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Quenching Activities of Common Hydrophilic and Lipophilic Antioxidants against Singlet Oxygen Using Chemiluminescence Detection System

Yasuhiro Nishida*, Eiji Yamashita and Wataru Miki

Institute for Food Science Research, Fuji Chemical Industry CO., Ltd., 55 Yokohoonji, Kamiichi, Toyama 930-0397, Japan

The singlet oxygen quenching activities among common hydrophilic and lipophilic antioxidants such as polyphenols, tocopherols, carotenoids, ascorbic acid, coenzyme Q10 and α-lipoic acid were recorded under the same test condition: the chemiluminescence detection system for direct $^1O_2$ counting using the thermodissociable endoperoxides of 1,4-dimethylnaphthalene as $^1O_2$ generator in DMF : $CDCl_3$ (9 : 1). Carotenoids exhibited larger total quenching rate constants than other antioxidants, with astaxanthin showing the strongest activity. α-Tocopherol and α-lipoic acid showed considerable activities, whereas the activities of ascorbic acid, CoQ10 and polyphenols were only slight; these included capsaicin, probucol, edaravon, BHT and Trolox. This system has the potential of being a powerful tool to evaluate the quenching activity against singlet oxygen for various hydrophilic and lipophilic compounds.

1. Introduction

Living organisms possess defense mechanisms against oxidative damage. One of the most important ways is using an antioxidants, such as ascorbic acid, polyphenols, coenzyme Q10 (CoQ10), tocopherols or carotenoids [2], for quenching and/or scavenging against reactive oxygen species (ROS).

Singlet oxygen ($^1O_2$) is a non-radical ROS with one of the strongest activities. It directly damages onto biological lipids, proteins and DNA, which are related to serious diseases such as diabetes, hypertension and cancer [1,2]. It would be valuable to search an effective quencher against $^1O_2$ and to develop its methodology.

Di Mascio [3] reported that lycopene showed the highest activity among carotenoids and tocopherols by Germanium photodiode detection system in EtOH : $CHCl_3$: H_2O (50 : 50 : 1) using the thermodissociable endoperoxides of a naphthalene derivative ($NDPO_2$) as a $^1O_2$ generator. One of the authors [4] evaluated the activities of marine carotenoids and α-tocopherol in two solvent systems, $CDCl_3$ and $CDCl_3$: CD_3OD (2 : 1) by the chemiluminescence detection system for direct $^1O_2$ counting using the thermodissociable endoperoxides of 1,4-dimethylnaphthalene (EDN) as $^1O_2$ generator. And it was found that astaxanthin showed the strongest activity.

The activities of carotenoids and tocopherols were revealed by both studies, whereas those of the other compound groups remain largely unknown. We therefore wanted to compare the activities of common antioxidants in nature such as ascorbic acid, polyphenols, α-lipoic acid, CoQ10 and others to those of carotenoids and tocopherols. Here we report the direct comparison of the quenching activities against $^1O_2$ among the antioxidants with not only lipophilic but hydrophilic property under the same test conditions.

*Corresponding author. E-mail: y-nishida@fujichemical.co.jp

Abbreviations:
BHT, butyleted hydroxytoluene; CoQ10, coenzyme Q10; EDN, endoperoxides of 1,4-dimethyl naphthalene; EGCG, epigallocatechin gallate; LDL, low-density lipoprotein; $NDPO_2$, naphthalene-1,4-dipropionate endoperoxide; $^1O_2$, singlet oxygen; QOL, quality of life; ROS, reactive oxygen species.

