Rapid paper

**Antioxidant Activity of Ubiquinol in Solution
and Phosphatidylcholine Liposome**

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Summary The antioxidant activities of ubiquinol and ubiquinone were measured in the free radical-mediated oxidations of methyl linoleate in solution and phosphatidylcholine liposomes in aqueous dispersion. Ubiquinol-10 suppressed the oxidation of methyl linoleate in hexane, although its reactivity toward peroxy radical was about 10 times less than that of α -tocopherol. Ubiquinone-10, on the contrary, did not show any antioxidant activity. On the other hand, ubiquinol-10 inhibited the oxidation of phosphatidylcholine liposomal membranes as efficiently as α -tocopherol. When both ubiquinol-10 and α -tocopherol were present, ubiquinol-10 decreased first and α -tocopherol decreased after all ubiquinol-10 was consumed in both solution and liposomes. These results and electron spin resonance (ESR) study suggest that ubiquinol-10 regenerates α -tocopherol by reducing α -tocopheroxyl radical.

Key Words ubiquinol, ubiquinone, α -tocopherol, antioxidant, lipid peroxidation

Ubiquinol (UQ) is a ubiquitous hydroquinone present in all of the membranes (1) and even in blood plasma (2). It is well known that UQ and its oxidized form, ubiquinone, play an important role in electron transfer in mitochondria membrane, but the function of UQ is not clear in other membranes and blood plasma. Since a compound which has a low oxidation potential like ascorbic acid and α -tocopherol is a good antioxidant and UQ has a lower oxidation potential than α -tocopherol, one may expect an antioxidant activity of UQ. Actually, hydroquinone is known as a good chain-breaking antioxidant (3), but it is not widely used as a commercial antioxidant because of its instability.

Compared with the enormous numbers of papers on antioxidant activity of vitamin E, only a few papers have been reported on that of UQ. A pioneering work by Mellors and Tappel (4) showed that UQ-6 was almost as effective as α -

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tocopherol in reducing 2,2-diphenyl-1-picrylhydrazyl, in inhibiting heme-catalyzed peroxidation of arachidonate emulsion, and in light-induced oxidation of mitochondria. Takayanagi *et al.* (5) also showed that the inhibition of ADP-Fe³⁺ induced oxidation of mitochondria was dependent on the concentration of UQ controlled by exogenous reductant like NAD(P)H and inhibitors of electron transport like rotenone and antimycin A. Furthermore, the administration of ubiquinone-10 protected rats from hepatic cellular damage caused by hepatic ischemia and reperfusion (6), probably due to the inhibition of lipid peroxidation by ubiquinol-10 formed from exogenous ubiquinone-10 *in vivo* (7). However, the antioxidant activity of UQ has not been quantitated; *i.e.*, the rate constant for the reaction of UQ with peroxy radical is not known. Therefore, we compared the ability of scavenging lipid peroxy radical by UQ-10 with that by α -tocopherol in a free radical-mediated lipid peroxidation system where lipid peroxy radical is a predominant chain carrier. The synergism of these two antioxidants was also examined.

The oxidation of methyl linoleate or soybean phosphatidylcholine (PC) was induced with 2,2'-azobis-2,4-dimethylvaleronitrile (AMVN) and was carried out (8,9) at 37°C under air in the absence or presence of UQ-10 and/or α -tocopherol. The formation of hydroperoxide of methyl linoleate and PC was followed by HPLC monitoring at 234 nm (10). An ODS and a silica gel columns were used with methanol/water (9:1 by volume) and methanol/40 mM sodium phosphate, monobasic (9:1 by volume) as the eluant, respectively. UQ-10 and α -tocopherol were measured by an HPLC equipped with an electrochemical detector (0.8 V) using octyl column and 50 mM sodium perchlorate in methanol/*tert*-butyl alcohol (9:1 by volume) as the eluant.

The upper panel of Fig. 1 shows a typical example of the formation of methyl linoleate hydroperoxide during the 0.2 mM AMVN-induced oxidation of 453 mM methyl linoleate in hexane at 37°C under air in the absence or presence of 3 μ M UQ-10 and/or 3 μ M α -tocopherol. In the absence of antioxidant, the oxidation of methyl linoleate proceeds smoothly without any induction period. When 3 μ M UQ-10 was added, the rate of oxidation was clearly reduced but this reduction was not as efficient as that observed when 3 μ M α -tocopherol was added. On the other hand, α -tocopherol gave a clear induction period. When 3 μ M each of both antioxidants were present, the induction period was prolonged to a sum of each induction period and, more interestingly, the inhibition of oxidation was as efficient as that by α -tocopherol throughout the whole induction period.

UQ-10 decreased during the oxidation and concomitantly ubiquinone-10 was formed (data not shown). A rapid oxidation took place after all UQ-10 was consumed. Furthermore, the addition of ubiquinone-10 did not inhibit the oxidation, as shown in Fig. 1. Therefore, we conclude that ubiquinone-10 is not an antioxidant in this system, although Mellors and Tappel (4) and other investigators (11-13) suggested that ubiquinone had some antioxidant activity. This is not surprising since ubiquinone has no active hydrogen to scavenge oxygen radical

Fig. 1. Formation of methyl linoleate hydroperoxide (LOOH) in 0.2 mM AMVN-induced oxidation of 453 mM methyl linoleate in the absence of antioxidant (IH) or presence of 3 μM ubiquinol-10 (UQ-10) and/or 3 μM α -tocopherol (VE) in hexane at 37°C under air. The effect of addition of 3 μM ubiquinone-10 was also examined. Lower panel shows the decrease of ubiquinol-10 and α -tocopherol when both were present in the system.

