QM-MM Investigation on Chorismate Synthase Enzyme Role

Narin Lawan*
Computational Simulation and Modelling Laboratory (CSML), Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.
*Author for correspondence; e-mail: Narin.Lawan@gmail.com

ABSTRACT
Chorismate is a key intermediate in the biosynthesis of many aromatic compounds, including the aromatic amino acids. Chorismate synthase (CS) is an enzyme responsible for the biosynthesis of chorismate. Although several proposals have been made, the role of this enzyme is still unclear. In this work, therefore, the QM-MM adiabatic mapping calculations at the AM1-CHARMM27 level were performed. The comparisons between potential energy surface (PES) of reaction in enzyme (QM-MM energy) and the PES of reaction without enzyme (QM energy) were made. The results showed that the first step of the reaction mechanism is proton transfer rather than phosphate elimination. Several mechanisms have been proposed that the CS enzyme stabilized transition state to catalyze the reaction. This work, however, observed that not only was transition state stabilized but also intermediate and product. In addition, the enzyme holds substrate and cofactor together in the appropriate orientation making the reaction easier to proceed.

Keywords: chorismate synthase, enzyme role, QM-MM method, transition state and product stabilization

1. INTRODUCTION
The chorismate synthase (CS) reaction is the conversion of 5-enolpyruvylshikimate-3-phosphate (EPSP) to chorismate. This reaction is catalyzed by CS enzyme as shown in Scheme 1. The fact that the CS enzyme exists in bacteria, apicomplexan parasites, plants, and fungi but is not present in mammals, makes it an attractive target for new antibiotics and herbicides[1]. The CS enzyme is a good candidate for development of new therapies against tuberculosis as well [2]. The development of a new generation of CS inhibitors would clearly benefit from knowledge of the structure and conformational flexibility of the CS enzyme active site, including its reaction mechanism. Various studies have been carried out on the structure[1-5] and mechanism [6-12] of CS.

X-ray structures of CS from Streptococcus pneumoniae cocrystallized with oxidized flavin mononucleotide (FMN) and
EPSP [1] and Mycobacterium tuberculosis (MTB) [2] were reported. A homology modeling and molecular dynamics of CS revealed that Arg129, Arg125, Arg327, Arg134 and Arg48 played important roles in substrate binding [3]. A predicted structure of CS from MTB with coenzyme FMN and substrate EPSP showed that the most of template interactions are preserved in MTB structure [4]. A homology modeling of the structure of Plasmodium falciparum chorismate synthase (PfCS) along with coenzyme FMN using crystal structure of Helicobacter pylori chorismate synthase (HpCS) as template exhibited a three layered β-α-β sandwich fold [5].

The CS reaction mechanism involving elimination of phosphate from substrate EPSP to form an intermediate is an important step to convert the substrate into product. 1,4 radical elimination of allylphosphate was found to be a model reaction of the conversion of shikimate to chorismate [7]. A mechanistic study of phosphate formation from EPSP catalyzed by Escherichia coli CS indicated that the phosphate is released in the rate-determining step of CS reaction [6]. Intermediate formation after initial loss of phosphate from EPSP was proposed proceed via two possible pathways involving formation of radical and cationic intermediates [1]. A series of substrate EPSP was prepared to provide structural and mechanistic studies of CS from Neurosporacassa. Measurements of a secondary deuterium kinetic isotope effect at C-3 with EPSP shown that both C-H and C-O cleavage are partially rate-determining [8]. A study of secondary β deuterium kinetic isotope effect in the CS reaction provided evidence that the C-O bond is cleaved first [9].

As discussed previously, the 1, 4 elimination of phosphate group and hydrogen atom from EPSP to obtain chorismate is a key step of the CS reaction. This elimination reaction requires monoanionic reduced FMN cofactor, which is bound in active site of CS, to involve in electron transfer of a radical transfer mechanism to facilitate breakage of C-O bond of substrate. Two histidine residues in active site of CS were found to play an important role in catalytic activity of the enzyme: His17 and His106. His106 protonates the monoanionic reduced FMN, whereas His17 protonates the leaving phosphate group of the EPSP substrate [10]. Evidence from a mutagenic analysis of an invariant aspartate residue at position 367 (Asp367) [11] and two invariant serine residues at position 16 (Ser16) and 127 (Ser127) [12] of the Neurosporacassa CS indicated that the carbonyl group of Asp367 participates in the deprotonation of N5 of the reduced flavin cofactor [11]. It was proposed that Ser16 and Ser127 form part of a proton relay system among the isoalloxazine ring of FMN, His106 and the phosphate group of EPSP, which is necessary for the short-lived intermediate formation [12].

