Theoretical Study of the Mechanism of Acetaldehyde Hydroxylation by Compound I of CYP2E1

Yong Wang,[†] Hongming Wang,[†] Yonghua Wang,[§] Chuanlu Yang,[†,‡] Ling Yang,[§] and Keli Han*[,†,‡]

State Key Laboratory of Molecular Reaction Dynamics, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, People's Republic of China, Department of Physics, Yantai Normal University, Yantai 264025, People's Republic of China, and Laboratory of Biotechnology, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, People's Republic of China

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Recent experimental studies revealed that cytochrome P450 2E1 (CYP2E1) could metabolize not only ethanol but also its primary product, acetaldehyde, accompanying the well-known acetaldehyde dehydrogenases (ALDH) in the metabolism of acetaldehyde. Mechanistic aspects of acetaldehyde hydroxylation by Compound I model active species of CYP2E1 were investigated by means of B3LYP DFT calculations in the present paper. Our study results demonstrate that acetaldehyde hydroxylation by CYP2E1 is in accord with the effectively concerted mechanisms both on the high quartet spin state (**HS**) and on the low doublet spin state (**LS**). The rate-limiting step is H-abstraction, and the activation energy is about 11.7∼14.0 kcal/mol on the quartet (doublet) reaction route, which is about one-half to one-third of that needed by methane hydroxylation. The phenomenon that the **HS** and **LS** reaction routes are both effectively concerted was shown for the first time to occur in *trans*-2-phenyl-*iso*-propylcyclopropane hydroxylation by Kumar et al. (see Figure 7 in the paper of Kumar, D.; de Visser, S. P.; Sharma, P. K.; Cohen, S.; Shaik, S. *J. Am. Chem. Soc.* **2004**, *126*, 1907) and was confirmed in our work of acetaldehyde hydroxylation by cytochrome P450. Theoretical exploration of the **HS** O-rebound barrier degradation is also presented in the present paper on the basis of Shaik's valence bond (VB) model.

I. Introduction

Cytochrome P450 2E1 (CYP2E1) is considered as one of the major human hepatic Cytochrome P450 enzymes and has been widely studied because of its clear relevance to alcoholism.[1] Recent experimental work revealed that CYP2E1 could catalyze not only ethanol but also acetaldehyde, the primary product of ethanol oxidation. Being more active than ethanol, acetaldehyde is considered to be responsible for many of the toxic effects caused by ethanol, such as acidosis and fatty livers. Through analysis of products formed from [$^2H_4$]-acetaldehyde with CYP2E1-containing reconstituted membrane vesicles, Terelius et al. revealed that acetate was the only detectable product in their experiment.[2] Guengerich has investigated this CYP2E1-catalyzed oxidation of acetaldehyde to acetic acid by kinetic deuterium isotope reactions.[3,4] All results mentioned above indicated that CYP2E1 is a NADPH-dependent MAOS (microsomal acetaldehyde-oxidizing system), accompanying the well-known NAD-dependent acetaldehyde dehydrogenases (ALDH) in the metabolism of acetaldehyde. These results also indicated that the mechanism of acetaldehyde hydroxylation by CYP2E1 follows probably the oxygen-rebound mechanism, the same as that of alkane hydroxylation.[5−6]

The mechanism of alkane hydroxylation mediated by CYP450 is probably one of the most disputed mechanisms in enzymology.[7−9] The conventional mechanism is that of Groves' rebound mechanism enunciated in 1976,[10,11] which is generally cited as the hydrogen-abstraction/oxygen-rebound mechanism. However, the results of ultrafast radical clock obtained by Newcomb et al. cast doubt on Groves' rebound mechanism[12,13] and initiated the so-called "rebound controversy".[9] To reconcile with this controversy, Shaik proposed his famous two-state reactivity (TSR) rebound mechanism in 1998.[14−16] In this mechanism, the reaction proceeds via two spin states; one is the high-spin (**HS**) quartet state, while the other is the low-spin (**LS**) doublet sate. The **HS** and **LS** energy routes are close to each other until the H-abstraction step is finished, generating the radical PorFeOH/Alk• intermediates, and then they bifurcate. In the **HS** profile, there is a significant energy barrier on the O-rebound step, which leads to the PorFeOH/Alk• intermediate having a long enough lifetime to be detected. In contrast, in the **LS** profile, the O-rebound step is effectively barrierless and the lifetime of the intermediate is ultrashort. This mechanism has been shown to occur for many C−H hydroxylation processes.[17−29] Ortiz de Montellano commented on this TSR rebound mechanism as "readily rationalizes the experimental data" and "is one of the most useful advances in the recent past".[30]

