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Electrochemical and Mechanistic Study of Superoxide Scavenging by Pyrogallol in N,N-Dimethylformamide through Proton-Coupled Electron Transfer

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Abstract: Scavenging of electrogenerated superoxide radical anion (O_2•−) by pyrogallol (PyH3) was investigated on the basis of cyclic voltammetry and in situ electrolytic electron spin resonance spectrum in N,N-dimethylformamide with the aid of density functional theory (DFT) calculations. Quasi-reversible dioxygen/O_2•− redox couple was modified by the presence of PyH3, suggesting that O_2•− was scavenged by PyH3 through proton-coupled electron transfer (PCET) involving two proton transfer and one electron transfer. DFT calculation suggested that the prereactive formation of a hydrogen-bond (HB) complex and the subsequent concerted-two-proton coupled electron transfer characterized by catechol moiety in PyH3 is plausible mechanism that embodies the superior kinetics of the O_2•− scavenging by PyH3 as shown in the electrochemical results. Furthermore, it was clarified that the three hydroxyl groups of PyH3 promote the formation of HB complex, in comparative analyses using related compounds, resulting in the promotion of the O_2•− scavenging.

Keywords: proton-coupled electron transfer; superoxide radical anion; antioxidants; cyclic voltammetry; electron spin resonance spectrum; pyrogallol

1. Introduction

Benzeno-1,2,3-triol (pyrogallol, PyH3) is one of the most sensitive organic compounds toward oxygen and causes autoxidation [1–6]. Its characteristic structure based on three hydroxyl (OH) groups substituted onto a benzene ring constitute some antioxidants, such as gallocatechin, epigallocatechin gallate, and prodelphinidin (the polymeric tannins composed of gallocatechin). It is well recognized that PyH3 is much more efficient antioxidants than benzeno-1,2-diol (catechol, CatH2). Therefore, numerous studies have investigated the antioxidant reaction mechanism with polyphenols containing PyH3 moiety experimentally and theoretically [1,5,7–10]. Three isometric benzenetriols (PyH3, benzene-1,2,4-triol, and benzene-1,3,5-triol) are differentiated by the position of OH groups. The larger number of OH groups on phenolic compounds is, the higher are the antioxidant properties, although these isomers show different reactivity depending on their positions of OH groups. Therefore, the antioxidant property of PyH3 is considered as due to electron donation derived from its π-conjugated quinoid structure, which directly reacts with any reactive oxygen species (ROS) such as superoxide radical anion (O_2•−); precursor of other ROS, and grand-state molecular oxygen (O_2) in the autoxidation. The antioxidant activity of PyH3 with its autoxidation involves complicated reaction mechanisms, though the mechanism between PyH3 and O_2 involves two-proton transfer (PT) and two-electron transfer (ET) forming pyrogallol-ortho-quinone (PyH) and hydroperoxide (H_2O_2).

Simultaneously, it is well recognized that the autoxidation is promoted in an alkali solution, showing that the primary deprotonation occurs forming corresponding anion (PyH2−) followed by the autoxidation reaction [2,3]. Furthermore, it is considered that
forming intermediate peroxiradical (ROS) such as hydroperoxiradical (HO$_2^\cdot$) followed by a free radical chain reaction is a main mechanism for the net autoxidation. O$_2$ is a moderately good electrophile that can accept electrons from PyH$_3$, rather than O$_2^\cdot$·. However, HO$_2^\cdot$ formed after protonation of O$_2^\cdot$· as a Brønsted base is a strong oxidant. Thus, the deprotonation and subsequent oxidation; i.e., PT and ET between PyH$_3$ and oxygen species involving O$_2$ and ROS, are closely related. Most of the reported papers on the antioxidant activity of PyH$_3$ are in aqueous solvents [2,3,6,11,12], and even under pH control, much proton is present. Among these papers, antioxidant reactions of PyH$_3$ involving the autoxidation and ROS scavenging were detected and measured in aqueous media, by increases in O$_2$ consumption or absorbance changes of the solution. Hence, the antioxidant reactions cannot be estimated separately, because several reactant species, i.e., PyH$_3$, PyH$_2^\cdot$, O$_2$, O$_2^\cdot$·, and HO$_2^\cdot$· coexist in the experimental solution. Thus, there are still some uncertain issues to be clarified regarding the antioxidant mechanism with the relationship between PT and ET, although, the plausible main mechanism for O$_2^\cdot$· scavenging by different reactants: PyH$_3$/PyH$_2^\cdot$, are simply denoted in Scheme 1, forming pyrogallol-ortho-quinone radical anion (PyH$^\cdot$)/radical dianion (Py$^{2\cdot}$) and H$_2$O$_2$.