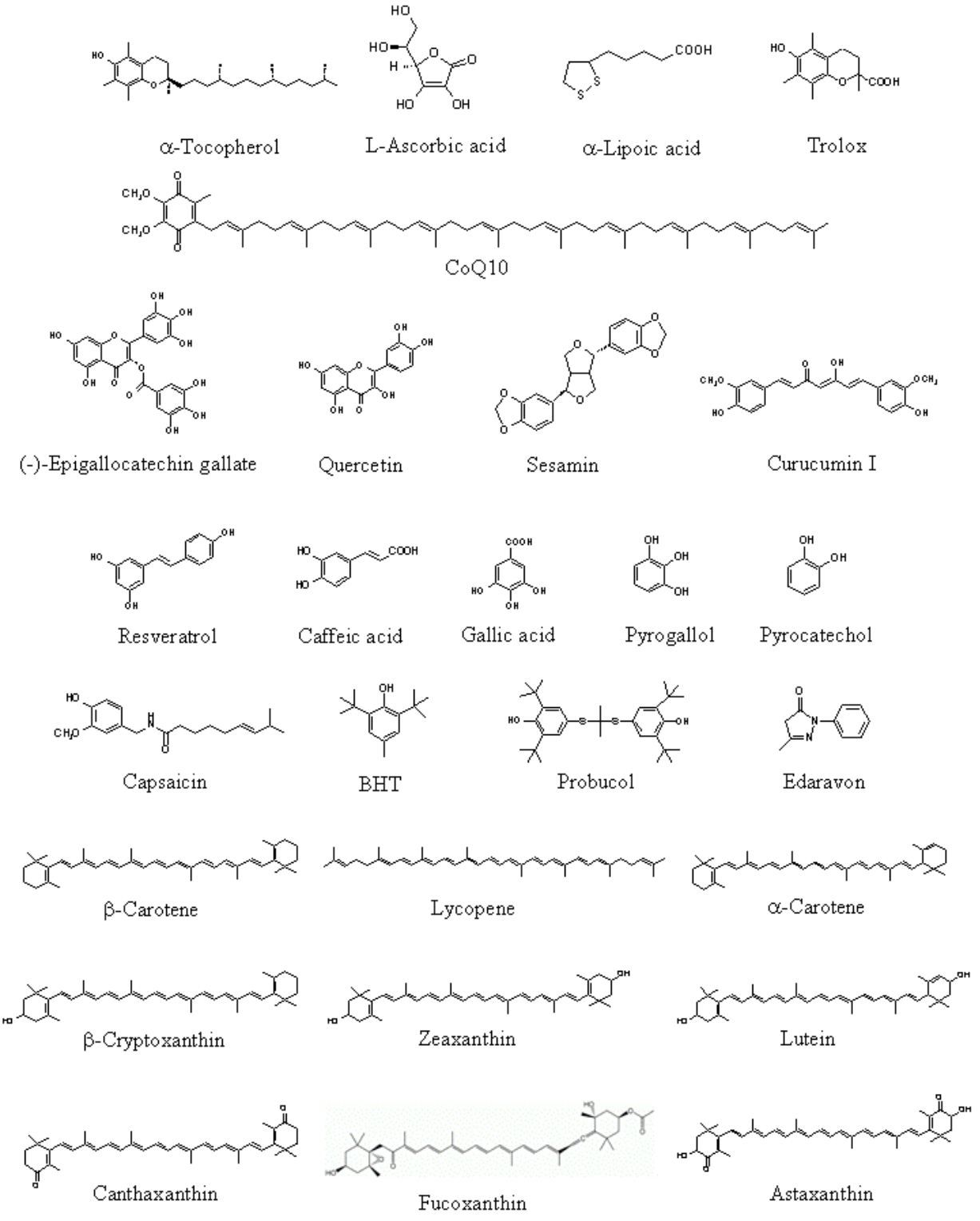

Fig. 1 Chemical structures of tested compounds

2. Experimental

2.1. Test compounds.

Astaxanthin, lutein, α-lipoic acid, ubiquinone-10 (CoQ10), caffeic acid, quercetin, resveratrol, gallic acid, pyrocatechol, pyrogallol, BHT and sesamin were purchased from Sigma-Aldrich (St. Louis, MO, USA), L(+)-ascorbic acid, α-tocopherol, probucol, canthaxanthin and lycopene from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), β-cryptoxanthin from Extrasynthase (Genay, France), Trolox and edaravon (MCI-186) from Cosmo Bio Co., Ltd. (Tokyo, Japan) and curcumin I, (-)-epigallocatechin gallate (EGCG) and capsaicin from Nagara Science Co., Ltd. (Gifu, Japan). β-Carotene was a gift from Prof. H. Hashimoto of Osaka City University. Fucoxanthin was extracted from the brown algae *Undaria pinnatifida* and *Laminaria japonica*. Recrystallization and/or chromatography of all these compounds resulted in obtaining a purity greater than 99%.

2.2. Measurement of $^1O_2$ quenching activity

As one of the authors previously reported [4], thermodissociable EDN prepared from 1,4-dimethylnapthalene (purchased from Sigma-Aldrich in St. Louis, MO, USA) was used as $^1O_2$ generator. EDN was dissolved with $CDCl_3$ and stored at below 0 ºC until used. It could release molecular oxygen in the singlet state ($^1\Delta_g$) at 37 ºC. Chemiluminescence emissions from $^1O_2$ were counted with a chemiluminescence detector, AccuFlex Lumi 400 (Aloka, Japan).

