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Effects of Metal Ions Distinguishing between One-Step Hydrogen- and Electron-Transfer Mechanisms for the Radical-Scavenging Reaction of (+)-Catechin

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A kinetic study of a hydrogen-transfer reaction from (+)-catechin (**1**) to galvinoxyl radical ($G^\bullet$) has been performed using UV−vis spectroscopy in the presence of $Mg(ClO_4)_2$ in deaerated acetonitrile (MeCN). The rate constants of hydrogen transfer from **1** to $G^\bullet$ determined from the decay of the absorbance at 428 nm due to $G^\bullet$ increase significantly with an increase in the concentration of Mg^{2+}. The kinetics of hydrogen transfer from **1** to cumylperoxyl radical has also been examined in propionitrile (EtCN) at low temperature with use of ESR. The decay rate of cumylperoxyl radical in the presence of **1** was also accelerated by the presence of scandium triflate [$Sc(OTf)_3$ ($OTf = OSO_2CF_3$)]. These results indicate that the hydrogen-transfer reaction of (+)-catechin proceeds via electron transfer from **1** to oxyl radicals followed by proton transfer rather than via a one-step hydrogen atom transfer. The coordination of metal ions to the one-electron reduced anions may stabilize the product, resulting in the acceleration of electron transfer.

Introduction

Catechins contained in green tea are a class of bioflavonoids that show significant antioxidative activity.[1−9] It has been suggested that catechins trap radical species by hydrogen atom transfer from its phenolic OH group on the B ring.[10,11] However, little is known about the elementary steps of hydrogen-transfer reactions from catechins to radical species. There are two possibilities in the mechanism of hydrogen-transfer reactions from phenolic anitioxidants to radical species, i.e., a one-step hydrogen atom transfer or electron transfer followed by proton transfer.[12] Kusu et al. have recently reported the quantitative relationship between the antioxidative activity of catechins and their oxidation potentials determined by flow-through column electrolysis.[13] The galloylated catechins having more negative oxidation potentials than those of nongalloylated catechins show more efficient antioxidative activity on NADPH-dependent microsomal lipid peroxidation than the nongalloylated catechins.[13] On the other hand, based on the gas-phase bond dissociation enthalpy and ionization potential calculated by density functional method, Wright et al. have concluded that in most hydrogen-transfer reactions of phenolic antioxidants, hydrogen-atom transfer will be dominant rather than electron transfer.[12] Thus, it is still not clear whether the hydrogen-transfer reaction of catechins proceeds via a one-step hydrogen atom transfer or electron transfer followed by proton transfer. It has previously been demonstrated that the effect of Mg^{2+} on the hydrogen-transfer rates from NADH (dihydronicotinamide adenine dinucleotide) analogues to aminoxyl or nitrogen radicals provides a reliable criterion for distinguishing between the one-step hydrogen atom transfer and the electron-transfer mechanisms.[14]

We report herein the effect of metal ions, such as Mg^{2+} and Sc^{3+}, on the rates of hydrogen transfer from (+)-catechin (**1**) to oxyl radical species, such as galvinoxyl and cumylperoxyl radicals. Cumylperoxyl radical, which is much less reactive than alkoxyl radicals, is known to follow the same pattern of relative reactivity with a variety of substrates.[15−17] The detailed kinetic studies provide valuable mechanistic insight into the mechanism of the antioxidative reactions of **1**: whether the reaction between **1** and oxyl radical species proceeds via one-step hydrogen atom transfer or via electron transfer.

Experimental Section

Materials. (+)-Catechin (**1**) was purchased from Sigma. Galvinoxyl radical ($G^\bullet$) was obtained commercially from Aldrich. $Mg(ClO_4)_2$ and acetonitrile (MeCN; spectral grade) were purchased from Nacalai Tesque, Inc., Japan, and used as received. Di-*tert*-butyl peroxide was obtained from Nacalai Tesque Co., Ltd., and purified by chromatography through alumina which removes traces of the hydroperoxide. Cumene was purchased from Wako Pure Chemical Ind. Ltd., Japan. Scandium trifluoromethanesulfonate, $Sc(OTf)_3$ ($OTf = OSO_2CF_3$, 99%) was obtained from Pacific Metals Co., Ltd. (Taiheiyo Kinzoku). Propionitrile (EtCN) used as solvent was purified and dried by the standard procedure.[18]

