ABSTRACT: It has been shown that the major reactive oxygen species (ROS) generated by the aqueous reaction of Fe(II) and H₂O₂ (i.e., the Fenton reaction) are high-valent oxoiron(IV) species, whereas the hydroxyl radical plays a role only in very acidic conditions. Nevertheless, when the Fenton reaction is conducted in phosphate buffer solutions, the resulting ROS turns into hydroxyl radical even in neutral pH conditions. The present density functional theory (DFT) study discloses the underlying principle for this phenomenon. Static and dynamic DFT calculations indicate that in phosphate buffer solutions, the iron ion is highly coordinated by phosphoric acid anions. Such a coordination environment substantially raises the pKₐ of coordinated water on Fe(III). As a consequence, the Fe(III)–OH intermediate, resulting from the reductive decomposition of H₂O₂ by ferrous ion is relatively unstable and will be readily protonated by phosphoric acid ligand or by free proton in solution. These proton-transfer reactions, which become energetically favorable when the number of phosphate coordination goes up to three, prevent the Fe(III)–OH from hydrogen abstraction by nascent •OH to form Fe(IV)═O species. On the basis of this finding, a ligand design strategy toward controlling the nature of ROS produced in the Fenton reaction is put forth. In addition, it is found that while phosphate buffers facilitate •OH radical generation in the Fenton reaction, phosphoric acid anions can act as •OH radical scavengers through hydrogen atom transfer reactions.

1. INTRODUCTION

The generation of reactive oxygen species (ROS) via the catalytic decomposition of hydrogen peroxide by ferrous ion is referred to as the Fenton reaction. The Fenton and Fenton-like reactions are ubiquitous and have a broad range of applications. For example, they take place in natural environments and have been considered to be a pathway of ROS generation in atmospheric clouds and in aquatic environments such as river and ocean. They also inevitably occur in the human body and have been thought to have connections with aging and many diseases, including neurodegenerative diseases, cardiovascular diseases, and cancers. In addition, the Fenton and Fenton-like reactions are listed as advanced oxidation processes that are promising techniques for wastewater treatment and environmental remediation. They can also be applied in organic synthesis and the preparation of graphene quantum dots. Recently, the application of Fenton chemistry in cancer diagnosis and therapy has been actively developed. An appealing strategy for cancer therapy called chemodynamic therapy, which is defined as in situ treatments using Fenton chemistry to selectively produce ROS in tumor sites, has been proposed.

The identity of ROS produced in the Fenton reaction has been a longstanding dispute. The mechanism of the Fenton reaction is rather complicated, as it can be affected by various factors such as the concentration of Fe(II) and H₂O₂, the pH condition, the nature of ligand and buffer, and so on. So far, experimental evidences have demonstrated that in non-coordinating buffer solutions, the high-valent oxoiron(IV) species is the major ROS produced by the Fenton reactions under the conditions of pH > 3, whereas the hydroxyl radical becomes dominant merely in more acidic conditions. The preference for the formation of oxoiron(IV) species has also been supported by density functional theory (DFT) studies. The DFT calculations further pointed out that the oxoiron(IV) aqua complexes can exist in three protonation states, [(H₂O)₅FeIV O]²⁺, [(H₂O)₄FeIV O(OH)]⁺, and [(H₂O)₃FeIV O(OH)₂], depending on the pH conditions. The oxidizing power of the oxo group in these complexes was found to be quite dissimilar and principally determined by the total charge of complexes. On the other hand, when the Fenton reaction is performed in coordinating phosphate buffer solutions, the resulting ROS switches to •OH radical even in a neutral pH for some unknown reason.

The capability of controlling the formation of hydroxyl radical or oxoiron(IV) species in the Fenton reaction is of importance, as these two reactive species have their own applications. The hydroxyl radical is powerful but nonselective and can be exploited in catalytic synthesis such as C–H bond
activation and epoxidation. Furthermore, phosphate buffer is one of the most commonly used buffer systems. The knowledge of how phosphate buffer influences the property and the reaction behavior of transition-metal ions is essential to the correct interpretation of the experimental results. Unfortunately, the relevant knowledge is very limited and even the nature of the coordination sphere of metal ions in phosphate buffers is not clear. This inspired me to conduct the DFT study aiming to determine the coordination sphere of ferrous iron in phosphate-buffered solution and to disclose how such a coordination environment alters the mechanism of the Fenton reaction.

2. COMPUTATIONAL METHODS

All the DFT calculations were achieved by using Gaussian 09 and Gaussian 16 program.54 Geometry optimization and vibrational frequency calculations were carried out in an aqueous environment described by the conductor-like polarizable continuum model (CPCM).56,57 Grimme’s D3 dispersion correction58 was added to improve the description of noncovalent interactions. To ensure that the wave functions obtained are lowest-energy solutions, the calculation of wave function stability test was followed for each stationary point. The thermal correction to the Gibbs free energy was made at standard conditions of 298.15 K and 5.55 M for H2O and 1 M for the remaining species. The setting of ultrafine grids was employed for numerical integrations.

