Structure and mechanism of the ThDP-dependent benzaldehyde lyase from *Pseudomonas fluorescens*

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Pseudomonas fluorescens is able to grow on *R*-benzoin as the sole carbon and energy source because it harbors the enzyme benzaldehyde lyase (BAL) that cleaves the acyloin linkage using thiamine diphosphate (ThDP) as a cofactor [1]. In the reverse reaction, this lyase catalyzes the carboligation of two aldehydes with high substrate and stereo-specificity.

The enzyme structure was determined by X-ray diffraction at 2.6 Å resolution at the EMBL oustation in Hamburg [2]. Phases were obtained by the incorporation of seleno-*L*-methionine (SeMet) and multiwavelength anomalous diffraction. BAL is a homotetramer of 4*563 amino acid residues. Each subunit binds one ThDP molecule using one Mg^{2+} ion and consists of the three domains Dom-α (residues 1-183), Dom-β (184-363) and Dom-γ (364-563). Residues involved in binding of the cofactor ThDP are located at the C-terminal ends of the β-strands of Dom-γ (phosphates and Mg^{2+}) and of Dom-α′ of a neighboring subunit (pyrimidine moiety) (Figure 1).

BAL belongs to a group of closely related ThDP-dependent enzymes. In all family members the ThDP molecules are fixed at their two ends in separate domains, suspending a rather mobile thiazole ring between them. While the residues binding the two ends of ThDP are well conserved, the lining of the active center pocket around the thiazole moiety varies greatly within the family. Accounting for the known reaction chemistry, the natural substrate *R*-benzoin could be modeled unambiguously into the active center of the reported benzaldehyde lyase. Due to its substrate spectrum and stereo-specificity, the enzyme extends the synthetic potential for carboligations appreciably.

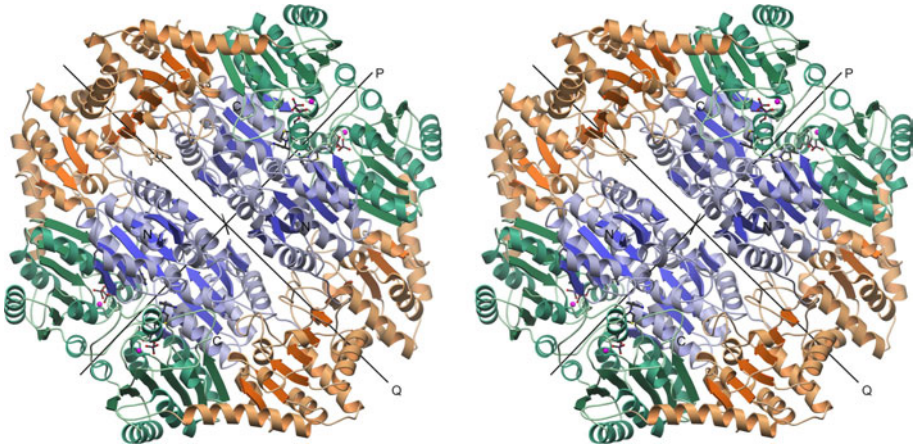

Figure 1: Stereo ribbon plot of the D_2-symmetric BAL tetramer.

The tetramer should be described as a dimer of dimers. Each tight dimer contains two active centers at its interface. ThDP is shown as a ball-and-stick model..

References

[1] P. Hinrichsen, I. Gomez and R.Vicuña, *Gene* **144**, 137 (1994)

[2] T. Mosbacher, M. Mueller and G.E. Schulz, FEBS Letters, accepted (2005)