ10 would be more protective against ONOO\(^-\)-induced lipid peroxidation than CoQ\(_2\) and idebenone in uninhibited BHM. For this we used PA, a conjugated polyunsaturated fluorescent fatty acid that loses its fluorescence upon peroxidation. Sequential additions of ONOO\(^-\) to BHM respiring on succinate caused step decreases in PA fluorescence (Fig. 5D, trace c), and MitoQ10 protected against this loss (Fig. 5D, trace a). Idebenone also protected against the loss of PA fluorescence (Fig. 5D, trace b); however, the protection was significantly less than that given by MitoQ10. The background decay of PA was unaffected by ubiquinones, suggesting that it is not related to lipid peroxidation (Fig. 5D, traces d–f).

CoQ\(_2\) behaved like idebenone, while MitoQ10 in the absence of succinate offered no protection (data not shown).

In summary, the reduced form of MitoQ10 is an effective antioxidant against ONOO\(^-\), and its slow oxidation by complex III makes it a more effective antioxidant than untargeted
ubiquinone analogs. Importantly, the respiratory chain can reduce MitoQ10 repeatedly recycling it back to its active antioxidant form after it has detoxified ONOO⁻/H₂O₂.

Ubiquinols Are Oxidized by Superoxide—Ubiquinones and ubiquinols as well as their partially protonated and reduced intermediates undergo a complex set of reactions with oxygen and O₂⁻. Oxygen can react with ubiquinols and ubisemiquinones to produce O₂⁻; conversely, O₂⁻ generation is dependent on deprotonation of ubiquinol to the ubiquinolate anion (UQ⁻). UQ, ubiquinone; UQ⁺, ubisemiquinone anion; UQH²⁻, ubisemiquinone radical. The deprotonation reaction occurs in the aqueous phase and is thus inhibited by ubiquinol hydrophobicity. B, ubiquinols are slowly oxidized by O₂⁻ in aqueous solution. Reduced MitoQ10 (50 μM) was incubated in KP buffer with 0.015 unit/ml xanthine oxidase at pH 7.8 (a) or pH 6.8 (b). Acetaldehyde (5 mM) and SOD (100 units/ml) were added as indicated. C, ubiquinols are rapidly oxidized by O₂⁻ in organic solvent. 25 μl of ~10 mM KO₂ in 10 mM 18-crown-6 ether (a) or 25 μl of 10 mM 18-crown-6 ether (b) or 50 μl of 1:1 ~10 mM KO₂ in 10 mM 18-crown-6 ether: H₂O incubated for 2 min (c) was added to 50 μM reduced MitoQ₁₀ in 2.5 ml of PBS-saturated octan-1-ol. 25 μl of 10 mM KO₂ in 10 mM 18-crown-6 ether was added to a 50 μM concentration of the reduced form of MitoQ₁₀ in 2.5 ml of KP, (pH 7.4) (d). Inset, before and after the addition of KO₂ to MitoQ₁₀ in trace a. KO₂ or equivalent additions were made as indicated.

Exogenous Ubiquinones as Therapies and Tools

Fig. 6. Consumption of O₂⁻ by ubiquinols. A, proposed reactions through which ubiquinols both generate and consume O₂⁻. Ubiquinol (UQH₂) directly consumes O₂⁻/H₂O₂ in the membrane or aqueous phase to generate H₂O₂. O₂⁻ generation is dependent on deprotonation of ubiquinol to the ubiquinolate anion (UQ⁻). UQ, ubiquinone; UQ⁺, ubisemiquinone anion; UQH²⁻, ubisemiquinone radical. The deprotonation reaction occurs in the aqueous phase and is thus inhibited by ubiquinol hydrophobicity. B, ubiquinols are slowly oxidized by O₂⁻ in aqueous solution. Reduced MitoQ₁₀ (50 μM) was incubated in KP buffer with 0.015 unit/ml xanthine oxidase at pH 7.8 (a) or pH 6.8 (b). Acetaldehyde (5 mM) and SOD (100 units/ml) were added as indicated. C, ubiquinols are rapidly oxidized by O₂⁻ in organic solvent. 25 μl of ~10 mM KO₂ in 10 mM 18-crown-6 ether (a) or 25 μl of 10 mM 18-crown-6 ether (b) or 50 μl of 1:1 ~10 mM KO₂ in 10 mM 18-crown-6 ether: H₂O incubated for 2 min (c) was added to 50 μM reduced MitoQ₁₀ in 2.5 ml of PBS-saturated octan-1-ol. 25 μl of 10 mM KO₂ in 10 mM 18-crown-6 ether was added to a 50 μM concentration of the reduced form of MitoQ₁₀ in 2.5 ml of KP, (pH 7.4) (d). Inset, before and after the addition of KO₂ to MitoQ₁₀ in trace a. KO₂ or equivalent additions were made as indicated.

ubiquinone analogs. Importantly, the respiratory chain can reduce MitoQ₁₀ repeatedly recycling it back to its active antioxidant form after it has detoxified ONOO⁻.