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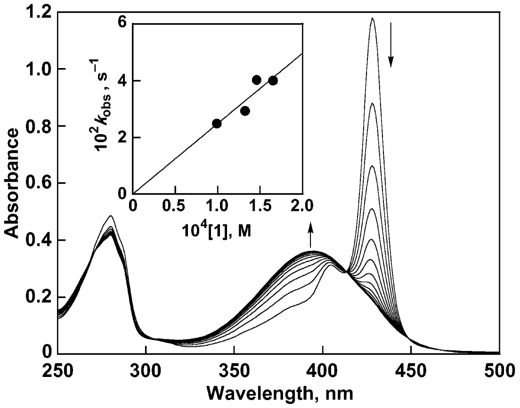


Figure 1. Spectral changes observed upon addition of **1** (1.5×10^{-4} M) to a deaerated MeCN solution of $G^\bullet$ (8.4×10^{-6} M) at 298 K (Interval: 10 s). Inset: plot of the pseudo-first-order rate constant (k_{obs}) vs the concentration of **1**.

Spectral and Kinetic Measurements. Typically, an aliquot of (+)-catechin (**1**; 2.0×10^{-2} M) in deaerated MeCN was added to a quartz cuvette (10 mm i.d.) which contained galvinoxyl radical ($G^\bullet$; 8.4×10^{-6} M) and $Mg(ClO_4)_2$ (0.1 M) in deaerated MeCN (3.0 mL). This led to a hydrogen-transfer reaction from **1** to $G^\bullet$. UV−vis spectral changes associated with this reaction were monitored using an Agilent 8453 photodiode array spectrophotometer. The rates of hydrogen transfer were determined by monitoring the absorbance change at 428 nm due to $G^\bullet$ ($\epsilon = 1.43 \times 10^5$ M^{-1} cm^{-1}). Pseudo-first-order rate constants were determined by a least-squares curve fit using an Apple Macintosh personal computer. The first-order plots of $\ln(A - A_\infty)$ vs time (A and A_∞ are denoted to the absorbance at the reaction time and the final absorbance, respectively) were linear until three or more half-lives with the correlation coefficient $\rho > 0.999$.

Kinetic measurements for the hydrogen-transfer reactions between **1** and cumylperoxyl radical were performed on a JEOL X-band spectrometer (JES-ME-LX) at 203 K. Typically, photoirradiation of an oxygen-saturated propionitrile (EtCN) solution containing di-*tert*-butyl peroxide (1.0 M) and cumene (1.0 M) with a 1000 W mercury lamp resulted in formation of cumylperoxyl radical ($g = 2.0156$), which could be detected at low temperatures. The g values were calibrated by using an Mn^{2+} marker. Upon cutting off the light, the decay of the ESR intensity was recorded with time. The decay rate was accelerated by the presence of **1** (1.0×10^{-4} M). Rates of hydrogen transfer from **1** to $PhCMe_2OO^\bullet$ were monitored by measuring the decay of the ESR signal of $PhCMe_2OO^\bullet$ in the presence of various concentrations of **1** in EtCN at 203 K. Pseudo-first-order rate constants were determined by a least-squares curve fit using a microcomputer. The first-order plots of $\ln(I - I_\infty)$ vs time (I and I_∞ are the ESR intensity at time t and the final intensity, respectively) were linear for three or more half-lives with the correlation coefficient, $\rho > 0.99$. In each case, it was confirmed that the rate constants derived from at least five independent measurements agreed within an experimental error of ±5%.

Results and Discussion

Effect of Mg^{2+} on Hydrogen Transfer from (+)-Catechin to Galvinoxyl Radical. Upon addition of (+)-catechin (**1**) to a deaerated acetonitrile (MeCN) solution of galvinoxyl radical ($G^\bullet$), the absorption band at 428 nm due to $G^\bullet$ disappeared immediately as shown in Figure 1. This indicates that hydrogen transfer from one of the OH groups on the B ring of **1** to $G^\bullet$ takes place to give catechin radical ($1^\bullet$) and hydrogenated $G^\bullet$ (GH) (eq 1).[19]