Up to the present, all reaction systems studied for C−H bond hydroxylation by CYP450 are a kind of inert C−H bond, and no investigations on active C−H bond activation such as acetaldehyde hydroxylation have been reported. So, one of the questions for weak acetaldehyde C−H bond hydroxylation mediated by CYP2E1 is whether it proceeds via the TSR mechanism similar to that of alkane hydroxylation.

* Author to whom correspondence should be addressed. E-mail: klhan@dicp.ac.cn.
† State Key Laboratory of Molecular Reaction Dynamics, Dalian Institute of Chemical Physics.
‡ Yantai Normal University.
§ Laboratory of Biotechnology, Dalian Institute of Chemical Physics.

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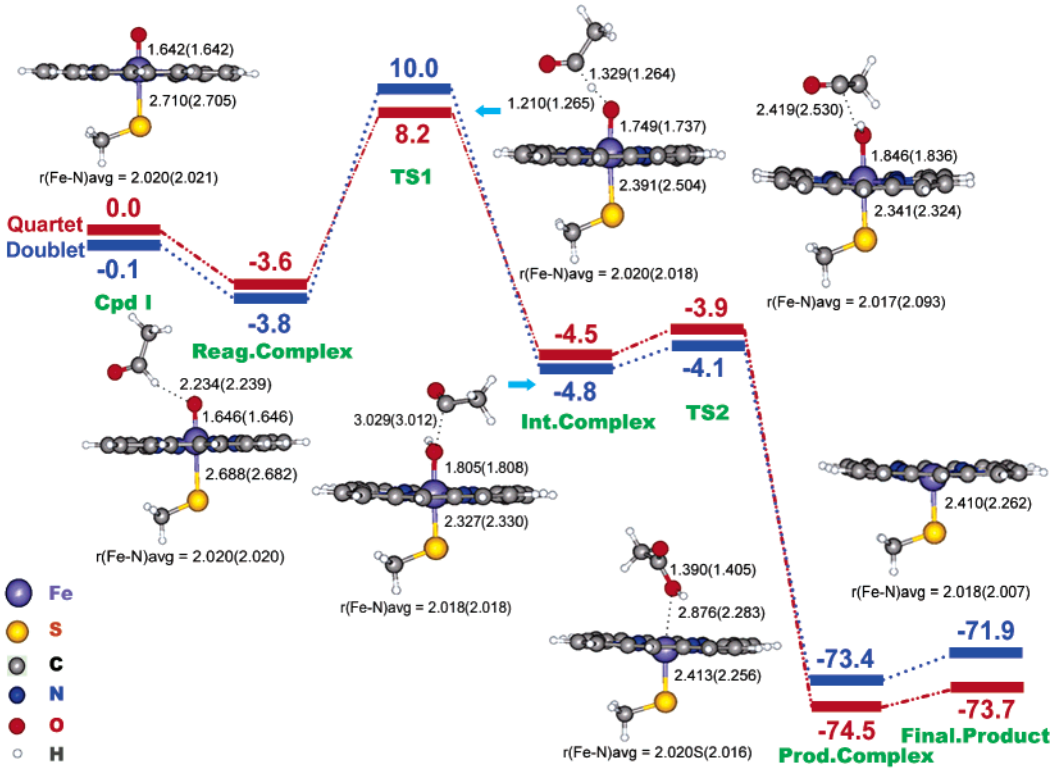

Figure 1. Energy profile (in kcal/mol) for acetaldehyde hydroxylation by the SMe-Cpd I model of CYP2E1 in the quartet (doublet) state with properties of various reaction species (in angstrom).

