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Development of Singlet Oxygen Absorption Capacity (SOAC) Assay Method. 3. Measurements of the SOAC Values for Phenolic Antioxidants

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ABSTRACT: Measurements of the singlet oxygen ($^1O_2$) quenching rates (k_Q (S)) and the relative singlet oxygen absortpion capacity (SOAC) values were performed for 16 phenolic antioxidants (tocopherol derivatives, ubiquinol-10, caffeic acids, and catechins) and vitamin C in ethanol/chloroform/D_2O (50:50:1, v/v/v) solution at 35 °C. It has been clarified that the SOAC method is useful to evaluate the $^1O_2$-quenching activity of lipophilic and hydrophilic antioxidants having 5 orders of magnitude different rate constants from 1.38×10^{10} M^{-1} s^{-1} for lycopene to 2.71×10^5 for ferulic acid. The logarithms of the k_Q (S) and the SOAC values for phenolic antioxidants were found to correlate well with their peak oxidation potentials (E_p); the antioxidants that have smaller E_p values show higher reactivities. In previous works, measurements of the k_Q (S) values for many phenolic antioxidants were performed in ethanol. Consequently, measurements of the k_Q (S) and relative SOAC values were performed for eight carotenoids in ethanol to investigate the effect of solvent on the $^1O_2$-quenching rate. The k_Q (S) values for phenolic antioxidants and carotenoids in ethanol were found to correlate linearly with the k_Q (S) values in ethanol/chloroform/D_2O solution with a gradient of 1.79, except for two catechins. As the relative rate constants (k_Q^{AO} (S)/$k_Q^{\alpha\text{-Toc}}$ (S)) of antioxidants (AO) are equal to the relative SOAC values, the SOAC values do not depend on the kinds of solvent used, if α-tocopherol is used as a standard compound. In fact, the SOAC values obtained for carotenoids in mixed solvent agreed well with the corresponding ones in ethanol.

KEYWORDS: *singlet oxygen, quenching rate, endoperoxide, tocopherols, catechins, caffeic acids, carotenoids, SOAC value, kinetic study, molar extinction coefficient*

INTRODUCTION

Singlet oxygen ($^1O_2$) has attracted much attention as a biological oxidant. In biological systems, $^1O_2$ is generated by the reaction of triplet sensitizers with molecular oxygen ($^3O_2$) (type II photosensitization reaction)[1,2] and by the biochemical reactions in cells and tissues exposed to oxidative stress.[3,4] $^1O_2$ reacts with many kinds of biological targets including lipids,[5] proteins,[1,2] and DNA.[6,7] Reactions with $^1O_2$ occur mainly by chemical reaction, inducing the degradation of biological systems. Natural antioxidants, including carotenoids and phenolic antioxidants, are widely present in foods, plants, and animals in high concentrations and may function as efficient $^1O_2$ quenchers in biological systems.[8−12]

In previous works,[13,14] kinetic studies of the quenching reaction of $^1O_2$ with eight kinds of carotenoids and α-tocopherol (α-Toc) were performed in ethanol/chloroform/D_2O (50:50:1, v/v/v) solution (abbreviated "mixed solvent") at 35 °C. The second-order rate constants (k_Q) for the reaction of carotenoids with $^1O_2$ were measured, using the competition reaction method, where endoperoxide was used as a $^1O_2$ generator and 2,5-diphenyl-3,4-benzofuran (DPBF) as an UV−vis absorption probe (see Scheme 1 in ref 14).

$$^1O_2 + \text{carotenoid} \xrightarrow{k_Q} {}^3O_2 + \text{carotenoid} \quad (1)$$

The rate constants, k_Q (S) and k_Q ($t_{1/2}$), were determined by analyzing the first-order rate constant (S) and the half-life ($t_{1/2}$) of the decay curve of DPBF, respectively, showing good accordance with each other. Measurements of the k_Q (S) and k_Q ($t_{1/2}$) values were also performed for tomato, carrot, and red paprika extracts containing high concentrations of carotenoids. From the results, a new assay method that can quantify the singlet oxygen absorption capacity (SOAC) of antioxidants, including carotenoids, α-tocopherol, and vegetable extracts, was proposed.[13,14] The relative SOAC value was defined in the following way.

$$\begin{aligned}&\text{relative SOAC value}\\ &= \{(t_{1/2}{}^{\text{sample}} - t_{1/2}{}^{\text{blank}})/(t_{1/2}{}^{\alpha\text{-Toc}} - t_{1/2}{}^{\text{blank}})\}\\ &\quad \times \{[\alpha\text{-Toc}]/[\text{sample}]\}\\ &= k_Q{}^{\text{sample}}/k_Q{}^{\alpha\text{-Toc}}\end{aligned} \quad (2)$$

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Table 1. k_Q^{AO} (S) and k_Q^{AO} ($t_{1/2}$) Values for Many Kinds of Antioxidants (AO) in Ethanol/Chloroform/D_2O Solution at 35.0 °C, Relative Rate Constants (k_Q^{AO} (S)/$k_Q^{\alpha\text{-Toc}}$ (S)), and Relative SOAC Values

antioxidant	k_Q^{AO} (S)[b]/M^{-1} s^{-1}	k_Q^{AO} ($t_{1/2}$)[b]/M^{-1} s^{-1}	k_Q^{AO} (S)/$k_Q^{\alpha\text{-Toc}}$ (S)	relative SOAC value[b]
α-Toc **1**	1.31×10^8	1.29×10^8	1.00	1.00
β-Toc **2**	9.30×10^7	8.34×10^7	0.710	0.806−0.922 (av 0.866)
γ-Toc **3**	8.44×10^7	7.09×10^7	0.644	0.666−0.764 (av 0.706)
δ-Toc **4**	4.11×10^7	3.86×10^7	0.314	0.369−0.394 (av 0.380)
tocol **5**	1.84×10^7	1.61×10^7	0.140	0.149−0.174 (av 0.162)
trolox **6**	4.20×10^7	3.66×10^7	0.321	0.327−0.369 (av 0.349)
$UQ_{10}H_2$ **7**	6.22×10^7	5.86×10^7	0.475	0.468−0.594 (av 0.521)
IE **8**	2.98×10^6	2.53×10^6	0.0227	0.0242−0.0278 (av 0.0258)
CA **9**	6.85×10^5	5.85×10^5	0.00523	0.00637- 0.00679 (av 0.00659)
FA **10**	2.71×10^5	2.08×10^5	0.00207	0.00217−0.00257 (av 0.00228)
EC **11**	8.31×10^6	7.85×10^6	0.0634	0.0724−0.0814 (av 0.0752)
EGC **12**	1.31×10^7	1.30×10^7	0.100	0.117−0.129 (av 0.122)
ECG **13**	4.94×10^6	3.61×10^6	0.0377	0.0367, 0.0383 (av 0.0375)
EGCG **14**	5.05×10^6	4.27×10^6	0.0385	0.0442, 0.0405 (av 0.0424)
4-MC **15**	4.96×10^6	4.12×10^6	0.0379	0.0421−0.0495 (av 0.0474)
4-MG **16**	1.34×10^5	1.05×10^5	0.00102	0.00114−0.00126 (av 0.00119)
Vit C	1.92×10^6	1.88×10^6	0.0147	0.016, 0.016 (av 0.016)
Lyc[a]	1.38×10^{10}	1.26×10^{10}	105	av 123
Ast[a]	1.17×10^{10}	1.08×10^{10}	89.3	av 109
β-Car[a]	1.08×10^{10}	1.02×10^{10}	82.4	av 95.8

[a]Values reported in ref 14. [b]The experimental errors in the rate constants (k_Q (S)^{AO} and k_Q^{AO} ($t_{1/2}$)) and relative SOAC values were estimated to be <10% (see refs 12 and 13).

where [α-Toc] and [sample] are molar concentrations of α-Toc and sample, respectively. α-Toc was used as a standard compound.

Many kinds of natural phenolic antioxidants, such as tocopherol homologues (α-, β-, γ-, δ-tocopherols), ubiquinol-10, caffeic acids (isoeugenol, caffeic acid, ferulic acid), and catechins (epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate), are included in foods, plants, and animals and may function as $^1O_2$ quenchers in biological systems. Therefore, in the present work, to ascertain that the SOAC assay method proposed for carotenoids is applicable to general phenolic antioxidants and vegetable extracts including phenolic antioxidants, measurements of k_Q (S), k_Q ($t_{1/2}$), and relative SOAC values were performed for the above phenolic antioxidants and related compounds (see Table 1) in mixed solvent at 35 °C. Measurement was also performed for vitamin C (Vit C), which is well-known as an important water-soluble antioxidant.

