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Quinone–Hydroquinone π-Conjugated Redox Reaction Involving Proton-coupled Electron Transfer Plays an Important Role in Scavenging Superoxide by Polyphenolic Antioxidants

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The proton-coupled electron transfer (PCET) from *p*-, *o*-, and *m*-dihydroxybenzenes (PQH_2, OQH_2, and MQH_2, respectively) to the hydroperoxy radical ($HO_2^{\bullet}$) derived from superoxide ($O_2^{\bullet-}$) is investigated. It is demonstrated that quinone–hydroquinone π-conjugated redox systems characterized by electron transfer in PQH_2 and OQH_2 moieties play an important role in scavenging $O_2^{\bullet-}$ by polyphenolic antioxidants.

Special attention has been directed to polyphenolic antioxidants such as flavonoids[1] that can prevent biomolecules from undergoing oxidative damage through free-radical-mediated reactions.[2] It is well recognized that phenolic antioxidants play a protective role by interrupting chain reactions, such as lipid peroxidation, ascribed to H-atom-transfer and single-electron-transfer reactions to intermediate radicals.[3,4] On the hand, $O_2^{\bullet-}$ is an important biological intermediate that is formed in living cells[5] and in particular is overproduced in tissues subjected to chronic infection and inflammation.[6] Hence, $O_2^{\bullet-}$ is known to cause oxidative DNA damage in living tissues under inflammatory conditions.[7] However, the $O_2^{\bullet-}$ scavenging mechanism of phenolic antioxidants remains unclear. So far, it is considered that the reaction of $O_2^{\bullet-}$ with acidic substrates such as phenols involves an initial proton transfer from the substrate to $O_2^{\bullet-}$ to give $HO_2^{\bullet}$, followed by rapid dismutation to give H_2O_2 and O_2. The substrate anion is oxidized by the O_2 from the dismutation process.[8] This mechanism is referred to as the superoxide-facilitated oxidation (SFO) reaction.[9] Neither the electron transfer between phenolic compounds and $O_2^{\bullet-}$ (or $HO_2^{\bullet}$) nor the nature of such reactions has been investigated. Recently, it has been demonstrated that nitrosodisulfonate radical as a model of $HO_2^{\bullet}$ readily oxidizes quercetin to a radical anion.[10] This result inspires research into the electron-transfer mechanism between $HO_2^{\bullet}$ and phenolic antioxidants against the SFO mechanism. This study proposes the PCET mechanism from phenolate ions to $HO_2^{\bullet}$ as an $O_2^{\bullet-}$ scavenging reaction and shows the structural properties and π-conjugation effects of positional isomers making up polyphenolic antioxidants on the PCET scavenging reaction of $O_2^{\bullet-}$.

The presence of MQH_2 induces an apparent effect upon the $O_2/O_2^{\bullet-}$ electrochemistry,[11] as shown in Figure 1a. The change from a monoelectronic to a bielectronic O_2 reduction process in the presence of phenol has been well documented.[8,12] In light of these results, it is rationalized that $O_2^{\bullet-}$ formation after the primary electron-transfer step associated with proton transfer from MQH_2 leads to the irreversible overall reduction of O_2 to H_2O_2, driven by the exergonic reduction of the resulting $HO_2^{\bullet}$. This is supported by the fact that addition of MQH_2 to a DMF solution containing equimolar sodium methylate does not affect the electrochemistry of O_2. In contrast, the presence of PQH_2 is not only associated with an apparent decrease in the reversibility of the $O_2/O_2^{\bullet-}$ redox couple but is also associated with the progressive growth of a new cathodic peak and two anodic peaks (A, B, and C in Figure 1b). Remarkably, the O_2 reduction remains a monoelectronic process despite full loss of the $O_2/O_2^{\bullet-}$ reversibility. This indicates that the $HO_2^{\bullet}$ produced in the electrochemical process is consumed immediately in the reaction with PQH^-, without being reduced on the electrode. The electron transfer from PQH^- to $HO_2^{\bullet}$ has been inferred from ESR and optically transparent thin-layer electrode (OTTLE) spectroelectrochemical measurements, which show the generation of $PQ^{\bullet-}$ despite the applied potentials corresponding to O_2 reduction (Figures 2a and 2b).[13] In addition, the new redox wave (peak A/B in Figure 1b) is assigned to reversible $PQ^{\bullet-}/PQ^{2-}$ electron transfers as supported by the fact that the OTTLE spectral change corresponds to the PQ^{2-} generation (Figure 2c), and that peak C in Figure 1b corresponds to oxidation of $PQ^{\bullet-}$ to PQ. The spectra and the redox potentials observed here were in complete agreement with those obtained from sequential reduction of PQ.[14] In the case of OQH_2, loss of $O_2/O_2^{\bullet-}$