II. Computational Model and Methods

With the purpose of gaining a theoretical insight into this vital enzyme reaction, we carried out B3LYP DFT calculations using the Gaussian03 program.[31] In our model system, two popular Compound I models, the six-coordinated ferryl-oxo species, $Fe^{4+}O^{2-}(C_{20}N_4H_{12})^{-}(SMe)^{-}$ [27−29,32−34] and $Fe^{4+}O^{2-}$-$(C_{20}N_4H_{12})^{-}(SH)^{-}$,[16−25] were both employed as the compound I model (SMe-Cpd I and SH-Cpd I) of CYP2E1, with acetaldehyde as the substrate. We employed the spin-unrestricted B3LYP as the method of choice[35] and the TZV (Fe)/D95(H, C, N, S, O) basis sets[36] for the SMe-Cpd I model system and the LACVP**(Fe)/6-31G**(H, C, N, S, O), LACVP** in brief,[37] basis sets for SH-Cpd I model system to optimize various reaction species without symmetry constraints. Single-point energy calculations were performed with higher basis sets. For the SMe-Cpd I model system, Wachters' all-electron basis set for Fe, McLean-Chandler basis set for S, and 6-311+G** basis set for other H, C, N, S, and O atoms were used.[36] For SH-Cpd I model system, LACV3P+*(Fe)/6-311+G*(H, C, N, S, O), LACV3P+* in brief, was employed.[37] These basis sets have been fully tested and successfully applied in Shaik's and Yoshizawa's studies. Transition states were ascertained by vibrational analysis with only one imaginary frequency mode. To evaluate the weak polarization effect of the protein environment, chlorobenzene ($\epsilon = 5.62$) was employed in the PCM solvation method. More computational details can be found in the Supporting Information section.

III. Results and Discussion

Figures 1 and 2 present the calculated energy profile together with the properties of various reaction species calculated with SMe-Cpd I and SH-Cpd I. Electronic aspects for these reaction species are listed in Tables 1 and 2. From Figures 1 and 2, we can see that the acetaldehyde hydroxylation reaction proceeds via two closely located spin states, one is the high-spin (**HS**) quartet state and the other is the low-spin (**LS**) doublet one. Initially, a C−H bond activation step is involved in both the **HS** and **LS** reaction routes. This is the rate-determining step, and the activation energy is about 11.8 (13.8) kcal/mol on the quartet (doublet) reaction route in the SMe-Cpd I model system and about 11.7 (14.0) kcal/mol in the SH-Cpd I model system, which is about one-half to one-third of that needed for alkane hydroxylation and which fits the barrier-bond energy correlation for the C−H hydroxylation as presented by Shaik et al. (the activation energy calculated with the function mentioned in ref 43 is 10.8 kcal/mol, see details in SI).[16,27,43] On this transition state (**TS1**), the length of H···O is about 1.210 Å (1.265 Å) [1.256 Å (1.253 Å) for the SH-Cpd I model system] and that of the H···C is 1.329 Å (1.264 Å) [1.296 Å (1.280 Å) for SH-Cpd I model system] in the SMe-Cpd I model system for the quartet (doublet) state. The configuration of the C···H···O is almost linear, and there is obviously a "polar effect" in these three-electron/three-center transition states (see Tables 1 and 2), so the C−H activation step is a H-atom abstraction one.[16] The shifting of a spin-down (up) electron from the σ_{C-H} orbital to the π_S ($a_{2u}+\sigma_S$) orbital occurs in the SMe-Cpd I (SH-Cpd I) model system and leads to the radical intermediate that possesses singly occupied π_{xz}*, π_{yz}*, and ϕ_C orbital (see Figures 3 and 4). The second step is an O-rebound one. An electron promotion from the ϕ_C orbital of the $CH_3CO^{\cdot}$ moiety to the σ_{z2}* (π_{xz}*) orbital of the FeO moiety takes place (Figure 3) on the **HS** (**LS**) route, which results in a significant spin density change in Fe from 1.74 to 1.09 for the doublet state and from 1.71 to 2.62 for the quartet state (see Table 1) in the SMe-Cpd I model system (while in the SH-Cpd I model system the spin density in Fe stays at about 1.0 in the doublet state and changes from 1.74 to 2.46 for the quartet state) and further results in the longer Fe−O and Fe−S bond lengths in the ^{4}**TS2** as compared to those of ^{2}**TS2** (Figure 1). A second electron transfer occurs from the

