非血红素铁(III)活化氧分子反应的自旋轨道耦合和零场分裂

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摘要: 采用密度泛函理论对原儿茶酚 3,4-双加氧酶(3,4-PCD)活化 O₂ 分子的反应机理进行了探讨. 初始复合物, 六重态⁶1 的超快形成主要归因于电子交换诱导系间穿越(EISC), Fe *d_{z²}*:O₂ *π*^{*}(z) 是主要的交换通道, 在 Fe—O 键长为 0.2487 nm 处, 交换重叠积分 $S_j = \langle d_{z^2} \alpha | \pi^*(z) \beta \rangle = 0.3758$. 从六重态⁶1 形成四重态中间体⁴1, 有两种效应共存, 即电子交换耦合作用和自旋轨道耦合(SOC)作用, 且相互竞争. 计算结果表明, 自旋轨道耦合(SOC)作用起主导因素(SOC=353.16 cm⁻¹). 至于 O—O 键的解离主要取决于儿茶酚(PCA)最高占据分子轨道(HOMO)的电子转移, 非血红素酶的铁中心仅承担 PCA 向 O₂ 电子转移的缓冲作用.

关键词: 原儿茶酚 3,4-双加氧酶; 自旋轨道耦合; 零场分裂; 反应机理

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Spin-Orbit Coupling and Zero-Field Splitting in Dioxygen Activation by Non-Heme Iron(III)

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Abstract: The mechanism of the O₂ activation by the protocatechuate 3,4-dioxygenase was investigated using density functional calculations. In the initial complex, the ultrafast formation of the sextet ⁶1 was probably the result of electron-exchange-induced intersystem crossing, and Fe *d_{z²}*:O₂ *π*^{*}(z) was the dominant exchange pathway, with an overlap of $S_j = \langle d_{z^2} \alpha | \pi^*(z) \beta \rangle = 0.3758$ at an Fe—O bond length of 0.2487 nm. Two coexisting effects, electron spin exchange coupling and spin-orbit coupling (SOC) in the sextet ⁶1, are responsible for formation of the quartet state ⁴1 from the sextet ⁶1. The exchange interaction competes with the SOC interaction as a driving force for spin conversion. The calculated results show that the latter is the dominant factor, because of the larger SOC constant (353.16 cm⁻¹). In cleavage of the O—O bond, electron transfer from the protocatechuate (PCA) highest occupied molecular orbital (HOMO) plays a vital role. The Fe center of the non-heme enzyme is a buffer to transfer an electron pair from the PCA HOMO to O₂.

Key Words: Protocatechuate 3,4-dioxygenase; Spin-orbit coupling; Zero-field splitting; Reaction mechanism

1 Introduction

The mononuclear non-heme iron enzymes are an important

group with a diverse range of chemical reactions including dioxygenation, hydroxylation, ring closure, oxidative desatura-

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tion, and aromatic ring cleavage.¹ Within this broad class, the oxygen-activating enzymes are one of the most extensively studied fields.¹⁻⁴ Most non-heme oxygenases catalyze O₂ activation using a high spin Fe(II) site through a redox process that also involves the substrate to provide the required number of electrons. By contrast, a small group of non-heme iron enzymes perform a high spin Fe(III) site to activate substrate for direct attack by O₂, such as the lipxygenases and intradiol dioxygenases.

Protocatechuate 3,4-dioxygenase (3,4-PCD) is one of the intradiol dioxygenase family. It catalyzes the ring cleavage of protocatechuate (PCA) to form β -carboxy-*cis,cis*-muconate, with the incorporation of both oxygen atoms from molecular oxygen. Based on different electronic descriptions of the enzyme-substrate (ES) complex, various mechanisms¹ have been proposed for the initial O₂ binding and activation steps, but they have not been definitively observed, therefore are not well understood. Mechanism for catechol ring cleavage by non-heme iron intradiol dioxygenases has been computationally investigated using density functional theory (DFT) by Borowski and Siegbahn.⁵ In 2003, Deeth and Bugg⁶ have also reported the studied results for the extradiol cleavage mechanism. But, in these investigations, the electronic structures and intersystem crossing processes of the initial O₂ binding complexes have not been discussed in detail.