Although the CS reaction has been intensively studied in both experimental and computational methods, the mechanism of the reaction remains unclear. There are various works which use computer simulations to solve chemical problems including material [13], enzyme [3-5, 14-16] and inhibitor [17]. In this work, comparisons between the potential energy surfaces (PESs) calculated by QM-MM and QM methods have been made in order to elucidate the role of the CS enzyme and to investigate the mechanism of the CS reaction.
2. METHODS

The protein structure was obtained from the Protein databank and has the PDB code 1QXO [1]. The x-ray structure 1QXO is the complex between CS, EPSP and oxidized FMN. Since the structure is tetramer and every chain has the same binding site, in this study chain A is focused on. All atom types in the topology files were assigned based on the CHARMM27 parameter set [18]. The atomic partial charges of flavin mononucleotide (FMNH) and EPSP were based on fitting point charges to the electrostatic potential (HF/6-31G* CHELPG) [14, 15]. The positions of hydrogen atoms were placed in the protein using the CHARMM procedure HBUILD [19], considering the predictions made by the WHATIF web interface [20]. The protein system was solvated and truncated, retaining all protein residues within 25 Å of the carbon atom C4 of EPSP (see Figure 1 for atom numbering). The QM region was defined as the EPSP molecule and part of FMNH molecule including flavin isoalloxazine ring. The FMNH was partitioned across the bond between atoms C1′ and C2′ using a link atom [21]. The QM region consisted of 63 atoms with a net charge of -4e. The system was minimized and then equilibrated at 300 K for 120 ps using the AM1-CHARMM27 method. The simulation was carried out in the presence of solvent so that conformations of the enzyme, substrate and water molecules correspond to their native states.

![Scheme 1. Synthesis reaction of chorismate catalyzed by CS (adapted from [4]).](image)

![Figure 1. Atom numbering in EPSP and FMNH molecules.](image)
Equilibrated structures at 40 ps (calculation number 1), 60 ps (calculation number 2) and 80 ps (calculation number 3) were used as starting structures of AM1-CHARMM27 adiabatic mapping calculations. The proposed mechanism of the CS reaction used for this simulation is shown in Scheme 2. Two reaction coordinates were defined: (i) the reaction coordinate for proton transfer \( R_{\text{H+ transfer}} = d(C2 \text{ of EPSP-H8 of EPSP}) - d(H8 \text{ of EPSP-N5 of FMNH}) \) and (ii) the reaction coordinate for phosphate elimination \( R_{\text{PO elimination}} = d(C4 \text{ of EPSP-O4' of EPSP}) \). The PES of the reaction was modeled by reaction restraining the two reaction coordinates simultaneously. \( R_{\text{H+ transfer}} \) was sampled at an interval of 0.2 Å for reaction coordinate values between -1.3 to 1.3 Å. \( R_{\text{PO elimination}} \) was run with an interval 0.1 Å for the reaction coordinate between 1.4 to 3.1 Å. The force constant for the reaction coordinate restraint in all simulations was 1000 kcal mol\(^{-1}\) Å\(^{-2}\). Comparisons between PES of QM-MM and QM calculations were made. Intermediate stabilization energy (ISE) and product stabilization energy (PSE) were calculated by subtracting the QM-MM energy from the QM energy of the corresponding state.

**Scheme 2.** Proposed mechanism of the CS reaction used for the QM-MM simulation. FMNH\(_2\) stands for reduced flavin mononucleotide.
3. RESULTS AND DISCUSSION

To compare PESs of the CS reaction in enzyme and in gas phase which were calculated using the AM1-CHARMM27 method with different starting equilibrated structures, the PESs are plotted in the same graph as shown in Figure 2. The result from calculation number 1 (Figure 2(a)) shows that the reaction in enzyme proceeds from reactant (labeled as 1) to intermediate (labeled as 2) then continues to product (labeled as 3). Likewise, the reaction in gas phase progresses from reactant (labeled as 1) to intermediate (labeled as 2') then yields product (labeled as 3'). It is clear that the PES of the reaction in enzyme is lower than that in gas phase. The PESs from calculation number 2 (Figure 2(b)) and 3 (Figure 2(c)) show the similar trend as calculation number 1. Intermediate stabilization energy (ISE) and product stabilization energy (PSE) of calculations numbered 1, 2 and 3 were extracted from the graphs in Figure 2. All values of the ISEs and the PSEs are summarized in Table 1. Noting that calculations numbered 1, 2 and 3 are calculations which increase in time of equilibrated starting structures. It can be seen that the longer equilibration time yields the higher ISE and PSE. This indicates that the CS reaction is facilitated by the CS enzyme. In addition, the reaction pathway seen in Figure 2 is evidence showing that the first step of the reaction mechanism is proton transfer rather than phosphate elimination.