Ubiquinols Are Oxidized by Superoxide—Ubiquinones and ubiquinols as well as their partially protonated and reduced intermediates undergo a complex set of reactions with oxygen and O₂⁻. Oxygen can react with ubiquinols and ubisemiquinones to produce O₂⁻; conversely, O₂⁻ generation is dependent on deprotonation of ubiquinol to the ubiquinolate anion (UQ⁻). UQ, ubiquinone; UQ⁺, ubisemiquinone anion; UQH²⁻, ubisemiquinone radical. The deprotonation reaction occurs in the aqueous phase and is thus inhibited by ubiquinol hydrophobicity. B, ubiquinols are slowly oxidized by O₂⁻ in aqueous solution. Reduced MitoQ₁₀ (50 μM) was incubated in KP buffer with 0.015 unit/ml xanthine oxidase at pH 7.8 (a) or pH 6.8 (b). Acetaldehyde (5 mM) and SOD (100 units/ml) were added as indicated. C, ubiquinols are rapidly oxidized by O₂⁻ in organic solvent. 25 μl of ~10 mM KO₂ in 10 mM 18-crown-6 ether (a) or 25 μl of 10 mM 18-crown-6 ether (b) or 50 μl of 1:1 ~10 mM KO₂ in 10 mM 18-crown-6 ether: H₂O incubated for 2 min (c) was added to 50 μM reduced MitoQ₁₀ in 2.5 ml of PBS-saturated octan-1-ol. 25 μl of 10 mM KO₂ in 10 mM 18-crown-6 ether was added to a 50 μM concentration of the reduced form of MitoQ₁₀ in 2.5 ml of KP, (pH 7.4) (d). Inset, before and after the addition of KO₂ to MitoQ₁₀ in trace a. KO₂ or equivalent additions were made as indicated.

Exogenous Ubiquinones as Therapies and Tools
To see if Reaction 3 could lead to a direct antioxidant effect of exogenous ubiquinones on \( O_2^- \) we first measured oxidation of the ubiquinol form of MitoQ10 at 275 nm by \( O_2^- \) generated from acetalddehyde and xanthine oxidase in aqueous buffer. Generation of \( O_2^- \) caused slow oxidation of reduced MitoQ10 that could be fully blocked by SOD (Fig. 6B). Although \( O_2^- \) can react with ubiquinol in aqueous buffer, spontaneous dismutation to hydrogen peroxide (\( H_2O_2 \)) appears to dominate as the rate of ubiquinol oxidation was low relative to the rate of \( O_2^- \) production. Reaction 3 is also likely to occur within phospholipid bilayers and could thereby provide a mechanism for detoxifying \( HO_2^- \) that is inaccessible to SOD. This would be expected to be important in tissues such as the heart, where cristae phospholipids occupy a volume similar to the aqueous mitochondrial matrix (62). To investigate this we added \( \sim 100 \mu M \) potassium superoxide (\( KO_2 \)) to a 50 \( \mu M \) concentration of the reduced form of MitoQ10 in PBS-saturated octan-1-ol. \( KO_2 \) rapidly oxidized reduced MitoQ10 (Fig. 6C, trace a). Oxidation of reduced MitoQ10 was specific to \( O_2^-/HO_2^- \) as it was not oxidized by carrier or by \( KO_2 \) that was previously decomposed to \( H_2O_2 \) (Fig. 6C, traces b and c). Furthermore, reduced MitoQ10 in aqueous buffer was not oxidized by \( KO_2 \) due to its rapid dismutation to \( H_2O_2 \) (Fig. 6C, trace d). Therefore these results show that ubiquinols are likely to be effective scavengers of \( O_2^- \) when it diffuses into phospholipid bilayers as \( HO_2^- \). While several variations of Reaction 3 with different protonation states of the reactants could contribute to this, their net effect would be similar: ubiquinol oxidation, \( H_2O_2 \) generation, and a lower steady-state concentration of \( O_2^-/HO_2^- \).