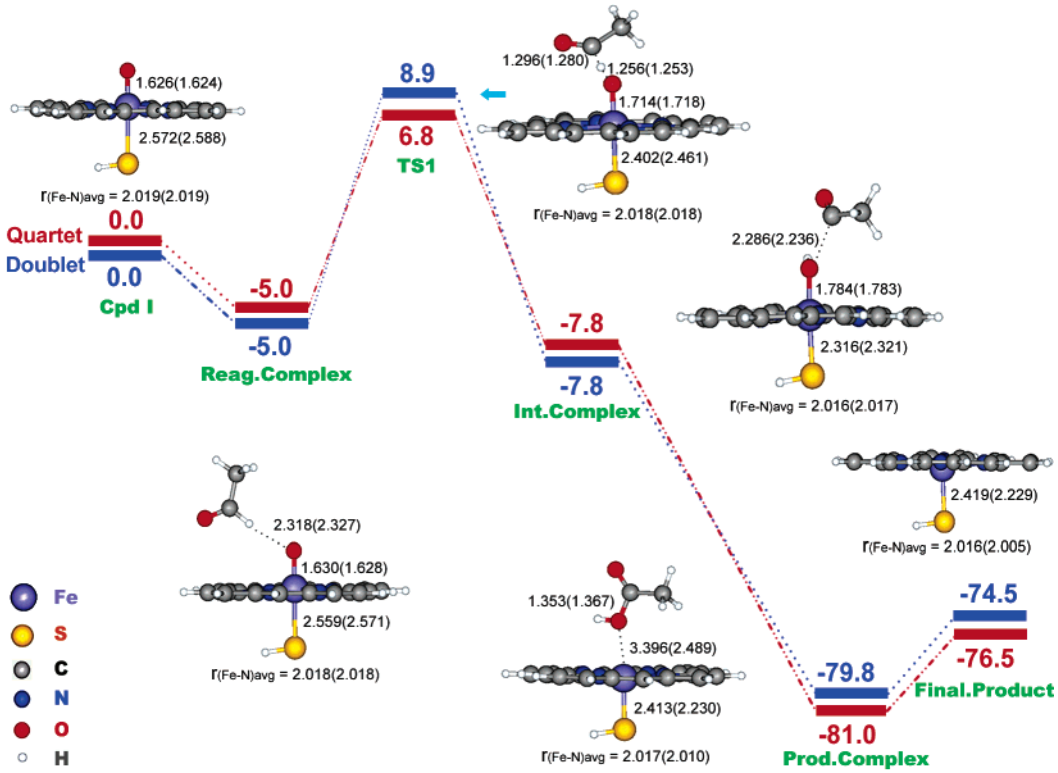

Figure 2. Energy profile (in kcal/mol) for acetaldehyde hydroxylation by the SH−Cpd I model of CYP2E1 in the quartet (doublet) state with properties of various reaction species (in angstrom).

