

UBIQUINONE-10 AS AN ANTIOXIDANT*

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ABSTRACT

Ubiquinol, the reduced form of coenzyme Q, is known as a lipid antioxidant. Its fully oxidized form, ubiquinone, would theoretically not have this activity. However, we report that ubiquinone-10, the oxidized form of coenzyme Q, demonstrated antioxidant activity in model studies against a lipid-soluble free radical generator, 2,2'-azobis(2,4-dimethylvaleronitrile). This was demonstrated at both 1:100 and 1:1,000 ratios of ubiquinone-10 to lipid (for both methyl linoleate and methyl linolenate). Ubiquinone-10 should therefore not be discounted as a possible antioxidant in more complex systems such as food.

PRACTICAL APPLICATIONS

Coenzyme Q (ubiquinol/ubiquinone) is an important component of electron transport in biological tissues and is also classified as a potent antioxidant. It is generally believed that the form that is active as an antioxidant is the reduced ubiquinol. In evaluating the loss of antioxidants in mackerel light and dark muscle tissues, it was observed that the oxidized form, ubiquinone, comprised over 87% of the total coenzyme Q in light muscle and over 93% in dark muscle and this observation did not change under anaerobic conditions. This study was done to determine if the oxidized form of coenzyme Q, ubiquinone, was capable of acting as an antioxidant. In this study, it is shown that it can function in this manner.

* The views expressed herein are those of the authors and do not necessarily reflect the views of the National Oceanic and Atmospheric Administration or any of its subagencies.

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This paper is dedicated to the memory of Herbert O. Hultin, a true gentleman, my friend, and inspirational mentor.

INTRODUCTION

Coenzyme Q or ubiquinone/ubiquinol is a vital electron and proton carrier which supports adenosine triphosphate synthesis in the mitochondrial inner membrane. In addition, the reduced form of coenzyme Q has been shown to act as a regeneratable reducing agent for the removal of free radicals and peroxides in model systems and in biological membranes (Frei *et al.* 1990; Ernster and Beyer 1991). Ubiquinol is capable of reducing both ferrylmyoglobin and metmyoglobin to oxymyoglobin (Mordente *et al.* 1993). Other studies have focused on the regeneration of other antioxidants by coenzyme Q (Kagan *et al.* 1990; McGwire *et al.* 1992). Suarna *et al.* (1993) and Cabrini *et al.* (1991) reported that ubiquinol (CoQH₂) appears to be lost before α -tocopherol, which is consistent with the observation by Mukai *et al.* (1990) that CoQH₂ is able to regenerate tocopherol.

Although the reduced form of coenzyme Q, ubiquinol, is generally considered to be the antioxidant, a patent in 1993 stated that it was found that the oxidized ubiquinone form had a significant antioxidation effect in food as well as cosmetic and pharmaceutical products and particularly those that were rich in polyunsaturated fatty acids (Bracco *et al.* 1993). This could be significant since it was shown that the majority of coenzyme Q in postmortem mackerel light and dark muscles was the oxidized form, ubiquinone (Petillo *et al.* 1998). The percentage of reduced coenzyme Q (ubiquinol) was only 12.8% in mackerel light muscle and 8.6% in dark muscle when expressed on a per gram of lipid basis. These figures changed only slightly to 12.5% for mackerel light muscle and 6.9% for mackerel dark muscle when the coenzyme Q contents were expressed on a wet weight basis. Coenzyme Q is a member of the electron transport system in the mitochondrial inner membrane and is present in high concentrations therein. Petillo *et al.* (1998) suggested that coenzyme Q may function as a prooxidant in mackerel dark muscle by producing some reactive oxygen species that escape the mitochondrial chain. The reduced ubiquinol content was closely and positively correlated with rancidity changes in the mackerel dark muscle.