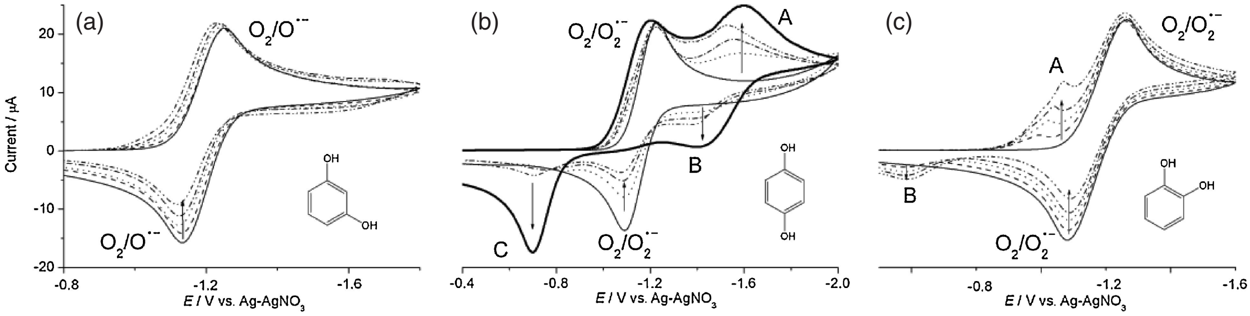

Figure 1. Cyclic voltammograms of 4.8×10^{-3} mol dm^{-3} O_2 in the absence and in the presence of MQH_2 (a), PQH_2 (b), and OQH_2 (c), observed at a scan rate of 0.1 V s^{-1} in DMF containing 0.1 mol dm^{-3} TPAP. Concentrations of QH_2 are 0, 1, 2, 3, and 5×10^{-3} mol dm^{-3}. A typical voltammogram in the presence of PQH_2 (15×10^{-3} mol dm^{-3}) was also illustrated by a bold solid line in Fig. b.

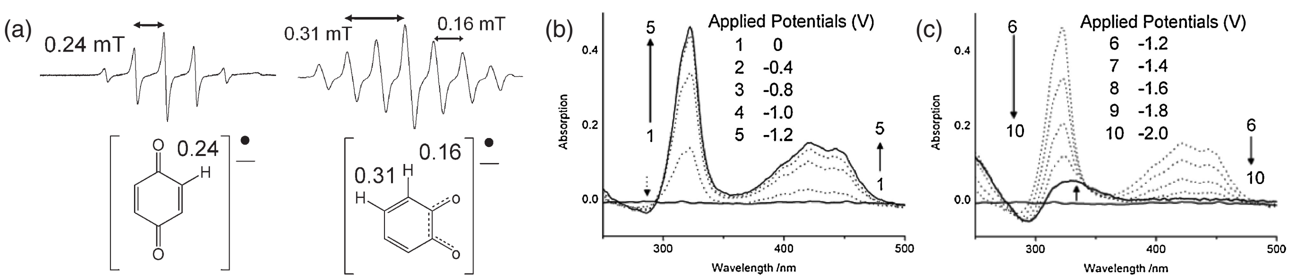

Figure 2. ESR spectra of the O_2 solution containing PQH_2 (left) and OQH_2 (right) obtained by the controlled-potential electrolysis at an applied potential of -1.2 V (a), and spectral change of the O_2 solution containing PQH_2 with the OTTL electrolyses over applied potentials corresponding to the $O_2^{\bullet -}$ generation (b) and over applied potentials corresponding to peak A in Figure 1b (c), observed in DMF containing 0.1 mol dm^{-3} TPAP. Concentrations of PQH_2 and OQH_2 are 1.0×10^{-3} mol dm^{-3}. The OTTL spectra were observed as differential spectra from those of neutral PQH_2.