**Ubiquinol Autoxidation Requires Deprotonation and Is Decreased by Ubiquinol Hydrophobicity**—While the ubiquinol form of MitoQ10 is not oxidized by complex III, like all other exogenous ubiquinols it can be oxidized directly by oxygen to form \( O_2^- \) in vitro. The transfer of electrons from ubiquinol to cyt c has been studied extensively (43, 66, 72). From this it can be concluded that the direct donation of an electron by ubiquinol to oxygen is unlikely (\( UQH_2^-/UQH_2^- \), \( E_m,7 > 850 \text{ mV} \) (66)). Instead, electron transfer from ubiquinol requires an initial deprotonation to a ubiquinolate anion (\( \rho K_7, 13.3 (66) \), and this is the likely electron donor to oxygen (\( UQH/UQH^- \), \( E_m,7 = 190 \text{ mV} \) (66); see Reactions 4 and 5).

\[
\text{UQH}_2^- + \text{HO}_2^- \rightarrow \text{UQH}^- + \text{H}_2\text{O}
\]

**REACTION 3**

The ubiqusemiquinone radical formed can also react with oxygen to form \( O_2^- \) or it can dismutate, but it is the initial reaction between the ubiquinolate and oxygen that is likely to be rate-limiting for autoxidation (Fig. 6A). To see if this model would account for ROS production by exogenous ubiquinols, we assessed the pH sensitivity of ubiquinol autoxidation by measuring the increase in ubiquinone absorption at 275 nm. There was negligible oxidation of the reduced form of MitoQ10 in aqueous solution at pH 6.8, but autoxidation increased a little at pH 7.8 and dramatically at pH 8.3 (Fig. 7A). Consistent with Fig. 6A, ubiquinol autoxidation was SOD-insensitive. To demonstrate that \( O_2^- \) generation occurred during ubiquinol autoxidation we used acetylated cyt c (cyt \( c_{acet} \)), which is readily reduced by one electron transfer from \( O_2^- \) (73) and ubisemiquinones (43, 72) but whose reduction and oxidation by the respiratory chain are limited (73). In aqueous buffer the ubiquinol, but not the ubiquinone, forms of CoQ and MitoQ10 reduced cyt \( c_{acet} \), and this reduction occurred primarily via \( O_2^- \) as the rate was 80–90% SOD-sensitive (data not shown). To demonstrate \( O_2^- \) generation during autoxidation of complex II-reduced MitoQ10, we measured cyt \( c_{acet} \) reduction in myxothiazol-inhibited BHM (Fig. 7B). This showed that reduction of MitoQ10 by complex II also caused cyt \( c_{acet} \) reduction and that this rate increased with pH from pH 6.8 to 8.3. This pH dependence was not due to changes in MitoQ10 reduction by complex II as this rate was identical at pH 6.8 and 8.3 (data not shown). Therefore \( O_2^- \) production from exogenous ubiquinols produced chemically or by mitochondrial respiration is pH-dependent, consistent with ubiquinol deprotonation being critical for autoxidation.

As deprotonation creates two charged species, autoxidation will predominantly occur in the aqueous phase rather than within phospholipid bilayers and its rate should be inversely proportional to ubiquinol hydrophobicity. To investigate this we measured ubiquinol autoxidation in myxothiazol-inhibited BHM using a range of MitoQ and non-targeted ubiquinone analogs with a spectrum of hydrophobicities (Fig. 7C). All the exogenous ubiquinones reduced cyt \( c_{acet} \) (Fig. 7C), and in all cases this rate of reduction was about \( \sim 50\% \) inhibitable by SOD (data not shown). The rate of \( O_2^- \) production from autoxidizing untargeted ubiquinones was inversely proportional to their octan-1-ol/PBS partition coefficients (Fig. 1B). The lowest levels of \( O_2^- \) were generated by the most hydrophobic ubiquinol, decyIQ, with the most water-soluble, idebenol, producing the most \( O_2^- \) and CoQ9 being intermediate. There was a similar inverse correlation with hydrophobicity for the MitoQ analogs from MitoQ9 to MitoQ15. MitoQ9 was an exception to this trend, possibly due to its slow reduction by complex II leading to a lower ubiquinol concentration (Fig. 3C). While this inverse relationship between autoxidation and hydrophobicity held within the two groups, the MitoQ analogs were less prone to autoxidation than more hydrophobic non-targeted ubiquinones (e.g. MitoQ10 versus idebenone). MitoQ analogs are charged cations, while untargeted ubiquinones are neutral, so partitioning into octan-1-ol may not accurately reflect binding to phospholipid bilayers. Therefore we measured the relative binding of MitoQ analogs and untargeted ubiquinones to mitochondrial membranes (Fig. 7D) and found that increased binding of MitoQ analogs to phospholipid bilayers could explain their lower rate of autoxidation when compared with equivalent untargeted ubiquinones.

