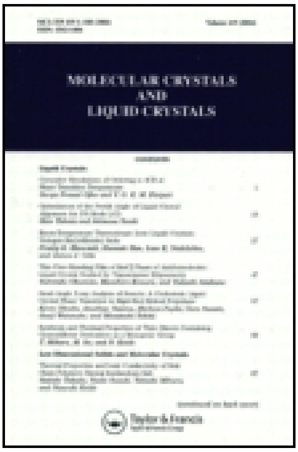

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Anti-Oxidative Properties of Astaxanthin and Related Compounds

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Anti-Oxidative Properties of Astaxanthin and Related Compounds

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Carotenoids have attracted attention in food and healthcare industries owing to their ability to quench ROS. Here, we present a method for the determination of anti-oxidative property against singlet oxygen ($^1O_2$), which plays an important role in skin aging. We quantified the bimolecular $^1O_2$ quenching rate constant, and the amount of $^1O_2$ molecules that can be quenched by a single anti-oxidative molecule before degradation, for five materials. We have defined the anti-oxidative property against $^1O_2$ as the product of these two constants. It was revealed that anti-oxidative properties of astaxanthin and lycopene were the highest among the materials investigated.

Keywords Anti-oxidative property; astaxanthin; carotenoid; ROS; singlet oxygen

1. Introduction

ROS such as superoxide, hydrogen peroxide, hydroxyl radical, singlet oxygen, and peroxides are chemically reactive molecules produced in the human body. Overproduction of ROS has been widely accepted to be involved in diseases such as arteriosclerosis, myocardial infarction, and cancer [1–4].

Among ROS, we focused on singlet oxygen ($^1O_2$), which plays an important role in skin aging. $^1O_2$ produced by exposure of skin to sunlight is considered to react with biomolecules, producing peroxides and ultimately leading to skin roughness and wrinkle. Once peroxides are formed, oxidation occurs continuously in a chain reaction manner, damaging skin cell membranes and collagen networks. Therefore, scavenging of $^1O_2$ is essential for maintaining wrinkleless skin. Anti-oxidative materials such as astaxanthin and lycopene, which can reduce harmful effect of ROS, have gained a great deal of attention in food and healthcare industries. However, most studies hitherto have aimed to quantify bimolecular quenching constants against $^1O_2$, and to the best of our knowledge, there are

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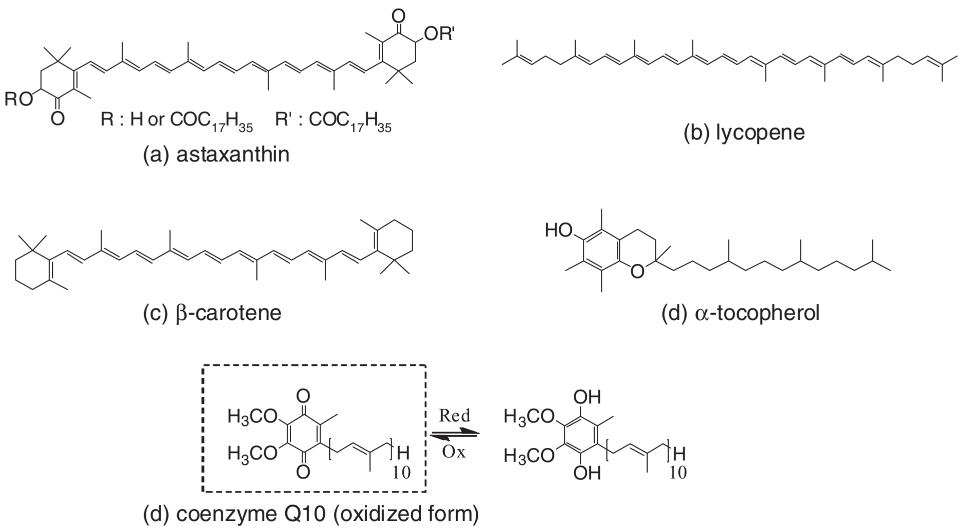

Figure 1. Structures of lipophilic anti-oxidative materials investigated in this study.

few reports that have dealt with the amount of $^1O_2$ molecules a single molecule can quench before degradation. In this report, we present a method for determination of quantitative anti-oxidative property against $^1O_2$ and the results of our measurements.