However, we know that the chemistry of transition metals and their compounds (especially, metal-containing enzymes complexes such as cytochromes P450 and non-heme iron) is strongly influenced by the availability of multiple low-lying electronic states in these species.⁷⁻⁹ Shaik and co-workers¹⁰ have proposed that multiple spin states play an important role in these reactions, with many of them involving what they have called “two-state reactivity (TSR)”. The initial complexes of the triplet ground state O₂ ($S=1$) bound to Fe(III) ($S=5/2$) of 3,4-PCD-PCA can have a total spin of $S=7/2$, $S=5/2$, or $S=3/2$, and form the different spin state complexes. This means that the reactions should involve spin-conserving and spin-inversion processes. These will result in the complexity of the reaction mechanism.

Therefore, detailed analyses of electronic structures and intersystem crossing processes in the initial complexes are very important in order to better understand the O₂ activation mecha-

nism. In present paper we computed electronic exchange coupling (J) and zero-field splitting (ZFS) using quasi-restricted theory at the DFT level¹¹ implemented with the program ORCA.¹² Then spin-orbit coupling (SOC) matrix elements were obtained by the approximate one-electron spin-orbit Hamiltonian.¹³ Some very meaningful conclusions have been obtained by these calculations.

2 Computational details

2.1 Step of the system

The activate-site geometric structure of 3,4-PCD-PCA was obtained from the averaged crystallographic coordinate of *P. putida* 3,4-PCD complex with PCA (PDB code 3PCA).¹⁴ Hydrogen atoms were placed at standard bond lengths and angles. Optimize calculations were performed on a model of the triplet state O₂ ($S=1$) bound to 3,4-PCD-PCA involving two Me-imidazoles to model His460 and His462, 4-Me-phenolate to model Tyr408, and a bidentate PCA in the fully deprotonated state, which is shown in Scheme 1.

Unrestricted calculations allow α and β electrons to occupy orbitals with different spatial localizations and, can describe the spin localized on two different paramagnetic centers. Therefore, density functional theory calculations were performed using the Gaussian 03 program¹⁵ with spin-unrestricted functional U-BP86 with 10% Hartree-Fock exchange and the Pople triple- ζ basis set, 6-311G(*d*) to optimize geometry of the active model described above. For comparison, calculations were also performed with the spin-unrestricted U-B3LYP functional with the LanL2DZ effective core potential basis set. Transition state structures were found to have one imaginary frequency, which correlated to the motion of the bond being broken or formed.

2.2 Calculations of zero-field splitting (ZFS) tensor

In the absence of nuclear spins and exchange interactions, the effective spin Hamiltonian of these interactions is usually written as

$$H_{\text{spin}} = H_{\text{Ze}} + H_{\text{ZFS}} = \beta_B g S + S D S \quad (1)$$

where H_{Ze} is Zeeman effect operator, H_{ZFS} is zero-field splitting operator, β_B is the Bohr magneton, B is the magnetic flux density, S is the effective spin operator, and g and D are the g -tensor and ZFS-tensor, respectively. H_{spin} acts on the basis functions $|SM\rangle$ with $M=S, S-1, \dots, -S$. If we choose a coordinate

Scheme 1 Active-site geometric structures of 3,4-PCD-PCA (PDB code 3PCA)

system that diagonalizes D , H_{ZFS} can be rewritten:

$$H_{\text{ZFS}} = D[S_z^2 - 1/3 S(S+1)] + E(S_x^2 - S_y^2) \quad (2a)$$

$$D = D_{zz} - 1/2(D_{xx} + D_{yy}), E = 1/2(D_{xx} - D_{yy}) \quad (2b)$$

Thus, the ZFS is uniquely defined by the parameters D and E and the tensor orientation. Typically, D and E/D are given in a coordinate system that fulfils the following condition:

$$0 \leq E/D \leq \frac{1}{3}$$

DFT calculations of the ZFS were carried out using quasi-restricted theory¹¹ by the ORCA program.¹² Previous studies¹¹ have investigated the dependences of the g -tensor and ZFS on the exchange-correlation functional. Thus, in this work, we obtain ZFS parameters (D and E) from additional single-point calculations using 6-311G(d) basis sets and the BP86 GGA functional. We use a recently described SOC operator that efficiently implements a spin-orbit mean-field (SOMF) method.¹⁶

