Antioxidant Properties of Coenzyme Q_{10} in Food Systems

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Antioxidant properties of coenzyme Q_{10} (ubiquinone 10, CoQ_{10}) and its reduced form, ubiquinol 10 ($CoQ_{10}H_2$), were determined in foods and the results discussed on the basis of radical exchange reactions occurring between these compounds and lipid radicals. CoQ_{10} does not react with peroxidizing lipids and therefore is not a food antioxidant. Although forming the corresponding semiquinone radical by reaction with peroxidizing lipid, $CoQ_{10}H_2$ is only a poor food antioxidant, as it is rapidly transformed into the corresponding inactive quinone in air. To be active as a food antioxidant, CoQ_{10} must be used in conjunction with a reducing agent such as vitamin C.

1. INTRODUCTION

Coenzyme Q is a group of homologous quinones [ubiquinones (I), Figure 1] widely distributed in animals, plants, and microorganisms. They are exceedingly versatile molecules functionally involved in a number of distinct, but related, cellular processes, for example, mitochondrial and bacterial electron transport (Lenaz, 1985). In addition to its multiple roles in energy metabolism, coenzyme Q as well as its reduced and isomerized forms, namely the ubiquinols (II) and ubichromenols (III) (Figure 1), have been implicated as biological antioxidants (Mellors and Tappel, 1966; Zamora et al., 1991; Landi et al., 1985; Beyer, 1990; Sugiyama et al., 1980; Booth et al., 1982; Takeshige et al., 1980; Ernster, 1984; Frei et al., 1990; Yamamoto et al., 1990; Kagan et al., 1990). As the majority of tissue ubiquinone molecules exist in the reduced ubiquinol form (Dallner, 1990), it can be assumed that this form is responsible for the antioxidant properties observed in vivo (Mellors and Tappel, 1966; Zamora et al., 1991; Landi et al., 1985; Beyer, 1990; Sugiyama et al., 1980; Booth et al., 1982; Takeshige et al., 1980; Ernster, 1984; Frei et al., 1990; Yamamoto et al., 1990; Kagan et al., 1990).

Antioxidant properties of coenzyme Q_{10} (CoQ_{10}) in food, cosmetic, and pharmaceutical products have recently been claimed (Bracco et al., 1989). Although it is known that ubiquinol reacts with the stable free radical diphenyl-*p*-picrylhydrazyl (Takeshige et al., 1980), there are no reports in the literature concerning the reaction of either ubiquinone of ubiquinol with radicals present in autoxidizing lipids. The aim of the present study was therefore to investigate the radical reactions of CoQ_{10} and its reduced form, ubiquinol 10 ($CoQ_{10}H_2$) in autoxidizing lipid and to determine the antioxidative properties of these substances in lipids.

