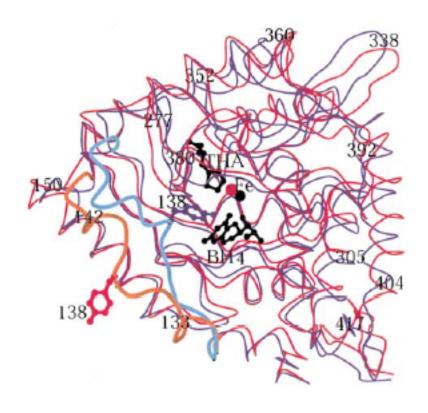
## Crystal Structure of the Ternary Complex of the Catalytic Domain of Human Phenylalanine Hydroxylase with Tetrahydrobiopterin and 3-(2-Thienyl)-L-alanine, and its Implications for the Mechanism of Catalysis and Substrate Activation

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## **Abstract:**

Phenylalanine hydroxylase catalyzes the stereospecific hydroxylation of phenylalanine, the committed step in the degradation of this amino acid. We have solved the crystal structure of the ternary complex (II)·BH<sub>4</sub>·THA) of the catalytically active Fe(II) form of a truncated form (DN1-102/DC428-452) of human phenylalanine hydroxylase ThPheOH), using the catalytically active reduced cofactor 6(R)-L-erythro-□,6,7,8-tetrahydrobiopterin (BH<sub>4</sub>) 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)·BH<sub>4</sub> triggers structural hanges 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. BH<sub>4</sub> is displaced 2.6 angstroms in the direction of Glu286 and the iron atom, relative to the Fe(II)·BH<sub>4</sub> structure, Thus changing its hydrogen bonding network. The active-site structure of the ternary complex gives new insight into the substrate specificity of the Anzyme, notably the low affinity for L-tyrosine. Furthermore, the structure as implications both for the catalytic mechanism and the molecular basis for the activation of the full-length tetrameric enzyme by its substrate. The Targe 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.

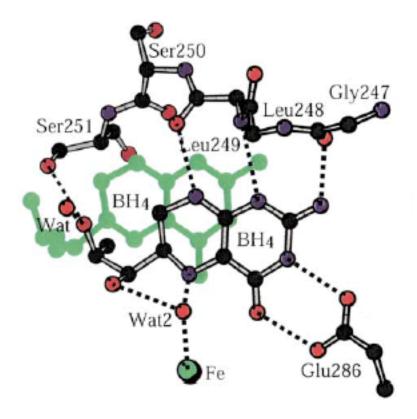
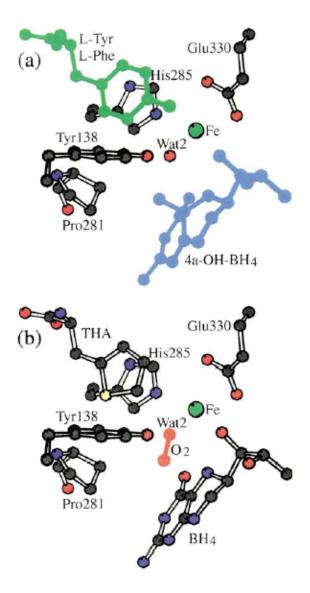


Figure 5. Stereo picture of the binding site of BH4 in the ternary Fe(II)·BH4·THA structure. Side-chains for Leu248 and Leu249 are omitted for clarity. All potential hydrogen bonds to the pterin moiety are shown as dotted lines. The green model of BH4 illustrates its position in the binary Fe(II)·BH4 complex when superimposed on the ternary structure using conserved active-site residues (His285, His290 and Fe).

Structural insight into the effect of single-point hutations of active-site residues Mutations in the human gene encoding the PheOH enzyme result in the autosomal recessively herited disease PKU/HPA, and more than 200 mutations have so far been identified. The sulting metabolic and clinical phenotypes range has everity from mild forms of non-PKU HPA to the severe forms of PKU. The structural basis of some of the metabolic and enzymatic phenotypes has been discussed recently in the light of the bur crystal structures of inactive Fe(III) forms of the enzyme, but without information on the mode for substrate binding and associated conformational hanges in the catalytic domain. Table 2 presents summary of the current information on the ported missense single-point mutations of active-site residues, involved in the binding of the duced pterin cofactor and the substrate. Of these thutations, 13 are found in nine residues involved in substrate binding. Only a few of these mutations have been expressed as recombinant enzymes. As expected, mutations for the BH4 and L-Phe,

respectively, and thus reduced atalytic 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.