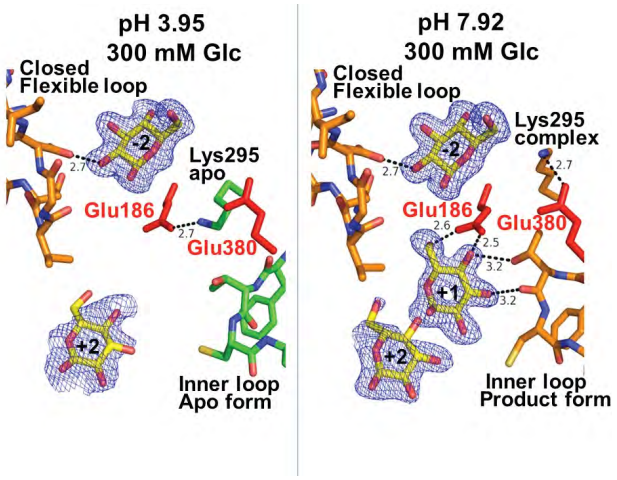

Keywords: β-amylase, enzyme reaction, enzyme/substrate complex

MS09-P09

Structure analysis of haloalkane dehalogenase DbeA ΔCl variant from *Bradyrhizobium elkanii* USDA94

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A novel enzyme, DbeA, belonging to the family of haloalkane dehalogenases (EC 3.8.1.5) was isolated from *Bradyrhizobium elkanii* USDA94. This haloalkane dehalogenase is closely related to DbjA enzyme from *Bradyrhizobium japonicum* USDA110 (71% sequence identity), but has different biochemical properties. DbeA is generally less active and has a higher specificity towards brominated and iodinated compounds than DbjA. The DbeA protein was crystallised using the sitting-drop vapour-diffusion method and the crystal structure of a DbeA enzyme has been solved and deposited at Worldwide Protein Data Bank under PDB ID 4k2a. The DbeA wt structure revealed the presence of two halide-binding sites. The first chloride-binding site is located in the active site in between two halide-stabilizing residues. The second halide-binding site is unique to DbeA and has not been previously reported in any other structure of this enzyme family. To elucidate the role of the second halide-binding site, a two-point variant DbeA ΔCl (I44L+Q102H) lacking this site was constructed and biochemically characterized [1]. Elimination of the second halide-binding site decreased the stability and catalytic activity, and dramatically altered the substrate specificity. The two-point substitution resulted in a shift of the substrate-specificity class, which is the first time this has been demonstrated for this enzyme family. Rational design of buried halide-binding sites represents a novel strategy for engineering of enzymes with modified catalytic properties.

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References:

1. Chaloupkova R, et al., Acta Crystallogr. D70, 1884-1897 (2014)

Keywords: Haloalkane dehalogenase, second halide binding site and halogenated compounds