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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 (O2•−) 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/O2•− redox couple was modified by the presence of PyH3, suggesting that O2•− was scavenged by PyH3 through proton-coupled electron transfer (PCET) involving two proton transfer and one electron transfer. DFT calculation suggested that the pre-reactive 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 O2•− 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 O2•− scavenging.

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

1. Introduction

Benzen-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, constitutes some antioxidants, such as gallocatechin, epigallocatechin gallate, and prodelphinidin (the polymeric tannins composed of galloccatechin). It is well recognized that PyH3 is a much more efficient antioxidant than benzene-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 the number of OH groups on phenolic compounds is, the higher the antioxidant properties are, 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 (O2•−), precursor of other ROS, and grand state molecular oxygen (O2) in the autoxidation. The antioxidant activity of PyH3 with its autoxidation involves complicated reaction mechanisms, though the mechanism between PyH3 and O2 involves two-proton transfer (PT) and two-electron transfer (ET) forming pyrogallol-ortho-quinone (PyH) and hydroperoxide (H2O2).
Simultaneously, it is well recognized that the autoxidation is promoted in an alkali solution, showing that the primary deprotonation occurs forming corresponding anion (PyH$_2^-$) followed by the autoxidation reaction [2,3]. Furthermore, it is considered that forming intermediate peroxiradical (ROS) such as hydroperoxiradical (HO$_2^*$) 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$_2^{*-}$. However, HO$_2^*$ formed after protonation of O$_2^{*-}$ as a Bronsted base is a strong oxidant. Thus, the deprotonation and subsequent oxidation, i.e., PT and ET between PyH$_2$ and 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 PCET [16–20], and sequential proton-loss electron transfer (SPLET) [21]. In the SFO mechanism, the initial PT from the substrate to O$_2^{*-}$ to give HO$_2^*$ is followed by rapid dismutation to give H$_2$O$_2$ and O$_2$. Then, the substrate anion

Scheme 1. Reaction schemes of O$_2^{*-}$ scavenging by (a) PyH$_3$ and (b) PyH$_2^-$, through proton-coupled electron transfer (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$_2^-$ increases electron density in the benzene ring and consequently increases its reactivity to electrophilic attack. Considering the 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 electrochemical oxidation (SFO) [13–15], hydrogen atom transfer (HAT) involving PCET [16–20], and sequential proton-loss electron transfer (SPLET) [21]. In the SFO mechanism, the initial PT from the substrate to O$_2^{*-}$ to give HO$_2^*$ is followed by rapid dismutation to give H$_2$O$_2$ and O$_2$. Then, the substrate anion

\[
\begin{align*}
\text{(a)} & \quad \text{PyH}_3 + O_2^{*-} \xrightarrow{\text{PCET}} \text{PyH}^- + HO_2^* + H_2O_2 \\
\text{(b)} & \quad \text{PyH}_2^- + O_2^{*-} \xrightarrow{\text{PCET}} \text{Py}^{2-} + H_2O_2
\end{align*}
\]
is oxidized by the O$_2$ formed in the dismutation process [15]. Conversely, the other two mechanisms involve direct oxidation by O$_2$$^•−$/HO$_2$$^•−$.

Considering the relationship between the structure of PyH$_3$ and the mechanism of O$_2$$^•−$/HO$_2$$^•−$ 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$$^•−$/HO$_2$$^•−$ is scavenged by polyphenols [20], diphenols (hydroquinone [22] and CatH$_2$ [23]), and monophenols [24,25], through a 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$$^•−$ scavenging. Therefore, CatH$_2$ moiety comprised in PyH$_3$ is expected to play through the concerted 2PCET, although the third OH group (3OH) gives a different chemical mechanism of PyH$_3$ from CatH$_2$.

In this study, we analyzed the reaction between PyH$_3$ and electrogenerated O$_2$$^•−$ comparately using some related compounds (Figure 1) in dehydrated N,N-dimethylformamide (DMF) by using electrochemistry and density functional theory (DFT) calculation. Accordingly, herein we present a mechanistic insight into PCET for the O$_2$$^•−$ scavenging reaction by PyH$_3$ that constitute some highly reactive antioxidants.

