World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:10, No:02, 2016

STD-NMR Based Protein Engineering of the Unique Arylpropionate-Racemase AMDase G74C

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Abstract : Enzymatic racemization allows the smooth interconversion of stereocenters under very mild reaction conditions. Racemases find frequent applications in deracemization and dynamic kinetic resolutions. Arylmalonate decarboxylase (AMDase) from Bordetella Bronchiseptica has high structural similarity to amino acid racemases. These cofactor-free racemases are able to break chemically strong CH-bonds under mild conditions. The racemase-like catalytic machinery of mutant G74C conveys it a unique activity in the racemisation of pharmacologically relevant derivates of 2-phenylpropionic acid (profenes), which makes AMDase G74C an interesting object for the mechanistic investigation of cofactor-independent racemases. Structure-guided protein engineering achieved a variant of this unique racemase with 40-fold increased activity in the racemisation of several arylaliphatic carboxylic acids. By saturation–transfer–difference NMR spectroscopy (STD-NMR), substrate binding during catalysis was investigated. All atoms of the substrate showed interactions with the enzyme. STD-NMR measurements revealed distinct nuclear Overhauser effects in experiments with and without molecular conversion. The spectroscopic analysis led to the identification of several amino acid residues whose variation increased the activity of G74C. While single-amino acid exchanges increased the activity moderately, structure-guided saturation mutagenesis yielded a quadruple mutant with a 40 times higher reaction rate. This study presents STD-NMR as versatile tool for the analysis of enzyme-substrate interactions in catalytically competent systems and for the guidance of protein engineering.

Keywords : racemase, rational protein design, STD-NMR, structure guided saturation mutagenesis
Conference Title : ICB 2016 : International Conference on Biocatalysis
Conference Location : Barcelona, Spain
Conference Dates : February 15-16, 2016