A theoretical study of the catalytic mechanism of oxalyl-CoA decarboxylase, an enzyme for treating urolithiasis†

Xiang Sheng,a Yongjun Liu*ab and Rui Zhangc

Oxalate is harmful to many organisms by forming insoluble precipitates with some metal cations. In humans, calcium oxalate is a major constituent of kidney stones leading to urolithiasis. Oxalobacter formigenes is a bacterium in most vertebrates and can regulate the homeostasis of oxalate. Replacement therapies of O. formigenes or related-enzymes are new strategies for treating oxalate-related diseases. Oxalyl-CoA decarboxylase (OXC) is an enzyme involved in the oxalate degradation in O. formigenes. In this paper, the catalytic mechanism of OXC was investigated by using the density functional theory (DFT) method. The most likely reaction pathway, detail of elementary steps, and roles of key residues were determined. Our calculation results indicate that the decarboxylation process can proceed rapidly, which agrees well with the experimental observation. In the protonation of the HDC-ThDP intermediate, the 4'-NH2 of ThDP is suggested to be the proton donor, which abstracts a proton from the nearby residue E121. The rate-limiting step is calculated to be the proton transfer from 4'-NH2 to the HDC-ThDP intermediate with an energy barrier of 21.8 kcal mol⁻¹. However, if this pathway is blocked by mutating residue E121, the reaction may follow another mechanism, in which Y483 acts as the proton donor and uses a water molecule as a mediator. These findings can explain the experimental observation that replacement of residues Y483 or E121 significantly reduces but does not completely abolish the activity of the enzyme. Our results may provide useful information for exploring the enzymatic mechanism and developing biocatalytic applications for treating the oxalate-related diseases.

1. Introduction

Oxalate is a compound that is naturally present in animals, plants and fungi. It can form insoluble precipitates with some metal cations, causing oxalate to be ecologically harmful to many organisms.1 In humans, calcium oxalate is a major constituent of kidney stones2 leading to urolithiasis. The increase of oxalate level has also been demonstrated to be associated with renal failure,3 cardiomyopathy4 and cardiac conductance disorders.5 However, humans and other mammals are unable to biodegrade oxalate, and this compound can only be excreted through urine or absorbed by intestine.6 As oxalate is mainly acquired from diets, one strategy to prevent oxalate-related diseases is to cut down the daily consumption of foods rich in oxalate,7 but its long-term effectiveness is still uncertain and such a diet would probably cause the deficiencies of some essential nutrients.8

Recently, an obligate anaerobe and gut-dwelling bacterium, Oxalobacter formigenes, has been proved to be directly relevant to the recurrence of kidney stone episodes.9 O. formigenes mainly exists in the gastrointestinal tracts of most vertebrates including humans, and uses oxalate as the sole energy source for their survival,10 which means O. formigenes can be used to regulate the homeostasis of oxalate, providing a new strategy for treating oxalate-related diseases. The lack of O. formigenes could increase the excretion of urinary oxalate and promote the formation of calculi.11 Strong evidences have supported that patients of urolithiasis with O. formigenes in their stool can lower the oxalate excretion in their urine.9b,12 Furthermore, traditional treatments are scarce and only achieve satisfactory results in a minority of patients.13 Thus, replacement therapies of O. formigenes or related-enzymes have attracted much attention,14 and colonization of O. formigenes has been proved to be effective in decreasing urinary oxalate levels15 and reducing the risk of stone recurrence.16 However, clinical studies showed that this colonization is not a permanent solution,17 which inspires interest to develop the enzyme-based therapies.

Two enzymes, formyl-CoA transferase (FRC)17 and oxalyl-CoA decarboxylase (OXC),18 are involved in the oxalate anaerobic
The cofactor ThDP maintains its V-like conformation, bridging a water molecule with residues I34, Y120 and Y483. R266, R408 and R555, and the oxalyl part interacts directly or via a bridging water molecule with residues I34, Y120 and Y483 or S553 greatly reduced the activity of enzyme, but these residues Y120 or S553 greatly reduced the activity of enzyme. Besides, some mutant experiments have been performed for understanding the catalysis of this enzyme. Recently, Lindqvist et al. obtained several crystal structures of OXC from O. formigenes. In which the diphosphate and 3'-phosphate of CoA moiety of substrate are positioned by residues R266, R408 and R555, and the oxalyl part interacts directly or via a bridging water molecule with residues I34, Y120 and Y483. The cofactor ThDP maintains its V-like configuration, which has been proved to be prerequisite for catalysis. A conserved glutamate residue (E56 in OXC from O. formigenes) forms a hydrogen bond with the 1'-N of ThDP, and is considered to play an important role in the activation process of ThDP. In addition, the 4'-NH2 of ThDP forms two hydrogen bonds with residues Y120 and E121. Besides, some mutant experiments have been performed for understanding the catalysis of this enzyme. For example, replacements of residues E56, Y120, E121, Y483 or S553 greatly reduced the activity of enzyme, but these mutants were not completely inactive, except E56A. Based on these structural and kinetic data, Lindqvist et al. proposed that no pocket residues directly participate in the proton transfer after ThDP activation, but a water molecule protonates the HDC-ThDP intermediate.