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

2. Materials and Methods

2.1. Chemicals

We obtained PyH$_3$ (98.0%), MoCatH$_2$ (99.0%), and CatH$_2$ (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 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)/ultraviolet-visible (UV-vis) spectral–measurements was spectrograde purity DMF (99.7%) available from Nacalai Tesque Inc. (Kyoto, Japan) and used as received. Tetrapropyrammonium 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/UV–vis 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 (Pt) counter electrode, and a silver/silver nitrate (Ag/AgNO$_3$) reference electrode (containing acetonitrile solution of 0.1 mol dm$^{-3}$ tetraethylammonium perchlorate and 0.01 mol dm$^{-3}$ AgNO$_3$; BAS RE-5) at 25 °C using BAS 100B electrochemical workstation, coupled to BAS electrochemical software to record data (Supplementary Materials, Table S1). In situ electrolytic ESR/UV–vis spectra were measured using a JEOL JES-FA200 X-band spectrometer/an OCEAN HDX spectrometer (OptoSirius Co., Ltd.). 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/an optically transparent thin-layer electrochemical (OTTLE) cell (path length: 1.0 mm) using a Pt mesh working electrode (Supplementary Materials, Figure S1). 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 air-bubbling the gas for ca. 2–3 min and the gas was passed over the solutions during the electrochemical and spectral 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.

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 ζ 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 O₂/O₂•⁻ in the Presence of PyH₃

In Figure 2, cyclic voltammograms (CV) of saturated O₂ (4.8 × 10⁻³ mol dm⁻³) in the presence of PyH₃ and related compounds (Figure 1a–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 (c) CatH₂ and CV in the presence of (d) PhOH, were already reported in our previous paper, though are shown here for comparison [22,24]. In aprotic solvents such as DMF, O₂ shows quasi-reversible redox at −1.284 V vs. the ferrocenium ion/ferrocene (Fc⁺/Fc) couple (Equation (1)) corresponding to generation of O₂•⁻ in the initial cathodic scan and reoxidation to the starting materials (O₂), in the returned anodic scan (1c/1a, bold lines in Figure 2), where O₂•⁻ 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 × 10⁻³ mol dm⁻³), supported that CVs of bubbled N₂ showed no peak over the potential range. The reactivity of PyH₃ estimated from a loss of reversibility of the CV is higher than the others. Thus, the loss of reversibility in the CVs of O₂/O₂•⁻ is caused by the acid–base reaction; the initial PT from the compounds to O₂•⁻ as a Brønsted base forming HO₂• (Equation (2)).
product radical/dianion (PyH\textsuperscript{3+})

duction (trode
cation
bielectronic
the
PhOH,
PhOH,
two
characteristics;
ortho/para-diphenol,
and (2) the OH proton for the second PT [20,22–24,30].

\[ \text{O}_2 + e^- \leftrightarrow \text{O}_2^{2-} \quad (E^\circ = -1.284 \text{ V vs. } \text{Fc}^+/\text{Fc}) \]  \hfill (1)

\[ \text{O}_2^{2-} + \text{PyH}_3 \rightarrow \text{HO}_2^* + \text{PyH}_2^- \quad (\text{the initial PT}) \]  \hfill (2)

\[ \text{HO}_2^* + e^- \rightarrow \text{HO}_2^- \quad (E^\circ = -0.4 \text{ to } -0.2 \text{ V vs. } \text{Fc}^+/\text{Fc}) \]  \hfill (3)

\[ \text{HO}_2^* + \text{PyH}_2^- \rightarrow \text{HO}_2^- + \text{PyH}_2^* \quad (\text{ET}) \]  \hfill (4)

\[ \text{HO}_2^- + \text{PyH}_2^* \rightarrow \text{H}_2\text{O}_2 + \text{PyH}^* \quad (\text{the second PT}) \]  \hfill (5)

Considering these results, we rationalized that O\textsubscript{2}^{2-} formation after the primary electrode process associated with PT from the OH group leads to the irreversible overall reduction of O\textsubscript{2} to H\textsubscript{2}O\textsubscript{2}, which is driven by the exergonic reduction of the resulting HO\textsubscript{2}^*/HO\textsubscript{2}^-. Therefore, the CV traces for O\textsubscript{2}/O\textsubscript{2}^{2-} 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 O\textsubscript{2}^{2-} scavenging. Figure 3 shows the plausible electrochemical mechanism for O\textsubscript{2}/O\textsubscript{2}^{2-} in the presence of (a) PyH\textsubscript{3} and (b) PhOH, summarizing Equations (1)–(5).

