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Kinetic study of the electron-transfer oxidation of the phenolate anion of a vitamin E model by molecular oxygen generating superoxide anion in an aprotic medium †

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Electron-transfer reduction of molecular oxygen (O_2) by the phenolate anion (**1**$^-$) of a vitamin E model, 2,2,5,7,8-pentamethylchroman-6-ol (**1H**), occurred to produce superoxide anion, which could be directly detected by a low-temperature EPR measurement. The rate of electron transfer from **1**$^-$ to O_2 was relatively slow, since this process is energetically unfavourable. The one-electron oxidation potential of **1**$^-$ determined by cyclic voltammetric measurements is sufficiently negative to reduce 2,2-bis(4-*tert*-octylphenyl)-1-picrylhydrazyl radical (DOPPH$^\bullet$) to the corresponding one-electron reduced anion, DOPPH$^-$, suggesting that **1**$^-$ can also act as an efficient radical scavenger.

Introduction

Vitamin E (α-tocopherol) is one of the most important biological phenolic antioxidants that can act as an efficient hydrogen-atom donor to active oxygen radicals, such as hydroxyl radical ($^\bullet$OH) and lipid peroxyl radical (LOO$^\bullet$), showing efficient antioxidative activities against oxidative stress in biological systems.[1] In addition to its protective radical-scavenging action, α-tocopherol is known to promote low-density lipoprotein (LDL) oxidation,[2] which is an important event in the development of atherosclerosis.[3] The oxidation of LDL may be catalyzed by metal ions in advanced atherosclerotic lesions[4–6] and α-tocopherol can act as a pro-oxidant *via* reduction of Cu(II) to Cu(I).[7] α-Tocopherol has also been reported to produce superoxide anion ($O_2^{\bullet-}$) in apoptosis-induced cells.[8] The apoptosis has been regarded as responsible for the cell toxicity of α-tocopherol.[9–11] Natural-occurring flavonoids such as (+)-catechin and quercetin are also known to scavenge active oxygen radicals efficiently.[12] However, there is also considerable evidence for the generation of reactive oxygen species (ROS) by such antioxidants under specific reaction conditions, such as in the presence of a base or metal ions.[13] We have recently reported that the dianion species of (+)-catechin and its derivative are generated under strongly basic conditions and act as a strong electron donor, which can reduce molecular oxygen (O_2) to $O_2^{\bullet-}$.[14,15] Yamashita *et al*. have reported that quercetin induces oxidative DNA damage and forms 8-oxo-dG by reacting with Cu(II).[16] With regard to the antioxidant and pro-oxidant properties of α-tocopherol, there have been a number of reports demonstrating the radical-scavenging ability.[17–21] However, whether generation of $O_2^{\bullet-}$ results from the reaction between α-tocopherol itself and O_2 or not has yet to be clarified.

The present work has been performed to clarify this point by determining the rate for the generation of $O_2^{\bullet-}$ in the reaction between the anion species of an α-tocopherol model, 2,2,5,7,8-pentamethylchroman-6-ol (**1H**), and O_2 under basic conditions in an aprotic medium. The radical scavenging ability of the α-tocopherol model anion *via* electron transfer reactions is also reported. Detailed spectroscopic and kinetic analyses provide valuable mechanistic insight into the $O_2^{\bullet-}$ formation by α-tocopherol as well as the radical scavenging ability.

Experimental

Materials

2,2,5,7,8-Pentamethylchroman-6-ol (**1H**) was purchased from Wako Pure Chemical Ind. Ltd., Japan. Tetra-*n*-butylammonium hydroxide (1.0 M in methanol) was obtained commercially from Aldrich and used as received. Tetra-*n*-butylammonium perchlorate (Bu_4NClO_4) used as a supporting electrolyte for the electrochemical measurements was purchased from Tokyo Chemical Industry Co., Ltd., Japan, recrystallized from ethanol, and dried under vacuum at 313 K. 2,2-Bis(4-*tert*-octylphenyl)-1-picrylhydrazyl radical (DOPPH$^\bullet$) was obtained commercially from Aldrich. Acetonitrile (MeCN; spectral grade) was purchased from Nacalai Tesque, Inc., Japan and used as received.