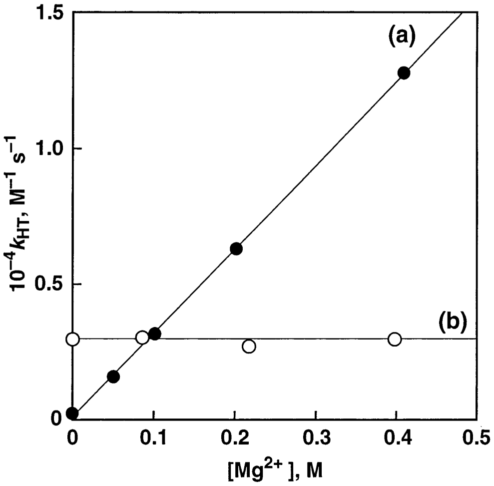
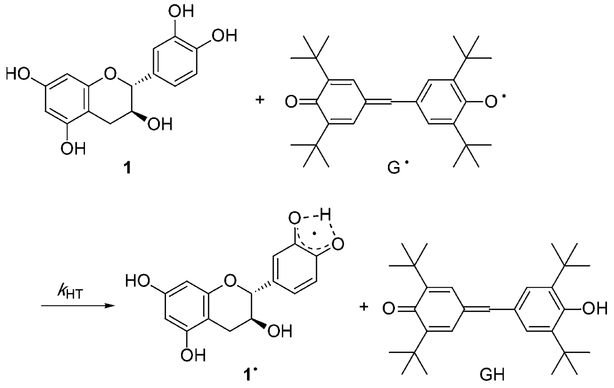


Figure 2. Plot of k_{HT} vs [Mg^{2+}] in the hydrogen transfer from (a) **1** to $G^\bullet$ and (b) **2** to $G^\bullet$ in the presence of $Mg(ClO_4)_2$ in deaerated MeCN at 298 K.



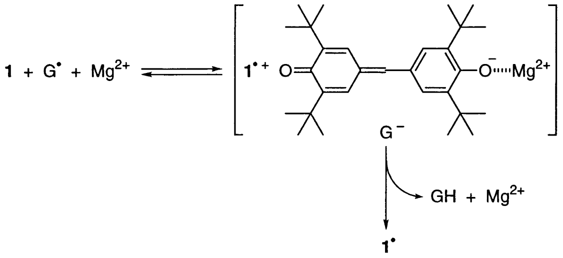
(1)

The decay of the absorbance at 428 nm due to $G^\bullet$ obeyed pseudo-first-order kinetics when the concentration of **1** was maintained at more than 10-fold excess of the $G^\bullet$ concentration. The observed pseudo-order-rate constant (k_{obs}) increases linearly with an increase in the concentration of **1** (inset of Figure 1). From the slope of the linear plot of k_{obs} vs the concentration of **1** is determined the second-order rate constant (k_{HT}) for the hydrogen transfer from **1** to $G^\bullet$ at 298 K as 2.3×10^2 M^{-1} s^{-1}. The $1^\bullet$ thus formed is known to be unstable and undergo disproportionation to produce the parent **1** and an *o*-quinone-type oxidized product of **1**.[20]

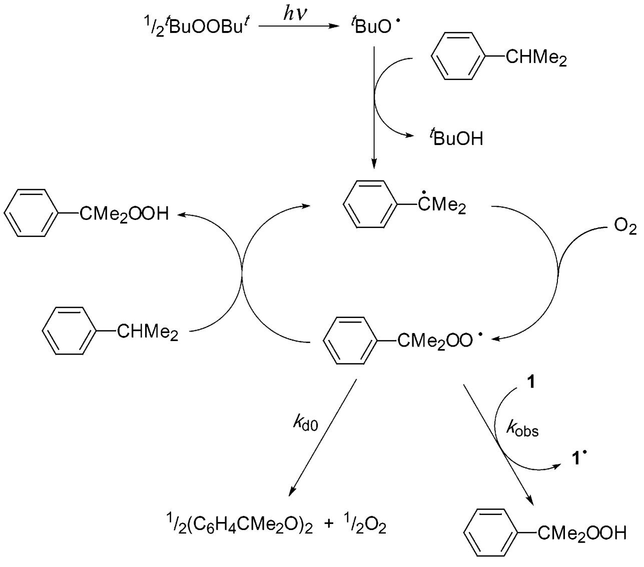
If the hydrogen transfer from **1** to $G^\bullet$ involves an electron-transfer process as the rate-determining step, the rate of hydrogen transfer would be accelerated by the presence of magnesium ion.[14] This is checked by examining an effect of Mg^{2+} on the hydrogen transfer rate from **1** to $G^\bullet$. When $Mg(ClO_4)_2$ is added to the **1**−$G^\bullet$ system, the rate of hydrogen transfer from **1** to $G^\bullet$ was significantly accelerated. The k_{HT} value increases linearly with increasing Mg^{2+} ion concentration as shown in Figure 2a (black circles). Thus, the hydrogen transfer may proceed via electron transfer from **1** to $G^\bullet$, which is accelerated by the presence of Mg^{2+}, followed by proton transfer from $1^{\bullet+}$ to G^- to yield $1^\bullet$ and GH as shown in Scheme 1. In such a case, the coordination of Mg^{2+} to the one-electron reduced species of $G^\bullet$ (G^-) may stabilize the product, resulting in the acceleration of electron transfer.