At present, a rough picture of the catalytic mechanism of OXC has been obtained. But open questions still remain unresolved. For example, a water molecule was suggested to protonate the HDC-ThDP intermediate, and this water molecule exists in the form of hydroxide anion in the post-decarboxylation intermediate complex. It has been known that, in some enzymes Zn2+ cation serves as Lewis acid to assist the water molecule to donate its proton. But in OXC, no Zn2+ cation or other strong Lewis acid exists in the catalytic site. Thus, it is interesting to know which residue plays this role. Besides, in some other ThDP-dependent enzyme, the 4'-NH2 of ThDP can function as a general acid/base to protonate the carbonyl group of substrate. Is it possible that the HDC-ThDP intermediate is protonated by the 4'-NH2? In addition, the specific roles of key residues in the active site, and the energetic information about the bond formation and cleavage are still not understood. An explicit description of the enzymatic mechanism is of particularly significance for exploring the enzymatic characterization and developing biocatalytic applications, and some valuable information cannot be acquired by experiments alone. Therefore, theoretical studies on the reaction mechanism at the atomistic level are still necessary. However, in contrast to the extensive studies on some other ThDP-dependent enzymes, such as pyruvate decarboxylase (PDC), acetohydroxyacid synthase (AHAS) and oxidoreductase (PFOR), so far there is no theoretical study on the catalytic mechanism of OXC.

Here, we present a density functional theory (DFT) study on the catalytic mechanism of OXC with cluster approach. In the past decades, the cluster approach has been widely applied in qualitatively elucidating the enzymatic reaction mechanisms. It should be noted that the combined quantum mechanics and molecular mechanics (QM/MM) method, which has been increasingly applied for investigating the fundamental problems of enzymology, should be the preferred strategy for this study. However, studies on different types of enzymes by using cluster approach have also shown that the calculation results with relative large cluster models were reliable and useful for qualitatively elucidating the enzymatic reaction mechanisms.

In addition, the cluster approach is easy to operate, therefore, the cluster approach was selected in the present study. Based on our calculations, the reaction pathways, the detailed energetic description of the entire reaction and the structures of involved species are presented, and the nature of proton donors and the roles of key pocket residues were determined as well.

2. Computational methods

2.1 Cluster models
The cluster models used in this work are constructed on the basis of recently obtained crystal structure of OXC from O. formigenes (PDB code: 2J7), which is a covalent HDC-ThDP intermediate after the decarboxylation of the α-oxalyl-CoA-ThDP intermediate. From the active site structure shown in Fig. 2, we can see that in this post-decarboxylation intermediate, a water molecule (W1) has taken the place of the previously formed CO2. Thus, it can be used to construct the computational model for investigating the second half-reaction. The selected atoms in our model are shown in ball and stick in Fig. 2, which contain the decarboxylated substrate–ThDP adduct HDC-ThDP, two water molecules (W1 and W2) and some key residues including E56, E121 and G426, which form hydrogen bonds with the ThDP moiety, and I34, Y120 and Y483 that interact with the CoA part directly or via water molecules.

![Scheme 1](image_url) The catalytic reaction of oxalyl-CoA decarboxylase (OXC).
Due to the crystal structure of OXC in complex with the substrate α-oxalyl-CoA and cofactor ThDP is still not available, according to the principle of microreversibility, we reversibly studied the first half-reaction by using an intermediate that the decarboxylation has been completed. To construct the starting structure for studying the first half-reaction, the water molecule W1 in the crystal structure (PDB code: 2JI7) was removed and the CO2 molecule was added manually. Thus, in fact, the initial structure for the first half-reaction is IM2, and that for the second half-reaction is IM3, as shown in Fig. 3.

