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Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations

(coenzyme Q/lipid peroxidation/α-tocopherol/heart disease)

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ABSTRACT **It is well known that ubiquinone-10 (coenzyme Q_{10}, ubiquinone 50) acts as an electron carrier of the respiratory chain in mitochondria. In this paper we show that ubiquinol-10, the reduced form of ubiquinone-10, also efficiently scavenges free radicals generated chemically within liposomal membranes. Ubiquinol-10 is about as effective in preventing peroxidative damage to lipids as α-tocopherol, which is considered the best lipid-soluble antioxidant in humans. The number of radicals scavenged by each molecule of ubiquinol-10 is 1.1 under our experimental conditions. In contrast to α-tocopherol, ubiquinol-10 is not recycled by ascorbate. However, it is known that ubiquinol-10 can be recycled by electron transport carriers present in various biomembranes and possibly by some enzymes. We also show that ubiquinol-10 spares α-tocopherol when both antioxidants are present in the same liposomal membranes and that ubiquinol-10, like α-tocopherol, does not interact with reduced glutathione. Our data together with previous work on the antioxidant function of ubiquinol reported in the literature strongly suggest that ubiquinol-10 is an important physiological lipid-soluble antioxidant.**

Ubiquinone (2,3-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinone), or coenzyme Q, is a lipid-soluble compound comprised of a redox-active quinoid nucleus and a hydrophobic side chain containing a number of monounsaturated *trans*-isoprenoid units. Ubiquinol is the two-electron reduction product of ubiquinone. The predominant form of ubiquinone in animals and humans is ubiquinone-10, containing 10 isoprenoid units in the side chain (this has also been called ubiquinone 50 for the total number of carbons in the side chain). The main function of ubiquinone in biology is to act as redox component of transmembrane electron transport systems, such as the respiratory chain of mitochondria. Ubiquinone has also been suggested to transport protons across the inner mitochondrial membrane by transverse diffusion, resulting in the conservation of energy ("protonmotive Q cycle") (1).

As early as 1961 Kaufmann and Garloff (2) suggested that ubiquinone exerts yet another function in biology: to act as a membrane antioxidant. In a pioneering study in 1966, Mellors and Tappel (3) showed that ubiquinol-6, in contrast to ubiquinone-6, is a good antioxidant and is slightly more efficient than α-tocopherol in inhibiting hemoglobin-catalyzed peroxidation of arachidonic acid. They also demonstrated that ubiquinone reduced to ubiquinol through the electron transport chain strongly inhibits lipid peroxidation in isolated mitochondria. This finding has been corroborated by other *in vitro* studies (4, 5). Booth *et al.* (6) showed that ubiquinol in phosphatidylcholine (PtdCho) liposomes strongly inhibits lipid peroxidation and suggested that in the inner mitochondrial membrane, ubiquinol is at least as active as α-tocopherol in removing free radicals because of its similar antioxidant efficiency and the 10-fold greater molar concentration of ubiquinone plus ubiquinol than of α-tocopherol.

Good evidence for *in vivo* antiperoxidative activity of ubiquinol came from studies with experimental endotoxemia in mice (7, 8). Endotoxin-induced lipid peroxidation in liver was found to be associated with drastic decreases in hepatic levels of ubiquinol-9, α-tocopherol, and glutathione (GSH). Injected ubiquinone-10, after accumulation in the liver and reduction to ubiquinol-10, prevented the decreases in endogenous antioxidants, completely suppressed lipid peroxidation, and markedly increased the survival rates of endotoxin-administered mice. Other findings indicating *in vivo* antioxidant activity of ubiquinol include decreased tissue levels of ubiquinol-9 and -10 and decreased levels of α-tocopherol in rat liver (9) and brain (10) following ischemia and reoxygenation and in spinal cord following impact trauma (11). Both postischemic reoxygenation and trauma injuries have been associated with oxidant stress (12). In addition, administered ubiquinone-10 has been shown to protect against acute postischemic hepatic (9, 13) and myocardial (14, 15) injury and against carbon tetrachloride-induced lipid peroxidation (16) in experimental animals.

There is some evidence for antioxidant activity of ubiquinol also in humans (17–19). For example, it has been shown that preoperative administration of ubiquinone-10 to patients can increase the tolerance of the heart to ischemia (17) and that patients with adult respiratory distress syndrome, a condition known to be associated with oxidant stress (12), have decreased plasma levels of ubiquinol-10 and very low plasma levels of ascorbate (18). There are some excellent recent reviews on the function of ubiquinol as antioxidant *in vitro* and *in vivo* (19–21).