Hydrogen Transfer from (+)-Catechin to Cumylperoxyl Radical. Direct measurements of the rates of hydrogen transfer

SCHEME 1



SCHEME 2



from (+)-catechin (**1**) to cumylperoxyl radical were performed in propionitrile (EtCN) at 203 K by means of ESR. The photoirradiation of an oxygen saturated EtCN solution containing di-*tert*-butylperoxide and cumene with a 1000 W mercury lamp results in formation of cumylperoxyl radical, which was readily detected by ESR. The cumylperoxyl radical is formed via a radical chain process shown in Scheme 2.[21−25] The photoirradiation of Bu^tOOBu^t results in the homolytic cleavage of the O−O bond to produce $Bu^tO^\bullet$,[26,27] which abstracts a hydrogen from cumene to give cumyl radical, followed by the facile addition of oxygen to cumyl radical. The cumylperoxyl radical can also abstract a hydrogen atom from cumene in the propagation step to yield cumene hydroperoxide, accompanied by regeneration of cumyl radical (Scheme 2).[28,29] In the termination step, cumylperoxyl radicals decay by a bimolecular reaction to yield the corresponding peroxide and oxygen (Scheme 2).[28,29] When the light is cut off, the ESR signal intensity decays, obeying second-order kinetics due to the bimolecular reaction in Scheme 2.

In the presence of **1**, the decay rate of cumylperoxyl radical after cutting off the light becomes much faster than that in the absence of **1**. The decay rate in the presence of **1** (1.0×10^{-4} M) obeys pseudo-first-order kinetics. This decay process is ascribed to the hydrogen transfer from **1** to cumylperoxyl radical (Scheme 2). The pseudo-first-order rate constants increase with increasing **1** concentration to exhibit first-order dependence on [**1**]. From the slope of the linear plot of k_{obs} vs the concentration of **1** is determined the second-order rate constant (k_{HT}) for the hydrogen transfer from **1** to cumylperoxyl radical as 6.0×10^2 M^{-1} s^{-1} in EtCN at 203 K.

Mg^{2+} shows little acceleration effect on the rate of hydrogen transfer from **1** to cumylperoxyl radical as shown in Figure 3.

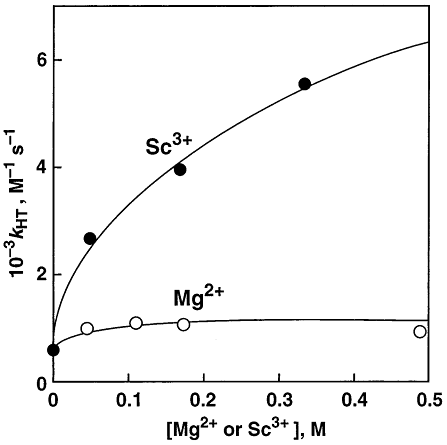
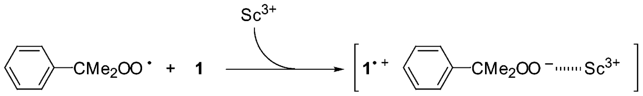


Figure 3. Plot of k_{HT} vs [Mg^{2+}] (white circles) and [Sc^{3+}] (black circles) in the hydrogen transfer from **1** to cumylperoxyl radical in the presence of $Mg(ClO_4)_2$ or $Sc(OTf)_3$ in EtCN at 203 K.

SCHEME 3



In contrast to the case of Mg^{2+}, the rate of hydrogen transfer from **1** to cumylperoxyl radical is enhanced significantly by the presence of $Sc(OTf)_3$ (OTf = triflate). Scandium ion has recently been reported to be the most effective promoter among various metal ions in metal ion-promoted electron-transfer reactions.[30] The k_{HT} values increase with increasing the scandium ion concentration, as shown in Figure 3. The acceleration effect of Sc^{3+} can be ascribed to the strong binding of Sc^{3+} to $PhCMe_2OO^-$, which results in a decrease in the free energy of the electron transfer as shown in Scheme 3. Thus, the electron transfer from **1** to cumylperoxyl radical becomes energetically feasible in the presence of Sc^{3+}. It should be noted that there is no interaction between cumylperoxyl radical and Sc^{3+}, since the *g* value of cumylperoxyl radical in the presence of Sc^{3+} is the same as the value in its absence.