2. EXPERIMENTAL PROCEDURES

2.1. Materials. CoQ_{10} was purchased from Kanegafuchi Chemical Industries Co., Ltd. (Osaka) and was used without further purification. $CoQ_{10}H_2$ was prepared by reduction of the corresponding quinone with zinc powder in glacial acetic acid (Cheng and Casida, 1970). The product was characterized by UV spectroscopy ($\lambda_{max} = 288$, $\epsilon = 4800$ at concentration 10^{-4} w/v in ethanol) and by ^1H NMR in $CDCl_3$ [δ 5.29 (s, OH), 5.27 (s, OH), 5.11 (m, 10 H olefin), 3.88 (s, 2 OCH_3), 3.34 (d, $J = 6.7$ Hz, Ar CH_2), 2.13 (s, Ar CH_3), 2.00 (br s, 18 CH_2 allyl), 1.77 (s, CH_3), 1.67 (s, CH_3), 1.59 (s élargi, 9 CH_3)]. Vitamin E (*dl*-α-tocopherol), vitamin C, and soybean lecithin (Topcithin) were obtained from Merck (Darmstadt, Germany), Fluka (Buchs, Switzerland), and Lukas Meyer (Hamburg, Germany), respectively. Tocopherol levels measured in the Topcithin by HPLC were α, 0.004%; β/γ,
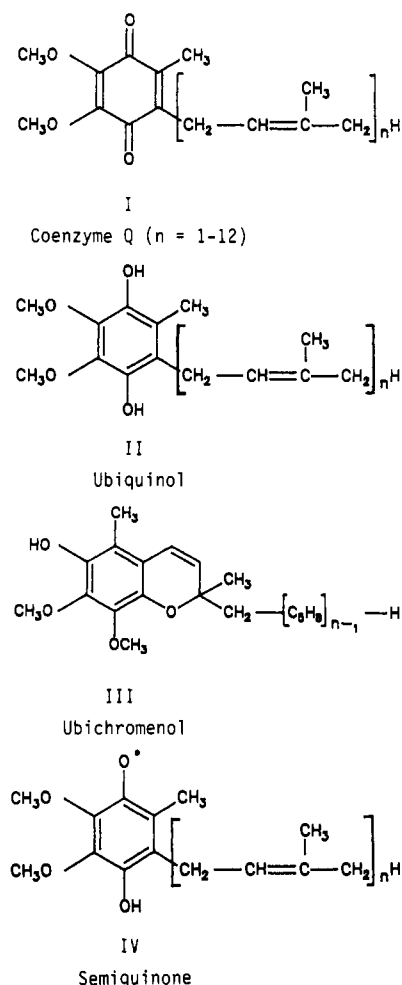

Figure 1. Structure of coenzyme Q and related compounds.

0.074%; and δ, 0.028%. Chicken fat liquid fraction (CFLF) was prepared according to a procedure already described (Lambelet et al., 1985). CFLF was oxidized in an open 20-mL beaker in an air circulation oven at 150 °C for 3 h. Fish oil was from Jahresfabrikker AS (Sandefjord, Norway). Samples without vitamin C were prepared by dissolving the chemicals directly into the lipid. Vitamin C containing samples were prepared by mixing a saturated solution of vitamin C in ethanol with the fat containing the liposoluble chemicals. Ethanol was evaporated at room temperature under a steady flux of dry nitrogen. The samples were stored and oxidized in Pyrex Petri dishes; for ESR analyses quartz sample tubes were used.

2.2. Headspace Gas Analyses. The headspace gas analyses for volatile, short, straight-chain hydrocarbons were performed
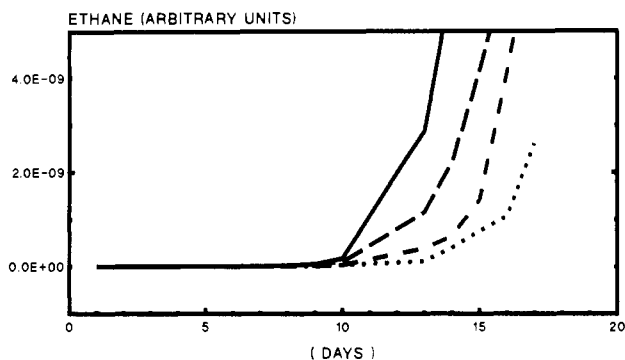
Figure 2. Ethane formation during storage at 37 °C of fish oil stabilized with CoQ_{10} and $CoQ_{10}H_2$. (—) Blank; (– – –) 0.1% CoQ_{10}; (- - -) 0.05% $CoQ_{10}H_2$; (···) 0.1% $CoQ_{10}H_2$.

Figure 3. Oxygen absorption during storage at 37 °C of fish oil stabilized with CoQ_{10} and $CoQ_{10}H_2$. (—) Blank; (– – –) 0.1% CoQ_{10}; (- - -) 0.05% $CoQ_{10}H_2$; (···) 0.1% $CoQ_{10}H_2$.

as previously described (Farr et al., 1986), on a 2-m alumina column, temperature programmed from 50 to 250 °C at a 10 °C/min heating rate, FID detector. Sampling was performed at regular intervals during a 2-month storage period at 37 °C. The analyses were run in duplicate, and mean values were reported. The statistical analyses of these types of results were shown to be very highly significant; the standard error of the means are at least 1 order of magnitude smaller than the results. Pentane and ethane, secondary products of linoleic and linolenic acid autoxidation, respectively, were used as markers for their oxidation. Residual oxygen concentration in headspace was determined in parallel (Farr et al., 1986). Both indicators point in the same direction: the higher the level of hydrocarbons formed, the lower the amount of residual oxygen in the headspace gas above the sample, indicating a higher degree of fat oxidation.

