Superoxide Anion Generation via Electron-Transfer Oxidation of Catechin Dianion by Molecular Oxygen in an Aprotic Medium

Ikuo Nakanishi,[†] Kiyoshi Fukuhara,* Kei Ohkubo,[††] Tomokazu Shimada,[†††] Hisao Kansui, Masaaki Kurihara,
Shiro Urano,[†††] Shunichi Fukuzumi,*[††] and Naoki Miyata*
Division of Organic Chemistry, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501
[†]*Japan Science and Technology Corporation (JST), 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501*
[††]*Department of Material and Life Science, Graduate School of Engineering, Osaka University, CREST,*
Japan Science and Technology Corporation (JST), 2-1 Yamada-oka, Suita, Osaka 565-0871
[†††]*Department of Applied Chemistry, Shibaura Institute of Technology, 3-9-14 Shibaura, Minato-ku, Tokyo 108-8548*

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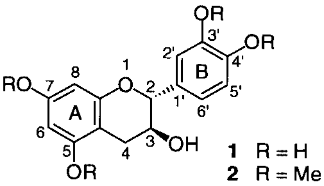
Superoxide anion ($O_2^{\bullet-}$) was generated via an electron-transfer oxidation of catechin dianion, which was produced in the reaction of catechin with two equivalents of methoxide anion, by molecular oxygen in acetonitrile. From the detailed spectroscopic and kinetic analyses was determined the rate constant for the formation of $O_2^{\bullet-}$ to be 5.8×10^{-2} mol^{-1} dm^3 s^{-1}.

Flavonoids, such as catechin, quercetin, etc., are known to act as an antioxidant, which reductively scavenges oxygen radicals, such as superoxide anion ($O_2^{\bullet-}$) and hydroxyl radical (•OH), which are responsible for various pathological events.[1] However, in spite of the antioxidative activities, flavonoids can also generate reactive oxygen species (ROSs) and induce oxidative DNA damage,[2] which causes carcinogenicity. Catechin and quercetin also induce copper-dependent DNA damage, where the ROSs are generated by the copper complex of these flavonids.[3] Catechin and quercetin are reported to enhance the DNA cleavage by •OH generated in the Fenton reaction.[4] However, very little is known about the mechanism of the ROS formation caused by flavonoids.

We report herein that $O_2^{\bullet-}$ is indeed produced by catechin under strongly basic conditions in acetonitrile (MeCN) via an electron transfer from catechin dianion to molecular oxygen. Detailed spectroscopic and kinetic analyses provide a valuable mechanistic insight into the $O_2^{\bullet-}$ formation by catechin dianion.



1 R = H
2 R = Me

When one equivalent of methoxide anion (MeO$^-$), produced in the reaction between tetra-*n*-butylammonium hydroxide and methanol, is added to an MeCN solution of catechin (**1**), the absorption band at 280 nm due to **1** shifted to 290 nm. When **1** was replaced by methylated catechin analogue (**2**),[5] where four OH groups on the A and B rings are substituted by four methoxy groups, no such spectral change was observed upon addition of MeO$^-$. These results indicate that the deprotonation of one of the four OH groups in **1** occurs to produce catechin anion (**1**$^-$). Since the first deprotonation of **1** is known to occur at the OH group at C-3' position on the B ring,[6] the reaction of **1** with MeO$^-$ is described as shown in Scheme 1.

Addition of two equivalents of MeO$^-$ to **1** resulted in an increase in the absorption band at 290 nm, accompanied by an increase in the absorbance at around 340 nm as a shoulder. This can be ascribed to the deprotonation of **1**$^-$ to form catechin dianion (**1**$^{2-}$) (Scheme 1). The second deprotonation is reported to occur at the OH group at C-7 position on the A ring as shown in Scheme 1.[6] The **1**$^{2-}$ thus formed is stable under anaerobic conditions. However, introduction of molecular oxygen (O_2) to the MeCN solution of **1**$^{2-}$ resulted in an increase in the absorption band at 430 nm as shown in Figure 1. Such a spectral change suggests that **1**$^{2-}$ is oxidized by O_2 to produce catechin radical anion (**1a**$^{\bullet-}$) and superoxide anion ($O_2^{\bullet-}$). In fact, the ESR spectrum having a *g* value of 2.0051 was observed for an O_2-saturated MeCN solution of **1** and two equivalents of MeO$^-$. The observed ESR spectrum can be simulated with the hyperfine splitting constants (a_H = 3.33, 1.50, 1.27, and 1.27 G), which are almost the same as those reported for the semiquinone radical anion form of catechin (**1b**$^{\bullet-}$) in NaOH-saturated dimethyl sulfoxide.[7] This indicates that an electron transfer from **1**$^{2-}$ to O_2 to produce **1a**$^{\bullet-}$ and $O_2^{\bullet-}$ is followed by a proton transfer from the OH group on the B ring in **1a**$^{\bullet-}$ to the A ring to give **1b**$^{\bullet-}$.[8,9]