In this scenario, the CV results recorded in the presence of (d) PhOH demonstrate type A (O\textsubscript{2}^{2-} is not scavenged) showing the appearance of a cathodic current ascribed HO\textsubscript{2}^*. Conversely, each of the CV results in the presence of (a) PyH\textsubscript{3}, (b) MoCatH\textsubscript{2}, and (c) CatH\textsubscript{2} demonstrate type B (scavenging of O\textsubscript{2}^{2-}/HO\textsubscript{2}^*). Then, the O\textsubscript{2}^{2-}/HO\textsubscript{2}^* scavenging by (c) CatH\textsubscript{2} was confirmed via in situ electrolytic ESR measurements of the CV solutions at an applied potential of -1.3 V corresponding to the O\textsubscript{2} reduction (Equation (1)) with ESR scanning during 4 min. With reference to (a) PyH\textsubscript{3} and (b) MoCatH\textsubscript{2}, ESR shows no signal and the CV shows type B with a large reactivity. The cathodic prepeaks (2c) appearing in Figure 2a-c are inferred to be assigned to reduction of the product radical/dianion (PyH^*/PyH^2−, MoCat^*/MoCat^2−, Cat^*/Cat^2−), although
the corresponding anodic peaks are observed only for (c). It is presumed that the ESR spectra for (a, b) were undetectable because the generated PyH\(^{\bullet-}\) and MoCat\(^{\bullet-}\) were further reduced to dianions (PyH\(^2\)- and MoCat\(^-\)) at the applied potential (−1.3 V) for the electrogeneration of O\(_2\)\(^{2-}\).

Figure 3. Plausible electrochemical mechanisms of O\(_2\)/O\(_2\)\(^{\bullet-}\) in the presence of (a) PyH\(_3\) and (b) PhOH in DMF. \(^1\) one-electron reduction of O\(_2\)/O\(_2\)\(^{\bullet-}\), \(^2\) the initial PT from acidic substrate to O\(_2\)\(^{\bullet-}\), \(^3\) one-electron reduction of HO\(_2\)\(^\bullet\)/HO\(_2\)^\(-\), \(^4\) ET from substrate anion to HO\(_2\)\(^\bullet\), \(^5\) the second PT to HO\(_2\)^\(-\). The net PCET reaction between PyH\(_3\) and O\(_2\)\(^{\bullet-}\) forming PyH\(^{\bullet-}\) and H\(_2\)O\(_2\) involves two PTs and one ET.

Next, in situ electrolytic UV-vis spectra for the CV solution containing PyH\(_3\) (1.0 \times 10^{-3} \text{ mol dm}^{-3}) using the OTTLE cell (Supporting Information, Figure S2) were measured in the absence of O\(_2\) under purging N\(_2\) and in the presence of O\(_2\) (Figure 4). The spectrum of PyH\(_3\) alone has a characteristic absorption band centered at 272 nm. Under the applied potential at 1.0 to −2.0 V vs. Fc\(^+\)/Fc without O\(_2\), the spectrum did not change where any potential was applied (data not shown), demonstrating that PyH\(_3\) is not electrolyzed without deprotonation. Conversely, the spectrum has changed in the presence of CH\(_3\)ONa (5.0 \times 10^{-3} \text{ mol dm}^{-3}) without applying a potential (red line). Since PyH\(_3\) is deprotonated by CH\(_3\)ONa as a Brønsted base, the spectrum will be attributed to PyH\(_2\^-\) or PyH\(^2\^-\). On the other side, the spectrum of PyH\(_3\) in the presence of saturated O\(_2\) (4.8 \times 10^{-3} \text{ mol dm}^{-3}) has changed, showing the appearance of an absorption band centered at 292 nm at applied cathodic potentials over −1.3 V corresponding electrogeneration of O\(_2\)\(^{\bullet-}\).
However, the HOMO (−0.2754) of the PT-forming H$_2$O$_2$ is lower than HOMO (−0.1648) of PyH$_3$ for subsequent ET. The controlled potential at −1.0, −1.2, −1.4, and −1.6 V vs. Fe$^+/\text{Fc}$ was applied to the solutions.

These spectral changes have demonstrated that the product of homogeneous reaction between PyH$_3$ and O$_2$•$^-$ is PyH$^+_2$, and the observed spectrum derives from PyH$^2^-$ via further 1-electron reduction of PyH$^+_2$ at the electrode. By analogy with the CV results, the initial PT (Equation (1)) and the following reactions including ET between HO$_2^*$ and PyH$_2^-$ (Equation (4)) rapidly undergo base-catalyzed oxidation. Since PyH$^+_2$ would be electrolyzed to PyH$_2^-$ at the electrode, the radical product was undetectable using the in situ electrolytic ESR system.