2.3. Electron Spin Resonance Spectroscopy. Radicals were generated (i) by reaction at 20–80 °C with peroxidizing lipids [CFLF in the presence of minute amounts of n-BuOH (or n-BuOD) or methyl linoleate]. Prior to ESR analyses, the samples were aerated by bubbling through with air for 10 min (Saucy et al., 1990). Radicals were also generated (ii) by photolysis at low temperature in situ in the cavity of the spectrometer using an Oriel (Stamford, CT) 500-W mercury arc lamp.

The ESR spectra were run on a Varian E109 Century Series Mark III spectrometer equipped with an X-band klystron and 100-MHz magnetic field modulation (Lambelet et al., 1985). The temperature of the samples was controlled by means of a Varian E-257 variable-temperature unit. The microwave frequency was measured using a HP 5342 frequency counter and the magnetic field with a Varian E-500-2 NMR gaussmeter calibrated with perylene cation radical (Wertz and Bolton, 1972).

3. RESULTS

3.1. Antioxidant Activities. The ethane formation and oxygen consumption in the headspace during storage at 37 °C of fish oil stabilized with various antioxidants are depicted in Figures 2–5. For all types of antioxidant pentane evolution during storage (data not shown) went in a parallel direction with ethane formation. Adding 0.1% CoQ_{10} to the oil enhanced its stability only marginally (Figures 2 and 3). $CoQ_{10}H_2$ protected the oil slightly better but remains a poor antioxidant.

Mixtures of 0.1% CoQ_{10}/1% Topcithin, 0.1% vitamin C/1% Topcithin, or 0.1% CoQ_{10}/0.05% vitamin E/1% Topcithin did not protect fish oil very efficiently (Figures 4 and 5). An important retardation of the oxidative degradation was, however, observed in the presence of a mixture of 0.1% CoQ_{10}/0.1% vitamin C/1% Topcithin. A further increase in stability was provided by the addition of 0.05% vitamin E to the CoQ_{10}/vitamin C/Topcithin mixture (Figures 4 and 5).

3.2. Radical Reactions. The ESR spectrum illustrated in Figure 6A was recorded at the beginning (within the first 10 min) of the photolysis of CoQ_{10} in n-hexane/n-
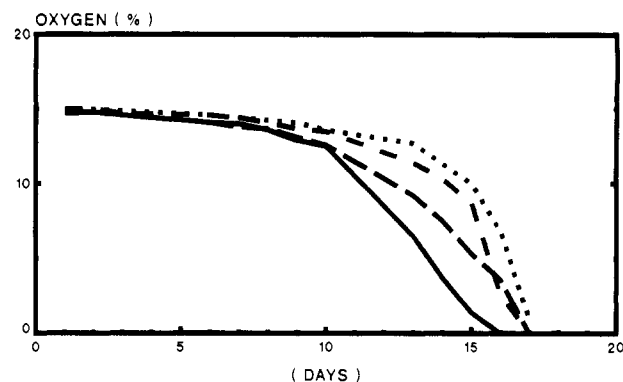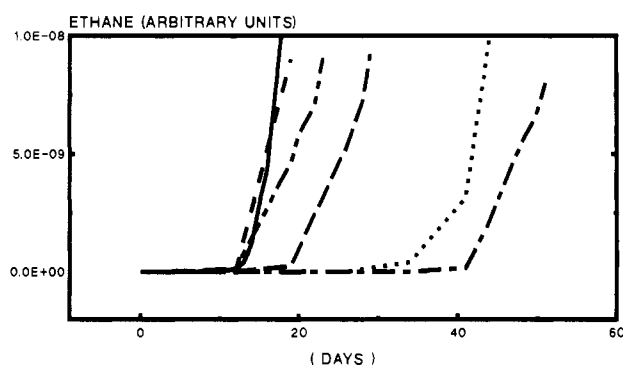