2.3 Spin-orbit coupling and exchange coupling calculations

Exchange coupling constants (J) are calculated using the Yamaguchi formula, which covers consistently the whole range of situations from the strong to the weak exchange coupling limit.^{17,18}

$$J = - \frac{E_{\text{HS}} - E_{\text{BS}}}{\langle S^2 \rangle_{\text{HS}} - \langle S^2 \rangle_{\text{BS}}} \quad (3)$$

where E_{HS} and E_{BS} account for the energies of the high-spin and broken-symmetry states, respectively; $\langle S^2 \rangle_{\text{HS}}$ and $\langle S^2 \rangle_{\text{BS}}$ denote the spin angular momentum calculated in the high-spin and broken-symmetry solutions, respectively. These calculations were performed with a development version of the ORCA program.¹²

In addition, in order to obtain the more detailed SOC matrix elements, the SOC calculations of the sextet and quartet states were studied using the approximate one-electron spin-orbit Hamiltonian (h)¹³ given in Eq.(4):

$$H_{\text{SO}} = \frac{\alpha^2}{2} \sum_i \sum_k \left(\frac{Z_k^*}{r_{ik}^3} \right) (S_i \cdot L_{ik}) = \sum_i h_i(Z^*), \quad \frac{\alpha^2}{2} = \frac{e^2 \hbar}{4\pi m_e c^2} \quad (4)$$

where L_{ik} and S_i are the orbital and spin angular momentum operators for electron i in the framework of the nuclei indexed k , respectively, and Z_k^* is the effective nuclear charge, r_{ik} is the distance of between each electron samples (i) and all nuclei (k). One-electron SOC calculations were carried out using the GAMESS program.¹⁹

3 Results and discussion

3.1 Initial complex electronic states

The optimized geometries and energetic data for the octet, sextet, and quartet electronic states are depicted in Fig.1, in order to keep the discussion more simple, the goal complex, denoted as 1, is initially formed as 3,4-PCD-PCA and O₂ collide end-on with each other, where the superscripts denote the spin multiplicities (⁸1, ⁶1, and ⁴1).

3.1.1 Octet state ($S=7/2$)

The singly occupied molecular orbitals (SOMOs) of ⁸1 are

plotted in Fig.2 and It clearly shows that O₂ has two unpaired electrons, O₂ $\pi^*(y)$ and $\pi^*(z)$, while five others reside on the ferrous Fe(III) center, namely occupy five d orbitals, thus forming a high-spin (HS) Fe(III) ($S=5/2$) combined ferromagnetically with a triplet O₂ ($S=1$). Therefore the octet ⁸1 is approximately understood as van der Waals complex, which involves O₂ in a end-on orientation with a long Fe—O bond length of about 0.2558 nm at the U-B3LYP/LanL2DZ level (0.2487 nm for U-BP86/6-311G*), as shown in Fig.1.

3.1.2 Sextet state ($S=5/2$)

We obtained a sextet ⁶1 complex as shown in Fig.1. Compared with ⁸1, the Fe—O bond length was shortened to 0.1906 nm for U-BP86/6-311G*, while the O—O bond length was increased to 0.1281 nm. These show that O₂ and Fe center has obvious chemically bonded effect in the sextet ⁶1 complex. The electronic structure of ⁶1 is schematically shown in Fig.3. It is seen that O₂ is a superoxide, having a singly occupied O₂ $\pi^*(y)$, which is vertical to the Fe—OO plane, while the other doubly occupied $\pi^*(z)$ [$\pi^*(z)$ is in Fe—OO plane] forms a two-electron bond with the iron d_{z^2} orbital. ⁶1 has an ferromagnetically coupling of the $S=1/2$ superoxo anion O₂⁻ with $S=2$ Fe(IV) center. ⁸1 as zero reference, the U-BP86 calculated relative energy of ⁶1 is $-12.14 \text{ kJ} \cdot \text{mol}^{-1}$.