In summary, all ubiquinols are autoxidized, but this requires an initial deprotonation to a ubiquinolate anion, which is unfavorable within the hydrophobic environment of a phospholipid bilayer. Therefore there are two major determinants of the degree to which ubiquinol will autoxidize: its extent of reduction and its hydrophobicity. Furthermore, the charged TPP moiety of MitoQ analogs may be a better way of lowering overall hydrophobicity and improving pharmacokinetics without enhancing the tendency to autoxidation, as it leads to adsorption onto phospholipid bilayers and insertion of the ubiquinol moiety into the hydrophobic core of the membrane.

**Ubiquinol Autoxidation Produces Superoxide, Which Can Dismutate to Hydrogen Peroxide or Consume Nitric Oxide**—In the above analysis, \( O_2^- \) generation during ubiquinol autoxidation was assessed using cyt \( c_{acet} \) reduction, but cyt \( c_{acet} \) can be reduced by \( O_2^- \) and by the ubiqusemiquinone radical (43, 66, 72, 73), both of which may form during ubiquinol autoxidation. Furthermore, even though the reduction of cyt \( c_{acet} \) was \( \sim 50\% \) SOD-sensitive, it is still difficult to conclude a dominant role for \( O_2^- \) as the steady-state concentration of the ubiqusemiquinone radical decreases as a consequence of consuming \( O_2^- \) by dismu-
In addition, cyt c acet itself may act as an O2 sink and thus distort the relative rates and routes of ubiquinol autoxidation. Therefore to confirm that autoxidation of exogenous ubiquinol produced O2, we used a H2O2 electrode to measure the production of H2O2 from the dismutation of O2 (4). Measurement of H2O2 is of further significance as MitoQ10 has been used as a radical scavenger in biological systems. In the present study, we investigated the autoxidation of ubiquinol in vitro and in intact mitochondria using MitoQ10 as a model compound. Our results provide new insights into the mechanism of autoxidation and the role of ubiquinol in oxygen metabolism.

**Fig. 7. Generation of O2•− by ubiquinols.**

A. Ubiquinol autoxidation increases with pH. Oxidation of the ubiquinol form of MitoQ10 in KP buffer at: pH 8.3 (a), pH 7.8 (b), or pH 6.8 (c). NaBH4-reduced MitoQ10 (50 μM) was added as indicated. SOD (100 units/ml) was added as indicated to trace a. B, ubiquinol-dependent reduction of cyt c rat increases with pH. a–d, reduction of cyt c rat by BHM supplemented with myxothiazol, KCN, rotenone, cyt c acet, and 10 μM MitoQ10 at: pH 8.3 (a), pH 7.8 (b), pH 7.3 (c), or pH 6.8 (d). C, increasing ubiquinol hydrophobicity decreases reduction of cyt c rat. a–g, reduction of cyt c rat by inhibited BHM supplemented with myxothiazol, KCN, rotenone, and cyt c acet and 10 μM idebenone (a), CoQ2 (b), decylQ (c), MitoQ3 (d), MitoQ5 (e), MitoQ10 (f), or MitoQ15 (g). Succinate was added as indicated. D, ubiquinol-dependent reduction of cyt c rat is partially SOD-sensitive. E, MitoQ analogs partition more strongly into BHM than into octan-1-ol. Ubiquinones (50 μM) were incubated in PBS (2 ml) containing BHM (500 μg of protein) at 37 °C for 5 min followed by centrifugation. The relative concentration in each phase was determined after extraction into octan-1-ol. BHM phospholipid volume was taken as 0.22 ± 0.01 ml/g protein. BHM/PBS partition coefficients are the means ± range (n = 2). E, ubiquinone autoxidation generates H2O2. a–c, H2O2 production from BHM supplemented with rotenone, cyt c, SOD (100 units/ml) and either myxothiazol (a), 50 μM CoQ2 (b), or myxothiazol and 50 μM CoQ2 (c). Succinate was added where indicated. F, ubiquinone autoxidation consumes NO. a and b, NO consumption by BHM (20 μg protein/ml) supplemented with rotenone, cyt c, and either ethanol (a) or 10 μM CoQ2 (b). DETA-NONOate, succinate, myxothiazol, and SOD were added as indicated.
shown to block a number of redox signaling and apoptotic pathways, but the mechanism remains unclear (35–37, 39–42). BHM alone did not produce H$_2$O$_2$ when inhibited by myxothiazol (Fig. 7E, trace a) nor did BHM supplemented with CoQ$_2$ and succinate (Fig. 7E, trace b). However, inhibition of ubiquinol oxidation with myxothiazol (Fig. 7E, trace c) or cyanide (data not shown) led to a build up of ubiquinol and subsequent H$_2$O$_2$ production. MitoQ$_{10}$ also produced H$_2$O$_2$ but did so in the absence of myxothiazol as it was already fully reduced (data not shown). Therefore autoxidation of ubiquinols does produce O$_2^*$ that will dismutate to H$_2$O$_2$ and this could be important in redox signaling.