2.1. Ligand-Exchange Reaction. The free energy changes of stepwise displacement of H2O by H2PO4− from [FeII(H2O)n]3+ were calculated to determine the component of ferrous complex in phosphate buffer solution. The accuracy of these computations largely depends on the performance of the DFT method in describing the metal–ligand bonding interactions and the performance of the continuum solvation model in estimating the hydration energies of species involved. To select a proper DFT method, the gas-phase energy change of a simplified ligand exchange reaction, [FeII(H2O)n]3+ + H2PO4− → [FeII(H2PO4)n]3+ + H2O, was calculated by some widely used exchange-correlation functionals (B3LYP59–61-D3, PBE062-D3, M0663–D3, PW6B9564-D3) in combination with 6-31+G(d) basis set. Among these functionals, the result of PW6B95-D3 most closely matches the reference value derived from the Brueckner coupled cluster BD(T)/Def2-TZVP calculations (rows 2–5 in Table S1). The effect of the basis set was also examined using the PW6B95-D3 functional, and it turned out that increasing the size of the basis set did not improve the result (rows 5–7 in Table S1). Regarding the continuum solvation model, three popular methods (integral equation formalism polarizable continuum model—IEFPCM65, CPCM,56,57 solvent model density—SMD66) were tested by calculating the hydration energies of H2O and H2PO4− for which the experimental values are available. The results show that the solvent model density (SMD) method overestimates the hydration energies of H2O and H2PO4− by 3 and 9 kcal/mol, respectively (Table S2). This is probably because the SMD method was parameterized using gas-phase-optimized geometries rather than aqueous-phase-optimized geometries adopted in the present study. By contrast, integral equation formalism polarizable continuum model (IEFPCM) and CPCM methods offer reasonable hydration energies of both species when aqueous-phase geometries are used. On the basis of these benchmark studies, CPCM/PW6B95-D3/6-31+G(d) method was selected to investigate the ligand-exchange reactions of ferrous complexes.

2.2. Quantum Molecular Dynamics. DFT molecular dynamics simulations were carried out using the atom-centered density matrix propagation (ADMP) approach.67,68 The temperature of the ADMP simulations was kept at 300 K by velocity scaling at every 10 steps. The number of steps in each simulation is 10,000, with the step size of 0.1 fs. The fictitious electronic mass was set to 0.1 amu. Molecular coordinates were collected at 20-step intervals for analysis.

2.3. Fenton Reaction and pH Estimation. The CPCM/PBE0-D3/6-31+G(d) method was used to study the mechanism of Fenton reactions. Our previous benchmark study has verified that this level of theory can nicely reproduce the Brueckner coupled cluster BD(T)/Def2-TZVP results for the relative energies of key Fenton intermediates.65 The pKa values of coordinated H2O of the iron complexes were evaluated by using the linear equation: pK_a = 2.0847ΔG_deprot − 47.59790, where ΔG_deprot is the deprotonation free energy, G(A−) − G(HA), computed by the CPCM/PBE0-D3/6-31+G(d) method. This equation has been demonstrated to be robust for predicting the pKa of the aqua complexes of the first-row transition-metal ions with the error being within 0.6 pKa unit.65

3. RESULTS AND DISCUSSION

3.1. Coordination Sphere of Fe(II). Phosphoric acid is a polyprotic acid with three pKa of 2.15, 7.20, and 12.32, respectively. For the sake of simplicity, here only the existence of dihydrogen phosphate ion was considered, which corresponds to the condition of pH ~ 5. To determine the coordination sphere of Fe(II) in pH 5 phosphate buffer solution, the free energy changes of successive replacements of H2O by H2PO4− for [FeII(H2O)n]3+ were evaluated by the CPCM/PW6B95-D3/6-31+G(d) method (Table 1). Other combinations of DFT functionals, solvation models, and basis sets were also applied to compute the ligand-exchange energies (Table S3). These calculations consistently reveal that all the six ligand-exchange processes are exergonic. However, it should be born in mind that these reaction free energies were derived from the calculations with the standard state concentrations of 5.5 M for H2O and 1 M for the remaining species. In realistic experiments, the typical concentrations of Fe(II) and phosphate buffer are 1 and 10 mM, respectively. Using these experimental concentrations and the equilibrium constants derived from the CPCM/PW6B95-D3/6-31+G(d) free energies, the percentage formation of each

| n  | ΔG | K_a | % formation |
|----|----|-----|-------------|
| 1  | −17.4 | 5.678 × 10^4 | 0 |
| 2  | −15.2 | 1.386 × 10^11 | 0 |
| 3  | −10.8 | 8.249 × 10^7 | 0 |
| 4  | −13.6 | 9.307 × 10^4 | 4 |
| 5  | −7.4  | 2.656 × 10^8 | 88 |
| 6  | −4.1  | 1.012 × 10^10 | 8 |

*[^{[Fe] = 1 mM; [buffer] = 10 mM.}]*
The major form of ferrous ions in phosphate buffers of pH 5 is $[\text{Fe}^{II}(\text{H}_2\text{PO}_4)_5(\text{H}_2\text{O})]^3^-$, with the percentage being ca. 90% (Table 1). The optimized structure of $[\text{Fe}^{II}(\text{H}_2\text{PO}_4)_5(\text{H}_2\text{O})]^3^-$ is depicted in Figure 1. It can be seen that the $[\text{Fe}^{II}(\text{H}_2\text{PO}_4)_5(\text{H}_2\text{O})]^3^-$ complex maintains a distorted octahedral geometry. Notably, numerous hydrogen bonds (H-bonds) are formed between $\text{H}_2\text{PO}_4^-$ ligands and between $\text{H}_2\text{PO}_4^-$ and $\text{H}_2\text{O}$ ligands in this structure (denoted by dotted line and label HB in Figure 1). These interligand H-bonding interactions are expected to partially relieve the electrostatic repulsion between the anionic ligands and thus provide a stabilization for the highly negatively charged six-coordinate complex.