Similarly, dehydroascorbic acid and tocopherolquinone, oxidized form of ascorbic acid and tocopherol, respectively, do not show antioxidant activity.

The length of the induction period is dependent on the stoichiometric number (n) of peroxy radical trapped by each antioxidant molecule. Since the n value for α -tocopherol is 2 (14), Fig. 1 suggests that n value for UQ-10 is smaller than 1.

The rate of oxidation (R_{inh}) during the induction period is expressed by the equation

$$R_{\text{inh}} = k_p \cdot [\text{LH}] \cdot R_i / n \cdot k_{\text{inh}} \cdot [\text{IH}]$$

where k_p , k_{inh} , $[\text{LH}]$, $[\text{IH}]$, and R_i are the rate constants for the propagation reaction and scavenging lipid peroxy radical by antioxidant, the concentrations of lipid and antioxidant, and the rate of chain initiation, respectively (8,9). Figure 2 shows the plot of R_{inh} against the reciprocal of antioxidant concentration in the AMVN-induced oxidation of methyl linoleate in hexane, and a good linear relationship was observed, as expected from the above equation. The ratio of the two

Fig. 2. Plot of rate of oxidation (R_{inh}) during the induction period against a reciprocal of the antioxidant (IH) concentration in the 0.2 mM AMVN-induced oxidation of 453 mM methyl linoleate in hexane at 37°C under air.

slopes shows the ratio of $n \cdot k_{inh}$ for two antioxidants. It was found that k_{inh} for UQ-10 was about 10 times less than that for α -tocopherol in hexane, assuming n value for UQ-10 is 1.

The lower panel in Fig. 1 shows the decrease of UQ-10 and α -tocopherol when both were present as antioxidants. As shown, UQ-10 decreased faster than α -tocopherol and the rate of disappearance of α -tocopherol increased after all UQ-10 was consumed. Since α -tocopherol can trap peroxy radical much faster than UQ-10, this result indicates the regeneration of α -tocopherol by the interaction of UQ-10 and α -tocopheroxyl radical. It was found by ESR that α -tocopheroxyl radical disappeared rapidly when it was reacted with UQ-10 as observed with ascorbic acid (15). On the other hand, α -tocopheroxyl radical did not react with ubiquinone-10. Mukai *et al.* also observed the disappearance of α -tocopheroxyl radical by UQ-10 using a stopped flow spectroscopy (16). Furthermore, Maguire *et al.* showed that the mitochondrial electron transport chain can reduce tocopheroxyl radical (17).

The upper panel of Fig. 3 shows the results of oxidation of 5.2 mM soybean PC liposome initiated with 1.0 mM AMVN in the absence or presence of 3 μ M UQ-10 and/or 3 μ M α -tocopherol at 37°C under air. The lower panel shows the decrease of α -tocopherol and UQ-10 during the oxidation when they were present together. Essentially the same results were obtained as those in homogeneous solution. Again, ubiquinone-10 did not show any significant antioxidant activity as shown in Fig. 3. However, UQ-10 suppressed the oxidation almost as efficiently as α -tocopherol in this liposomal system. The insert shows that the ratio of the rate of oxidation during the induction period inhibited by UQ-10 to that inhibited by α -tocopherol is about 3. As discussed earlier, this ratio represents the ratio of $n \cdot k_{inh}$ for two antioxidants. Therefore, k_{inh} for two antioxidants were almost the same, assuming n value for α -tocopherol and UQ-10 as 2 and 1, respectively. It has been

Fig. 3. Formation of soybean phosphatidylcholine hydroperoxide (PC-OOH) in 1.0 mM AMVN-induced oxidation of 5.2 mM soybean phosphatidylcholine multilamellar liposome dispersed in 0.1 M NaCl containing 100 μ M EDTA in the absence of antioxidant (IH) or presence of 3 μ M ubiquinol-10 (UQ-10) and/or 3 μ M α -tocopherol (VE) at 37°C under air. The effect of addition of 3 μ M ubiquinone-10 was also examined. Insert shows the early events in a magnified scale. Lower panel shows the decrease of ubiquinol-10 and α -tocopherol when both were present in the system.

shown (18) that α -tocopherol reduces its antioxidant activity in liposome compared with homogeneous solution. Therefore, when both UQ-10 and α -tocopherol are present in the liposomal membrane, lipid peroxy radical may be trapped not only by α -tocopherol but also by UQ-10 and, additionally, UQ-10 spares α -tocopherol by reducing α -tocopheroxyl radical. Interestingly, the rate of disappearance of UQ-10 was faster when it was present with α -tocopherol than when it was present alone (data not shown).

In summary, UQ-10 has been clearly shown to scavenge lipid peroxy radical, and its activity is almost as efficient as α -tocopherol in liposomal membrane. Furthermore, UQ-10 can interact with α -tocopheroxyl radical to regenerate α -tocopherol. These data indicate an important role of UQ-10 as lipid-soluble antioxidant in biomembranes.

Note added in proof

After we submitted this paper, two papers on antioxidant activity of ubiquinol came out; Kagan, V. E., Serbinova, E. A., Koynova, G. M., Kitanova, S. A., Tyurin, V. A., Stoytchev, T. S., Quinn, P. J., and Packer, L. (1990): Antioxidant action of ubiquinol homologues with different isoprenoid chain length in biomembranes. *Free Rad. Biol. Med.*, **9**, 117–126, and Frei, B., Kim, M. C., and Ames, B. N. (1990): Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations. *Proc. Natl. Acad. Sci. USA*, **87**, 4879–4883. We also learned that Dr. R. Stocker *et al.* found antioxidant activity of ubiquinol (*Proc. Natl. Acad. Sci. USA*, in press).

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