Figure 4. Ethane formation during storage at 37 °C of fish oil stabilized with various antioxidant mixtures. (—) Blank; (– – –) 0.1% CoQ_{10}/1% Topcithin; (- - -) 0.1% vitamin C/1% Topcithin; (– ·· –) 0.1% CoQ_{10}/0.05% vitamin E/1% Topcithin; (···) 0.1% CoQ_{10}/0.1% vitamin C/1% Topcithin; (– · –) 0.1% CoQ_{10}/0.05% vitamin E/0.1% vitamin C/1% Topcithin.
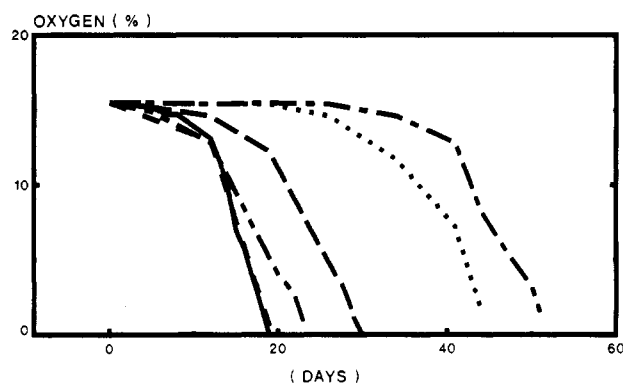

Figure 5. Oxygen absorption during storage at 37 °C of fish oil stabilized with various antioxidant mixtures. (—) Blank; (– – –) 0.1% CoQ_{10}/1% Topcithin; (- - -) 0.1% vitamin C/1% Topcithin; (– ·· –) 0.1% CoQ_{10}/0.05% vitamin E/1% Topcithin; (···) 0.1% CoQ_{10}/0.1% vitamin C/1% Topcithin; (– · –) 0.1% CoQ_{10}/0.05% vitamin E/0.1% vitamin C/1% Topcithin.

BuOH solution at –40 °C. This spectrum, characterized by a g factor of 2.0047, could be interpreted in terms of a quartet (a = 4.7 G) of doublets (a = 1.7 G). The features of the spectrum were slowly changing as the UV irradiation proceeded further. A broad asymmetrical triplet was thus recorded following 2 h of photolysis. Photolyzing CoQ_{10} in n-hexane/n-BuOD solution led to the disappearance of the hyperfine splitting associated with the 1.7-G coupling and to the observation (Figure 6B) of tiny hyperfine splittings (coupling smaller than 0.5 G). The central line appearing in this spectrum can be assigned to a second paramagnetic species formed during photolysis of CoQ_{10}. No paramagnetic species could be detected during the
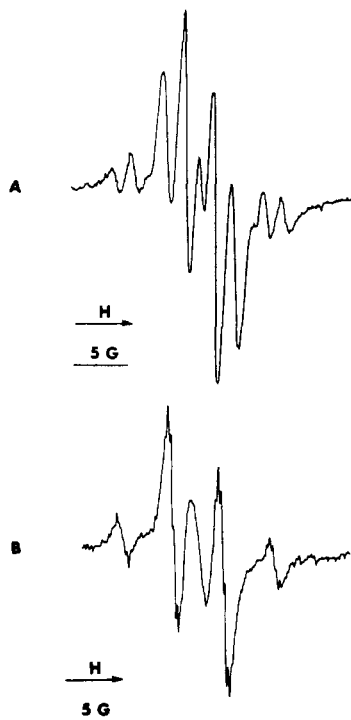
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Figure 6. ESR spectra recorded at the beginning of UV irradiation of CoQ$_{10}$ in *n*-hexane in the presence of minute amounts of (A) *n*-BuOH and (B) *n*-BuOD. Spectra were recorded at -50 °C with 2-mW incident power and 1-G modulation amplitude (A) and at -40 °C with 1-mW incident power and 0.32-G modulation amplitude (B).
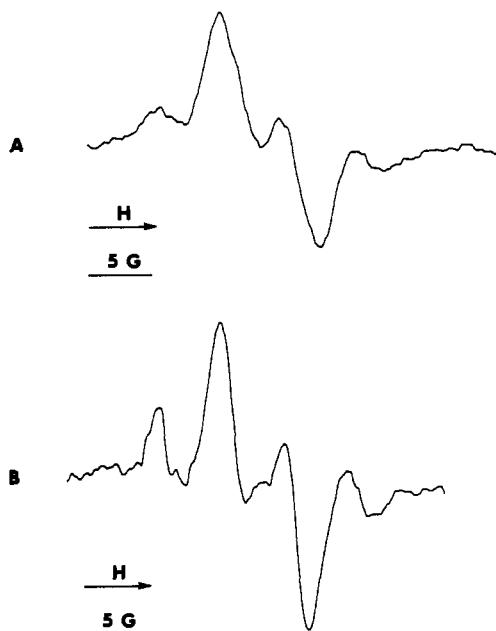