DFT molecular dynamics simulations with the CPCM/PW6B95-D3/6-31G(d) method at $T = 300$ K were carried out to see if $[\text{Fe}^{II}(\text{H}_2\text{PO}_4)_5(\text{H}_2\text{O})]^3^-$ is still stable when thermal fluctuations are taken into account. Figure 2 represents the time evolution of the metal–ligand bond distances for the simulation starting from the optimized geometry. The results showed that all the six Fe(II)–ligand bond lengths displayed an oscillating behavior and no sign of ligand dissociation was observed within a 1 ps simulation. The average value and the standard deviation of the metal–ligand bond distances are summarized in Table S4. As can be seen, the Fe(II)–water bond ($\text{FeL}_1$) exhibits a larger bond distance and fluctuation compared to the Fe(II)–phosphate bonds ($\text{FeL}_{2-6}$), reflecting the fact that the bonding between Fe(II) and $\text{H}_2\text{O}$ is weaker. Furthermore, the fluctuation of H-bonds between ligands was found to be relatively large, especially those involving $\text{H}_2\text{O}$. Figure 3 clearly indicates that the H-bonds between water and phosphate ligands (HB$_{1-3}$) are more fragile than the H-bonds between phosphate ligands (HB$_{4-9}$); this can also be seen from the bond length data summarized in Table S4, which shows that in general the average bond distance and the deviation of HB$_{4-9}$ are smaller than those of HB$_{1-3}$. In fact, the H-bonds between the phosphate ligands are relatively robust and should play a role in stabilizing the structure of $[\text{Fe}^{II}(\text{H}_2\text{PO}_4)_5(\text{H}_2\text{O})]^3^-$. Since the computational cost of ADMP dynamics simulations with hybrid functionals is expensive, especially for the open-shell systems like the ferrous ion complex investigated in this work, a longer simulation is not allowed with current computing facilities in our laboratory. Instead, another two 1 ps ADMP dynamics simulations of $[\text{Fe}^{II}(\text{H}_2\text{PO}_4)_5(\text{H}_2\text{O})]^3^-$, starting from different initial geometries and kinetic energies, were carried out (Figures S1 and S2). In general, the dynamic
behaviors of Fe(II)−phosphate bonds and H-bonds between ligands in the three trajectories are similar. In all simulations, the Fe(II)−phosphate bonds vibrated with a moderate amplitude and were never actually broken. However, the dissociation of water ligand was detected in one of the trajectories (FeL1 in Figure S2). This, in turn, leaves an open site for subsequent catalytic reaction with hydrogen peroxide.

One would think that a total charge of $-3$ for the ferrous complex with five phosphoric acid ligands is very unlikely. Notwithstanding, there are indeed few examples of highly negatively charged ferrous complex in literature, let us recall a well-known case of ferrocyanide $[\text{Fe}^{II}(\text{CN})_6]^{4-}$. While this complex bears a total charge of $-4$ with six anionic cyanide ligands, it is very stable and not to release free cyanide in aqueous solution.

### 3.2. Fenton Reaction of $[\text{Fe}^{II}(\text{H}_2\text{PO}_4)_5(\text{H}_2\text{O}_2)]^{3-}$

In light of the foregoing results, the Fenton reaction in pH 5 phosphate buffer was assumed to be initiated by the reaction of $[\text{Fe}^{II}(\text{H}_2\text{PO}_4)_5(\text{H}_2\text{O})]^{3-}$ with $\text{H}_2\text{O}_2$, leading to the formation of the reactant complex $[\text{Fe}^{II}(\text{H}_2\text{PO}_4)_5(\text{H}_2\text{O}_2)]^{3-}$. Figure 4 represents the free energy profile for the Fenton reaction calculated by the CPCM/PBE0-D3/6-31+G(d) method. This level of theory has been shown to be faithful in predicting the relative energies of the two critical intermediates in the Fenton reaction, namely the hydroxyl radical and the oxoiron(IV) species.53 In addition, the reaction was calculated at a high-spin quintet state ($S = 2$), which has been manifested to be the ground state for the aqueous Fenton reaction.43,47,52 In fact, all the intermediates shown in Figure 4 have been reoptimized at singlet and triplet states. The results showed that these low-spin intermediates were substantially higher than the quintet state counterparts by 13−43 kcal/mol, indicating that the phosphate coordination does not cause the reversal of the spin states for the Fenton reaction.

Figure 4. PBE0-D3/6-31+G(d) free energy profile for the Fenton reaction in phosphate buffer ($L = \text{H}_2\text{PO}_4^-$ and $L' = \text{HPO}_4^{2-}$). The gray line represents the result in nonbuffered aqueous solution, namely, the $[\text{Fe}^{II}(\text{H}_2\text{O})_5(\text{H}_2\text{O}_2)]^{2+}$ model.