TABLE 1: Calculated Mulliken Charge and Spin Densities for Various Moieties in the SMe-Cpd I Model System

	doublet state						quartet state					
	Fe	O	SMe	Por	H	MeCO	Fe	O	SMe	Por	H	MeCO
Cpd I	1.70	−0.32	−0.16	−1.22	0.12	−0.12	1.12	−0.32	−0.17	−0.60	0.12	−0.12
	1.24[a]	**0.91**	**−0.82**	**−0.33**	**0.00**	**0.00**	**1.14**	**0.91**	**0.79**	**0.16**	**0.00**	**0.00**
reac.	1.29	−0.13	−0.19	−0.90	0.21	−0.28	1.29	−0.13	−0.19	−0.89	0.21	−0.28
	1.31	**0.86**	**−0.80**	**−0.36**	**0.00**	**0.01**	**1.16**	**0.87**	**0.78**	**0.17**	**0.00**	**0.01**
TS1	1.14	−0.19	−0.33	−0.87	0.43	−0.18	1.02	−0.39	−0.22	−0.75	0.51	−0.18
	1.96	**0.17**	**−0.39**	**−0.32**	**−0.01**	**−0.41**	**1.28**	**0.63**	**0.49**	**0.05**	**−0.01**	**0.54**
inter.	0.84	−0.09	−0.23	−0.73	0.24	−0.02	1.07	−0.36	−0.21	−0.71	0.25	−0.04
	1.74	**0.25**	**0.14**	**−0.15**	**−0.03**	**−0.94**	**1.71**	**0.25**	**0.20**	**−0.14**	**0.04**	**0.94**
TS2	1.25	−0.23	−0.18	−0.99	0.17	0.00	1.36	−0.23	−0.24	−1.06	0.20	−0.03
	1.70	**0.24**	**0.11**	**−0.12**	**0.00**	**−0.93**	**1.89**	**0.13**	**0.18**	**−0.14**	**0.01**	**0.94**
prod.	1.88	−0.20	−0.31	−1.76	0.36	0.03	1.90	−0.22	−0.41	−1.44	0.28	−0.11
	1.09	**0.00**	**0.03**	**−0.12**	**0.00**	**0.00**	**2.62**	**0.00**	**0.52**	**−0.15**	**0.00**	**0.00**
final.	1.28	−0.23	−0.27	−1.00	0.27	−0.04	1.16	−0.23	−0.33	−0.83	0.27	−0.04
	1.07	**0.00**	**0.02**	**−0.09**	**0.00**	**0.00**	**2.51**	**0.00**	**0.52**	**−0.03**	**0.00**	**0.00**

[a] Number in bold is data of spin density, all data are computed with UB3LYP/TZV(Fe) and D95(H,C,N,S,O).

acetaldehyde radical to the Fe (Fe+thiolate) moiety on the doublet (quartet) step. The subsequent step is an exothermic one, generating the acetic acid product complex and then releasing the acetic acid and restoring to the resting state.

From the analysis mentioned above, we can see that there are obvious differences between acetaldehyde hydroxylation and alkane hydroxylation mediated by CYP450. The C−H bond activation of acetaldehyde hydroxylation is less endergonic (about 11.0∼13.0 kcal/mol) than that of the alkane hydroxylation and is about one-half to one-third of that needed for methane hydroxylation. The weaker the C−H bond being activated, the lower the energy demand for the radical-type **HS** route,[14] so transition states for the O-rebound step have just very small energy barriers in both routes (0.6 kcal/mol for **HS** and 0.7 kcal/mol for **LS**), and they are virtually barrierless in the SMe-Cpd I model system (in the SH-Cpd I model system they are absolutely barrierless). The alkane hydroxylation has a significant O-rebound barrier on the **HS** route and the reaction is stepwise, whereas on the **LS** route it is concerted and barrierless, so the reaction is spin-state sensitive. For the acetaldehyde hydroxylation, the rebound transition states are virtually barrierless in both the **HS** and **LS** routes, so the reaction proceeds in an effectively concerted O-rebound pattern, and the reaction is nearly spin-state insensitive. The phenomenon that both the **HS** and **LS** reaction routes are effectively concerted was first discovered in *trans*-2-phenyl-*iso*-propylcyclopropane hydroxylation by Kumar et al. (see Figure 7 in ref 21) and was reproduced here in our acetaldehyde hydroxylation.

