Atom Tunneling in the Hydroxylation Process of Taurine/α-Ketoglutarate Dioxygenase (TauD) identified by QM/MM Simulations

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Abstract

TauD dioxygenase is one of the most studied α-ketoglutarate dependent dioxygenases (αKGDs), involved in several biotechnological applications. We investigated the key step in the catalytic cycle of the αKGDs, the hydrogen transfer process, by a QM/MM approach (B3LYP/CHARMM22). Analysis of the charge and spin densities during the reaction demonstrates that a concerted mechanism takes place, where the H atom transfer happens simultaneously with the electron transfer from taurine to the Fe=O cofactor. We found quantum tunneling of the hydrogen atom to increase the rate constant by a factor of 40 at 5°C. As a consequence a quite high KIE value of close to 60 is obtained, which is consistent with the experimental value.
Introduction

Dioxygenases are non-heme iron enzymes that donate both atoms of molecular oxygen to one or more substrates. In the latter case α-ketoglutarate is often required as a co-substrate. Thus α-ketoglutarate dependent dioxygenases (αKGDs) are a very important subgroup of dioxygenases. They are crucial for several biotechnological applications, e.g., the biosynthesis of collagen (P4H enzymes), the repair of DNA or RNA (AlkB repair enzymes) or the synthesis of antibiotics (as fosfomycin or viomycin). The basic catalytic mechanism of most αKGDs is similar, the general scheme is outlined in Figure 1. Taurine/α-ketoglutarate Dioxygenase (TauD) is one of the best studied αKGDs. It is involved in the formation of aminoacetaldehyde and sulfite from taurine in bacteria like *E. coli.* We focus this study on the H transfer step of the taurine hydroxylation process in TauD and compare our outcome to previous theoretical work as well as to experimental data.

Figure 1: Schematic representation of catalytic cycle of the α-ketoglutarate dependent dioxygenases (αKGDs).
A proposed catalytic cycle for αKGDs can be found in Figure 1. For the TauD enzyme, the substrate (R-H) corresponds to taurine and the ligand (L) to Asp₁₀₁. It catalyzes the reaction shown in Figure 2.

The whole catalytic cycle (Figure 1) can be divided in three main steps: the succinate formation, the taurine hydroxylation and the release of succinate. During the first step, the $O_2$ and the α-ketoglutarate are linked to the Fe$^{II}$ center, while the taurine is situated in the surrounding. The α-ketoglutarate reacts with the $O_2$ forming succinate, an oxo-iron complex, and CO$_2$. The mechanisms for $O_2$ activation and the decarboxylation process are relatively well understood, see Figure 1. In addition, several studies agree that its rate-limiting barrier is lower than 14 kcal mol$^{-1}$. In the second step, the taurine is hydroxylated by the oxo-iron complex. The rate-limiting step is the initial H atom transfer (HAT). Previous studies show that the subsequent OH rebound proceeds with a low activation energy.

Experimentally, several crystal structures of E. coli TauD including iron have been reported. Even though all of those correspond to the state before the decarboxylation process, the oxo-iron complex has been characterized as well. Bollinger et al. confirmed a +4 oxidation state for Fe by EPR spectroscopy. They also computed reaction rate constants of the whole catalytic cycle and estimated that the rate limiting step is the release of the by-product succinate. In addition, at 278 K, a kinetic isotope effect (KIE) of 58 (interpreted as a lower limit) was found for the H atom transfer.

The final products of the taurine hydroxylation were indirectly identified by chromatography. The unstable 1-hydroxy-2-aminoethanesulfonic acid is decomposed to aminoacetaldehyde and sulfite (Figure 2). Leisinger et al. failed to detect aminoacetaldehyde in their
chromatography-mass spectra experiments because the aminoacetaldehyde undergoes polymerization reactions. Therefore, they carried out experiments adding alcohol dehydrogenase and NADH. In the latter case, the product of the reduction of aminoacetaldehyde (i.e. the 2-aminoethanol species) was monitored by chromatography.