2. Experimental

Materials

Figure 1 shows the structures of the anti-oxidative materials investigated in this report. Astaxanthin, lycopene, Coenzyme Q10 (CoQ10), α-tocopherol, β-carotene, methylene blue and carbon tetrachloride were purchased from Wako Pure Chemical *etc*, and used without further purification.

Coenzyme Q10 (CoQ10) may exist in three redox states, namely fully oxidized (ubiquinone), partially reduced (semiquinone), and fully reduced (ubiquinol). In this study, we used the fully oxidized form (ubiquinone), since CoQ10 mainly exists as ubiquinone in cosmetics.

Measurements of Anti-Oxidative Properties

Measurement of the electronic absorption spectrum was conducted with UVPC-2500 spectrometer (Shimadzu). For detection of singlet oxygen, emission spectra in the near-infrared region were obtained using a home-made apparatus, consisting of a spectrometer (Ritsu), a xenon lamp (MAXCUTE 302, Asahi Spectra Co., Ltd.), and a Ge detector (EO-817L, North Coast Scientific). By adjusting the optical density of the sample to 0.1–0.3, re-absorption and other undesirable optical effects were avoided. Degradation product of anti-oxidative materials was quantified by LC-10AD type HPLC system (Shimadzu) using Capcellpack C18 UG120 type column (SHISEIDO).

$^1O_2$ was produced by photosensitization, a known photophysical method [5], and methylene blue was used as a photosensitizer. Sample solution was typically prepared by the following procedure. Methylene blue and anti-oxidative material were dissolved in ethanol, a good solvent for these solutes, and then diluted with carbon tetrachloride (CCl_4) to prescribed concentrations (for example, 25 μM methylene blue and 3 μM astaxanthin). Excitation of methylene blue was performed by GLG He-Ne laser (633 nm, NEC). As an example, when a sample containing 25 μM methylene blue (absorptivity 0.9) is irradiated with 633 nm light (2 mW) for 600 s, the accumulated amount of $^1O_2$ generated is calculated to be 2.9 μmol, using the quantum yield of $^1O_2$ generation in this system [6]. Calculation for this particular example can be written as equation (1).

$$2 \times 10^{-3}\,(\mathrm{W})^{*}600(\mathrm{s})^{*}0.9\,(\mathrm{absorptivity})/189 \times 10^{3}\,(\mathrm{J/mol})^{*}0.5\,(\mathrm{quantum\ yield}) = 2.9\,(\mu\mathrm{mol}) \quad (1)$$

3. Results And Discussion

3.1 Definition of Anti-Oxidative Property Against $^1O_2$

Since carotenoids are not produced *de novo* and the amounts of these molecules are limited in the human skin, we focused on two parameters when evaluating anti-oxidative materials. Namely, the quenching rate constant against $^1O_2$, and the amount of $^1O_2$ molecules a single anti-oxidative molecule can quench before degradation. We have defined the "anti-oxidative property against $^1O_2$" as the product of these two parameters (equation 2).

[Anti-oxidative property against $^1O_2$] = [$^1O_2$ quenching rate constant] * [Amount of $^1O_2$ molecules a single anti-oxidative molecule quenches before degradation] (2)

It is noteworthy that the anti-oxidative properties are influenced by the polarity of the solvent used in the measurement system [7–10]. Lipophilic anti-oxidative materials investigated in this report are assumed to end up in lipid membranes and other lipidic fractions of the body when they are provided as cosmetics and supplements. Hence, anti-oxidative properties were measured in carbon tetrachloride solution, whose polarity (dielectric constant; $\varepsilon = 2.2$) is similar to lipids.