Jørgensen *et al.* (1985) showed that the ubiquinone content in the absence of substrates in isolated pigeon heart mitochondria could be as high as 70–90% of the total coenzyme Q and that 15–20% of the total coenzyme Q content was still in the oxidized form under the strongest reducing conditions. Schnurr *et al.* (1996) reported a reaction of lipoxygenase with mitochondrial membranes both *in vitro* and *in vivo*. The total lipoxygenase-mediated oxygen uptake was much higher than could be predicted based on the uptake of oxygen by the lipids of the mitochondria of beef heart. Evidence suggested that ubiquinone was a necessary condition for this extra oxygen consumption. Roginsky *et al.* (1996) reported a similar phenomenon in a model system

suggesting that there was electron transfer from ascorbate to the ubiquinone forming both the semiquinone and ascorbyl radicals. They suggested that the semiquinone radical reacted with oxygen which was the cause of the high oxygen consumption. The extra oxygen consumption in the presence of ubiquinone was particularly high at low oxygen concentrations. Petrucci *et al.* (2000) suggested that ubiquinone could promote dismutation of superoxide radical and thus raised the question as to whether ubiquinones could mimic superoxide dismutase. The high content of coenzyme Q in mitochondria may be of particular importance in the dark muscles of pelagic fish species such as mackerel where mitochondria comprise about 45% of the volume of the cell (Bone 1978). In this work, we will show that ubiquinone-10 in an aprotic medium at 37C could reduce the rate of oxidation of both methyl linoleate and methyl linolenate exposed to a soluble free radical generator.

MATERIALS AND METHODS

Materials

Methyl linoleate and methyl linolenate were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Hexane and isopropanol (2-propanol) were purchased from Fisher Scientific (Pittsburgh, PA). 2,2'-azobis (2,4-dimethylvaleronitrile) (AMVN) was obtained from Monomer-Polymer & Dajac Laboratories (Feasterville, PA). Coenzyme Q₁₀ (ubiquinone-50) was purchased from Sigma Chemical Co. (St. Louis, MO).

Methods

A simple model system was developed to determine the antioxidant activity of the oxidized form of coenzyme Q, ubiquinone. The model was a modification of a study used to compare the antioxidant activities among a group of carotenoids (Terao 1989). The model consisted of an unoxidized lipid (methyl linoleate), a lipid-soluble oxidizing agent, AMVN and the lipid-soluble antioxidant (ubiquinone-10) in a solution of hexane/isopropanol. Two hundred μL of a solution of ubiquinone-10 (50 $\mu\text{mol}/\text{mL}$ hexane) was added to 8.8 mL of a mixture of hexane/isopropanol (1:1) containing 1,000 μmol methyl linoleate. Oxidation was initiated by adding 1.0 mL of AMVN (100 $\mu\text{mol}/\text{mL}$ hexane). The molar ratio was 100:10:1 for lipid, oxidant and antioxidant, respectively. Another trial was done using a molar ratio of 1,000:100:1. The mixtures were incubated in a shaking water bath at 37C under air in the dark. Samples were periodically withdrawn and methyl linoleate peroxide accumulation was monitored as the increased absorbance by

conjugated dienes at 235 nm. Other trials were done using methyl linoleate as the lipid substrate. Only one trial was run for each substrate.

RESULTS

The results of the methyl linoleate trial are presented graphically in Fig. 1 and those of methyl linolenate in Fig. 2. Oxidized coenzyme Q – ubiquinone-10 – functioned as an antioxidant at both 1:100 and 1:1,000 (ubiquinone-10 : substrate) ratios. Some differences were noted between the substrates. There was very little autooxidation, i.e., without AMVN, of methyl linoleate, as shown in Fig. 1. Ubiquinone-10, at a ratio of 1:100, reduced the rate of oxidation of AMVN-initiated linoleate oxidation to a rate as low as that of autooxidation. The effectiveness of the antioxidant at a 1:1,000 ratio was not as great but still significantly reduced the AMVN-catalyzed oxidation of methyl linoleate compared with a sample without the antioxidant.

Methyl linolenate had an autooxidation rate some six times greater than that of methyl linoleate. This was determined by comparing the slopes of