3.1.3 Quartet state ($S=3/2$)

For the quartet state ⁴1, in contrast with the sextet ⁶1 case, one pronounced difference is that the Fe(IV) center has two singly occupied orbitals (d_{xy} and π_{xz} , $S=1$), that is, ⁴1 differs from ⁶1 by a spin-flip of the a singly occupied orbital from $\sigma_{x^2-y^2}$ to d_{yz} , consequently, d_{yz} is doubly occupied. O₂ ($S=1/2$) is still a superoxide with a singly occupied $\pi^*(y)$ and a doubly occupied $\pi^*(z)$ orbitals, and coupled ferromagnetically with the Fe(IV) ($S=1$), thus formed an intermediate-spin $S=3/2$ complex, ⁴1. The U-BP86 calculated energy difference is $9.21 \text{ kJ} \cdot \text{mol}^{-1}$ relative to the ⁶1 state.

3.2 Intersystem crossing process of initial complexes

As has been already discussed before, the triplet O₂ ($S=1$) mixes with Fe(III) ($S=5/2$) resulting in the different spin states with a total spin of $S=7/2$, $S=5/2$, or $S=3/2$, and smaller energy differences among them. Moreover, we found that the spin states from the octet to quartet state are changed with the decrease of the Fe—O bond length (from 0.2487 to 0.1895 nm at the U-BP86/6-311G* level), and the sextet ⁶1 is the most stable as shown in Fig.4. In this respect, the interesting question is raised: how is the intersystem crossing of the different spin states happened?

3.2.1 Exchange coupling (J)

Let us turn to discuss this question now, starting from the octet state ⁸1. To identify some main atomic orbital interactions, the main antiferromagnetic orbital interactions of ⁸1 were also inferred from overlaps calculated from the broken-symmetry wave function (U-BP86/6-311G*), the results calculated are plotted in Fig.5. In general, the broken-symmetry orbitals of

Fig.1 Selected bond lengths (nm) and energies (listed under the structures) obtained from the key point optimizations at the U-BP86/6-311G(*d*) and U-B3LYP/LanL2DZ levels (in the parentheses)

2^* is the second intermediate, TS is transition state, P is product.

different spin indexes (i.e., α or β) are not orthogonal to each other and are localized on Fe(ϕ_α) or $O_2(\phi_\beta)$. Therefore, to this extent, the overlaps $S_{ij} = \langle \phi_j | \phi_i \rangle$ are intimately related to the strength of antiferromagnetic spin coupling. As seen from Fig.5, the overlap between the localized Fe d_{z^2} and $O_2 \pi^*(z)$, 129α and 129β , is considerably better and $S_{ij} = \langle 129\alpha | 129\beta \rangle = 0.3758$ at the Fe—O distance of 0.2487 nm. The low spin cou-

pling between 129α and 129β electron pair is therefore strong enough to lead to a Fe—O bonding. By contrast, the other overlap $S = \langle 130\alpha | 130\beta \rangle$ of the Fe d_{xz} and $O_2 \pi^*(y)$ is much weaker and $S_{ij} = 0.0868$. Thus the magnitude of the overlaps strongly suggested that the first Fe $d_{z^2} : O_2 \pi^*(z)$ was clearly dominant ex-

Fig.2 Electronic configuration of the octet state 8_1

Fig.3 Occupied active natural orbitals of 4_1 at the BP86/6-311G(*d*) level

Fig.4 Schematic representation of calculated reaction paths at the B3LYP/LanL2DZ level

change pathway, whereas $\text{Fe } d_{yz}:\text{O}_2 \pi^*(y)$ contributed to a lesser extent and was fairly comparable. At the same time, we found that the overlaps are increased with the decrease of the $\text{Fe}-\text{O}$ bonding, and the change of the exchange coupling constant J is very similar to that of the overlaps, the J values are increased from -20.8 to -81.4 cm^{-1} with the distance of the $\text{Fe}-\text{O}$ bonding from 0.2487 to 0.2200 nm . These calculations provided a detailed approximation to antiferromagnetism induced by $\text{Fe } d_{yz}:\text{O}_2 \pi^*(z)$ delocalization.