reversibility without change to a bielectronic process was observed in the cyclic voltammograms of O_2 (Figure 1c), and the $OQ^{\bullet -}$ generation ascribed to reaction of OQH^- with $HO_2^{\bullet}$ was confirmed by ESR spectra (Figure 2a), in analogy with the case of PQH_2. As the $OQ^{\bullet -}/OQ^{2-}$ redox potential is more positive than the $O_2/O_2^{\bullet -}$ potential, it is deduced that the new cathodic peak (peak A in Figure 1c) may be ascribed to the re-reduction of $OQ^{\bullet -}$ to OQ^{2-}. Thus, the new anodic peak (peak B in Figure 1c) may be due to electrochemical oxidation of OQ^{2-} to $OQ^{\bullet -}$.[15] These experimental results indicate that the hydroxy groups located in the *p*- and *o*-positions play an important role in the electron-transfer reaction with $HO_2^{\bullet}$.

By analogy with the SFO mechanism, $O_2^{\bullet -}$ formed after the initial electron uptake at the electrode protonates the PQH_2, to afford $HO_2^{\bullet}$ and PQH^-. $HO_2^{\bullet}$ rapidly undergoes reaction with PQH^- (eq 1) in preference to either heterogeneous reduction at the potential or a disproportionation reaction.

$$HO_2^{\bullet} + PQH^- \rightarrow H_2O_2 + PQ^{\bullet -} \quad (1)$$

It is rationalized that reaction (1) involves PCET rather than single-electron transfer, as suggested by recent theoretical investigations.[3,16,17] Within this framework, the occurrence of a fast ECC (electrochemical–chemical–chemical) sequence is attributed to the exergonic interaction between $HO_2^{\bullet}$ and PQH^- and the thermodynamic stability of the ensuing $PQ^{\bullet -}$. Prior to PCET from PQH^- to $HO_2^{\bullet}$, the couple will form a prereaction hydrogen-bonded complex involving OH of PQH^- and one of the O lone pairs on $HO_2^{\bullet}$. UB3LYP/6-31+G(d) calculation results indicate that the complex is nearly planar and is predicted to lie 131.0 kJ mol^{-1} lower in energy than the separated reactants. This situation is expected given that the PCET reaction involves three atomic centers and occurs through the migration of the proton of PQH^- across the hydrogen bond to an O lone pair on $HO_2^{\bullet}$, as is shown in Figure 3.[3,16] The reaction of OQH_2 with $O_2^{\bullet -}$ is explained by the same ECC mechanism. However, a crucial difference in the PCET reaction for MQH_2 seems to be the low stability of the resulting $MQ^{\bullet -}$. The UB3LYP/6-31+G(d) calculated H° value of $MQ^{\bullet -}$ is 57.6 and 27.6 kJ mol^{-1} higher than that of $PQ^{\bullet -}$ and $OQ^{\bullet -}$, indicating that π-conjugation stability in $MQ^{\bullet -}$ is less than that in either $PQ^{\bullet -}$ or $OQ^{\bullet -}$. The radical anions are an intermediate in the quinone–hydroquinone electron-transfer systems conjugated with proton transfers. This implies that the quinone–hydroquinone π-conjugated redox systems characterized by electron transfer in the PQH_2 and OQH_2 moieties play an important role in the scavenging of $O_2^{\bullet -}$ by polyphenolic antioxidants.

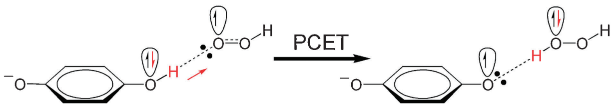

Figure 3. Schematic showing the plausible mechanism for PCET between PQH^- and $HO_2^{\bullet}$. Proton transfer occurs between O lone-pair σ-type orbitals that are nominally in the plane of the molecular framework. Electron transfer between O π-type orbitals that are orthogonal to the molecular framework accompanies the proton transfer.

In conclusion, it has been demonstrated that PQH_2 and OQH_2 moieties are essential to scavenge $O_2^{\bullet -}$ via PCET. It is suggested that the antioxidant action of flavonoids relates to a planar preference of the ensuing radicals that allows extended electronic delocalization between adjacent rings.[18] In this respect, natural polyphenolic antioxidants characterized by PQH_2 and OQH_2 moieties may have strong activity in scavenging $O_2^{\bullet -}$ in association with stabilization of $PQ^{\bullet -}$ and $OQ^{\bullet -}$ by adjacent rings.

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