

Abstract No. Chan0203

**On the Catalytic Role of Glu-60 in *Thermoanaerobacter Brockii* Alcohol Dehydrogenase**

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Beamline(s): X9B

*Thermoanaerobacter Brockii* alcohol dehydrogenase (TbADH) is a bacterial, NADP<sup>+</sup>-linked, zinc dependent enzyme. TbADH and its analogues alcohol dehydrogenase from *Clostridium beijerinckii* (CbADH) have been crystallized and their three-dimensional structures with and without NADP<sup>+</sup> were studied by X-ray crystallography (1). The crystal structures show that unlike most other alcohol dehydrogenases, the conserved residue, Glu-60, is directly coordinated to the catalytic zinc ion in the wild type enzyme and hence should be considered as a key residue to enzyme catalysis. Yet, the coordination of Glu-60 to the catalytic zinc ion is not conclusive in the crystal structure of TbADH+NADP<sup>+</sup> complex (1). In order to study the role of Glu-60 residue in TbADH catalysis we have performed site directed mutagenesis and structural spectroscopic studies on the mutated enzymes in their apo-, holo-, and inhibited forms. Specifically, we have replaced the Glu-60 residue in TbADH to alanine (E60A-TbADH) and aspartate (E60D-TbADH). Surprisingly, the E60A mutation failed to completely abolish the enzyme activity and reduced it by only 30-50% while the E60D mutation reduced the activity by 90%. Structural determination of the local environment of the catalytic zinc ion in E60A-TbADH and E60D-TbADH by X-ray absorption fine structure (EXAFS), circular dichroism (CD), and fluorescence spectroscopy reveal distinct differences among the various complexes of wild type TbADH and the analogous complexes of the Glu-60 mutants. Based on these results we propose that the conserved Glu-60 residue in TbADH plays a dynamic role in mediating TbADH catalysis via water molecule during turnover.