In view of all the evidence for an antioxidant role of ubiquinol, relatively little is known about the mechanism by which it acts as antioxidant in biomembranes and about whether ubiquinol-10 acts alone or in concert with other physiological antioxidants. Therefore, we investigated the hydrogen-donating, antiperoxidative activity of physiological concentrations of ubiquinol-10 in a well-defined model system of membranous lipid peroxidation and the possible interaction of ubiquinol-10 with α-tocopherol, ascorbate, and GSH.

MATERIALS AND METHODS

Materials. Ubiquinone-10, *d,l*-α-tocopherol, L-ascorbic acid, GSH, sodium borohydride, soybean PtdCho, silica gel (100–200 mesh), isoluminol, and microperoxidase MP-11

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Abbreviations: AMVN, 2,2′-azobis(2,4-dimethylvaleronitrile); GSH, glutathione; *n* value, number of radicals trapped by each molecule of an antioxidant; $O_2^{\cdot-}$, superoxide radicals; PtdCho, phosphatidylcholine; t'_{inh}, length of the induction period as determined by our method.

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were purchased from Sigma. 2,2'-Azobis(2,4-dimethylvaleronitrile) (AMVN) was obtained from Polysciences, and Chelex 100 (200–400 mesh, sodium form) was from Bio-Rad. All other chemicals used were of the highest purity commercially available. PtdCho was purified by silica gel chromatography (22). AMVN was recrystallized from methanol. Phosphate-buffered saline (136 mM NaCl/2.6 mM KCl/1.4 mM KH_2PO_4/8.0 mM Na_2PO_4, pH 7.4) was treated with Chelex 100 to remove trace amounts of transition metal ions.

Preparation of Ubiquinol-10. Ubiquinone-10 in methanol was quantitatively reduced to ubiquinol-10 by addition of an equimolar amount of freshly prepared sodium borohydride in methanol. After 5 min at 4°C, an aliquot of this mixture was subjected to HPLC on a semipreparative LC-18 column (25 cm × 10 mm I.D.) (Supelco) with methanol as eluant at a flow rate of 7.0 ml/min; the eluate was monitored at 210 nm. Ubiquinol-10, which was eluted as a single peak with a retention time of 26 min, was collected and immediately used.

Preparation and Incubation of Multilamellar Liposomes. An aliquot of a stock solution of PtdCho (64 mM in benzene) was mixed with 2 ml of methanol, an aliquot of 2 mM AMVN in benzene, and, if appropriate, an aliquot of freshly prepared ubiquinol-10 in methanol or α-tocopherol in ethanol or both. The mixture was dried under vacuum in a rotary evaporator to give a thin homogenous film. Phosphate-buffered saline was added, and liposomes were prepared by shaking. The final concentrations were 20 mM PtdCho, 0.2 mM AMVN, and 5 or 10 μM ubiquinol-10 or α-tocopherol. Where indicated, the saline buffer contained 50 μM ascorbate or 50 μM GSH. Incubations were started by placing the tubes containing the reaction mixtures under air into a shaking 50°C waterbath. AMVN is a lipid-soluble azo compound that thermally decomposes and thereby generates radicals at a known and constant rate (23), which initiates formation of PtdCho hydroperoxides in the liposomes. Since the concentration of AMVN has to be kept low to avoid disrupting the membrane structure, the incubation temperature has to be raised to 50°C to obtain a sufficient rate of radical production. The elevated temperature neither affects the liposome structure nor impairs the efficacy of antioxidants (22).

Determination of PtdCho Hydroperoxides. HPLC was used to determine PtdCho hydroperoxides, with detection at 234 nm (22).

Determination of α-Tocopherol, Ubiquinol-10, and Ubiquinone-10. A 50-μl aliquot was withdrawn from the liposomal incubation mixture and extracted with 200 μl of ice-cold methanol and 1 ml of ice-cold hexane. After brief centrifugation at 13,600 × *g*, 0.8 ml of the upper hexane phase was removed and evaporated to dryness under reduced pressure. The sample was dissolved either in 100 μl of ethanol or 150 μl of methanol/*tert*-butyl alcohol (1:1 by volume).

α-Tocopherol in 50 μl of the ethanol solution was quantitated by reversed-phase HPLC with electrochemical detection (24). Ubiquinone-10 and ubiquinol-10 were measured by HPLC with UV and chemiluminescence detection (25). Briefly, 100 μl of the methanol/*tert*-butyl alcohol solution was subjected to reversed-phase HPLC. The eluate was analyzed at 210 nm (for quantitation of ubiquinone-10) and then mixed with an alkaline reaction solution containing isoluminol and microperoxidase. Under these conditions ubiquinol-10 generates light, which is measured in a fluorometer used as a photon detector (25). Ubiquinol-10 and ubiquinone-10 were eluted from the HPLC column as single peaks with retention times of 7.2 and 10.0 min, respectively. A small amount (5–10%) of ubiquinol-10 was oxidized to ubiquinone-10 during work-up. The data shown in Figs. 1 *Right* and 4 (see *Results*) have been corrected accordingly.