NO' is a signaling molecule produced by NO' synthases, and their activity may be associated with mitochondria and is important for mitochondrial biogenesis (75–77). NO' interacts with mitochondria by reversibly inhibiting complex IV (78), as well as affecting the function of proteins via S-nitrosylation of cysteine residues (79). Therefore we next investigated the interaction of exogenous ubiquinols with NO', which may react directly with ubiquinols to produce NO' or with ubiquinol-generated O$_2^*$ to form ONOO$^-$ (80). For this the steady-state NO' concentration produced by the NO' donor, DETA-NONOate, in the presence of BHM was measured using an NO' electrode (Fig. 7F). DETA-NONOate gave a steady-state NO' concentration of $-400$ nM after 8–10 min that persisted for a further 10–20 min (Fig. 7F, trace a). BHM supplemented with CoQ$_2$ consumed NO' at an increased rate upon addition of succinate, and addition of myxothiazol led to the complete depletion of NO' that could be partially reversed by SOD (Fig. 7F, trace b). MitoQ$_{10}$ also consumed NO' to a similar extent, but as it is present in the reduced form, it consumed NO' rapidly even in the absence of myxothiazol (data not shown). Therefore the autoxidation of exogenous ubiquinols generated by mitochondrial respiration does lead to the formation of O$_2^*$ that can react with NO' or dismutate to H$_2$O$_2$.

MitoQ Analogs Cause Efflux of Hydrogen Peroxide from Mitochondria, but Only MitoQ$_3$ Damages Aconitase—The above findings show that all exogenous ubiquinols have the potential to autoxidize and form O$_2^*$ in aqueous solution. To determine whether ubiquinol autoxidation led to significant O$_2^*$ generation within intact mitochondria, we measured the efflux of H$_2$O$_2$ from isolated rat heart mitochondria in the presence of 1 mM exogenous ubiquinol. H$_2$O$_2$ efflux is due to the dismutation of O$_2^*$ to H$_2$O$_2$, which can then diffuse through the mitochondrial inner membrane (81) and be detected using Amplex Red and horseradish peroxidase (Fig. 8A). A significant rate of H$_2$O$_2$ efflux was observed in the presence of rotenone and a 1 mM concentration of either MitoQ$_3$, MitoQ$_5$, or MitoQ$_{10}$ (Fig. 8A, traces b–d). As expected, the presence of exogenous SOD had no effect on H$_2$O$_2$ efflux from MitoQ$_3$ (data not shown). In BHM exogenous ubiquinols inhibit the horseradish peroxidase detector with other mechanisms of H$_2$O$_2$ production. Isolated rat heart mitochondria (0.2 mg protein$^{-1}$) were incubated at 27 °C with Amplex Red, horseradish peroxidase, SOD, and either ethanol (a), 1 mM MitoQ$_3$ (b), 1 mM MitoQ$_5$ (c), 1 mM MitoQ$_{10}$ (d), or 1 mM MitoQ$_{15}$ (e). Succinate was added where indicated. The c, aconitase inactivation was plotted as time versus the natural logarithm of activity and given a linear fit. The slope of this line corresponds to the pseudo-first order rate constant of aconitase inactivation. Data are the mean ± S.D. of three independent experiments. Statistical significance was calculated relative to G3P alone using a Student’s one-tailed t test. *p < 0.05, ***, p < 0.001.
tion system (data not shown), consequently the accumulation of ubiquinol may cause the apparent decrease in H$_2$O$_2$ efflux with time. Therefore, the initial rate of H$_2$O$_2$ efflux is the best indication of autoxidation, and this is significantly lower for the more hydrophobic MitoQ analogs. To gauge the relative magnitude of MitoQ$_{10}$ autoxidation, we compared it with other known mechanisms for generating H$_2$O$_2$ efflux at 37 °C (Fig. 8 B). This showed that H$_2$O$_2$ generation by 1 μM MitoQ$_{10}$ was equivalent to high micromolar concentrations of the redox cycler Paraquat and greater than the proton motive force-dependent and rotenone-sensitive efflux of H$_2$O$_2$ from succinate-energized mitochondria (82) (Fig. 8B).