<table>
<thead>
<tr>
<th>Calculation number</th>
<th>Intermediate stabilization energy (ISE) (kcal/mol)</th>
<th>Product stabilization energy (PSE) (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42.0</td>
<td>28.1</td>
</tr>
<tr>
<td>2</td>
<td>44.5</td>
<td>32.0</td>
</tr>
<tr>
<td>3</td>
<td>50.8</td>
<td>97.5</td>
</tr>
</tbody>
</table>

Figure 2. Comparisons of PESs in enzyme (the lower PES, calculated by AM1/CHARMM27 method) and in gas phase (the upper PES, calculated by AM1 method); (a) calculation number 1, (b) calculation number 2 and (c) calculation number 3. Labeled numbers in the graphs are defined as follows: 1 represents reactant, 2 and 2' represent intermediate in enzyme and in gas phase, and 3 and 3' represent product in enzyme and in gas phase.
Stabilization energy surfaces (SESs) of the three calculations are shown in Figure 3. The ISE (labeled as 2*) and the PSE (labeled as 3*) were plotted relative to reactant stabilization energy (RSE)(labeled as 1*) so that the RSE is 0.0 kcal/mol. In Figure 3(a), as the reaction proceeds the proton transfer step, the stabilization energy (SE) increases from RSE (0.0 kcal/mol) to ISE (42.0 kcal/mol). When the reaction continues from intermediate to product, the SE slightly decreases from ISE (42.0 kcal/mol) to PSE (28.1 kcal/mol). The feature of the SES from calculation number 2 (Figure 3(b)) is relatively similar to that of calculation number 1 but the SE is slightly different. The ISE of this calculation is 44.5 kcal/mol. The PSE energy is 32.0 kcal/mol. Interestingly, in calculation number 3 (Figure 3(c)), as the reaction proceeds the proton transfer step, the SE increases from RSE (0.0 kcal/mol) to ISE (50.8 kcal/mol). Thereafter, the reaction proceeds from intermediate to product with significant increase of the SE from ISE (50.8 kcal/mol) to PSE (97.5 kcal/mol). The dramatic increase of the PSE in this calculation could be attributed to the effect of equilibration time. The longer equilibration could make active site orientation of the CS enzyme to be more appropriate position for product binding.

Figure 3. Stabilization energy surfaces (SESs) calculated by AM1-CHARMM27; (a) calculation number 1 (b) calculation number 2 and (c) calculation number 3. 1*, 2* and 3* represent reactant state, intermediate state and product state, respectively.
The potential of stabilization of the CS enzyme could be due to electrostatic interaction and/or hydrogen bonds of surrounding residues in the active site of the enzyme with the EPSP substrate and the FMNH cofactor (see Figure 4) as found in chorismate mutase enzyme reaction [15, 16].

Figure 4. Orientation of substrate, cofactor and surrounding residues in the active site of the CS enzyme, the structure was generated from equilibration using AM1-CHARMM27 molecular dynamics simulation.

4. CONCLUSIONS

In this work, PESs of the CS reaction calculated by QM-MM and QM methods are compared in order to evaluate the role of CS enzyme and to investigate mechanism of the CS reaction. The AM1-CHARMM27 calculations show that the enzyme not only stabilizes the transition state but also stabilizes the intermediate and product of the reaction. The mechanism of this catalysis proceeds via the proton transfer as the first step followed by the phosphate elimination. Furthermore, the CS enzyme holds the EPSP substrate and the FMNH cofactor near each other at the appropriate angle in order to enhance the rate of this reaction. These CS enzyme roles will be useful for inhibitor design in the future.

ACKNOWLEDGEMENTS

The author thanks Adrian J. Mulholland, Frederick R. Manby, Kara E. Ranaghan, and Stephen Macrae for useful discussions, the Department of Chemistry, Chiang Mai University, for the provision of research facilities.

REFERENCES

dynamics study of chorismate Synthase from *Shigella flexneri*, *J. Mol. Graph.*, 2006; 25(4): 434-441.