The protonation states of all ionizable residues involved in the cluster models were determined according to the experimental condition, and their pK_a values were predicted by the PROPKA 3.1 program. After adding hydrogen atoms, both of two cluster models contain 133 atoms. In our calculations, the HDC-ThDP and all residues were truncated so that only important peptide backbones or side chains were included. During the optimizations, the truncated atoms were kept fixed to their crystallographic positions to prevent unrealistic movements of the groups, and the fixed atoms were marked with asterisks in the corresponding figures. It can be seen that only a few atoms were fixed in the models during the optimizations, including the terminal carbon atoms of truncated CoA part and residues I34, E56, Y120, E121, G426, and Y483 as well as the terminal carbon and oxygen atoms of truncated ThDP moiety.

Since there are extensive experimental and computational investigations on the activation mechanism of ThDP elsewhere, the activation process was not considered in our calculations. Based on the active site of used crystal structure (PDB code: 2JI7), we assume that residue Y483 may act as the acid to donate its proton of phenolic hydroxyl group to the water molecule W1, which has already donated its proton to C_2a atom of HDC-ThDP intermediate. In addition, the 4^-NH_2 may also act as the proton donor. Thus, for the second half-reaction, according to the different proton donors, two reaction pathways (Path-1 and Path-2) were considered, as shown in Fig. 3, in which the water molecule W1 and the 4^-NH_2 respectively serve as the proton donor. In Path-2, after the proton transfer from 4^-NH_2 of ThDP to C_2a atom, the reaction will undergo two elementary reactions, including the proton transfer from E121 to 4^-NH, and the cleavage of C_2–C_2a bond. Since the proton transfer process may occur prior or later to the cleavage of C_2–C_2a bond, therefore, two possible reaction pathways (Path-2A and Path-2B) were considered for Path-2.

2.2 Computational details

All quantum chemical calculations presented here were carried out by employing the Gaussian 09 program package. All geometrical optimizations were conducted with the hybrid meta-GGA density functional M06-2X and the 6-31G(d,p) basis set, and all single-point calculations were performed with the larger basis set 6-311+G(2d,2p). To consider the effect of surrounding protein environment on the energetics that was not included in the computational models, we used the polarizable-continuum model (PCM) to calculate the single-point energies at the same level for each species on the above optimized geometries. In this solvation model, the solvent is represented by a constant dielectric medium surrounding a cavity containing the solute. The empirical dielectric constant of the enzyme environment is chosen to be 4, which has been used in many studies. Harmonic frequency calculations were performed with the 6-
31G(d,p) basis set to obtain zero-point vibrational energies (ZPE) and to confirm the nature of stationary points. Forcing the truncation atoms to their crystallographic positions during the geometrical optimizations gives rise to a few small negative eigenvalues, typically in the order of 10 cm\(^{-1}\). These frequencies are very small and do not contribute significantly to the zero-point energies and thus can be tolerated. Intrinsic reaction coordinate (IRC) calculations were performed to confirm connection of transition states to two relevant minima.

3. Results and discussion

3.1 Reaction pathways

For ThDP, the characteristic torsion angles \(\Phi_t\) defined by atoms C5'–C3, 5'-N3–C2 and \(\Phi_p\) defined by C4'–C5'–C3, 5'-N3 have been suggested to be important parameters for describing the relative orientation of the two rings.\(^{35}\) Tables 1 and 2 list the values of \(\Phi_t\) and \(\Phi_p\) of all species, which indicate that the so-called “V” conformation of ThDP is well kept during the catalytic reaction. To clearly elucidate the catalytic cycle, the first and second half-reactions are respectively discussed in the following sections. All reported energies are obtained at the level of M06-2X/6-311++G(2d,2p)//M06-2X/6-31G(d,p) in the solvent phase (PCM, \(\epsilon = 4\)).

