MS5-P7 Structural insights into malonyl-CoA reductase of 3-hydroxypropionate cycle

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The novel crystal structures of C-terminal part of Malonyl CoA reductase (MCR) from *Chloroflexus aurantiacus* and its NADPH bound form have been determined in 2.05 Ă and 2.11 Ă, respectively. NADPH bound structure has a considerable conformational change compared to the native MCR. It forms an unusual hairpin structure near the NADPH binding site at the interface between the enzyme and solvent. The residues forming the hairpin structure are disordered at the native MCR. Furthermore, there is no significant difference between these two structures. NADPH binding site involves many consecutive hydrophobic residues which are necessary for nucleotide binding. Moreover, NADPH interacts with the polar residues which may play a crucial role in catalytic activity. These novel structures will elucidate the catalytic mechanism of MCR.

Keywords: protein crystallography, 3-HP cycle, carbon fixation, Malonyl CoA reductase

MS5-P8 The first X-ray structure of prokaryotic dipeptidyl peptidase III

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Dipeptidyl peptidase III (DPP III) is a widely distributed cytosolic zinc peptidase from the M49 family, which hydrolyses dipeptides from the N-termini of its peptide substrates. Until now, most studies on DPP III were done on orthologs from eukaryotic organisms, showing that DPP III participates in the intracellular protein catabolism and oxidative stress response. The knowledge about prokaryotic DPP III is very scarce, particularly data on its catalytic mechanism, physiological function and structural characteristics are lacking. We have been studying two bacterial orthologs: DPP III from the human gut symbiont *Bacteroides thetaiotaomicron* (*Bt*) and from *Caldithrix abyssi* (*Ca*), which inhabits hydrothermal vents. Both proteins were produced in *Escherichia coli* and then purified. Diverse crystallization screens were applied and wild-type *Ca*DPP III crystals were obtained. Crystallization experiments with wild-type *Bt*DPP III failed probably due to charge heterogeneity. In order to overcome this problem, specific amino acid replacements (all cysteines were replaced by serines) were introduced and crystals of the variant protein were grown. Datasets for both DPP III proteins were collected at BESY II, Germany. Up to date, all efforts to solve the structures of the two proteins using molecular replacement method and anomalous dispersion of the zinc ions, were unsuccessful. Therefore, selenomethionine labeled *Bt*DPP III, non-cysteine variant, was prepared and the crystals were obtained in the same crystallization condition as the non-labelled protein. Diffraction data up to 1.8 Å resolution were collected at Elettra Trieste, Italy, and the first prokaryotic DPP III structure was solved using single-wavelength anomalous dispersion of the selenium atoms. *Bt*DPP III crystallized in space group $P3_121$ with unit cell dimensions a=103.5 Å and c=141.6 Å and one molecule per asymmetric unit. The overall fold is typical for a member of the M49 family with two domains separated by a wide cleft. The upper domain is mostly helical with a tetracoordinated zinc ion, while the lower domain contains mixed secondary structure elements with a five-stranded β-barrel core. The main structural difference between *Bt*DPP III and eukaryotic DPP III is the absence of a 30 amino acid loop in upper domain. In human DPP III this loop contains the ETGE motif which is supposed to be responsible for binding KEAP1, a repressor protein from the Keap1-Nrf2 signaling pathway.