Even though TauD is one of the most frequently studied αKGD, most of the theoretical work was based on gas-phase calculations where the model used for simulating the enzyme is greatly truncated and the effect of the environment is neglected or approximated by simple implicit models. The effect of the protein environment was incorporated in the QM/MM calculations by Visser et al., who performed an energetic study of the hydroxylation process in the triplet, quintet and septet states. In this work, we focus our QM/MM study on the H atom transfer process and restrict ourselves to the quintet state, which is well established as the high spin ground state, with the aim of clarifying the importance of the tunneling as well as the charge/spin rearrangement that takes place during that rate-limiting step.

Computational Details

The model of the enzyme c was obtained from one of the monomers (chain B) of the PDB structure 1OS7 which contains taurine and iron. We added the oxo group to the iron and replaced the α-ketoglutarate by succinate (Succ) in order to model the oxo-iron complex (see Figure 1).

Before performing the QM/MM calculations, we solvated and equilibrated the system by MD simulations. The NAMD code with a modified version of the CHARMM22 force field was employed for that purpose. 5 simulation runs of 4 ns each, starting from random distribution of the initial velocities, were performed. We selected 8 snapshots with the shortest (Fe)O–H distances as starting points for the QM/MM optimizations of the reactant states.
The QM/MM calculations were performed in the ChemShell suite\textsuperscript{39–41} using electrostatic embedding, in which the MM charges polarize the QM part. The MM part used the CHARMM22\textsuperscript{33–38} force field in DL\_POLY,\textsuperscript{12} while the QM calculations were done with TURBOMOLE 7.0.\textsuperscript{43} Geometry optimizations (minima and transition states) were done with DL-FIND\textsuperscript{14} in ChemShell. Transition state searches in, depending on the snapshot, typically 1600 degrees of freedom were performed with a superlinearly convergent\textsuperscript{45} variant of the dimer method.\textsuperscript{16–18} For the QM part (see Figure 3), we used the B3LYP functional\textsuperscript{49} as implemented in TURBOMOLE 7.0.\textsuperscript{43} We have chosen that functional because was repeatedly successfully employed in previous DFT studies on similar systems by other groups.\textsuperscript{18,19,29,30} A comparison to other density functionals is provided in table S1 of the Supporting Information. All the QM calculations were carried out at the B3LYP/def2-TZVP level with the exception of the scan for analyzing the HAT process of snapshot 1, where the optimizations along the path were done at the B3LYP/def2-SVP level followed by single point calculations and IBO and NBO analysis\textsuperscript{50,51} at the B3LYP/def2-TZVP level.

Figure 3: Schematic representation of the QM part in the QM/MM calculations.

Thermal averages for the energy barriers ($\Delta E^i_{av}$) have been calculated according to:\textsuperscript{52,53}

$$\Delta E^i_{av} = -RT \ln \left( \frac{1}{n} \sum_{i=1}^{n} \exp \left( \frac{-\Delta E^i}{RT} \right) \right) \tag{1}$$

where $n$ is the number of snapshots, $\Delta E^i$ are the energy barriers of the different snapshots,
R is the ideal gas constant, and \( T = 278 \, \text{K} \) (5 °C, as in experiments). \(^{27}\)

Rate constants and KIEs were calculated using transition state theory (TST) taking into account the contribution of atom tunneling by means of the Eckart barrier. \(^{34}\) Description of the methodology can be found in the Supporting Information.

## Results and Discussion

### Structure and Energetics

TauD is an enzyme with a quite flexible atomic structure, which results in pronounced differences between the 8 snapshots taken from the MD simulations. Barriers and reaction energies (exothermicities) relative the the reactant complexes of each snapshot are given in Table 1. The barriers including zero point energy (ZPE) differences \((\Delta E^0_i)\) vary between 12.6 and 16.8 kcal mol\(^{-1}\). The thermally averaged energy barrier, according to equation (1), including ZPE is \(E^{0,\dagger}_{av} = 13.3\) kcal mol\(^{-1}\). Without ZPE the thermally averaged barrier is \(\Delta E^{\dagger}_{av} = 18.4\) kcal mol\(^{-1}\).