3.1.1 The first half-reaction. Although the calculations were started from the intermediate IM2 and the corresponding reactant and transition state structures were derived from IM2, to conveniently elucidate the reaction mechanism, we still discuss the first half-reaction by the forward order, i.e., the ligation of C2 atom of ThDP and C2a atom of substrate oxalyl-
Table 1 The characteristic torsion angles $\Phi_\nu$ and $\Phi_\tau$ (°) for the orientation of the two ThDP rings, and the lengths (Å) of some key hydrogen bonds in the species of the first half-reaction

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>TS1</th>
<th>IM1</th>
<th>TS2</th>
<th>IM2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Phi_\nu$</td>
<td>-79</td>
<td>-74</td>
<td>-65</td>
<td>-68</td>
<td>-69</td>
</tr>
<tr>
<td>$\Phi_\tau$</td>
<td>74</td>
<td>100</td>
<td>111</td>
<td>109</td>
<td>105</td>
</tr>
<tr>
<td>$d_{W2,I34}$</td>
<td>2.13</td>
<td>2.26</td>
<td>2.15</td>
<td>2.18</td>
<td>2.12</td>
</tr>
<tr>
<td>$d_{W2,Y120}$</td>
<td>2.03</td>
<td>1.92</td>
<td>1.96</td>
<td>1.89</td>
<td>1.83</td>
</tr>
<tr>
<td>$d_{W2,G426}$</td>
<td>2.00</td>
<td>2.09</td>
<td>1.996</td>
<td>2.02</td>
<td>2.03</td>
</tr>
<tr>
<td>$d_{Y120-O}$</td>
<td>1.86</td>
<td>1.73</td>
<td>1.59</td>
<td>1.56</td>
<td>1.48</td>
</tr>
<tr>
<td>$d_{E56,N}$</td>
<td>1.71</td>
<td>1.73</td>
<td>1.73</td>
<td>1.70</td>
<td>1.67</td>
</tr>
<tr>
<td>$d_{G426,N}$</td>
<td>1.96</td>
<td>1.93</td>
<td>1.93</td>
<td>1.96</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Table 2 The characteristic torsion angles $\Phi_\nu$ and $\Phi_\tau$ (°) for the orientation of the two ThDP rings, and the lengths (Å) of some key hydrogen bonds in the species of Path-1 and Path-2 of the second half-reaction

<table>
<thead>
<tr>
<th></th>
<th>Path-1 IM3</th>
<th>TS3-1 IM4-1</th>
<th>TS4-1 P-1</th>
<th>Path-2A IM3</th>
<th>TS3-2 IM4-2</th>
<th>TS4-2A IM5-2A</th>
<th>TS5-2A P-2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Phi_\nu$</td>
<td>-69</td>
<td>-70</td>
<td>-79</td>
<td>-86</td>
<td>-86</td>
<td>-84</td>
<td>-69</td>
</tr>
<tr>
<td>$\Phi_\tau$</td>
<td>104</td>
<td>100</td>
<td>107</td>
<td>106</td>
<td>102</td>
<td>102</td>
<td>105</td>
</tr>
<tr>
<td>$d_{W2,I34}$</td>
<td>2.06</td>
<td>2.12</td>
<td>2.11</td>
<td>2.09</td>
<td>2.26</td>
<td>2.59</td>
<td>2.32</td>
</tr>
<tr>
<td>$d_{W2,Y120}$</td>
<td>1.78</td>
<td>1.77</td>
<td>1.78</td>
<td>2.10</td>
<td>2.67</td>
<td>2.64</td>
<td>2.36</td>
</tr>
<tr>
<td>$d_{W2,G426}$</td>
<td>2.02</td>
<td>2.23</td>
<td>2.20</td>
<td>1.92</td>
<td>1.82</td>
<td>1.57</td>
<td>1.65</td>
</tr>
<tr>
<td>$d_{Y120-O}$</td>
<td>1.43</td>
<td>1.43</td>
<td>1.50</td>
<td>1.80</td>
<td>1.91</td>
<td>2.00</td>
<td>1.90</td>
</tr>
<tr>
<td>$d_{E56,N}$</td>
<td>1.65</td>
<td>1.61</td>
<td>1.60</td>
<td>1.65</td>
<td>1.67</td>
<td>1.65</td>
<td>1.62</td>
</tr>
<tr>
<td>$d_{G426,N}$</td>
<td>1.97</td>
<td>1.97</td>
<td>2.01</td>
<td>1.95</td>
<td>1.97</td>
<td>1.97</td>
<td>1.97</td>
</tr>
</tbody>
</table>

Fig. 4 Optimized geometry of the reactant R. The hydrogen bonds are shown with bond length in angstrom. The fixed terminal atoms in the model are labeled by asterisks.