Therefore the formation of $^6\text{1}$ from $^8\text{1}$ is most likely due to the electron exchange induced enhanced intersystem crossing (EISC).²⁰ Certainly, for antiferromagnetic exchange, the energy gap between $^6\text{1}$ and $^8\text{1}$ is also very important. The energy gap between these two states is about $12.14 \text{ kJ}\cdot\text{mol}^{-1}$, one might expect that EISC would be faster. The electron exchange interaction between Fe(III) ($S=5/2$) and O_2 ($S=1$) serves as the first-order perturbation that drives EISC. The magnitude of this perturbation, and thus the overall intersystem crossing rate, depends strongly on the electronic overlap between the orbitals that contribute to the singly occupied molecular orbitals (SOMO) of the Fe(III) ($S=5/2$) center and O_2 ($S=1$).

In addition, antiferromagnetic exchange coupling can lead to the partly forming of $^4\text{1}$ due to the weaker overlap $S=\langle 130\alpha|130\beta\rangle$. We also noted that the quartet wave function can have some admixture of the sextet wave function. The effect was more noticeable, the expectation value of the total spin operator after annihilation, $\langle S^2 \rangle=5.47$ was far from the value expected for a pure quartet state, $\langle S^2 \rangle=3.75$. Based on the following expressions for the spin expectation values of the contaminated quartet state:¹⁸

$$\begin{aligned} (S_4^{\text{BS}})^2 &\approx C_6^2 S_6(S_6+1) + C_4^2 S_4(S_4+1) \\ C_6^2 + C_4^2 &\approx 1 \end{aligned} \quad (5)$$

where C is the configuration coefficient, S is the total spin operator, subscripts 6 and 4 denote sextet state and quartet state, respectively. From Eq.(5) we obtain the contribution of the quartet state which is about 67% of the mixing states. This reason is that a second-order term of ZFS, SOC) introduces some angular momentum into the sextet state. These mechanisms above are described in Fig.3.

3.2.2 Zero-field splitting (ZFS)

The net effects for ZFS are to introduce a splitting of the $2S+1$, in the absence of an external magnetic field. This will main-

Fig.5 Pathways of the exchange coupling from $^8\text{1}$ to $^6\text{1}$ or $^4\text{1}$

ly attribute to the ZFS's two contributions:¹¹ (a) a first-order term, the direct dipolar spin-spin (SS) interaction between pairs of electrons and (b) a second-order term, arising from SOC. Thus an analysis and interpretation of the ZFS is imperative in this study.

BP86 calculations were performed to determine the signs of the D and E values for $^6\text{1}$. The ZFS parameters [$D=D_z-1/2(D_{xx}+D_{yy})$, $E=1/2(D_{xx}-D_{yy})$] are calculated, $D=+8.589\text{ cm}^{-1}$ and $E/D=+0.068$. The more detailed results of the calculated ZFS using the quasi-restricted DFT method are shown in Table 1. From the results in Table 1, the major contribution arises from the second-order SOC contribution (around 97.5% of D), while the SS contributions are essentially negligible. Concerning the SOC part, it contains four significant contributions: $\alpha\rightarrow\alpha$ ($D=0.332\text{ cm}^{-1}$), $\beta\rightarrow\beta$ ($D=0.240\text{ cm}^{-1}$), $\alpha\rightarrow\beta$ ($D=8.138\text{ cm}^{-1}$), and $\beta\rightarrow\alpha$ ($D=-0.336\text{ cm}^{-1}$). However the major contribution comes from the $\alpha\rightarrow\beta$ spin-flip excitation. This excitation contributes around 77.2% of the D_{SOC} value, corresponding to the spin-pairing $\Delta S=-1$ (i.e., sextet $\rightarrow$ quartet) transitions. The second larger SOC contribution arises from the $\beta\rightarrow\alpha$ spin-raising $\Delta S=+1$ (sextet $\rightarrow$ octet) excitation (around 4% of D_{SOC}) corresponding to ligand-to-metal charge-transfer transitions. The remaining two SOC contributions come from the spin allowed $\Delta S=0$ (sextet $\rightarrow$ sextet) ligand-field excitations and have usually been solely held responsible for the ZFS. However, these contributions are only around 7% compared to the D_{SOC} value.