Furthermore, measurements of the k_Q (S), k_Q ($t_{1/2}$), and relative SOAC values were performed for eight carotenoids in ethanol and/or ethanol/THF (4:1, v/v) solutions. In previous works,[9−11,13−15] mixed solvent was used for the measurements of the k_Q (S) values for carotenoids and vegetable extracts including high concentrations of carotenoids, because carotenoids generally show low (or very low) solubility in ethanol. On the other hand, as general phenolic antioxidants are easily soluble in ethanol, measurements of k_Q (S) values were performed in ethanol, in previous works.[16−20] It will be interesting to compare the k_Q (S) values obtained in mixed solvent with those in ethanol because it is difficult to find a solvent in which all types of antioxidants are soluble. From the results, it has been clarified that the SOAC assay method is applicable to extensive and wide-ranging natural antioxidants having 5 orders of magnitude different $^1O_2$-quenching rates.

MATERIALS AND METHODS

Materials. D-α-, β-, γ-, and δ-tocopherol (α-, β-, γ-, δ-Toc) and tocol were obtained from Eisai Food Chemicals Co. Ltd., Japan. Isoeugenol (IE), caffeic acid (CA), and ferulic acid (FA) were obtained from Nacalai Tesque, Japan. Astaxanthin (Ast), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) were obtained from Funakoshi Co. Ltd., Japan. Trolox (Fluka Chemika) is commercially available. Lutein (Lut), β-cryptoxanthin (β-Cry), zeaxanthin (Zea), and capsanthin (Cap) were obtained from Extrasynthese (Genay, France). α- and β-carotene (α-, β-Car), lycopene (Lyc), 4-methylcatechol (4-MC), uric acid (UA), and vitamin C (Vit C) were obtained from Wako Chemicals, Japan. 4-Methyl gallate (4-MG) and DPBF were obtained from Tokyo Kasei Organic Chemicals, Japan. 3-(1,4-Epidioxy-4-methyl-1,4-dihydro-1-naphthyl)propionic acid (endoperoxide, EP) was obtained from Wakenyaku Co. Ltd., Japan. The result of the measurement of the UV spectrum of EP indicates that the powder sample of EP includes 95% EP and 5% EP-precursor unreacted.[13]

Ubiquinone-10 (UQ_{10}) was kindly supplied by Kaneka Corp. Ubiquinol-10 ($UQ_{10}H_2$) was prepared by the reduction of UQ_{10} with sodium hydrosulfite in *n*-hexane under a nitrogen atmosphere.[21−26] It was recrystallized from ethanol/petroleum ether solution: mp 46−47 °C. Ubiquinol-10 as prepared was kept under vacuum in a refrigerator at −20 °C.

In the present work, measurements of the $^1O_2$-quenching activity of 16 phenolic antioxidants were performed. These antioxidants were classified into four groups ((i) tocopherols (or tocopherol derivatives), (ii) ubiquinol-10, (iii) caffeic acids, and (iv) catechins) for the sake of convenience. The four groups i−iv include (i) α-, β-, γ-, and δ-Toc, tocol, and trolox; (ii) $UQ_{10}H_2$; (iii) IE, CA, and FA; and (iv) EC, EGC, ECG, EGCG, and two related compounds (4-MC and 4-MG), respectively. The names of the groups are used in the text and Figures 4−7.

Measurements of Rate Constants (k_Q) and Molar Extinction Coefficient (ε_{max}). Measurements of rate constants (k_Q) were performed in ethanol/chloroform/D_2O (50:50:1, v/v/v) (mixed solvent), ethanol, and ethanol/THF (4:1, v/v) solutions by using a Shimadzu UV−vis spectrophotometer (UV-1800), equipped with a six-channel cell positioner and an electron-temperature control unit (CPS-240A). All of the measurements were performed at 35.0 ± 0.5

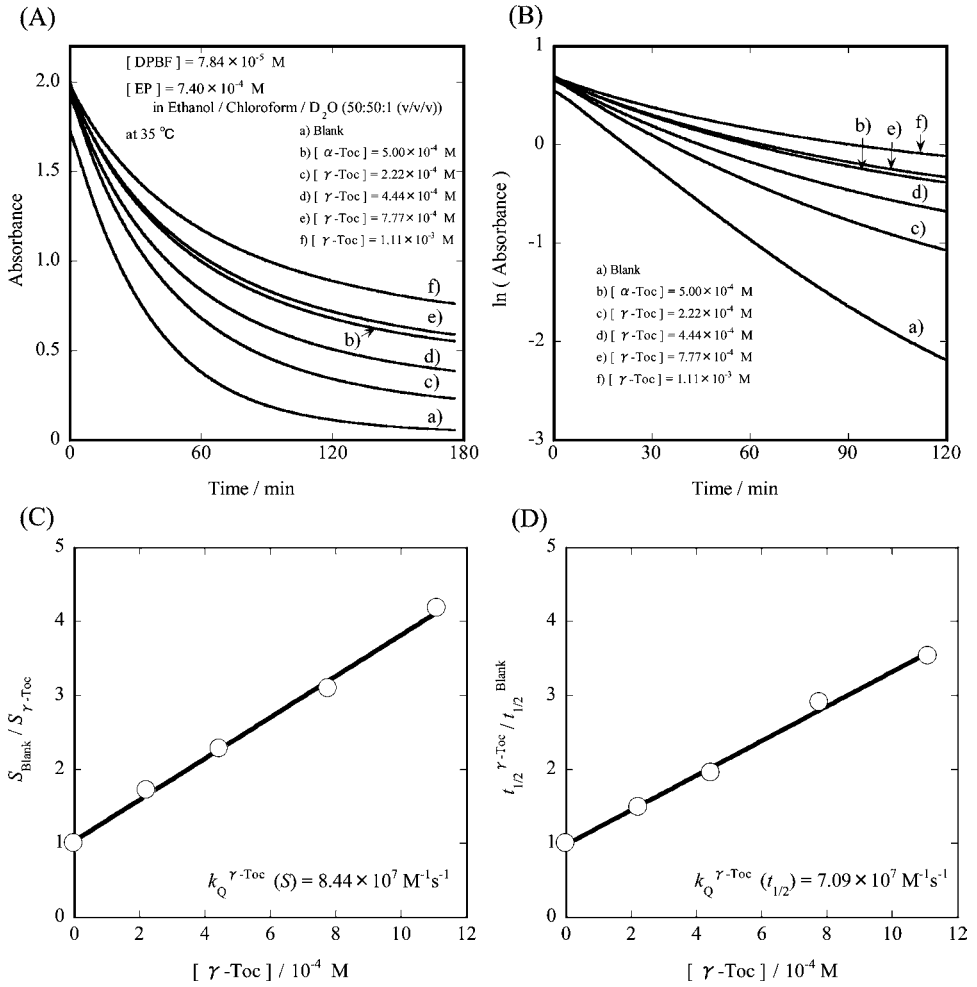

Figure 1. (A) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1O_2$ in the absence and presence of sample (α- or γ-tocopherol) in ethanol/chloroform/D_2O solution at 35 °C. $[DPBF]_{t=0} = 7.84 \times 10^{-5}$ M and $[EP]_{t=0} = 7.40 \times 10^{-4}$ M. The values of $[\alpha\text{-Toc}]_{t=0}$ and $[\gamma\text{-Toc}]_{t=0}$ are shown in panel A. (B) Plot of ln(absorbance) versus t. (C) Plot of $S_{blank}/S_{\gamma\text{-Toc}}$ versus [γ-Toc]. (D) Plot of $t_{1/2}^{\gamma\text{-Toc}}/t_{1/2}^{blank}$ versus [γ-Toc].

°C. Detailed experimental conditions used for measurements were described in a previous work.[13] The values of molar extinction coefficient (ε_{max}) of carotenoids were determined, using Lambert–Beer's equation (absorbance = ε_{max} [carotenoid]).