Figure 5. PBE0-D3/6-31+G(d) optimized geometries and selected bond lengths for the intermediates and transition states in the Fenton reaction.
Starting from the reactant complex \([\text{Fe}^{III}(\text{H}_2\text{PO}_4)_6(\text{H}_2\text{O})_3]^{3-} (\text{RC})\), the subsequent step is the homolysis of the \(\text{HO}--\text{OH}\) bond to generate the ferric intermediate \([\text{Fe}^{IV}(\text{H}_2\text{PO}_4)_5(\text{OH})]^{3-}/\text{OH} (\text{INT1OH}/\text{OH})\) via the transition state \(\text{TS}_{\text{INT}}\). This process is characterized by an activation energy of 11.8 kcal/mol and slightly exergonic by 0.6 kcal/mol. Through the rotation of the \(\text{OH}\) groups, this ferric intermediate is transformed into a more stable conformation, \(\text{INT2OH}/\text{OH}\), in which the \(\text{OH}\) group of a phosphoric acid ligand acts as a H-bond donor to stabilize the hydroxide group on Fe(III) (Figures 4 and 5). This H-bond is fairly strong with the \(\text{OH}--\text{OH}\) distance being 1.46 Å, rendering \(\text{INT2OH}/\text{OH}\) lower in energy than \(\text{INT1OH}/\text{OH}\) by 7.4 kcal/mol. From \(\text{INT2OH}/\text{OH}\) there are two possible reaction pathways. The nascent \(\text{OH}\) radical could attack and abstract the hydrogen atom of the hydroxide group on Fe(III) to generate the ferryl-oxo intermediate \([\text{Fe}^{IV}(\text{H}_2\text{PO}_4)_5(\text{O})]^{3-}/\text{OH} (\text{INT3O} / \text{OH})\) via the transition state \(\text{TS}_{\text{INT}}\) (blue path in Figure 4). The activation energy of this step is 7.7 kcal/mol, and the formation of \(\text{INT3O} / \text{OH}\) is exergonic by 8.4 kcal/mol. Alternatively, the hydroxide group on Fe(III) can be immediately protonated by the phosphoric acid ligand via the transition state \(\text{TS}_{\text{INT}}\) resulting in the formation of the ferric iron complex of \([\text{Fe}^{III}(\text{H}_2\text{PO}_4)_4(\text{HPO}_4)(\text{H}_2\text{O})]^{3-}\) and \(\text{OH} (\text{INT3OH} / \text{OH})\) (red path in Figure 4). This interligand proton transfer is barrier-free and the formation of \(\text{INT3OH} / \text{OH}\) is exergonic by 12.8 kcal/mol and, therefore, is more favorable than the formation of \(\text{INT3O} / \text{OH}\) in terms of both kinetics and thermodynamics. The further dissociation of \(\text{OH}\) from \(\text{INT3OH} / \text{OH}\) was found to be slightly exergonic by 1 kcal/mol. These results rationalize the experimental observation that the major Fenton oxidant in phosphate buffered solutions starting from \([\text{Fe}^{II}(\text{H}_2\text{O})_5(\text{H}_2\text{O}_2)]^{2+}\) are Fe(III) and Fe(II) complexes. In that computational study, the authors demonstrated that the coordinated phosphoric acid assists the production of \(\text{H}_2\) by delivering proton to metal hydride.

It should be mentioned that the electronic potential energy surface along the reaction coordinate of the interligand proton transfer is quite flat. As a consequence, although the \(\text{TS}_{\text{INT}}\) was indeed a transition state on the electronic potential energy surface, it became lower in energy than its precursory intermediate \(\text{INT2OH}/\text{OH}\) upon zero-point energy and free energy corrections. To provide a more solid evidence that the interligand proton transfer can be considered as a barrierless process under thermal fluctuations, ADMP dynamics simulation with the CPCM/PBE0-D3/6-31G(d) method was carried out for \([\text{Fe}^{III}(\text{H}_2\text{PO}_4)_5(\text{OH})]^{3-}\) starting from the optimized geometry. The simulation revealed that the proton transfer from \(\text{H}_2\text{PO}_4^-\) to \(\text{HO}^\text{-}\) immediately occurred at 0.15 ps (Figure 6), supporting the statement that the process is barrierless.

For comparison, the results of the Fenton reaction in nonbuffered solutions starting from \([\text{Fe}^{III}(\text{H}_2\text{O})_5(\text{H}_2\text{O})]^{2+}\) are also illustrated in Figure 4 (gray line). In general, the coordination of \(\text{H}_2\text{PO}_4^-\) to iron was found to facilitate the Fenton reaction by systematically lowering the energies of the transition states and the intermediates along the reaction coordinate. This can be attributed to the stronger bonding between \(\text{H}_2\text{PO}_4^-\) and iron in the Fe(III) and Fe(IV) intermediates than in the Fe(II) reactant complex. A closer look further reveals that the \(\text{H}_2\text{PO}_4^-\) coordination provides a somewhat larger stabilization for the Fe(III) intermediate (decreasing from 8.8 to −0.6 kcal/mol for \(\text{INT1OH}/\text{OH}\)) than for the Fe(IV) intermediate (decreasing from −3.3 to −8.4 kcal/mol for \(\text{INT3OH} / \text{OH}\)). It should be borne in mind that although the oxidation state of iron in ferryl-oxo species is conventionally assigned as +4, the strong covalent character of Fe(IV)−O bond makes the actual charge of iron in the ferryl-oxo intermediate substantially less positive than that in the ferric intermediate. This is evidenced by the natural population analysis (NPA) showing that the atomic charge of iron is 1.367 and 1.102 au in \(\text{INT1OH}/\text{OH}\) and \(\text{INT3OH}\) respectively. As a consequence, the bonding between the iron and phosphoric acid anions should be somewhat stronger in the former than in the latter.

