ANALYTICAL SCIENCES JULY 2015, VOL. 31

Application of Electrochemiluminescence for the Evaluation of the Antioxidant Capacity of Some Phenolic Compounds Against Superoxide Anion Radicals

Masanori MATSUOKA and Jiye JIN†

Department of Chemistry, Faculty of Science, Shinshu University, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan

This paper for the first time reports on novel and non-enzymatic method for studying the free radical-scavenging properties of phenolic compounds against superoxide anion radicals ($O_2{\cdot}^-$) by using the cathodic electrochemiluminescence (ECL) of lucigenin (Luc^{2+}). The ECL of Luc^{2+} at a glassy carbon (GC) electrode is observed in an aeration electrolytic solution (pH 7), which is believed to be due to the reaction of a one-electron reduced form of Luc^{2+} (*i.e.* a radical cation, $Luc{\cdot}^+$) with *in situ* electrogenerated $O_2{\cdot}^-$. The ECL intensity is dependent on the concentration of dissolved oxygen, and is suppressed dramatically by superoxide dismutase (SOD), a typical $O_2{\cdot}^-$ scavenger. Since the coexisting hydrogen peroxide (H_2O_2) has no influence on the cathodic ECL of Luc^{2+}, it is thus suggested that the ECL signal specifically reflected the $O_2{\cdot}^-$ concentration level generated at the electrode surface. When phenolic compounds were added into the solution, this resulted in the inhibition of ECL signals due to the elimination of $O_2{\cdot}^-$. The ECL inhibition rate measured at each concentration was compared against the SOD equivalent (U mL^{-1}), and the relative antioxidant efficiency, K_{ao} (U $mmol^{-1}$ equivalent SOD), was used to evaluate the antioxidant activity of some phenolic compounds, including flavonoids, in this study. Structurally different water-soluble phenols were compared, and those compounds containing to catechol skeletal structure are found to present the higher antioxidant capacity.

Keywords Superoxide anion, phenolic compounds, antioxidant capacity, electrochemiluminescence, lucigenin

(Received December 15, 2014; Accepted March 4, 2015; Published July 10, 2015)

Introduction

Phenolic compounds, such as flavonoids (*e.g.* quercetin and rutin) and hydroxycinnamic acid derivatives (*e.g.* caffeic acid), are known to possess free-radical scavenging properties that can scavenge harmful reactive oxygen species (ROS) through donating electrons with more or less efficiency, and have gained much interest as natural antioxidants.[1-3] Since the presence of these compounds in foods may prevent the development of many diseases, including atherosclerosis and cancer, there has been significant interest in developing new methods for estimating the total antioxidant capacity of these compounds, and to elucidate the relationship between the chemical structure and the antioxidant activities over the past few years.[4,5] ROS are various forms of activated oxygen, including superoxide anion radicals ($O_2{\cdot}^-$), hydroxyl radicals (·OH) and non-free radical species (H_2O_2). Because different ROSs have their own characteristics regarding the generation mechanism, lifetime and chemical reactivity, the development of a method capable for evaluating the antioxidant capacity against a certain ROS is necessary.

$O_2{\cdot}^-$ is formed by the one-electron reduction of dioxygen (O_2), which is abundantly present in nature. It appears to be a particularly interesting species because it has a longer lifetime than ·OH, and because of various possible reaction pathways that make $O_2{\cdot}^-$ a good candidate for probing mechanisms.[6] A few analytical methods have been developed to determine the scavenging capacity specifically toward the $O_2{\cdot}^-$. These assays are generally based on $O_2{\cdot}^-$ generation using an enzyme system, for example, the hypoxanthine/xanthine oxidase system. The inhibition reaction by the antioxidant substrate is usually measured by spectrophotometry,[7] amperometry,[8] and chemiluminescent techniques.[9] Nevertheless, since some flavonoids, like quercetin, may inhibit the radical formation by interfering with the enzyme,[10] the development of analytical protocols based some non-enzymatic reactions are greatly desired.

Recently, Abasq *et al.* proposed an electrochemical method with a non-enzymatic reaction for evaluating the antioxidant capacities of some phenolic compounds.[11,12] The method was based on the kinetics of the reaction of the antioxidant phenols with $O_2{\cdot}^-$. A cyclic voltammetric technique was used to generate $O_2{\cdot}^-$ by the reduction of molecular oxygen in aprotic media. In the same experiment the consumption of the radical was directly measured by the anodic current decay of the superoxide anion radical oxidation in the presence of increasing concentrations of antioxidant substrate. However, the approach could only be applicable in an aprotic media.[12]

The electrogenerated chemiluminescence (ECL) of lucigenin (Luc^{2+}) has been studied in both aqueous and non-aqueous media at the solid electrodes. In the cathodic process, the