RESULTS

To investigate the antioxidant activity of physiological concentrations of ubiquinol-10 in PtdCho liposomes, we first determined the concentration of ubiquinone-10 plus ubiquinol-10 in membranes of human tissues using the data in refs. 26 and 27 and an average molecular weight for phospholipids of 790 g/mol. We calculated the molar ratios of ubiquinone-10 plus ubiquinol-10 to phospholipids in human skeletal muscle, heart, and liver to be about 1:3000, 1:200, and 1:1700, respectively. Therefore, we decided to use ubiquinol-10 in our experiments at 1/2000th the concentration of PtdCho.

When PtdCho liposomes (20 mM) in aqueous dispersion were exposed to radicals generated within the liposomal membranes by 0.2 mM AMVN at 50°C under air in the absence of an antioxidant, PtdCho was peroxidized immediately at a constant rate (Fig. 1 *Left*). Ubiquinol-10 or α-tocopherol incorporated into the liposomes at 1/2000th the concentration of PtdCho initially markedly inhibited lipid peroxidation (Fig. 1 *Left*). In the presence of ubiquinol-10 and α-tocopherol, the amounts of PtdCho hydroperoxides formed after 60 min of incubation were decreased by 79.8 ± 4.8% (mean value ± SD; three separate experiments) and 71.6 ± 5.9% (three experiments), respectively, compared with the amounts of PtdCho hydroperoxides formed in the absence of these antioxidants. Thus, under our experimental conditions, ubiquinol-10 traps free radicals and thereby inhibits lipid peroxidation with about equal efficacy as α-tocopherol. Ubiquinone-10 showed no antioxidant activity (Fig. 1 *Left*).

The length of the induction period—i.e., the time during which lipid peroxidation is inhibited—was shorter in the presence of ubiquinol-10 than in the presence of α-tocopherol (Fig. 1 *Left*). During the induction period, ubiquinol-10 (Fig. 1 *Right*) and α-tocopherol (not shown) were oxidized completely. Ubiquinol-10 was oxidized quantitatively to ubiquinone-10 (Fig. 1 *Right*), as confirmed by indistinguishable chromatographic behavior and UV absorbance spectra of the single oxidation product of ubiquinol-10 and an authentic

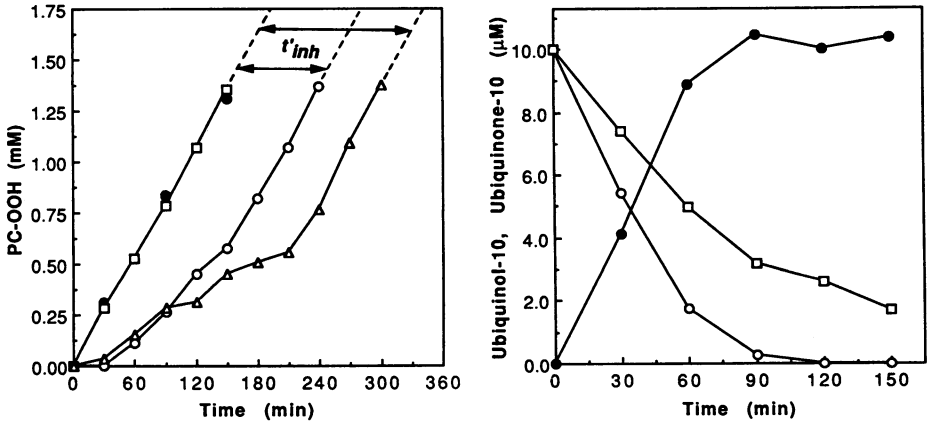

FIG. 1. Antioxidant activity of ubiquinol-10 and α-tocopherol in PtdCho liposomes. (*Left*) AMVN-induced formation of PtdCho hydroperoxides (PC-OOH) in the absence of an additive (control) (□) and in the presence of 10 μM ubiquinone-10 (●), 10 μM ubiquinol-10 (○), or 10 μM α-tocopherol (△). (*Right*) AMVN-induced consumption of ubiquinol-10 (○) and formation of ubiquinone-10 (●) in PtdCho liposomes. The oxidation of 10 μM ubiquinol-10 in PtdCho liposomes incubated in the absence of AMVN is also shown (□).

standard of ubiquinone-10. After the complete oxidation of ubiquinol-10 or α-tocopherol, the induction period came to an end, and lipid peroxidation proceeded at the same rate as in the control incubation without antioxidants (Fig. 1 *Left*). In the absence of AMVN, ubiquinol-10 autoxidized at a considerable rate (Fig. 1 *Right*).