3.2 $^1O_2$ Quenching Rate Constant

There are several methods to produce $^1O_2$, either chemically or photophysically. However, chemical methods, such as thermal degradation of endoperoxides and the reaction between hydrogen peroxide and hypochlorite, are not adequate for the accurate measurement of $^1O_2$ quenching rate constant, because of the complexity of the system [11]. Therefore we chose photosensitization, a known photophysical method, owing to the simplicity of the system and the high reproducibility of the measurement [5].

Figure 2 shows the scheme for the generation of $^1O_2$ by photosensitization and quenching by anti-oxidative materials. After photoexcitation of the photosensitizer, intersystem crossing to the lowest excited triplet state (T_1) occurs immediately. $^1O_2$ is generated by energy transfer from the photosensitizer in the T_1 state to the ground state triplet oxygen ($^3O_2$), which is spontaneously dissolved in the solution. $^1O_2$ then deactivates to the ground state through emission and/or quenching by anti-oxidative materials. We observed this process by monitoring the near-infrared emission spectra. By using a transient absorption technique, we confirmed that under our experimental conditions, anti-oxidative materials do not interact with the T_1 state of the photosensitizer.

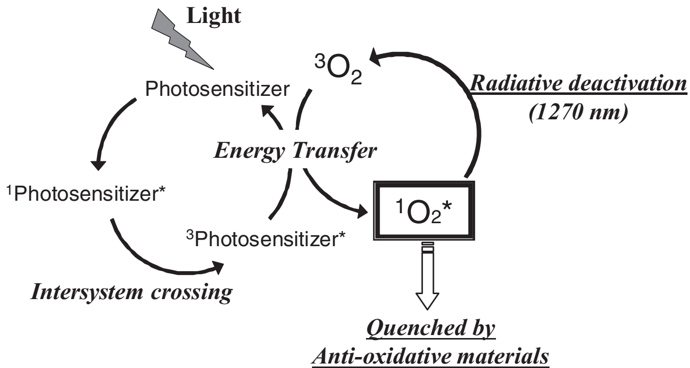

Figure 2. Schematic illustration of $^1O_2$ generation by photosensitization method, and $^1O_2$ quenching by anti-oxidative materials.

Typical spectra of $^1O_2$ emission derived by photosensitization, and Stern-Volmer plots for the five anti-oxidants are shown in Fig. 3. Emission of $^1O_2$ observed around 1270 nm showed a decrease in intensity as the amount of added astaxanthin increased (Fig. 3 (a)). The ratio of emission intensity (I_0/I) was plotted as a function of anti-oxidative material concentration in Fig. 3(b) (Stern-Volmer plot [12]). The rate constant of $^1O_2$ quenching was obtained from the slope of the plot, and the rate constant of astaxanthin (9×10^9 M^{-1} s^{-1}) was in agreement with the former reported value [13].

The quenching rate constants of lycopene (11×10^9 M^{-1} s^{-1}) and astaxanthin (9×10^9 M^{-1} s^{-1}) were highest among the materials investigated. These values were comparable to the diffusion controlled rate constant derived from Smoluchowski-Stokes-Einstein equation, indicating that $^1O_2$ quenching by astaxanthin and lycopene is a highly efficient process [14].