\[ \text{Scheme 1.} \text{ Reaction schemes of O}_2^\cdot\text{· scavenging by (a) PyH}_3\text{ and (b) PyH}_2^\cdot, through PCET involving two PTs and one ET.} \]

Nasr et al. reported the electrochemical oxidation of PyH$_3$ in acidic aqueous solution [12]. In their pioneering work, the voltammetric results showed that PyH$_3$ oxidation occurs in the same potential region as that of phenol (PhOH). Conversely, the initial deprotonation of PyH$_3$ forming PyH$_2^\cdot$ increases electron density in the benzene ring and consequently increase its reactivity to electrophilic attack. Considering above, the deprotonation first occurs in an aqueous buffer media, then, the oxidation processes occur either directly on the electrode surface or can be mediated by O$_2$ (autoxidation) and other oxygen species electrogenerated at the anode surface, implying that it is the main pathway for the oxidation of PyH$_3$ by oxygen species in natural environments such as living body. Several reaction mechanisms for ROS scavenging by phenolic antioxidants such as PyH$_3$ are known, including the superoxide-facilitated oxidation (SFO) [13–15], hydrogen–atom transfer (HAT) involving proton-coupled electron transfer (PCET) [16–20], and sequential proton-loss electron transfer (SPLET) [21]. In the SFO mechanism, the initial proton transfer (PT) from the substrate to O$_2^\cdot$· to give HO$_2^\cdot$· is followed by rapid dismutation to give H$_2$O$_2$ and O$_2$. Then, the substrate anion is oxidized by the O$_2$ formed in the dismutation process [15]. Conversely, the other two mechanisms involve direct oxidation by O$_2^\cdot$·/HO$_2^\cdot$·.

Considering the relationship between structure of PyH$_3$ and the mechanism of O$_2^\cdot$·/HO$_2^\cdot$· scavenging, quinone–hydroquinone π-conjugation is inferred to play a role in
a PCET mechanism. In our previous studies, it has been reported that O$_2^\cdot$-/HO$_2^\cdot$ is scavenged by polyphenols [20], diphenols (hydroquinone [22] and CatH$_2$ [23]), and monophenols [24,25], through PCET mechanism. In these studies, a concerted two-proton-coupled electron transfer (2PCET) involving two PTs and one ET is a plausible reaction pathway for CatH$_2$ moiety based on the energetics and kinetics for successful O$_2^\cdot$- scavenging. Therefore, CatH$_2$ moiety comprised in PyH$_3$ is expected to play through the concerted 2PCET, although PyH$_3$ has three adjacent OH groups. It is presumed that the third OH group (3OH) gives a different chemical mechanism of PyH$_3$ from CatH$_2$, but its relationship with O$_2^\cdot$- scavenging through PCET is unclear.

In this study, we analyzed the reaction between PyH$_3$ and O$_2^\cdot$- electrogenerated from O$_2$ comparatively using some related compounds (Figure 1) in dehydrated N,N-dimethylformamide (DMF), focusing on the O$_2^\cdot$- scavenging in relation to the PCET with a deprotonation by electrochemistry and density functional theory (DFT) calculation.

![Figure 1. Structures of PyH$_3$ and the related compounds considered in this study. (a) PyH$_3$, (b) CatH$_2$, (c) PhOH, and (d) 3-methoxybenzene-1,2-diol (MoCatH$_2$).](image)

2. Materials and Methods

2.1. Chemicals

We obtained PyH$_3$ (98.0%), CatH$_2$ (99.0%), and MoCatH$_2$ (99.0%), from Sigma-Aldrich Inc (Tokyo, Japan), and purified by repeated sublimation under reduced pressure immediately before use. PhOH (99.5%) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), at the best available grade and was used as received. Dinitrogen (N$_2$) gas (99.0%) and dioxygen (O$_2$) gas (99.0%) were purchased from Medical Sakai Co., Ltd. (Gifu, Japan), and were used as received. The solvent for electrochemical and electron spin resonance (ESR) spectral-measurements was spectrograde purity DMF (99.7%) available from Nacalai Tesque Inc. (Kyoto, Japan) and used as received. Tetrapropylammonium perchlorate (TPAP) was prepared as described previously [26] and used as a supporting electrolyte for DMF. Ferrocene (Fc), used as a potential reference compound, was commercially available from Nacalai Tesque Inc. and used as received.