Since the rate of lipid peroxidation during the induction period in the presence of ubiquinol-10 was not linear (see Fig. 1 *Left*), we were unable to determine the length of the induction period by the conventional method (28). As a measure of it, we instead used the distance between the control curve in the absence of an antioxidant and the parallel curve of uninhibited lipid peroxidation after the induction period in the presence of an antioxidant had come to its end (t'_{inh}; see Fig. 1 *Left*). From the data of Fig. 1 *Left* and another two identical experiments, t'_{inh} values for ubiquinol-10 and α-tocopherol were found to be 84 ± 8 min (three experiments) and 149 ± 10 min (three experiments), respectively. The shorter t'_{inh} for ubiquinol-10 indicates that the number of radicals trapped by each molecule of ubiquinol-10 (its *n* value) is lower than α-tocopherol's *n* value. Since the *n* value of α-tocopherol is known to be 2.0 (23, 28) and the *n* value of an antioxidant is directly proportional to the length of the induction period it produces, the *n* value of ubiquinol-10 can be calculated to be (84 min/149 min) × 2.0 = 1.1 under our experimental conditions.

It has been shown previously that α-tocopherol in lipid bilayers can be regenerated from the α-tocopheroxyl radical by reduction with ascorbate at the water/lipid interface (29). Such synergistic interaction does not take place between α-tocopherol and water-soluble GSH (22, 30), another important physiological antioxidant. We investigated whether ubiquinol-10 in liposomal membranes can interact with ascorbate or GSH in the aqueous medium. First, we sought to confirm the cooperative antioxidant activity of α-tocopherol and ascorbate in our experimental system. We found that 50 μM ascorbate inhibits AMVN-induced oxidation of PtdCho only slightly (see Fig. 2) because ascorbate present in the aqueous phase has no access to the radicals generated within the liposomes. When 5 μM α-tocopherol was incorporated into the liposomes, t'_{inh} was 78 min. This induction period was extended to 163 min in the additional presence of 50 μM ascorbate, and the rate of lipid peroxidation during the induction period was lowered by ≈70%. These results show that there is a strong synergistic antiperoxidative effect of ascorbate and α-tocopherol in our experimental system.

We did not observe such synergism between ubiquinol-10 and ascorbate (Fig. 2). On the contrary, in the presence of ascorbate the length of the induction period for ubiquinol-10 decreased. The oxidation of ubiquinol-10 was accordingly faster in the presence of ascorbate than in its absence (not shown). When ascorbate was replaced by GSH, there was no adverse effect on the antiperoxidative potency of ubiquinol-10 (Fig. 3). GSH had little inhibitory effect on AMVN-induced oxidation of PtdCho whether or not ubiquinol-10 was present in the liposomes.

Finally, we investigated the antioxidant activity of ubiquinol-10 and α-tocopherol present in the same lipid bilayer. The combined presence of ubiquinol-10 and α-tocopherol, each at 1/4000th the concentration of PtdCho, very strongly inhibited AMVN-induced lipid peroxidation (Fig. 4); the effect was much stronger than with each antioxidant alone at 1/2000th the concentration of PtdCho (see Fig. 1 *Left*). Ubiquinol-10 efficiently spared α-tocopherol: α-tocopherol was oxidized only after a major portion of ubiquinol-10 had been oxidized (Fig. 4). As expected, α-tocopherol was oxidized at a lower rate than ubiquinol-10, reflective of α-tocopherol's higher *n* value.

DISCUSSION

In this paper we have used a lipid-soluble radical initiator in liposomal membranes to study the antiperoxidative activity of ubiquinol-10. The radical initiator initially generates carbon-centered radicals, which rapidly react with molecular oxygen to form peroxyl radicals in the membrane (23). These free radicals initiate radical chain oxidation of the liposomal lipids. A compound capable of donating hydrogen atoms to the initiating or propagating radicals will trap these radicals and thereby inhibit lipid peroxidation.

We found that in this assay system (*i*) ubiquinol-10 at 1/2000th the concentration of PtdCho (i.e., at about physiological concentrations found in human tissues; refs. 26 and 27), traps free radicals and inhibits lipid peroxidation with similar efficiency as α-tocopherol (see Fig. 1 *Left*); (*ii*) the *n* value of ubiquinol-10 is 1.1; (*iii*) ubiquinol-10 is quantitatively oxidized to ubiquinone-10 during its antiperoxidative action; (*iv*) ubiquinone-10 exerts no antioxidant activity (Fig. 1 *Left*); (*v*) ubiquinol-10 spares α-tocopherol (Fig. 4); (*vi*) ubiquinol-10 is not recycled by ascorbate (see Fig. 2); and (*vii*) there is no synergism between ubiquinol-10 and GSH (Fig. 3).