The optimized structure of reactant R is shown in Fig. 4, in which many hydrogen bonds are formed among the water molecule W2, cofactor ThDP, substrate oxalyl-CoA and pocket residues. Specifically, the carboxyl and carbonyl groups of oxalyl-CoA form two hydrogen bonds with residues Y483 and Y120; W2 forms three hydrogen bonds with the amino group of I34 backbone, carboxyl group of E121 side chain and phenolic hydroxyl group of Y120 side chain; the 4'-NH$_2$ of ThDP forms two hydrogen bonds with residues E121 and G426, and 1'-N atom of ThDP forms one hydrogen bond with residue E56. All these hydrogen bond lengths are shorter than 2.2 Å, which may play important role in stabilizing the intermediates and transition states in the subsequent reaction.

The optimized structures of reactant (R), intermediates (IM1 and IM2) and transition states (TS1 and TS2) are shown in Fig. 5. The reactive C$_4$ atom of ThDP thiazolium ring positions a distance ($r_1$) of 2.96 Å from the C$_{2g}$ of oxalyl-CoA, implying this structure is favorable for the covalent addition of substrate with ThDP. From R to TS1, $r_1$ is shortened from 2.96 Å to 2.12 Å. Downhill from TS1 to IM1, this distance is further decreased to 1.54 Å, indicating the z-oxalyl-CoA/ThDP intermediate has been formed, which can be characterized by the sp$^3$ hybridized C$_{2a}$ carbon atom and bond length of C$_{2g}$O$_{2g}$ (1.33 Å). In IM1, the hydrogen bonds (shown in Fig. 5) between Y483 and the carboxyl group of oxalyl-CoA part are well kept, which means that Y483 may play significant role in the following decarboxylation. Pervious experimental studies have suggested that in the adduct of ThDP and oxalyl-CoA (IM1), the carbonyl oxygen atom had abstracted a proton from the 4'-NH$_2$ of ThDP which...
existed as a neutral hydroxyl group.\textsuperscript{35,36,37} However, in our optimized structure of IM1, this oxygen atom is still in the form of hydroxyl anion. To further verify our calculation result, by using the optimized structure of IM1 as the starting structure, we scanned the energy profile of this proton transfer process from 4′-NH\textsubscript{2} to the hydroxyl anion, as shown in Fig. 6. In our calculation, the corresponding reaction coordinate was defined by the distance between the hydrogen atom of 4′-NH\textsubscript{2} and the oxygen atom O\textsubscript{2a} of hydroxyl anion in a step size of 0.04 Å. It is found that the energy was continuously increased with the reaction coordinate changing from 1.65 Å to 1.01 Å, and no saddle point was successfully recognized. Thus, the O\textsubscript{2a} hydroxyl group in this intermediate is confirmed to be a hydroxyl anion rather than a neutral hydroxyl. The stability of hydroxyl anion can be attributed to its hydrogen bond with residue Y120. In the following step from IM1 to IM2 via TS2, the bond length of C\textsubscript{2a}–C\textsubscript{2b} is increased from 1.64 Å to 3.00 Å via 2.13 Å, and the hydrogen bond between Y483 and the broken carboxyl group still maintained (bond lengths of 1.83 Å in TS2 and 2.08 Å in IM2). Thus, residue Y483 only acts in stabilizing the formed CO\textsubscript{2}. The O\textsubscript{2a} hydroxyl group in IM2 was also calculated to be a hydroxyl anion by scanning the energy profile of proton transfer (see Fig. S1†).

The calculated energy profile for the first half-reaction is shown in Fig. 7. It can be seen that the formation of α-oxalyl-CoA-ThDP intermediate corresponds to a low energy barrier of 4.4 kcal mol\textsuperscript{−1}, and the subsequent decarboxylation suffers a low energy barrier of 5.7 kcal mol\textsuperscript{−1}. Since both of the two energy barriers are quite low, the first half-reaction is calculated to be easy to occur, which is in agreement with the previous proposals for OXC and other ThDP-dependant decarboxylases that the decarboxylation proceeds rapidly.\textsuperscript{36,37} The relative energy of intermediate IM2 is 9.4 kcal mol\textsuperscript{−1} lower than that of R, indicating the intermediate IM2 is stable and the first half-reaction is exothermic.

To examine the influence of protein electrostatic environment on the energy barriers, we also calculated the single point energies in gas for each species on the optimized geometries. The relative energies are given in the brackets in Fig. 7. Compared with that in enzyme surrounding, for each step, minor differences can be observed on the energy barriers. Therefore, the enzyme environment can influence this enzymatic reaction. The same effects were found in the following second half-reaction.