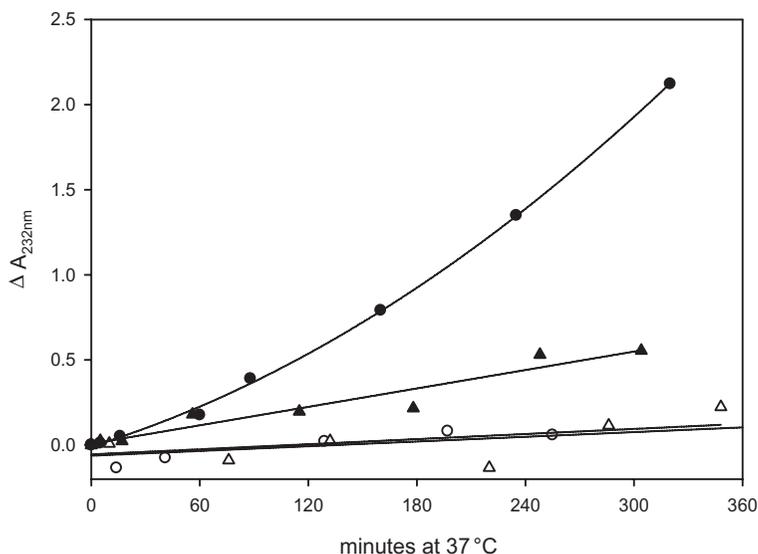


FIG. 1. FORMATION OF CONJUGATED DIENES FROM METHYL LINOLEATE (○); METHYL LINOLEATE AND AMVN (●) METHYL LINOLEATE, AMVN AND UBIQUINONE-10 (100:100:1, △); AND METHYL LINOLEATE, AMVN AND UBIQUINONE-10 (1,000:100:1, ▲) IN HEXANE AT 37°C

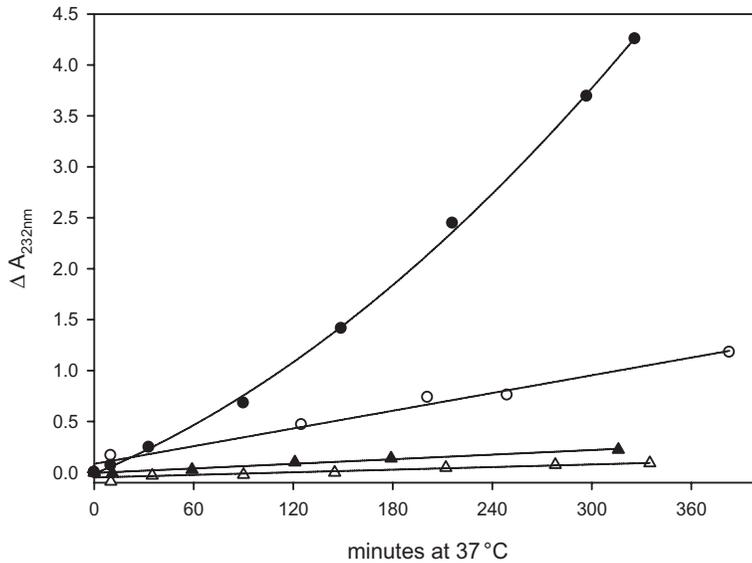


FIG. 2. FORMATION OF CONJUGATED DIENES FROM METHYL LINOLENATE (○); METHYL LINOLENATE AND AMVN (●); METHYL LINOLENATE, AMVN AND UBIQUINONE-10 (100:10:1, △); AND METHYL LINOLENATE, AMVN AND UBIQUINONE-10 (1,000:100:1, ▲) IN HEXANE AT 37C

the linear regression curves (r value for methyl linoleate = 0.79; for methyl linolenate = 0.99) for the two substrates. The rate of AMVN-initiated oxidation without ubiquinone was approximately twice as great for methyl linolenate as that for methyl linoleate as determined by comparing the initial slopes of the quadratic curves from the regression equations ($R^2 = 0.999$ for both substrates). Ubiquinone-10 was an effective inhibitor at ratios of both 1:100 and 1:1,000 with methyl linolenate. These results suggest that ubiquinone-10 is a more effective antioxidant with fatty acids containing more than two double bonds, which is in line with the observations of Bracco *et al.* (1993).

DISCUSSION

A major potential pool of ubiquinol is present as ubiquinone, the oxidized form, in pigeon heart mitochondria (Jørgensen *et al.* 1985) and in fish dark muscle (Petillo *et al.* 1998). The reducing systems, succinate and internal nicotinamide adenine dinucleotide coupled to oxidative phosphorylation, needed to recycle ubiquinone back to ubiquinol become progressively weaker

