BIOTRANS
LA ROCHELLE 2023

N°199 / OC / PC
TOPIC(s) : Artificial enzymes and de-novo enzyme design

Boronic-Acid Catalysis in an Artificial Enzyme

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PURPOSE OF THE ABSTRACT

Due to their unparalleled activities, selectivities and benign operating conditions, enzymes will play an important role in the transition towards greener chemical manufacture[1]. However, their reaction scope still lags behind that of chemical catalysts, a problem that is addressed through the creation of artificial enzymes: protein scaffolds equipped with abiological catalytic moieties[2]. Here we disclose the design and evolution of an artificial enzyme exhibiting boronic-acid catalysis using a low-valent boron species as catalytic moiety (Figure 1)[3]. We mutated a non-enzymatic protein with a promiscuous hydrophobic pore to incorporate a genetically encoded boronic-acid containing unnatural amino acid[4]. This new side chain catalyses oxime-formation between alpha-hydroxy ketones and hydroxylamine. The protein scaffold boosts activity, as well as providing selectivity in the transformation, allowing a kinetic resolution to be performed. By screening cell-free lysates from site-saturation libraries in a plate-reader based assay, we performed several rounds of directed evolution and identified a triple mutant with greatly improved activity and E value over 100. Through 11B NMR spectroscopy, we could characterise the hybridisation state of the catalytic residue and its binding to several ligands. These adducts could also be observed with mass spectrometry. X-ray crystallography revealed a large structural change in the protein induced by the unnatural catalytic residue. This study paves the way for realisation of many new biocatalytic activities exploiting boronic acid catalytic residues.

FIGURES

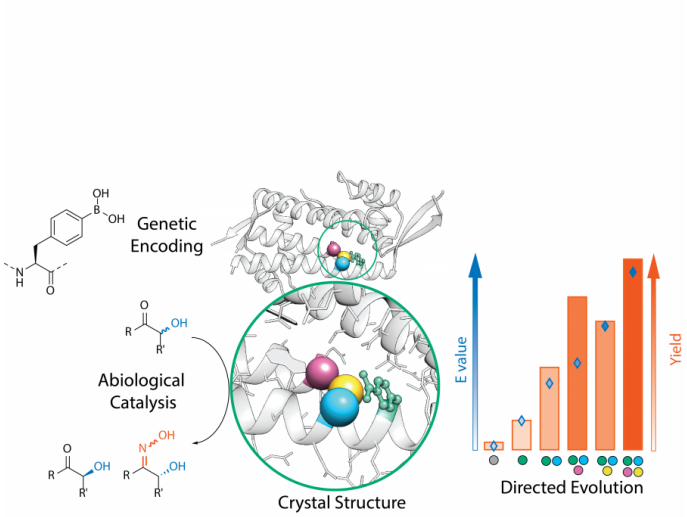

FIGURE 1

Figure 1

Design and crystal structure of a boronic-acid based artificial enzyme with genetically encoded boronic-acid residue catalysing oxime formation of alpha-hydroxy ketones and its subsequent directed evolution to optimise the protein environment surrounding

FIGURE 2

KEYWORDS

Artificial Enzyme | Unnatural Amino Acid | Directed Evolution | Crystallography

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