Analyses of the Second-Order Rate Constants (k_Q^{AO} (S) and k_Q^{AO} ($t_{1/2}$)) and SOAC Values. The rate constant $k_Q^{AO}(S)$ for the reaction of $^1O_2$ with an antioxidant (AO) was determined by eq 3[13,27,28]

$$S_{blank}/S_{AO} = 1 + \{k_Q^{AO}(S)[AO]\}/k_d \quad (3)$$

where S_{blank} and S_{AO} are slopes of the first-order plots (that is, ln(absorbance) vs t plots) of the disappearance of DPBF in the absence and presence of antioxidant, respectively (see Figure 1A,B). k_d is the rate of natural deactivation of $^1O_2$ in ethanol/chloroform/D_2O ($k_d = 3.03 \times 10^4$)[10] and ethanol ($k_d = 8.3 \times 10^4$ s^{-1}).[12] The value of k_d in ethanol/THF (4:1, v/v) solution was tentatively determined by assuming the relationship $k_d = \{4k_d(EtOH) + k_d(THF)\}/5 = \{4 \times (8.3 \times 10^4) + 1 \times (5.0 \times 10^4)\}/5 = 7.64 \times 10^4$ s^{-1}, where the k_d values for ethanol (k_d (EtOH) = 8.3×10^4 s^{-1}) and THF (k_d (THF) = 5.0×10^4 s^{-1}) were used for the calculation.[12] Equation 3 indicates that the $k_Q^{AO}(S)$ value can be obtained from S_{blank}/S_{AO} versus [AO] plot (see Figure 1C).[13]

We can easily obtain eq 4, by substituting the relationship for the first-order reaction ($t_{1/2}^{AO} = \ln 2/S_{AO}$) into eq 3

$$t_{1/2}^{AO}/t_{1/2}^{blank} = 1 + \{k_Q^{AO}(t_{1/2})[AO]\}/k_d \quad (4)$$

where $t_{1/2}^{blank}$ and $t_{1/2}^{AO}$ are the half-lives of DPBF in the absence and presence of antioxidant, respectively. Equation 4 indicates that the $k_Q^{AO}(t_{1/2})$ value can be obtained from $t_{1/2}^{AO}/t_{1/2}^{blank}$ versus [AO] plot (see Figure 1D).[13]

As proposed in a previous work,[13] the relative SOAC value for antioxidant was defined as follows:

$$\begin{aligned}&\text{relative SOAC value (based on molar concentration unit (M = mol/L))}\\ &= \{(t_{1/2}^{AO} - t_{1/2}^{blank})/(t_{1/2}^{\alpha\text{-Toc}} - t_{1/2}^{blank})\} \times \{[\alpha\text{-Toc}]\,(M)/[AO]\,(M)\}\\ &= k_Q^{AO}\,(M^{-1}\,s^{-1})/k_Q^{\alpha\text{-Toc}}\,(M^{-1}\,s^{-1}) \end{aligned} \quad (5)$$

Equation 5 indicates that the SOAC value corresponds to the ratio ($k_Q^{AO}/k_Q^{\alpha\text{-Toc}}$) of the quenching rate of singlet oxygen (k_Q^{AO}) by antioxidant to that ($k_Q^{\alpha\text{-Toc}}$) by α-Toc. α-Toc is used as a standard compound of SOAC assay.[13] According to eq 5, the SOAC value was determined by the measurement of the half-life of DPBF.

RESULTS

Measurements of the $^1O_2$-Quenching Rates (k_Q (S) and k_Q ($t_{1/2}$)) and SOAC Values for Tocopherols, Ubiquinol-10, and Caffeic Acids in Ethanol/Chloroform/D_2O Solution. Measurements of k_Q (S), k_Q ($t_{1/2}$), and

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Table 2. Employed Concentrations, First-Order Decay Rates (S), and Half-Lives ($t_{1/2}$) of Blank (DPBF Only), α-Tocopherol, and Antioxidants (AO) ((a) γ-Tocopherol, (b) Ferulic Acid, (c) Epicatechin, and (d) Epicatechin gallate) and Relative SOAC Values in Ethanol/Chloroform/D_2O Solution

	blank	α-Toc	AO-1	AO-2	AO-3	AO-4
			γ-Tocopherol			
concn (M)	0	5.19×10^{-4}	2.22×10^{-4}	4.44×10^{-4}	7.77×10^{-4}	1.11×10^{-3}
S_{AO} (s^{-1})	0.0255	0.0088	0.0148	0.0112	0.0083	0.0061
$t_{1/2}$ (min)	26.6	71.0	39.6	51.9	77.4	94.0
relative SOAC value			0.685	0.666	0.764	0.710 (av 0.706)
			Ferulic Acid			
concn (M)	0	5.04×10^{-4}	2.42×10^{-2}	4.85×10^{-2}	9.69×10^{-2}	1.21×10^{-1}
S_{AO} (s^{-1})	0.0360	0.0124	0.0282	0.0243	0.0191	0.0170
$t_{1/2}$ (min)	26.4	60.5	30.6	33.6	40.6	44.5
relative SOAC value			0.00257	0.00219	0.00217	0.00221 (av 0.00228)
			Epicatechin			
concn (M)	0	5.28×10^{-4}	4.63×10^{-3}	6.94×10^{-3}	9.25×10^{-3}	1.16×10^{-2}
S_{AO} (s^{-1})	0.0315	0.0113	0.0140	0.0116	0.0094	0.0074
$t_{1/2}$ (min)	23.0	62.8	48.7	61.4	73.5	94.2
relative SOAC value			0.0736	0.0734	0.0724	0.0814 (av 0.0752)
			Epicatechin Gallate			
concn (M)	0	4.99×10^{-4}	9.11×10^{-4}	1.37×10^{-3}	2.73×10^{-3}	4.55×10^{-3}
S_{AO} (s^{-1})	0.0509	0.0169	0.0431	0.0404	0.0348	0.0290
$t_{1/2}$ (min)	14.7	38.6	17.0	17.8	19.5	23.1
relative SOAC value			0.0516	0.0472	0.0367	0.0383 (av 0.0375)

SOAC values were performed for 10 kinds of phenolic antioxidants, including tocopherol derivatives (α-, β-, γ-, and δ-Toc, tocol, trolox), ubiquinol-10, and caffeic acids (IE, CA, FA) (see Table 1). Figure 1A shows an example of measurement of the reaction between DPBF and EP in the absence ((a) blank) and presence of antioxidants (AO) ((b) [α-Toc] = 5.00×10^{-4} M, (c) [γ-Toc] = 2.22×10^{-4} M, (d) [γ-Toc] = 4.44×10^{-4} M, (e) [γ-Toc] = 7.77×10^{-4} M, (f) [γ-Toc] = 1.11×10^{-3} M) in mixed solvent at 35 °C. This mixed solvent was used by several investigators[9−11,13−15] to measure the $^1O_2$-quenching rate (k_Q) of many carotenoids. The disappearance of DPBF at λ_{max} = 413 nm due to the chemical reaction with $^1O_2$ was observed. The values of first-order decay rate constant (S_{blank}, $S_{\alpha\text{-Toc}}$, $S_{\gamma\text{-Toc}}$) (see Table 2a) were estimated by analyzing the decay curve of DPBF, as shown in Figure 1B. The analysis of the decay curve was performed at ∼5 < t < ∼60 min. This is an important condition to obtain the correct rate constant (k_Q) for antioxidants.[13] The values of half-life ($t_{1/2}^{\alpha\text{-Toc}}$, $t_{1/2}^{\gamma\text{-Toc}}$, $t_{1/2}^{blank}$) were calculated carefully according to the method described in a previous work.[13] The values obtained are summarized in Table 2a.

Plots of $S_{blank}/S_{\gamma\text{-Toc}}$ and $t_{1/2}^{\gamma\text{-Toc}}/t_{1/2}^{blank}$ versus [γ-Toc] are shown in Figure 1, panels C and D, respectively. The $k_Q^{\gamma\text{-Toc}}$ (S) and $k_Q^{\gamma\text{-Toc}}$ ($t_{1/2}$) values obtained by using eqs 3 and 4 are 8.44×10^7 and 7.09×10^7 M^{-1} s^{-1}, respectively. As the measurements were performed for one concentration of α-Toc and four concentrations of γ-Toc (AO-1−AO-4), we can determine four sets of relative SOAC values, using eq 5 (see Table 2a). The relative SOAC values (0.666−0.764, av = 0.706) obtained for γ-Toc are similar to each other and agree well with the ratio of the quenching rate constant of γ-Toc to that of α-Toc ($k_Q^{\gamma\text{-Toc}}$ (S)/$k_Q^{\alpha\text{-Toc}}$ (S) = 0.644), as expected from eq 5. Similar measurements were performed for β- and δ-Toc, tocol, trolox, and ubiquinol-10. The rate constants (k_Q^{Toc} (S)) obtained are listed in Table 1.

On the other hand, in the case of FA, the rate constant (k_Q (S)) is 2−3 orders of magnitude smaller than that of γ-Toc (see Table 1). Consequently, we had to use 2−3 orders of magnitude higher concentrations to obtain reliable k_Q (S) and SOAC values, as anticipated from eqs 3 and 5, respectively (see Table 2b). We could not obtain a reliable SOAC value when the difference between the half-lives of antioxidant and blank ($t_{1/2}^{AO} - t_{1/2}^{blank}$) (that is, the value of a numerator in eq 5) was smaller than ∼5 min (deta not shown). The rate constants and the SOAC values obtained for caffeic acids (IE, CA, and FA) are summarized in Table 1.

The $^1O_2$-quenching rates (k_Q (S) and k_Q ($t_{1/2}$)) and SOAC values obtained for phenolic antioixants are summarized in Table 1, together with those reported for three carotenoids in previous works.[13,14] As listed in Table 1, the SOAC values obtained for six tocopherol derivatives, ubiquinol-10, and three caffeic acids show a fair agreement with the ratios of the quenching rate constants (k_Q^{AO} (S)/$k_Q^{\alpha\text{-Toc}}$ (S)). The result indicates that the definition of eq 5 is useful for the estimation of the SOAC value of the phenolic antioxidants, which have 2−5 orders of magnitude smaller SOAC values (that is, $^1O_2$-quenching rates (k_Q (S) and k_Q ($t_{1/2}$))) than those of carotenoids.