To determine whether this level of intramitochondrial O$_2$ production was damaging, we incubated intact yeast mitochondria with MitoQ analogs and studied their effects on aconitase activity, the iron-sulfur center of which is particularly sensitive to O$_2$ (60). The pseudo-first order rate constant for aconitase inactivation within mitochondria was taken as an indication of the steady-state matrix O$_2$ concentration (Fig. 8C). Aconitase inactivation responded appropriately to factors that decrease O$_2$ (no substrate, uncoupler) or increase O$_2$ (Paraquat), therefore this system can detect variations in endogenous O$_2$ level (Fig. 8C). Addition of 1 μM MitoQ$_{10}$ caused a small but statistically significant increase in aconitase inactivation above that of substrate alone. In contrast, 1 and 5 μM MitoQ$_{10}$ decreased the rate of aconitase inactivation, but this was due to mild uncoupling as decyl-TPP gave a similar result (Fig. 8C and data not shown). Therefore, although MitoQ analogs can generate O$_2$ within mitochondria, this rate appears too low to cause significant damage to O$_2$-sensitive mitochondrial enzymes.

Exogenous Ubiquinones Do Not React with Peroxides—MitoQ$_{10}$ blocks H$_2$O$_2$-induced apoptosis and cell death, but the details of how this is achieved remain unclear (29, 41, 42). H$_2$O$_2$ can cause oxidative damage through Fenton chemistry with Fe$^{3+}$ or Cu$^+$ as well as being a diffusible signaling molecule that reacts with protein thiols (4, 5). As direct reaction of H$_2$O$_2$ or alkyl peroxides with ubiquinones and ubiquinols has not been reported under physiological conditions, it was important to clarify whether they react with MitoQ$_{10}$ and other exogenous ubiquinones. A lack of direct reaction of the ubiquinol form of MitoQ$_{10}$ with H$_2$O$_2$ was confirmed by $^1$H NMR measurement of the oxidation of 10 mM MitoQ$_{10}$ in acidified D$_2$O. Even incubation with 10 mM H$_2$O$_2$ under air over several days did not result in H$_2$O$_2$-sensitive oxidation of reduced MitoQ$_{10}$ (data not shown).

As many of the signaling effects of H$_2$O$_2$ within mitochondria occur through its interactions with thiols, we next determined whether MitoQ$_{10}$ could affect the oxidative reaction of reactive biological thiols by H$_2$O$_2$. To do this we incubated the ubiquinol form of MitoQ$_{10}$ in the presence of reduced GSH and glutathione peroxidase and exposed it to a flux of H$_2$O$_2$ generated by glucose oxidase (0.006 units/ml) and 10 mM glucose in the presence of glutathione peroxidase (0.02 units/ml$^{-1}$), 500 μM GSH, NADPH, and glutathione reductase. Reduced MitoQ$_{10}$ was added as indicated. B, the mitochondrial PTP is triggered by tBHP. Liver mitochondria were incubated in the presence of succinate, rotenone, calcium green-5N and either no additions (a), 5 μM tBHP (b), or 5 μM tBHP and 500 μM Ca$^{2+}$ (c). 5 μM Ca$^{2+}$ was added where indicated. C, induction of the PTP by tBHP is not blocked by MitoQ$_{10}$. Liver mitochondria were incubated in the presence of succinate, rotenone, calcium green-5N, 1 μM MitoQ$_{10}$, and either no addition (a), 5 μM tBHP (b), or 5 μM tBHP and 500 nM CsA (c). 5 μM Ca$^{2+}$ was added where indicated.