3.3. \(\text{pK}_a\) Variation with the Number of \(\text{H}_2\text{PO}_4^-\) Coordination. At first glance, it seems surprising that the proton transfer from \(\text{H}_2\text{PO}_4^-\) to Fe(III)−OH (i.e., \(\text{INT2OH}/\text{OH} \Rightarrow \text{INT3OH} / \text{OH}\)) is thermodynamically favorable, if one considers the fact that the \(\text{H}_2\text{PO}_4^-\) \((\text{pK}_a = 7.2)\) is considerably less acidic than the coordinate water of \([\text{Fe}^{III}(\text{H}_2\text{O})_5]^3+\) \((\text{pK}_a = 2.2)\). Since the proton-transfer step plays a critical role in prohibiting the Fe(IV)−O formation, detailed analysis on how the \(\text{pK}_a\) of the coordinated \(\text{H}_2\text{O}\) in \([\text{Fe}^{III}(\text{H}_2\text{PO}_4)_5(\text{H}_2\text{O})]^{3-}\) varies with the number of \(\text{H}_2\text{PO}_4^-\) coordination, \(n\), was carried out. The results show that the \(\text{pK}_a\) of the coordinated water monotonically increases from 2.2 to 7.5 until \(n = 3\), then becomes slow, and reaches 8.9 at \(n = 5\) (Table 2). The enhancement of \(\text{pK}_a\) upon \(\text{H}_2\text{PO}_4^-\) coordination is caused by two factors. One is the electron transfer from \(\text{H}_2\text{PO}_4^-\) to the metal center, which, in turn, increases the electron density on iron and thus reduces the acidity of the coordinated water. This effect can be seen at the \(n = 0−3\) region, where the atomic charge on Fe decreases from 1.449 to 1.370 au. The other factor is the electrostatic repulsion between the resulting hydroxide anion and \(\text{H}_2\text{PO}_4^-\) ligands that increases the deprotonation energy and hence the \(\text{pK}_a\) of coordinated water. This electrostatic effect is expected to be augmented with the number of \(\text{H}_2\text{PO}_4^-\) ligand and is responsible for the increase in \(\text{pK}_a\) at the \(n = 3−5\) region in which the ligand-to-metal electron transfer is saturated (Table 2). The influence of the \(\text{H}_2\text{PO}_4^-\) ligand on the acidity of Fe(III) is relatively moderate in comparison to that of the \(\text{OH}^\text{-}\) ligand. This can be evidenced by the fact that the first \(\text{pK}_a\) increment caused by the \(\text{H}_2\text{PO}_4^-\) substitution is 2.1 \((n = 0\text{ to }1)\).
The calculations without the explicit H2O reveal that both H2PO4− and HPO42− could efficiently scavenge the •OH radical. Both HAT reactions were highly exergonic and possessed a very small activation barrier (Table 3); the transition state for the HPO42− system cannot even be located, as a potential energy surface scan manifested a continuous descent of energy along the reaction coordinate of HAT. However, when explicit H2O molecules are incorporated in the calculations, the situation is somewhat different. Although the •OH radical scavenging reactions of H2PO4− and HPO42− are still feasible, now the activation barrier is increased and the exergonicity is reduced and becomes less efficient compared to that of tert-butyl alcohol (Table 3). The reduction of •OH radical scavenging activity of H2PO4− and HPO42− in explicit solvation model is ascribed to the charge transfer from anionic solutes to H2O solvent molecules, the effect that is not taken into account in implicit solvation model. The natural population analysis indicates that about −0.032 and −0.250 |e| negative charges are, respectively, transferred from H2PO4− and HPO42− to H2O solvent molecules; the delocalization of the negative charge is intuitively expected to stabilize anions and, therefore, diminish their ability to be oxidized. By contrast, the effect of charge transfer between solute and solvent molecules is negligible for neutral tert-butyl alcohol; the NPA charge on tert-butyl alcohol in explicit solvation model is only 0.001 au, very close to neutral. As a result, the incorporation of explicit H2O displays a relatively minor influence on the computed energies of the HAT reaction for tert-butyl alcohol (Table 3).

The present calculations indicate that even though the Fenton reaction in phosphate buffers results in the •OH radical formation, the reaction of •OH with phosphates can act to remove it from the reaction mixture. However, it does not mean that this system cannot be used as reactive species because almost all organic compounds react with •OH with a faster rate than phosphates.75,76

### 3.4. •OH Radical Quenching by H3PO4 and HPO42−

The effect of buffer solutions on the generation of •OH radical in the Fenton reaction has been investigated by spin trapping experiments. It was found that the generation of •OH was significantly reduced in coordinating phosphate buffer compared to that in non-coordinating N-(2-hydroxyethyl)-piperazine-N’-ethanesulfonic acid or 3-(N-morpholino) propanesulfonic acid buffers.4 This experimental observation seems to be in conflict with the present computational result revealing that the coordination of H2PO4− to Fe(II) facilitates the Fenton reaction both in kinetic and thermodynamic aspects (Figure 4). One possible explanation for the discrepancy is that the phosphate buffer does not inhibit the Fenton reaction but instead scavenges hydroxyl radicals. To test this possibility, the reactions of hydrogen atom transfer (HAT) from H2PO4− and HPO42− to •OH radical were simulated by models with and without explicit water molecules. For comparison, the corresponding reaction of tert-butyl alcohol, a common scavenger for •OH radical, was computed as well. The calculated activation energies and reaction energies are given in Table 3, and the optimized transition state structures are depicted in Figure 7.

### 3.5. Ligand Design Strategy for ROS Control

On the basis of these DFT results, a strategy to control the nature of

### Table 2. Atomic Charge qFe (au) and pK_a of [FeIII(H2PO4)(H2O)]6− and Activation and Reaction Free Energies (kcal/mol) of the Proton Transfer from H2PO4− to OH− in [FeIII(H2PO4)3(OH)(H2O)]2−

| n     | qFe | pK_a | ΔGaf | ΔGam |
|-------|-----|------|------|------|
| 0     | 1.449 | 2.2 (pK_a) | 6.3 (pK_a) | N.A. | N.A. |
| 1     | 1.413 | 4.3 | 4.0 | 5.4 |
| 2     | 1.386 | 6.0 | 2.7 | 2.7 |
| 3     | 1.370 | 7.5 | 0.0 | −0.4 |
| 4     | 1.370 | 8.5 | −0.9 | −3.0 |
| 5     | 1.392 | 8.9 | −0.5 | −2.7 |

Experimental data from Dalton Trans. 2009, 10223.

→ 1 in Table 2), substantially smaller than the pK_a increment of 4.1 caused by the OH− substitution derived from the experimental pK_a = 2.2 and pK_a = 6.3 for [FeIII(H2O)]6−.

