Can the peroxosuccinate complex in the catalytic cycle of taurine/α-ketoglutarate dioxygenase (TauD) act as an alternative oxidant?†

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Density functional theoretical studies on the catalytic properties of the peroxosuccinate intermediate in the catalytic cycle of taurine/α-ketoglutarate dioxygenase suggest that it cannot act as a second oxidant.

Dioxygenase enzymes are a large class of enzymes that utilize molecular oxygen on an iron center.[1] Probably the largest class of those is the α-ketoglutarate dioxygenases (αKGD), which use α-ketoglutarate (αKG) as a cofactor. These enzymes are involved in many important biochemical processes, such as in vancomycin synthesis, herbicide degradation, and DNA and RNA base repair *via* AlkB repair enzymes.[2] One of the most commonly studied αKGD enzymes is taurine/αKGD (TauD), which is found in *E. coli*.[3] The enzyme contains a central iron atom that is held in position by interactions with the side chains of two histidine and an aspartic acid residue. During the catalytic cycle of the enzyme, αKG and O_2 bind to the iron center and the system is converted into an oxoiron species with the release of CO_2 and succinate. Since the oxygen activation process occurs very fast, there is only limited information available about the catalytic cycle of the enzyme following the dioxygen binding step. Theoretical studies into the catalytic cycle of TauD identified several local minima between the dioxygen bound species and the oxoiron species, such as a superoxo bicyclic complex and a peroxosuccinate-iron complex.[4] These studies predicted the oxoiron species to be the most stable intermediate in the catalytic cycle and indeed experimental studies were able to trap and characterize it although its lifetime was very short.[5]

In heme and nonheme chemistry there are ongoing debates whether possible alternative species can act as oxidants of substrates.[6–10] For instance, cytochrome P450 mutants continued to react with alkenes to form epoxides even while the generation of the oxoiron form was prevented.[6] Based on these studies it was concluded that the precursor of the oxoiron species in the catalytic cycle, namely the hydroperoxoiron complex must be responsible for this and act as an alternative oxidant instead of the oxoiron species. These experimental conclusions were cast in doubt by density functional theoretical (DFT) calculations on model systems, which reasoned that the reactions proceeded on two competing spin state surfaces (two-state reactivity) rather than by two-oxidants.[11] Since, on each spin state surface the barrier for hydroxylation/epoxidation of a substrate is different, the final product distributions are determined by the population of these spin states.[11] By contrast, recent DFT studies on a nonheme iron complex showed that the $Fe^{III}OOH$ and $Fe^{IV}{=}O$ species are competitive oxidants of epoxidation and *cis*-diol reactions.[9] Thus, in some chemical systems there are several competitive oxidants that influence the product distributions as well as kinetic isotope effects, while in others two spin states of the same species masquerade as different oxidants. In order to find out whether there are possible alternative oxidants in the enzyme TauD, we have investigated the oxygenation properties of catalytic cycle intermediates prior to the formation of the oxoiron species. Recently we calculated the competitive hydroxylation and epoxidation mechanisms of propene by the oxoiron complex of TauD, which was found to be an aggressive and efficient oxidant as compared to oxoiron heme complexes.[12] Nevertheless, if the precursor of the oxoiron species has a long enough lifetime it may still act as a second oxidant.

We use commonly accepted procedures for our calculations,[12] which we will briefly summarize here. The calculations employ the UB3LYP hybrid density functional method[13] in combination with a double-ζ quality (LACVP) basis set on iron which includes an Electron Core Potential (ECP) while all other atoms are described by a 6-31 G basis set.[14] All structures were fully optimized (without constraints) in the Jaguar 5.5 program package,[15] and a subsequent analytical frequency calculation in Gaussian-03 verified that the structures were local minima with real frequencies only or transition state structures with one imaginary frequency.[16] Generally, minor energy differences between Jaguar and Gaussian results are obtained.[17] To test the effect of the basis set on the relative energies, we ran single point calculations with a triple-ζ quality (LACV3P+*) basis set on iron and 6-311+G* basis set on the rest of the atoms.[14] All energies reported in this work were taken from the single point LACV3P+* data with ZPE and environmental corrections at the LACVP level of theory. The effect of the environment on the relative energies was tested with the self-consistent reaction field (SCRF) model as implemented in Jaguar 5.5 using a dielectric constant (ε) of 5.7 with a probe radius of 2.72.

