Atomistic Modeling of Catalytic Function of Fatty Acid Photodecarboxylase

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Abstract: Fatty Acid Photodecarboxylase (FAP) is a natural photoenzyme that catalyzes the light-driven conversion of fatty acids into hydrocarbons, making it a promising candidate for sustainable biofuel production. Previous studies combining biophysical, biochemical, and computational approaches have elucidated key aspects of the FAP photocycle and revealed several unexpected reaction intermediates, including bicarbonate intermediate [1]. To clarify mechanistic aspects of the reaction intermediates, we studied bicarbonate formation in FAP, demonstrated experimentally at cryo temperatures using molecular dynamics (MD) and QM/MM simulations. Modeling shows that the fate of CO_2 in the FAP active site is controlled by temperature-dependent proton transfer and water dynamics in the catalytic site. At low temperature, catalytic residue Arg451, involved in the substrate proton transfer remains deprotonated and activates a nearby water molecule, whose hydroxide attacks CO_2 to form bicarbonate that becomes trapped in the active site. At room temperature, however, a nearby residue Cys432 rapidly reprotonates Arg451, preventing water activation; consequently CO_2 is directly released from the enzyme without forming bicarbonate, consistent with TRIR and isotope experiments [2]. Furthermore, using the flavin anion radical ($FAD^{\bullet-}$) we then probed the structure and dynamics of the FAP complex devoid of the substrate, the state responsible for FAP photodegradation. MD simulations of FAP with $FAD^{\bullet-}$ reveals an extended solvation of the catalytic site near the flavin cofactor without substrate with R451 rotated away from the FAD. These results were further corroborated by experimental observation of the formation of electron solvated species in the within extended water network near flavin [3]. Finally, we investigated the conformational flexibility of the flavin cofactor, focusing on the so-called "butterfly" bending motion that has been suggested to play a role in flavoprotein function. Using potential of mean force (PMF) calculations with selected mutations and principal component analysis (PCA), we identified residues that contribute to FAD bending and conformational dynamics. Our results demonstrate that the flavin bending strongly depends on the protein environment. In addition, our metadynamics simulations demonstrate that CO_2 exits the protein through a specific tunnel, and that mutations along this pathway could potentially affect the rate of product release. Together, these studies will deepen our mechanistic understanding of FAP catalysis while guiding rational engineering to optimize catalytic efficiency.

Bibliography :

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