Measurements of the $^1O_2$-Quenching Rates (k_Q (S) and k_Q ($t_{1/2}$)) and SOAC Values for Catechins and Vitamin C in Ethanol/Chloroform/D_2O Solution. Measurements of k_Q (S), k_Q ($t_{1/2}$), and relative SOAC values were performed for catechins (EC, EGC, ECG, EGCG, and related compounds (4-MC and 4-MG)) and Vit C in mixed solvent (see Table 1). EC, EGC, ECG, and EGCG are well-known as representative polyphenolic antioxidants. For example, the results obtained for EC are shown in Figure 2. The disappearance of DPBF at λ_{max} = 413 nm due to the chemical reaction with $^1O_2$ in the absence and presence of EC is shown in Figure 2A. ln(absorbance) versus t plots (see Figure 2B) indicate that the decay of DPBF for EC also follows first-order kinetics at ∼5 < t < ∼60 min. The values of S_{EC}, $S_{\alpha\text{-Toc}}$, S_{blank}, $t_{1/2}^{EC}$, $t_{1/2}^{\alpha\text{-Toc}}$, and $t_{1/2}^{blank}$ obtained are listed in Table 2c. S_{blank}/S_{EC} and $t_{1/2}^{EC}/t_{1/2}^{blank}$ versus [EC] plots are shown in

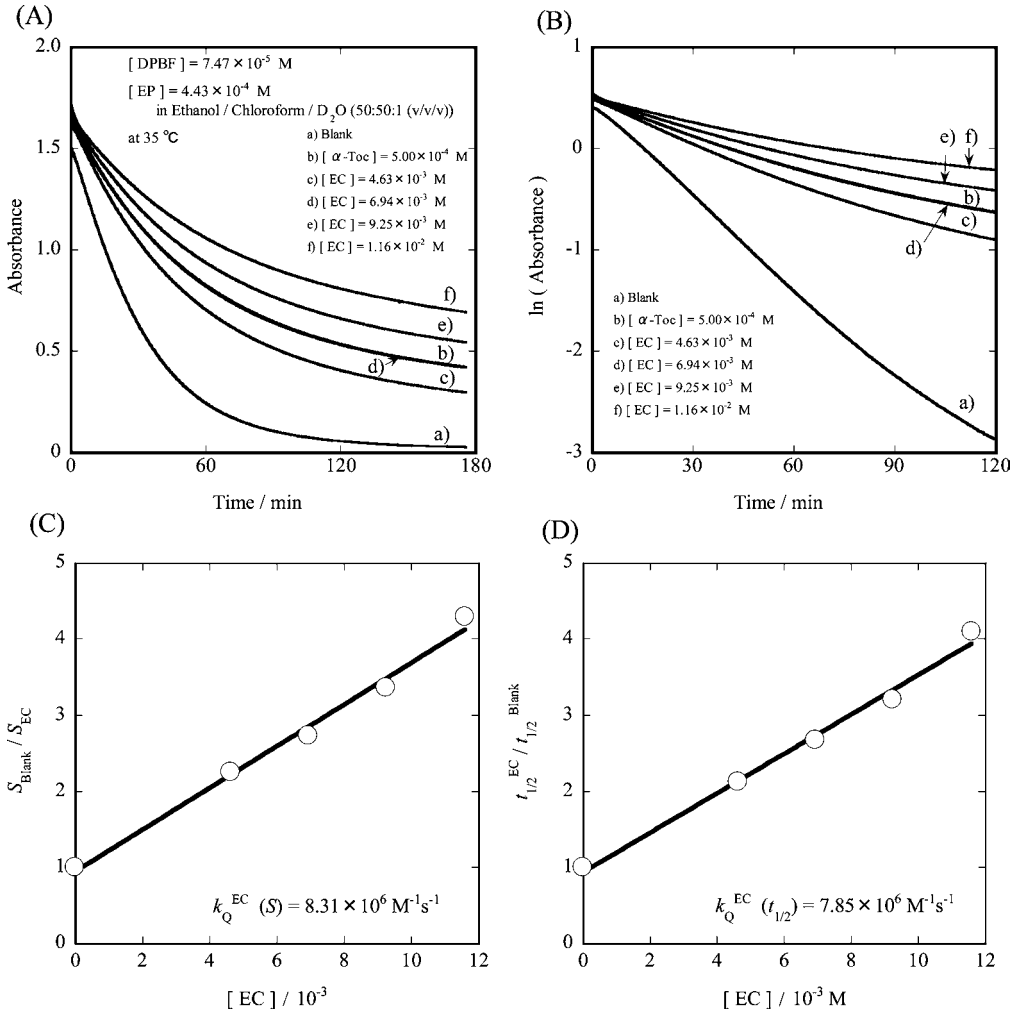

Figure 2. (A) Change in absorbance of DPBF at 413 nm during the reaction of DPBF with $^1O_2$ in the absence and presence of sample (α-tocopherol or epicatechin) in ethanol/chloroform/D_2O solution at 35 °C. $[DPBF]_{t=0} = 7.47 \times 10^{-5}$ M and $[EP]_{t=0} = 4.43 \times 10^{-4}$ M. The values of $[\alpha\text{-Toc}]_{t=0}$ and $[EC]_{t=0}$ are shown in panel A. (B) Plot of ln(absorbance) versus t. (C) Plot of S_{blank}/S_{EC} versus [EC]. (D) Plot of $t_{1/2}^{EC}/t_{1/2}^{blank}$ versus [EC].

Figure 2, panels C and D, respectively. The k_Q^{EC} (S) and k_Q^{EC} ($t_{1/2}$) values obtained are 8.31×10^6 and 7.85×10^6 M^{-1} s^{-1}, respectively (see Table 1). The linear dependence of S_{blank}/S_{EC} and $t_{1/2}^{EC}/t_{1/2}^{blank}$ values on [EC] suggests that the effects of the interactions between EC molecules included in solution are negligible, although relatively high concentrations of EC ($4.63 \times 10^{-3} \sim 1.16 \times 10^{-2}$ M) were used for the measurements (see Table 2c). Furthermore, the relative SOAC values (av 0.0752) were determined, using eq 5, and are listed in Table 1.

Similar measurements were performed for EGC, ECG, EGCG, 4-MC, and 4-MG by varying the concentrations of the antioxidants. For instance, in the cases of EGC, 4-MC, and 4-MG, similar SOAC values were obtained for four concentrations of the antioxidants, being independent of the concentrations of the antioxidants. The rate constants (k_Q^{AO} (S) and k_Q^{AO} ($t_{1/2}$)) and the minimum and maximum SOAC values (and average SOAC values) obtained are listed in Table 1.

In the case of ECG (and EGCG), we can expect that the k_Q^{AO} (S), k_Q^{AO} ($t_{1/2}$), and relative SOAC values for ECG (and EGCG) are higher than those for EC (and EGC), because ECG (and EGCG) includes EC (and EGC) and 4-MG moieties in a molecule. However, the values obtained for ECG and EGCG were smaller than those for EC and EGC, respectively, as listed in Table 1. To ascertain the reliability of the values obtained, measurements were repeated twice by varying the concentrations of ECG and EGCG. However, similar small k_Q (S) values were obtained for ECG (4.44×10^6 M^{-1} s^{-1}) and EGCG (4.52×10^6 M^{-1} s^{-1}), indicating that the small values obtained are reliable.

Furthermore, in the case of ECG, notable decreases of the SOAC values (0.0516, 0.0472, 0.0367, and 0.0383) (see Table 2d) were observed with increasing concentrations of ECG (9.11×10^{-4}, 1.37×10^{-3}, 2.73×10^{-3}, and 4.55×10^{-3} M, respectively), because, if the concentrations of ECG are low ([ECG] = 9.11×10^{-4} and 1.37×10^{-3} M), the differences between the half-lives for ECG and blank ($t_{1/2}^{ECG} - t_{1/2}^{blank}$) are smaller than ~5 min and, thus, we cannot obtain reliable SOAC values. At higher concentrations of ECG ([ECG] = 2.73×10^{-3} and 4.55×10^{-3} M), similar SOAC values (0.0367 and 0.0383, av 0.0375) were obtained. The SOAC values obtained for $t_{1/2}^{ECG} - t_{1/2}^{blank} > \sim 5$ min agree well with the relative rate constant (k_Q^{AO} (S)/$k_Q^{\alpha\text{-Toc}}$ (S) = 0.0377), as expected from eq 5 (see Table 1). Similar results were obtained for EGCG. The SOAC values of EGCG obtained for $t_{1/2}^{EGCG} - t_{1/2}^{blank} > \sim 5$ min are listed in Table 1.

