Poster Presentation

MS009.P33

Structure and mechanism of the amidase from Geobacilus pallidus

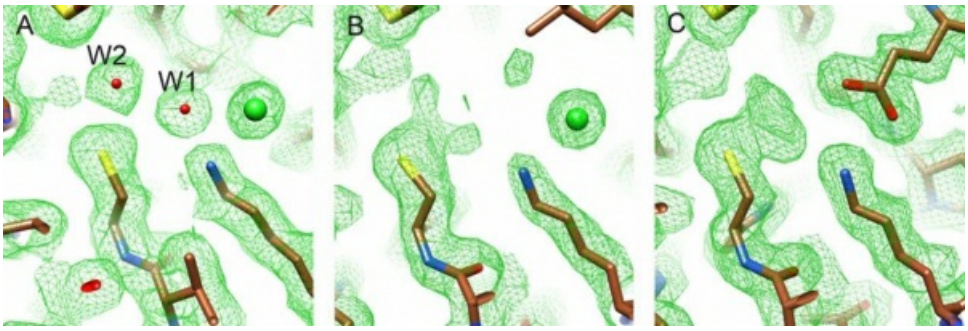
Brandon William Weber[1], Bryan Trevor Sewell[1]
[1]*Electron Microscope Unit, University Of Cape Town, Cape Town, South Africa*
E-mail: brandon.weber@uct.ac.za

The amidase from Geobacillus pallidus and its close homologues are the best characterized amidases of the nitrilases superfamily. The structure has been used to identify the active site residues and assign their roles in the putative reaction sequence. In essence, the amide substrate is positioned to undergo a nucleophilic attack on the carbonyl carbon by its interactions with a triad of residues comprising two glutamates and a lysine as well as backbone interactions (1).

The residue making the attack is a cysteine - however this cysteine is always oxidised during the x-ray diffraction experiment and this has impaired the visualization and interpretation of the active site (2). A water molecule is certainly responsible for this oxidation, but the location of this water in the active site remains undetermined experimentally and this has led to uncertainty about the mechanism. This is however not the only source of uncertainty. At least one mechanistic proposal calls for one of the glutamates to act as a general base and abstract the hydrogen from the attacking cysteine (3). Although the enzyme is inactivated by mutating the glutamate in question (2,3), the thio-ester intermediate can still be formed with certain substrates. Indeed a number of different reactions involving nucleophilic attack by the cysteine have been observed in the absence of this glutamate - strongly suggesting that it does not provide assistance at this early stage. The case would be strengthened by knowing the location of the cysteine's hydrogen. Locating this hydrogen would also help in understanding the source of the hydrogen that is added to the amine of the substrate amide.

One possibility is that this comes from the cysteine directly without an intermediate transferring group. Such a mechanism would call for the nitrogen lone pair to emerge peri-planar to the direction of the nucleophilic attack - this would require assistance from both active site glutamates to orientate the amine appropriately. Knowledge of the precise arrangement of the hydrogen atoms in the intact active site would provide a firm basis on which to argue the case. Current work is focused on producing crystals suitable for neutron diffraction in order to address this question.

[1] Kimani, S. W. et al. (2007). Acta Crystallogr. Sect. D-Biol. Crystallogr. 63, 1048-1058.
[2] Weber, B.W. et al. (2013). J Biol Chem. 288(40), 28514-28523.
[3] Hung, C.L. et al. (2007). J Biol Chem. 282(16), 12220-12229.



Keywords: amidase mechanism, thio-ester, cysteine oxidation