The differences between the snapshots are mainly caused by the different numbers of water molecules interacting with the active center and therefore the different number of hydrogen bonds. Of these 8 possible paths, the reaction will predominantly proceed via the lowest-barrier paths, which is why we discuss these in more detail.

Snapshots 1, 2, and 3 provide the lowest (and rather similar) barriers (see Figure 4). One of the main differences between snapshots 1 and 3 is the orientation of the Asp\(_{101}\) group, which establishes H-bonds with different residues. In snapshot 1, the carboxyl group of Asp\(_{101}\) points to Trp\(_{248}\), while in snapshot 3 it interacts with Arg\(_{270}\). In addition, in snapshots 2 and 3 a second water molecule is present in the reactive center. Snapshots 2 and 3 are quite similar, however, the interaction between the residues Asp\(_{101}\) and Arg\(_{270}\) established in snapshot 3 causes a TS with a stronger bending of the H\(_\alpha\)-O-Fe angle. While there are other small differences, it seems clear that a water molecule linking the O of the
Fe=O moiety and the Asn$_{95}$ (directly or through another water) is crucial for low energies barriers. The inclusion of that water molecule in the QM model changes the reaction barriers in less than 1 kcal mol$^{-1}$.

Figure 4: Schematic representation of the TS structures for snapshots 1 to 4. Important bonds (in Å) and angles (in °) are shown for clarity. See also Table 2.

The reaction coordinate can very well be described by the difference of distances $d$(C-$H_\alpha$)$-d$(H$_\alpha$-O) of the transferred hydrogen atom $H_\alpha$ to its donor and acceptor. In Table 2 one can see that snapshot 3 shows an early TS, $d$(C-$H_\alpha$)$-d$(H$_\alpha$-O) = $-0.031$ Å, while the TS in snapshot 2 is late, $d$(C-$H_\alpha$)$-d$(H$_\alpha$-O) = $0.104$ Å, just as the TS of most other snapshots. Besides the barrier, this also influences the barrier frequency and through that the crossover.
temperature \( T_c \) (see Supporting Information), which is with 292 K for snapshot 3 significantly lower than for the other snapshots, see Table 1.

In snapshot 5 we find, in contrast to other snapshots, that the non-bonding interactions change from the minimum to the TS (see Figure 5). In the TS, two water (MM) molecules are in the reactive center. As a consequence, more H-bonds are established: two between the water molecules and the carboxyl group of the Asp\textsubscript{94} and a third one between the water molecules. The corresponding reactant structure has only one water molecule in the proximity of the H\textsubscript{\alpha}-O-Fe frame. In addition, it has the largest H\textsubscript{\alpha}-O distance found in this study (2.48 Å). That is, probably, due to the weak interaction established between Fe=O and the Arg\textsubscript{270} (2.31 Å), which disappears in the TS. Despite these catalytic effects of the protein environment, the barrier is with 19.0 kcal mol\(^{-1}\) comparably large.

Figure 5: Schematic representation of the reactant state (RS) and transition state (TS) for snapshot 5. Important bonds (in Å) are shown for clarity. See also Table 2.

In this study we focus on potential energy differences. Since entropic changes might alter the results, we estimated the changes in the vibrational entropy, \( T \Delta S = 0.44 \text{ kcal mol}^{-1} \) at 300 K for snapshot 1 using the harmonic approximation. It is clear that this is a crude approximation, especially for soft modes of the protein. Nevertheless, we assume that the entropic contribution to the chemical step is negligible. This is in line with previous results on several different enzymes.\(^{155}\)
Tunneling and kinetic isotope effects (KIE)

At room temperature, the hydrogen atom transfer in TauD is dominated by tunneling. This is apparent by the large crossover temperature $T_c$ found for all snapshots. In all cases except snapshot 3, it is clearly above room temperature, see Table 1. Generally, the larger a barrier is, the larger is the curvature of the potential at the barrier top and, consequently, the larger is $T_c$. For most snapshots of our study, this is fulfilled, but even the snapshot with the smallest barrier, snapshot 1, shows a $T_c$ of 355.5K = 82.4°C. At 5°C atom tunneling enhances the rate constant by a factor of $\kappa = 39.6$ for snapshot 1.