Figure 7. ESR spectra recorded during the reaction of 1% CoQ$_{10}$H$_2$ with peroxidizing CFLF in the presence of minute amounts of (A) *n*-BuOH and (B) *n*-BuOD. Spectra were recorded at 40 °C with 185-mW incident power and 2.5-G modulation amplitude (A) and at 40 °C with 180-mW incident power and 2.5-G modulation amplitude (B).

reaction at 20 °C of 1% CoQ$_{10}$ with peroxidizing CFLF in the presence of a minute amount of *n*-BuOH. Under the same conditions, 1% CoQ$_{10}$H$_2$ gave a rather broad quartet ESR spectrum (Figure 7A). This spectrum is characterized by a hyperfine coupling constant of about 5 G and a *g* factor of 2.004. Carrying out the reaction in the presence of *n*-BuOD gave a similar but slightly better resolved ESR spectrum (Figure 7B).
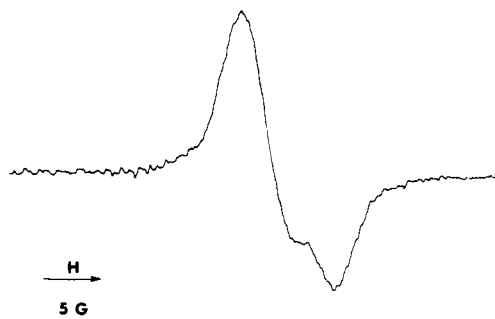

Figure 8. ESR spectrum recorded during the reaction of 0.1% vitamin C/1% Topcithin mixture in methyl linoleate at 20 °C. Spectrum was recorded with 10-mW incident power and 2-G modulation amplitude.

Neither CoQ$_{10}$ (0.1%) alone nor CoQ$_{10}$ (0.1%) in admixture with Topcithin (1%) gave rise to the observation of a paramagnetic species during their reaction with methyl linoleate. An unsymmetrical singlet at *g* = 2.005 (Figure 8) was recorded during the reactions of either vitamin C (0.1%)/Topcithin (1%) or vitamin C (0.1%)/Topcithin (1%)/CoQ$_{10}$ (0.1%) mixture with methyl linoleate at 20 °C. This spectrum could be observed during several hours provided the temperature of the reacting mixture was kept below 50 °C.

4. DISCUSSION

The type of radicals which can be produced from CoQ$_{10}$ and CoQ$_{10}$H$_2$ were first determined on the basis of experiments performed with one or the other substance alone. The radical reactions of CoQ$_{10}$ in the presence of vitamin C and a phospholipid were then investigated with the view to understanding the important antioxidant properties of this mixture.

4.1. Structure of Radicals. Apart from O-demethylation to the 2- and 3-hydroxyubiquinone (Cheng and Casida, 1970), ubiquinones undergo reduction reactions such as transformation into the parent ubiquinols or cyclization to the ubichromenol derivative when photolyzed in solution (Cheng and Casida, 1970; Moore and Folkers, 1965; Hales and Case, 1981). Reduction reactions take place via the intermediate semiquinone radical (Hales and Case, 1981).