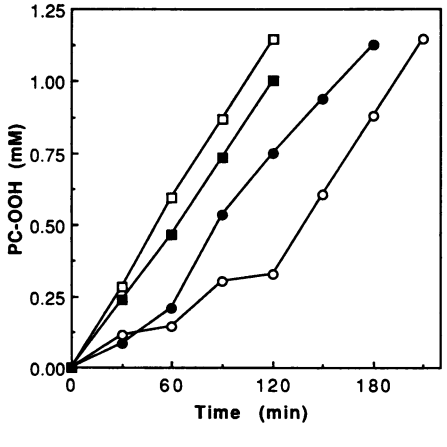

FIG. 2. Inhibition of AMVN-induced peroxidation of PtdCho liposomes by ubiquinol-10 in the presence of ascorbate. Formation of PtdCho hydroperoxides (PC-OOH) in the absence of an additive (control) (□) and in the presence of 50 μM ascorbate (■), 10 μM ubiquinol-10 (○), or 50 μM ascorbate together with 10 μM ubiquinol-10 (●).

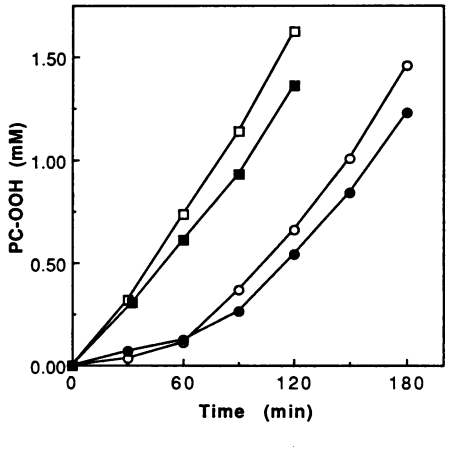

FIG. 3. Inhibition of AMVN-induced peroxidation of PtdCho liposomes by ubiquinol-10 in the presence of GSH. Formation of PtdCho hydroperoxides (PC-OOH) in the absence of an additive (control) (□) and in the presence of 50 μM GSH (■), 10 μM ubiquinol-10 (○), or 50 μM GSH together with 10 μM ubiquinol-10 (●).

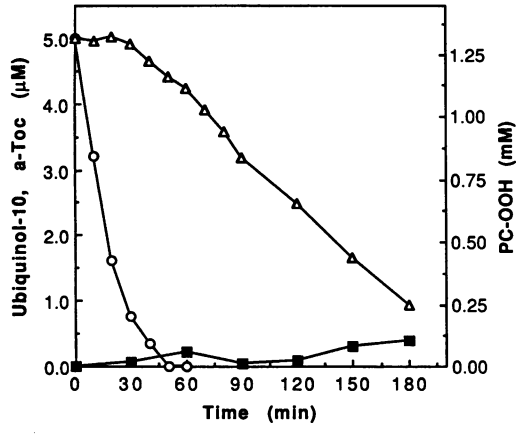

FIG. 4. Sparing effect of ubiquinol-10 on α-tocopherol (a-Toc) present in the same liposomal membranes. Consumption of 5 μM ubiquinol-10 (○) and 5 μM α-tocopherol (△) and lipid peroxidation (■) in PtdCho liposomes exposed to AMVN.

Since our assay measures hydrogen-donating activity, the mechanism by which ubiquinol-10 acts as antioxidant is presumably as follows:

$$Q_{10}H_2 + L(OO)^{\cdot} \rightarrow Q_{10}^{\cdot -} + H^+ + L(OO)H, \quad [1]$$

where $Q_{10}H_2$ represents ubiquinol-10 with the two phenolic hydrogen atoms; $Q_{10}^{\cdot -}$, the ubisemiquinone-10 radical; and $L(OO)^{\cdot}$, the AMVN-derived or PtdCho (peroxyl) radicals. The ubisemiquinone-10 radical can disproportionate to ubiquinone-10 (Q_{10}) and ubiquinol-10 (19) or might scavenge another (peroxyl) radical:

$$2Q_{10}^{\cdot -} + 2H^+ \rightarrow Q_{10}H_2 + Q_{10} \quad [2]$$

$$Q_{10}^{\cdot -} + H^+ + L(OO)^{\cdot} \rightarrow Q_{10} + L(OO)H. \quad [3]$$