in nonliving tissue, as these Krebs's cycle metabolites are consumed. Although the primary function of ubiquinol as an antioxidant is to donate H atoms, there was a question of the ability of ubiquinone to act as an antioxidant. This was tested in a system previously used (Terao 1989) to evaluate the antioxidative capacity of carotenoids. An antioxidant such as ubiquinol donates H atoms to a radical species and thus removes the radical from any initiation reactions. After donating an H atom, ubiquinol becomes semiubiquinone or ubiquinone (after donating both H atoms). Ubiquinone could possibly function as a free radical scavenger. A free radical scavenger, although not as efficient an antioxidant as an H donor, reacts with free radicals and lowers their activation energy; the reactivities of free radicals are thus reduced. In addition, free radical scavengers may interact with other antioxidants and provide mutual sparing, as described earlier and demonstrated by researchers with various model membrane studies. One such study supports the observations of Terao (1989) who reported that astaxanthin and canthaxanthin could be as effective as α -tocopherol as a radical-trapping antioxidant (Palozza and Krinsky 1992a) while another demonstrated the synergistic action between β -carotene and α -tocopherol (Palozza and Krinsky 1992b). However, the nonconjugated arrangement of the double bonds in the ubiquinone tail would suggest that it would be a relatively poor free radical scavenger and thus may work poorly under good food storage conditions. Nevertheless, it could have the ability to be effective under processing conditions such as heating.

Other studies report no such synergism, yet describe individual antioxidant behavior of ubiquinone and β -carotene (Cabrini *et al.* 1986). Ubiquinone-10 cannot donate hydrogen to free radicals, so the antioxidant activity of ubiquinone-10 requires that another mechanism be involved. Cabrini *et al.* (1986) suggests that the antioxidant behavior of ubiquinone could be due to its ability to (1) scavenge singlet oxygen; and (2) structurally affect the lipid bilayer so as to inhibit hydroperoxide decomposition by metal-catalyzed reactions which promote spontaneous lipid peroxidation. In addition, one or more of the following may be possible. Ubiquinone may function in a manner as described for β -carotene, the antioxidative ability of which is thought to be due to radical addition to its conjugated polyene system (Krinsky 1993). Terao (1989), in comparing the relative antioxidant activity among carotenoids, found that it was the oxo group at the 4 (4') position and not the hydroxyl group at the 3 (3') position of astaxanthin which made this carotenoid even more effective than β -carotene in his model system. Terao surmised that the "electron-withdrawing character of oxygen atoms substantially reduces the unpaired electron density on the carbon skeleton (of the astaxanthin) resulting in the decrease of the reactivity of the carbon-centered radical toward molecular oxygen." Ubiquinone-10 also has an oxo group at the 4-position. Unlike β -carotene, ubiquinone does not possess a highly conjugated side chain.

Ubiquinone-10 possesses approximately the same number of double bonds as β -carotene, astaxanthin or canthaxanthin but is at least twice as long and therefore more flexible. This flexible side chain may facilitate reversible reduction of quinone groups and conversion to chromenol via cyclization with an isoprene unit (Lehninger 1964). Another possibility involves the physical interaction between ubiquinone and the methylated fatty acid due to van der Waals forces which hampers the formation of the conjugated diene. Also, physical interaction between ubiquinone and AMVN could prevent the production of initiating radicals by AMVN. In either case, ubiquinone may be simply interfering with the acceptance of a radical by the methylated fatty acid or the production of a radical by AMVN in an indirect way.

Results presented in this paper show that ubiquinone can behave as an antioxidant. The possibility that it can function this way in food products should be explored since the oxidized form predominates in fish muscle, a product highly sensitive to lipid oxidation.

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REFERENCES

- BONE, Q. 1978. Locomotor muscle. In *Fish Physiology* Vol VII Locomotion (W.S. Hoar and D.J. Randall, eds.) pp. 361–424, Academic, New York, NY.
- BRACCO, U., LÖLIGER, J. and SAUCY, F. 1993. *Protection of a food, cosmetic or pharmaceutical product against oxidation*. U.S. Patent No. 5,258,179. <http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnethtml%2FPTO%2Fsearch-bool.html&r=9&f=G&l=50&co1=AND&d=PTXT&s1=5258179&OS=5258179&RS=5258179> (accessed November 2, 1993).
- CABRINI, L., PASQUALI, P., TADOLINI, B., SECHI, A.M. and LANDI, L. 1986. Antioxidant behavior of ubiquinone and β -carotene incorporated in model membranes. *Free Radic. Res.* 2, 85–92.

