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Quantum mechanics study on synthetic model of copper-containing quercetin 2,4-dioxygenase

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ABSTRACT

We report a computational study on the dioxygenation reaction of the substrate flavonolate (fla) by a synthetic model complex $[Cu^{2+}(idpa)(fla^{-})]^{+}$ (idpa = 3,3′-iminobis(*N*,*N*-dimethylpropylamine) and related species mimicking quercetin 2,4-dioxygenases. It is found that the reaction mechanism obtained for the present biomimetic complexes is substantially different from the plausible enzymatic reaction. All model complexes favor a single electron transfer from flavonolate to dioxygen over a valence tautomerism $[Cu^{2+}(idpa)(fla^{-}) \leftrightarrow Cu^{+}(idpa)(fla^{\cdot})]$, and a subsequent intersystem crossing and a ring-closure lead to a formation of a 1,2-dioxetane intermediate instead of undergoing a direct formation of a precursor endoperoxide. The generation of the 1,2-dioxetane intermediate is shown to be the rate-determining step and inclusion of a carboxylate co-ligand can enhance the reactivity, rendering this process barrier-free. We have also proposed for the first time a pathway, which can circumvent a non-enzymatic reaction by involving conversion from the 1,2-dioxetane to the endoperoxide with lower barriers.

1. Introduction

Oxygenation reactions are of considerable interest in academic and industrial research, and much effort has been directed at understanding how to activate C–H and C–C bonds and developing catalysts to make these reactions feasible under mild conditions [1]. The conversion of aromatic compounds to water-soluble products is expected to be useful from an environment point of view [2]. Dioxygenases basically activate dioxygen and catalyze a dioxygenation reaction, in which two oxygen atoms are incorporated and the subsequent aromatic C–C bond cleavage occurs [3–5]. This group of enzymes employ transition metals (typically Fe) or redox organic cofactors for catalysis [6,7]. A specific group of ring-cleaving dioxygenases, called cofactor-free dioxygenase, can also catalyze O_2 incorporation without any cofactor [8–10]. Here, we focus on quercetin (3,3′,4′,5,7-pentahydroxyflavone) (Que) 2,4-dioxygenases (2,4-QDs), which cause the scission of the C2–C3 and C3–C4 bonds of Que to provide the phenolic carboxylic acid ester and carbon monoxide as depicted in Scheme 1 [11–16].

2,4-QDs can contain several metal ions as its cofactor that influence the activity of the protein. The fungal 2,4-QD from *Aspergillus japonicus* uses a Cu^{2+} ion, while bacterial 2,4-QDs from *Bacillus subtilis* are capable of incorporating several divalent metal ions (Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+}) [13–16]. It is noteworthy Fe^{2+} might not be the correct cofactor for 2,4-QD as well as redox-inactive Zn^{2+} [13,14].

Concerning the mechanism of the enzymatic reaction for the well-studied Cu^{2+}-containing 2,4-QD [11,12,17–32], a valence tautomerism $[Cu^{2+}(Que^{-}) \leftrightarrow Cu^{+}(Que^{\cdot})]$, caused by one electron transfer from the substrate anion (Que^{-}) to the cupric ion, is considered as a trigger reaction. Then, the proximal oxygen atom (O_p) of dioxygen (see Scheme 2) may attack on the enzyme-substrate (ES) complex and leads to a formation of either an ES–O_2 complex with the Cu–O_p–O_d bond (path A) or that with the C2–O_p–O_d bond (path B) [11].

For bacterial 2,4-QDs in which Co^{2+} or Ni^{2+} is employed as a cofactor, direct electron transfer from the activated Que^{-} to dioxygen (path C) may occur in analogy with cofactor-free dioxygenases [9,10]. The subsequent radical ($Que^{\cdot}$)-radical(O_2^{-}) coupling via intersystem crossing leads to an ES–O_2 complex, because Co^{2+} and Ni^{2+} are expected to be redox-inactive in this process [14]. From path A, a concomitant attack of the distal oxygen atom (O_d) on the C2 atom of $Que^{\cdot}$ generates an alkylperoxo intermediate, which might proceed through nucleophilic attack on the C4 atom to yield an endoperoxide intermediate (**EP**) with the C2–O_p–O_d–C4 bond. Finally, the C2–C3 and C3–C4 bonds would be cleaved