3.1.2 The second half-reaction. The calculations for the second half-reaction started from the covalent HDC-ThDP intermediate (PDB code: 2JI7), in which the formed CO\textsubscript{2} was replaced by a water molecule (W1). The optimized structure is shown in Fig. 8. It was found that both of the hydrogen atoms of W1 and 4′-NH\textsubscript{2} of ThDP are not far from the C\textsubscript{2a} atom of HDC-ThDP, and the two distances are 2.41 and 2.26 Å, respectively, which means both of W1 and 4′-NH\textsubscript{2} of ThDP are possible proton donors. Therefore, two reaction pathways (Path-1 and Path-2) were considered for the second half-reaction. In Path-1 the water molecule W1 acts as the proton donor, while in Path-2 the 4′-NH\textsubscript{2} serves as the proton donor. Fig. 8 shows that many hydrogen bonds of IM3 are similar with those of the reactant R. Except the hydrogen bond between the carboxyl group of substrate and residue Y483, all the other hydrogen bonds in R are well maintained. In IM3, W1 forms hydrogen bonds with residues Y483 and I34 with lengths of 1.74 and 2.00 Å (d\textsubscript{9} and

Fig. 6 Energy profile and corresponding structures of the proton transfer from 4′-NH\textsubscript{2} of ThDP to the O\textsubscript{2a} atom of substrate. The key bond distances are shown in angstrom.

Fig. 7 Energy profile for the first half-reaction in solvent phase (PCM, ϵ = 4). The ZPE-corrected relative energies (in kcal mol\textsuperscript{−1}) are obtained at the M06-2X/6-311+g(2d,2p)//M06-2X/6-31G(d,p) level. The values in brackets are obtained at the same level in gas phase.

Fig. 8 Optimized geometry of the starting structure (IM3) of the second half-reaction. The hydrogen bonds are shown with bond length in angstrom. The fixed terminal atoms in the model are labeled by asterisks.
endothermic.

much higher than that of IM3, implying the reaction is highly
possible. In addition, the relative energy of the product P-1 is
is a little high for an enzymatic reaction, but this pathway is still

Fig. 11. In the transition state TS3-2, the distance \((r_6)\) between the hydrogen atom of W1 and the C2\(_{a}\) atom of HDC-ThDP is 2.41 Å, which agrees with that in the
crystal structure with \(r_6\) of 2.70 Å.\(^{18}\) In transition state TS3-1, \(r_6\) is shortened to 1.35 Å and the length \((r_3)\) of O–H bond of W1 is
elongated to 1.30 Å. Meanwhile, the proton of phenolic hydroxyl
group is concertedly shifting to W1 with the length \((r_7)\) of
phenolic C–O bond changing to 1.30 Å. In IM3-1, the concerted
proton transfers have been completely finished, which can be
characterized by the \(r_6\) of 1.11 Å and distance \((r_4)\) of 1.00 Å
between the phenolic hydroxyl hydrogen atom and W1 oxygen
atom. The following cleavage of C2\(_{a}\)–C2\(_{a}\) bond is the final step of
reaction. From IM4-1 to P-1 via TS4-1, the length of C2\(_{a}\)–C2\(_{a}\) bond
\((r_7)\) is increased from 1.54 Å to 3.14 Å via 2.32 Å. In this
elementary step, the C2\(_{a}\) atom gradually becomes to sp\(^3\)
hybridized.

The calculated energy profile of Path-1 is shown in Fig. 10.
The highest point of the energy profile is 28.3 kcal mol\(^{-1}\), which
is a little high for an enzymatic reaction, but this pathway is still
possible. In addition, the relative energy of the product P-1 is
much higher than that of IM3, implying the reaction is highly
endothemic.

In Path-2, as shown in Fig. 3, after the proton transfer from
4′-NH\(_2\) of ThDP to C2\(_{a}\) atom to generate IM4-2, the subsequent
proton transfer from E121 to 4′-NH may occur prior to (Path-2A)
or later (Path-2B) than the cleavage of C2\(_{a}\)–C2\(_{a}\) bond. The
optimized structures of species to generate IM4-2 are shown in
Fig. 11. In the transition state TS3-2, the distance \((r_6)\) between
the hydrogen atom of 4′-NH\(_2\) and C2\(_{a}\) atom is 1.32 Å and the

Fig. 9 Optimized geometries at the M06-2X/6-31G(d,p) level for
various species involved in Path-1 of the second half-reaction. The key
bond distances are shown in angstrom.

length \((r_7)\) of N–H bond has been elongated to 1.41 Å. From TS3-
2 to IM4-2, \(r_7\) further decreased to 1.10 Å, indicating the
completion of proton transfer.

