BACTERIAL MULTICOMPONENT MONOOXYGENASES FOR THE BIOSYNTHESIS OF ANTIOXIDANTS OF INDUSTRIAL INTEREST

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Abstract

Antioxidants are compounds that reduce, neutralize and prevent the damage induced in the cells by free radicals (ROS). Tyrosol and hydroxytyrosol belong to a class of natural phenolic antioxidants whose role in the prevention of diseases such as cancer and cardiovascular diseases is rapidly emerging. Unfortunately, either the purification of these compounds from natural sources or their chemical synthesis is difficult. As a consequence much effort has been dedicated in the last years to the development of bioconversion processes to produce these molecules. In this perspective the bacterial multicomponent monooxygenase ToMO has been recently used for the biosynthesis of tyrosol and hydroxytyrosol using 2-phenylethanol, a cheap and commercially available aromatic molecule, as the starting compound. Due to the inability of the wt type enzyme to convert 2-phenylethanol into the correct tyrosol isomer, a computational model was used which analyzed the interactions between ToMO active-site residues and the substrate. We found that residue F176 is the major steric hindrance for the correct positioning of the reaction intermediate leading to tyrosol production into the active site of the enzyme. Several mutants were designed and prepared, and we found that the combination of different mutations at position F176 with mutation E103G allows ToMO to convert up to 50% of 2-phenylethanol into tyrosol in 2 h. Based on the positive results obtained for this biocatalytic process, ToMO recombinant system was used to synthesize novel antioxidant catechols using several low-added-value aromatic compounds as starting material. The starting molecules were chosen on the basis of their similarity with the natural aromatic substrates of ToMO and were docked into the active site of ToMOA, where the hydroxylation reaction takes place. The starting compounds that have been the focus of our attention are 2-phenoxoethanol, 2,3 dihydrobenzofuran, phtalan and indanol. The different catechols obtained with the use of ToMO or its mutant E103G/F176A were purified and identified by mass spectrometry and NMR analysis. The antioxidant capacity of the dihydroxylated derivatives of 2-phenoxoethanol, 2,3-dihydrobenzofuran and 2-indanol was qualitatively evaluated with radical scavenging assays using DPPH (α, α-diphenyl-β-picrylhydrazyl). In all cases the new catechols produced were able to oxidize the stable DPPH radical. In conclusion, the ability of ToMO to produce catechols confirms the versatility of this multicomponent enzymatic system and the possibility to produce new potential antioxidants not found in nature.