2.2. Electrochemical and In situ Electrolytic ESR Spectrum Measurements

Cyclic voltammetry was performed using a three-electrode system comprising a 1.0-mm diameter glassy carbon (GC) working electrode, a coiled platinum counter electrode, and an Ag/AgNO$_3$ reference electrode (containing acetonitrile solution of 0.1 mol dm$^{-3}$ tetra-n-butylammonium perchlorate and 0.01 mol dm$^{-3}$ AgNO$_3$; BAS RE-5) at 25 °C using BAS 100B electrochemical workstation, coupled to a BAS electrochemical software to record data (Supplementary Materials, Table S1). In situ electrolytic ESR spectra were measured 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 (Supplementary Materials, Figure S1). Samples were prepared in a glove box completely filled with N$_2$ gas to prevent contamination by moisture. The DMF solution containing 0.1 mol dm$^{-3}$ TPAP as a supporting electrolyte was saturated with O$_2$ by air-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 $O_2$ at a constant level. The equilibrium concentration of $O_2$ was calculated as $4.8 \times 10^{-3}$ mol dm$^{-3}$.

2.3. Calculation

All solution phase calculations were performed at the DFT level with the Becke three-parameter Lee–Yang–Parr (B3LYP) hybrid functional as implemented in Gaussian 16 Program package [27]. This functional was chosen because it has been shown to give good geometries of the reactants, products and transition states (TS) in PCET reactions between phenolic compounds and free radicals [28]. Geometry optimization, vibrational frequency calculations, the intrinsic reaction coordinate (IRC) calculations, and population analysis of each compound was performed by employing the standard split-valence triple $\zeta$ basis sets augmented by the polarization 3df,2p and diffusion orbitals 6-311+G(3df,2p). The solvent contribution of DMF to the standard Gibbs free energies was computed employing the polarized continuum model (PCM) at the default settings of the Gaussian 16, which is widely employed in the description of the thermodynamic characteristics of solvation. The zero-point energies and thermal correction, together with entropy, were used to convert the internal energies to standard Gibbs energy at 298.15 K. The natural bond orbital (NBO) technique was used for electron and spin calculations in population analysis [29].

3. Results

3.1. Cyclic Voltammetry and ESR Analysis of $O_2/O_2^*$ in the Presence of PyH$_3$

In Figure 2, CVs of saturated $O_2$ ($4.8 \times 10^{-3}$ mol dm$^{-3}$) in the presence of PyH$_3$ and related compounds (Figure 1(a–d)) in DMF, and ESR spectra of the CV solutions (b) obtained via in situ electrolytic ESR system are demonstrated. CV and ESR in the presence of (b) CatH$_2$ and CV in the presence of (c) PhOH, were already reported in our previous paper, although, are shown for comparison [22,24]. In aprotic solvents such as DMF, $O_2$ shows quasi-reversible redox (Equation (1)) corresponding to generation of $O_2^*$ in the initial cathodic scan and reoxidation to the starting materials ($O_2$), in the returned anodic scan (1c/1a, bold lines in Figure 2), where $O_2^*$ is not particularly reactive toward aprotic DMF. The reversible CVs investigated here were all modified to irreversible one by the presence of any compounds (a–d) with concentration dependency (0 to 3.0, $5.0 \times 10^{-3}$ mol dm$^{-3}$), supported that CVs of bubbled N$_2$ showed no peak over the potential range. The reactivity of PyH$_3$ estimated from a loss of reversibility of the CV is higher than the others. Thus, the loss of reversibility in the CVs of $O_2/O_2^*$ is caused by the acid-base reaction; the initial PT from the compounds to $O_2^*$ acts as a Bronsted base forming HO$_2^*$ (Equation (2)).

