

Excerpt from J. Mol. Biol. (2002) 320, 1095–1108:

Crystal Structure of the Ternary Complex of the Catalytic Domain of Human Phenylalanine Hydroxylase with Tetrahydrobiopterin and 3-(2-Thienyl)-L-alanine, and its Implications for the Mechanism of Catalysis and Substrate Activation

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Abstract:

Phenylalanine hydroxylase catalyzes the stereospecific hydroxylation of L-phenylalanine, the committed step in the degradation of this amino acid. We have solved the crystal structure of the ternary complex (Fe(II)·BH₄·THA) of the catalytically active Fe(II) form of a truncated form (DN1–102/DC428–452) of human phenylalanine hydroxylase (hPheOH), using the catalytically active reduced cofactor 6(R)-L-erythro-2,6,7,8-tetrahydrobiopterin (BH₄) and 3-(2-thienyl)-L-alanine (THA) as a substrate analogue. The analogue is bound with the thiophene ring stacking against the imidazole group of His285 (average interplanar distance 3.8 angstroms) and with a network of hydrogen bonds and hydrophobic contacts. Binding of the analogue to the binary complex Fe(II)·BH₄ triggers structural changes throughout the entire molecule. The largest change occurs in the loop region comprising residues 131–155. This loop is refolded, bringing the hydroxyl oxygen atom of Tyr138 closer to the iron atom and into the active site. BH₄ is displaced 2.6 angstroms in the direction of Glu286 and the iron atom, relative to the Fe(II)·BH₄ structure, thus changing its hydrogen bonding network. The active-site structure of the ternary complex gives new insight into the substrate specificity of the enzyme, notably the low affinity for L-tyrosine. Furthermore, the structure has implications both for the catalytic mechanism and the molecular basis for the activation of the full-length tetrameric enzyme by its substrate. The large conformational change, moving Tyr138 from a surface position into the active site, may reflect a possible functional role for this residue.

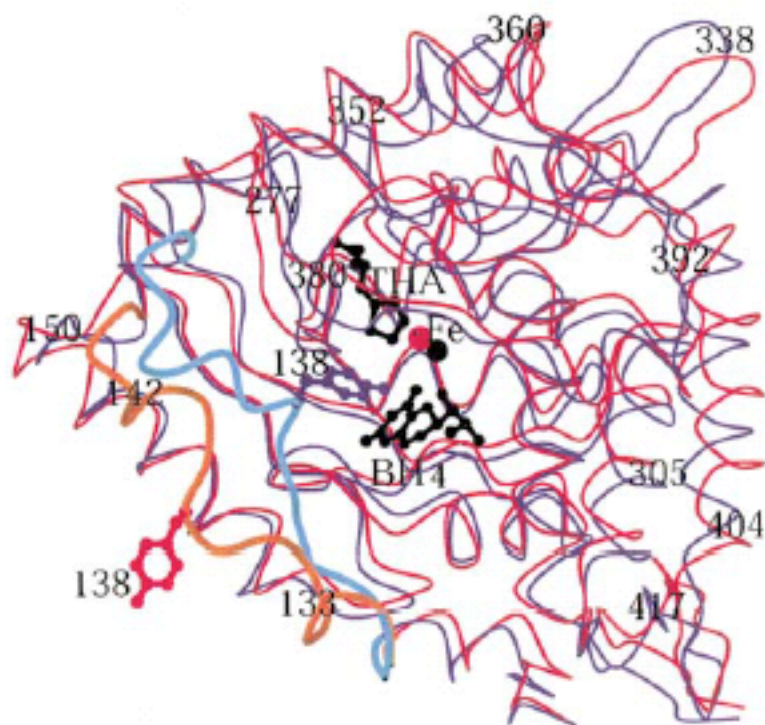


Figure 3. Picture of the ternary Fe(II)·BH₄·THA complex (blue/cyan) superimposed on the binary Fe(II)·BH₄ complex (red/orange) (PDB entry 1J8U). Black ball-and-stick models of BH₄, THA and iron are shown for the ternary structure. BH₄ in the binary structure is omitted for clarity.

respectively, and thus reduced catalytic efficiency in steady-state kinetic analyses. In addition, some of these mutations affect the overall structure and stability of the enzyme.

† http://data.mch.mcgill.ca/pahdb_new/

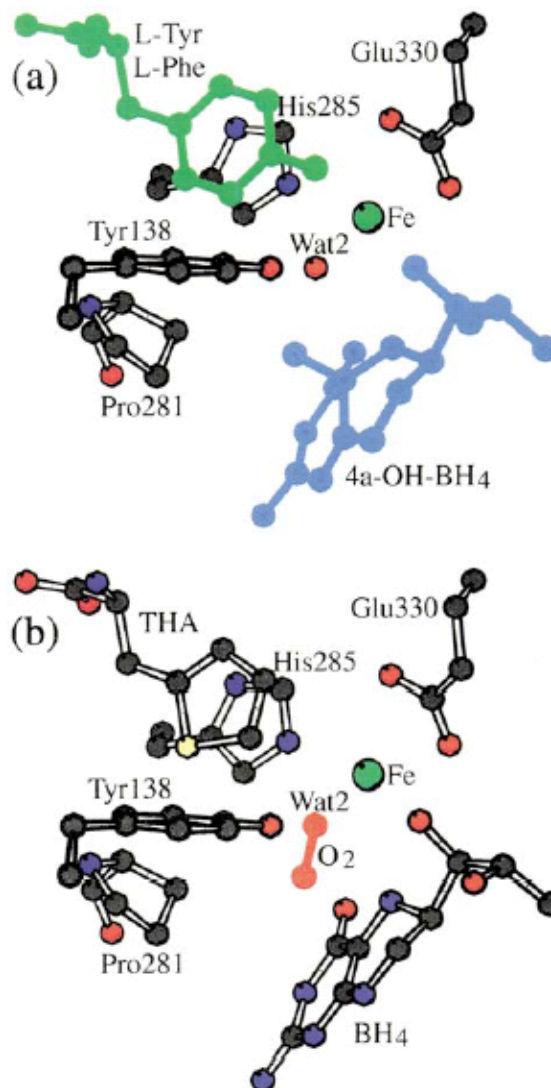


Figure 7. (a) Picture of the active site of the ternary structure. THA is replaced by the modelled L-Phe and L-Tyr (green), and BH4 is replaced by the superpositioned hydroxylated cofactor (4a-OH-BH4; blue). (b) Picture of the active site of the ternary structure with a proposed binding site of dioxygen (red). One of the oxygen atoms is positioned at Wat2, while the other atom is positioned in the direction of the C4a attached hydroxyl oxygen atom in 4a-OH-BH4 when superimposed on BH4.

Protein Data Bank accession numbers

Atomic coordinates and structure factors have been deposited at the Protein Data Bank (PDB), Research Collaboratory for Structural Bioinformatics (RCSB), with accession number 1KW0. The coordinates will remain privileged until 28th January 2003.