As many of the signaling effects of H$_2$O$_2$ within mitochondria occur through its interactions with thiols, we next determined whether MitoQ$_{10}$ could affect the oxidative reaction of reactive biological thiols by H$_2$O$_2$. To do this we incubated the ubiquinol form of MitoQ$_{10}$ in the presence of reduced GSH and glutathione peroxidase and exposed it to a flux of H$_2$O$_2$ generated by glucose oxidase. The H$_2$O$_2$ from glucose oxidase oxidized GSH to GSSG catalyzed by glutathione peroxidase, and GSSG was detected through NADPH consumption by glutathione reductase (Fig. 9A). Neither the reduced nor oxidized forms of MitoQ$_{10}$ (Fig. 9A and data not shown) affected GSSG oxidation (Fig. 9A) confirming that exogenous ubiquinones neither directly scavenge H$_2$O$_2$ nor interfere with the reaction of H$_2$O$_2$ with biological thiols. We next investigated the induction of the PTP by Ca$^{2+}$ and tBHP as this involves oxidation of critical protein thiols (83). PTP opening in Ca$^{2+}$-loaded rat liver mitochondria was induced by 5 μM tBHP and led to the release of accumulated Ca$^{2+}$ (Fig. 9B, trace b). This process could be blocked by CsA (Fig. 9B, trace c). When the experiment was repeated in the presence of either 1 μM (Fig. 9C) or 5 μM MitoQ$_{10}$ (data not shown), PTP opening was still triggered by 5 μM tBHP (Fig. 9C, trace b) and blocked with CsA (Fig. 9C, trace c). The extent of Ca$^{2+}$ uptake was decreased compared to the system without MitoQ$_{10}$, but the kinetics of PTP induction were similar. Thus MitoQ$_{10}$ cannot scavenge alkyl peroxides and prevent PTP opening caused by thiol oxidation.

In summary, MitoQ$_{10}$ does not react directly with peroxides or affect the interaction of peroxides with biological thiols. Therefore the potent blocking of exogenous peroxide-dependent reactions by MitoQ$_{10}$ (29, 41, 42) occurs downstream of the peroxide itself. As MitoQ$_{10}$ blocks lipid peroxidation (29, 30), it is likely that the effects of MitoQ$_{10}$ on exogenous peroxides are due to chain termination of lipid peroxidation, which is probably induced by Fenton chemistry of H$_2$O$_2$ in combination with ferrous iron.

**DISCUSSION**

This study has clarified the redox, antioxidant, and pro-oxidant properties of a series of mitochondria-targeted and
untargeted exogenous ubiquinones. While CoQ1, CoQ2, decylQ, CoQ6, and idebenone could all restore respiration in mitochondria lacking CoQ, MitoQ10 could not. The ability of the CoQ analogs to restore respiration was consistent with their fast reduction by ubiquinone reductases and their rapid oxidation by complex III. In contrast, none of the MitoQ analogs could act as electron carriers in respiration because they were not oxidized by complex III. While all the MitoQ analogs rapidly migrate through the mitochondrial inner membrane (30), the hydrophobic core is still a significant activation energy barrier for their transport, albeit a far lower one than that for equivalent hydrophilic cations (32, 33). Furthermore, although MitoQ10 has a similar octan-1-ol/PBS partition coefficient to idebenone, MitoQ analogs are amphipathic and consequently not evenly distributed within phospholipid bilayers (Fig. 1C) (32, 33). Thus, for MitoQ analogs the steady-state concentration of the ubiquinone moiety at a particular distance from the membrane surface will be related to the length of the alkyl chain. This is in marked contrast to the untargeted ubiquinones, which will be freely soluble within the hydrophobic core. This affinity of MitoQ analogs for the surface of the phospholipid bilayer is simply demonstrated by its limited solubility in cyclohexane, an organic solvent that mimics the membrane core: MitoQ10 formed a separate, orange-red, oily phase, with only some slight discoloration of the cyclohexane. In contrast, idebenone was freely soluble up to at least 4 mM in cyclohexane, as were MitoQ10 and idebenone in octan-1-ol, a more polar solvent that mimics the membrane surface. Therefore, the significantly decreased reactivity of all MitoQ analogs with complex III may be a consequence of the low concentration of their ubiquinone moieties in the active sites of complex III (Fig. 10A) (64, 84). These are near the center of the phospholipid bilayer, and for MitoQ analogs to dock into them, the TPP cation must be located in the hydrophobic core (Fig. 10A). In addition, enzyme shape and dimerization may further increase the effective distance between these binding sites and the surface of the phospholipid bilayer (64, 84). Therefore, the steady-state concentration of MitoQ analogs within the membrane core is decreased relative to other ubiquinones, and this is likely to explain their low reactivity with complex III.