† To whom correspondence should be addressed.
E-mail: jin@shinshu-u.ac.jp

coupling reaction between an *in situ* electrogenerated radical species, such as one-electron reduced lucigenin ($Luc^{\cdot+}$) and $O_2^{\cdot-}$, would lead to the formation of a dioxetane-type intermediate, and finally, to generate chemiluminescence in neutral or relatively weak alkaline solutions (pH 7 – 10).[13-16] Lucigenin ECL has been used for a diverse range of analytical applications, including the detection of $O_2^{\cdot-}$ production,[17-19] riboflavin and isatin based on the enhancement of ECL signals.[20,21] Recently, Dai *et al.* proposed a novel analytical method for the determination of bisphenol A based on its strong inhibition phenomenon to the Luc^{2+} ECL response.[22] The inhibition effect was suggested to be due to the antioxidant properties of bisphenol A. This implies that if an antioxidant is added into the Luc^{2+}/O_2 system, the ECL intensity would be inhibited and the degree of ECL decrease would depend on both the antioxidant capacity and the concentration. Up till now, however, the potential use of ECL on an antioxidant capacity study has not been well investigated. Herein, we report on our original results for evaluating the antioxidant capacity of phenolic compounds specifically against $O_2^{\cdot-}$ by using cathodic ECL of Luc^{2+}. ECL might have more promising applicative values than the protocols based on an enzymatic reaction because it can avoid problems due to the inhibition of enzyme activity by phenolic compounds.

Experimental

Chemicals

All reagents were of analytical grade and used as received. Lucigenin (bis-*N*-methylacridiniumnitrate) and superoxide dismutase (SOD) of bovine erythrocytes (5140 U/mg) were purchased from Nacalai Tesque (Kyoto, Japan). The phenolic compounds, catechol, 2,3-dihydroxy benzoic acid (2,3-DHBA), 2,4-dihydroxy benzoic acid (2,4-DHBA), 2,5-dihydroxy benzoic acid (2,5-DHBA) and salicylic acid were purchased from Wako Pure Chemical Industries (Osaka, Japan); protocatechuic acid, catechin and rutin were purchased from Nacalai Tesque (Kyoto, Japan); caffeic acid, benzoic acid, and quercetin were purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). A solution containing 0.1 M of KNO_3, 5 mM Na_2HPO_4 and 5 mM KH_2PO_4 (pH was adjusted to 7) was used as a supporting electrolyte in this study. All solutions were prepared with distilled water purified by a WS200 distillation system (Yamato Scientific Co., Tokyo, Japan).

Apparatus

Voltammetry was performed with a Model 660 electrochemical workstation (CH Instruments, Austin, TX) or an EG&G/PAR 263A potentiostat/galvanostat. A conventional three-electrode cell was used, with a Pt wire serving as the counter electrode and an Ag/AgCl reference electrode (RE-1, BAS Japan). A glassy carbon disk (Tokai Carbon Co., Tokyo, Japan) with diameter of 3 mm was used as a working electrode. The dissolved oxygen was measured with a polarographic oxygen sensor (Model DO-5509, LUTRON Electronic, Taiwan). ECL spectra were measured using an Ocean Optics USBFL-2000 spectrometer.

The ECL measurement system was described in our previous study.[23] The measurements were conducted in an electrochemical cell by using a salt-bridge system to separate the working and counter electrodes. The electrode surface of the working electrode was 0.5 mm distance from the optical window, and an H7732-10 PMT photosensor module equipped with a C7319 signal preamplifier unit (Hamamatsu Photonics, Shizuoka, Japan) was placed in front of the optical window to detect the light emitted from the electrode. The electrode potential was controlled by an EG&G/PAR 263A potentiostat/galvanostat, and a C7169 power supply unit (Hamamatsu Photonics) was used for driving the photosensor module. High-purity argon (Ar, 99.99%) and oxygen (O_2, 99.5%) were used for the removal or saturation of O_2 in solutions, respectively.