† Electronic supplementary information (ESI) available: the cyclic voltammogram of **1**$^-$ and the experimental EPR spectrum of **1**$^\bullet$ with the computer simulation spectrum. See http://www.rsc.org/suppdata/ob/b3/b306758k/

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Spectral and kinetic measurements

Since the phenolate anion of **1H** ($\mathbf{1}^-$) is readily oxidized by molecular oxygen (O_2), reactions were carried out under strictly deaerated conditions for generation of $\mathbf{1}^-$. A continuous flow of Ar gas was bubbled through a MeCN solution containing **1H** (2.0×10^{-4} M) in a square quartz cuvette (10 mm i.d.) with a glass tube neck for 10 min. The neck of the cuvette was sealed to ensure that air would not leak into the cuvette by using a rubber septum. A microsyringe was used to inject Bu_4NOMe ($0–4.0 \times 10^{-4}$ M), which was also deaerated, into the cuvette to produce $\mathbf{1}^-$. UV–vis spectral changes associated with this reaction were monitored using an Agilent 8453 photodiode array spectrophotometer. The reaction of $\mathbf{1}^-$ with O_2 was carried out by adding a stock solution of $\mathbf{1}^-$ to an MeCN solution of O_2 in the cuvette. The concentrations of O_2 in the solution were adjusted by purging with Ar, air, or O_2 for 10 min prior to the measurements ($[O_2] = 0$, 2.7×10^{-3}, or 1.3×10^{-2} M), respectively. The oxygen concentration was determined by the photooxidation of 10-methyl-9,10-dihydroacridine with oxygen in the presence of $HClO_4$ in MeCN as reported previously.[22] The rates of electron transfer from $\mathbf{1}^-$ to O_2 were determined by monitoring the absorbance change at 325 nm due to $\mathbf{1}^-$. Pseudo-first-order rate constants (k_{obs}) were determined by least-squares curve fitting using a personal computer. The first-order plots of $\ln(A_\infty - A)$ *vs.* time (where A_∞ and A denote the final absorbance and the absorbance at a given reaction time, respectively) were linear for three or more half-lives, with a correlation coefficient of $\rho > 0.999$.

Cyclic voltammetry

The cyclic voltammetry measurements were performed on an ALS-630A electrochemical analyzer in deaerated MeCN containing 0.10 M Bu_4NClO_4 as a supporting electrolyte. The Pt working electrode (BAS) was polished with BAS polishing alumina suspension and rinsed with acetone before use. The counter electrode was a platinum wire. The measured potentials were recorded with respect to an $Ag/AgNO_3$ (0.01 M) reference electrode. The $E_{1/2}$ values (*vs.* $Ag/AgNO_3$) were converted to those *vs.* SCE by adding 0.29 V.[23] All electrochemical measurements were carried out at 298 K under an atmospheric pressure of Ar.

EPR measurements

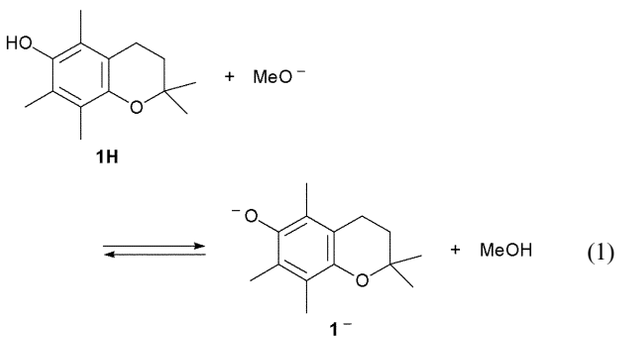
To an oxygen-saturated MeCN solution was added the stock MeCN solution of $\mathbf{1}^-$ (8.3×10^{-4} M) in a quartz EPR tube (4.5 mm i.d.) and the solution was immediately frozen by liquid nitrogen. The EPR spectrum of $O_2^{\bullet-}$ was taken in a frozen MeCN solution at 77 K using a JEOL X-band spectrometer (JES-FA100) under nonsaturating microwave power conditions. The magnitude of modulation was chosen to optimize the resolution and the signal-to-noise (S/N) ratio of the observed spectra. The g values were calibrated precisely with a Mn^{2+} marker which was used as a reference.

