

Spectroscopic Characterization of Co^{II}-substituted TauD

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Taurine/ α -ketoglutarate (α KG) dioxygenase (TauD) is a mononuclear non-heme Fe^{II} dependent enzyme that couples α KG decarboxylation with hydroxylation of taurine to provide sulfite as a sulfur source to *Escherichia coli*. Spectroscopic and crystallographic work has firmly established the mechanistic steps that precede oxygen interaction with this archetypal α KG-dependent dioxygenase, and an Fe^{IV}-oxo species has been identified as an intermediate following O₂ addition. In this study, we substitute Co^{II} for Fe^{II} to provide a spectroscopic probe of the early steps of the mechanism. In the absence of substrates Co^{II} substituted enzyme exhibits an electronic spectrum with a broad and roughly symmetrical single peak between 450 and 600 nm with a maximum at 520 nm and ϵ_{520} of 40 M⁻¹ cm⁻¹, consistent with a six-coordinate environment as expected for the metal site ligated by two histidines, one aspartate and three water molecules. The α -ketoacid likely binds directly to the Co^{II} center through its carboxylate and carbonyl moieties, thus displacing two water molecules in the same manner as for Fe^{II}, while maintaining a six-coordinate Co^{II} center as shown by the lack of spectral perturbation. The primary substrate binds near the active site and promotes dissociation of the last water ligand, as shown by the spectrum with λ_{max} at 570 nm and ϵ_{570} of 130 M⁻¹ cm⁻¹. EPR spectra are consistent with the decrease in coordination number as taurine binds, resulting a sharp signal with g = 6.3, 4.3 and 2.0. Unlike the case for the Fe^{II}-bound enzyme, UV-Visible and EPR spectra demonstrate that the five-coordinate Co^{II} did not react with oxygen or hydrogen peroxide. The spectroscopic features associated with Co^{II} substituted TauD may help in characterization of this and other α KG /non-heme Fe^{II} dioxygenases.