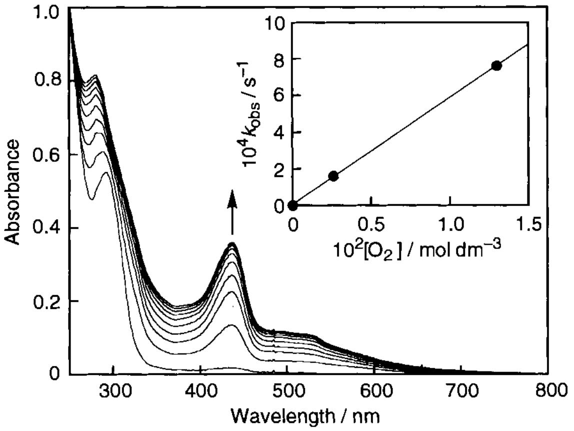
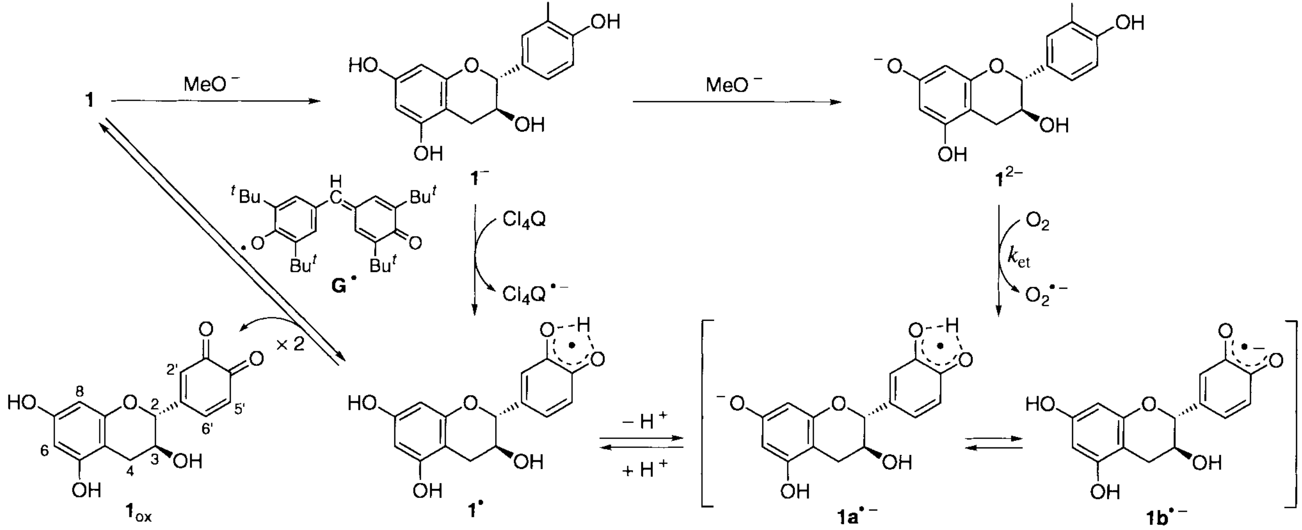


Figure 1. Spectral changes in the reaction of 1^{2-} (1.3×10^{-4} mol dm^{-3}) with O_2 (1.3×10^{-2} mol dm^{-3}) in MeCN at 298 K (600 s interval). Inset: Plot of k_{obs} vs $[O_2]$.

The accompanied formation of $O_2^{\bullet-}$ in the electron-transfer oxidation of **1**$^{2-}$ by O_2 was confirmed by a low-temperature ESR method. The characteristic ESR signal having $g_{//}$ value of 2.175 due to $O_2^{\bullet-}$ was observed for an O_2-saturated MeCN solution of **1** and two equivalents of MeO$^-$ at 113 K as shown in Figure 2.[10]

Scheme 1.