It is worth noting that the pK_a of the coordinated water becomes larger than that of H2PO4− at n ≥ 3, implying that the interligand proton transfer from H2PO4− to Fe(III)−OH will turn into a spontaneous process at this point. To verify this prediction, the evolution of the energy for the proton-transfer reaction with n was calculated as well. It can be seen that both the activation and the reaction free energies of the proton transfer reaction decrease with increasing n and the reaction turns into barrier-free and exergonic at n ≥ 3, in harmony with the variation of pK_a (Table 2). Moreover, the pK_a data in Table 2 implies that the hydride group of INT2OH+/OH− can also be protonated by the proton in the aqueous solution to form the [FeIII(H2PO4)(H2O)]2− and •OH radicals, which serve as an alternative route to block the formation of INTa•. The free energy of this protonation process is dependent on the pH value of the solution and can be estimated by the linear equation: ΔG_prot = RT ln10 (pH − pK_a).73 Taking the values of pH = 5 and T = 298.15 K, which are the simulated conditions here, and the pK_a = 8.9 for [FeIII(H2PO4)(H2O)]2− (Table 2), the protonation of INT2OH/INTa/INTb by the aqueous proton was calculated to be exergonic by 5.3 kcal/mol, comparable to the interligand proton transfer, which is exergonic by 4.8 kcal/mol (Figure 4), in thermodynamics.

### 3.4. •OH Radical Quenching by H3PO4 and HPO42−

The effect of buffer solutions on the generation of •OH radical in the Fenton reaction has been investigated by spin trapping experiments. It was found that the generation of •OH was significantly reduced in coordinating phosphate buffer compared to that in non-coordinating N-(2-hydroxyethyl)-piperazine-N’-ethanesulfonic acid or 3-(N-morpholino) propanesulfonic acid buffers.4 This experimental observation

### Table 3. PBE0-D3/Def2-TZVP//PBE0-D3/6-31+G(d) Activation and Reaction Free Energies (kcal/mol) for Hydrogen Atom Transfer Reactions

| reaction                                | without explicit H2O | with explicit H2O |
|-----------------------------------------|----------------------|------------------|
| H3PO4− + •OH → HPO42− + H2O       | 2.4                  | 7.8              |
| HPO42− + •OH → PO43− + H2O         | N.A.                 | 5.0              |
| (CH3)3COH + •OH → (CH3)2CHCOH + H2O | 3.8                  | 1.4              |

"Inclusion of 10 explicit H2O molecules in H2PO4− and HPO42− systems and five explicit H2O molecules in (CH3)2COH system."
ROS generated in the Fenton reaction was proposed. For a ligand able to promote the production of hydroxyl radical and suppress the formation of oxoiron(IV) species in the Fenton reaction should consist of two critical components. First, it must possess multiple anionic coordination sites. The purpose is to raise the pK_a of coordinated water on Fe(III), or in other words, to enhance the basicity of Fe(III)–OH. Second, the ligand should be furnished with dangling protic functional groups in the second coordination sphere. These protic functional groups can serve as a readily available proton source to protonate Fe(III)–OH, which, in turn, obstructs the further transformation to Fe(IV)=O.

4. CONCLUSIONS

DFT calculations have shown that the iron ion in phosphate buffers is highly coordinated by phosphate ions. Such a coordination environment significantly raises the pK_a of Fe(III) and, thus, inhibits the formation of the conjugate base Fe(III)–OH and the following transformation to Fe(IV)=O in a wide pH range extending to the basic site. Similar effect is expected to occur on other metal ions in phosphate buffers. This finding calls for caution against assigning unverified M=OH species (M = metals) in phosphate buffer solutions or even in other coordinating buffers, since conventional pK_a data for metal ions in aqueous solutions can be inappropriate in such buffer systems. Moreover, the calculations demonstrate that on the one hand the coordination of phosphate ions to Fe(II) can facilitate the Fenton reaction and promote the generation of •OH radicals, on the other hand, phosphate ions have the capability to scavenge •OH radicals, albeit not as efficiently as the commonly used scavengers such as tert-butyl alcohol. This result suggests that (i) intracellular fluids, which comprise a relatively high concentration of phosphate ions, provide a basic protection against •OH radicals and (ii) phosphate buffer is not a good choice for the study of •OH radical. The present study not only advances our ability to precisely control the Fenton oxidation process and expand its applications but also has a broad impact on the aqueous chemistry of transition-metal ions.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b02023.

Energy change of [Fe^{II}(H_2O)_6]^{2+} + H_2PO_4^- → [Fe^{III}HPO_4]^{2-} + H_2O (Table S1); Hydration energy of H_2O and H_2PO_4^- (Table S2); Free energy change of successive H_2O exchange by H_2PO_4^- for [Fe^{II}(H_2O)_6]^{2+} (Table S3); and ADMP dynamics simulation results (Table S4 and Figures S1 and S2); Theoretical (S^*) values (Table S5) (PDF)

AUTHOR INFORMATION

Corresponding Author
*E-mail: hychen@kmu.edu.tw.

ORCID

Hsing-Yin Chen: 0000-0003-3948-8915

Notes

The author declares no competing financial interest.

ACKNOWLEDGMENTS

I thank the Ministry of Science and Technology of Taiwan for financial support (MOST 107-2113-M-037-002 and MOST 106-2632-M-037-001).

