Phasing the *Bacillus brevis* α-acetolactate decarboxylase (ALDC) using S-SAD at BM14, ESRF

Shabir Najmudin and Vilmos Fülöp

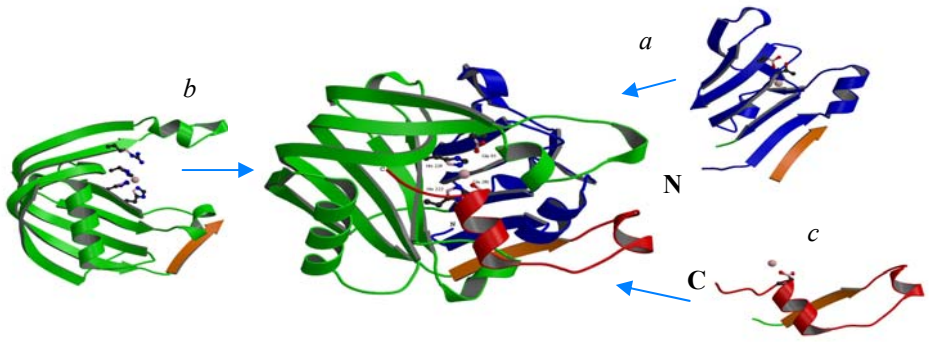
Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, England

α-Acetolactate decarboxylase has the unique ability to decarboxylate both enantiomers of acetolactate to give a single enantiomer of the decarboxylation product, (*R*)-acetoin. The enzyme decarboxylates the normal substrate (*S*)-α-acetolactate. It then catalyses tertiary ketol rearrangement of the *(R)*-enantiomer with the migration of the carboxylate group. Because this degenerate rearrangement proceeds via a transition state with a *syn* arrangement of the oxygen functions, the product is (*S*)-α-acetolactate which is then decarboxylated in the normal way. The enzyme also catalyses the decarboxylation of (*S*)-α-acetohydroxybutyrate. (*S*)-α-Acetolactate and (*S*)-α-acetohydroxybutyrate, the products of decarboxylation of β-ketocarboxylates are the biosynthetic precursors of the essential branch-chain amino acids valine and isoleucine respectively. α-Acetolactate decarboxylase has found practical application in brewing where it can be used to speed maturation by catalysing the non-oxidative decarboxylation of α-acetolactate thereby avoiding the oxidative decarboxylation to biacetyl, which gives an off-odour to beer.

A gene coding for α-acetolactate decarboxylase from *Bacillus brevis* (ATCC 11031) was cloned and overexpressed in *Bacillus subtilis*. The enzyme was purified in two steps to homogeneity prior to crystallisation (Najmudin *et al*., 2003).

A S-SAD dataset was collected at the BM14, ESRF On an ALDC crystal grown in 6 mM $CdCl_2$, 20% PEG 2000 MME, 0.1M Tris, pH 9.0. ALDC has 6 methionines out of 260 amino acid residues.

540° of data were collected with a Δφ of 0.5° giving a highly redundant dataset – 28.4-fold redundancy for all data (14.3 for the outer resolution shell at 2.3Å). HKL2000 was used data processing. Location of anomalous scatterers, phasing and density modification were carried out using SHELXD and SHELXE. A single fully occupied cadmium and seven partially occupied sulphur/cadmium sites were found. The contrast and connectivity figures of merit for the correct heavy-atom enantiomer ($P3_221$) were 0.42 and 0.89, respectively, as opposed to 0.25 and 0.85, respectively for the wrong hand. About 60% of the structure was autotraced by RESOLVE and REFMAC using the phases obtained after SHELXE. Currently the structure is being refined at 1.1Å resolution.



Structure of ALDC (central molecule) and it's dissected components, *a*, *b* and *c*. ALDC is a 2 domain α/β metalloprotein and exhibits a novel fold with no significant structural similarity to any other protein. The N-terminal domain comprises a 7 β-strand mixed β-sheet (*b*). This domain is completed by a short C-terminal tail (*c*). In between the two termini, the second, C-terminal domain is a β-cylinder comprising 5 anti-parallel β-strands and a long α-helix (*b*). It provides the three highly conserved histidines, which coordinate the Zn^{2+} ion. The coordination of the metal is completed by a conserved glutamate from the C-terminal tail (*c*) and two water molecules.

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Reference: Najmudin, S., Andersen, J. T., Patkar, S. A., Borchert, T. V., Crout, D. H. G. and Fülöp, V. (2003). *Acta. Cryst*. D **59**, 1073-1075.