We tried to measure the SOAC value for Vit C and uric acid, which are well-known as representative hydrophilic antiox-

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idants. The rate constants and SOAC values obtained for Vit C are listed in Table 1. The solubility of Vit C is low in mixed solvent, and the SOAC value decreases with increasing concentrations of Vit C, as observed for ECG and EGCG. The SOAC values obtained for $t_{1/2}^{\text{Vit C}} - t_{1/2}^{\text{blank}} > \sim 5$ min are listed in Table 1. Uric acid was insoluble in mixed solvent, and thus measurements of the rate constant and SOAC value were unsuccessful.

As listed in Table 1, a fair agreement between the relative rate constant ($k_Q^{\text{AO}}(S)/k_Q^{\alpha\text{-Toc}}(S)$) and the average SOAC value was obtained for many phenolic and polyphenolic antioxidants. The result indicates that the method of the analysis used for estimating the SOAC value is reasonable. The relative SOAC values obtained for many antioxidants in mixed solvent are shown as a bar graph in Figure 3.

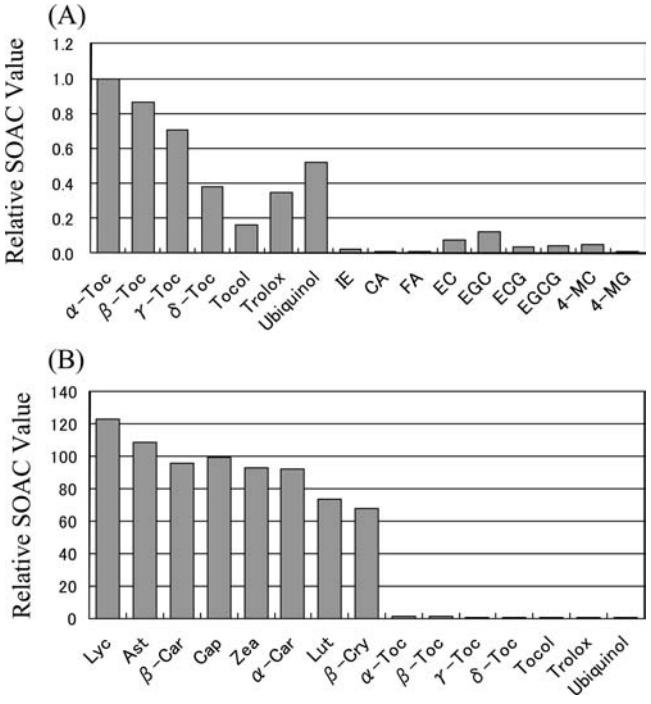

Figure 3. Comparison of the relative SOAC values for (A) tocopherol derivatives, ubiquinol-10, caffeic acids, catechins, and vitamin C and for (B) carotenoids, tocopherol derivatives, and ubiquinol-10 in ethanol/chloroform/D_2O solution.

UV−Vis Absorption Spectra of Eight Carotenoids in Ethanol and/or Ethanol/THF (4:1, v/v) Solutions. Measurements of UV−vis absorption spectra have been performed for many carotenoids in organic solvents.[15,29] Although the values of the wavelengths of absorption maxima (λ_{max}) were reported for many carotenoids, the carotenoids for which molar extinction coefficients (ε_{max}) were determined are very limited, because the solubility of carotenoids is generally low (or very low) in organic solvents and varies remarkably depending on the kind of solvent. Futhermore, commercially available carotenoids are very expensive.

In a previous work, measurements of UV−vis absorption spectra of eight carotenoids were performed in mixed solvent, and the correct λ_{max} and ε_{max} values were determined by repeating the measurements three times as reported.[13] The values reported are listed in Table 3. The $^1O_2$-quenching rates ($k_Q(S)$ and $k_Q(t_{1/2})$) and SOAC values of the eight carotenoids were determined, using the ε_{max} values obtained.

In the present work, measurements of the λ_{max} and ε_{max} values were performed for six carotenoids (Lyc, Ast, β-Car, Cap, α-Car, and β-Cry) in ethanol. The λ_{max} and ε_{max} values obtained for β-Car, Cap, α-Car, and β-Cry are summarized in Table 3, together with those reported for β-Car, Zea, and Lut in ethanol.[29] As the solubilities of Lyc and Ast in ethanol are very low, we could determine only the λ_{max} values for these carotenoids. Consequently, measurements of the ε_{max} values were performed in ethanol/THF (4:1, v/v) solution. Measurements of the ε_{max} values for α- and β-Car were also performed in ethanol/THF, to compare the values with the corresponding ones in ethanol (see Table 3). Using the ε_{max} values for carotenoids in ethanol and ethanol/THF, measurements of the rate constants ($k_Q(S)$ and $k_Q(t_{1/2})$) and SOAC values were performed.

Measurements of the $^1O_2$-Quenching Rates ($k_Q(S)$ and $k_Q(t_{1/2})$) and SOAC Values for Eight Carotenoids in Ethanol and/or Ethanol/THF (4:1, v/v) Solutions. In previous works,[16−20] measurements of the rate constant ($k_Q(S)$) for many phenolic antioxidants were performed in ethanol solution, because the solubilities of these antioxidants are generally very high in ethanol. Consequently, in the present work, measurements of the $k_Q(S)$, $k_Q(t_{1/2})$, and SOAC values were performed for six carotenoids (β-Car, Cap, Zea, α-Car, Lut, and β-Cry) in ethanol to compare the values with those reported for phenolic antioxidants. The values obtained are summarized in Table 4.

Similar measurements were performed for Lyc and Ast in ethanol/THF (4:1, v/v) solution, because the solubilities of these carotenoids are very low in ethanol and we could not determine the concentration of carotenoids, as described above. Measurements of the rate constants and SOAC values were also performed for α- and β-Car in ethanol/THF solution to compare with those obtained in ethanol (see Table 4).

DISCUSSION

The SOAC Assay Method Is Applicable to Antioxidants Having 5 Orders of Magnitude Different SOAC Values, That Is, $^1O_2$-Quenching Rates. Recently, $^1O_2$-quenching rate constants ($k_Q(S)$ and $k_Q(t_{1/2})$) and relative SOAC values were determined for eight carotenoids, α-tocopherol, and three kinds of vegetable extracts containing high concentrations of carotenoids in ethanol/chloroform/D_2O solution (mixed solvent).[13,14] The relative rate constants ($k_Q^{\text{AO}}(S)/k_Q^{\alpha\text{-Toc}}(S)$ and $k_Q^{\text{AO}}(t_{1/2})/k_Q^{\alpha\text{-Toc}}(t_{1/2})$) and relative SOAC values, which were determined using three different analytical methods (see eqs 3, 4, and 5, respectively), agreed well with each other. The result indicates that these methods are available to assess the $^1O_2$-quenching activity of carotenoids, α-tocopherol, and vegetable extracts.

The $k_Q(S)$, $k_Q(t_{1/2})$, and SOAC values for eight carotenoids and α-tocopherol decrease in the order

$$\text{Lyc} > \text{Ast} > \beta\text{-Car} \sim \text{Cap} \sim \text{Zea} \sim \alpha - \text{Car} > \text{Lut} > \beta\text{-Cry} \gg \alpha\text{-Toc} \quad (6)$$

However, for instance, the SOAC value of Lyc is only 1.82 times larger than that of β-Cry.[14] The difference among the SOAC values of eight carotenoids is not remarkable, and the individual ratio is less than twice. On the other hand, the SOAC

Table 3. UV−Vis Absorption Maxima (λ_{max}) and Molar Extinction Coefficients (ε_{max}) of the Carotenoids in Ethanol/Chloroform/D_2O, Ethanol, and/or Ethanol/THF Solutions

carotenoid	λ_{max}/nm (ε_{max}/M^{-1} cm^{-1})	λ_{max}/nm (ε_{max}/M^{-1} cm^{-1})	λ_{max} (mixed solv) − λ_{max} (EtOH)/nm	ε_{max} (EtOH)/ε_{max} (mixed solv)
	mixed solvent	EtOH		
Lyc	479	473	6	(av 1.07)
		low solubility		
	(160000)[a]	(171000)[b]		
Ast	486	479	7	(av 1.07)
		low solubility		
	(124000)[a]	(133000)[b]		
β-Car	459	452	7	1.02
	(133000)[a]	(136000)		1.06
		(lit.140400)[c]		
Cap	481	475	6	1.08
	(106000)[a]	(114000)		
Zea	459	452	7	1.12
	(129000)[a]	(lit. 144300)[c]		1.09
		(lit. 140900)[c]		
α-Car	453	446	7	0.971
	(138000)[a]	(134000)		
Lut	452	446	6	1.15
	(126000)[a]	(lit. 144800)[c]		
β-Cry	459	452	7	1.11
	(95100)[a]	(106000)		
				av 1.07
	mixed solvent	EtOH/THF		ε_{max} (EtOH/THF)/ε_{max} (mixed solv)
Lyc	479	475	4	1.11
	(160000)[a]	(177000)		
Ast	486	480	6	1.03
	(124000)[a]	(128000)		
β-Car	459	455	4	1.05
	(133000)[a]	(140000)		
α-Car	453	448	5	1.07
	(138000)[a]	(147000)		
				av 1.07

[a]Values reported in ref 13. [b]Values tentatively estimated, using eq 3. [c]Values reported in ref 29.

value of Lyc is 123 times larger than that of α-Toc, as listed in Table 1 and as shown in Figure 3A.

