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The Mechanism of Action of Flavin-Dependent Halogenases

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PURPOSE OF THE ABSTRACT

To rationally engineer the substrate scope and selectivity of flavin-dependent halogenases (FDHs), it is essential to first understand the reaction mechanism and substrate interactions in the active site. FDHs have long been known to achieve regioselectivity through an electrophilic aromatic substitution at C7 of the natural substrate Trp, but the precise role of a key active-site Lys residue remains ambiguous. Formation of hypochlorous acid (HOCl) at the co-factor-binding site is by direct reaction of molecular oxygen and a single chloride ion with reduced FAD and flavin hydroxide, respectively. HOCl is then guided 10 Å into the halogenation active-site. Lys79, located in this site, has been proposed to direct HOCl towards Trp C7 through hydrogen bonding or direct reaction with HOCl to form a -NH2Cl+ intermediate. Here, we present the most likely mechanism for halogenation based on MD simulations and active-site DFT 'cluster' models of FDH PrnA in complex with its native substrate L-tryptophan, hypochlorous acid and FAD co-factor. MD simulations with different protonation states for key active-site residues suggest that Lys79 directs HOCl through hydrogen bonding, which is confirmed by calculations of the reaction profiles for both proposed mechanisms.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

BIBLIOGRAPHY

1. Wolfenden, R.; Snider, M. J., The depth of chemical time and the power of enzymes as catalysts. Acc. Chem. Res. 2001, 34, 938-945.

2. Coin, I.; Beyermann, M.; Bienert, M., Solid-phase peptide synthesis: from standard procedures to the synthesis of difficult sequences. Nat. Protoc. 2007, 2, 3247-3256.

3. V, M., Roche's fuzeon challenge. Chem. Eng. News 2005, 83.

4. Dong, C. J.; Flecks, S.; Unversucht, S.; Haupt, C.; van Pee, K. H.; Naismith, J. H., Tryptophan 7-halogenase (PrnA) structure suggests a mechanism for regioselective chlorination. Science 2005, 309 (5744), 2216-2219.

Poor, C. B.; Andorfer, M. C.; Lewis, J. C., Improving the stability and catalyst lifetime of the halogenase RebH by directed evolution. Chembiochem 2014, 15, 1286-1289.

5. Karabencheva-Christova, T. G.; Torras, J.; Mulholland, A. J.; Lodola, A.; Christov, C. Z., Mechanistic insights into the reaction of chlorination of tryptophan catalyzed by tryptophan 7-halogenase. Sci. Rep. 2017, 17395-17410 .

6. Ainsley, J.; Mulholland, A. J.; Black, G. W.; Sparagano, O.; Christov, C. Z.; Karabencheva-Christova, T. G., Structural insights from molecular dynamics simulations of tryptophan 7-halogenase and tryptophan 5-halogenase. ACS Omega 2018, 3, 4847-4859.

7. Yeh, E.; Cole, L. J.; Barr, E. W.; Bollinger, J. M.; Ballou, D. P.; Walsh, C. T., Flavin redox chemistry precedes

substrate chlorination during the reaction of the flavin-dependent halogenase RebH. Biochemistry 2006, 45 , 7904-7912.

Yeh, E.; Blasiak, L. C.; Koglin, A.; Drennan, C. L.; Walsh, C. T., Chlorination by a long-lived intermediate in the mechanism of flavin-dependent halogenases. Biochemistry 2007, 46, 1284-1292.

8. Barker, R. D.; Yu, Y.; De Maria, L.; Johannissen, L. O.; Scrutton, N. S. Mechanism of Action of Flavin-Dependent Halogenases. ACS Catalysis 2022, 12, 15352-15360. DOI: 10.1021/acscatal.2c05231.