![Figure 2](image)

Figure 2. CVs of $4.8 \times 10^{-3}$ mol dm$^{-3}$ $O_2$ in the presence of (a) PyH$_3$, (b) CatH$_2$, (c) PhOH, and (d) MoCatH$_2$, in DMF containing 0.1 mol dm$^{-3}$ TPAP recorded with a GC electrode (1.0 mm) at a scan rate of 0.1 V s$^{-1}$. Concentrations ($\times 10^{-3}$ mol dm$^{-3}$) are (a) 0 (black), 1.0 (blue), 2.0 (green), and 3.0 (red), and (b–d) 0, 1.0, 2.0, 3.0, and 5.0, (the concentration changes are shown by arrows). ESR spectra obtained via in situ controlled-potential electrolysis (at −1.3 V vs Fc/Fc) of the CV solutions (b).

With the generation of HO$_2^*$, bielectronic CVs were observed derives from the reduction of HO$_2^*$ (Equation (3)) as shown in Figure 2c, cathodic current 2c. Conversely, in the presence of (a) PyH$_3$, (b) CatH$_2$, and (d) MoCatH$_2$, the bielectric CVs don’t appear due to the scavenging of HO$_2^*$ by the subsequent ET (Equation (4)) from the deprotonated anion (PyH$_3^-$, CatH$_2^-$, and MoCatH$^-$), where a cathodic prepeak appeared. In our previous
study, the ET involved in the PCET mechanism for successful O₂•⁻ scavenging required two structural characteristics: 1) the quinone–hydroquinone π-conjugated structure characterized by ortho/para-diphenol, and 2) the OH proton for the second PT [20,22–24,30].

\[ O_2 + e^- \leftrightarrow O_2^{* -} \quad (E^* = -1.284 \text{ V vs } \text{Fc/Fc}) \]  
\[ O_2^{* -} + \text{PyH}_3 \rightarrow \text{HO}_2^{*} + \text{PyH}_2^+ \text{ (the initial PT)} \]  
\[ \text{HO}_2^{*} + e^- \rightarrow \text{HO}_2^- \quad (E^* = -0.4 \text{ to } -0.2 \text{ V vs } \text{Fc/Fc}) \]  
\[ \text{HO}_2^{*} + \text{PyH}_2^+ \rightarrow \text{HO}_2^- + \text{PyH}_3 \quad \text{(ET)} \]  
\[ \text{HO}_2^- + \text{PyH}_3 \rightarrow \text{H}_2\text{O}_2 + \text{PyH}^- \text{ (the second PT)} \]

Considering these results, we rationalized that O₂•⁻ formation after the primary electrode process associated with PT from the OH group leads to the irreversible overall reduction of O₂ to H₂O₂, which is driven by the exergonic reduction of the resulting HO₂•⁻/HO₂⁻. Therefore, the CV traces for O₂/O₂•⁻ in the presence of phenolic compounds are divided into two typical curves: type A, an irreversible two-electron process observed in electro–chemical–electro reactions (Equations (1)–(3)), and type B, an irreversible one-electron process (Equations (1), (2), (4), and (5)) leading to O₂•⁻ scavenging. Figure 3 shows the plausible electrochemical mechanism for O₂/O₂•⁻ in the presence of (a) PyH₃ and (b) PhOH, summarizing Equations (1)–(5).

![Figure 3](image-url) Plausible electrochemical mechanisms of O₂/O₂•⁻ in the presence of (a) PyH₃ and (b) PhOH in DMF. ¹one-electron reduction of O₂/O₂•⁻, ²the initial PT from acidic substrate to O₂•⁻, ³one-electron reduction of HO₂•⁻/HO₂⁻, ⁴ET from substrate anion to HO₂•⁻, ⁵the second PT to HO₂⁻. The net PCET reaction between PyH₃ and O₂•⁻ forming PyH⁺ and H₂O₂ involves two PTs and one ET.