Direct Hydrogen Atom Transfer vs Electron Transfer. Judging from the one-electron oxidation potential of **1** ($E°_{ox}$ vs SCE = 0.12 V),[13] which is higher than the one-electron reduction potential of $G^\bullet$ ($E°_{red}$ vs SCE = 0.05 V), the free energy change of electron transfer from **1** to $G^\bullet$ is positive [$\Delta G°_{et}$ (in eV) $= e(E°_{ox} - E°_{red}) > 0$, where *e* is the elementary charge], and thereby the electron-transfer step is endergonic. In such a case, the overall rate of hydrogen transfer (k_{HT}), which consists of electron-transfer and proton-transfer steps, would be slower than the initial electron-transfer rate (k_{et}). The maximum k_{et} value is evaluated from the $\Delta G°_{et}$ value by eq 2, where it is assumed that the activation free energy ($\Delta G^{\ddagger}_{et}$) is equal to $\Delta G°_{et}$ (no additional barrier is involved), *Z* is the frequency factor taken as 1×10^{11} M^{-1} s^{-1}, and k_B is the Boltzmann constant.[31]

$$k_{et} = Z \exp(-\Delta G°_{et}/k_B T) \qquad (2)$$

Figure 4 shows a plot of log k_{HT} versus $\Delta G°_{et}$, calculated by eq 2 (denoted by ET line). The k_{HT} value of the **1**−$G^\bullet$ system (black circle) is much smaller than the corresponding maximum k_{et} value. This indicates that the hydrogen transfer proceeds via electron transfer followed by proton transfer as shown in Scheme 1 rather than a direct one-step hydrogen atom transfer. The hydrogen transfer from the vitamin E model, 2,2,5,7,8-pentamethyl-6-chromanol (**2**), to $G^\bullet$ (eq 3) is known to proceed via a

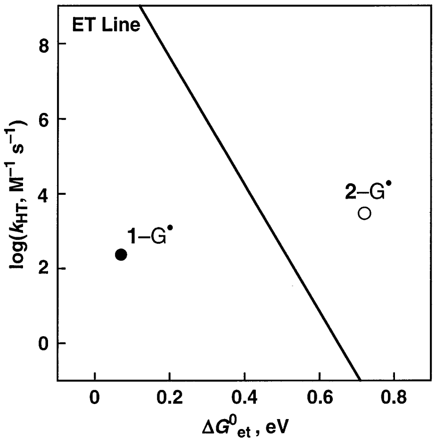
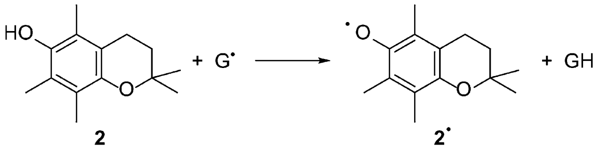


Figure 4. Plot of the rate constant of hydrogen transfer from **1** or **2** to G• (logk_{HT}) vs the free energy of electron transfer from **1** or **2** to G• ($\Delta G°_{et}$). The solid line shows the dependence of the calculated rate constant of electron transfer (k_{et}) on $\Delta G°_{et}$ based on eq 2, see text.

direct one-step hydrogen atom transfer.[32] In such a case, no effect of Mg^{2+} on the k_{HT} values is observed as shown in Figure 2b (white circles), demonstrating sharp contrast with the case of the electron-transfer reaction from **1** to G•. The k_{HT} value of



(3)

the **2**−G• system is much larger than the estimated k_{et} value as shown in Figure 4 (white circle). This confirms that the hydrogen transfer proceeds via a direct one-step hydrogen atom transfer rather than via electron transfer.

The $E°_{red}$ value of cumylperoxyl radical determined directly by the cyclic voltammetry is located at 0.65 V vs SCE. Thus, the electron transfer from **1** ($E°_{ox}$ vs SCE = 0.12 V) to cumylperoxyl radical is energetically feasible ($\Delta G°_{et} < 0$). In such a case, the acceleration of the rate of electron transfer from **1** to G• was observed by the presence of metal ions.

In conclusion, the hydrogen transfer from (+)-catechin to galvinoxyl and cumylperoxyl radicals proceeds via electron transfer followed by proton transfer. These results suggest that a hydrogen-transfer reaction from catechins to hydroxyl radical, which is the most powerful reactive oxygen species having a very high reduction potential, proceeds via electron transfer followed by proton transfer.

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