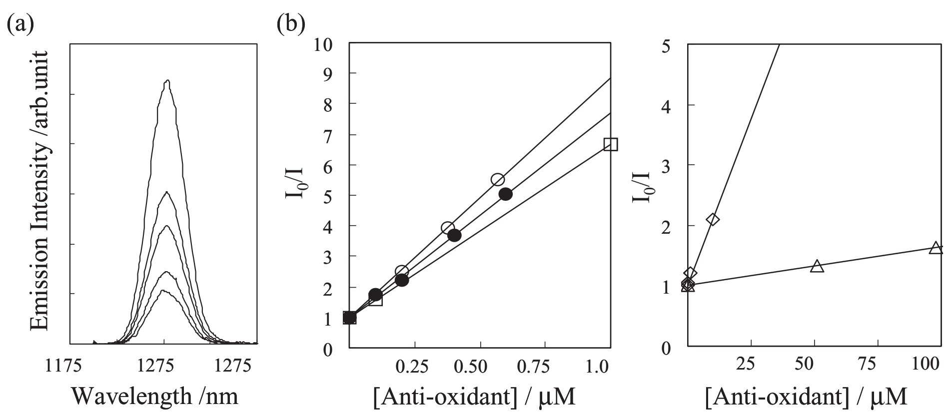

Figure 3. (a) Emission spectra of $^1O_2$ quenched by addition of astaxanthin. The concentrations of astaxanthin were 0, 0.1, 0.2, 0.4, and 0.6 μM, in the order of decreasing emission intesity. (b) Stern-Volmer plots for astaxanthin (closed circle), lycopene (open circle), β-carotene (open square), α-tocopherol (open diamond), and coenzyme Q10 (oxidized form) (open triangle).

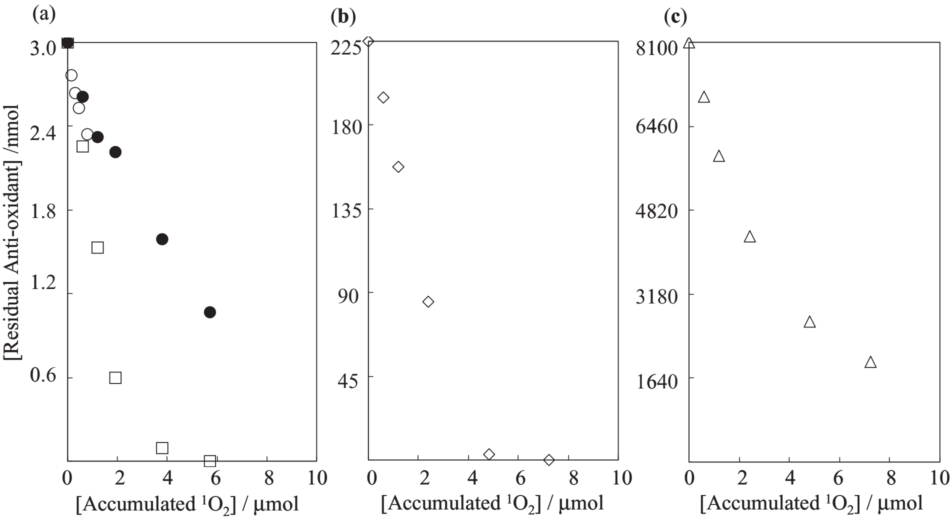

Figure 4. Degradation curves upon generation of $^1O_2$ for (a) astaxanthin (closed circle), lycopene (open circle), β-carotene (open square), (b) a-tocopherol (open diamond), and (c) CoQ10 (oxidized form) (open triangle).

3.3 Amount of Quenched $^1O_2$ Molecules

The amount of $^1O_2$ molecules a single anti-oxidative molecule quenches before degradation was defined as shown in equation 3.

[Amount of $^1O_2$ molecules a single anti-oxidative molecule quenches before degradation] = [Accumulated amount of $^1O_2$ generated by photosensitization] / [Amount of degraded anti-oxidative molecules] (3)

The accumulated amounts of $^1O_2$ molecules generated by photosensitization were calculated from the number of photons absorbed by photosensitizers and the quantum yield of $^1O_2$ generation in this system (further details are described in the EXPERIMENTAL section). The amounts of degraded anti-oxidative molecules were quantified by HPLC.

