Oxygen Acitvation by Variant H99A of Taurine:α-Ketoglutarate Dioxygenase

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Taurine:α-ketoglutarate dioxygenase (TauD) from *Escherichia coli* catalyzes the hydroxylation of taurine (2-aminoethane-1-sulfonic acid) at C1 by coupling reductive activation of O_2 at its Fe(II) cofactor with oxidative decarboxylation of α-ketoglutarate. Our recent studies have characterized an Fe(IV)-oxo intermediate, termed **J**, that accumulates to high levels upon reaction of the quaternary TauD•Fe(II)•αKG•taurine complex with O_2. In addition, several other intermediates, which are kinetically masked in the reaction with the normal substrates and the wild-type protein, have been proposed. Current efforts are aimed at using modified reaction constituents (substrate or protein variants) to promote accumulation of these intermediate complexes for their spectroscopic characterization. To probe the properties of the equatorial histidine ligand (H99) in the mechanism of O_2 activation we have produced a TauD variant with this histidine changed to alanine (TauD-H99A), with the intent to rescue the activity by addition of imidazole.

Surprisingly, TauD-H99A retains 35% of the activity of wild-type TauD in the absence of imidazole. During the TauD-H99A•Fe(II)•αKG•taurine reaction with O_2 an intermediate is observed with spectroscopic properties similar to those determined for **J**. Kinetic and spectroscopic studies will be presented on this poster.