The optimized structures of species for the next two steps,
including the proton transfer from E121 to 4′-NH and the
cleavage of C2\(_{a}\)–C2\(_{a}\) bond, are shown in Fig. 12. In IM4-2, the
length \((r_7)\) of C2\(_{a}\)–C2\(_{a}\) bond is 1.55 Å and the distance \((r_{11})\) of
hydrogen bond between the hydrogen atom of E121 carboxyl
group and nitrogen atom of 4′-NH is 2.12 Å. In Path-2A, the
proton transfer occurs firstly to form IM5-2A in which \(r_{11}\)
changes to 1.02 Å from 1.24 Å in TS4-2A. Then the C2\(_{a}\)–C2\(_{a}\) bond
broke down to generate P-2A via TS5-2A, and the distance \((r_7)\) of
C2\(_{a}\)–C2\(_{a}\) bond changes accordingly from 1.55 Å to 3.27 Å via 2.27 Å.
In the other pathway Path-2B, from IM4-2, the bond distance
\((r_7)\) of C2\(_{a}\)–C2\(_{a}\) is firstly increased to 2.28 Å in TS4-2B and further
lengthened to 3.04 Å in IM5-2B. Then the following proton
transfer occurs to complete the reaction to generate P-2B. By
comparing the products of the two pathways, some structural
differences can be found between P-2A and P-2B, which may be
the main reason leading to different total energies of P-2A and
P-2B.

The calculated energy profiles of Path-2 are shown in Fig. 13.
The first step to generate IM4-2 corresponds to a high energy
barrier of 21.8 kcal mol\(^{-1}\). In the following reactions, both in
Path-2A and Path-2B, the energy barriers for the proton
transfers from E121 to 4′-NH are calculated to be ~20 kcal mol\(^{-1}\),
and those of C2\(_{a}\)–C2\(_{a}\) bond cleavages are much lower (3.2 and 3.9 kcal
mol\(^{-1}\) in Path-2A in Path-2B, respectively). Due to the products
P-2A and P-2B are derived from different pathways, a ~4.0 kcal
mol\(^{-1}\) energy difference was found. But from energy point of
view, the overall energy barrier of Path-2A is higher than that of
Path-2B, thus the later pathway should be more possible.
Moreover, the overall barrier of Path-1 is 6.5 kcal mol\(^{-1}\) higher
that of Path-2B, therefore, Path-2B is the most favorable reac-
tion pathway.

In general, based on our calculation results, the OXC-
catalyzed reaction was suggested to contain five elementary
steps as in Path-2B: (1) the ligation of ThDP with the substrate
oxalyl-CoA; (2) the decarboxylation of \(\alpha\)-oxalyl-CoA-ThDP intermediate and the liberation of CO2; (3) the proton transfer from \(4'-\text{NH}_2\) to \(\text{O}_{2\alpha}\) atom in Path-2. The key bond distances are shown in angstrom.

Although Path-2B is the most favorable pathway, if Path-2B is blocked, the reaction may follow Path-1. However, as mentioned above, Path-1 corresponds to a higher overall energy barrier than Path-2B and is highly endothermic, the enzymatic activity may be reduced at some extent. In the previous mutant studies, replacement of some residues was proved to significantly reduce but not completely abolish the activity, supporting our calculation results. The authors also suggested that no side chains of the enzyme directly participate in proton transfer except the glutamic acid (Glu-56). However, our calculations revealed that both of residues Y483 and E121 are involved in the catalytic reaction. In Path-1, Y483 donates its proton of phenolic hydroxyl group to W1 which donates a proton to \(\text{C}_{2\alpha}\) atom. In Path-2B, E121 acts as a proton donor in the second half-reaction. Thus, we can conclude that the significant but not completely loss of enzymatic activities of mutants of E121Q, E121A, Y483A and Y483F may be attribute to that, in these mutants the reaction follow different pathways. In other words, if E121 was mutated, Y483 can act as the proton donor, and vice versa.

