

P013 The 3-His iron center – a distinct motif of catalytic function in non-heme metal dependent enzymes.

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The 2-His-1-carboxylate facial triad is a widespread and well characterised motif in the class of non-heme metal dependent enzymes. Much less is known about the 3-His metal center as alternative metal environment. We used the diketone-cleaving enzyme Dke1, a non-heme Fe(II) dependent dioxygenase belonging to the superfamily of cupins, as model system for structure-function relationships. By site-directed mutagenesis we remodelled the 2-His-1-carboxylate active site and investigated the mutants in term of metal binding and reactivity. We checked for “native” activities with diketones as well as for selected reported activities for 2-His-1-carboxylate enzymes.

The Cupin superfamily is class of proteins with a broad variety in terms of primary structure and activity. However, the tertiary structure is conserved and consists of a typical beta-sheet rich “jelly roll” motif. Comparison of structural data of Cupin proteins reveals the metal binding to be very similar making it almost impossible to draw conclusion about a proteins metal selectivity or reactivity. To gain more insight into this class of enzymes we isolated and expressed a so far uncharacterised protein of the strain *Burkholderia xenovorans*. We identified the enzyme to be capable of degrading beta-diketone structures with a 3-His metal center. EXAFS studies gave valuable insights into the active site structure of the enzyme.