Ubiquinol-10 and ubisemiquinone-10 radicals can also undergo autoxidation (19):

$$Q_{10}H_2 + O_2 \rightarrow Q_{10}^{\cdot -} + 2H^+ + O_2^{\cdot -} \quad [4]$$

$$Q_{10}^{\cdot -} + O_2 \rightarrow Q_{10} + O_2^{\cdot -}. \quad [5]$$

The superoxide radicals ($O_2^{\cdot -}$) formed in these reactions can further oxidize ubiquinol-10 (19):

$$Q_{10}H_2 + O_2^{\cdot -} \rightarrow Q_{10}^{\cdot -} + H_2O_2. \quad [6]$$

Reactions **1** and **3** are radical-trapping (i.e., antiperoxidative) reactions, whereas in reactions **4–6** ubiquinol-10 is "wasted." Under our experimental conditions (incubation at 50°C under air), a substantial portion of ubiquinol-10 is "wasted" by autoxidation (Fig. 1 *Right*) and does not contribute to radical-trapping. Thus, our estimate of the *n* value of ubiquinol-10 of 1.1 is quite conservative. It is very likely that *in vivo* in tissues, where the temperature and the oxygen partial pressure are considerably lower than in our experimental system, ubiquinol-10 undergoes much less autoxidation and, therefore, has a higher *n* value.

In the presence of ascorbate, the antioxidant potency of ubiquinol-10 is diminished. Obviously, ascorbate contributes to "wasting" of ubiquinol-10. It is possible that under our experimental conditions ascorbate autoxidizes, thereby generating $O_2^{\cdot -}$, which then oxidizes ubiquinol-10 (reaction **6**). For the same reasons as mentioned above, these reactions are much less likely to occur *in vivo*. Despite the relatively unfavorable conditions in our experiments, ubiquinol-10 always exerted antiperoxidative activity and never promoted lipid peroxidation. In this context it is noteworthy that the site of $O_2^{\cdot -}$ production by the mitochondrial respiratory chain appears not to be ubiquinol, as originally thought (31), but a nonheme iron-sulfur function of the NADH dehydrogenase complex (4, 21, 32). Thus, ubiquinol-10 acts as antioxidant and not as prooxidant both in our liposomal system (see also ref. 6) and in mitochondrial membranes (3–5).

An important finding of the present study is the sparing effect of ubiquinol-10 on α-tocopherol. This effect appears not to be due to much more rapid scavenging of free radicals by ubiquinol-10, since we (see above) and others (3, 6) have shown that ubiquinol is about as effective as α-tocopherol in inhibiting lipid peroxidation. The sparing effect could reflect site-specific antioxidant action within the membrane of the two antioxidants in our experimental system. Ubiquinol-10, owing to its long hydrophobic side chain, is primarily located in the central plane of the lipid bilayer (33), whereas the redox-active chromanol moiety of α-tocopherol is located near the bilayer surface (34). Like ubiquinol-10, AMVN is very lipophilic and can be expected to initiate lipid peroxidation deep in the membrane. Thus, because of proximity, ubiquinol-10 is likely to trap AMVN-derived and lipid (peroxyl) radicals before the peroxyl radicals diffuse towards the bilayer surface (35), where they can then be scavenged by α-tocopherol. Such antioxidant protection by ubiquinol-10 and α-tocopherol at different sites within the membrane might also account for the virtually complete suppression of PtdCho peroxidation during the induction period in the presence of both antioxidants, as opposed to only partial suppression by each antioxidant alone (compare Figs. 1 *Left* and 4).

Alternatively, or additionally, the sparing effect could be due to recycling of α-tocopherol by ubiquinol-10. Indeed, it has been suggested recently that ubiquinone reduced to ubiquinol by electron carriers in mitochondrial and microsomal membranes regenerates α-tocopherol from the α-tocopheroxyl radical (36–38). The same mechanism has been suggested for the reduction in mitochondria of α-tocopherolquinone, a two-electron oxidation product of α-tocopherol, to α-tocopherolhydroquinone (39). There is also evidence for sparing of α-tocopherol by ubiquinol-10 *in vivo*—namely, the observations that depletion of hepatic α-tocopherol in endotoxin-administered mice and in rats after hepatic ischemia and reoxygenation is prevented by preadministered ubiquinone-10 after its reduction to ubiquinol-10 in the liver (8, 9).

Besides protecting cellular membranes, ubiquinol-10 might also play a role in protecting lipoproteins against peroxidative damage. Ubiquinone is distributed among all the lipoprotein classes (40) and is present in human blood plasma mainly in its reduced form—i.e., as ubiquinol-10 (25). In plasma exposed to oxidants generated by activated neutrophils, ubiquinol-10 is oxidized and α-tocopherol is not (18), suggesting that ubiquinol-10 forms the first line of antioxidant defense in lipoproteins.

The data of this study and all the evidences for antioxidant function of ubiquinol published over the last 3 decades strongly suggest that ubiquinol-10 contributes significantly to antioxidant defenses in biology, complementing the antioxidant activities of α-tocopherol by scavenging free lipid radicals and, possibly, by recycling α-tocopherol. The notion that ubiquinol-10, like α-tocopherol, should be considered an important physiological lipid-soluble antioxidant is buttressed by the following observations.