### 3.2 Role of some key residues

Some hydrogen bonds are well kept during the reaction. The lengths of these hydrogen bonds in all species are given in Tables 1 and 2. The water molecule W2 always forms hydrogen bonds with surrounding residues I34, Y120 and E121, and the hydrogen bonds formed by E56 and G426 with ThDP are also

**Fig. 11** Optimized geometries at the M06-2X/6-31G(d,p) level for various species involved in the proton transfer from \(4'-\text{NH}_2\) of ThDP to \(\text{O}_{2\alpha}\) atom in Path-2. The key bond distances are shown in angstrom.

**Fig. 12** Optimized geometries at the M06-2X/6-31G(d,p) level for various species involved in the proton transfer from E121 to \(4'-\text{NH}\) and the cleavage of \(\text{C}_2-\text{C}_{2\alpha}\) bond in Path-2A and Path-2B. The key bond distances are shown in angstrom.

**Fig. 13** Energy profiles for Path-2A and Path-2B of the second half-reaction in solvent phase (PCM, \(\varepsilon = 4\)). The ZPE-corrected relative energies (in kcal mol\(^{-1}\)) are obtained at the M06-2X/6-311++G(2d,2p)/M06-2X/6-31G(d,p) level.
very stable. This indicates their roles in stabilizing the active site pocket.

For residue Y120, previous mutant studies showed that its replacement by phenylalanine or alanine residues can significantly influence the activities of the enzyme. Tables 1 and 2 show that, in the optimized reactant R the hydrogen bond distance \( d_1 \) between the hydrogen atom of phenolic hydroxyl group of Y120 and carbonyl oxygen atom of oxalyl-CoA is 1.86 Å, which are further decreased in the following intermediates, for example, they are 1.48 and 1.43 Å in IM2 and IM3, respectively. To further explore the role of Y120, we optimized the structures of mutant Y120A for proton transfers from W1 or 4'-NH2 to C2a atom in Path-1 and Path-2, and the corresponding energy profiles are shown in Fig. 14. After mutation of Y120, the energy barriers for the proton transfers from W1 or 4'-NH2 to C2a atom are both largely increased (23.6 and 28.5 kcal mol\(^{-1}\) for Path-1 and Path-2, respectively). These calculation results imply that the loss of hydrogen bond between Y120 and substrate can greatly raise the energy barriers of the reaction. Thus, the residue Y120 plays an important role in stabilizing the hydroxyl anion during the reaction.

4. Conclusion

In this paper, the catalytic mechanism of OXC has been investigated by using DFT method with cluster models. Based on our calculations, the most possible reaction pathway, the detail of each elementary step, and the roles of some key residues have been determined. Our calculation results indicate that the first-half reaction, which includes the ligation of ThDP with the substrate oxalyl-CoA and the decarboxylation of \( \alpha \)-oxalyl-CoA-ThDP intermediate and the liberation of \( \mathrm{CO}_2 \), can proceeds rapidly, which agrees well with the experimental observation. In the second-half reaction which contains the protonation of HDC-ThDP intermediate and the cleavage of its C2a-C2a bond, the 4'-NH2 of ThDP is suggested to be the proton donor, which abstracts a proton from the nearby residue E121. At the M06-2X/6-311++G(2d,2p)//M06-2X/6-31G(d,p) level of theory, the rate-limiting step is calculated to be the proton transfer from 4'-NH2 to the C2a atom of HDC-ThDP intermediate with an energy barrier of 21.8 kcal mol\(^{-1}\). However, if this pathway is blocked by mutating some residues, the reaction may follow the mechanism as Path-1, in which Y483 acts as the proton donor and uses a water molecule as mediator. These findings can explain the experimental observation that replacement of residues Y483 or E121 significantly reduces but does not completely abolish the activity of enzyme. During the reaction, in the intermediates with the C2a atom in sp\(^3\) hybridization, the O2a always exists in its hydroxyl anion form and forms hydrogen bonds with residue Y120 and/or 4'-NH2 of ThDP. Besides, water molecule W2 always forms hydrogen bonds with the surrounding residues I34, Y120 and E121, and residues G56 and G426 always form stable hydrogen bonds with ThDP, which plays important roles in stabilizing the intermediates and transition states. Our results may provide useful information for exploring the enzymatic mechanism and developing biocatalytic applications on treating the oxalate-related diseases.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (21173129, 21373125, 31200048).

References


32 Y. Zhao and D. G. Truhlar, 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 functional, Theor. Chem. Acc., 2006, 120, 215.