Notably, the CV and spectral results demonstrated that (a) PyH$_3$ with its two OH groups can scavenge O$_2$•$^-$ 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) PyH$_3$ is similar to that of (c) CatH$_2$ (Scheme 1), however their reactivities are different.

3.2. Change in HOMO–LUMO Energies upon PCET between PyH$_3$ and O$_2$•$^-$ in DFT Analyses

DFT calculations with the frontier molecular orbital analysis were performed to aid the mechanistic analysis of O$_2$•$^-$ scavenging by PyH$_3$ in DMF. Figure 5 shows HOMO–LUMO changes upon PCET between PyH$_3$/PyH$_2^-$ and O$_2$•$^-$.

After the initial PT, some reactant species, i.e., PyH$_3$, PyH$_2^-$, O$_2$•$^-$, and HO$_2^*$, coexist in the solution. The singly occupied molecular orbital (SOMO) energy (Hartree) for HO$_2^*$ (−0.3142) is much lower than HOMO energies of PyH$_3$ and PyH$_2^-$; thus, the electron acceptor will be HO$_2^*$, not O$_2$•$^-$.

Considering that CV in DMF revealed that HO$_2^*$ formed after the initial PT is scavenged (Figure 2a), the electron donor will be PyH$_2^-$, for which the downhill energy relationship is indicated by the bold red line. Thus, this change in HOMO–LUMO (SOMO) energies upon PT between PyH$_3$ and O$_2$•$^-$ forming PyH$_2^-$ and HO$_2^*$ is reasonable for subsequent ET. Next, the HOMO–LUMO relationship between the products after ET (i.e., PyH$_2^*$, and HO$_2^-$) is reversed, which is rational for orbital energies in the reverse ET (red dotted line). However, the HOMO (−0.2754) of the PT-forming H$_2$O$_2$ is lower than

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**Figure 4.** UV–vis spectral changes in PyH$_3$ (1.0 × 10$^{-3}$ mol dm$^{-3}$) solutions in the absence (black bold) and presence (red bold) of CH$_3$ONa (5.0 × 10$^{-3}$ mol dm$^{-3}$) and in situ electrolytic UV–vis spectra of PyH$_3$ in the presence of saturated O$_2$. The controlled potential at −1.0, −1.2, −1.4, and −1.6 V vs. Fe$^+/\text{Fc}$ was applied to the solutions.

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The important factors in determining the sequential processes shown in this scheme are PCET following components.

In the following pathway shown in the lower rectangle, both PT3 (357.9) and ET2 (28.2) ∆G° [20,23]. Therefore, it is expected that the 2PCET between PyH3 and O2•− occurs in a similar concerted manner through the HB formed between two OH groups of PyH3, forming the product complex (PC) [20,23]. Therefore, it is expected that the 2PCET between PyH3 and O2•− occurs in a similar concerted manner through the HB formed between two OH groups of PyH3 and O2•−.

3.3. Free Energy Calculations of PCET between PyH3 and O2•−

For a mechanistic analysis of the O2•− scavenging by PyH3 in DMF, DFT calculations were performed at the (U)BSLYP/PCM/6-311+G(3df,2p) level. In Figure 6, the equilibrium schemes and standard Gibbs free energy changes (∆G°/kJ mol−1, 298.15 K) of the six diabatic electronic states for the PCET involving two PTs and one ET between (a) PyH3 and O2•−, and between (b) O2•− and PyH− formed after the initial deprotonation are shown. The important factors in determining the sequential processes shown in this scheme are the ∆G°'s for the individual reactions; the acid–base interaction and redox potentials of the components. In Figure 6a, ET1 (∆G° = 405.3 kJ mol−1) is strongly endergonic, thus, PT1 (17.9) forming PyH− and HO2•− 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 PyH3 and O2•− without generating high-energy intermediates,
which we refer to as concerted 2PCET reactions, is another feasible pathway [23,28]. For a successful \( \text{O}_2^{**} \) 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 \( \text{PyH}_2^- \) and \( \text{O}_2^{**} \) shown in Figure 6b is also plausible, in case that the initial deprotonation of \( \text{PyH}_3 \) 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\( ^{**} \)) as a product of the net reaction involving three PTs and one ET from \( \text{PyH}_3 \).