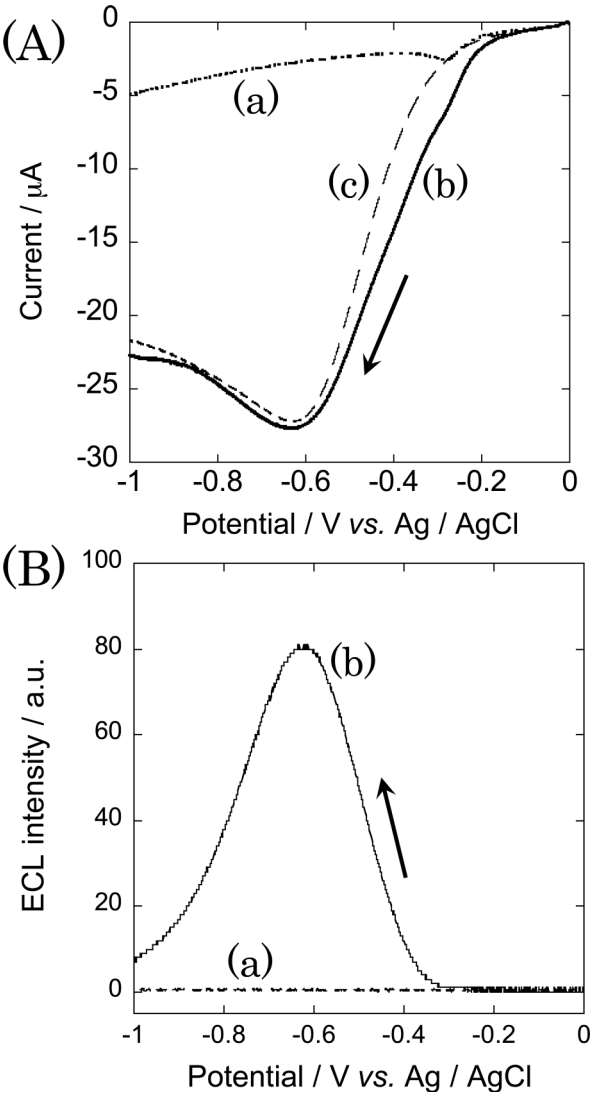

Fig. 1 Linear sweep voltammograms (A) and corresponding ECL-potential curves (B) of 50 μM Luc^{2+} at a GC electrode in a supporting electrolyte containing 0.1 M of KNO_3, 5 mM Na_2HPO_4 and 5 mM KH_2PO_4 (pH 7). (a) Ar saturated solution; (b) O_2 saturated solution; (c) O_2 saturated solution in the absence of Luc^{2+} (only voltammogram). Arrows show the potential sweep direction. Scan rate is 50 mV/s.

Results and Discussion

ECL of Luc^{2+} on GC electrodes

Figure 1 shows simultaneous linear sweep voltammograms (A) and the corresponding ECL responses (B) of 50 μM Luc^{2+} on a GC electrode in an aqueous solution containing 0.1 M KNO_3 (pH 7). The dotted curves (a) are the responses measured in an O_2 free (argon saturated) solution, whereas the solid lines are the responses in an O_2 saturated solution. In the absence of

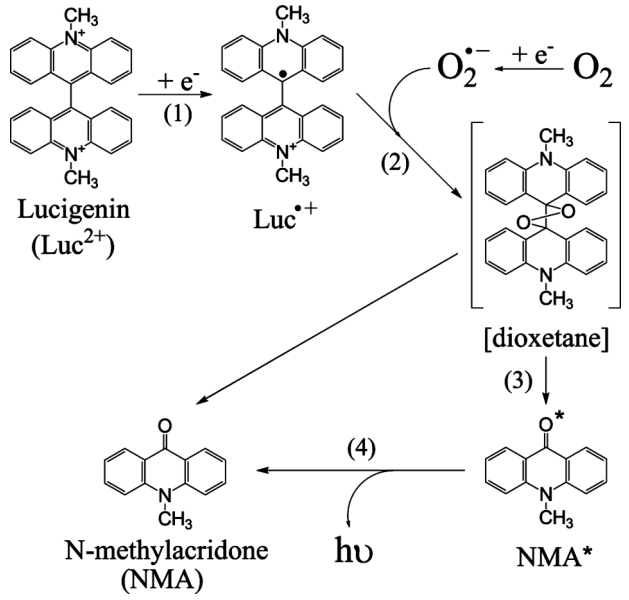

Fig. 2 Cathodic ECL scheme of the Luc^{2+}/oxygen system.

O_2, a reduction peak was observed at –0.3 V *vs.* Ag/AgCl (Fig. 1A). This could be due to the reduction of Luc^{2+}. The electrode reaction involved a one-electron reduction of Luc^{2+} to produce an intermediate radical, $Luc^{\cdot+}$, and was subsequently reduced to Luc^0 at the electrode surface.[15,16] The produced Luc^0 could be confirmed at about +0.45 V *vs.* Ag/AgCl in a subsequent anodic potential scan in a cyclic voltammogram (not shown here). However, there was no ECL response that could be observed during the reduction process of Luc^{2+} in the absence of O_2. In the O_2 saturated solution, a much larger reductive wave appeared at around –0.6 V *vs.* Ag/AgCl, which was attributed to the O_2 reduction, as is shown in Fig. 1(A)-c. The electrochemical reduction steps for O_2 involve a one-electron reduction of O_2 to $O_2^{\cdot-}$, and the subsequent one-electron reduction to produce of H_2O_2.[24,25] A distinct ECL signal was detected at the electrode potential coinciding with the reduction potential of O_2, as can be seen in Fig. 1(B).

The cathodic ECL of Luc^{2+} has been studied, and was suggested to involve 3 reaction steps: (1) a one-electron reduction of Luc^{2+} to produce radical $Luc^{\cdot+}$; (2) $Luc^{\cdot+}$ then reacts with a superoxide anion radical ($O_2^{\cdot-}$) and yields an extremely unstable dioxetane-type intermediate; (3) the decomposition of this intermediate provides an excited state of *N*-methylacridone (*NMA), which will be the primary emitter, emitting at *ca.* 452 nm (reaction 4).[13-16] It is worthy of note that the ECL spectrum with a broad band at 510 nm was observed in our experiment, which suggested that the light emission originated from the excited triplet state $*Luc^{2+}$ through the energy-transfer process from *NMA to Luc^{2+}, which fluoresced at longer wavelengths at 510 nm. The ECL reaction scheme for the Luc^{2+}/O_2 system is summarized in Fig. 2.

