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Exploring the active site of benzaldehyde lyase by modeling and mutagenesis

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Abstract

Benzaldehyde lyase (BAL) is a thiamin diphosphate-dependent enzyme, which catalyzes the breakdown of (*R*)-benzoin to benzaldehyde. In essence, this is the reverse of the carboligation reaction catalyzed by benzoylformate decarboxylase (BFD). Here, we describe the first steps towards understanding the factors influencing BFD to form a C−C bond under conditions wherein BAL will cleave the same bond. What are the similarities and differences between these two enzymes that result in the different catalytic activities? The X-ray structures of BFD and pyruvate decarboxylase (PDC) were used as templates for modeling benzaldehyde lyase. The model shows that a glutamine residue, Gln113, replaces the active site histidines of BFD and PDC. Replacement of the Gln113 by alanine or histidine reduced the value of k_{cat} for lyase activity by more than 200-fold. The residues in BFD interacting with the phenyl ring of benzoylformate have similarly positioned counterparts in BAL but Ser26, the residue known to interact with the carboxylate group of benzoylformate, has been replaced by an alanine (Ala28). The BAL A28S variant exhibited 7% of WT activity in the BAL assay but, in the most intriguing result, this variant was able to catalyze the decarboxylation of benzoylformate. Conversely, the BFD S26A variant was unable to cleave benzoin.

Keywords: Thiamin diphosphate; Carboligation; Decarboxylation; Catalysis; Interconversion; (*R*)-benzoin

1. Introduction

Benzaldehyde lyase (E.C. 4.1.2.38) was initially isolated from *Pseudomonas fluorescens* biovar I, a strain which was able to grow on the lignin model compound, anisoin, as sole carbon source [1]. Crude extracts of this strain were found to convert anisoin and benzoin to anisaldehyde and benzaldehyde, respectively. Purified benzaldehyde lyase (BAL) was subsequently shown to cleave the α-hydroxy linkage of (*R*)-benzoin to give two molecules of benzaldehyde (Fig. 1). BAL has a requirement for thiamin diphosphate (ThDP) and catalytic activity, which is lost by treatment with EDTA, can be restored by addition of 1 mM $MgCl_2$, $MnSO_4$ or $CaSO_4$ [1]. Later, BAL was cloned, sequenced and shown to have some sequence identity with the ThDP-dependent enzymes, acetolactate synthetase (ALS) and pyruvate decarboxylase (PDC) [2].

PDC also has ~20% sequence identity [3] with another ThDP-dependent enzyme, benzoylformate decarboxylase (BFD; EC 4.1.1.7), which catalyzes the non-oxidative cleavage of benzoylformate to benzaldehyde and carbon dioxide (Fig. 1). In addition to their main metabolic functions both BFD and PDC possess carboligase activity, i.e., they are able to catalyze carbon−carbon bond formation and, consequently, both enzymes have found use in stereospecific chemoenzymatic syntheses [4,5]. Interestingly, one of the most efficient reactions catalyzed by BFD is the benzoin reaction (Fig. 1), wherein two molecules of benzaldehyde are converted to (*R*)-benzoin [6]. This, of course, is the reverse of the reaction catalyzed by benzaldehyde lyase.

It is generally accepted that the catalytic cycle of ThDP-dependent enzymes involves the initial attack of the ThDP ylide [7] to produce an adduct which subsequently breaks down to form an enamine. In the absence of an acceptor aldehyde the product is released and the ylide is regenerated. However, if an acceptor aldehyde is present, the enamine is able undergo carbon−carbon bond formation (Fig. 2). This mechanism suggests that BAL also should be able to catalyze C−C bond formation, so it was not surprising when BAL, in the presence of an excess of benzaldehyde, was shown to produce (*R*)-benzoin almost quantitatively [8]. Subsequently,

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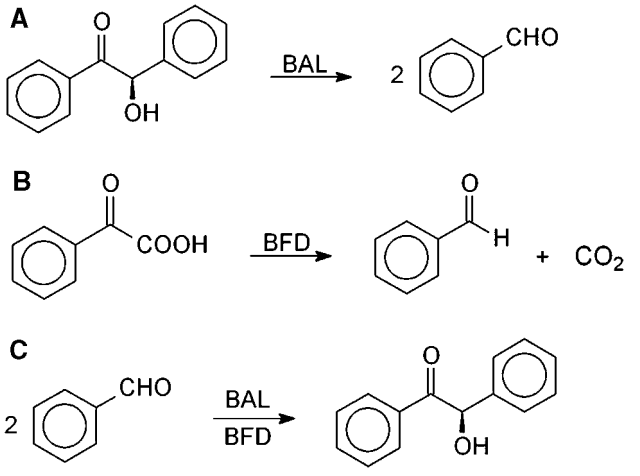

Fig. 1. (A) BAL catalyzed cleavage of (*R*)-benzoin, (B) BFD catalyzed decarboxylation of benzoylformate and (C) and the carboligation reaction catalyzed by both BAL and BFD.