Fig. 1 shows the potential energy surface for the activation of molecular oxygen by TauD. Thus, molecular oxygen binds the iron center of TauD (**1**) and subsequently *via* a barrier TS_A forms a stable superoxo bicyclic structure **2**. Thereafter, release of carbon dioxide from **2** produces the peroxosuccinate-iron complex (**3**) *via* a transition state TS_B. The final step leading to the oxoiron species (**4**) is the breaking of the peroxo dioxygen bond in **3** *via* a barrier

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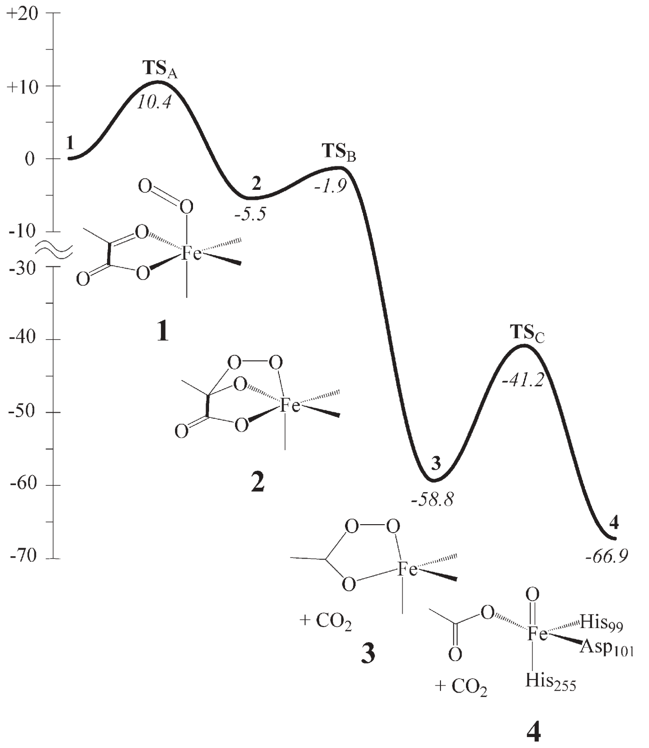

Fig. 1 Potential energy profile for the activation of molecular oxygen by a model of TauD. All energies are in kcal mol^{-1} relative to **1**.

TS$_C$. Only a small barrier (TS$_B$) of 3.6 kcal mol^{-1} exists between **2** and **3**, which implies that the bicyclic intermediate will have a very short lifetime and is unlikely to act as a second oxidant. Moreover, conversion of **2** to **3** is irreversible since more than 50 kcal mol^{-1} is gained in the process. The peroxosuccinate complex (**3**) splits further into the oxoiron species and succinate *via* a barrier (TS$_C$) of 17.6 kcal mol^{-1}. The difference in energy between TS$_C$ and **4** calculated here is almost identical to the value obtained by Siegbahn *et al.*[4] As **3** and **4** are relatively close in energy, they may exist in thermal equilibrium and consequently, both **3** and **4** will have a finite lifetime and can act as oxygenation catalysts toward substrates. To test this hypothesis, we ran calculations on the hydroxylation and epoxidation reactions of propene by **3** and compared the results with earlier work using **4** as the oxidant.[12]