Figure 3(A) shows the effect of the dissolved oxygen concentration on the Luc^{2+} ECL responses. The ECL intensity decreased with the decrease of dissolved oxygen, and nearly no ECL peak was observed in an Ar saturated solution. This means that the ECL signal is proportional to the $O_2^{\cdot-}$ concentration *in situ* generated at the electrode surface. The influence of hydrogen peroxide (H_2O_2) on the Luc^{2+} cathodic ECL process was examined, as is shown in Fig. 3(B). It can be seen that the coexisting H_2O_2 up to a concentration level of 200 μM has

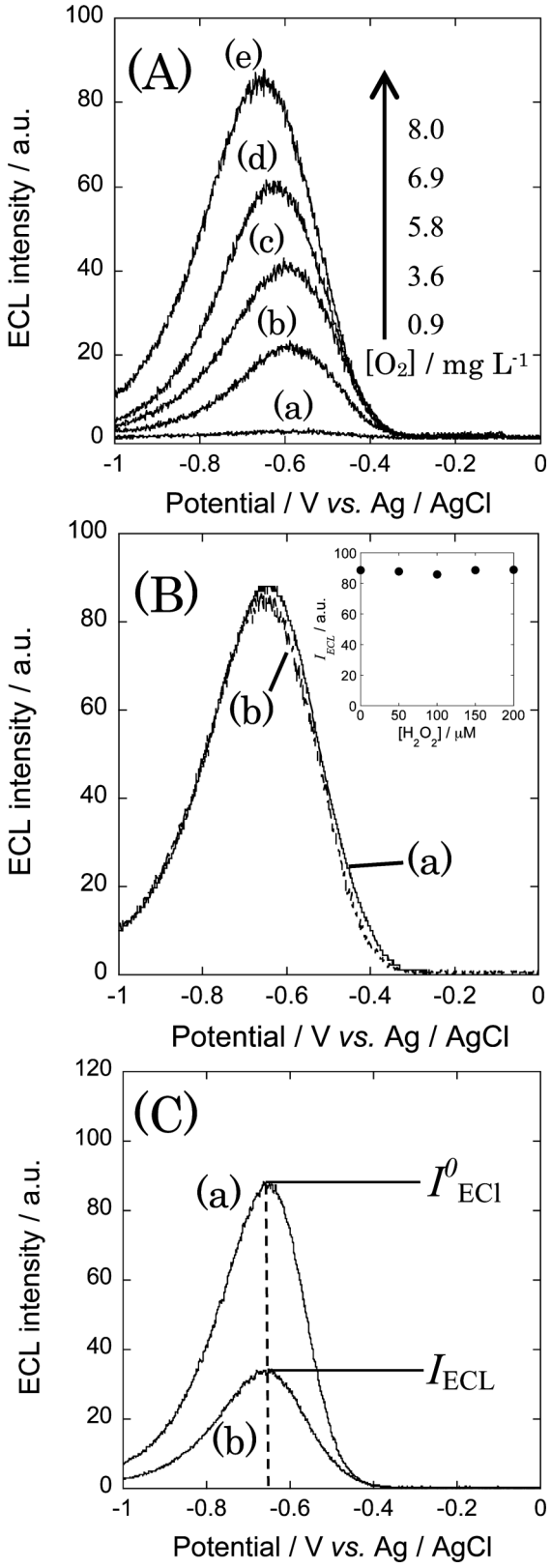

Fig. 3 (A) Effect of the dissolved oxygen concentration on the cathodic ECL responses at a GC electrode. The concentrations of dissolved oxygen were measured with a polarographic oxygen sensor. (B) Effect of the coexisting H_2O_2 on cathodic ECL of 50 μM lucigenin in an air saturated PBS (pH 9.0). Solid line (a): without H_2O_2: dotted line (b): in the presence of 50 μM H_2O_2. Inset: the dependence of H_2O_2 concentration on ECL intensity. (C) Inhibition of ECL response by addition of 0.1 U mL^{-1} of SOD. The other conditions were the same as in Fig. 1.