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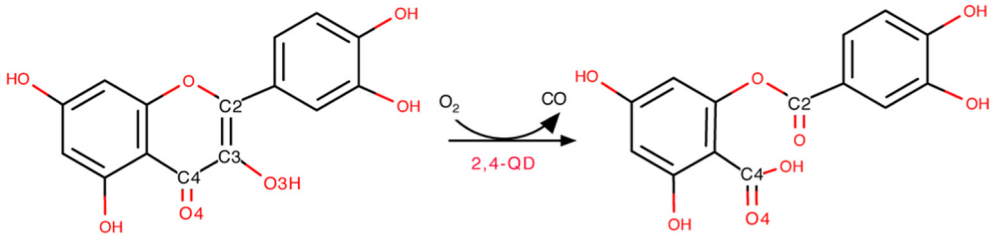
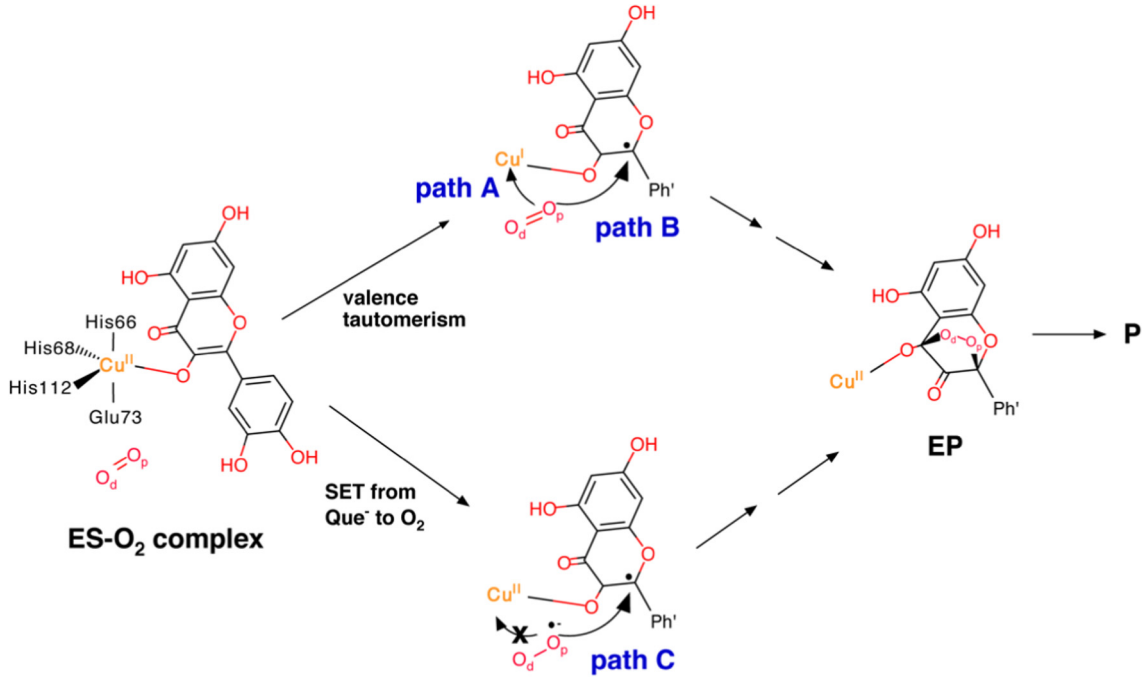


Scheme 1. Cleavages of the C2–C3 and C3–C4 bonds of quercetin (Que) catalyzed by 2,4-QDs.



Scheme 2. O_2 attack on Cu^+ (path A) or $Que^{\cdot}$ (path B) after the valence tautomerism, and a single-electron transfer from Que^- to O_2 (path C). Except for the ES-O_2 complex, amino residues are omitted and the catechol moiety is represented as Ph' for simplicity.

upon the O_p–O_d bond scission, and a resulting product complex (**P**) is obtained. The precursor **EP** can also be generated from path B and C by direct nucleophilic attack of the O_d atom on the C4 atom. However, theoretical studies using both an active-site model and the entire system supported path A, insofar as the copper-containing 2,4-QD is concerned [28,30] in a similar way to Fe^{3+}-containing intradiol dioxygenases and their model complexes [33–36]. Very recently, we found that an energetically unstable intermediate with the C2–O_p–O_d bond in path B ends up being an alkylperoxo intermediate by creating the Cu–O_d–O_p–C2 bond [30]. No reaction pathway via path C was found because the ES complex significantly favors path B over path C.

On the other hand, synthetic model complexes with a series of divalent metal ions (Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}) show an enzyme-like stoichiometric dioxygenation reactivity [23,25,26]. These biomimetic studies used synthetic metal flavonolate (fla^-) complexes. While the ES complex has the monodentate coordination in 2,4-QD, the model complexes basically have the bidentate coordination of flavonolate. It may cause a non-enzymatic reaction through not **EP** but a 1,2-dioxetane in the presence of 1,10-phenanthroline as a ligand [31]. The use of another ligand such as 3,3′-iminobis(*N*,*N*-dimethylpropylamine) (idpa) and heating leads to a $[Cu^{2+}(idpa)(fla^-)]^+$ complex (**1**) (Fig. 1), which can react with dioxygen to decompose flavonolate catalytically [20]. The use of Zn^{2+} in place of Cu^{2+}, namely a $[Zn^{2+}(idpa)(fla^-)]^+$ complex (**2**), also provides the same product as the enzymatic reaction, albeit with the stoichiometric reaction [21]. Barhács et al. reported that the activation enthalpies at 373 K were 14–18 and 17–19 kcal/mol for **1** and **2**, respectively [20,21]. Besides, inclusion of a carboxylate co-ligand such as the triphenylacetate anion (tpa^-) into **1**, $[Cu^{2+}(idpa)(fla^-)(tpa^-)]$ (**3**), further enhances the reactivity probably because the steric hindrance caused by the bulky ligand changes the bidentate coordination to the monodentate fashion [32]. Since the dioxygenation reaction can occur in **2** unlike a Zn^{2+}-containing 2,4-QD, another reaction mechanism without involving a valence tautomerism, more specifically path C might be considered.

Regarding these facts, there might be critical differences in reaction pathways between the protein and model complexes, and thus a better understanding of the reaction mechanisms of synthetic model complexes will provide many insights into the dioxygenation reaction. In this study, we have focused on the model complexes **1**–**3** (see Fig. 1) and explored their reaction mechanisms by means of the spin-unrestricted density functional theory (UDFT) calculations. The roles of metal ions (Cu^{2+} and Zn^{2+}) and co-ligand (tpa^-) in dioxygenation reactivity are discussed in comparison with experimental results. To the best of our knowledge, this is the first study of the oxidative cleavage of flavonolate in synthetic model complexes of 2,4-QD.

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