The Crystal Structure of XC1258 from *Xanthomonas campestris*: A Putative Nit Protein with an Arsenic Adduct in the Active Site

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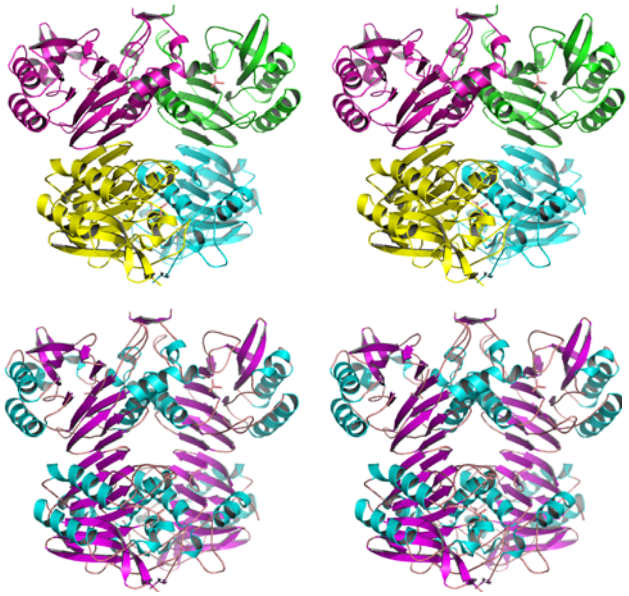
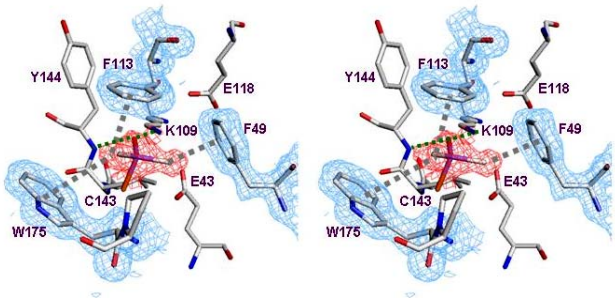
The CN-hydrolase superfamily proteins are involved in a wide variety of non-peptide carbon-nitrogen hydrolysis reactions, characterized by a thiol acylenzyme intermediate formed through the attack of a cyano or carbonyl carbon by a novel conserved catalytic triad of Glu-Lys-Cys, to produce important natural products such as auxin, biotin, precursors of antibiotics etc. We have determined the crystal structure of XC1258, a putative CN-hydrolase protein from the plant pathogen *Xanthomonas campestris* pv. *campestris*. to a resolution of 1.73 Å using the two-wavelength MAD approach. Interestingly, a cacodylate or dimethylarsinic acid compound was found to situate perfectly in the active region, forming a strong arsenic adduct with the active cysteine residue. This observation entails a common CN-hydrolase reaction mechanism and suggests that its activity could be inhibited by the dimethylarsinic compound through a sulfur-arsinic covalent bond.

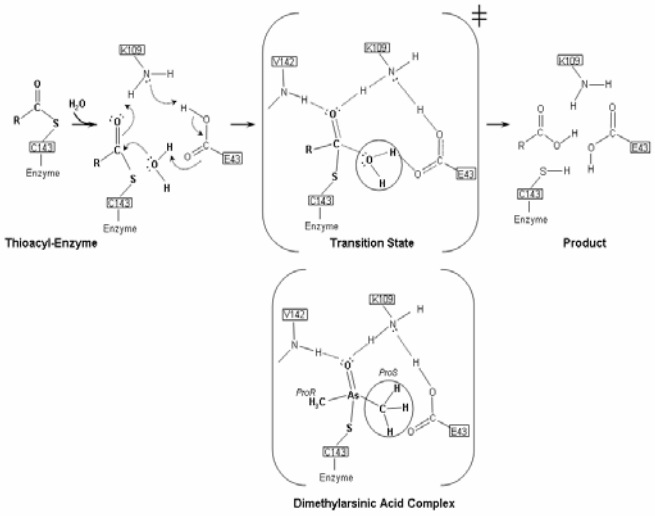


The above figure shows: a) The stereo riboon picture of the XC1258 tetramer. Each monomer is shown in different colors. b) The stereo riboon picture of the XC1258 tetramer shown in different perspective. α-helices were shown in yan, the β-strands in purple, and the random coil in brown. The four β-sheets comprising 12 β-strands is clearly revealed.

The stereo view of the active site complexed with dimethyl arsenic acid is shown below. The H-bonds were drawn in dotted green lines, while un-conventional C-H$^{\cdots}$ π bonds in dotted gray lines. Detailed electron density map was clearly observed for the dimethyl arsenic adduct (shown in red). Unlike the catalytic cavity in DCase, that of XC1258 is filled mainly with hydrophobic residues.



The bottom figure shows the proposed mechanism for the hydrolysis of thio-acyl enzyme intermediates. The dimethyl arsenic acid complex resembles the transition state for hydrolysis. The electron movements were shown in curved arrows, and the partial bonds formed or broken by dotted lines. the $^{ProS}CH_3$ group (enclosed in circle) in the dimethyl arsenic complex can be substituted by a H_2O molecule to initiate the hydrolysis process.



The XC1258 structure reported here represents a new variant of CN-hydrolase domain without a lid. Its complex structure with the dimethylarsenic adduct in the active site further reveals the common hydrolysis mechanism of CN-hydrolase superfamily protein.

Chin, *et al.* "The crystal structure of XC1258 from *Xanthomonas campestris*: A putative procaryotic Nit protein with an arsenic adduct in the active site", Proteins **69**, 665-671 (2007).