Figure 4 shows the results of the measurements, plotting residual anti-oxidative materials against the accumulated amount of $^1O_2$ molecules generated. Under initial conditions, concentrations of the anti-oxidative materials were high enough to quench $^1O_2$ completely. When the accumulated amount of $^1O_2$ generated was 1 μmol, only about 1 nmol of carotenoids were degraded (Fig. 4(a)). On the contrary, more than 50 nmol of α-tocopherol, and about 1500 nmol of CoQ10 (oxidized form) were degraded (Fig. 4(b), (c)). Table 1 shows the amount of $^1O_2$ quenched by a single anti-oxidative molecule before degradation, estimated from the initial slope of the plot in Fig. 4 and equation (3).

3.4 Comparison of Anti-oxidative Property Against $^1O_2$

Table 1 shows the anti-oxidative properties of lipophilic anti-oxidative materials measured in this report. The anti-oxidative properties of astaxanthin and lycopene were the highest among the five materials investigated. This is assumed to be because these carotenoids quench $^1O_2$ mainly through photophysical processes, not through chemical processes

Table 1. Anti-oxidative properties of lipophilic anti-oxidative materials investigated in this study

Materials	$^1O_2$ quenching rate constant (10^9 M^{-1} s^{-1})	Number of quenched molecules (per molecule)	Anti-oxidative property (Arb.unit)
astaxanthin	9	1900	17100
lycopene	11	900	9900
β-carotene	7	800	5600
α-tocopherol	0.1	20	2
coenzyme Q10 (Oxidized form)	0.009	1	0.009

accompanying degradation of the materials. Detailed studies on quenching mechanisms of lipophilic anti-oxidative materials are now under investigation.

4. Conclusion

We presented a method for determination of quantitative anti-oxidative properties against $^1O_2$. The anti-oxidative property against $^1O_2$ was defined as the product of bimolecular $^1O_2$ quenching rate constant and the accumulated amount of $^1O_2$ molecules quenched by a single anti-oxidative molecule before degradation. Anti-oxidative properties of astaxanthin and lycopene were the highest among the materials investigated.

Acknowledgments

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References

[1] Ooyanagi, Y. (1989). *Kasseisannso to byouki*, Kagaku-Dojin Publishing Company, INC: Kyoto, Japan.
[2] Nakano, M. *et al.* (1988). *Kasseisannso*, Kyoritsu Shuppan Co. Ltd.: Tokyo, Japan.
[3] Niki, E. *et al.* (1994). *Kousannkabussitu*, Gakkaishuppann: Tokyo, Japan.
[4] Kurashige, Y., Okazoe, Y., Okimasu, E., Ando, Y., Mori, M., Miki, W., Inoue, M. & Uchiumi, K. (1989). *Cyto-protection & Biology.*, *7*, 383.
[5] Turro, N. J., (1978). *Modern Molecular Photochemistry*, The Benjamin/Cummings Publishing Company: California, USA.
[6] Tanielian, C., Goden, L., & Wolff, C. (1984). *J. Photochem., 25, 117.*
[7] Kobayashi, M., & Sakamoto, Y. (1999). *Biotech. Lett.*, *21*, 265.
[8] DiMascio, P.; Kaiser, S., & Sies, H. (1989). *Arch. Biochem. Biophys.*, *274*, 532.
[9] Miki, W. (1991). *Pure Appl. Chem.*, *63*, 141.
[10] Goto, S., Kogure, K., Abe, K., Kimata, Y., Kitahama, K., Yamashita, E., & Terada, H. (2001). *Biochim. Biophys. Acta.*, *1512*, 251.
[11] Wasserman, H. H., & Murry R. W. (1979). *Singlet Oxygen*, Academic Press: London, UK.
[12] Stern, O., & Volmer, M. (1919). *Physic. Z.*, *20*, 183.
[13] Shimidzu, N.; Goto, M., & Miki,W. (1996). *Fisheries Sci.*, *62*, 134.
[14] Murov, S. L. *et al.* (1993). *Handbook of Photochemistry*, Marcel Dekker, INC.: New York, USA.