In the present work, measurements of the k_Q (S), k_Q ($t_{1/2}$), and SOAC values were performed for 16 kinds of phenolic and polyphenolic antioxidants and vitamin C in mixed solvent. As listed in Table 1 and as shown in Figure 3, these values decrease in the order

$$\begin{aligned}\text{carotenoids} \gg{}& \alpha\text{-Toc} > \beta\text{-Toc} > \gamma\text{-Toc} > UQ_{10}H_2 \\ &> \text{Trolox} \sim \delta\text{-Toc} > \text{Tocol} > \text{EGC} > \text{EC} \\ &> \text{EGCG} > \text{ECG} \sim 4 - \text{MC} > \text{IE} > \text{Vit C} \\ &> \text{CA} > \text{FA} > 4 - \text{MG} \end{aligned} \quad (7)$$

As listed in Table 5, the values of k_Q (S) vary from 1.38×10^{10} to 7.31×10^9 M^{-1} s^{-1} for carotenoids,[13,14] from 1.31×10^8 to 1.84×10^7 M^{-1} s^{-1} for tocopherol derivatives and ubiquinol-10, from 2.98×10^6 to 2.71×10^5 M^{-1} s^{-1} for caffeic acids, and from 1.31×10^7 to 4.94×10^6 M^{-1} s^{-1} for catechins and related compounds. For example, the k_Q (S) value (1.38×10^{10} M^{-1} s^{-1}) of Lyc is about 5 orders of magnitude larger than that (2.71×10^5 M^{-1} s^{-1}) of FA. Furthermore, a fair agreement between the relative rate constant (k_Q^{AO} (S)/$k_Q^{\alpha\text{-Toc}}$ (S)) and the SOAC value was observed for each antioxidant (see Table 1), as expected from eq 5. In fact, the relative SOAC value (= 123) for Lyc is about 5 orders of magnitude larger than that (= 0.00228) for FA. The result indicates that the SOAC assay method is applicable to evaluation of the rate constants (k_Q^{AO} (S)) and SOAC values for lipophilic and hydrophilic antioxidants having very different $^1O_2$-quenching activities.

Correlation between log k_Q (S) (and log SOAC Value) and Peak Oxidation Potential (E_P) for Phenolic Antioxidants. In the present work, measurements of the k_Q (S) values were performed for 16 phenolic and polyphenolic

Table 4. Second-Order Rate Constants (k_Q^{AO} (S) and k_Q^{AO} ($t_{1/2}$)), Relative Rate Constants (k_Q^{AO} (S)/$k_Q^{\alpha\text{-Toc}}$ (S)), Relative SOAC Values of Carotenoids in Ethanol and/or Ethanol/THF (4:1, v/v) Solutions, and Relative SOAC Values in Ethanol/Chloroform/D_2O

carotenoid	k_Q^{AO} (S)[a]/M^{-1} s^{-1}	k_Q^{AO} ($t_{1/2}$)[a]/M^{-1} s^{-1}	k_Q^{AO} (S)/$k_Q^{\alpha\text{-Toc}}$ (S)	relative SOAC value[a]	
				EtOH	mixed solv
		In EtOH			
α-Toc	2.06×10^8		1.00	1.00	
Lyc	low solubility				123[b]
Ast	low solubility				109[b]
β-Car	1.71×10^{10}	1.44×10^{10}	83.0	66.6−75.5 (av 71.1)	95.8[b]
Cap	1.79×10^{10}	1.71×10^{10}	86.9	69.8−89.1 (av 78.6)	99.3[b]
Zea	1.82×10^{10}	1.60×10^{10}	88.3	75.5−107.4 (av 87.0)	92.8[b]
α-Car	1.92×10^{10}	1.67×10^{10}	93.2	78.0−88.1 (av 83.7)	92.4[b]
Lut	1.76×10^{10}	1.61×10^{10}	85.4	67.0−83.7 (av 74.5)	73.8[b]
β-Cry	1.54×10^{10}	1.41×10^{10}	74.8	55.6−83.6 (av 68.0)	67.6[b]
		In EtOH/THF			
Lyc	2.14×10^{10}	1.77×10^{10}	103	86.0−96.7 (av 92.2)	
Ast	1.64×10^{10}	1.44×10^{10}	79.6	65.1−69.2 (av 68.0)	
β-Car	2.31×10^{10}	2.10×10^{10}	112	82.8−102.8 (av 92.6)	
α-Car	2.52×10^{10}	2.14×10^{10}	122	77.8−100.7 (av 89.8)	

[a]The experimental errors in the rate constants (k_Q (S)^{AO} and k_Q^{AO} ($t_{1/2}$)) and relative SOAC values were estimated to be <10% (see refs 13 and 14). [b]The relative SOAC values in ethanol/chloroform/D_2O solution reported in ref 14.

Table 5. k_Q (S) Values for Many Kinds of Antioxidants in Ethanol/Chloroform/D_2O and Ethanol Solutions at 35.0 °C, Ratio of the Rate Constants (k_Q (S) (EtOH)/k_Q (S) (Mixed Solvent)), and Oxidation Potentials (E_p)

antioxidant	k_Q (S)/M^{-1} s^{-1}[a] (mixed solvent)	k_Q (S)/M^{-1} s^{-1}[a] (EtOH)	k_Q (S) (EtOH)/k_Q (S) (mixed solvent)	E_p/mV vs SCE
α-Toc **1**	1.31×10^8	2.06×10^8	1.57	860
β-Toc **2**	9.30×10^7	1.53×10^8	1.64	920
γ-Toc **3**	8.44×10^7	1.38×10^8	1.64	930
δ-Toc **4**	4.11×10^7	6.48×10^7	1.58	990
tocol **5**	1.84×10^7	2.80×10^7	1.52	1050
trolox **6**	4.20×10^7	8.06×10^7	1.92	890
$UQ_{10}H_2$ **7**	6.22×10^7	1.32×10^8	2.12	930
IE **8**	2.98×10^6	3.48×10^6	1.17	1200
CA **9**	6.85×10^5	1.23×10^6	1.80	1260
FA **10**	2.71×10^5	4.28×10^5	1.58	1330
EC **11**	8.31×10^6	1.32×10^7	1.59	1070
EGC **12**	1.31×10^7	1.72×10^7	1.31	980
ECG **13**	4.94×10^6	7.81×10^7	15.8	840
EGCG **14**	5.05×10^6	1.47×10^8	29.1	820
4-MC **15**	4.96×10^6	6.70×10^6	1.35	1080
4-MG **16**	1.34×10^5	2.06×10^5	1.54	1370
Vit C	1.92×10^6	low solubility		
Lyc	1.38×10^{10}	low solubility		
Ast	1.18×10^{10}	low solubility		
β-Car	1.08×10^{10}	1.71×10^{10}	1.58	
Cap	1.06×10^{10}	1.79×10^{10}	1.69	
Zea	1.05×10^{10}	1.82×10^{10}	1.73	
α-Car	9.76×10^9	1.92×10^{10}	1.97	
Lut	9.24×10^9	1.76×10^{10}	1.90	
β-Cry	7.31×10^9	1.54×10^{10}	2.11	
			av 1.67	

[a]The experimental errors in the rate constants (k_Q (S) (mixed solvent) and k_Q (S) (EtOH)) were estimated to be <10% (see refs 12 and 13).

antioxidants in mixed solvent. The values are summarized in Table 5, together with the k_Q (S) values in ethanol and the peak oxidation potentials (E_p) in acetonitrile solution reported in previous works.[16−20]

The values of log k_Q (S) for phenolic and polyphenolic antioxidants in ethanol have been plotted against E_p. As shown in Figure 4, a plot of log k_Q (S) versus E_p is linear over most of the range with a slope of −(5.4 ± 0.4) V^{-1} (correlation coefficient = −0.97). The antioxidants that have smaller E_P values show higher reactivities (that is, $^1O_2$-quenching activities). The result suggests that the transition state in the above $^1O_2$-quenching reaction by antioixdants has the property of a charge-transfer intermediate.[16,17,28] In fact, for instance, the existence of the cation radicals of α-, β-, γ-, and δ-tocopherol model compounds was ascertained by electron spin resonance (ESR) and electron nuclear double-resonance (ENDOR) studies.[30]

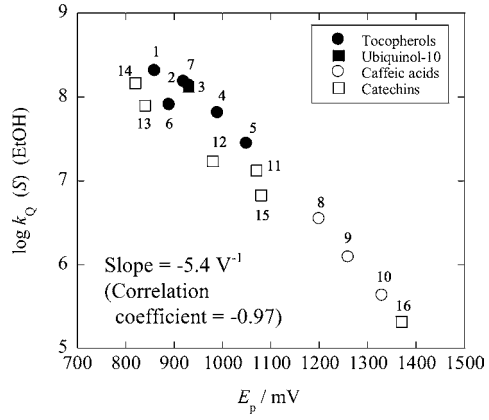

Figure 4. Plot of log k_Q (S) (EtOH) versus E_p for tocopherol derivatives (●), ubiquinol-10 (■), caffeic acids (○), and catechins (□). The numbers correspond to those in Tables 1 and 5.

