NOTE

Comparison of Antioxidative Activities of Ubiquinol-4 and α -Tocopherol against Methyl Linoleate Peroxidation and Their Cooperative Effect

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A comparison was made of the antioxidative activity of ubiquinol-4 (H_2CoQ_4) and α -tocopherol (α -TOC) in methyl linoleate system by gas chromatography (GC) and measuring the oxygen consumption of the head space gas. The synergistic effect of H_2CoQ_4 on α -TOC was also studied. At various concentrations, the activity of H_2CoQ_4 was weaker than that of α -TOC; 0.7% H_2CoQ_4 addition showed the same effect as 0.33% α -TOC addition. When present together, the efficiency was about the sum of their individual efficiencies, and synergistic effect of H_2CoQ_4 toward α -TOC could not be detected.

1 Introduction

As a kind of lipid-soluble compounds existed in biomembranes, ubiquinone and its reduced form ubiquinol have been considered to be important antioxidants beyond α -TOC^{(1)~(3)}. It seems to be more significant because ubiquinones are present at higher concentrations than α -TOC and are constantly being regenerated by the electron transfer system in mitochondria⁽¹⁾. Recently, Packer's group has reported that although tocopherols are much stronger membrane antioxidants than naturally occurring ubiquinols (ubiquinones), the later can synergistically enhance enzymic NADH- and NADPH-dependent recycling of tocopherols by electron transport in mitochondria and microsomes^{(4),(5)}. These reports are limited to the model membranes, little is known about the effect of ubiquinone (ubiquinol) on the autoxidation of edible oil in bulk phase compared with α -TOC. In the present paper, we investigated the antioxidant efficiencies of α -TOC and H_2CoQ_4 and their synergistic effect against methyl linoleate peroxidation to discuss the possible application of ubiquinone-4 as an oral administration antioxidant. We choose ubiquinone-4 because its structure is similar to α -TOC in phenyl chain and it is said to be more effective than ubiquinone-10 against the vitamin E deficiency, although ubiquinone-9 and ubi-

Scheme-1

quinone-10 are the major ubiquinone analogues existed in biomembranes of animals and they can be biosynthesized by themselves^{(6)~(8)}. These observations imply its spare or substitutive effect for α -TOC. Moreover, the molecular weight of H_2CoQ_4 is about the same of α -TOC, which is favorable to compare their antioxidative activities in weight addition level (Scheme-1).

2 Experimental

2.1 Material

dl- α -TOC (99.8%) and ubiquinone-4 were generous gifts of Eisai Co. Ltd., Tokyo. Methyl linoleate (>99% pure) was prepared according to reference 9. Reduced CoQ_4 solution was prepared immediately prior to use by reduction in ethanol with $NaBH_4$, followed by extraction with hexane

(separated into two layers by degassed distilled water). The solution was kept under N_2 atmosphere and protected from light.

2.2 Methods

For one test, 1 g of methyl linoleate containing α -TOC and/or H_2CoQ_4 was put into a 15-mL glass test tube and sealed it with a W-type rubber cap, then stored at 60°C in the dark. Absorbed oxygen level in the head space of the test tube was measured by gas chromatography with a thermconductivity detector⁹⁾.

3 Results

3.1 Comparison of antioxidative activities of α -TOC and H_2CoQ_4

The amounts of oxygen absorption in test tubes are shown in Fig. -1. It is considered to reach a plateau when oxygen absorption was over 2.0 mL¹⁰⁾, the days were used to compare the antioxidant activity. Methyl linoleate containing 0.18% α -TOC was oxidized within 6 d compared with 2 d of α -TOC free substrate oil. About double amount of supplementation (0.33%) didn't enhance the activity, and about six-times amount of supplementation prolonged the period only to 8 d (Fig. -1 a). These results indicate that an optimum concentration of α -TOC exists to obtain an effective antioxidative activity against methyl linoleate peroxidation. On the other hand, H_2CoQ_4 gave a weaker activity as comparison with α -TOC. 0.3% of H_2CoQ_4 addition

Fig.-1 Antioxidant activities of α -TOC and H_2CoQ_4 against methyl linoleate autoxidation during storage at 60°C.

