Importance of Proton-Coupled Electron Transfer from Natural Phenolic Compounds in Superoxide Scavenging

Tatsushi Nakayama and Bunji Uno*

Gifu Pharmaceutical University; Daigaku-nishi, Gifu 501–1196, Japan.
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The superoxide $O_2^-$ scavenging reaction of (+)-catechin (Cat), quercetin (Que), rutin, and $\alpha$-tocopherol ($\alpha$-TOH) as natural phenolic compounds is investigated on the basis of electrochemical and ESR spectral measurements with the aid of density functional theory (DFT) calculations. Reversibility of the $O_2^–/O_2^-$ redox couple is significantly affected by the presence of the phenolic compounds. The catechol moiety of Cat, Que, and rutin plays an essential role in concerted proton-coupled electron transfer (PCET) to HO$_2^-$ derived from $O_2^-$. On the other hand, the presence of $\alpha$-TOH causes sequential electron and proton transfers to HO$_2^-$ to give H$_2$O$_2$ and the corresponding $\alpha$-benzoquinone radical anions. These electron transfers in the presence of the phenolic compounds are inferred from the ESR spectral measurements. The DFT calculation results suggest that the $O_2^-$ scavenging reaction of the natural phenolic compounds proceeds efficiently with the one-step concerted PCET or sequential PCET mechanism.

Key words  proton-coupled electron transfer; superoxide; natural phenolic compound; cyclic voltammetry; ESR spectrum

For many natural phenolic compounds, such as polyphenols directly provided by human diet, their beneficial properties have been primarily related to antioxidant activities, which are associated with their redox behavior. Therefore, free radical scavenging by natural polyphenols has been widely studied experimentally and theoretically from thermodynamic perspectives, such as redox potentials and energy calculations. Currently, phenolic antioxidants are well known to play a protective role by interrupting chain reactions, such as lipid peroxidation, ascribed to H-atom transfer (HAT) involving concerted proton-coupled electron transfer (PCET), sequential PCET, and sequential proton loss electron transfer (SPLET) to intermediate radicals. In a solvent that supports dissociation of phenols the SPLET mechanism is considered to be significant compared with the HAT mechanism. On the other hand, concerted PCET distinguished from pure HAT as it involves concerted proton and electron transfer in one kinetic step using several molecular orbitals. Furthermore, it is well known that PCET mechanisms play an essential role in a broad range of energy conversion processes in chemistry and biology. However, the theoretical aspects of the free radical scavenging mechanism of phenolic antioxidants remains an important subject for the accurate prediction of free radical scavenging activity in physiological environments.

As a free radical, $O_2^-$ is an important biological intermediate in living cells and is overproduced in tissues subjected to chronic infection and inflammation. $O_2^-$ causes oxidative DNA damage in living tissues under inflammatory conditions. To data, the reaction of $O_2^-$ with acidic substrates such as phenols has been considered to involve an initial proton transfer from the substrate to $O_2^-$ followed by rapid dismutation to give H$_2$O$_2$ and O$_2$. The substrate anion is oxidized by the O$_2$ from the dismutation process. This mechanism is referred to as the base-catalyzed autoxidation process. Neither the electron transfer between phenolic compounds and O$_2^-$ nor the nature of such reactions has been investigated because, unlike general peroxides, the oxidation power of O$_2^-$ is not extremely large. Recently, it has been demonstrated that quinone–hydroquinone $\pi$-conjugated redox systems characterized by PCET in hydroquinone and catechol moieties play an important role in electron transfer to $O_2^-$. This result inspired our research into the direct $O_2^-$ scavenging mechanism based upon the PCET reaction of natural phenolic antioxidants.

In this study, the electron transfer reaction from flavonoids and $\alpha$-tocopherol (\(\alpha\)-TOH) as a representative natural antioxidant phenolic compound (Fig. 1) to $O_2^-$ has been investigated electrochemically. We show that the $O_2^-$ scavenging reaction involves PCET.

**Experimental**

**Chemicals** Natural products used in this study were (+)-catechin (Cat), quercetin (Que), rutin, and $\alpha$-TOH, which

![Fig. 1. Chemical Structures of (+)-Catechin (Cat) (a), Quercetin (Que) (b), Rutin (c), and $\alpha$-Tocopherol ($\alpha$-TOH) (d)](image-url)
were commercially available from Nacalai Tesque Inc. and used as received without further purification. The solvent for electrochemical and ESR spectral measurements was spectrograde purity N,N-dimethylformamide (DMF) available from Nacalai Tesque, Inc. and used as received. Tetrapropylammonium perchlorate (TPAP) was prepared as described previously39) and used as a supporting electrolyte for DMF. Ferrocene (Fc), used as a potential reference compound, was commercially available from Nacalai Tesque Inc. (Osaka, Japan) and purified by repeated sublimation under reduced pressure immediately prior to use.