(*i*) Although ubiquinol-10 might have a lower *n* value than α-tocopherol and cannot be recycled by ascorbate, it appears to be equally reactive with free radicals as α-tocopherol and, most importantly, can be recycled by the mitochondrial respiratory chain. Thus, the recycling of ubiquinol-10 is dependent on a source of reducing equivalents that is inexhaustible in respiring organisms.

(*ii*) Like α-tocopherol, ubiquinol is present in all membranes of eukaryotic cells (26, 41) and all lipoprotein classes (40). The molar ratio of ubiquinone plus ubiquinol to α-tocopherol in the inner mitochondrial membrane is about 10:1 (3), close to or lower than 1:1 in other biomembranes (37) and about 1:20 in lipoproteins.

(*iii*) Besides the various dehydrogenases feeding electrons into the respiratory chain at the ubiquinone level, a NADH:(quinone-acceptor) oxidoreductase (EC 1.6.99.5) in the inner mitochondrial membrane (42), an NAD(P)H-dependent enzyme in microsomal membranes (37, 43), and an electron transport chain in plasma membranes (44) are also likely to be capable of recycling ubiquinol. In addition, there are enzymes in the cytosol and the mitochondrial matrix that can potentially regenerate ubiquinol from the ubisemiquinone radical or ubiquinone, respectively—namely, superoxide dismutase (45) or NAD(P)H:(quinone-acceptor) oxidoreductase (EC 1.6.99.2) and NADPH:(quinone-acceptor) oxidoreductase (EC 1.6.99.6) (42). There is no evidence for direct enzymatic recycling of α-tocopherol in biological systems (36, 43).

(*iv*) Ubiquinol, unlike α-tocopherol (2), is an integral component of virtually all living cells (26). For example, aerobic microorganisms have ubiquinone and ubiquinol but no α-tocopherol in their membranes. Yet, these organisms thrive under atmospheric oxygen pressure and obviously cope successfully with oxidant stress. Thus, it seems that ubiquinol is the first lipid-soluble antioxidant in evolution and is of universal importance for antioxidant defenses in biology.