The reaction of propene with the peroxosuccinate complex (**3**) is concerted *via* a C–O bond activation transition state (TS$_D$) leading to epoxide products (**5**) or *via* a hydrogen abstraction barrier (TS$_E$) to form propenol products (**6**). Both reactions (Fig. 2) are concerted mechanisms and lead to products directly after crossing the barriers. Note that the imaginary frequency for the hydrogen abstraction (TS$_E$) is only *i*498.8 cm^{-1}, whereas the hydrogen abstraction barrier by the oxoiron species gave a value of *i*1055.1 cm^{-1}.[12] This difference will lead to a substantial lowering of the kinetic isotope effect for the replacement of hydrogen by deuterium in the peroxosuccinate complex with respect to the oxoiron species. Nevertheless, in the gas-phase the epoxidation barrier is the lower of the two with a barrier of 24.6 kcal mol^{-1}. These barriers are much higher than the energy needed to break the O–O bond to form the oxoiron complex *via* TS$_C$ (Fig. 1) by 7.0 and 12.0 kcal mol^{-1}. As such, the peroxosuccinate complex is unlikely to participate in hydroxylation or epoxidation reactions and will predominantly react to form the oxoiron complex. Previous calculations showed that a dielectric constant and hydrogen bonding interactions raise the epoxidation and hydroxylation barriers by 1–2 kcal mol^{-1}.[12] Here a dielectric constant has an effect of less than 1 kcal mol^{-1} on the barrier heights (Fig. 2), so we do not expect the ordering of the barriers to change in a more polar environment as is the case in the enzyme.

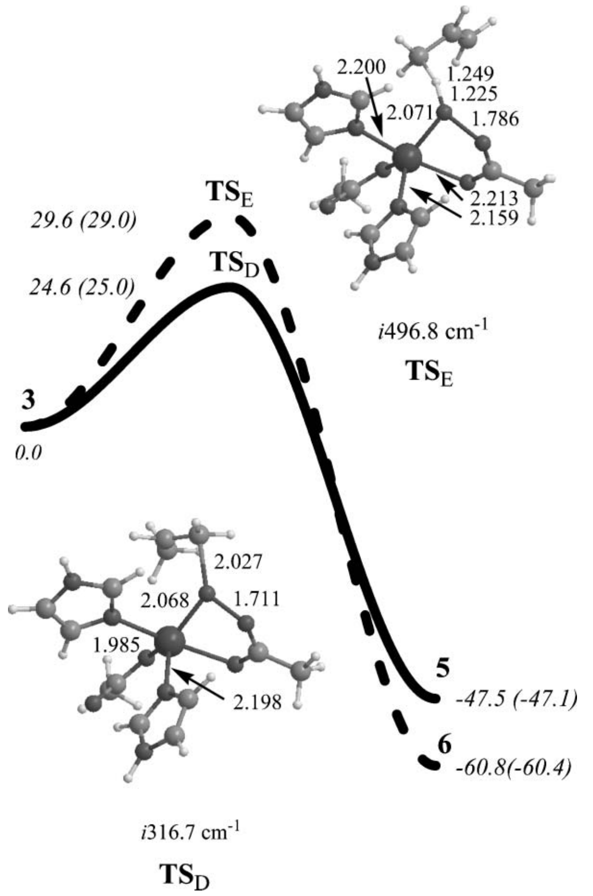

Fig. 2 Potential energy profile for the hydroxylation and epoxidation of propene by the peroxosuccinate complex (**3**) and optimized geometries of TS$_D$ and TS$_E$. All bond lengths are in Ångstroms and energies (in kcal mol^{-1}) are relative to **3**. In parentheses are energies in a dielectric constant of $\varepsilon = 5.7$ and out of parentheses are the gas-phase results.

In conclusion, DFT studies on the catalytic properties of the oxoiron *versus* the peroxosuccinate complex in the catalytic cycle of TauD indicate that the peroxosuccinate is a sluggish oxidant which is unlikely to compete with the oxoiron complex. Thus, similarly to the hydroperoxo iron complex in heme enzymes, where DFT calculations found it to be a sluggish oxidant as compared to the oxoiron species,[11] also in the catalytic cycle of TauD the precursor of the oxoiron species cannot compete with the efficiency of the oxoiron complex to hydroxylate and epoxidize substrates.

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