The EPR spectrum of $\mathbf{1}^\bullet$ produced in the reaction between $\mathbf{1}^-$ (5.0×10^{-4} M) and DOPPH$^\bullet$ (5.0×10^{-5} M) in deaerated MeCN was measured using a LABOTEC LLC-04B EPR sample tube at 298 K. Computer simulation of the EPR spectra was carried out using Calleo ESR Version 1.2 program (Calleo Scientific Publisher) on a personal computer.

Results and discussion

Generation of the phenolate anion of a vitamin E model

When a vitamin E model **1H** (2.0×10^{-4} M) was treated with methoxide anion (MeO^-) ($0–4.0 \times 10^{-4}$ M) produced in the reaction between tetra-*n*-butylammoniumu hydroxide (Bu_4NOH) and methanol in deaerated acetonitrile (MeCN), the absorption band at 294 nm due to **1H** decreased, accompanied by an increase in the absorption band at 325 nm with clear isosbestic points at 271 and 302 nm as shown in Fig. 1. Such a red-shift of the absorption band is indicative of formation of the phenolate anion.[24] The spectral change is completed by addition of one equivalent of MeO^- as shown in the inset of Fig. 1. This indicates that **1H** reacts with MeO^- to produce the phenolate anion $\mathbf{1}^-$ [eqn (1)]. The resulting $\mathbf{1}^-$ is stable under anaerobic conditions.



(1)

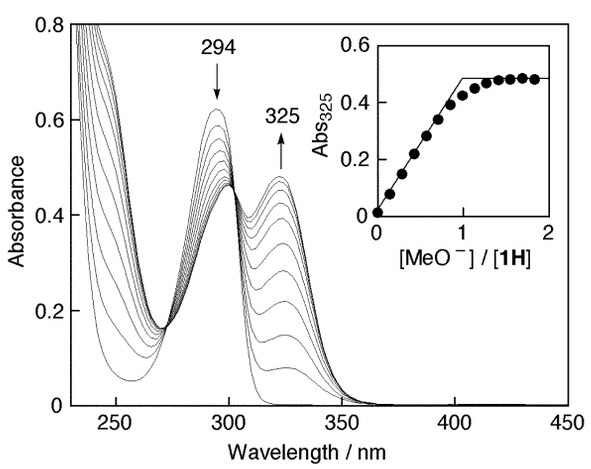
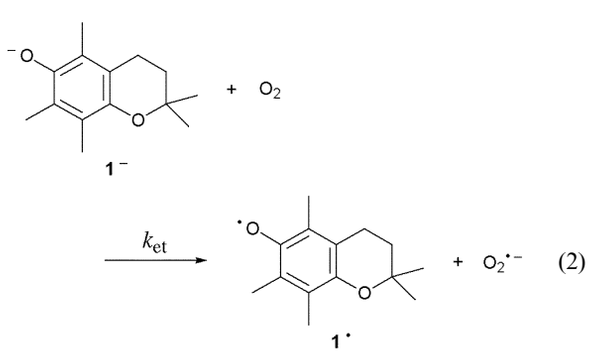


Fig. 1 Spectral change observed upon addition of MeO^- ($0–2.0 \times 10^{-4}$ M) to a deaerated MeCN solution of **1H** (2.0×10^{-4} M) at 298 K. Inset: plot of the absorbance at 325 nm (Abs_{325}) *vs.* $[MeO^-]/[\mathbf{1H}]$.