Similarly, the values of log k_Q (S) for these antioxidants in mixed solvent have been plotted against E_p. As shown in Figure 5, log k_Q (S) of antioxidants correlates well with E_p with a slope of −(5.7 ± 0.3) V^{-1} (correlation coefficient = −0.98), except

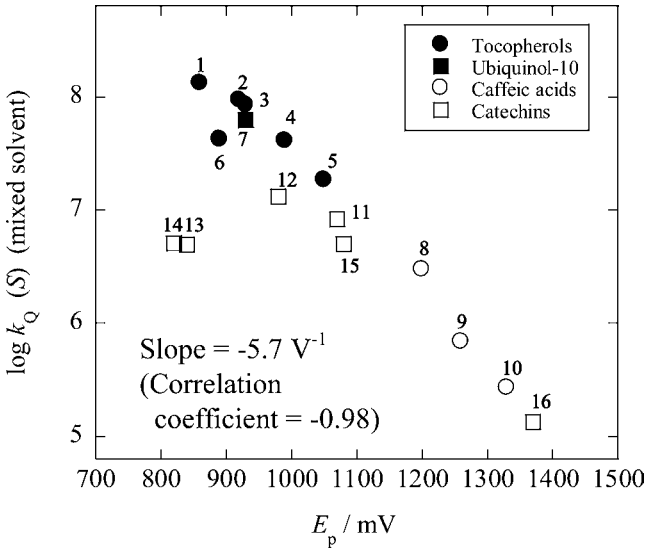

Figure 5. Plot of log k_Q (S) (mixed solvent) versus E_p for tocopherol derivatives (●), ubiquinol-10 (■), caffeic acids (○), and catechins (□). The numbers correspond to those in Tables 1 and 5.

for ECG and EGCG. Furthermore, log (SOAC value) versus E_p plots are shown in Figure 6, indicating that log (SOAC value) also correlates well with E_p with a slope of $-(5.6 \pm 0.3)$ V^{-1} (correlation coefficient = −0.98), except for ECG and EGCG.

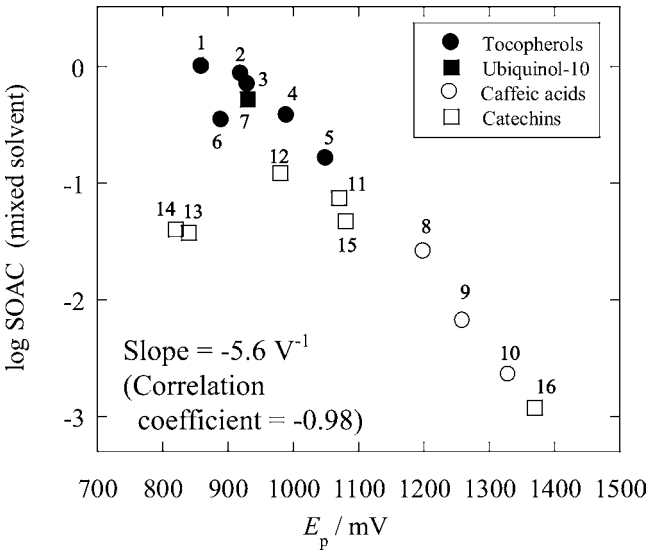

Figure 6. Plot of log SOAC versus E_p for tocopherol derivatives (●), ubiquinol-10 (■), caffeic acids (○), and catechins (□). The numbers correspond to those in Tables 1 and 5.

As listed in Table 5, the k_Q (S) values of catechins in ethanol decrease in the order EGCG > ECG > EGC > EC > 4-MC > 4-MG, as expected from the molecular structures of these compounds and as discussed in a previous work.[18] The ECG having EC and 4-MG moieties in the molecule showed a larger k_Q (S) value than EC. Similarly, the EGCG having EGC and 4-MG moieties showed a larger k_Q (S) value than EGC. On the other hand, the k_Q (S) values of catechins and related compounds in mixed solvent decreased in the order EGC > EC > EGCG ~ ECG ~ 4-MC > 4-MG. Abnormally small k_Q (S) values were obtained for ECG and EGCG (see Table 5). It may be due to a change of the steric structure of ECG (and EGCG) in mixed solvent, and the structural change would be induced by the hydrogen bond due to 1 vol % of D_2O molecules included in mixed solvent. However, the detailed reason why ECG and EGCG show smaller k_Q (S) values than EC and EGC, respectively, in mixed solvent is not clear at present. It will be better to use ethanol solvent for evaluating the k_Q (S) and SOAC values of polyphenolic antioxidants such as catechins and green and black tea extracts including high concentrations of catechins.[17,31−33]

Comparison between the $^1O_2$-Quenching Rates (k_Q (S)) (and Relative SOAC Values) Obtained for Many Kinds of Antioxidants in Ethanol/Chloroform/D_2O and Ethanol Solutions. Measurements of the $^1O_2$-quenching rate constants (k_Q (S)) were performd for 16 phenolic antioxidants and six carotenoids in mixed solvent and ethanol, as listed in Table 5. As described above, it was found that the logarithm of the $^1O_2$-quenching rates (k_Q (S) (EtOH) and k_Q (S) (mixed solvent)) of 16 phenolic antioxidants in ethanol and mixed solvent correlates well with their oxidation potential (E_p). Therefore, the k_Q (S) (EtOH) values have been plotted against the k_Q (S) (mixed solvent) ones. As shown in Figure 7A, the k_Q (S) (EtOH) values were found to correlate linearly with the k_Q (S) (mixed solvent) values (correlation coefficient = 0.99), except for ECG and EGCG. The ratios of k_Q (S) (EtOH) to k_Q (S) (mixed solvent) were estimated to be 1.66 ± 0.05 from the gradient in Figure 7A.

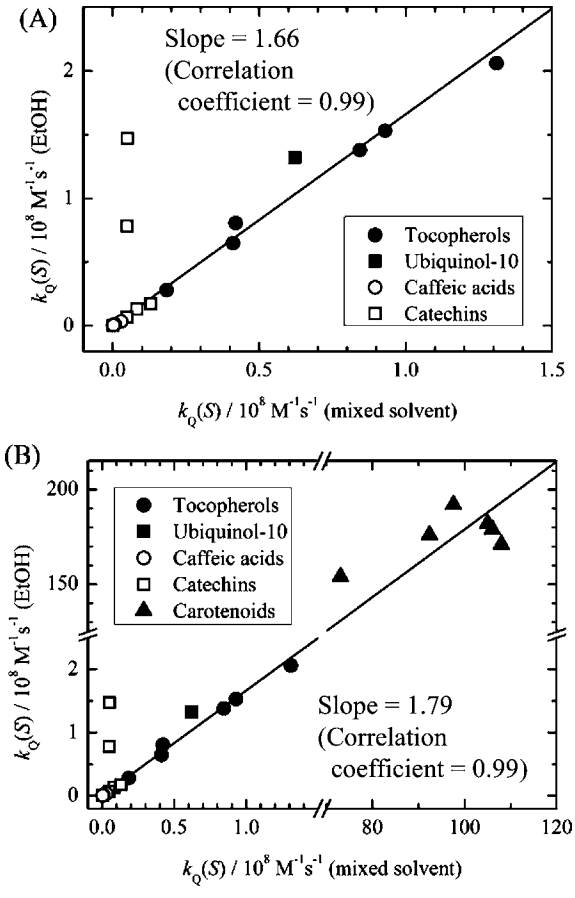

Figure 7. (A) Plot of k_Q (S) (EtOH) versus k_Q (S) (mixed solvent) for tocopherol derivatives (●), ubiquinol-10 (■), caffeic acids (○), and catechins (□). (B) Plot of k_Q (S) (EtOH) versus k_Q (S) (mixed solvent) for 16 phenolic antioxidants and six carotenoids (▲).

