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Regular Article

Computational Study on the Reaction Mechanism of LigW-Catalyzed Carboxylation of Monohydroxybenzoic Acids

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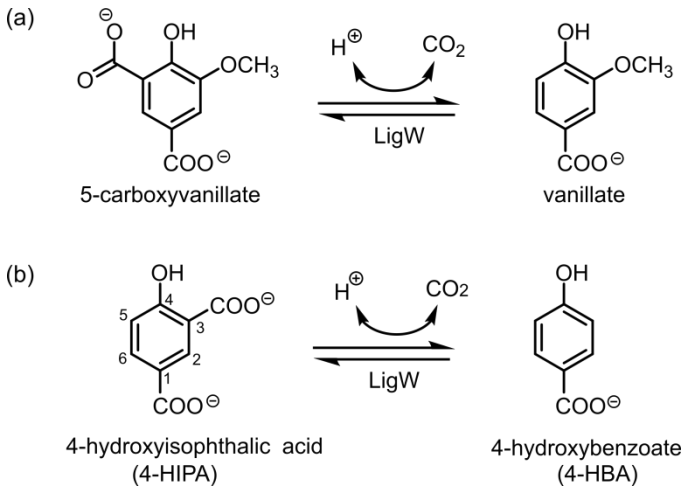
Abstract: Enzymatic carboxylation of phenols via the Kolbe-Schmitt reaction represents a promising sustainable strategy for CO_2 fixation and synthesis of high-value chemicals. This study investigates the reaction mechanism of 5-carboxyvanillate decarboxylase (LigW)-catalyzed carboxylation of non-natural monohydroxybenzoic acids, employing the quantum chemical cluster approach. First, we investigate the carboxylation mechanism of 4-hydroxybenzoate (4-HBA) to produce 4-hydroxyisophthalate (4-HIPA), a dicarboxylic acid with potent antioxidant and neuroprotective applications. The calculations reveal that the CO_2-binding mediates the preferred binding mode of the substrates and the incorporation of CO_2 to the active site favors the mode beneficial for the following reaction. The chemical reaction is initiated by the formation of a carbon-carbon bond between CO_2 and 4-HBA, followed by the rate-limiting proton transfer from the active site residue Asp314 to the resulting intermediate of the first step with a calculated barrier of 19.2 kcal/mol. Additionally, the potential of LigW in catalyzing the carboxylation of 3-hydroxybenzoate (3-HBA) is evaluated, and the calculations show that the reaction is energetically unfeasible due to the prohibitively high barrier of chemical steps. These mechanistic insights, together with the previous studies on the natural substrate, provide important information for the rotational design of LigW variants for industrial biocatalysis and CO_2 utilization.

Key words: carboxylation, reaction mechanism, quantum chemical cluster approach, biocatalysis, decarboxylase.

1. Introduction

As environmental challenges such as rising carbon dioxide (CO_2) levels intensify, developing efficient CO_2 fixation strategies has become critical. Enzymatic approaches, particularly those utilizing carboxylating enzymes, offer a promising avenue for converting CO_2 into valuable chemical products [1-3]. 5-Carboxyvanillate decarboxylase (LigW), an enzyme playing a key role in microbial lignin degradation, holds significant potential as a biotechnological tool for carbon utilization as it has been established to display carboxylation activity, in addition to its natural decarboxylation activity [4].

The reaction catalyzed by LigW in nature, the decarboxylation of 5-carboxyvanillate (Scheme 1a), has been studied using computational and experimental approaches [5-7]. It was proposed that the decarboxylation reaction proceeds through a two-step mechanism, requiring the formation of a C5-protonated intermediate before the carbon-carbon bond cleavage and the subsequent formation of the product and CO_2.



Scheme 1. LigW-catalyzed interconversion (a) between 5-carboxyvanillate and vanillate and (b) between 4-hydroxyisophthalate (4-HIPA) and 4-hydroxybenzoate (4-HBA).