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1. Mitchell, P. & Moyle, J. (1982) in *Functions of Quinones in Energy Conserving Systems*, ed. Trumpower, B. L. (Academic, New York), pp. 553–575.
2. Kaufmann, H. P. & Garloff, H. (1961) *Fette Seifen Anstrichmittel* **63,** 331–344.
3. Mellors, A. & Tappel, A. L. (1966) *J. Biol. Chem.* **241,** 4353–4356.
4. Takeshige, K., Takayanagi, R. & Minakami, S. (1980) in *Biomedical and Clinical Aspects of Coenzyme Q*, eds. Yamamura, Y., Folkers, K. & Ito, Y. (Elsevier/North Holland, Amsterdam), Vol. 2, pp. 15–25.
5. Landi, L., Cabrini, L., Sechi, A. M. & Pasquali, P. (1984) *Biochem. J.* **222,** 463–466.
6. Booth, R. F. G., Galanopoulou, D. G. & Quinn, P. J. (1982) *Biochem. Int.* **5,** 151–156.
7. Sugino, K., Dohi, K., Yamada, K. & Kawasaki, T. (1987) *Surgery* **101,** 746–752.
8. Sugino, K., Dohi, K., Yamada, K. & Kawasaki, T. (1989) *Surgery* **105,** 200–206.
9. Marubayashi, S., Dohi, K., Yamada, K. & Kawasaki, T. (1984) *Biochim. Biophys. Acta* **797,** 1–9.
10. Yoshida, S., Abe, K., Busto, R., Watson, B. D., Kogure, K. & Ginsberg, M. D. (1982) *Brain Res.* **245,** 307–316.
11. Lemke, M., Frei, B., Ames, B. N. & Faden, A. I. (1990) *Neurosci. Lett.* **108,** 201–206.
12. Cross, C. E. (1987) *Ann. Intern. Med.* **107,** 526–545.
13. Marubayashi, S., Dohi, K., Ezaki, H., Hayashi, K. & Kawasaki, T. (1982) *Surgery* **91,** 631–637.
14. Sugiyama, S., Ozawa, T., Kato, T. & Suzuki, S. (1980) *Am. Heart J.* **100,** 829–837.
15. Tominaga, R., Kouda, Y., Tanaka, J., Nakano, E., Ando, H., Ueno, Y. & Tokunaga, K. (1983) *J. Surg. Res.* **34,** 111–117.
16. Beyer, R. E. (1988) *Free Rad. Biol. Med.* **5,** 297–303.
17. Tanaka, J., Tominaga, R., Yoshitoshi, M., Matsui, K., Komori, M., Sese, A., Yasui, H. & Tokunaga, K. (1982) *Ann. Thor. Surg.* **33,** 145–151.
18. Cross, C. E., Forte, T., Stocker, R., Louie, S., Yamamoto, Y., Ames, B. N. & Frei, B. (1990) *J. Lab. Clin. Med.* **115,** 396–404.
19. Beyer, R. E. & Ernster, L. (1990) in *Biochemistry, Bioenergetics and Clinical Aspects of Ubiquinone*, ed. Lenaz, G. (Taylor & Francis, London), in press.
20. Ozawa, T. (1985) in *Coenzyme Q*, ed. Lenaz, G. (Wiley, New York), pp. 441–456.
21. Beyer, R. E., Nordenbrand, K. & Ernster, L. (1987) *Chem. Scr.* **27,** 145–153.
22. Stocker, R. & Peterhans, E. (1989) *Biochim. Biophys. Acta* **1002,** 238–244.
23. Barclay, L. R. C., Locke, S. J., MacNeil, J. M., Van Kessel, J., Burton, G. W. & Ingold, K. U. (1984) *J. Am. Chem. Soc.* **106,** 2479–2481.
24. Pascoe, G. A., Duda, C. T. & Reed, D. J. (1987) *J. Chromatogr.* **414,** 440–448.
25. Frei, B., Yamamoto, Y., Niclas, D. & Ames, B. N. (1988) *Anal. Biochem.* **175,** 120–130.
26. Ramasarma, T. (1985) in *Coenzyme Q*, ed. Lenaz, G. (Wiley, New York), pp. 67–81.
27. Lentner, C., ed. (1981) *Geigy Scientific Tables* (CIBA-Geigy, Basel), Vol. 1, p. 221.
28. Niki, E., Saito, M., Yoshikawa, Y., Yamamoto, Y. & Kamiya, Y. (1986) *Bull. Chem. Soc. Jpn.* **59,** 471–477.
29. Niki, E., Kawakami, A., Yamamoto, Y. & Kamiya, Y. (1985) *Bull. Chem. Soc. Jpn.* **58,** 1971–1975.
30. Barclay, L. R. C. (1989) *J. Biol. Chem.* **263,** 16138–16142.
31. Cadenas, E., Boveris, A., Ragan, C. I. & Stoppani, A. O. M. (1977) *Arch. Biochem. Biophys.* **180,** 248–257.
32. Takeshige, K. & Minakami, S. (1979) *Biochem. J.* **180,** 129–135.
33. Ulrich, E. L., Girvin, M. E., Cramer, W. A. & Markley, J. L. (1985) *Biochemistry* **24,** 2501–2508.
34. Perly, B., Smith, I. C., Hughes, L., Burton, G. W. & Ingold, K. U. (1985) *Biochim. Biophys. Acta* **819,** 131–135.
35. Barclay, L. R. C. & Ingold, K. U. (1981) *J. Am. Chem. Soc.* **103,** 6478–6485.
36. Maguire, J. J., Wilson, D. S. & Packer, L. (1989) *J. Biol. Chem.* **264,** 21462–21465.
37. Kagan, V., Serbinova, E. & Packer, L. (1990) *Biochem. Biophys. Res. Commun.*, in press.
38. Kagan, V. E., Serbinova, E. A., Koyona, G. M., Kitanova, S. A., Tyurin, V. A., Stoytchev, T. S., Quinn, P. J. & Packer, L. (1990) *Free Rad. Biol. Med.*, in press.
39. Bindoli, A., Valente, M. & Cavallini, L. (1985) *Biochem. Int.* **10,** 753–761.
40. Elmberger, P. G., Kalen, A., Brunk, U. T. & Dallner, G. (1989) *Lipids* **24,** 919–930.
41. Kalen, A., Norling, B., Appelkvist, E. L. & Dallner, G. (1987) *Biochim. Biophys. Acta* **926,** 70–78.
42. Frei, B., Winterhalter, K. H. & Richter, C. (1986) *Biochemistry* **25,** 4438–4443.
43. Packer, L., Maguire, J. J., Mehlhorn, R. J., Serbinova, E. & Kagan, V. E. (1989) *Biochem. Biophys. Res. Commun.* **159,** 229–235.
44. Morre, D. J., Crane, F. L., Sun, I. L. & Navas, P. (1987) *Ann. N.Y. Acad. Sci.* **498,** 153–171.
45. Cadenas, E., Mira, D., Brunmark, A., Lind, C., Segura-Aguilar, J. & Ernster, L. (1988) *Free Rad. Biol. Med.* **5,** 71–79.