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Similarly, the k_Q (S) (EtOH) values for 16 phenolic antioxidants and 6 carotenoids have been plotted against the k_Q (S) (mixed solvent) ones. As shown in Figure 7B, the k_Q (S) (EtOH) values were also found to correlate linearly with the k_Q (S) (mixed solvent) ones (correlation coefficient = 0.99), except for ECG and EGCG. The ratios of k_Q (S) (EtOH) to k_Q (S) (mixed solvent) were estimated to be 1.79 ± 0.04 from the gradient in Figure 7B (see eq 8).

$$k_Q\ (S)\ (\mathrm{EtOH}) = 1.79 k_Q\ (S)\ (\text{mixed solvent}) \quad (8)$$

The ratio (1.79) for phenolic antioxidants and carotenoids is similar to that (1.66) for phenolic antioxidants. The result shows that the ratios of the rate constants (k_Q (S) (EtOH)/k_Q (S) (mixed solvent)) for many kinds of antioxidants are intrinsically constant and do not depend on the kinds of antioxidants, even if (i) the antioxidants vary from carotenoids to phenolic antioxidants and (ii) the rate constants (k_Q (S)) vary by 5 orders of magnitude from 10^{10} to 10^5 M^{-1} s^{-1}.

The ratios of the rate constants obtained in ethanol to those in mixed solvent (k_Q (S) (EtOH)/k_Q (S) (mixed solvent)) are listed in Table 5. For example, the ratios obtained for α-, β-, γ-, and δ-Toc, tocol, and trolox are 1.57, 1.64, 1.64, 1.58, 1.52, and 1.92, respectively, showing a good agreement to each other. The average value of the ratio for all of the phenolic antioxidants is 1.60, except for ECG (15.8) and EGCG (29.1). Furthermore, the ratios for six carotenoids (β-Car, Cap, Zea, α-Car, Lut, and β-Cry) are 1.58, 1.69, 1.73, 1.97, 1.90, and 2.11 (average value 1.83), respectively. The average value of the ratios obtained for all of the antioxidants (including phenolic antioxidants and carotenoids) is 1.67, showing a good agreement with the above gradient (1.79, eq 8), as expected.

$$k_Q\ (S)\ (\mathrm{EtOH}) = 1.67 k_Q\ (S)\ (\text{mixed solvent}) \quad (9)$$

Furthermore, the result indicates that the relative rate constants (k_Q^{AO} (S)/$k_Q^{\alpha\text{-Toc}}$ (S)) of many antioxidants in ethanol and mixed solvent do not depend on the solvents used and are basically constant. Consequently, if we can determine the quenching rate k_Q (S) (EtOH), we can presume the quenching rate k_Q (S) (mixed solvent) using eq 9 and vice versa.

As eq 5 indicates, the relative rate constants of antioxidants are equal to the relative SOAC values. In fact, a fair agreement between the relative rate constants and the relative SOAC values was observed for phenolic antioxidants (see Table 1) and for carotenoids (see Table 2 in ref 14). Therefore, if we use α-Toc as a standard compound, relative SOAC values for general antioxidants will be independent of the kinds of solvent. In fact, the relative SOAC values obtained for six carotenoids in mixed solvent agreed well with the corresponding ones in ethanol, as listed in Table 4.

The solubilities of antioxidants (and food and plant extracts) in organic solvents will be different from each other. However, the relative SOAC value will show a constant value in different solvents for each antioxidant (and food and plant extracts). The results of the present study suggest that the SOAC assay method may be applicable to not only general antioxidants and food and plant extracts but also extracts from biological systems, including lipophilic and hydrophilic antioxidants having different solubilities.

Comparison between the λ_{max} (and ε_{max}) Values for Eight Carotenoids Obtained in Ethanol/Chloroform/D_2O and Ethanol Solutions. Measurements of UV−vis absorption spectra of eight carotenoids in mixed solvent were performed in a previous work (see Table 3).[13] In the present work, measurements of UV−vis absorption spectra of six carotenoids were performed in ethanol solution. The λ_{max} and ε_{max} values obtained are listed in Table 3, together with those reported for β-Car, Zea, and Lut.[29]

By comparing the λ_{max} values of eight carotenoids in ethanol with those in mixed solvent, the former shows blue shift by 6−7 nm from the latter (see Table 3). The ε_{max} values of Ast (ε_{max} = 124000 M^{-1} cm^{-1}), β-Car (133000), Zea (129000), α-Car (138000), and Lut (126000) in mixed solvent are similar to each other. Lyc (ε_{max} = 160000 M^{-1} cm^{-1}) shows a larger value (and Cap (ε_{max} = 106000) and β-Cry (95100) show smaller values) than those for the above five carotenoids. The ε_{max} values in mixed solvent decrease in the order

$$\text{Lyc} > \alpha\text{-Car} \sim \beta\text{-Car} \sim \text{Zea} \sim \text{Lut} \sim \text{Ast} > \text{Cap} > \beta\text{-Cry} \quad (10)$$

Similar results were obtained for the ε_{max} values of carotenoids in ethanol, although we could not determine the ε_{max} values for Lyc and Ast because of their low solubilities. The ε_{max} values of carotenoids in ethanol decrease in the order

$$\alpha\text{-Car} \sim \beta\text{-Car} \sim \text{Zea} \sim \text{Lut} > \text{Cap} > \beta\text{-Cry} \quad (11)$$

By comparing the ε_{max} values of six carotenoids in ethanol with those in mixed solvent, the former shows 1.04−1.15 times larger values than the latter, except for α-Car (0.971), as listed in Table 3. The values of ε_{max} in ethanol were plotted against ε_{max} in mixed solvent. As shown in Figure 8, the ε_{max} (EtOH) values were found to correlate linearly with the ε_{max} (mixed solvent) ones (correlation coefficient = 0.86), although the numbers of carotenoids measured were limited. The ratios of ε_{max} (EtOH) to ε_{max} (mixed solvent) were estimated to be 1.07 ± 0.03 from the gradient in Figure 8.

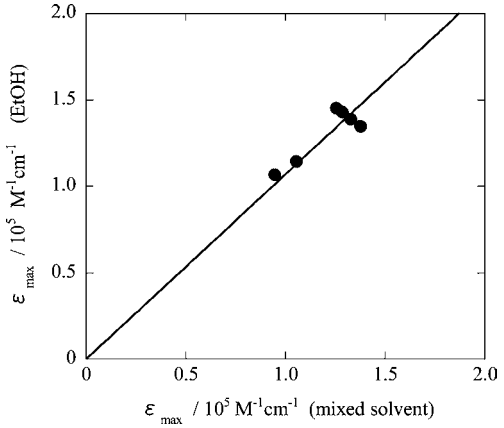

Figure 8. Plot of ε_{max} (EtOH) versus ε_{max} (mixed solvent) for carotenoids.

Consequently, if we could determine the ε_{max} (mixed solvent) values, we can presume the ε_{max} (EtOH) values using eq 12.

$$\varepsilon_{max}\ (\mathrm{EtOH}) = 1.07 \varepsilon_{max}\ (\text{mixed solvent}) \quad (12)$$

The ε_{max} (EtOH) values of Lyc and Ast in ethanol were tentatively estimated to be 171000 and 133000 M^{-1} cm^{-1}, respectively, using eq 12, although we could not determine the ε_{max} values in ethanol.

Furthermore, the ε_{max} values for β-Car (140000 M^{-1} cm^{-1}) and α-Car (147000) in EtOH/THF (4:1, v/v) are similar to those for β-Car (136000) and α-Car (134000) in ethanol, respectively. The ratios of the ε_{max} values of four carotenoids in EtOH/THF to those in mixed solvent (ε_{max} (EtOH/THF)/ε_{max} (mixed solvent)) are 1.03−1.11 (average 1.07), as listed in Table 3. Such a comparison for the λ_{max} and ε_{max} values of a series of carotenoids in organic solvents has not been performed, as far as we know.

In a previous work, a new assay method that can quantify the SOAC of antioxidants, including eight carotenoids and three vegetable extracts (red paprika, carrot, and tomato), was proposed. Many kinds of phenolic and polyphenolic antioxidants are included in foods, plants, and animals and may function as $^1O_2$ quencher in biological systems. Therefore, in the present work, measurements of the $^1O_2$-quenching rates (k_Q (S)) and the relative SOAC values were performed for 16 kinds of phenolic and polyphenolic antioxidants and Vit C in ethanol/chloroform/D_2O solution at 35 °C, by using a competition reaction method, to ascertain the validity of the SOAC assay method proposed for carotenoids. It has been clarified that the SOAC assay method is applicable to evaluation of the $^1O_2$-quenching activity of lipophilic and hydrophilic antioxidants having 5 orders of magnitude different rate constants from 1.38×10^{10} to 2.71×10^5 M^{-1} s^{-1}. Measurements of the SOAC values for many food and plant extracts including high concentrations of phenolic and polyphenolic antioxidants are now in progress in our laboratory.

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