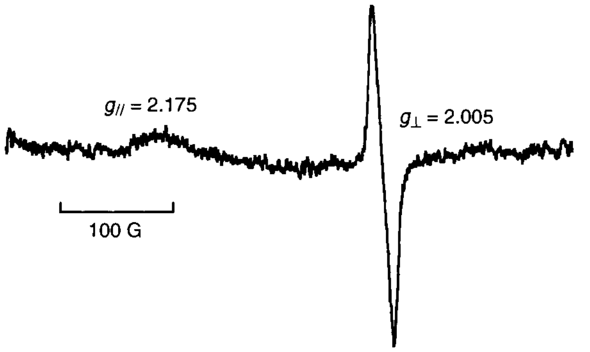


Figure 2. ESR spectrum of $O_2^{\bullet-}$ generated in the reaction of $\mathbf{1}^{2-}$ (2.0×10^{-2} mol dm^{-3}) with O_2 (1.3×10^{-2} mol dm^{-3}) in MeCN at 298 K and measured at 113 K.

The increase of the absorbance at 430 nm due to $\mathbf{1b}^{\bullet-}$ obeyed pseudo-first-order kinetics under conditions where the O_2 concentration was maintained at more than a 10-fold excess of the $\mathbf{1}^{2-}$ concentration. The pseudo-first-order rate constant (k_{obs}) increases linearly with an increase in the O_2 concentration as shown in the inset of Figure 1. From the slope of the linear plot of k_{obs} vs $[O_2]$ was obtained the second-order rate constant of electron transfer (k_{et}) from $\mathbf{1}^{2-}$ to O_2 as 5.8×10^{-2} mol^{-1} dm^{3} s^{-1}. The relatively small k_{et} value indicates that electron transfer from $\mathbf{1}^{2-}$ to O_2 is endergonic to produce $\mathbf{1a}^{\bullet-}$, followed by a rapid intramolecular proton transfer to yield $\mathbf{1b}^{\bullet-}$ (Scheme 1).[11] It was confirmed that $\mathbf{1b}^{\bullet-}$ was also formed in the reaction of $\mathbf{1}^{2-}$ with tetramethyl-*p*-benzoquinone which has a similar one-electron reduction potential (E^0_{red} vs SCE = –0.84 V)[12] to O_2 (E^0_{red} vs SCE = –0.87 V).[13]

On the other hand, **1** acts as an efficient radical scavenger, since hydrogen transfer from **1** (1.0×10^{-3} mol dm^{-3}) to galvinoxyl radical (G•; 1.0×10^{-3} mol dm^{-3}) occurs to give $\mathbf{1}^{\bullet}$ which undergoes disproportionation to yield **1** and $\mathbf{1}_{ox}$.[14] The same product ($\mathbf{1}_{ox}$) was obtained via the protonation of $\mathbf{1b}^{\bullet-}$ with trifluoroacetic acid (CF_3COOH) as well as via the one-electron oxidation of $\mathbf{1}^{-}$ with *p*-chloranil (Cl_4Q) as shown in Scheme 1.

In conclusion, the present study has demonstrated that $O_2^{\bullet-}$ is generated via the electron transfer-oxidation of catechin dianion by O_2 in MeCN. The dual roles of catechin acting as both an antioxidant and an ROS generator are ascribed to the radical scavenging ability of the neutral form and the ability to reduce oxygen in the dianion form.

References and Notes

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5 Synthesis of **2** will be reported elsewhere.

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9 The generated $\mathbf{1b}^{\bullet-}$ may be more stable than $\mathbf{1a}^{\bullet-}$, since the heat of formation value for $\mathbf{1b}^{\bullet-}$ calculated by the PM3 method is 24.7 kJ mol^{-1} smaller than that for $\mathbf{1a}^{\bullet-}$. For the PM3 method, see: J. J. P. Stewart, *J. Comput. Chem.*, **209**, 221 (1989).

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11 This indicates that the oxidation potential of $\mathbf{1}^{2-}$ may be more positive than the reduction potential (E^0_{red} vs SCE) of O_2 (–0.87 V in MeCN) (ref 13). However, the strong adsorption of electrolyzed products of $\mathbf{1}^{2-}$ on the electrode has precluded the direct determination of the oxidation potential of $\mathbf{1}^{2-}$ by the cyclic voltammetry.

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14 NMR data for $\mathbf{1}_{ox}$: ^{1}H NMR (400 MHz, CD_3CN) δ 2.50 (dd, *J* = 8.4 and 16.0 Hz, H-4), 2.88 (dd, *J* = 5.4 and 16.0 Hz, H-4), 3.47 (bs, 3-OH), 4.03 (m, H-3), 4.57 (d, *J* = 7.2 Hz, H-2), 5.93 (d, *J* = 2.2 Hz, H-6), 5.99 (d, *J* = 2.2 Hz, H-8), 6.37 (m, H-2'), 6.40 (d, *J* = 10.2 Hz, H-5'), 7.23 (dd, *J* = 2.4 and 10.2 Hz, H-6').