**Electrochemical and Spectroelectrochemical Measurements** Cyclic voltammetry was performed with a three-electrode system comprising a glassy carbon (GC) working electrode, a coiled platinum counter electrode, and an Ag/AgNO₃ reference electrode (containing CH₃CN solution of 0.1 mol dm⁻³ tetrabutylammonium perchlorate and 0.01 mol dm⁻³ AgNO₃; BAS RE-5) at 25°C using a BAS 100B electrochemical workstation, coupled to a Dell Optiplex760 PC using BAS electrochemical software to record and analyze

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**Fig. 2.** Cyclic Voltammograms of O₂/O₂⁻ in the Absence and Presence of Cat (a), Que (b), and Rutin (c) in DMF Containing 0.1 m TPAP on a Glassy Carbon (GC) Electrode at a Scan Rate of 100 mV s⁻¹. The values described in this figure denote the concentrations of the polyphenols. The ESR spectra in this figure are obtained by the controlled-potential electrolysis at an applied potential of −1.3 V vs. Fc⁺/Fc.

**Fig. 3.** Cyclic Voltammograms of O₂/O₂⁻ in the Absence and Presence of p-Dimethoxybenzene (a), 4-Methoxyphenol (b), and α-TOH (c) in DMF Containing 0.1 dm⁻³ TPAP on a GC Electrode at a Scan Rate of 100 mV s⁻¹. The values described in this figure denote the concentrations of the additives. In (a) all the voltammograms are completely overlapped.
the data.

In situ electrochemical ESR spectra were observed using a JEOL JES-FA200 X-band spectrometer. The controlled potential electrolysis was performed at room temperature in an electrochemical ESR cell using a 0.5-mm-diameter straight Pt wire sealed in a glass capillary as a working electrode.

Samples were prepared in a glove box completely filled with N₂ gas to prevent contamination by moisture. The DMF solution containing 0.1 mol dm⁻³ TPAP as a supporting electrolyte was saturated with O₂ by bubbling the gas for ca. 2–3 min and the gas was passed over the solutions during the electrochemical and ESR measurements to maintain the concentration of O₂ at a constant level. The equilibrium concentration of O₂ was calculated as 4.8×10⁻³ mol dm⁻³.

Calculation All solution-phase calculations were performed using Gaussian 09 Program package. Geometry of each compound and radical or ionic structure was optimized using density functional theory (DFT) method with B3LYP functional. Calculations were performed by employing the standard split-valence triple-ζ 6-311+G(d) basis sets augmented by the polarization d and diffusion orbitals 6-311+G(d). Solvent contribution of DMF to the standard Gibbs formation energies was computed employing the integral equation formalism polarized continuum model (IEF-PCM) method that is widely employed in the description of the thermodynamic characteristics of solvation. The PCM calculations were performed using default settings of Gaussian 09 Program package. The zero-point energies and thermal correction, together with the entropies, were used to convert the internal energies to standard Gibbs energy at 298.15 K.

Results and Discussion

Cyclic Voltammetry of O₂ in the Presence of Flavonoids

The elimination reaction of O₂⁻ with flavonoids is discussed on the basis of electrochemical measurements. Figure 2 shows the voltammetric behaviors of the O₂⁻/O₂⁺ redox couple with flavonoid concentrations. In reduction of O₂⁻ in an aprotic solvent such as DMF the cathodic peak corresponding to the generation of O₂⁻ is followed in the subsequent anodic scan by one good defined anodic peak which is associated with regenerating the starting materials (O₂), showing the reversible redox wave (solid line, Fig. 2). The presence of anodic peak suggests that O₂⁻ is moderately stable, at least on the cyclic voltammetric time-scale. On the other hand, loss of reversibility in the cyclic voltammograms of O₂ was observed without change in a monocyclic process in the presence of all flavonoids investigated here. The results shown in Fig. 2 are the same as those obtained previously for catechol (OQH₂⁻) because the flavonoid compounds used here include an OQH₂ moiety. This indicates that the OQH₂⁻ moiety in the flavonoids is involved in the elimination of O₂⁻. The new cathodic peak appeared in more positive potentials than the O₂/O₂⁺ redox. This was ascribed to the reduction of the o-benzoquinone radical anion (OQ⁻) to OQ⁻⁻ because the OQ⁻⁻/OQ⁻ redox potential is more positive than the O₂/O₂⁺ potential. These voltammetric behaviors have been well documented our previous work. The OQ⁻⁻ generation ascribed to the proton and electron transfer reaction from OQH⁻ to HO₂ was further confirmed by ESR spectra, as shown in Fig. 2. The ESR spectra of OQ⁻ were obtained by controlled-potential electrolysis of the O₂ solution containing the polyphenols at an applied potential of −1.3 V corresponding to the O₂ reduction.