The main experimental indication of tunneling is the H/D kinetic isotope effect. Since the mass of deuterium is twice that of protium and tunneling is extremely sensitive to the mass of the tunneling particle, a significant KIE can be observed. At 5°C, we calculate a KIE of 59 for snapshot 1, which compares excellently to the value of 58 found experimentally. While the exact agreement is fortuitous, we find similar values for the low-barrier snapshots 2, 4, and 5. As observed in Table 1 these snapshots present similar barrier heights (12.6-13.4 kcal mol$^{-1}$) and similar crossover temperatures (around 350-370 K). Snapshot 3 shows a lower KIE, although the barrier is close to the previous cases. In the latter case, the crossover temperature is smaller, which is related to the geometry of the TS. Snapshot 3 has an early (d(C-H$_\alpha$)<d(H$_\alpha$-O))) and more angular TS, which influences the curvature of the reaction, and therefore, the tunneling efficiency. For the other cases (snapshots 6 to 8), the high-barrier snapshots show larger KIEs as expected. The dependency of KIE with $T$ is shown in Figure 6. In addition, in Figure S1 the increase of the rate constants by the tunneling effect is displayed.

Mechanism of the HAT

Formally, a hydrogen atom is abstracted from a singlet ($S = 0$) taurine moiety to O of the iron-oxo complex, which originally is in a quintet ($S = 2$) state. After the HAT, the remaining taurine radical is in a doublet state ($S = 1/2$) coupled antiferromagnetically to
Figure 6: Temperature dependence of KIEs for the 8 snapshots selected in the study.

The Fe-OH species in a sextet state \((S = 5/2)\). These formal spin states are nicely confirmed by the spin densities projected on individual atoms of snapshot 1 depicted in Figure 7. The taurine spin density changes from zero to \(-1\), corresponding to a spin-down \(S = 1/2\) state after the HAT. While in the reactant \(\text{Fe}=\text{O}\) state the spin is distributed between iron and oxygen, it is mostly centered on the iron in the product \(\text{Fe}-\text{OH}\) state. The small spin density on the ligands is caused by the partially covalent bonding of the iron to its ligands. From Figure 7 it is clear that the changes in spin density occur almost exactly concerted with the atomic movement. At the transition state, about half of the final spin density is accumulated at the taurine. Thus, no intermediate charge separation can be observed, the reaction is a clear HAT.

The same conclusion can be drawn from the analysis of the atomic charges during the reaction. In Figure S2, we can observe that an electron transfer from the iron atom to the ligands (\(\text{His}_{99}, \text{His}_{255}, \text{Asp}_{101}, \text{succinate}\)) happens during the H migration. This metal to ligand charge transfer takes place mostly between \(-0.3\ \text{Å} \) and \(0.2\ \text{Å}\). On the other hand, the O seems to abstract electrons from the taurine, since the decrease in the partial charge of the O matches with the increase in the partial charge of the taurine (note that \(H_\alpha\) is plotted independently).
Figure 7: IBO spin densities along the H-abstraction reaction path for snapshot 1. The distance difference $d(C-H_\alpha) - d(H_\alpha-O)$ has been used as reaction coordinate (being $H_\alpha$ the abstracted H from the taurine). The reaction coordinate was computed at the B3LYP/def2-TZVP//B3LYP/def2-SVP level. The vertical violet line shows the position of the TS during the scan.