BAL too has been shown to have considerable utility in enantioselective synthesis [9–11].

The X-ray structures of PDC from *Z. mobilis* [12] and *S. cerevisiae* [13,14], and that of BFD from *P. putida* [3], are available. These show that, overall, the architectures of the two decarboxylases are comparable. The residues involved in ThDP binding are similar and there is some positional conservation of other residues. For example, each active site contains two histidine residues located in similar positions relative to the protein backbone and the cofactor [3]. In both BFD [15] and PDC [16–18], the two histidines have been implicated in catalysis. However, unexpectedly, the histidines are located on different regions of the polypeptide chain [3]. We have been interested in the evolutionary relationship between PDC and BFD and have had some success in interconverting the activities of the two enzymes [19]. Differences in active sites notwithstanding, the PDC-BFD interconversion is, ultimately, an exercise in altering the substrate specificities of two decarboxylases.

The relationship, evolutionary or otherwise, between BAL and BFD is not so clear. Both catalyze reactions producing benzaldehyde, and it is intriguing to us that the two enzymes are readily able to catalyze the same carboligation reaction using benzaldehyde as substrate. This suggests that the two enzymes are likely to have a similar array of residues involved in substrate binding and catalysis. However, the possibility of BAL having decarboxylase activity or BFD having lyase activity is largely unexplored. In this report, we confirm that, under normal circumstances, neither enzyme is

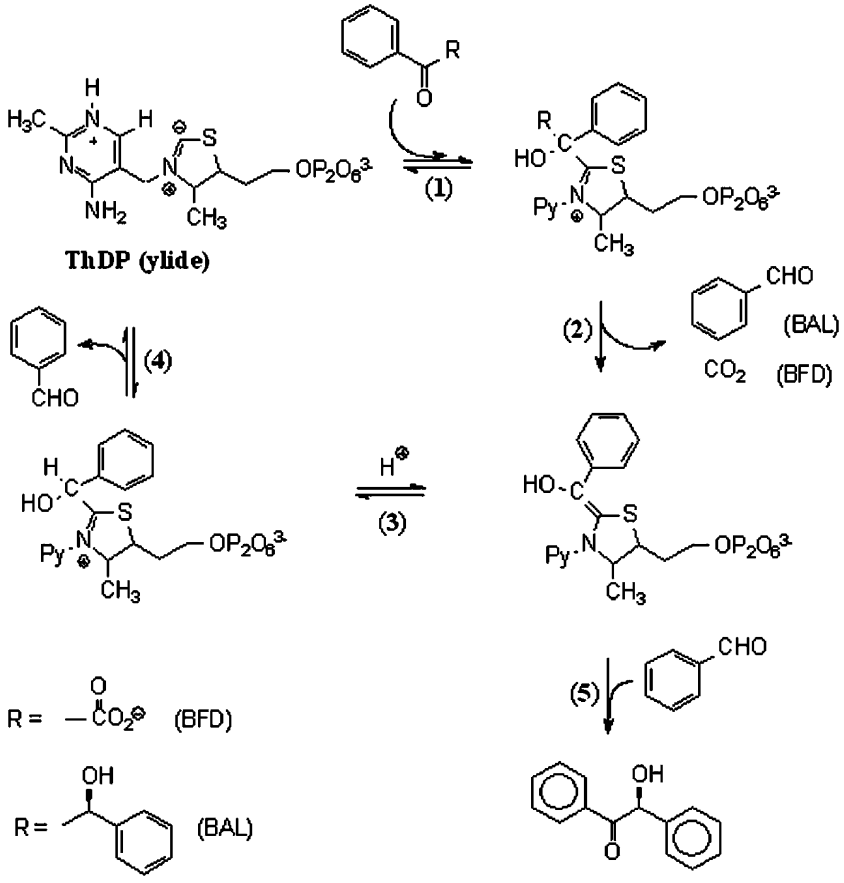

Fig. 2. Mechanism of ThDP-dependent enzymes such as BAL and BFD. In the first step the ThDP ylide reacts with either (*R*)-benzoin or benzoylformate forming a tetrahedral intermediate. In the second step either benzaldehyde (BAL) or CO2 (BFD) is eliminated, resulting in the formation of an enamine. In the absence of a second substrate the enamime is then protonated (step 3) and benzaldehyde is subsequently released (step 4). However, if excess benzaldehyde is present, carboligation takes place (step 5) resulting in the formation of (*R*)-benzoin.