Poster Presentation

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Haloalkane dehalogenases as a subject for crystallographic studies

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An integrated view of protein structure, solvation, dynamics, and function is emerging, where proteins are considered as dynamically active machines and internal protein motions are closely linked to function such as enzyme catalysis. It is widely believed that hydration is necessary for enzyme catalytic function [1]. Dry enzymes are non-functional and below a threshold hydration level, enzymes are inactive. Hydration has been observed to play a functional role not only in enzyme catalysis, but also in protein folding, protein architecture, conformational stability, protein dynamics, protein plasticity, ligand binding and the selectivity of specific interactions. The elucidation of protein-water interactions at molecular level is thus necessary for a complete understanding of protein functionality. Motion and flexibility within enzymes play important functional role in the formation of catalytically competent configurations. During the catalytic cycle, the enzyme molecule passes through different states and each state is associated with a different active site conformation. Rapid transition between the different conformational states is therefore mandatory for the maximum enzyme activity. The role of conformational flexibility has been well established in connection with the accessibility of the active site, the binding of substrates and ligands, and release of products, stabilization and trapping of intermediates, orientation of the substrate into the binding cleft or adjustment of the reaction environment.

Our model systems including haloalkane dehalogenases DhaAwt from Rhodococcus rhodochrous NCIMB 13064 [2] and its variant DhaA31 with evolved catalytic efficiency towards anthropogenic compound 1,2,3-trichloropropane (TCP) [3] and several LinB variants with modified access tunnels, have been investigated by structural methods such as X-ray and neutron crystallography, time-resolved crystallography and hydrogen-deuterium exchange mass spectrometry. Based on carefully designed experiments and by combination of the information obtained from different, but complementary, techniques we will be able to get inside into (i) conformational changes of selected enzymes upon their interactions with substrates, (ii) location of hydrogen atoms inside the enzyme active site and the access tunnels and (iii) protonation state of catalytic residues of the enzymes during their catalytic cycles.

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