Table-1 Cooperative effect of α -tocopherol and ubiquinol-4 against methyl linoleate peroxidation. Each value indicates the number of days required to reach a plateau on oxygen uptake curve.

Compound added		α -Tocopherol		
		0	0.18%	0.33%
Ubiquinol-4	0	2	6	6
	0.3%	2	8	8
	0.7%	6	12	12

didn't inhibit the peroxidation of methyl linoleate. Above twice as amount of addition (0.7%) reached the same efficacy of 0.18% or 0.33% of α -TOC addition (Fig. -1 b).

3.2 Investigation of cooperative effect of H_2CoQ_4 to α -TOC

When α -TOC was coexisted with H_2CoQ_4 , the efficacy was about the sum of individual efficacy of them. To understand it easily, we showed the result in Table-1. For example, 0.33% of α -TOC increased the storage period to 6 d, while 0.7% of H_2CoQ_4 also increased it to 6 d. Coexistence of 0.33% of α -TOC and 0.7% of H_2CoQ_4 increased the period to 12 d. No synergistic effect was observed.

4 Discussion

Under our experimental conditions (incubation at 60°C under air), a substantial portion of H_2CoQ_4 might be consumed by autoxidation of itself and could not play the role of an antioxidant¹¹⁾, i. e., the real amount of antioxidative H_2CoQ_4 was lower than that expressed in Fig.-1 and Table-1. Its weaker antioxidative efficacy than α -TOC may be derived from the above reason. The mechanism by which H_2CoQ_4 and other reduced coenzyme Q act as antioxidants remains unsolved thoroughly, Frei *et al.* have suggested that they trap free radicals and inhibit the propagation reaction of lipid peroxidation at least by their hydrogen-donating abilities similar to α -TOC, although they cannot be recycled by ascorbate, most importantly, they can be recycled by the mitochondrial respiratory chain¹¹⁾. Another possible mechanism is suggested that they can react with ADP-perferryl ions or with superoxide directly and thus interfere with the initiation of lipid peroxidation¹⁰⁾.

Kagan *et al.* have reported that the antioxidant effects of ubiquinones in microsomes and mitochondria are mediated by tocopherol recycling, which is NADH- and NADPH-dependent⁴⁾. In our systems used, absence of the regeneration of α -TOC by ubiquinone may be due to the absence of NADH- and NADPH-dependent recycling system. During our paper was reviewed, we read Yamamoto *et al.*'s report which suggests that ubiquinol-10 can regenerate α -TOC by reducing α -tocopheroxyl radical¹³⁾, but they did not show direct evidence. It shouldn't ignore that ubiquinol is more easily autoxidized than α -TOC. Administration of ubiquinone inhibited ethanol- and carbon tetrachloride-stimulated lipid peroxidation of rats¹⁴⁾, and in elderly animals ubiquinone levels dropped drastically in heart, kidney, and highly aerobic (red) muscles¹⁵⁾. On the basis of results *in vivo* and in bulk phase, ubiquinone shows a possible application in oral administration as an antioxidant which can spare and substitute for α -TOC.

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リノール酸メチル自動酸化に対する α -トコフェロールとユビキノール-4 の酸化防止力の比較及び共存効果

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リノール酸メチルに対する α -トコフェロール, ユビキノール-4 の酸化防止力は GC 法の酸素吸収量によって比べられた。同時にユビキノール-4 の α -トコフェロールへの相乗作用も調べられた。ユビキノール-4 の酸化防止力は α -トコフェロールより弱く, 0.7% 添加量のユビキノール-4 の酸化防止力は 0.33% 添加量の α -トコフェロールのそれに相当した。また, 共存した場合の酸化防止強度はそれぞれの和であった。つまり, ユビキノール-4 の相乗作用は認められなかった。