The role of partial structures of flavonoids in their antioxidant capacity has been investigated experimentally and theoretically over the past years. It is well recognized that the OH group at C-3 position on the C ring and the OQH₂⁻ moiety (B-ring) play an important role in the free radical scavenging activities of Que derivatives (flavonols). It has been reported that reaction of Que with O₂⁻ is triggered by proton transfer from Que to yield dismutated products of O₂⁻ and deprotonated Que at the OH group at C-3 position on the C ring, which allows quercetinase-like dioxygenation to give the corresponding depside in high yield through an SFO reaction.

Cyclic Voltammetry of O₂ in the Presence of α-TOH

Figure 3 shows typical cyclic voltammograms of O₂ in the presence of acidic and nonacidic additives. The reversible wave of the O₂/O₂⁻ redox couple is not affected by the pres-
ence of nonacidic compounds such as \( p \)-dimethoxybenzene (Fig. 3a). However, the presence of acidic \( p \)-methoxyphenol induces an apparent effect on the \( O_2/O_2^- \) electrochemistry (Fig. 3b). The redox behaviors in the presence of acidic phenolic compounds have been well documented.\(^{34-36,42}\) According to the results obtained in the literature, an increase and a decrease in the cathodic and anodic currents, respectively, and a positive shift of the redox potential (Fig. 3b) can be related to the irreversible two-electron reduction of \( O_2 \). A fast protonation from the phenol to produce electrically neutral \( HO_2^- \) precedes the second electron-transfer reaction, causing the potential inversion corresponding to the case when the addition of the second electron is thermodynamically more facile than that of the first one. Consequently, \( O_2^- \) formation after the primary electrode process associated with proton transfer from the phenol leads to the irreversible overall reduction of \( O_2 \) to \( H_2O_2 \), driven by the exergonic reduction of the resulting \( HO_2^- \). This mechanism is supported by the fact that addition of the phenol to a DMF solution containing equimolar sodium methylate does not affect the electrochemistry of \( O_2 \). On the other hand, the presence of \( \alpha \)-TOH is only associated with an apparent decrease in the reversibility of the \( O_2/ O_2^- \) redox couple. A monoelectronic process with the disappearance of the reoxidation wave (Fig. 3c) indicates that the

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**Fig. 5.** Four Diabetic Electronic States and the Standard Gibbs Energy Changes (\( \Delta G^\circ \)) for the PCET Reaction between \( HO_2^- \) and Cat (a), and between \( HO_2^- \) and Que (b)

PT and ET denote proton and electron transfers, respectively. The values for PT and ET reactions and concerted PCET denote the \( \Delta G^\circ \) values in kJ mol\(^{-1}\) calculated by the B3LYP/PCM/6-311+G(d) method. The values marked with an asterisk were calculated using the Gibbs energies of \( Cat^+ \) and \( Que^+ \) for Figs. (a) and (b), respectively.
HO_2· produced in the electrochemical process in the presence of acidic α-TOH is consumed immediately in the reaction with the α-TO· or α-TOH, without being reduced on the electrode.

The electron transfer from α-TO· or α-TOH to HO_2· has been inferred from ESR spectral measurements. Figure 4a shows the ESR spectrum of the O_2 solution containing α-TOH obtained by the controlled-potential electrolysis at an applied potential of −1.3 V corresponding to the O_2 reduction. The ESR spectrum simulated for the structure of α-TO· (Fig. 4c) well reproduces the experimental ESR spectrum, as shown in Figs. 4a, b. Therefore, it is clear that the reaction product of O_2·− with α-TOH is α-TO·. As shown in Fig. 4c, the UB3LYP/6-311+G(d) optimized structure of α-TO· is similar to that of the p-benzoquinone radical anion rather than those of the general phenoxy radicals. The α-TOH redox is expected to act as the quinone–hydroquinone π-conjugated redox system characterized by PCET. These experimental and theoretical results indicate that the hydroxyl group in α-TOH play an important role in the electron-transfer reaction with HO_2·.

**Mechanisms for the O_2·− Scavenging Reaction by Natural Phenolic Compounds**

It is considered that PCET reaction after initial proton transfer from phenolic compounds to O_2·− is important in efficient O_2·− scavenging. The PCET reaction involving the transfer of one electron and one proton from phenolate anion to HO_2· can be described in terms of the four diabatic electronic states depicted in Fig. 5. The standard Gibbs energy changes (ΔG°) calculated with the DFT method for the PCET process in the O_2·− scavenging reaction by Cat and Que are also given in Fig. 5. The important factors in the determination of the stepwise processes shown in this scheme are the acid–base interaction and the redox potentials of the components. Since the deprotonation of Cat is well known to firstly occur at the OH group at C-3 position on the B ring and secondly occur at C-7 position on the A ring, the structures of Cat− and Cat_2− are described as shown in Fig. 5a. It is also accepted that oxidation of Cat_− to produce Cat_2− is
followed by an intramolecular proton transfer to give Cat$_2$$^\cdot$.

