Coenzyme Q10 as a Front-Line Antioxidant against Oxidative Stress

Yorihiko Yamamoto*

School of Bionics, Tokyo University of Technology, 1404-1 Katakura, Hachioji, Tokyo 192-0982, Japan

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Summary Coenzyme Q10 was discovered as an essential cofactor of mitochondrial ATP production in 1957. Coenzyme Q10 is ubiquitously present in other biomembranes and even in lipoproteins. Reduced form of coenzyme Q10 (ubiquinol-10) is believed to work as antioxidant in these places. When human plasma was incubated under aerobic conditions, ubiquinol-10 depleted after the depletion of ascorbate but α -tocopherol remained unchanged, indicating that ascorbate and ubiquinol-10 are front-line antioxidants against oxygen radicals. The redox status of plasma coenzyme Q10 is a good indicator of oxidative stress since oxidative stress is defined as a disturbance in the prooxidant-antioxidant balance in favor of the former. We therefore developed a simple and reliable method for the simultaneous detection of both oxidized and reduced forms of coenzyme Q10. We applied this method to patients plasma and demonstrated an increase of oxidative stress in patient's with hepatitis, cirrhosis, and hepatoma, in LEC rats (animal model of liver cancer), in newborn babies, in patients with Parkinson disease, and others.

Key Words: vitamin E, vitamin C, oxidative stress marker

Introduction

Crane *et al.* discovered a lipid-soluble, orange compound from bovine heart mitochondria in 1957 [1] and named the compound coenzyme Q because it is an essential cofactor of ATP production and possesses quinone structure. At the same time, Morton also recognized quinones which are ubiquitously present in animals, plants, and even in bacteria and name them ubiquinones [2]. Soon these two compounds were found to be identical and Folkers *et al.* characterized the chemical structure [3].

Lengths of isoprenoid side chain are different with species. Humans have coenzyme Q10 with 10 isoprenoid units. The oxidized and reduced forms of

coenzyme Q10 are called as ubiquinone-10 and ubiquinol-10, respectively (Fig. 1).

Humans biosynthesize coenzyme Q10, because coenzyme Q10 is essential for ATP production and an important antioxidant. However, tissue concentration of coenzyme Q10 decreases with age [4]. In human heart, for example, 30 and 50% reduction of coenzyme Q10 content were observed at ages of 40's and 80's, respectively, when compared with the value at 20's. Therefore, it is reasonable that supplementation of coenzyme Q10 is popular in USA and European countries, and lately in Japan. Now the coenzyme Q10 market is expanding rapidly.

Antioxidant Activity of Ubiquinol-10

In 1966, Mellors and Tappel showed that ubiquinol-6 was almost as effective as α -tocopherol in reducing 2,2-diphenyl-1-picrylhydrazyl, in inhib-

* To whom correspondence should be addressed.
Tel/Fax: +81-426-37-2918

times less than that of α -tocopherol (Fig. 3) [9]. On the other hand, ubiquinol-10 inhibited the oxidation of phosphatidylcholine liposomal membranes as effi-

Fig. 4. Formation of soybean phosphatidylcholine hydroperoxide (PC-OOH) during the aerobic oxidation of 5.2 mM soybean phosphatidylcholine multilamellar liposome dispersed in 0.1 M NaCl containing 100 μ M EDTA initiated with 1.0 mM 2,2'-azobis(2,4-dimethylvaleronitrile) in the absence of antioxidant or the presence of 3 μ M ubiquinol-10 ($\text{CoQH}_2\text{-10}$) and/or 3 μ M α -tocopherol (VE) in hexane at 37°C. The effect of addition of 3 μ M ubiquinone-10 (CoQ-10) was also measured. Lower panel shows the decrease of $\text{CoQH}_2\text{-10}$ and VE when both antioxidants were present in the system.

ciently as does α -tocopherol (Fig. 4) [9]. This may be ascribed to that ubisemiquinone radical, one electron oxidation product of ubiquinol, reacts with oxygen to give hydroperoxyl radicals which continue oxidation of methyl linoleate in hexane (Fig. 5). On the contrary, hydroperoxyl radicals migrate to aqueous phase in liposomal oxidation and do not participate in lipid-phase oxidation (Fig. 5). This is consistent with the stoichiometric number of peroxy radical trapped by ubiquinol-10 is about 1 [9]. When both ubiquinol-10 and α -tocopherol were present, ubiquinol-10 decreased first and α -tocopherol decreased after all of ubiquinol-10 were consumed in both solution and liposomes (Figs. 3 and 4) [9]. These results and ESR study suggest that ubiquinol-10 regenerates α -tocopherol by reducing α -tocopheroxyl radical [9, 13]. No significant antioxidant activity of ubiquinone-10 was observed (Figs. 3 and 4) [9].

