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Unveiling the structural determinants for substrate recognition and catalysis of bacterial C-glycoside oxidases

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PURPOSE OF THE ABSTRACT

C-glycosides are a class of natural products with biological activities but are recalcitrant to degradation, making their catabolic pathway a topic of interest. C-glycoside 3-oxidases (G3Oxs) are a newly identified group of bacterial flavo-oxidases from the glucose-methanol-choline (GMC) superfamily, which catalyze the oxidation of C-glycosides with the concomitant reduction of O2 to H2O2. Physiologically, this oxidative step is followed by the C-C cleavage through the action of a deglycosylation complex, which releases the sugar and the free aglycone. Despite the high sequence similarity between G3Oxs and the well-studied pyranose-2-oxidases (P2Oxs), which oxidize monosaccharides at the C2 position, these two groups belong to distinct phylogenetic clades. This work shows that a bacterial G3Ox, previously known as AsP2Ox [1], has a 4-order magnitude higher catalytic efficiency (kcat/Km) to the C-glycoside mangiferin compared to D-glucose, which is the preferred substrate of fungal P2Ox. NMR analysis shows that mangiferin is oxidized to 3-keto-mangiferin, and we have renamed AsP2Ox to PsG3Ox [2]. The crystal structure was solved, showing a higher similarity to previously characterized bacterial monomeric G3Oxs [3] than to fungal tetrameric P2Oxs. The combination of mutagenesis, X-ray studies, molecular simulations, and substrate dockings revealed (i) a substrate loop located inside the active site that modulates substrate regioselective recognition, and (ii) a new structural motif, an insertion segment, with a key role for the catalysis. The joint articulation of these two regions is essential for the access and proper accommodation of the substrates in the active site. This work advances our understanding of the structure-function relationships among this family of enzymes and the mechanisms underlying the different substrate specificity between P2Ox and G3Ox.

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FIGURES

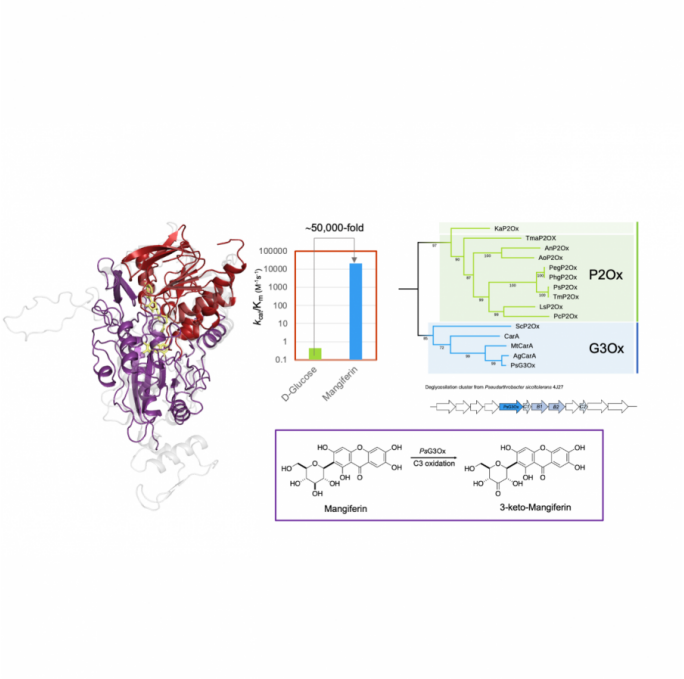

FIGURE 1

Glycoside 3-oxidases: New Bacterial Enzymes With Distinctive Phylogenetic, Functional And Structural Features

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FIGURE 2

KEYWORDS

Flavoenzymes | Carbohydrate oxidases | Auxiliary active family AA3 | Glycosides

BIBLIOGRAPHY

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