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 PT1 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 \( \text{PyH}_3 \) (concerted: –39.6, total: –21.6). Notably, both the \( \Delta G^\circ \) values (concerted and total) for \( \text{PyH}_3 \) are larger than those for CatH\(_2\) and MoCatH\(_2\), showing a lower reactivity of PyH\(_3\). The effect of the substituted group of MoCatH\(_2\), PyH\(_3\), and CatH\(_2\), on the \( \text{O}_2^{**} \) scavenging through the PCET is primarily considered to be due to the electron-donating ability (–\( \text{OCH}_3 \) > –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; \( \text{PyH}_2^- \), \( \text{PyH}^2^- \), and its trianion (Py\( ^{3-} \)), consequently suppressing their reactivities to electrophilic attack. Thus, these \( \Delta G^\circ \) values confirm that the PCET mechanism in Figure 6a alone cannot explain the reason for the higher reactivity of \( \text{PyH}_3 \) than the others toward electrogenerated \( \text{O}_2^{**} \) 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 \( \text{O}_2^{**} \) scavenging by \( \text{PyH}_3 \); PT–concerted PCET and 2PCET between \( \text{PyH}_3 \) and \( \text{O}_2^{**} \), and 2PCET between \( \text{PyH}_2^- \) and \( \text{O}_2^{**} \).

Figure 6. Six diabatic electronic states and the \( \Delta G^\circ \) values for PCET between (a) \( \text{PyH}_3 \) and \( \text{O}_2^{**} \), and (b) \( \text{PyH}_2^- \) and \( \text{O}_2^{**} \), involving two PTs and one ET in DMF. The \( \Delta G^\circ \) (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. \( \Delta G^\circ \) values (kJ mol\(^{-1}\), 298.15 K) for PCET between \( \text{O}_2^{*\ -} \) and phenolic compounds (PyH\(_3\), PyH\(_2\)\(^-\), CatH\(_2\), and MoCatH\(_2\)) in DMF, calculated using DFT at the (U)B3LYP/PCM/6-311+G (3df,2p) level.

| Compounds  | PT1 | PT2   | PT3   | PT4   | ET1 | ET2 | ET3 | Concerted | Total  
|------------|-----|-------|-------|-------|-----|-----|-----|-----------|--------
| PyH\(_3\)  | 17.9| -359.0| 357.9 | -67.8 | 405.3| 28.2| -397.5| -39.6     | -21.6  
| PyH\(_2\)\(^-\) | 88.5| -221.5| 454.1 | -29.5 | 253.5| -56.5| -540.1| -          | 2.4    
| CatH\(_2\) | 19.4| -364.2| 390.6 | -78.7 | 406.8| 23.0| -446.3| -55.7     | -36.2  
| MoCatH\(_2\)| 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 \( \Delta G^\circ \)s for two PTs and one ET.

3.4. Potential Energy Surfaces of the PCET between PyH\(_3\) and \( \text{O}_2^{*\ -} \)

For gaining deeper insight into the PCET mechanism for the \( \text{O}_2^{*\ -} \) scavenging by PyH\(_3\) 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 started with an analysis of potential energy scanning for the stable HB complexes (PRC, intermediate HB complex, and PC) along the PCET reaction (Figure 7a). Then, optimized structures of plausible PRC (PyH\(_3\)--O\(_2^{*\ -}\)) formed from the FRs via two HBs (step i) were obtained, resulting in a lower \( \Delta G^\circ \) by 81.2 kJ mol\(^{-1}\) (set as zero). Next, an energy profile (\( \Delta G^\circ \), kJ mol\(^{-1}\)) along the IRC for the 2PCET involving concerted two PTs and one ET forming the PC (PyH\(^*\)\(_2\)--H\(_2\)O\(_2\)) was obtained (step ii). The IRC shows that a 2PCET occurs between PyH\(_3\) and \( \text{O}_2^{*\ -} \) in one kinetic process via the TS of a low activation energy (\( E_a \)) at 53.9 kJ mol\(^{-1}\), without generating any intermediates such as HO\(_2^{*}\), HO\(_2\)\(^-\), PyH\(_2\)\(^-\), and PyH\(_2^{*}\).