The $\Delta S=-1$ state (sextet is flipped to quartet state) is found to make significant contributions to D_{SOC} , with the primary contribution arising from the single-determinant spin-paired states within the single occupied sets of orbitals, $\sigma_{x^2-y^2}$, π_{xz}^* , d_{xy} , and d_{yz} (see Fig.3). Namely, D_{xx} ($\sigma_{x^2-y^2}\uparrow\rightarrow d_{yz}\downarrow$), D_{yy} ($\sigma_{x^2-y^2}\uparrow\rightarrow\pi_{xz}^*\downarrow$), and D_{zz} ($\sigma_{x^2-y^2}\uparrow\rightarrow d_{xy}\downarrow$), vital spin-flip excitation. Applying the angular momentum operator to this four orbitals generates three nonzero SOC integrals: $\langle\sigma_{x^2-y^2}|l_x|d_{yz}\rangle$, $\langle\sigma_{x^2-y^2}|l_y|\pi_{xz}^*\rangle$, and $\langle\sigma_{x^2-y^2}|l_z|d_{xy}\rangle$ which make the largest contribution to the SOC and lead to the larger values of $D_{xx}(=8.8\text{ cm}^{-1})$, $D_{zz}(=16.8\text{ cm}^{-1})$, and $D_{yy}(=7.6\text{ cm}^{-1})$. It identifies the origin of large ZFS as spin-orbit coupling to low-lying $\Delta S=-1$ state, and shows that the quartet $^4\text{1}$ can arise from normal spin-orbit induced intersystem crossing as compared with the weaker exchange coupling.

3.2.3 Spin-orbit coupling

Table 1 Contributions to the calculated zero-field splitting between spin-orbit coupling and spin-spin for the sextet state

Method	Individual contributions	D/cm^{-1}	E/cm^{-1}
BP86-QR-SOMF/ TZVP	spin-orbit-coupling	$\alpha\rightarrow\alpha$	0.332
		$\beta\rightarrow\beta$	0.240
		$\alpha\rightarrow\beta$	8.138
		$\beta\rightarrow\alpha$	-0.336
		total	8.374
	spin-spin	coulomb	0.180
		exchange	0.035
		total	0.215

Based on the above analysis, the sextet $^6\text{1}$ from $^8\text{1}$ is fast formed via EISC, following the system will well change its spin multiplicities from the sextet state to the quartet ground state $^4\text{1}$ by SOC in the O_2 gradual approach to Fe center process. However, a spin-forbidden transition requires an effect of SOC that provides a major mechanism for the intersystem crossing process.²¹⁻²⁴ Therefore, we must inspect the orbital relationships which promote the SOC matrix elements.

The ROHF orbitals for the construction of the quartet and sextet CASCI wave functions to be used in the SOC evaluation have been generated by the sextet ROHF calculation. At the CAS(7, 6) level the dominant determinants of the MCSCF wave functions have configuration interaction (CI) coefficients of 0.98 and 0.17 for the sextet and 0.86 and -0.48 for the quartet state (configurations of less than 0.1 have not been listed). Thus the permissible approximation of the sextet wave function by a single configuration (0.98) enables us to analyze the SOC matrix elements. The quartet configuration is described as shown in Eq.(6),

$$|^4\psi\rangle = 0.86|^4d_{yz}^2\pi^*(y)^1d_{xy}^1\pi_{xz}^*\rangle - 0.48|^4d_{yz}^1\pi^*(y)^1d_{xy}^2\pi_{xz}^*\rangle \quad (6)$$

Nonzero elements of the p -components ($p=x, y, z$) of the SOC matrix, $\langle^6\psi(M_s)|H_{\text{SO}}|^4\psi(M_s)\rangle_p$, are always proportional to the function, F_p , as given in Eq.(7).