Above results suggest that ubiquinol-10 could be a front-line antioxidant against oxygen radicals *in vivo*. In fact, ubiquinol-10 decreased first when human plasma was oxidized with a lipid-soluble radical initiator while ascorbate decreased first when oxidized with a water-soluble radical initiator (Fig. 6) [14]. When human plasma was incubated at 37°C under aerobic conditions, ubiquinol-10 depleted after the depletion of ascorbate (Fig. 7) [15]. However, no significant decay in α -tocopherol was seen (Fig. 7) [15]. A significant increase in cholesteryl ester hydroperoxides was observed after the depletion of ascorbate and ubiquinol-10 (Fig. 7), indicating that α -tocopherol without ubiquinol-10 and ascorbate did not suppress the formation of lipid hydroperoxide. Bowry and Stocker showed that α -tocopheroxyl radicals act as a chain carrier in this type of lipid

Fig. 5. Roles of ubiquinol-10 ($\text{CoQH}_2\text{-10}$) in the oxidation of biological lipids (LH) to prevent the formation of lipid hydroperoxides (LOOH) and their reaction pathways. 1. Scavenge peroxy radicals ($\text{LOO}\cdot$), 2. reduce α -tocopheroxyl radical ($\text{VE}\cdot$), and 3. transfer radicals in lipid phase into aqueous phase.

Fig. 6. Changes in plasma antioxidant levels (mole fraction) during the aerobic oxidation of human plasma initiated with a water-soluble initiator (10 mM 2,2'-azobis(2-amidinopropane) dihydrochloride, AAPH) and a lipid-soluble initiator (10 mM 2,2'-azobis(2,4-dimethylvaleronitrile), AMVN) at 37°C. VC, vitamin C; UA, uric acid; BR, unconjugated bilirubin.

Fig. 7. Changes in plasma antioxidant levels during the aerobic oxidation of human plasma in the absence (upper panel) or presence (lower panel) of 5 μM cupric chloride at 37°C. CE-OOH, cholesteryl ester hydroperoxide; PC-OOH, phosphatidylcholine hydroperoxide.

oxidation and called this oxidation process as tocopherol-mediated peroxidation (TMP) [16]. Ascorbate and ubiquinol-10 are the most important, biological anti-TMP reagents to reduce α -tocopheryl radical to α -tocopherol [15, 17].

Marker of Oxidative Stress

The redox status of plasma coenzyme Q10 is a good indicator of oxidative stress since oxidative stress is defined as a disturbance in the prooxidant-antioxidant balance in favor of the former and ubiquinol-10 is a front-line antioxidant against oxidative stress as described above. We therefore developed a simple and reliable method for the simultaneous detection of plasma ubiquinol-10 and ubiquinone-10 [18]. Figure 8 shows HPLC system for the detection of plasma ubiquinol-10 and ubiquinone-10. Plasma coenzyme Q10 is necessary to be extracted by an organic solvent such as hexane. Therefore, analytical procedure is as follows.

Stored plasma (50 μl) was mixed vigorously with 250 μl of cold methanol and 500 μl of cold hexane in a 1.5 ml-polypropylene tube. After centrifugation at 10,000 $\times g$ for 3 min at 4°C, 5 μl of hexane layer (corresponding to 0.5 μl of plasma) was injected immediately and directly onto HPLC equipped with two guard columns (Type Supelguard LC-ABZ, 5 μm, 20 $\times$ 4.6 mm i.d., Supelco, Tokyo), an analytical column (Type Supelcosil LC-8, 5 μm, 250 $\times$ 4.6 mm i.d., Supelco), a reduction column (Type RC-10-1, Irica, Kyoto), and an amperometric electrochemical detector (ECD, Model Σ985, Irica). The oxidation potential for ECD was 600 mV on a glassy carbon electrode. The mobile phase consisted of 50 mM sodium perchlorate in methanol/*tert*-butyl

Fig. 8. HPLC system for the detection of plasma ubiquinol-10 and ubiquinone-10.

Fig. 9. Typical HPLC chromatograms of hexane extract from a normal subject monitored by a UV detector and an amperometric electrochemical detector. FC, free cholesterol; Ch20:4, cholesteryl arachidonate; Ch18:2, cholesteryl linoleate; Ch18:1, cholesteryl oleate.

alcohol (85/15, v/v) delivered at a flow rate of 0.8 ml/min.

Typical HPLC chromatograms are shown in Fig. 9, indicating that coenzyme Q₁₀ is mostly present in the reduced form. The detection limit of plasma ubiquinol-10 and ubiquinone-10 is about 4 nM with excellent reproducibilities. Free cholesterol and cholesteryl esters are also measured at their absorbance at 210 nm as shown in Fig. 9. Table 1 summarizes the results obtained from 31 male donors in the

Table 1. Plasma levels of cholesterol and antioxidants in healthy humans.

