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Unlocking the catalytic potential of an unusual thiamine diphosphate (ThDP)-dependent enzyme

AUTHORS
Lucrezia LANZA / UNIVERSITY OF FREIBURG, ALBERTSTRASSE 25, FREIBURG IM BREISGAU

PURPOSE OF THE ABSTRACT
Thiamine diphosphate (ThDP)-dependent enzymes are valuable biocatalysts for the synthesis of chiral building blocks needed in the pharmaceutical and chemical industries. Their substrate promiscuity, high stereo- and enantioselectivity, and ability to catalyse carbon–carbon bond formation, make them extremely attractive in this field.

One of the peculiar features of these enzymes is that they have overall little sequence similarity but share a similar structural organization that favours the same ThDP cofactor binding and chemistry. There is one conserved motif that has been commonly identified among this diverse class of enzymes: the ThDP binding motif characterized by a -GDG-(X)26-28-NN- sequence [1]. This motif is involved in the correct positioning of the cofactors (ThDP and a divalent cation) in the active site and is therefore crucial for the catalytic activity of the enzymes [2]. Notably, some exceptions to this conserved motif exist in nature, but, to the best of our knowledge, have not been characterized yet.

Here we present a novel ThDP-dependent enzyme with an unusual ThDP-binding motif. By combining in silico analysis, mutagenesis studies, and structural characterization, we investigate the mechanistic role of this uncommon motif. Furthermore, using biosynthetic gene cluster analysis we speculate on the physiological substrates of the enzyme and characterize it in terms of basic biochemical parameters, substrate promiscuity, and stereoselectivity. Finally, we exploit the enzyme for unusual product scopes with respect to biocatalytic applications.

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FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Biocatalysis | Thiamine diphosphate-dependent enzymes | Carboligation

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