The ESR spectrum depicted in Figure 6A can be ascribed to the neutral semiquinone IV (Figure 1, *n* = 10). Indeed, the 4.7-G splitting is consistent with the methyl group in position ortho with regard to the deprotonated oxygen and the 1.6-G splitting with the remaining hydroxyl proton (Foster et al., 1978). This assignment is confirmed by the disappearance of the 1.7-G splitting observed in the presence of *n*-BuOD. The semiquinone radical IV is, however, not the only paramagnetic species produced by UV irradiation of CoQ$_{10}$. The presence of a line in the center of the spectra of Figure 6 as well as the abnormally high central lines in the spectrum of Figure 6A indicates the formation of a second radical characterized by a narrower ESR spectrum. Except for the smallest hyperfine coupling (1.7 G) which is not resolved in the CFLF, the features of the ESR spectra depicted in Figures 6 and 7 are similar. This result indicates that the radicals formed during the decomposition of CoQ$_{10}$H$_2$ in peroxidizing lipids are the same as those produced at the beginning of the photolysis of CoQ$_{10}$, e.g., principally the semiquinone IV. The asymmetrical singlet recorded during the reaction of either the Topcithin/vitamin C or the Topcithin/vitamin C/CoQ$_{10}$ mixture with autoxidizing lipids (Figure 8) can be understood as an immobilized ascorbyl radical (Neubacher, 1984).

4.2. Origin of Antioxidant Activities. Most primary food antioxidants are either phenols or amines (Löliger, 1989). As CoQ$_{10}$ does not possess any labile hydrogen atom, it is not surprising that this compound has no antioxidant activity in foods. The fact that no positive ESR response can be obtained during the reaction of CoQ$_{10}$ with peroxidizing lipids confirms that ubiquinone is not (or is a poor) a scavenger of peroxy radicals (Yamamoto et al., 1990) under our experimental conditions. CoQ$_{10}$H$_2$ is, on the contrary, known as a good scavenger of lipid radicals (Takeshige et al., 1980; Frei et al., 1990; Yamamoto et al., 1990; Kagan et al., 1990). Present results show that CoQ$_{10}$H$_2$ actually reacts with lipid peroxy radicals to form semiquinone radicals by a hydrogen atom exchange reaction. On this basis CoQ$_{10}$H$_2$ would be expected to be a fairly good antioxidant, which is, however, not observed experimentally. This apparent discrepancy can be explained by the fact that, unlike other food antioxidants of the phenol type, CoQ$_{10}$H$_2$ is spontaneously oxidized in air into the corresponding inactive quinone.

The observation of the vitamin C radical during the reaction of CoQ$_{10}$/vitamin C/Topcithin mixture with peroxidizing lipids does not account for its important antioxidant activity. Indeed, the vitamin C radical is also observed with the vitamin C/Topcithin mixture (Figure 8), which is a poor antioxidant (Figures 4 and 5). The good antioxidant properties of the CoQ$_{10}$/vitamin C/Topcithin mixture (Figures 4 and 5) are to be related to the presence of CoQ$_{10}$ in the system. Although CoQ$_{10}$H$_2$ is not recycled by ascorbate (Frei et al., 1990), it can be hypothesized that CoQ$_{10}$ is reduced by the vitamin C into active compounds capable of trapping peroxy radicals and forming nonradical products. In other words, good inhibition of lipid autoxidation will take place under conditions favoring CoQ$_{10}$ reduction (Beyer, 1990).

4.3. Conclusion. Neither CoQ$_{10}$ nor CoQ$_{10}$H$_2$ is a good food antioxidant. The latter, although possessing phenol groups which favor antioxidant properties, is too unstable in air to be active. Ubiquinone is, on the contrary, a potent antioxidant when used in conjunction with a substance able to reduce it. In this way "active forms" of CoQ$_{10}$ are formed directly in the lipid medium and can therefore react with highly reactive lipid radicals. Vitamin C dispersed in the lipid by use of an emulsifier is such a reducing substance. To obtain protection of the lipid against oxidative damages, CoQ$_{10}$ should not be used as such but with substances able to produce reduced products which are the only active antioxidant forms of CoQ$_{10}$.

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