We present here two possible factors of the **HS** O-rebound barrier degradation, comparing to alkane hydroxylation (methane, for example) in terms of Shaik's valence bond (VB) model.[9,38]

$$\Delta E_{\mathrm{reb}} = fG - B$$

$$G_{\mathbf{HS(LS)}} = \mathrm{IP} - \mathrm{EA}_{\mathrm{FeOH}} + E_{\mathrm{el}}(+\Delta E_{\chi^*\rightarrow\sigma^*}) \qquad (1)$$

TABLE 2: Calculated Mulliken Charge and Spin Densities for Various Moieties in the SH-Cpd I Model System

	doublet state						quartet state					
	Fe	O	SH	Por	H	MeCO	Fe	O	SH	Por	H	MeCO
Cpd I	0.50	−0.07	−0.05	−0.38	0.06	−0.06	0.49	−0.06	−0.05	−0.38	0.06	-0.06
	1.19[a]	**−0.50**	**−0.59**	**0.90**	**0.00**	**0.00**	**1.08**	**0.44**	**0.54**	**0.94**	**0.00**	**0.00**
reac.	0.50	−0.02	−0.05	−0.42	0.13	−0.13	0.48	−0.02	0.06	−0.41	0.13	-0.13
	1.23	**−0.54**	**−0.55**	**0.86**	**0.00**	**0.00**	**1.16**	**0.17**	**0.78**	**0.87**	**0.00**	**0.01**
TS1	0.38	−0.11	−0.01	−0.53	0.29	−0.01	0.41	−0.17	0.01	−0.54	0.31	-0.02
	0.99	**−0.46**	**−0.47**	**0.60**	**−0.04**	**0.38**	**1.18**	**0.23**	**0.42**	**0.70**	**−0.02**	**0.49**
inter.	0.40	−0.18	0.03	−0.58	0.30	0.03	0.40	−0.23	0.05	−0.58	0.32	0.04
	1.01	**0.29**	**0.48**	**0.20**	**−0.03**	**−0.95**	**1.74**	**−0.11**	**0.13**	**0.26**	**0.03**	**0.94**
prod.	0.27	−0.41	0.04	−0.44	0.35	0.19	0.45	−0.33	−0.15	−0.47	0.36	0.14
	1.15	**−0.10**	**−0.04**	**0.00**	**0.00**	**0.00**	**2.46**	**0.06**	**0.48**	**0.00**	**0.00**	**0.00**
final.	0.37	−0.34	−0.02	−0.47	0.32	0.15	0.53	−0.37	−0.16	−0.47	0.32	0.15
	1.20	**−0.13**	**−0.07**	**0.00**	**0.00**	**0.00**	**2.46**	**0.06**	**0.48**	**0.00**	**0.00**	**0.00**

[a] Number in bold is data of spin density, all data are computed with UB3LYP/LACVP**.