REFERENCES

(1) Deguillaume, L.; Leriche, M.; Desboeufs, K.; Mailhot, G.; George, C.; Chaumerliac, N. Transition Metals in Atmospheric Liquid Phases: Sources, Reactivity, and Sensitive Parameters. Chem. Rev. 2005, 105, 3388–3431.
(2) Deguillaume, L.; Leriche, M.; Chaumerliac, N. Impact of Radical Versus Non-radical Pathway in the Fenton Chemistry on the Iron Redox Cycle in Clouds. Chemosphere 2005, 60, 718–724.
(3) Deguillaume, L.; Desboeufs, K. V.; Leriche, M.; Long, Y.; Chaumerliac, N. Effect of Iron Dissolution on Cloud Chemistry: from Laboratory Measurements to Model Results. Atmos. Pollut. Res. 2010, 1, 220–228.
(4) White, E. M.; Vaughan, P. P.; Zepp, R. G. Role of the Photo-Fenton Reaction in the Production of Hydroxyl Radicals and Photobleaching of Colored Dissolved Organic Matter in a Coastal River of the Southeastern United States. Aquat. Sci. 2003, 65, 402–414.
(5) Kepp, K. P. Bioinorganic Chemistry of Alzheimer’s Disease. Chem. Rev. 2012, 112, 5193–5239.
(6) Kozlowski, H.; Luczkowski, M.; Remelli, M.; Valensin, D. Copper, Zinc and Iron in Neurodegenerative Diseases (Alzheimer’s, Parkinson’s and Prion Diseases). Coord. Chem. Rev. 2012, 256, 2129–2141.
(7) Savelieff, M. G.; Nam, G.; Kang, J.; Lee, H. J.; Lee, M.; Lim, M. H. Development of Multifunctional Molecules as Potential Therapeutic Candidates for Alzheimer’s Disease, Parkinson’s Disease, and Amyotrophic Lateral Sclerosis in the Last Decade. Chem. Rev. 2019, 119, 1221–1322.
(8) Sawicki, K. T.; Chang, H. C.; Ardehali, H. Role of Heme in Cardiovascular Physiology and Disease. J. Am. Heart Assoc. 2015, 4, No. e001138.
(9) Muñoz-Bravo, C.; Gutiérrez-Bedmar, M.; Gómez-Aracena, J.; García-Rodríguez, A.; Navajas, J. F. C. Iron: Protector or Risk Factor
(10) Torti, S. V.; Torti, F. M. Iron and Cancer: More Ore to be Mined. Nat. Rev. Cancer 2013, 13, 342–355.

(11) Jiang, L.; Akatsuka, S.; Nagai, H.; Chew, S. H.; Ohara, H.; Okazaki, Y.; Yamashita, T.; Yoshikawa, Y.; Yasui, H.; Ikuta, K.; Sasaki, K.; Kohgo, Y.; Hirano, S.; Shinohara, Y.; Kohyama, N.; Takahashi, T.; Toyokuni, S. Iron Overload Signature in Chrysole-Induced Malignant Mesothelioma. J. Pathol. 2013, 228, 366–377.

(12) Sun, H.; Zhang, C.; Cao, S.; Sheng, T.; Dong, N.; Xu, Y. Fenton Reactions Drive Nucleotide and ATP Syntheses in Cancer. J. Mol. Biol. 2018, 10, 448–459.

(13) Kang, Y. W.; Hwang, K. Y. Effects of Reaction Conditions on the Oxidation Efficiency in the Fenton Process. Water Res. 2000, 34, 2786–2790.

(14) Deng, Y.; Englehardt, J. D. Treatment of Landfill Leachate by the Fenton Process. Water Res. 2006, 40, 3683–3694.

(15) Bautista, P.; Mohedano, A. F.; Gilarranz, M. A.; Casas, J. A.; Rodriguez, J. J. Application of Fenton Oxidation to Cosmetic Wastewater Treatment. J. Hazard. Mater. 2007, 143, 128–134.

(16) Lucas, M. S.; Peres, J. A. Removal of COD from Olive Mill Wastewater by Fenton's Reagent: Kinetic Study. J. Hazard. Mater. 2009, 168, 1253–1259.

(17) Babuonnusamni, A.; Muthukumar, K. A Review on Fenton and Improvements to the Fenton Process for Wastewater Treatment. J. Environ. Chem. Eng. 2014, 2, S57–S72.

(18) Wang, N.; Zheng, T.; Zhang, G.; Wang, P. A Review on Fenton-like Processes for Organic Wastewater Treatment. J. Environ. Eng. 2016, 4, 762–787.

(19) Usman, M.; Byrne, J. M.; Chaudhary, A.; Orsetti, S.; Hanna, K.; Ruby, C.; Kappler, A.; Haderlein, S. B. Magnette and Gee Rust: Synthesis, Properties, and Environmental Applications of Mixed-Valent Iron Minerals. Chem. Rev. 2018, 118, 3251–3304.

(20) Oloso, V. N.; Que, L., Jr. Bioinspired Nonheme Iron Catalysts for C–H and C=C Bond Oxidation: Insights into the Nature of the Metal-Based Oxidants. Acc. Chem. Res. 2015, 48, 2612–2621.

(21) Kim, C.; Chen, K.; Kim, J.; Que, L., Jr. Stereosepecific Alkane Hydroxylation with H2O2 Catalyzed by an Iron(II)–Tris(2-pyridylmethyl)amine Complex. J. Am. Chem. Soc. 1997, 119, 5964–5965.

(22) Chen, M. S.; White, M. C. A Predictably Selective Aliphatic C–H Oxidation Reaction for Complex Molecule Synthesis. Science 2007, 318, 783–787.

(23) Talsi, E. P.; Bhatikar, K. P. Chemo- and Stereoselective C–H Oxidations and Epoxidation/cis-dihydroxylation with H2O2, Catalyzed by Non-heme Iron and Manganese Complexes. Coord. Chem. Rev. 2012, 256, 1418–1434.

(24) Wang, B.; Boms, M. Artificial Metallooxidases from Cyclodextrin Diacids. Chem. - Eur. J. 2017, 23, 13766–13775.

(25) Zhou, X.; Zhang, Y.; Wang, C.; Wu, X.; Yang, Y.; Zheng, B.; Wu, H.; Guo, S.; Zhang, J. Photo-Fenton Reaction of Graphene Oxide: A New Strategy to Prepare Graphene Quantum Dots for DNA Cleavage. ACS Nano 2012, 6, 6592–6599.

