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Preview

Harnessing a versatile monooxygenase GorA to synthesize *N*-hydroxy compounds

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In *Cell Reports Physical Science*, Maier et al. unveil a biocatalytic approach for synthesizing *N*-hydroxy compounds by integrating the flavin-dependent monooxygenase GorA into an enzymatic cascade with the decarboxylase GorB and a formate dehydrogenase-driven cofactor recycling system. This work showcases GorA's substrate scope and establishes a biocatalytic synthetic route for valuable *N*-hydroxy compounds.

N-Hydroxy compounds are found in bioactive molecules, but their chemical synthesis is challenging. These compounds, such as (chiral) hydroxylamines, are highly valuable for their reactivity in forming secondary metabolites, such as the histonedeacetylase inhibitor trichostatin A and hydroxamate siderophores.[1] Chemical synthesis of *N*-hydroxy compounds often involves amine oxidation or nitro/oxime reduction, which can lead to side reactions and require harsh conditions or toxic chemicals.[2] In nature, *N*-hydroxy compounds are synthesized by enzymes such as flavin-dependent *N*-hydroxylating monooxygenases (NMOs),[3] cytochrome P450 monooxygenases,[4] *N*-nitrosating metalloenzymes, and nitroreductases.[5] Among these, NMOs (EC 1.14.13.x) stand out for their high selectivity, mild redox potential, and ability to hydroxylate symmetric diamines without overoxidation. By contrast, P450s require complex electron-transport systems, and nitroreductases reduce hydroxylamines to amines.

In *Cell Reports Physical Science*, Maier et al. present a comprehensive view of the NMO GorA,[6] which is part of a desferrioxamine synthesis cluster from *Gordonia rubripertincta* CWB2,[7] by focusing on its kinetic properties, substrate specificity, and application in biocatalytic cascades for the synthesis of *N*-hydroxy compounds, particularly *N*-hydroxytriazenes. This work contributes significantly to the field of biocatalysis and the application of *N*-hydroxylating enzymes in producing valuable chemical intermediates.

GorA stands out for its broad substrate scope, which is uncommon among NMOs.[7] This enzyme efficiently hydroxylates diamines, such as cadaverine (Cad) and putrescine (Put), and shows activity with 2-methylcadaverine (2-MeCad), cystamine (CA), agmatine (Agm), and diethylenetriamine (DETA). Kinetic studies have shown GorA's preference for Cad (K_m = 0.011 mM; k_{cat}/K_m = 30 s^{-1} mM^{-1}) over Put (K_m = 0.81 mM; k_{cat}/K_m = 0.3 s^{-1} mM^{-1}), and the high coupling rates (89% for Cad; 56% for Put) ensure efficient NADPH use and minimize H_2O_2 byproduct formation from hydroperoxyflavin uncoupling.[3,8] Substrate inhibition at Cad concentrations > 0.5 mM underscores the need to optimize reaction conditions, such as in a cascade fashion, to control the substrate concentration for GorA.

In the study by Maier et al., liquid chromatography coupled to mass spectrometry (LC-MS/MS) analysis confirmed hydroxylation products for Cad, Agm, and CA with Agm's primary amine (not its guanidinium group) as the hydroxylation site, suggesting GorA's tolerance for structural variations beyond the α-carbon. 2-MeCad and DETA products were not detected despite substrate depletion, possibly as a result of instability or alternative transformations. This broad substrate scope makes GorA more versatile than other NMOs, such as SpMO from *Shewanella putrefaciens*, which is more substrate specific.[9]

The authors further designed a three-enzyme cascade (Figure 1) to produce OH-Cad and OH-Put from lysine (Lys) and ornithine (Orn). GorB first decarboxylates Lys and Orn to produce the corresponding *N*-hydroxydiamines Cad and Put, respectively, which GorA then hydroxylates while a mutant NADP-dependent formate dehydrogenase (FDH M7, mutated for cofactor specificity and stability)[10] regenerates NADPH, addressing issues with cofactor degradation. Starting with 5 mM substrate, the cascade yielded 2.5 mM OH-Cad and 3.6 mM OH-Put within 24 h. LC-MS/MS revealed rapid Lys consumption and highest Cad levels within 1 h, although OH-Cad showed signs of degradation, suggesting instability under prolonged reaction times. The conversion of Orn was less efficient, most likely because of the suboptimal kinetic parameters for Put. The use of formate as an electron donor for recycling NADPH with FDH M7 ensured a cost-effective system, and GorB was compatible under these reaction conditions.

Furthermore, the authors carried out the chemoenzymatic synthesis of *N*-hydroxytriazenes, which exhibit anti-inflammatory and other bioactivities. The biocatalytically produced OH-Cad and OH-Put were coupled with 4-sulfobenzenediazonium chloride (SBD) to form 3-hydroxy-3-(5-aminopentane)-1-(4-sulfonyl) phenyltriazene (OH-CadPT) and 3-hydroxy-3-(4-aminobutane)-1-(4-sulfonyl) phenyltriazene (OH-PutPT), respectively. Initial coupling attempts using unpurified hydroxylated intermediates yielded only trace products, most likely because of side reactions. To address this, the authors developed an extraction method using 80% 4-nonylphenol in octane at pH 11.5, which enabled >70% OH-Cad recovery. Back extraction at acidic pH yielded a 60% total extraction rate, and LC-MS confirmed OH-Cad purity.

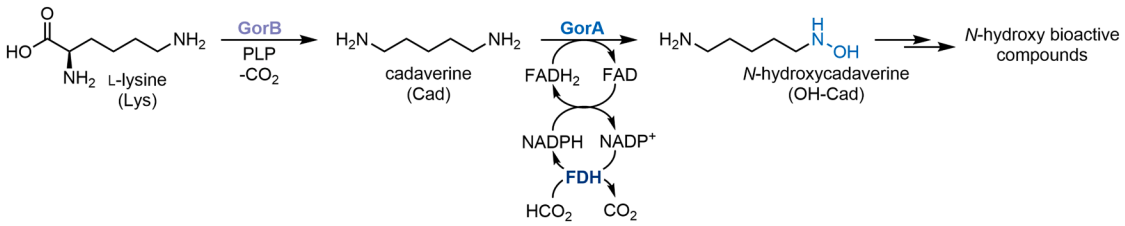

Figure 1. GorA-mediated enzymatic cascade for producing *N*-hydroxy compounds, illustrated with Lys as an example
GorB is a pyridoxal 5'-phosphate (PLP)-dependent decarboxylase that produces the diamine (Cad) *in situ* for the hydroxylation. GorA is coupled to the NADP-dependent FDH mutant M7 to recycle NADPH.

This study highlights the potential of the *N*-hydroxylating monooxygenase enzyme GorA to synthesize *N*-hydroxy compounds, offering an alternative to chemical methods. Its broad substrate scope enables access to novel *N*-hydroxy derivatives, and the cascade's integration with chemical coupling steps facilitates the production of bioactive molecules. The remaining challenges, including OH-Cad instability and the lack of detectable products for some substrates, could be addressed through optimized enzyme and substrate loading and other analytical methods. Expanding the cascade to include N–N coupling or acylation steps could further diversify the bioactive compounds produced to enhance applications in drug discovery and synthesis.

In summary, Maier et al. have demonstrated the synthetic potential and application of GorA by leveraging its promiscuity in an enzymatic cascade to produce valuable *N*-hydroxy compounds and their derivatives. This work not only advances our understanding of NMOs but also sets a foundation for scalable, biocatalytic synthetic strategies.

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DECLARATION OF INTERESTS

The author declares no competing interests.

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