However, the proton transfer and electron transfer from Cat$^-$ to HO$_2^-$ are totally inefficient due to the high instability of Cat$_2^\cdot$ (H$_2$O$_2^-$) and Cat$^-$ (HO$_2^-$), respectively, as shown in Fig. 5a. The high energy of Cat$_2^\cdot$ well explains the fact that O$_2^-$ are generated via an electron transfer oxidation of Cat$^-$ by molecular oxygen.$^{34,45}$ Therefore, the proton and electron are transferred in one kinetic step corresponding to following the diagonal of the rectangle in Fig. 4a, labeled concerted PCET, avoiding generation of the high-energy intermediates.$^{46}$ On the other hand, the deprotonation of Que firstly occurs at the OH group at C-4$^\cdot$ position on the B ring and secondly occur at C-3 position on the C ring, as shown in Fig. 5b.$^{47}$ The proton transfer and electron transfer between Que$^-$ and HO$_2^-$ are both uphill reactions, as shown in Fig. 5b. Therefore, the concerted PCET reaction occurs primarily by the proton transfer from OOH$_2^-$ in Que to O$_2^-$ and proceeds efficiently to yield Que$_2$$^\cdot$ and HO$_2^-$, avoiding high-energy intermediates. The concerted PCET proceeds efficiently even in the case of weak acid–base interaction and uphill redox potentials.

The detailed PCET mechanism of the OOH$_2^-$ moiety in the flavonoids is as follows. Concerted PCET occurs in the hydrogen-bonded complex between OOH$^-$ and HO$_2^-$, in which the proton transfer occurs along the hydrogen bond to a lone pair $\alpha$-type orbital on the oxygen atom of OOH$^-$ that is nominally in the plane of the molecular framework. This transfer is coupled to the electron transfer that occurs from the $\pi$-type lone-pair of the oxygen atom of OOH$^-$ to the singly occupied molecular orbital of HO$_2^-$.

The scavenging reaction of O$_2^-$ with $\alpha$-TOH occurs primarily by the proton transfer from acidic $\alpha$-TOH to basic O$_2^-$, followed by the PCET reaction of HO$_2^-$ to give H$_2$O$_2$. Four diabatic electronic states used in the PCET theory for the O$_2^-$ scavenging reaction by $\alpha$-TOH are depicted in Fig. 6. Since anodic oxidation of $\alpha$-TO$^\cdot$ produced in a basic solution of aprotic media is well known to yield $\alpha$-TOH at much more negative potential ($E^{\alpha\to\alpha}$ vs. Fe/Fe$^\cdot$) than that of $\alpha$-TOH ($E^{\alpha\to\alpha}$ vs. Fe/Fe$^\cdot$)$^{48,49}$ a direct electron transfer from $\alpha$-TO$^\cdot$ to HO$_2^-$, labeled ET on an upper side of the rectangle in Fig. 6, is thermodynamically preferred. Therefore, the electron transfer from $\alpha$-TO$^\cdot$ to HO$_2^-$ occurs, followed by the proton transfer from another $\alpha$-TOH to the HO$_2^-$. The PCET reaction occurs in the hydrogen-bonded complex composed of HO$_2^-$, $\alpha$-TOH, and $\alpha$-TO$^\cdot$, corresponding to moving along the edges of the rectangle, as shown in Fig. 6.

Conclusion

It has been demonstrated that the catechol moiety in the polyphenols and the hydroxyl group in $\alpha$-TOH are essential to scavenge O$_2^-$ via PCET. The antioxidant action of flavonoids is suggested to relate to a planar preference of the ensuing radicals that allows extended electronic delocalization between adjacent rings.$^{13}$ In this respect, natural polyphenolic antioxidants characterized by a catechol moiety may exhibit strong activity in scavenging O$_2^-$ in association with stabilization of O$_2^-$ by adjacent rings. On the other hand, $\alpha$-TOH shows the strong effect of the O$_2^-$ scavenging despite the single hydroxyl group. The redox property of $\alpha$-TOH is considered as the quinone–hydroquinone electron transfer system involving PCET. We note that all the experiments and results in this work were designed and conducted under aprotic conditions. However, the results and findings shown here appear as promising to analyze the O$_2^-$ scavenging reaction by natural phenolic compounds in lipid bilayer membranes and neutral plasma.$^{23}$ Several original and review articles on the chemical, biological, theoretical, and electrochemical aspects of PCET have been published over the past decade.$^{17–30}$ PCET mechanisms are well known to play an essential role in a broad range of energy conversion processes in chemistry and biology.$^{20–23}$

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Conflict of Interest The authors declare no conflict of interest.

References and Notes

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