(26) Bai, H.; Jiang, W.; Katchev, G. P.; Saith, W. A.; Bythell, B. J.; Jarvis, J. M.; Marshall, A. G.; Robinson, R. A. S.; Star, A. Insight into the Mechanism of Graphene Oxide Degradation via the Photo-Fenton Reaction. J. Phys. Chem. C 2014, 118, 10519–10529.

(27) Huo, M.; Wang, L.; Chen, Y.; Shi, J. Tumor-Selective Catalytic Nanomedicine by Nanocatalyst Delivery. Nat. Commun. 2017, 8, No. 557.

(28) Lee, K. T.; Lu, Y. J.; Mi, F. L.; Burnouf, T.; Wei, Y. T.; Cui, S. C.; Chang, E. Y.; Lu, S. Y. Catalase-Medulated Heterogeneous Fenton Reaction for Selective Cancer Cell Eradication: SnFe2O4 Nanocrystals as an Effective Reagent for Treating Lung Cancer Cells. ACS Appl. Mater. Interfaces 2017, 9, 1273–1279.

(29) Fu, L. H.; Qi, C.; Lin, J.; Huang, P. Catalytic Chemistry of Glucose Oxidase in Cancer Diagnosis and Treatment. Chem. Soc. Rev. 2018, 47, 6454–6472.
(50) Ensing, B.; Baerends, E. J. Reaction Path Sampling of the Reaction Between Iron(II) and Hydrogen Peroxide in Aqueous Solution. J. Phys. Chem. A 2002, 106, 7902–7910.

(51) Yamamoto, N.; Koga, N.; Nagaoka, M. Ferry–Oxo Species Produced from Fenton’s Reagent via a Two-Step Pathway: Minimum Free-Energy Path Analysis. J. Phys. Chem. B 2012, 116, 14178–14182.

(52) Pettit, A. S.; Pennifold, R. C. R.; Harvey, J. N. Electronic Structure and Formation of Simple Ferryloxo Complexes: Mechanism of the Fenton Reaction. Inorg. Chem. 2014, 53, 6473–6481.

(53) Lu, H. F.; Chen, H. F.; Kao, C. L.; Chao, I.; Chen, H. Y. A Computational Study of the Fenton Reaction in Different pH Ranges. Phys. Chem. Chem. Phys. 2018, 20, 22890–22901.

(54) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; et al. Gaussian 09, revision D.01; Gaussian, Inc.: Wallingford, CT, 2013.

(55) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; et al. Gaussian 16, revision A.03; Gaussian, Inc.: Wallingford, CT, 2016.

(56) Barone, V.; Cossi, M. Quantum Calculation of Molecular Energies and Energy Gradients in Solution by a Conductor Solvent Model. J. Phys. Chem. A 1998, 102, 1995–2001.

(57) Cossi, M.; Rega, N.; Scalmani, G.; Barone, V. Energies, Structures, and Electronic Properties of Molecules in Solution with the C-PCM solvation Model. J. Comput. Chem. 2003, 24, 669–681.

(58) Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. A Consistent and Accurate Ab Initio Parametrization of Density Functional Dispersion Correction (DFT-D) for the 94 Elements H–Pu. J. Chem. Phys. 2010, 132, No. 154104.

(59) Becke, A. D. Density-Functional Thermochemistry. III. The Role of Exact Exchange. J. Chem. Phys. 1993, 98, No. 5648.

(60) Lee, C.; Yang, W.; Parr, R. G. Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density. Phys. Rev. B 1988, 37, No. 785.

(61) Miechlic, B.; Savin, A.; Stoll, H.; Preuss, H. Results Obtained with the Correlation-Energy Density Functionals of Becke and Lee, Yang and Parr. Chem. Phys. Lett. 1989, 157, 200–206.

(62) Adamo, C.; Barone, V. Toward Reliable Density Functional Methods Without Adjustable Parameters: The PBE0 Model. J. Chem. Phys. 1999, 110, No. 6158.

(63) Zhao, Y.; Truhlar, D. G. The M06 Suite of Density Functionals for Main Group Thermochemistry, Thermochemical Kinetics, Noncovalent Interactions, Excited States, and Transition Elements: Two New Functionals and Systematic Testing of Four M06-Class Functionals and 12 Other Functionals. Theor. Chem. Acc. 2008, 120, 215–241.

(64) Zhao, Y.; Truhlar, D. G. Design of Density Functionals That Are Broadly Accurate for Thermochemistry, Thermochemical Kinetics, and Nonbonded Interactions. J. Phys. Chem. A 2005, 109, 5656–5667.

(65) Scalmani, G.; Frisch, M. J. Continuous Surface Charge Polarizable Continuum Models of Solvation. I. General Formalism. J. Chem. Phys. 2010, 132, No. 114110.

(66) Marenich, A. V.; Cramer, C. J.; Truhlar, D. G. Universal Solvation Model Based on Solute Electron Density and a Continuum Model of the Solvent Defined by the Bulk Dielectric Constant and Atomic Surface Tensions. J. Phys. Chem. B 2009, 113, 6378–6396.

(67) Iyengar, S. S.; Schlegel, H. B.; Millam, J. M.; Voth, G. A.; Scuseria, G. E.; Frisch, M. J. Ab Initio Molecular Dynamics: Propagating the Density Matrix with Gaussian Orbitals. II. Generalizations Based on Mass-Weighting, Idempotency, Energy Conservation and Choice of Initial Conditions. J. Chem. Phys. 2001, 115, No. 10291.

(68) Schlegel, H. B.; Millam, J. M.; Iyengar, S. S.; Voth, G. A.; Scuseria, G. E.; Daniels, A. D.; Frisch, M. J. Ab Initio Molecular Dynamics: Propagating the Density Matrix with Gaussian Orbitals. J. Chem. Phys. 2001, 114, No. 9758.