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Primary determinants for substrate preference and regioselectivity of Burkholderia thailandensis lipoxygenase

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PURPOSE OF THE ABSTRACT

Lipoxygenases (LOXs) are enzymes that catalyze regioselective dioxygenation of polyunsaturated fatty acids (PUFAs) into fatty acid hydroperoxides (FAHPs). The regioselective dioxygenation of PUFAs opens up a number of interesting applications in the food and chemical industries as the position of the hydroperoxide group in FAHPs will determine which products, with which functionalities can be derived from them. The regioselectivity of LOXs is regulated by certain structural features, including the depth of the substrate-binding pocket [1] and the position of the migration channel that shuttles molecular oxygen to the active site [2]. LOXs produced by bacteria have gained more attention in the past few years, because they have been reported to be active towards a broad range of PUFAs [3]. However, their specific activity towards each of these PUFAs can vary. Currently, there is no report on the structural features that determine the substrate preference of bacterial LOXs. In this study, we focused on understanding the structural determinants of substrate specificity in Burkholderia thailandensis LOX which has shown a high activity at pH 6 at 20-30 °C. This enzyme preferentially used ω-6 PUFAs as substrates and catalyzed the dioxygenation regioselectivity at ω-5 position. Mutations were performed on specific residues, that were proposed to play a role in the substrate preference and regioselectivity, i.e. Leu445, Phe446 and Ala431. Mutation Leu445Ala changed the substrate specificity of the enzyme from ω-6 to ω-3 PUFAs, while mutations Phe446Val and Ala431Gly enabled the enzyme to utilize both ω-6 and ω-3 PUFAs equally. Mutation Leu445Ala changed the regioselectivity of the enzyme from ω-5 to ω-2 carbon atom on ω-3 PUFAs as the substrate, while mutations Phe446Val and Ala431Gly made the enzyme less regioselective. These findings provide insights into specific residues involved in the substrate preference and regioselectivity of Burkholderia thailandensis LOX. Manipulating them may allow the synthesis of different FAHPs and therefore stimulate the exploitation of bacterial LOXs as a green alternative in a wide range of applications.

FIGURES

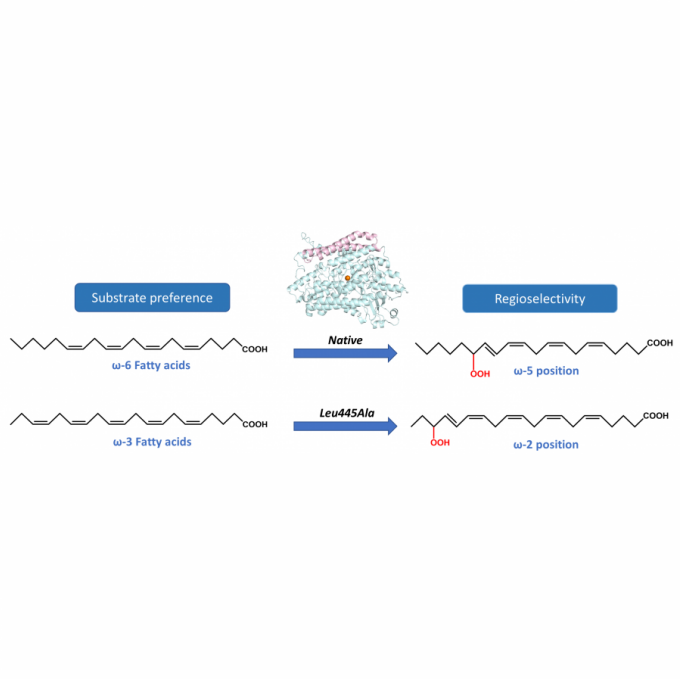

FIGURE 1

The effect of mutation on substrate preference and regioselectivity of B. thailandensis LOX

FIGURE 2

KEYWORDS

lipoxygenase | regioselectivity | substrate preference | mutagenesis

BIBLIOGRAPHY

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