

## **Spectroscopic Studies of Inhibited Alcohol Dehydrogenase from *Thermoanaerobacter Brockii*: Proposed Structure for the Catalytic Intermediate State**

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*Thermoanaerobacter brockii* alcohol dehydrogenase (TbADH) catalyzes the reversible oxidation of secondary alcohols to the corresponding ketones using NADP<sup>+</sup> as the cofactor. The active site of the enzyme contains a zinc ion that is tetrahedrally coordinated by four protein residues. The enzymatic reaction leads to the formation of a ternary enzyme-cofactor-substrate complex; and catalytic hydride ion transfer is believed to take place directly between the substrate and cofactor at the ternary complex. Although crystallographic data of TbADH and other alcohol dehydrogenases as well as their complexes are available, their mode of action remains to be determined. It is firmly established that the zinc ion is essential for catalysis. However, there is no clear agreement about the coordination environment of the metal ion and the competent reaction intermediates during catalysis. We used a combination of X-ray absorption, Circular Dichroism (CD) and Fluorescence spectroscopy, together with structural analysis and modeling studies to investigate the ternary complexes of TbADH that are bound to a transition state analogue inhibitor. Our structural and spectroscopic studies indicated that the coordination sphere of the catalytic zinc site in TbADH undergoes conformational changes when it binds the inhibitor and forms a penta-coordinated complex at the zinc ion. These studies provide the first active site structure of bacterial ADH bound to a substrate analogue. Here, we suggest the active site structure of the central intermediate complex and more specifically, propose the substrate-binding site in TbADH.