In this scenario, the CV results recorded in the presence of (c) PhOH demonstrate type A (O₂•⁻ is not scavenged) showing the appearance of a cathodic current ascribed HO₂•⁻. Conversely, each of the CV result in the presence of (a) PyH₃, (b) CatH₂, and (d) MoCatH₂ demonstrates type B (scavenging of O₂•⁻/HO₂•⁻). Then, the O₂•⁻/HO₂•⁻ scavenging by (b) CatH₂ was confirmed via in situ electrolytic ESR measurements of the CV solutions at an applied potential of −1.3 V corresponding to the O₂ reduction (Equation (1)) with ESR.
scanning during 4 minutes. With reference to (a) PyH₃ and (d) MoCatH₂, ESR shows no signal and the CV shows type B with a large reactivity. The cathodic prepeaks (2c) appearing in Figure 2(a, b, and d) are inferred to be assigned to reduction of the product radical/anion (PyH⁺/PyH⁻, Cat⁺/Cat, MoCat⁺/MoCat), although the corresponding anodic peaks are observed only for (b). It is presumed that the ESR spectra for (a, d) were undetectable because the generated PyH⁺ and MoCat⁺ were further reduced to dianions (PyH²⁻ and MoCat⁻) at the applied potential (~1.3 V) for the electrogeneration of O₂⁻. Notably, the CV and ESR results demonstrated that (a) PyH₃ with its CatH₂ moiety and two OH groups can scavenge O₂⁻ through the PCET involving two PTs and one ET, whereas the role of the 3OH group is unclear. These results imply that the reaction mechanism of (a) PyH₃ is similar to that of (b) CatH₂ (Scheme 1), however their reactivities are different.

3.2. Change in HOMO–LUMO Energies upon PCET Between PyH₃ and O₂⁻ in DFT Analyses

DFT calculations with the frontier molecular orbital analysis were performed to aid the mechanistic analysis of O₂⁻ scavenging by PyH₃ in DMF. Figure 4 shows HOMO−LUMO changes upon PCET between PyH₃/PyH⁻ and O₂⁻. After the initial PT, some reactant species, i.e., PyH₃, PyH⁻, O₂⁻, and HO₂⁻ coexist in the solution. The SOMO energy (Hartree) for HO₂⁻ (−0.3142) is much lower than HOMO energies of PyH₃ and PyH⁻. Thus, the electron acceptor will be HO₂⁻, not O₂⁻. Considering that CV in DMF revealed that HO₂⁻ formed after the initial PT is scavenged (Figure 2a), the electron donor will be PyH⁻, for which the downhill energy relationship is indicated by the bold red line. Thus, this change in HOMO−LUMO energies upon PT between PyH₃ and O₂⁻ forming PyH⁻ and HO₂⁻ is reasonable for subsequent ET. Next, the HOMO−LUMO relationship between the products after ET (i.e., PyH⁺, and HO₂⁻) is reversed, which is rational for orbital energies in the reverse ET (red dotted line). However, the HOMO (−0.2754) of the PT-forming H₂O₂ is lower than HOMO (−0.1648) of HO₂⁻, making the reverse ET impossible. Thus, the subsequent PT is dominant in determining the ET direction.

![Figure 4](image-url)  
Figure 4. Change in HOMO–LUMO energies (Hartree) upon PCET between PyH₃ and O₂⁻ with its corresponding chemical species in DMF, calculated using the DFT-(U)B3LYP/PCM/6-311+G(3df,2p).

Alternatively, the HOMO–LUMO relationship for O₂⁻ scavenging by PyH₂ preformed by the initial deprotonation indicates that the similar PCET involving two PTs and one ET occurs; the downhill ET (blue bold line) and reverse ET (blue dotted line). As reported previously, 2PCET occurs between CatH₂ moiety and O₂⁻ after the formation of the pre-reactive hydrogen-bond (HB) complex from the free reactants (FR), and the proton and electron are concertedly transferred in one kinetic step via a TS forming the product.
complex (PC) [20,23]. Therefore, it is expected that the 2PCET between PyHs and O$_2$$^\cdot$ occurs in a similar concerted manner through the HB formed between two OH groups of PyHs and O$_2$$^\cdot$.

