Poster Presentation

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A crystallographic study of the Toho-1 β-lactamase acylation mechanism

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β-lactam antibiotics have been used effectively over several decades against many types of highly virulent bacteria. The predominant cause of resistance to these antibiotics in Gram-negative bacterial pathogens is the production of serine β-lactamase enzymes. A key aspect of the class A serine β-lactamase mechanism that remains unresolved and controversial is the identity of the residue acting as the catalytic base during the acylation reaction. Multiple mechanisms have been proposed for the formation of the acyl-enzyme intermediate that are predicated on understanding the protonation states and hydrogen-bonding interactions among the important residues involved in substrate binding and catalysis of these enzymes. For resolving a controversy of this nature surrounding the catalytic mechanism, neutron crystallography is a powerful complement to X-ray crystallography that can explicitly determine the location of deuterium atoms in proteins, thereby directly revealing the hydrogen-bonding interactions of important amino acid residues. Neutron crystallography was used to unambiguously reveal the ground-state active site protonation states and the resulting hydrogen-bonding network in two ligand-free Toho-1 β-lactamase mutants which provided remarkably clear pictures of the active site region prior to substrate binding and subsequent acylation [1,2] and an acylation transition-state analog, benzothiophene-2-boronic acid (BZB), which was also isotopically enriched with 11B. The neutron structure revealed the locations of all deuterium atoms in the active site region and clearly indicated that Glu166 is protonated in the BZB transition-state analog complex. As a result, the complete hydrogen-bonding pathway throughout the active site region could then deduced for this protein-ligand complex that mimics the acylation tetrahedral intermediate [3].

[1] *Tomanicek, S.J., Blakeley, M.P., Cooper, J., Chen, Y., Afonine, P.V., Coates, L. (2010). Neutron diffraction studies of a class A β-lactamase Toho-1 E166A/R274N/R276N triple mutant. J. Mol. Biol. 396, 1070-1080.,* ***[2]*** *Tomanicek, S.J., Weiss, K.L., Wang, K.K., Blakeley, M.P., Cooper, J., Chen, Y., Coates, L. (2011). The active site protonation states of perdeuterated Toho-1 β-lactamase determined by neutron diffraction support a role for Glu166 as the general base in a,* ***[3]*** *Tomanicek, S.J., Standaert, R., Weiss, K.L., Ostermann, A., Schrader, T., Ng, J.D., Coates, L. Neutron and X-ray crystal structures of a perdeuterated enzyme inhibitor complex reveal the catalytic proton network of the Toho-1 β-lactamase for the acylatio*

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