One hundred eighty micro litters of $CDCl_3$ or a mixture of $CDCl_3$: CD_3OD (2:1) or DMF containing 0.01 to 50,000 μM of each compound was placed in a thermostated glass tube (12φ X 75 mm) at 37 ºC. Chemiluminescence counting was started just after addition of the EDN in $CDCl_3$ at the final concentration of 50 mM, and was counted for 60 seconds. Both chemiluminescence counts of a control (S_0) without any test compound, and a sample (S) with the identical test compound were recorded. The total quenching constant, generally for total quenching by chemical reaction and/or physical quenching, $k_T = k_q + k_r$, was analyzed on a Stern-Volmer plot, which is based on the following equation [5],

$$S_0/S = 1 + k_T \cdot k_d^{-1}[Q] \quad (1)$$

where k_q is the physical quenching rate constant, k_r is the chemical reaction rate constant, k_d is the first-order decay rate constant of singlet oxygen in the solvent, and [Q] is concentration of the test compounds. Total quenching constant k_T was used to evaluate the activity of the each test compound.

3. Results and Discussion

Within the range of the concentrations actually tested each antioxidant was dissolved in the solvent at 37 ºC.

Fig. 2 showed the Stern-Volmer plots of astaxanthin, β-carotene, lycopene, α-tocopherol and α-lipoic acid in $CDCl_3$, $CDCl_3$: CD_3OD (2 : 1) and DMF : $CDCl_3$ (9 : 1). A high linearity meaning more than 0.9 of r^2 value was observed in the each plot. The plots of other test compounds were similar to those (data not shown).

Total quenching constant ($k_T = k_q + k_r$) of each test compound is shown in Table 1. The activities of canthaxanthin (in $CDCl_3$), α-carotene, β-cryptoxanthin, fucoxanthin (in $CDCl_3$), lycopene, lutein (in $CDCl_3$: CD_3OD (2 : 1)), α-tocopherol (in $CDCl_3$: CD_3OD (2 : 1)), CoQ10 and α-lipoic acid were additionally recorded by the same method as the former study [4] and a similar tendency was observed. Briefly, carotenoids showed stronger $^1O_2$ quenching activities than α-tocopherol as well as CoQ10 and α-lipoic acid which are recognized as common antioxidants.

In the case of carotenoids, a number of conjugated double bonds including C=C and C=O were found to contribute the quenching activity. In $CDCl_3$ represented

as the lipophilic system, lycopene showed the largest value. And in $CDCl_3$: CD_3OD (2 : 1) with more hydrophilicity, astaxanthin did so.

Both hydrophilic and lipophilic common antioxidants were directly compared in the new system using DMF : $CDCl_3$ (9 : 1). All carotenoids exhibited larger k_T value than other antioxidants. Moreover, astaxanthin showed the strongest activity among carotenoids tested. The hydroxyl groups in the carotenoid molecule were found to contribute slightly to the activity in the solvent, while the carbonyl groups were also found in $CDCl_3$: CD_3OD (2 : 1). The values of α-tocopherol and α-lipoic acid were relatively large. Ascorbic acid, CoQ10 and polyphenols such as EGCG represented as catechins, quercetin as flavonoids, curcumin as curcumnoids, resveratrol as stilbenoids, gallic acid as tannins, sesamin as lignan, pyrocatechol, caffeic acid and pyrogallol had weaker activities. Weaker activities were also noted in capsaicin, probucol and edaravon as medicines, BHT used as an antioxidant food additive and Trolox (6-hydroxy-2,5,7,8-tetramethyl- chroman-2-carboxylic acid) which is a reference substance for ORAC (Oxygen Radical Absorbance Capacity) value. They might rather be singlet oxygen quenchers than free radical scavengers against superoxide anion or hydroxyl radicals. This system has the potential of being a powerful tool to evaluate the quenching activity against singlet oxygen for various hydrophilic and lipophilic compounds.