3.3. Free Energy Calculations of PCET Between PyHs and O$_2$$^\cdot$

For a mechanistic analysis of the O$_2$$^\cdot$ scavenging by PyHs in DMF, DFT calculations were performed at the (U)B3LYP/PCM/6-311+G(3df,2p) level. In Figure 5, the equilibrium schemes and standard Gibbs free energy changes ($\Delta$$G^\circ$/kJ mol$^{-1}$, 298.15 K) of the six diabatic electronic states for the PCET involving two PTs and one ET between (a) PyHs and O$_2$$^\cdot$, and between (b) O$_2$$^\cdot$ and PyH$_2$ formed after the initial deprotonation are shown. The important factors in determining the sequential processes shown in this scheme are the $\Delta$$G^\circ$s for the individual reactions; the acid–base interaction and redox potentials of the components. In Figure 5a, ET1 ($\Delta$$G^\circ$ = 405.3 kJ mol$^{-1}$) is strongly endergonic, thus, PT1 (17.9) forming PyH$_2$ and HO$_2$$^\cdot$ must primarily occur, as shown in the CV result. In the following pathway shown in the lower rectangle, both PT3 (357.9) and ET2 (28.2) are uphill endergonic, suggesting that the sequential PCET does not proceed but the concerted PCET (−39.5) is a thermodynamically feasible pathway. Alternatively, one-step one-electron transfer concerted with sequential two-proton transfer after initial formation of the HB complexes between PyHs and O$_2$$^\cdot$ without generating high energy intermediates, which we refer to as concerted 2PCET reactions, is another feasible pathway [23,28]. For a successful O$_2$$^\cdot$ scavenging in either PCET pathway, the second PT coupled to ET is necessary, as reported in our previous studies [25,31]. On the other side, a PCET reaction between PyH$_2$ and O$_2$$^\cdot$ shown in Figure 5b is also plausible, in case that the initial deprotonation of PyH$_2$ will partially occur in an aprotic DMF solution. Although, since both PT1 (88.5) and ET1 (253.5) are uphill, the only feasible pathway is 2PCET forming quinone-radical-dianion (Py$^\cdot$) as a product of the net reaction involving three PTs and one ET from PyHs.

For a comparative study, the $\Delta$$G^\circ$ values of the PCET pathways for CatH$_2$ and MoCatH$_2$ were calculated (Table 1). From a thermodynamic viewpoint, the total values of $\Delta$$G^\circ$ for the net PCET were obtained from the sum of the values for the two PTs and one ET. If the PCET occurs along a pathway involving the unfeasible single PT/ET (PT1, PT3, ET1, and ET2), the total values cannot embody the energetic driving force because the $\Delta$$G^\circ$ for the unfeasible PT/ET has been summed in it. However, since the concerted PCET (ET2–PT4/PT3–ET3) after the initial PT is endergonic for both CatH$_2$ (−55.7) and MoCatH$_2$ (−79.1), the total values (−36.2 and −32.9) can embody the exergonic driving force through PT1–concerted PCET pathway, similar to PyH$_2$ (concerted: −39.6, total: −21.6). Notably, both the $\Delta$$G^\circ$ values (concerted and total) for PyH$_2$ are larger than those for CatH$_2$ and MoCatH$_2$, showing a lower reactivity of PyH$_2$. The effect of the substituted group of MoCatH$_2$, PyH$_2$, and CatH$_2$, on the O$_2$$^\cdot$ scavenging through the PCET is primarily considered to be due to the electron-donating ability (-OCH$_3$ > -OH > -H) increasing electron density in the benzene ring, known as the Hammett equation [32]. Additionally, the intramolecular HB formed at the 3OH group strongly stabilizes the negatively-charged-deprotonated species along the PCET; PyH$_2$, PyH$_2$$^+$, and Py$^\cdot$, consequently suppressing their reactivities to electrophilic attack. Thus, these $\Delta$$G^\circ$ values confirm that the PCET mechanism in Figure 5a alone cannot explain the reason for the higher reactivity of PyH$_2$ than the others toward electrogenerated O$_2$$^\cdot$ shown in the CVs (Figure 2). As a result of the comparative analyses of the $\Delta$$G^\circ$ values, the involvement of three reaction pathways is plausible for efficient O$_2$$^\cdot$ scavenging by PyH$_2$; PT–concerted PCET and 2PCET between PyH$_2$ and O$_2$$^\cdot$, and 2PCET between PyH$_2$ and O$_2$$^\cdot$. 
Figure 5. Six diabatic electronic states and the ΔG° values for PCET between (a) PyH3 and O2•−, and (b) PyH2− and O2•−, involving two PTs and one ET in DMF. The ΔG° (kJ mol−1, 298.15 K) for the (PT1-PT4) and ET (ET1-ET3) were calculated using DFT-(U)B3LYP/PCM/6-311+G(3df,2p) method.