Electron-transfer oxidation of the phenolate anion of a vitamin E model by O_2

Introduction of molecular oxygen (O_2) to the MeCN solution of $\mathbf{1}^-$ resulted in a decrease in the absorption band at 325 nm due to $\mathbf{1}^-$ as shown in Fig. 2. This spectral change suggests that $\mathbf{1}^-$ is oxidized by O_2 to produce phenoxyl radical $\mathbf{1}^\bullet$ and superoxide anion ($O_2^{\bullet-}$) [eqn. (2)]. The formation of $O_2^{\bullet-}$ was confirmed by a low-temperature EPR. A characteristic EPR signal of $O_2^{\bullet-}$ having a $g_{||}$ value of 2.087 was observed for an O_2-saturated MeCN solution of **1H** and one equivalent of MeO^- at 77 K as shown in Fig. 3.



(2)

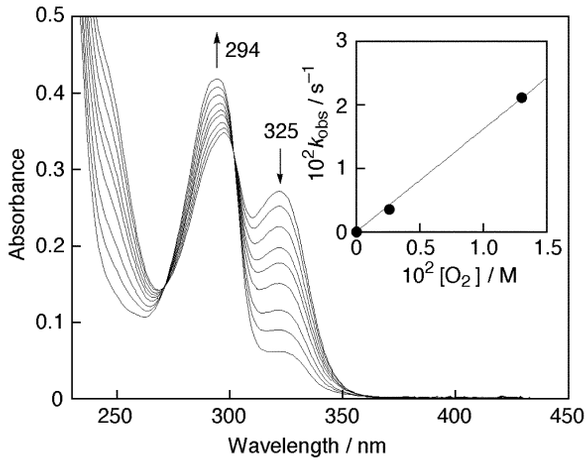


Fig. 2 Spectral change observed in the reaction of $\mathbf{1}^-$ (1.0×10^{-4} M) with O_2 (1.3×10^{-2} M) in MeCN at 298 K (30 s intervals). Inset: plot of k_{obs} *vs.* [O_2].

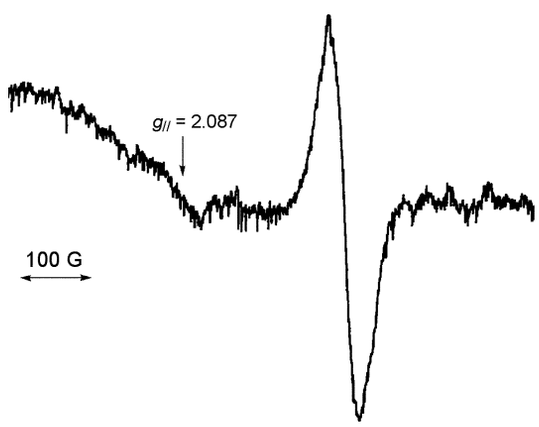


Fig. 3 EPR spectrum of $O_2^{\bullet -}$ generated in the reaction of $\mathbf{1}^-$ (8.3×10^{-4} M) with O_2 (1.3×10^{-2} M) in MeCN at 298 K and measured at 77 K.

On the other hand, the EPR signal of $\mathbf{1}^\bullet$ could not be detected in the reaction between $\mathbf{1}^-$ and O_2 at 298 K because of the instability of $\mathbf{1}^\bullet$ (*vide infra*). It is known that the phenoxyl radical species derived from α-tocopherol and its derivatives decompose *via* a bimolecular disproportionation reaction to produce the parent tocopherols and the corresponding two-electron oxidized products in the absence of O_2.[21,25] In the presence of O_2, radical coupling reactions of phenoxyl radical species of α-tocopherol and its derivatives with O_2 are known to occur rapidly to produce a wide variety of oxidized products,[26] which have yet to be identified.