Overall, astaxanthin exhibited the most potent singlet oxygen quenching activity among the compounds tested in this study because it showed a stable superior property under the three different conditions. Astaxanthin is widely distributed in fish and shellfish, crustaceans, zoo- and phyto-planktons, bacteria and so on, particularly in marine organisms. In fact, the first isolation and identification was accomplished in 1938 from the lobster, *Astacus gammarus* [6], and numerous studies were carried out over a long period. It is reported that the biological activity of astaxanthin originated from potent $^1O_2$ quenching and lipid peroxidation suppressing activities [7]. Various human benefits for human health have been recognized to date: immunomodulation [8], anti-stress [9], anti-inflammation [10], LDL cholesterol oxidation suppression [11], enhanced skin health [12], improved semen quality [13], attenuation of eye fatigue [14], sports performance and endurance enhancement [15], limitations on exercised induced muscle damage [16], limitations of diabetic nephropathy [17], improvement of hypertension [18] and metabolic syndrome [19]. Astaxanthin obviously plays an important role in promoting QOL to prevent diseases and maintain a healthy life.

Further study is needed for the evaluation of lipid peroxidation suppressing activity among common hydrophilic and lipophilic antioxidants based on this experiment.

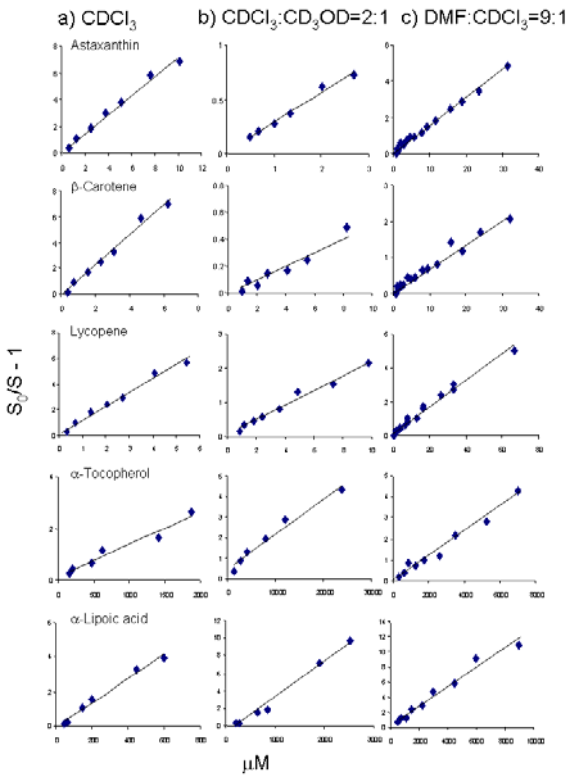

Fig.2 Stern-Volmer plots of some tested compounds

Table 1. Total singlet oxygen quenching rate constants

Compound	Tested Concentration (μM)	k_T ($10^9 M^{-1}s^{-1}$)		
		$CDCl_3$	$CDCl_3/CD_3OD$ (2:1)	DMF/$CDCl_3$(9:1)
Astaxanthin	0.01-15	2.2	1.8	5.4
Canthaxanthin	0.01-15	2.2	1.3	2.0
α-Carotene	0.01-15	0.66	0.23	0.93
β-Carotene	0.01-15	2.2	0.28	1.1
β-Cryptoxanthin	0.01-15	2.0	0.27	1.7
Fucoxanthin	0.01-15	0.29	0.075	0.97
Lycopene	0.01-15	3.0	1.4	3.4
Lutein	0.01-15	0.61	0.26	2.1
Zeaxanthin	0.01-15	2.0	0.73	3.4
L-Ascorbic acid	20-50,000	-	-	0.00089
α-Tocopherol	10-20,000	0.020	0.0039	0.049
α-Lipoic acid	10-10,000	0.056	0.038	0.072
Ubiquinone-10	10-3,000	0.0019	0.0021	0.0068
BHT	10-10,000	-	-	0.0040
Caffeic acid	10-10,000	-	-	0.0023
CurcuminI	10-10,000	-	-	0.0036
(-)-Epigallocatechingallate	10-6,000	-	-	0.0096
Gallic acid	10-10,000	-	-	0.0023
Pyrocatechol	10-10,000	-	-	0.0055
Pyrogallol	10-10,000	-	-	0.0055
Quercetin	10-10,000	-	-	0.0018
Resveratrol	10-10,000	-	-	0.0018
Sesamin	10-5,000	-	-	0.0012
Capsaicin	10-10,000	-	-	0.0021
Probucol	10-10,000	-	-	0.00044
Edaravon	10-10,000	-	-	0.0067
Trolox	10-20,000	-	-	0.011

4. Acknowledgments

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