Table 1. ΔG° values (kJ mol−1, 298.15 K) for PCET between O2•− and phenolic compounds (PyH3, PyH2−, CatH2, and MoCatH2) in DMF, calculated using DFT at the (U)B3LYP/PCM/6-311+G(3df,2p) level.

| Compounds     | PT1  | PT2   | PT3    | PT4    | ET1  | ET2    | ET3    | Con concerted | Totalb |
|---------------|------|-------|--------|--------|------|--------|--------|-------------|--------|
| PyH3          | 17.9 | −359.0| 357.9  | −67.8  | 405.3| 28.2   | −397.5 | −39.6       | −21.6  |
| PyH2−         | 88.5 | −221.5| 454.1  | −29.5  | 253.5| −56.5  | −540.1 | -           | 2.4    |
| CatH2         | 19.4 | −364.2| 390.6  | −78.7  | 406.8| 23.0   | −446.3 | −55.7       | −36.2  |
| MoCatH2       | 46.0 | −335.4| 379.5  | −92.4  | 394.8| 13.3   | −458.6 | −79.1       | −32.9  |

1 Total values involve the sum of ΔG’s for two PTs and one ET.

3.4. Potential Energy Surfaces of the PCET between PyH3 and O2•−

For gaining deeper insight into the PCET mechanism for the O2•− scavenging by PyH3 in DMF, potential energy surfaces were investigated at the (U)B3LYP/PCM/6-311+G(3df,2p) level of theory. It is assumed that the reaction involves three elementary steps: i) formation of the prereactive HB complex (PRC) from the FRs, ii) reaction to the PC via a TS, and iii) dissociation of the PC yielding free products (FP). Furthermore, the structural and electronic changes during the reaction were analyzed with the NBO calculations. First, we start with an analysis of potential energy scanning for the stable HB complexes (PRC, intermediate HB complex, and PC) along the PCET reaction (Figure 6a). Then, optimized structures of plausible PRC (PyH3−O2•−) formed from the FRs via two HBs (step i) were obtained, resulting in a lower ΔG° by 81.2 kJ mol−1 (set as zero). Next, an energy profile (ΔG°, kJ mol−1) along the IRC for the 2PCET involving concerted two PTs and one ET forming the PC (PyH3−H2O2) was obtained (step ii). The IRC shows that a 2PCET occurs between PyH3 and O2•− in one kinetic process via the TS of a low activation energy (Ea) at 53.9 kJ mol−1, without generating any intermediates such as H2O2•, H2O2•−, PyH2−, and PyH2−.

Figure 6b shows changes in O−H bond distances (OH1: black line, and OH2: red line) with the number of electrons on the Ï•-orbital of PyH3 moiety along the IRC (blue circle). Then, spin density distributions localizing the atoms consisting of the radical before and after the TS along the 2PCET are demonstrated, showing that the radical localized on O2•− in the initial PRC was transferred to PyH2− in the resulting PC. Changes in the spins on...
the electron donor side (PyH$_3$) and acceptor side (O$_2$•) are in good correlation with the changes in the π-electron of the PyH$_3$. Furthermore, careful observation of changes in structures and OH$^+$/OH$^-$ in the IRC indicates that the π-electron transfer occurs simultaneously with sequential lengthening of the two O–H bond distances of PyH$_3$. The first step of the reaction is the attraction of one phenolic proton (H$^+$) by O$_2$•. This attraction results in nearly complete deprotonation of PyH$_3$ and transfer of one-half of the π-electron from PyH$_3$ to O$_2$• in the TS. Movement of the second proton (H$^+$) accelerates the ET from the TS forward and leads to formation of the PC as the resulting reaction system. The results also demonstrate that the electronic state of the TS is characterized by the delocalization of the radical anion over the HB complex of the components.

For a comparative study, potential energy surfaces for the PCET with IRC and TS between O$_2$• and the other compounds; CatH$_3$, MoCatH$_3$, and the anion: PyH$_3$–, were investigated. Similar 2PCET mechanisms for CatH$_3$ and MoCatH$_3$ were obtained without generating intermediates (Supplementary Data, Figure S2–S3), but was not obtained for PyH$_3$–. Then, the Δ$G^\circ$ values of the complexes (FR, PC, and FP) and the $E_a$ values, for PyH$_3$, CatH$_3$, and MoCatH$_3$ (Geometries of the TS are shown in Supplementary Materials, Table S3–S5), are listed in Table 2. Notably, there is almost no difference in each $E_a$ values (PyH$_3$: 53.9, CatH$_3$: 52.5, and MoCatH$_3$: 50.7), and either of those is as low as the hydrogen-bonding energy. Conversely, there are about 10-20 kJ mol$^{-1}$ differences between the Δ$G^\circ$ values for forming each of the PRC from the FRs (PyH$_3$: 81.2, CatH$_3$: 71.6, and MoCatH$_3$: 61.4). Thus, the Δ$G^\circ$ values for the formation of the PRC from the FRs (step i), rather than the kinetics of the 2PCET reaction to the PC via a TS (step ii), embody the superior O$_2$•-scavenging ability of PyH$_3$ with a good correlation with the CV results in DMF. Considering the IRC results together with the CV (Figure 2) and the Δ$G^\circ$ results (Figure 5), the 2PCET between O$_2$• and PyH$_3$– formed after the deprotonation of PyH$_3$ is not feasible. In an aprotic DMF solution, the initial reaction between PyH$_3$ and O$_2$• will be the formation of the PRC stabilized at ~81.2 kJ mol$^{-1}$ via two HBs, rather than the initial PT (PT1 in Figure 2a: 17.9 kJ mol$^{-1}$) or a deprotonation (proton loss in the solution).