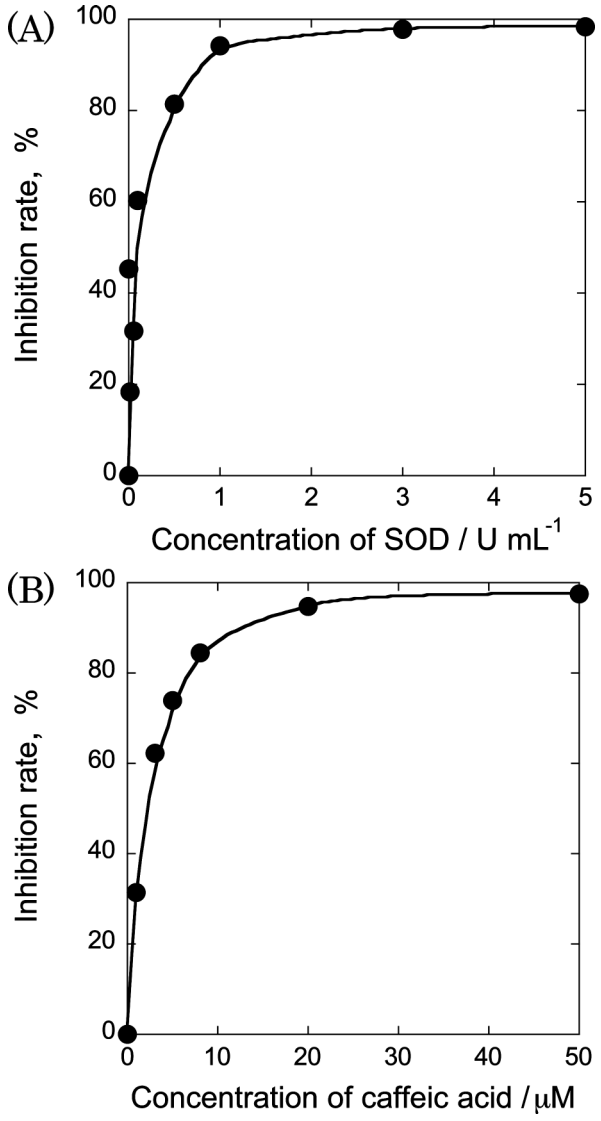

Fig. 4 Dependence of SOD (A) and the caffeic acid (B) concentrations on the ECL inhibition rate (%). ECL measurements were conducted in O_2 saturated solutions containing 50 μM of Luc^{2+} and a 0.1 M KNO_3 supporting electrolyte (pH 7).

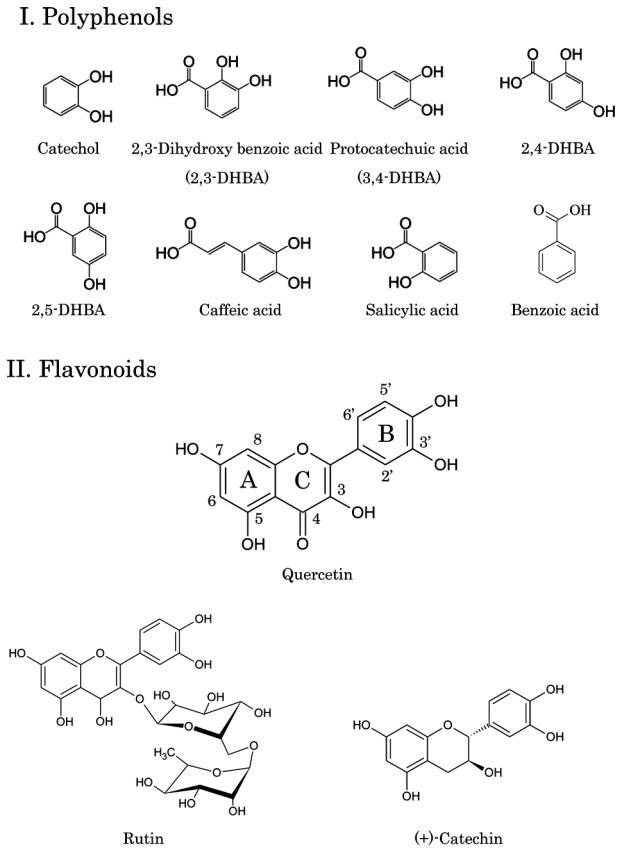

Fig. 5 Chemical structures of the phenolic compounds investigated in this study.

almost no influence on the ECL response. We had confirmed the ECL specifically responded to the $O_2{\cdot}^-$ concentration level at the electrode surface.

In Fig. 3(C), it was found that the intensity of the ECL signal was inhibited by about 30% upon the addition of 0.1 U mL^{-1} of SOD into the solution. The SOD is known as one of the most important antioxidative enzymes that can catalyze the dismutation of $O_2{\cdot}^-$ into hydrogen peroxide and molecular oxygen. The antioxidant activity of SOD can be evaluated from the ECL inhibition rate (%), which can be calculated from the following equation:

$$\text{ECL inhibition rate (\%)} = (I_{ECL}^0 - I_{ECL})/I_{ECL}^0 \times 100\%, \quad (1)$$

where I_{ECL}^0 is the ECL intensity measured without SOD, and I_{ECL} is that measured in the presence of SOD. Figure 4(A) shows that an increase in the concentrations of SOD enhances the scavenging activity against $O_2{\cdot}^-$, and therefore increases the ECL inhibition rate (%).

Quenching of the ECL signals by phenolic compounds

Typical phenolic compounds that possess antioxidant activity can be naturally classified into two big groups, flavonoids and nonflavonoids.[26] In this study, the flavonoid compounds (quercetin, catechin and rutin) and some phenolic derivatives with different hydroxylated positions (caffeic acid, protocatechuic acid, catechol, 2,3-DHBA, 2,4-DHBA, 2,5-DHBA, benzoic acid, salicylic acid) were investigated as model compounds (Fig. 5). Because some of flavonoids and phenolic derivatives undergo autoxidation, and are unstable under a higher pH condition, the experiments were conducted at neutral pH (= 7) in this study.