A related explanation may account for the increasing reduction of MitoQ analogs by complex II and G3PDH on increasing the length of the carbon chain connecting the TPP and ubiquinone moieties (Fig. 3, D and E). The ubiquinone reduction site of complex II is close to the membrane surface, and it is possible to dock the ubiquinone moiety of MitoQ10 into the active site.
without the TPP cation dipping into the membrane (Fig. 10B). In contrast, the alkyl linkers for MitoQs and MitoQs appear to be too short to span the distance from the active site to the membrane surface (Fig. 10, C and D). This offers a plausible explanation for the greater reactivity of MitoQ10 with complex II compared with MitoQs and MitoQs, although with complex II it is not possible to exclude a contribution from hydrophobicity or steric hindrance. If this explanation of the observed reactivities is correct, it may lead to the development of alkyl TPP derivatives as useful probes of membrane proteins. For example, MitoQ10 was poorly reactive with complex I but did react well with G3PDH, suggesting that the ubiquinone binding site of G3PDH may be closer to the membrane surface, while that of complex I is nearer the hydrophobic core of the membrane.

The effects of MitoQ analogs are not due to their ability to complement respiration. In contrast, untargeted ubiquinones should be able to supplement respiratory defects in vivo provided they can be delivered to mitochondria within cells. In our experiments only the ubiquinones with isoprenoid tails, CoQ6, CoQ8, and CoQ10, could restore non-fermentative growth in CoQ-deficient yeast, while the exogenous ubiquinones with simple saturated carbon chains were ineffective. As this difference is not due to poor electron transfer in the respiratory chain, there may be lower mitochondrial accumulation of the saturated relative to the isoprenoid exogenous ubiquinones. This is not due to differences in passive diffusion as CoQ6 is saturated relative to the isoprenoid exogenous ubiquinones.

The autoxidation of ubiquinols is well established, but its nature varies with the ubiquinone used and the conditions applied. In cell damage and signaling (95). This work shows that while antioxidation of exogenous ubiquinones generates subtoxic levels of ROS, this lack of antioxidation-dependent damage is consistent with clinical trials using idebenone that have shown it is well tolerated for at least 2 years (28, 88–90), even though it has a high tendency to autoxidize in vitro (71). While the focus of studies on ROS is often their toxicity, it is possible that antioxidation of exogenous ubiquinones can be beneficial in vivo as it is associated with a large increase in membrane associated antioxidant capacity, against both lipid peroxidation and O2−, and can lead to the induction of antioxidative enzymes and antioxidative pathways via hormesis (4, 91). The complex nature of ROS interactions is illustrated by the paradoxical fact that 20 μM H2O2, antioxidation-resistant ascorbate derivatives, and MitoQ10 all extend the lifespan of cultured cells and decrease telomere shortening (39, 91). Therefore, it remains unclear whether exogenous ubiquinone antioxidation is detrimental or beneficial.

MitoQ10 has been shown to block a number of redox signaling pathways as well as cell death induced by exogenous H2O2 (29, 41, 42); however, the details of the processes affected are uncertain. MitoQ10 does not react significantly with H2O2 or other alkyl peroxides (Fig. 9) but may be very effective against lipid peroxidation (29, 30). This suggests that the effects of MitoQ10 against H2O2 are primarily due to blocking the consequences of OH· presumably formed from iron-catalyzed Fenton chemistry. The presence OH· within mitochondria results in lipid peroxidation, which has a number of deleterious effects on mitochondrial function and leads to breakdown products, such as the reactive aldehyde 4-hydroxy-2-nonenal, which may have a role in cell damage and signaling (95). This work shows that while MitoQ10 could interact with ROS in several ways in vivo, the most likely way is that it blocks the effects of exogenous H2O2 by acting downstream of H2O2 to inhibit peroxidative chain reactions.

Here we have investigated how a series of exogenous ubiquinone analogs interact with oxidative phosphorylation and ROS. We found that while the type of reactions that MitoQ and untargeted ubiquinone analogs underwent with ROS were similar, ubiquinone hydrophobicity dramatically affected the extent of these reactions. This work clearly shows that the antioxidation reactions of exogenous ubiquinones will predominantly occur within phospholipid bilayers, while the pro-oxidant reactions require an aqueous environment. Therefore, the relative rates of these reactions can be fine-tuned by hydrophobicity, allowing a rational approach to the design of therapeutically useful compounds that can interact with the mitochondrial respiratory chain. This occurred because the TPP cation decreased the oxidation of MitoQ analogs by complex III. Thus MitoQ analogs function as antioxidation tools while other short-chain ubiquinones can also be oxidative phosphorylation substrates. When used in combination they may provide a way for separating cause and effect in...
mitochondrial DNA diseases, Parkinson disease, and aging where both a decline in oxidative phosphorylation and an increase in ROS may overlap to cause pathology.

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