Similar analyses of the atomic charges and spin densities with the NBO approach during the reaction can be found in Figure S3.

Comparison to previous theoretical studies

Our results are overall in good agreement with the ones predicted in previous DFT calculations (gas-phase or including the environment by simple models)\cite{18,19,29,30}. A recent gas-phase study by Kim\textit{ et al.}\cite{30} using methylethanesulfonate ($\text{CH}_3\text{CH}_2\text{SO}_3\text{CH}_3$) or $\text{CH}_4$ as models for the taurine substrate showed that the barriers with the B3LYP functional are similar to those obtained with the M06 functional (slightly higher). They are, overall, similar to our barriers.

In gas-phase studies, the HAT process was divided in two steps:\cite{18,19,30} a) the formation of a “preparatory stage” interpreted as a very electrophilic Fe(III)-oxyl species, and b) the H transfer. They found most of the electron transfer to take place before the transition state. This may be due to the neglect of the protein environment, since Visser\textit{ et al.}\cite{31} found a
concerted HAT process in a QM/MM model in agreement with our results. They, however, observe a lower barrier (∼7 kcal mol\(^{-1}\) with B3LYP/6-31G/MM\(^{12,15,17,31}\)) than we and Kim et al. (∼13 kcal mol\(^{-1}\)).\(^{30}\) This may be because Visser et al.\(^{31}\) model taurine as closed, i.e. including a H-bond between the NH\(_3^+\) and the SO\(_4^-\) groups of the taurine, in contrast to X-ray structure and our results.

Conclusions

We have studied the HAT process in TauD by QM/MM simulations. We found a concerted mechanism, i.e. hydrogen is abstracted along with its electron from taurine by a Fe=O species to form Fe-OH. The reaction is significantly enhanced by atom tunneling, which increases the rate constant by a factor of 39.6 at 5°C. This causes a KIE of about 60, which is in excellent agreement with the experimental value of about 58. We find an average barrier of 13.3 kcal mol\(^{-1}\) including ZPE.

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Supporting Information Available

The following files are available free of charge.

The following files are available free of charge.

- supporting_info.pdf:
Detailed explanation of the computational details (It includes the energy comparison in table S1).

Description of the topology and parameter files.

Results: Energy comparison (Table S1) Tunneling effect (Figure S1). HAT process explanation (Figures S2 and S3).

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Graphical TOC Entry
Table 1: Relative energies with respect to the reactant complexes (in kcal mol$^{-1}$) computed at the B3LYP/def2-TZVP level for the 8 selected snapshots. Crossover temperatures (in K) and KIEs (at 278 K) are also included.

| Snapshot | $\Delta E_i$ | $\Delta E_i^0$ | $T_c$  | KIE (278 K) |
|----------|--------------|----------------|-------|-------------|
| Snapshot 1 | 0.0          | 0.0            |       |             |
| RS       |              |                |       |             |
| TS       | 17.7         | 12.6           | 355.5 | 59          |
| PS       | -0.3         | -2.1           |       |             |
| Snapshot 2 | 0.0          | 0.0            |       |             |
| RS       |              |                |       |             |
| TS       | 18.2         | 12.9           | 363.0 | 68          |
| PS       | -0.8         | -2.9           |       |             |
| Snapshot 3 | 0.0          | 0.0            |       |             |
| RS       |              |                |       |             |
| TS       | 18.2         | 13.2           | 292.1 | 28          |
| PS       | -5.0         | -6.8           |       |             |
| Snapshot 4 | 0.0          | 0.0            |       |             |
| RS       |              |                |       |             |
| TS       | 18.6         | 13.4           | 374.7 | 80          |
| PS       | 0.5          | -1.5           |       |             |
| Snapshot 5 | 0.0          | 0.0            |       |             |
| RS       |              |                |       |             |
| TS       | 19.0         | 14.0           | 336.7 | 52          |
| PS       | 2.5          | 0.2            |       |             |
| Snapshot 6 | 0.0          | 0.0            |       |             |
| RS       |              |                |       |             |
| TS       | 19.8         | 16.8           | 381.3 | 101         |
| PS       | 4.3          | 3.7            |       |             |
| Snapshot 7 | 0.0          | 0.0            |       |             |
| RS       |              |                |       |             |
| TS       | 20.9         | 15.6           | 386.0 | 128         |
| PS       | 5.0          | 2.8            |       |             |
| Snapshot 8 | 0.0          | 0.0            |       |             |
| RS       |              |                |       |             |
| TS       | 21.1         | 15.7           | 381.8 | 125         |
| PS       | 5.8          | 4.1            |       |             |
Table 2: Selected geometry parameters for the stationary points of the different snapshots computed at the B3LYP/def2-TZVP level.