Upon the addition of caffeic acid into the Luc^{2+}/O_2 ECL system, it resulted in a concentration-dependent inhibition of ECL similar to that for SOD (Fig. 4(B)). Apart from caffeic acid (pK_{a1} = 4.47, pK_{a2} = 8.32),[27] other phenolic compounds, such as quercetin (pK_{a1} = 7.19, pK_{a2} = 9.36),[28] catechin (pK_{a1} = 8.85, pK_{a2} = 9.97),[27] rutin (pK_{a1} = 4.47, pK_{a2} = 8.32),[29] catechol (pK_{a1} = 9.43),[28] and protocatechuic acid (pK_{a1} = 4.38, pK_{a2} = 8.74),[27] also exhibited a significant ECL inhibitory effect. Quenching of the ECL signals is suggested to be due to their potentials to scavenge $O_2{\cdot}^-$ produced at the electrode surface by phenolic compounds. The reaction of $O_2{\cdot}^-$ with phenolic compounds, for example catechin, has been suggested to be due to the scheme shown in Fig. 6.

Most flavonoids are not dissociated at pH 7. It can act as an antioxidant by donating an electron to the $O_2{\cdot}^-$ which is accompanied with a proton-transfer process to produce a flavonoid phenoxyl radical (reaction (6) in Fig. 6). It is subsequently deprotonated to a flavonoid anion radical because

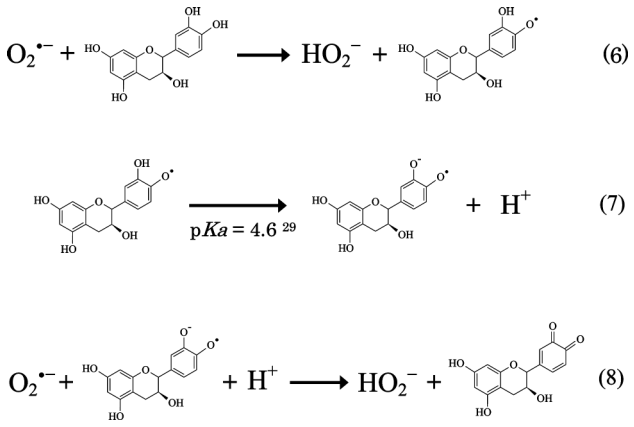

Fig. 6 Reaction scheme between catechin and $O_2^{\cdot -}$.

of a relatively low pK_a (= 4.6)[29] of the flavonoid phenoxyl radical (reaction (7) in Fig. 6). The flavonoid anion radical can act as a secondary antioxidant by donating an electron to the $O_2^{\cdot -}$ to yield corresponding quinone-type metabolites (oxidized form of catechin) according to reaction (8) in Fig. 6. The reaction mechanisms for the scavenging of $O_2^{\cdot -}$ by phenolic derivatives are considered to be the same as that for catechin, although caffeic acid and protocatechuic acid existed as monoanions at pH 7 due to deprotonation of the carboxylic acid group.

As shown in reaction (8), the reaction can accompany some side reactions, such as a dimerization reaction, and is able to generate hydrogen peroxide (H_2O_2) due to the autoxidative behavior of the phenolic compound. The presence of H_2O_2 usually affects the results of the antioxidant activity in many chemiluminescence assays.[30] The ECL method, however, provides information about the radical scavenging effect specifically toward $O_2^{\cdot -}$. The relative capacity of phenolic compounds to scavenge $O_2^{\cdot -}$ could thus be evaluated from the degree of the inhibition rate of ECL signals.

Evaluation of antioxidant efficiency of phenolic compounds by ECL

The ECL inhibition rates (%) depended on both the antioxidant capacity and the concentration of the phenolic compound, which were examined at different concentration levels for each phenolic compound. In this study, the ECL inhibition rate (%) measured at each concentration was compared against the SOD equivalent (U mL^{-1}) using the data in Fig. 4(A), and the relative antioxidant efficiency, K_{ao} (U mmol^{-1} equivalent SOD) was used to evaluate the antioxidant activity of phenolic compounds. It is shown in Fig. 7 that a good linear relationship between the SOD equivalent (U mL^{-1}) and the concentration of tested phenolic compounds were obtained in a range of 0 ~ 50 μmol L^{-1}. The slopes of the plot are defined as the relative antioxidant efficiency, K_{ao} (SOD U mmol^{-1}), for each of the phenolic compounds, *i.e.* the larger is value of K_{ao}, the higher is the antioxidant capacity. The 50%-inhibition concentrations (IC_{50}) are often used to evaluate the antioxidant active; *i.e.* the lower is the IC_{50} value, the higher is the activity the phenolic compound. These values were easy calculated by a linear regression of the plots in Fig. 7, and are listed in Table 1, along with the K_{ao} values. As expected, K_{ao} or IC_{50} depends on the position of the hydroxylation and the chemical nature of the substituents present on the phenolic compound.