A recent study demonstrated the use of LigW in catalyzing the carboxylation reaction of 4-hydroxybenzoate (4-HBA) to synthesize 4-hydroxyisophthalate (4-HIPA) (Scheme 1b), laying the groundwork for its biocatalytic application in CO_2 fixation [8]. The bio-carboxylation of aromatic and phenolic compounds is an environmental-friendly method for producing high-value aromatic carboxylic acids, in particular the dicarboxylic acid, which has been recognized for its potential application as a novel cell-protective antioxidant [9,10]. A number of other decarboxylases from the same family as LigW, namely the amidohydrolase superfamily, have also been proven to display carboxylation activities on aromatic and phenolic compounds [1-3,11]. These reactions align with the mechanistic principles of the traditional chemical Kolbe–Schmitt reaction, adapted thus as the Bio-Kolbe-Schmitt reaction in biological systems. Despite extensive studies having been performed on these enzymes [1-3,11,12], the mechanism of the LigW-catalyzed carboxylation of the non-natural monohydroxybenzoate 4-HBA remains unsolved, and the critical transition states and intermediates in the reaction and key amino acid residues regulating its activity are yet to be identified.

Detailed understanding of the reaction mechanism of LigW, especially the factors controlling its activity toward different substrates, is critical for optimizing its use in biocatalysis applications. In the present study, using the quantum chemical cluster approach, which is a powerful computational method for modeling enzymatic reactions [6,12-16], the mechanisms of the LigW-catalyzed carboxylation of monohydroxybenzoic acids are investigated. First, the binding mode of 4-HBA and the reaction pathway for its carboxylation are identified, and the corresponding energy profile is obtained. Subsequently, the feasibility of 3-hydroxybenzoate (3-HBA), a compound structurally similar to 4-HBA, was evaluated to probe the catalytic versatility and substrate selectivity of LigW. The computational insights provide a theoretical foundation for enhancing the applications of LigW in industrial biocatalysis.

2. Computational methods

2.1 Technical details

The B3LYP hybrid density functional method [17,18], incorporating D3 (BJ) dispersion corrections [19,20], was applied to all calculations presented in this study, as implemented in the Gaussian 16 program [21]. Geometry optimization was performed with 6-31g (d,p) basic sets for C, N, O, and H and LANL2DZ pseudopotential [22] for Mn. At the same level of theory as the geometry optimization, single-point energies were calculated using the SMD solvation model with $\varepsilon = 4$ [23]. Single-point calculations on the optimized structures were performed with LANL2DZ for Mn and the larger basis set 6-311+G (2d,2p) for the other atoms, ensuring improved accuracy in the energies. The zero-point energies (ZPEs) were calculated at the same level of geometry optimization. All reported values of the energies presented in the current study are the large basis set energies, corrected for ZPEs and solvation effects. The entropy contribution from binding or releasing a small gas molecule in the computational model was estimated by the translational entropy of the unbound molecule, following the previous studies [6,24-28]. The entropy of CO_2, calculated as 11.1 kcal/mol at room temperature, was incorporated into the energy of enzyme-CO_2 adduct formation. For the spin state of Mn^{2+}, the high-spin sextet state in the enzyme-substrate complex is energetically much favored over the low-spin doublet and quartet states by more than 30 kcal/mol. This trend is consistent with that reported in the previous computational study on the LigW-catalyzed reaction of the natural substrate [7]. Therefore, only the sextet state was considered for Mn^{2+} in the mechanistic study.

2.2 Model setup

Following the previous computational study on the LigW-catalyzed decarboxylation of the natural substrate [6], a large cluster model of the active site is here designed. The model was constructed on the basis of the high-resolution crystal structure of LigW from *Novosphingobium aromaticivorans*, with 5-nitrovanillate (5-NV) bound to the active site (PDB ID: 4QRN) [5]. In the current study, 5-NV was manually replaced by either 4-hydroxybenzoate (4-HBA) or 3-hydroxybenzoate (3-HBA) as substrates. In addition to the substrate, the model includes the Mn^{2+} cation and its coordinating ligands (Glu19, His188, Asp314, and Wat1), and the amino acids forming the active site (Leu47, Tyr51, Arg58, Thr90, Ser91, Tyr186, Gly207, Ala208, Ile209, Phe212, Val239, Gly240, His241, Glu244, Arg252, Arg265, Ser289, and Tyr317). Nine additional crystallographic water molecules were also incorporated. For both substrates, the hydroxyl and carboxyl groups of 4-HBA and 3-HBA are assumed to deprotonate upon binding to the active site, due to the coordination to Mn^{2+} or the hydrogen bond interactions with Tyr51 and Arg265. Amino acids were truncated as shown in the corresponding figures, and hydrogen atoms were added manually to saturate the truncated atoms. To prevent unrealistic movements during geometry optimizations, certain atoms were constrained to their crystallographic positions, as specified in Figure S1. Both 4-HBA and 3-HBA models comprise 301 atoms and have a total charge of +1.