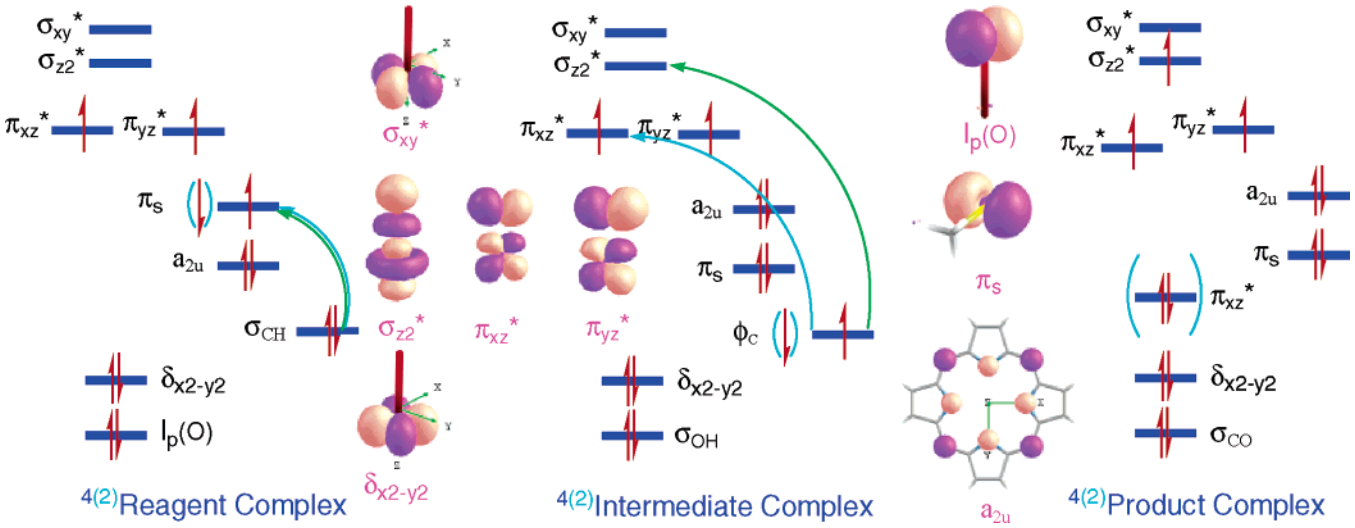

Figure 3. Orbital diagram showing the electronic change in the quartet (doublet) state in the SMe-Cpd I model system with key orbital fragments.

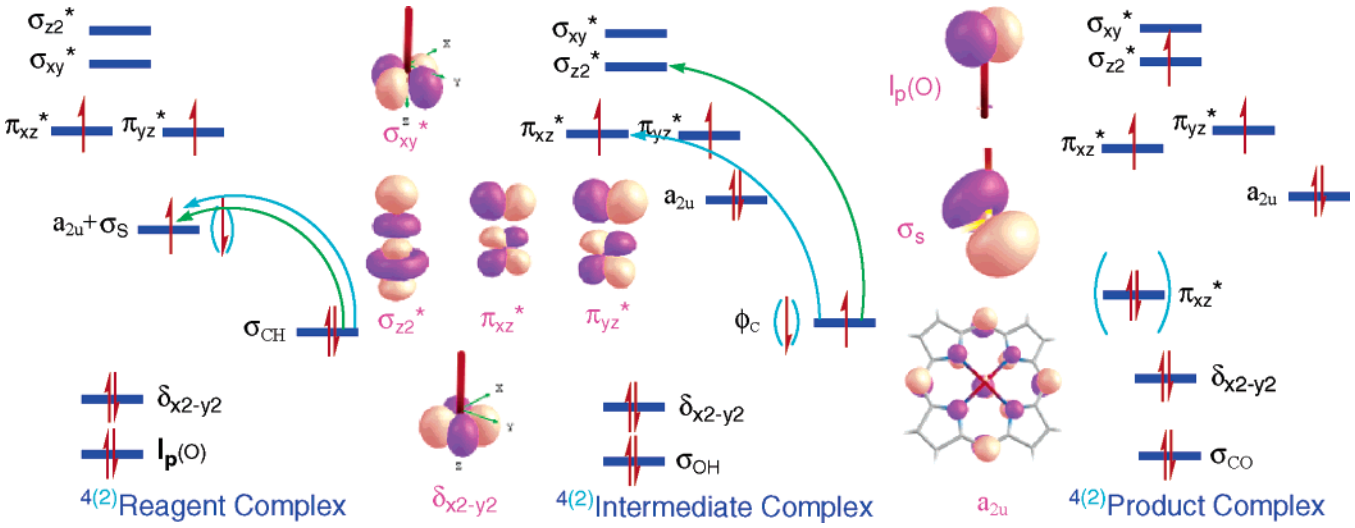

Figure 4. Orbital diagram showing the electronic change in the quartet (doublet) state in the SH-Cpd I model system with key orbital fragments.