Figure 7b shows changes in O--H bond distances (OH\(_1\): black line, and OH\(_2\): red line) with the number of electrons on the \( \pi \)-orbital of PyH\(_3\) 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 O\(_2^{*\ -}\) in the initial PRC was transferred to PyH\(^*\) 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 \( \pi \)-electron of the PyH\(_3\). Furthermore, careful observation of changes in structures and OH\(_1^2\)/OH\(_2^2\) in the IRC indicates that the \( \pi \)-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\(_1^1\)) by O\(_2^{*\ -}\). This attraction results in nearly complete deprotonation of PyH\(_3\) and transfer of one-half of the \( \pi \)-electron from PyH\(_3\) to O\(_2^{*\ -}\) in the TS. Movement of the second proton (H\(_2^2\)) 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.
Table 2. \( \Delta G^0 \) and \( E_a \) values (kJ mol\(^{-1}\), 298.15 K) for 2PCET between \( O_2^{-} \) and the compounds (PyH\(_3\), CatH\(_2\), and MoCatH\(_2\)) in DMF, calculated using DFT at the (U)B3LYP/PCM/6-311+G(3df,2p) level.

<table>
<thead>
<tr>
<th>Reactants</th>
<th>( ^1 ) FR</th>
<th>TS ( (E_a) )</th>
<th>PC</th>
<th>FP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PyH(_3) (+O(_2^{-}))</td>
<td>81.2</td>
<td>53.9</td>
<td>−29.4</td>
<td>68.9</td>
</tr>
<tr>
<td>CatH(_2) (+O(_2^{-}))</td>
<td>71.6</td>
<td>52.5</td>
<td>−20.9</td>
<td>45.3</td>
</tr>
<tr>
<td>MoCatH(_2) (+O(_2^{-}))</td>
<td>61.4</td>
<td>50.7</td>
<td>−28.4</td>
<td>38.5</td>
</tr>
</tbody>
</table>

\( ^1 \Delta G^0 \) values (kJ mol\(^{-1}\)) of PRC were set as a zero point.

Taken together, these findings indicate that the \( O_2^{-} \) scavenging by PyH\(_3\) 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 6a. In Figure 8,
the net mechanism of the O$_2^\bullet^-$ scavenging by PyH$_3$ in DMF is shown. In the 2PCET mechanism, ET occurs between oxygen-$\pi$-orbitals orthogonal to the molecular framework, then, PT occurs between oxygen-$\sigma$-orbitals along the HBs [23]. It is presumed that the higher reactivity of PyH$_3$ with O$_2^\bullet^-$ than that for CatH$_2$, is due to the sequential reactions; the initial formation of the PRC, followed by the 2PCET.

![Figure 8](image-url) Plausible mechanism and the $\Delta G$ and $E_a$ values (kJ mol$^{-1}$, 298.15 K) for the PCET pathways between PyH$_3$ and O$_2^\bullet^-$ involving the initial formation of PRC followed by concerted 2PCET in DMF. The $\Delta G$ and $E_a$ values were calculated using DFT-(U)B3LYP/PCM/6-311+G(3df,2p) method.

4. Conclusions

In conclusion, we have investigated the O$_2^\bullet^-$ scavenging by PyH$_3$ through the PCET in DMF. As a result, we have clarified;

- PyH$_3$ scavenges O$_2^\bullet^-$ through the 2PCET involving concerted two PTs and one ET, in a similar mechanism for CatH$_2$;
- the net mechanism involves the initial formation of PRC followed by concerted 2PCET;
- the 3OH group thermodynamically promotes the formation of PRC via two HBs but does not promote the latter 2PCET, resulting in an effective O$_2^\bullet^-$ scavenging ability of PyH$_3$.

Although the results presented in this manuscript are for a chemical reaction in aprotic DMF solvent, the PCET theory is adaptable to biological processes in such as a lipid bilayer. Therefore, we hope that the findings obtained in this study will provide evidence for the mechanistic actions of O$_2^\bullet^-$ scavenging by various antioxidants involving PyH$_3$ moiety.

Supplementary Materials: The following is available online at https://www.mdpi.com/article/10.3390/electrochem3010008/s1, Table S1: CV parameters, Tables S2–S5: $\Delta G$ values for the PCET in various solvents, Table S6: Calculated geometry of complexes, Figure S1: In situ electrolytic ESR/UV-vis system, Figure S2: Energy profiles along IRC of 2PCET between CatH$_2$ and O$_2^\bullet^-$, Figure S3: Energy profiles along IRC of 2PCET between MoCatH$_2$ and O$_2^\bullet^-$.

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