The decrease in the absorbance at 325 nm due to $\mathbf{1}^-$ obeyed pseudo-first-order kinetics under conditions where the O_2 concentration (1.3×10^{-2} M)[22] was maintained at more than a 10-fold excess relative to the $\mathbf{1}^-$ concentration. The pseudo-first-order rate constant (k_{obs}) increases proportionally with increasing O_2 concentration, as shown in the inset of Fig. 2. The slope of the linear plot of k_{obs} *vs.* [O_2] gave the second-order rate constant of the electron transfer (k_{et}) from $\mathbf{1}^-$ to O_2 as 6.7×10^{-1} M^{-1} s^{-1}. The relatively small k_{et} value suggests that the electron transfer from $\mathbf{1}^-$ to O_2 is endergonic, where the one-electron oxidation potential of $\mathbf{1}^-$ (E^0_{ox}) is more positive than the one-electron reduction potential of O_2 (E^0_{red} *vs.* SCE = −0.87 V)[27] (*vide infra*). The rate constant of the disproportionation reaction of $\mathbf{1}^\bullet$ has been determined as 2.7×10^3 M^{-1} s^{-1} in deaerated MeCN at 298 K.[21] This value is much larger than the k_{et} value. In such a case, the electron transfer from $\mathbf{1}^-$ to O_2 becomes the rate-determining step, followed by the rapid disproportionation reaction of $\mathbf{1}^\bullet$. Such a follow-up reaction makes it possible to produce $O_2^{\bullet -}$ *via* the endergonic electron transfer. However, the detection of $\mathbf{1}^\bullet$ becomes extremely difficult.

Oxidation potentials of a vitamin E model and its phenolate anion

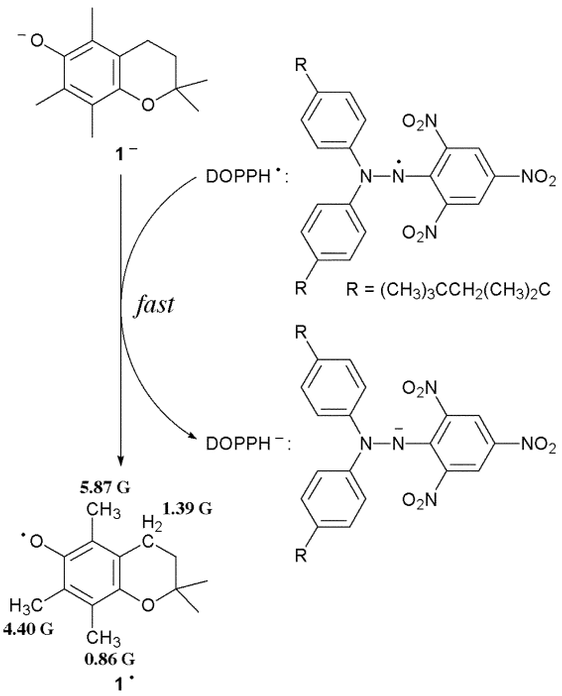
As mentioned above, the one-electron oxidation potential of $\mathbf{1}^-$ (E^0_{ox}) may be more positive than the one-electron reduction potential of O_2 (E^0_{red} *vs.* SCE = −0.87 V), leading to the electron transfer from $\mathbf{1}^-$ to O_2 an endergonic process [$\Delta G^0_{et} = e(E^0_{ox} - E^0_{red}) > 0$, where ΔG^0_{et} is the free energy change of electron transfer and e is the elementary charge]. Thus, the cyclic voltammetry was performed to determine the one-electron oxidation potential of $\mathbf{1}^-$ in deaerated MeCN containing 0.1 M Bu_4NClO_4 as a supporting electrolyte. At a low scan rate (0.10 V s^{-1}), an oxidation peak current of $\mathbf{1}^-$ was observed with the smaller reduction peak current in a deaerated MeCN solution containing **1H** (2.0×10^{-3} M), MeO^- (2.0×10^{-3} M), and Bu_4NClO_4 (0.1 M). The smaller reduction peak current of $\mathbf{1}^\bullet$ generated by the electrochemical oxidation as compared to the oxidation peak current of $\mathbf{1}^-$ is ascribed to the instability of $\mathbf{1}^\bullet$, which decomposes *via* the bimolecular disproportionation reaction (*vide supra*). At a fast scan rate (2.0 V s^{-1}), the CV becomes reversible (Fig. S1 †). From the midpoint of the redox peaks of the CV of $\mathbf{1}^-$ was determined the oxidation potential of $\mathbf{1}^-$ (E^0_{ox} *vs.* SCE) as −0.50 V, which is significantly more positive than the E^0_{red} value of O_2 (−0.87 V) as expected above. This result is consistent with the relatively small k_{et} value. Since the one-electron oxidation of **1H** is known to occur at E^0_{ox} *vs.* SCE = 0.77 V, the deprotonation of the phenolic OH group in **1H** to form $\mathbf{1}^-$ resulted in the very largely negative shift (1.27 V) of the oxidation potential. Thus, the electron transfer reduction of O_2 becomes possible by the deprotonation of **1H**. A negative shift of the oxidation potential by deprotonation was also observed between (+)-catechin and its anion species. The oxidation potential of (+)-catechin is E^0_{ox} *vs.* SCE = 1.18 V,[28] while that of the anion is located at 0.12 V.[29] In this case, however, no $O_2^{\bullet -}$ is produced by the reaction of the catechin anion with O_2.[30]