One factor is the ionization potential energy (IP) of the intermediate radical (Acet• or alk•). From eq 1, we can see that the smaller the IP quantity, the lower the rebound barrier. The IP (165.4 kcal/mol) of the acetaldehyde radical is lower than that of the methyl (227.3 kcal/mol), so this will cause the acetaldehyde hydroxylation to possess a lower O-rebound barrier on the **HS** route. The other factor is the exothermicity of the rebound phase. The more exothermic the rebound, the smaller the *f* factor which is proportional to the O-rebound barrier (see eq 1). The acetaldehyde hydroxylation is more exothermic than methane hydroxylation (68.8 kcal/mol for acetaldehyde and 60.5 kcal/mol for methane), so acetaldehyde hydroxylation has a lower O-rebound barrier than that of the methane hydroxylation in the **HS** route.

Though the SMe-Cpd I model system and the SH-Cpd I model system give similar reaction energy profiles in the gas phase, such as the H-abstraction activation energy and the concerted **HS** and **LS** reaction routes, they are obviously

different in electronic structure. For the SMe-Cpd I case, the electronic ground states are the $^{2,4}\Pi_S$ states with the $\pi_{xz}{}^{*1}\pi_{yz}{}^{*1}\pi_S{}^1$ arrangement, in which there are large electronic spin densities locating on the thiolate ligand but nearly none on the close-shell porphyrin ring. On the contrary, in the SH-Cpd I case, the electronic ground states are the $^{2,4}A_{2u}$ states with the $\pi_{xz}{}^{*1}\pi_{yz}{}^{*1}a_{2u}{}^1$ arrangement, in which spin densities are distributing on the strongly coupled open-shell porphyrin ring and on the σ_S orbital of the thiolate ligand. Recently, QM/MM studies in which a real protein environment is considered have given evidence supporting the $^{2,4}A_{2u}$ ground states,[39−41] so that although the SMe-Cpd I model system gives acceptable O-rebound transition states, it is less appropriate than the SH-Cpd I to be taken as the model of cytochrome P450.

Meanwhile, Shaik and co-workers usually regard the rebound step to have more than just two states, that is, it has multistate reactivity (MSR).[41,42] In styrene epoxidation, Kumar et al. have pointed out the existence of another radical intermediate with $^4\mathbf{I}_{rad}(Fe^{III})$ in a $\pi_{xz}{}^{*2}\pi_{yz}{}^{*1}a_{2u}{}^1\phi_{Alk}{}^1$ arrangement.[42] In this state, the **HS** state has a significant barrier for the rebound step, augmented by polarity effects. If the $^4\mathbf{I}_{rad}(Fe^{III})$ indeed has a significant barrier for rebound in acetaldehyde hydroxylation by P450, then the standard TSR picture will fit this process as well. This potential reaction route is important, but we will stay away from it with this kind of study, because it is beyond our computational ability.

IV. Conclusions

Mechanistic aspects of acetaldehyde hydroxylation by Compound I model active species of CYP2E1 were investigated using B3LYP DFT calculations in the present paper on the basis of Shaik's TSR O-rebound mechanism. Our study demonstrates that acetaldehyde hydroxylation by CYP2E1 favors an effectively concerted pattern on both the high quartet spin state and the low doublet spin state. The rate-limiting step is the H-abstraction step, and the activation energy is about 11.7∼14.0 kcal/mol in this reaction route, which is about one-half to one-third of that needed by methane hydroxylation. Theoretical insight into the **HS** O-rebound barrier degradation was presented on the basis of Shaik's valence bond (VB) model. Meanwhile, our study presents a good theoretical proof for the existence of concerted **HS**/**LS** routes in C−H bond hydroxylation by cytochrome P450, which enriches the paradigms of Shaik's TSR theory in organometallic chemistry.

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Supporting Information Available: Ionization potential energy analysis on the basis of Shaik's valence bond (VB) model, detailed aspects of the O-rebound transition state in the SMe-Cpd I model system, and Cartesian coordinates for various reaction species. This material is available free of charge via the Internet at http://pubs.acs.org.

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