- CABRINI, L., STEFANELLI, C., FIORENTINI, D. and LANDI, L. 1991. Ubiquinol prevents α -tocopherol consumption during liposome peroxidation. *Biochem. Int.* 23, 743–749.
- ERNSTER, L. and BEYER, R.E. 1991. Antioxidant functions of coenzyme q: Some biological and pathophysiological implications. In *Biomedical and Clinical Aspects of Coenzyme Q*, Vol 6 (K. Folkers, G.P. Littarru and T. Yamagami, eds.) pp. 45–58, Elsevier, Amsterdam, the Netherlands.
- 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.
- JØRGENSEN, B.M., RASMUSSEN, H.N. and RASMUSSEN, U.F. 1985. Ubiquinone reduction pattern in pigeon heart mitochondria. *Biochem. J.* 229, 621–629.
- KAGAN, V.E., SERBINOVA, E.A., KOYNOVA, G.M., KITANOVA, S.A., TYURIN, V.A., STOYTCHEV, S., QUINN, P.J. and PACKER, L. 1990. Antioxidant action of ubiquinol homologues with different isoprenoid chain lengths in biomembranes. *Free Radic. Biol. Med.* 9, 117–126.
- KRINSKY, N.I. 1993. Actions of carotenoids in biological systems. *Ann. Rev. Nutr.* 13, 561–587.
- LEHNINGER, A.L. 1964. *The Mitochondrion: Molecular Basis of Structure and Function*, pp. 72–74, W.A. Benjamin, Inc., New York, NY.
- MCGWIRE, J.J., KAGAN, V., ACKRELL, B.A.C., SERBINOVA, E. and PACKER, L. 1992. Succinate-ubiquinone reductase linked recycling of α -tocopherol in reconstituted systems and mitochondria: Requirement for reduced ubiquinol. *Arch. Biochem. Biophys.* 292, 47–53.
- MORDENTE, A., MARTORANA, G.E., SANTINI, S.A., MIGGIANO, G.A.D., PETITTI, T., GIARDINA, B., BATTINO, M. and LITTARRU, G.P. 1993. Antioxidant effect of coenzyme Q on hydrogen peroxide-activated myoglobin. *Clin. Investig.* 71, S92–S96.
- MUKAI, K., KIKUCHI, S. and URANO, S. 1990. Stopped-flow kinetic study of the regeneration reaction of tocopheroxyl radical by reduced ubiquinone-10 in solution. *Biochim. Biophys. Acta* 1035, 77–82.
- PALOZZA, P. and KRINSKY, N.I. 1992a. Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. *Arch. Biochem. Biophys.* 297, 291–295.
- PALOZZA, P. and KRINSKY, N.I. 1992b. β -carotene and α -tocopherol are synergistic antioxidants. *Arch. Biochem. Biophys.* 297, 184–187.
- PETILLO, D., HULTIN, H.O., KRZYNOWEK, J. and AUTIO, W.R. 1998. Kinetics of antioxidant loss in mackerel light and dark muscle. *J. Agric. Food Chem.* 46, 4128–4137.

- PETRUCCI, R., GIORGINI, E., DAMIANI, E., CARLONI, P., MARROSU, G., TRAZZA, A., LITTARRU, G.P. and GRECI, L. 2000. A study on the interactions between coenzyme Q₀ and superoxide anion. Could ubiquinones mimic superoxide dismutase (SOD)? *Res. Chem. Intermed.* *26*, 269–282.
- ROGINSKY, V.A., MOHR, D. and STOCKER, R. 1996. Reduction of ubiquinone-1 by ascorbic acid is a catalytic and reversible process controlled by the concentration of molecular oxygen. *Redox Rep.* *2*, 55–62.
- SCHNURR, K., HELLWING, M., SEIDEMANN, B., JUNGBLUT, P., KÜHN, H., RAPOPORT, S.M. and SCHEWE, T. 1996. Oxygenation of biomembranes by mammalian lipoxygenases: The role of ubiquinone. *Free Radic. Biol. Med.* *20*, 11–21.
- SUARNA, C., HOOD, R.L., DEAN, R.T. and STOCKER, R. 1993. Comparative antioxidant activity of tocotrienols and other natural lipid-soluble antioxidants in a homogenous system, and in rat and human lipoproteins. *Biochim. Biophys. Acta* *1166*, 163–170.
- TERAO, J. 1989. Antioxidant activity of β -carotene-related carotenoids in solution. *Lipids* *24*, 659–661.