Unraveling the Regioselective Reaction Mechanism of Gentisic Acid Catalyzed by GDO Enzyme

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ABSTRACT

Gentisate 1,2-dioxygenase (GDO), a ring-fission non-heme dioxygenase enzyme, displays a unique regioselective reaction with gentisic acid (GTQ) in the presence of molecular oxygen. Classical molecular dynamics simulations were carried out for both the wild-type GDO and its mutated variants, Asp174Glu and Asp174Ala, revealing the presence of three active water molecules at the enzyme's active site, pivotal in facilitating the oxidative cleavage of an aromatic C–C bond in the GTQ substrate [1]. Additionally, employing quantum mechanics/molecular mechanics (QM/MM) calculations, we unveiled three distinct reaction mechanisms explaining the regioselective oxidation of GTQ by the GDO enzyme. The formation of the main product, maleylpyruvate, via pathway A emerged as the most favorable mechanism, with a rate-determining barrier of 21.4 kcal mol^{-1}. Our study underscores the essential role of active water molecules in stabilizing the O_2 molecule and aiding in O–O and C–C bond cleavage, while also highlighting the crucial anchoring function of Asp174 in the enzymatic cycle. Moreover, upon introducing the G106A mutation to the wild-type enzyme, a significant change in catalytic activity was observed with two different substrates, salicylate and GTQ, attributable to the presence of a hydrogen bond network with water and the 5'-OH group of GTQ, which is absent in salicylate [2]. Long-range classical molecular dynamics simulations of both the wild-type GDO and its G106A mutant variant, each complexed with two different substrates, confirmed the existence of an inter molecular hydrogen bond network which is aligned with previous findings.

References

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