$$F_p = 2^{-1/2} \times 0.86 \langle\sigma_{x^2-y^2}|H_{\text{SO},k}|d_{yz}\rangle - 2^{-1/2} \times 0.48 \langle\sigma_{x^2-y^2}|H_{\text{SO},k}|d_{xy}\rangle \quad (7)$$

To further understand the efficient SOC, it is very important that the SOC matrix elements $\langle\sigma_{x^2-y^2}|H_{\text{SO},k}|d_{yz}\rangle$ and $\langle\sigma_{x^2-y^2}|H_{\text{SO},k}|d_{xy}\rangle$ are discussed. Because the SOC constant (ζ_{Fe}) is an order of magnitude greater than that of oxygen, making it a reasonable approximation to consider only the Fe contribution when discussing spin-orbit mixing with quartet states. Thus

$$\langle\text{SOC}\rangle = \eta C_0 C_{j1} \sum_p \zeta_{\text{Fe}} \langle d_{x^2-y^2} | L_{\text{Fe},p} | d_{yz} \rangle \langle \theta_2 | S_p | \theta_4 \rangle + \eta C_0 C_{j2} \sum_p \zeta_{\text{Fe}} \langle d_{x^2-y^2} | L_{\text{Fe},p} | d_{xy} \rangle \langle \theta_2 | S_p | \theta_3 \rangle \quad (8)$$

where the η term is the M_s -dependent weight weighing factor, and $\theta=\alpha$ and/or β . In the present case, for the sextet state, the fundamental open-shell configuration has one dominant coeffi-

Table 2 Calculated SOC matrix elements (cm^{-1}) in the $^4\text{1}$ structure

State 1	State 2	$\langle H_{\text{SO}} \rangle_r$	$\langle S_1=5/2; M_{S1} H_{\text{SO}} S_2=3/2; M_{S2} \rangle$	SOC
6A	4A	$r=z$	$\langle M_{S1}=3/2 H_{\text{SO}} M_{S2}=3/2 \rangle = -86.17$	353.16
			$\langle M_{S1}=1/2 H_{\text{SO}} M_{S2}=1/2 \rangle = -105.55$	
			$\langle M_{S1}=-1/2 H_{\text{SO}} M_{S2}=-1/2 \rangle = -105.55$	
			$\langle M_{S1}=-3/2 H_{\text{SO}} M_{S2}=-3/2 \rangle = -86.17$	
		$r=x, y$	$\langle M_{S1}=5/2 H_{\text{SO}} M_{S2}=3/2 \rangle = 141.21; 44.23$	
			$\langle M_{S1}=-5/2 H_{\text{SO}} M_{S2}=-3/2 \rangle = 141.21; -44.23$	
			$\langle M_{S1}=3/2 H_{\text{SO}} M_{S2}=1/2 \rangle = 109.38; 34.26$	
			$\langle M_{S1}=-3/2 H_{\text{SO}} M_{S2}=-1/2 \rangle = 109.38; -34.26$	
			$\langle M_{S1}=1/2 H_{\text{SO}} M_{S2}=1/2 \rangle = 77.34; 24.22$	
			$\langle M_{S1}=-1/2 H_{\text{SO}} M_{S2}=-1/2 \rangle = 77.34; -24.22$	
			$\langle M_{S1}=-1/2 H_{\text{SO}} M_{S2}=-3/2 \rangle = 44.65; 13.99$	
			$\langle M_{S1}=1/2 H_{\text{SO}} M_{S2}=3/2 \rangle = 44.65; 13.99$

Fig.6 Electronic evolution during the O—O bond cleavage

cient, i.e., $C_0=0.98\approx 1$, while coefficients of quartet states are $C_{11}=0.86$ and $C_{12}=-0.48$, respectively.