|                  | d(Fe-O) (Å) | d(O-H$_\alpha$) (Å) | d(C-H$_\alpha$) (Å) | $\angle$(Fe-O-H$_\alpha$) (°) | $\angle$(C-H$_\alpha$-O) (°) |
|------------------|-------------|----------------------|----------------------|-------------------------------|-------------------------------|
| Snapshot 1       |             |                      |                      |                               |                               |
| RS               | 1.624       | 2.060                | 1.090                | 141.92                        | 164.26                        |
| TS               | 1.781       | 1.228                | 1.291                | 144.86                        | 169.70                        |
| PS               | 1.873       | 0.974                | 3.097                | 111.59                        | 81.27                         |
| Snapshot 2       |             |                      |                      |                               |                               |
| RS               | 1.623       | 2.081                | 1.089                | 153.11                        | 164.63                        |
| TS               | 1.771       | 1.203                | 1.307                | 152.02                        | 171.98                        |
| PS               | 1.867       | 0.971                | 3.164                | 118.07                        | 108.92                        |
| Snapshot 3       |             |                      |                      |                               |                               |
| RS               | 1.626       | 2.214                | 1.089                | 149.36                        | 156.55                        |
| TS               | 1.775       | 1.284                | 1.253                | 140.83                        | 165.83                        |
| PS               | 1.850       | 0.977                | 2.794                | 117.97                        | 109.74                        |
| Snapshot 4       |             |                      |                      |                               |                               |
| RS               | 1.624       | 2.135                | 1.092                | 155.90                        | 176.55                        |
| TS               | 1.774       | 1.200                | 1.320                | 154.48                        | 168.81                        |
| PS               | 1.912       | 0.967                | 2.896                | 108.59                        | 106.98                        |
| Snapshot 5       |             |                      |                      |                               |                               |
| RS               | 1.634       | 2.482                | 1.092                | 140.44                        | 161.44                        |
| TS               | 1.820       | 1.256                | 1.283                | 140.74                        | 165.43                        |
| PS               | 1.914       | 0.966                | 2.545                | 112.76                        | 136.35                        |
| Snapshot 6       |             |                      |                      |                               |                               |
| RS               | 1.622       | 2.140                | 1.090                | 146.14                        | 157.86                        |
| TS               | 1.772       | 1.207                | 1.317                | 149.73                        | 168.05                        |
| PS               | 1.887       | 0.965                | 2.435                | 115.89                        | 136.80                        |
| Snapshot 7       |             |                      |                      |                               |                               |
| RS               | 1.620       | 2.185                | 1.092                | 157.80                        | 168.68                        |
| TS               | 1.759       | 1.183                | 1.340                | 159.15                        | 177.38                        |
| PS               | 1.876       | 0.969                | 2.746                | 115.23                        | 100.25                        |
| Snapshot 8       |             |                      |                      |                               |                               |
| RS               | 1.618       | 2.288                | 1.092                | 142.33                        | 150.92                        |
| TS               | 1.749       | 1.176                | 1.338                | 155.11                        | 175.24                        |
| PS               | 1.866       | 0.969                | 2.892                | 117.76                        | 105.43                        |