Efficient radical-scavenging reaction by the phenolate anon of the vitamin E model

When 2,2-bis(4-*tert*-octylphenyl)-1-picrylhydrazyl radical (DOPPH$^\bullet$) is used as an oxidant for $\mathbf{1}^-$ instead of O_2, an electron transfer from $\mathbf{1}^-$ to DOPPH$^\bullet$ occurred very rapidly in deaerated MeCN at 298 K to produce $\mathbf{1}^\bullet$ and $DOPPH^-$ (Scheme 1). This is consistent with the largely negative free energy change of electron transfer from $\mathbf{1}^-$ (E^0_{ox} *vs.* SCE = −0.47 V) to DOPPH$^\bullet$ (E^0_{red} *vs.* SCE = 0.18 V). Thus, electron transfer from $\mathbf{1}^-$ to DOPPH$^\bullet$ is much faster than the bimolecular disproportionation of $\mathbf{1}^\bullet$ and too rapid to be monitored using a stopped-flow technique. In such a case, it becomes possible to detect the resulting $\mathbf{1}^\bullet$ by EPR. The characteristic EPR spectrum having a g value of 2.0047 was observed in the reaction of $\mathbf{1}^-$ with DOPPH$^\bullet$ (Fig. S2a †) and well reproduced by the computer simulation (Fig. S2b †) using the hyperfine coupling (hfc) values shown in Scheme 1.

Since the one-electron reduction potentials of a variety of peroxyl radicals[31–35] are known to be more positive than the E^0_{red} value of DOPPH$^\bullet$, peroxyl radicals may also be scavenged *via* electron transfer from $\mathbf{1}^-$ to peroxyl radicals.

In conclusion, the electron-transfer reduction of O_2 by the vitamin E model **1H** under basic conditions takes place to produce superoxide anion ($O_2^{\bullet -}$) in MeCN, where the phenolate anion of **1H** ($\mathbf{1}^-$) acts as an actual electron donor to O_2. The one-electron oxidation potential of $\mathbf{1}^-$ is sufficiently negative to reduce DOPPH$^\bullet$ to $DOPPH^-$. This suggests that the phenolate anion of α-tocopherol can also act as an efficient radical scavenger *via* the electron transfer reactions.



Scheme 1 Reductive DOPPH•-scavenging reaction *via* electron-transfer from $\mathbf{1}^-$ to DOPPH• and the hfc values of the resulting $\mathbf{1}^{\bullet}$.

Acknowledgements

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