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The Active Site Chemistry Of Factor Inhibiting HIF-1, Coordination, Bonding, And Reaction

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Abstract
Oxygen is vital for aerobic life. A shortage of oxygen can produce disastrous outcomes to cellular functions. To avoid this, eukaryotic cells developed an oxygen sensing machinery of which the key component is hypoxia inducible factor (HIF). The function of HIF is controlled by two α-ketoglutarate-dependent non-heme iron dioxygenases, HIF-1 prolyl

hydroxylase (PHD) and HIF-1 asparaginyl hydroxylase (FIH-1). FIH-1 is the focus of this study. When oxygen is in short supply, FIH-1 cannot hydroxylate HIF. HIF then binds on DNA and turns on genes which control energy production, oxygen uptake, angiogenesis and cell growth. When the concentration of oxygen is optimal, FIH-1 hydroxylates the C-terminal activation domain (CTAD) of HIFα and turns-off the transcriptional machinery. Studying FIH-1 will not only help to provide a clear image about the reaction mechanism of FIH-1, but also guide the way in how to modulate the hypoxic responses. The three topics of concentration for FIH-1 are active site coordination, functionality, and hydrogen bond networking.

The iron center in the FIH-1 active site is octahedral in the resting state, coordinated by a His_2 Asp facial triad, bidentate α-ketoglutarate, and an axial water ligand. In the consensus mechanism, the water molecule leaves the active site when primary substrate, HIFα, binds with FIH-1 and generates a five coordinated iron center. The results shown in this thesis indicate that the five coordinated iron center occurs even without HIFα binding. This leads to an uncoupled and suicide reaction termed auto-hydroxylation.

The auto-hydroxylation reaction is studied in the functionality assays, which occurs when oxygen is sufficient and in the absence of HIFα. In this aberrant reaction, FIH-1 will use one oxygen atom to cleave α-ketoglutarate and use the other oxygen atom to hydroxylate Trp296. This auto-hydroxylation reaction leads to chromophore formation, that is shown to be iron and α-ketoglutarate dependent, and sensitive to reductant and preservative. Since the reaction condition for FIH autohydroxylation, planty of oxygen but absent of HIF-1α, does exist in normoxic environment, it is important to understand this reaction. The detail of this reaction provides insights not only into how FIH-1 controls O_2 activation, but also how cells deal with reactive oxygen species.

The active site of FIH-1 has a hydrogen bond network, which involves Asn803 on HIFα and four residues from FIH-1: Gln205, Arg238, Gln239, and Gln294. FIH-1 residues involved in hydrogen bonding are mutated into alanine one by one, and the impact of these mutations are analyzed. The results suggest that Gln205 and Gln294 act as a decelerator for O_2 activation, and Arg238 and Gln239 act as an accelerator to prime the Fe (II) for reaction with O_2 following HIF binding.

FIH-1 is crucial for oxygen regulation in human cells. The results of these experiments provide detailed knowledge about FIH-1, which provides broader insight into the control of oxidation chemistry, and may aid in the therapeutic targeting in multiple diseases that are associated with hypoxic pathways.