It is shown in Table 1 that flavonoid compounds (quercetin, rutin and catechin) exhibited relatively higher antioxidant activity with a K_{ao} value from 6.80×10^{-2} to 2.00×10^{-2} U mmol^{-1} SOD equivalent. In general, the antioxidant activity of flavonoids depends on the structure and the substitution pattern of the hydroxyl groups. The resulting antioxidant-derived radical is governed by its ability to stabilize and to delocalize the unpaired electron of the semiquinone anion radical in reaction (8) in Fig. 6.[31] Rutin is a naturally occurring flavonol consisting of aglycone quercetin and a rutinoside moiety at position 3 of the C ring (Fig. 5). This may block its C-3 hydroxyl group, which would result in a reducing of the antioxidant activity, in comparison with quercetin. Indeed, quercetin has the structure like that of catechol in the B ring, and the presence of the 3-OH group attached to the 2,3-double bond adjacent to the 4-carbonyl in the C ring. With this structure, the hydrogen bond between the OH group in the B ring and the 3-OH group in the C ring would make molecule structure planar. This planarity is expected to have a higher electron delocalization, which would stabilize the π system of the flavonoid phenoxyl radical.[32] Meanwhile, for catechin,

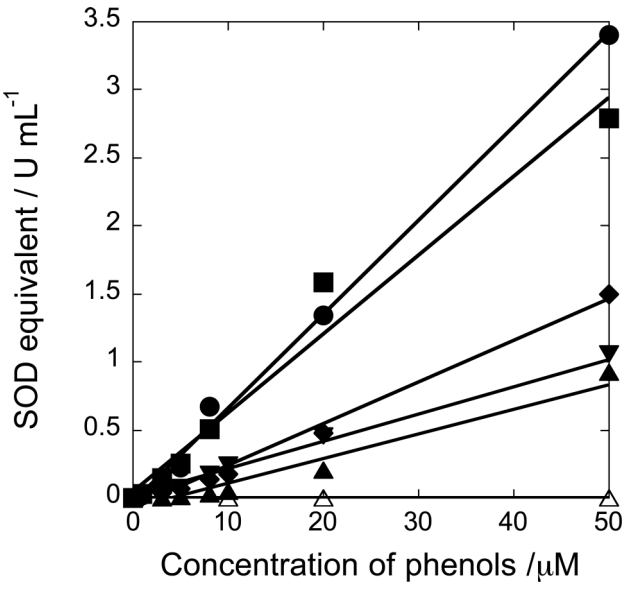

Fig. 7 Relationship between the SOD equivalent and the concentration for some selected phenolic compounds. The slope of the plot is defined as the antioxidant efficiency (K_{ao}, U mmol^{-1}) (●) Quercetin, (■) caffeic acid, (◆) rutin, (▲) catechol, (▼) catechin, (△) salicylic acid.

Table 1 K_{ao} and IC_{50} values of some phenolic compounds

Compound	K_{ao}/U mmol	IC_{50}/mM
Quercetin	6.80×10^{-2}	2.56
Caffeic acid	5.91×10^{-2}	1.92
Rutin	2.84×10^{-2}	5.80
Catechin	2.03×10^{-2}	5.72
Protocatechuic acid	2.36×10^{-2}	6.9
Catechol	1.70×10^{-2}	14.1
2,5-DHBA	7.0×10^{-4}	>1000
2,4-DHBA	5.0×10^{-4}	>1000
2,3-DHBA	2.0×10^{-4}	>1000
Salicylic acid	0.00	>1000
Benzoic acid	0.00	>1000

electronic delocalization may occur separately on rings B and A because of the absence of a conjugated 2,3 double bond in the C ring.[32] This may result in a reduction of the antioxidant activity in comparison with that of quercetin and rutin.

Although flavonoids have been the focus of most dietary antioxidant studies, the antioxidant properties of nonflavonoid phenolic compounds have also been investigated in a number of radical scavenging assays. Phenolic compounds with the catechol moiety (caffeic acid, protocatechuic acid, catechol) present higher antioxidant activities. Caffeic acid is known as cinnamic acid, which is present in many plants. Caffeic acid exhibits a higher antioxidant activity because of additional conjugation in the propenoic side chain, which would facilitate electron delocalization, by resonance, between the aromatic ring and the propenoic group for the semiquinone anion radical ($Q^{\cdot -}$). However, for compounds without the catechol moiety (2,4-DHBA, 2,5-DHBA, benzoic acid, salicylic acid), very low K_{ao} values were measured. Because the K_{ao} values for those compounds are extremely small, IC_{50} could not be evaluated meaningfully. In the case of 2,3-DHBA, which has the catechol moiety, but shows very low antioxidant activity, we considered that the steric effect caused by the COOH group interferes with the reaction at the catechol moiety, and reduces the antioxidant activity.

Conclusions

In this study, we described the possibility to determine antioxidant capacity using the ECL method for the first time. It has been demonstrated that the ECL signals of Luc^{2+} would be suppressed in the presence of phenolic compounds due to the elimination of $O_2^{\cdot -}$ by these compounds. The ECL inhibition rate (%) measured at each concentration was compared against the SOD equivalent (U mL^{-1}), and the relative antioxidant efficiency, K_{ao} (U $mmol^{-1}$ equivalent SOD), was used to evaluate the antioxidant activity of phenolic compounds in this study. It is notable that $O_2^{\cdot -}$ was electrogenerated by the one-electron reduction of dioxygen and therefore its generation involves neither enzymes nor chelating metals, which are usually used in methods testing the superoxide scavenging capacity. Consequently, this method measures specifically the radical scavenging efficiency of phenolic compounds toward $O_2^{\cdot -}$ in aqueous solution.

