Using saturation mutagenesis to explore substrate specificity and catalysis in benzoylformate decarboxylase

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Abstract

Benzoylformate decarboxylase (BFDC) from *Pseudomonas putida* and pyruvate decarboxylase (PDC) from *Zymomonas mobilis* are thiamin diphosphate (ThDP)-dependent enzymes. The two share a common three-dimensional structure and catalyze a similar chemical reaction, i.e., decarboxylation of 2-keto acids. However, they vary significantly in their substrate utilization pattern. In particular, BFDC has extremely limited activity with pyruvate, while PDC has no activity with benzoylformate. Both enzymes also catalyze stereospecific carboligation reactions that are of commercial interest, again with a different range of substrates. In order to identify similarities and differences on a molecular level, and to reveal factors responsible for substrate specificity and enantioselectivity, the X-ray structures BFDC and PDC were compared. Residues identified in this process were subjected to site-directed mutagenesis. The results show that, although it was not possible to simply interchange substrates, it was possible to engineer enzymes that had distinctly different substrate specificities while retaining excellent kinetic activity. However, it also became apparent that a more general approach was needed. Towards this end we developed a screening procedure for BFDC to enable us to use saturation mutagenesis to examine residues involved in substrate specificity. During the development of the methodology it became clear that it was possible to use this approach to explore residues involved in catalysis by BFDC. Here we describe the unexpected results obtained using saturation mutagenesis on putative catalytic residues. In addition we report towards converting BFDC into an efficient pyruvate decarboxylase.