Substance	Mean $\pm$ SD
FC (mM)	1.14 $\pm$ 0.23
CE (mM)	3.28 $\pm$ 0.64
Vitamin E (μ M)	21.8 $\pm$ 7.5
CoQH ₂ -10 (nM)	704 $\pm$ 205
CoQ-10 (nM)	32 $\pm$ 13
CoQH ₂ -10 + CoQ-10 (nM)	737 $\pm$ 275
%CoQ-10	4.5 $\pm$ 1.3

Male, $n=31$, age = 29.1 $\pm$ 7.3. Abbreviations: FC, free cholesterol; CE, cholesteryl esters, %CoQ-10 (%) = CoQ-10 / (CoQH₂-10 + CoQ-10).

range of 22–45 years old. It was found that percentages of oxidized form of coenzyme Q₁₀ (%CoQ-10) in total coenzyme Q₁₀ is 4.5% [21], indicating that plasma coenzyme Q₁₀ exists mostly in the reduced form.

Increase in Oxidative Stress as Measured by Redox Status of Coenzyme Q₁₀

Human liver cancer often develops after the onset of chronic hepatitis and the subsequent cirrhosis. Measurement of oxidative stress at each stage is of interest since oxidative stress has been suggested to be a causative factor in cancer. However, only a limited number of papers have documented the occurrence of oxidative stress in these liver patients. Therefore, we applied above described method to plasma obtained from patients with hepatitis, cirrhosis, and hepatoma [19].

Profiles of patients and control are given in Table 2, indicating that average age is about 60 and patients are mostly infected by C virus. A significant decrease in plasma ascorbate level was observed in patient groups as compared with normal subjects (Table 2). However, it is possible that the lowered

Table 2. Subject profiles and plasma levels of water-soluble antioxidants.

	Number	Male/female	Age (year)	B virus/ C virus
Normal	16	12/4	57.7±16.6	
Hepatitis	28	18/10	59.5±10.7	3/25
			0.4832	
Cirrhosis	16	10/6	60.4±13.0	1/15
			0.4552	
Hepatoma	20	15/5	65.7±7.0	2/18
			0.2414	

	Uric acid (μM)	Vitamin C (μM)	Unconjugated bilirubin (μM)
Normal	328±65	52.7±46.7	4.1±2.6
Hepatitis	279±79	15.3±15.0	3.2±2.0
	0.0560	0.0003	0.1426
Cirrhosis	282±86	20.6±15.8	5.7±4.1
	0.1331	0.0108	0.1851
Hepatoma	275±118	15.3±21.6	5.6±3.6
	0.1425	0.0009	0.2499

Mean±SD. Numbers in *italic* show *p* values when compared with normal subjects as determined by Mann-Whitney test.

Fig. 10. Box and whisker plots of plasma ubiquinone-10 percentage (%CoQ-10) in total amounts of ubiquinone-10 and ubiquinol-10 among normal subjects and patients with chronic active hepatitis, liver cirrhosis, and hepatocellular carcinoma (hepatoma). Significant differences compared with normal subjects were analyzed by non parametric Mann-Whitney test.

level of plasma ascorbic acid in these patients is caused by the shortage of ascorbate intake.

We adopted our method for the measurement of plasma ubiquinol-10 and ubiquinone-10. The ratio of ubiquinone-10 to ubiquinol-10 should be one of

Fig. 11. Box and whisker plots of plasma vitamin E to total cholesterol among normal subjects and patients with chronic active hepatitis, liver cirrhosis, and hepatocellular carcinoma (hepatoma). No significant differences compared with normal subjects were observed when analyzed by non parametric Mann-Whitney test.

the most reliable markers of oxidative stress since it is a direct product of redox imbalance. Figure 10 shows that patients with chronic active hepatitis, liver cirrhosis, and hepatocellular carcinoma had significantly higher %CoQ-10 value than normal subjects. These results indicate that oxidative stress is evident after the onset of hepatitis and the subsequent cirrhosis and liver cancer. A significant increase in the ratio of plasma ubiquinone-9 to ubiquinol-9 was also observed in LEC rat (animal model of liver cancer) after the onset of hepatitis. On the other hand, vitamin E/total cholesterol ratio remained unchanged among four groups as shown in Fig. 11 as expected from Fig. 7.

We also demonstrated an increase in oxidative stress in newborn babies [21], and in patients with Parkinson disease [22].

Concluding Remarks

This review focused on antioxidant activity of coenzyme Q10. Because of its ability of scavenging peroxyl radicals and reducing α -tocopheroxyl radical, the reduced form of coenzyme Q10 (ubiquinol-10) is a front-line antioxidant *in vivo*. This also authorize that redox status of coenzyme Q10 is a good marker of oxidative stress. If the readers want to know more about coenzyme Q10, a recent review by Turunen *et al.* [23] is highly recommended.

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