Table 2. Δ$G^\circ$ and $E_a$ values (kJ mol$^{-1}$, 298.15 K) for 2PCET between O$_2$• and the compounds (PyH$_3$, CatH$_3$, and MoCatH$_3$) in DMF, calculated using DFT at the (U)B3LYP/PCM/6-311+G(3df,2p) level.
This study will provide evidence for the biological involvement of (U)B3LYP/PCM/6 Figure 7. Plausible mechanism and the ΔG° involving the initial formation of PRC followed by -311+G(3df,2p) method. Conflicts of Interest: The authors declare no conflict of interest. References

4. Conclusions

In conclusion, we have investigated the O2•− scavenging by PyH3 through the PCET in DMF. As a result, we have clarified:

- PyH3 scavenges O2•− through the 2PCET involving concerted two PTs and one ET, in a similar mechanism for CatH2.
- the 3OH group thermodynamically promotes the formation of PRC via two HBs, resulting in an effective O2•− scavenging ability of PyH3.

Although the results presented in this manuscript are for a chemical reaction in aprotic DMF solvent rather than a biological system, the PCET theory is adaptable to biological processes involving both protic and aprotic conditions in such as a lipid bilayer. Therefore, we hope that the findings obtained in this study will provide evidence for the biological mechanistic actions of O2•− scavenging by antioxidants involving PyH3 moiety.

Supplementary Materials: The following is available online at www.mdpi.com/xxx/s1, Table S1: CV parameters, Figure S1: In situ electrolytic ESR system, Table S2-S5: ΔG° values for the PCET in various solvents, Figure S2: Energy profiles along IRC of 2PCET between CatH2 and O2•−, Figure S3: Energy profiles along IRC of 2PCET between MoCatH2 and O2••−, Tables S6: Calculated geometry of complexes.

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ΔG° values (kJ mol⁻¹) of PRC were set as a zero point.

Taken together, these findings indicate that the O2•− scavenging by PyH3 in DMF is governed by the concerted 2PCET after forming PRC via two HBs, which corresponds to a moving along the red diagonal line of the two rectangles shown in Figure 5a. In Figure 7, the net mechanism of the O2•− scavenging by PyH3 in DMF is shown. In the 2PCET mechanism, ET occurs between oxygen-π-orbitals orthogonal to the molecular framework, then, PT occurs between oxygen-σ-orbitals along the HBs [23]. It is presumed that the higher reactivity of PyH3 with O2•− than that for CatH2, is due to the sequential reactions; the initial formation of the PRC, followed by the 2PCET.

| Reactants       | 1 FR | TS (E°) | PC  | FP  |
|-----------------|------|---------|-----|-----|
| PyH3 (+O2•−)    | 81.2 | 53.9    | −29.4 | 68.9 |
| CatH2 (+O2•+)   | 71.6 | 52.5    | −20.9 | 45.3 |
| MoCatH2 (+O2•+) | 61.4 | 50.7    | −28.4 | 38.5 |

$^{1}$ΔG° values (kJ mol⁻¹) of PRC were set as a zero point.

Figure 7. Plausible mechanism and the ΔG° and E° values (kJ mol⁻¹, 298.15 K) for the PCET pathways between PyH3 and O2•− involving the initial formation of PRC followed by concerted 2PCET in DMF. The ΔG° and E° values were calculated using DFT-(U)B3LYP/PCM/6-311+G(3df,2p) method.
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