Therefore:

$$\begin{aligned} \langle d_{x^2-y^2} | L_{Fe,p} | d_{yz} \rangle \langle \theta_2 | S_p | \theta_3 \rangle &= \langle d_{x^2-y^2} | L_{Fe,x,y} | d_{yz} \rangle \langle \alpha | S_{x,y} | \beta \rangle + \\ &\langle d_{x^2-y^2} | L_{Fe,z} | d_{yz} \rangle \langle \alpha | S_z | \alpha \rangle \end{aligned} \quad (9)$$

Here, only the first term is nonzero value, the electron shift from $d_{x^2-y^2}$ to d_{yz} creates a non-zero angular momentum in the L_x -direction, resulting in the larger SOC matrix elements as listed in Table 2. The second term is zero value due to the mismatch for the $d_{x^2-y^2} \rightarrow d_{yz}$ transition with L_z angular momentum direction. Similarly, the larger z -direction SOC comes from $\langle d_{x^2-y^2} | L_{Fe,z} | d_{yz} \rangle \langle \alpha | S_z | \alpha \rangle$ coupling of the second term in Eq.(9). These analyses are in good agreement with the SOC calculations of the approximate one-electron spin-orbit Hamiltonian (see Table 2) and D -tensor of ZFS,²⁵ namely there exist the larger SOC matrix elements in L_x - and L_z -directions, resulting in the SOC constant of 353.16 cm^{-1} . These also further indicated that the significant contributions to D arise from the $\Delta S=-1$ spin-flip transition, and the quartet state 41 is produced by a spin-orbit coupling intersystem crossing.

3.3 Cleavage process of the O—O bond

Structural parameters of the transition state (^4TS) on the quartet surface are collected in Fig.1. The O—O bond cleavage pathway is shown in Fig.6. The O—O bond activation takes place through a precursor intermediate 42 and transition state (^4TS), to afford a cleavage product ^4P . The ^4TS has the feature of partially broken O—O bond (0.1687 nm), in which the activation barrier is $111.37 \text{ kJ} \cdot \text{mol}^{-1}$ with respect to 42 . ^4TS is characterized as a transition structure by one imaginary frequency of $839.7i \text{ cm}^{-1}$, and the vibrational vector corresponds to the expected components of the reaction coordinate, i.e., breaking of the O—O bond.

Schematic of the frontier molecular orbitals which participate in the three-electron transfer process upon the O—O bond

cleavage is shown in Fig.6. As can be seen from Fig.6, the doubly occupied PCA π orbital is the HOMO in the complex, one electron from the doubly occupied PCA π orbital can be donated directly to the $\text{O}_2 \pi^*$ orbital to form the distorted O—C_{PCA} bond. Two electrons are transferred to the $\text{O}_2 \pi^*$ orbital generating a Fe(IV)-peroxide. There is the strong covalent interaction between the PCA HOMO and Fe d_{xz} orbitals in the α manifold, the second electron of the PCA HOMO is transferred to Fe, at the same time the transfer of an Fe d electron into the $\text{O}_2 \sigma^*$ orbital to break the O—O bond is accomplished. Finally, cleavage of the O—O bond leads to formation of an Fe(IV)-oxo. Therefore, the transfer of these three electrons (one α and two β) shows that the Fe center acts as a buffer to transfer an electron pair from PCA π orbital to the triplet O_2 in the spin forbidden reaction.

4 Conclusions

The O_2 activating mechanism by non-heme iron enzyme, 3,4-PCD-PCA, has been studied using theoretical calculations. The electronic structure origins and intersystem crossing of the different spin states (a total spin of $S=7/2$, $S=5/2$, or $S=3/2$) were discussed by the broken-symmetry method and SOC mechanism. The octet state 81 is stable with the aid of the electrostatic interaction, while the ultrafast formation of 61 is most likely due to EISC. As for the formation of the quartet state 41 from the sextet 61 , there coexist the two effects, electron spin exchange coupling and spin-orbit coupling in the sextet 61 . As a driving force of spin conversion the exchange interaction competes with spin-orbit coupling interaction. The calculated results show that the latter is the dominant factor due to the larger SOC constant (353.16 cm^{-1}). The doublet 21 optimization is failure using various DFT methods, all optimizations can not be converged due to the energy fluctuation.

The O—O bond activation takes place through a precursor intermediate 42 and transition state (^4TS), to afford a cleavage

product 'P. The electronic transfer of the PCA HOMO is the vital role for the cleavage of the O—O bond. The Fe center of non-heme enzyme is a buffer to transfer an electron pair from PCA HOMO orbital to the O₂ in the reaction.

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