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Biochemical Characterization and Structure Elucidation of a New Cytochrome P450 Decarboxylase

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Abstract : Alkenes have an economic appeal, especially in the biofuels field, since they are precursors for drop-in biofuels production, which have similar chemical and physical properties to the conventional fossil fuels, with no oxygen in their composition. After the discovery of the first P450 CYP152 OleTJE in 2011, reported with its unique property of decarboxylating fatty acids (FA), by using hydrogen peroxide as a cofactor and producing 1-alkenes as the main product, the scientific and technological interest in this family of enzymes vastly increased. In this context, the present work presents a new decarboxylase (OleTRN) with low similarity with OleTJE (32%), its biochemical characterization, and structure elucidation. As main results, OleTRN presented a high yield of expression and purity, optimum reaction conditions at 35 °C and pH from 6.5 to 8.0, and higher specificity for oleic acid. Besides that, structure-guided mutations were performed and according to the functional characterizations, it was observed that some mutations presented different specificity and chemoselectivity by varying the chain-length of FA substrates from 12 to 20 carbons. These results are extremely interesting from a biotechnological perspective as those characteristics could diversify the applications and contribute to designing better cytochrome P450 decarboxylases. Considering that peroxygenases have the potential activity of decarboxylating and hydroxylating fatty acids and that the elucidation of the intriguing mechanistic involved in the decarboxylation preferential from OleTJE is still a challenge, the elucidation of OleTRN structure and the functional characterizations of OleTRN and its mutants contribute to new information about CYP152. Besides that, the work also contributed to the discovery of a new decarboxylase with a different selectivity profile from OleTJE, which allows a wide range of applications.

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