Acknowledgements

Financial Aid under the dean's discretion of Faculty of Science, Shinshu University is greatly appreciated.

References

1. M. Carocho and I. C. F. R. Ferreira, *Food Chem. Toxicol.*, **2013**, *51*, 15.
2. C. Kaur and H. C. Kapoor, *Int. J. Food Sci. Technol.*, **2001**, *36*, 703.
3. N. Balasundram, K. Sundram, and S. Samman, *Food Chem.*, **2006**, *99*, 191.
4. A. M. Pisoschi and G. P. Negulescu, *Biochem. Anal. Biochem.*, **2011**, *1*, 106.
5. C. A. Rice-Evans, N. J. Miller, and G. Paganga, *Free Radicals Biol. Med.*, **1996**, *20*, 933.
6. F. Auchere and F. Rusnak, *J. Biol. Inorg. Chem.*, **2002**, *7*, 664.
7. N. S. C. Gaulejac, C. Provost, and N. Vivas, *J. Agric. Food Chem.*, **1999**, *47*, 425.
8. S. Ignatov, D. Shinshniashvili, B. Ge, F. W. Scheller, and F. Lisdat, *Biosens. Bioelectron.*, **2002**, *17*, 191.
9. S. Yamaguchi, N. Kishikawa, K. Ohyama, Y. Ohba, M. Kohno, T. Masuda, A. Takadate, K. Nakashima, and N. Kuroda, *Anal. Chim. Acta*, **2010**, *665*, 74.
10. P. Cos, L. Ying, M. Calomme, J. P. Hu, K. Cimanga, B. Poel, L. Pieters, A. J. Vlietinch, and D. V. Berghe, *J. Nat. Prod.*, **1998**, *61*, 71.
11. C. Bourvellec, D. Hauchard, A. Darchen, J. L. Burgot, and M. L. Abasq, *Talanta*, **2008**, *75*, 1098.
12. A. Renè, M. L. Abasq, D. Hauchard, and P. Hapiot, *Anal. Chem.*, **2010**, *82*, 8703.
13. T. Okajima and T. Ohsaka, *Luminescence*, **2003**, *18*, 49.
14. Y. G. Sun, H. Cui, and X. Q. Lin, *J. Lumin.*, **2001**, *92*, 205.
15. K. D. Legg and D. M. Hercules, *J. Am. Chem. Soc.*, **1969**, *91*, 1902.
16. K. D. Legg, D. W. Shive, and D. M. Hercules, *Anal. Chem.*, **1972**, *44*, 1650.
17. Y. Li, H. Zhu, and P. Kuppusamy, *J. Biol. Chem.*, **1998**, *273*, 2015.
18. M. Rost, E. Karge, and W. Klinger, *J. Biolumin. Chemilumin.*, **1998**, *13*, 355.
19. S. I. Liochev and I. Fridovich, *Free Radicals Biol. Med.*, **1998**, *25*, 926.
20. C. Zhang and H. Qi, *Spectrochim. Acta, Part A*, **2011**, *78*, 211.
21. C. Zhang and H. Qi, *Luminescence*, **2004**, *19*, 21.
22. H. Dai, S. Zhang, Y. Lin, Y. Ma, L. Gong, G. Xu, M. Fu, X. Lia, and G. Chen., *Anal. Methods*, **2014**, *6*, 4746.
23. J. Jin, F. Takahashi, T. Kaneko, and T. Nakamura, *Electrochim. Acta*, **2010**, *55*, 5532.
24. R. J. Taylor and A. A. Humffray, *J. Electroanal. Chem.*, **1975**, *64*, 85.
25. B. H. J. Bielski and A. O. Allen, *J. Phys. Chem.*, **1977**, *81*, 1048.
26. C. A. Rice-Evans, N. J. Miller, and G. Paganga, *Trends Plant Sci.*, **1997**, *2*, 152.
27. F. Z. Erdemgil, S. Sanli, N. Sanli, G. Özkan, J. Barbosa, J. Guiteras, and J. L. Beltrán, *Talanta*, **2007**, *72*, 489.
28. J. M. Herrero-Martinez, M. Sanmartin, M. Rosés, E. Bosch, and C. Ràfols, *Electrophoresis*, **2005**, *26*, 1886.
29. S. V. Jovanovic, S. Steenken, M. Tosic, B. Marjanovic, and M. G. Simic, *J. Am. Chem. Soc.*, **1994**, *116*, 4846.
30. R. Kahl, A. Weimann, S. Weinke, and A. G. Hildebrandt, *Arch. Toxicol.*, **1987**, *60*, 158.
31. E. G. Bakalbassis, N. Nenadis, and M. Tsimidou, *J. Am. Oil Chem. Soc.*, **2003**, *80*, 459.
32. S. A. B. E. Acker, M. J. Groot, D. J. Berg, M. N. J. L. Tromp, G. D. Kelder, W. J. F. Vijgh, and A. Bast, *Chem. Res. Toxicol.*, **1996**, *9*, 1305.