

Protein Tyrosine Phosphatase 1B (PTP1B)

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In the insulin-signaling pathway, PTP1B is a negative regulator of the tyrosine phosphorylation cascade. Protein tyrosine phosphatase 1B is a therapeutic target for type II diabetes therefore its inhibition can lead to a possible treatment for type II diabetes. PTP1B belong to the large PTP family of over forty members that are receptor like and transducing enzymes that catalyzes the dephosphorylation of phosphotyrosine residues. The PTPase signature motif also known as the PTP loop includes is made up of the following residues (I/V)HCXAGXGR(S/T)G that includes the active site Cys215. The PTP loop can be seen in Figure 1 (F2 in the PYMOL

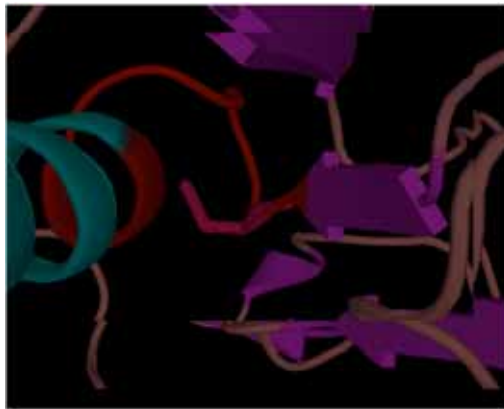


Figure 1. (F2) Showing the PTP loop (residues 214-222)

presentation). The PDB structure that was chosen to view PTP1B was PDB entry 1SUG. It has a resolution of 1.95 angstrom containing 321 residues with a R-factor of 0.188 compared to a R-free of 0.203, which is reasonable because the difference between the values is less than 3%. There are 251 water molecules in the crystal structure that doesn't seem reasonable. Lastly, the occupancy of the atoms was presented as one in the PDB header that states that the atoms are in their expected positions at all times. PTP1B is a 37 kD protein that consists of 8 alpha helices and 12 beta sheets. The ramachandran plot

showing the positions of the secondary structures of PTP1B shows that the secondary structures mainly lie in their expected regions. This can be seen in Figure 2.

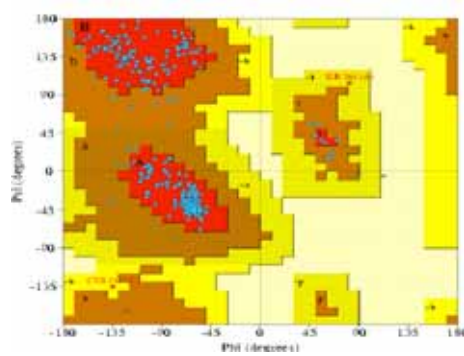


Figure 2. Showing the phi-psi angles of the secondary structure elements of PTP1B.

The effect of allosteric inhibition on PTP1B was investigated and it was found that allosteric inhibition inhibits the closure of the catalytic WPD loop (Trp179, Pro180, Asp181). Active site ligands and inhibitors interact with the side chains of the WPD loop. In the presence of allosteric inhibitors, the WPD loop changes conformation compared to the Apo form of PTP1B that can be seen in the overlay of both structures in Figure 3 (F4). The WPD loop is closed (active) in the Apo structure where it is accessible to binding substrate and the WPD loop is open in the allosteric inhibited structure.

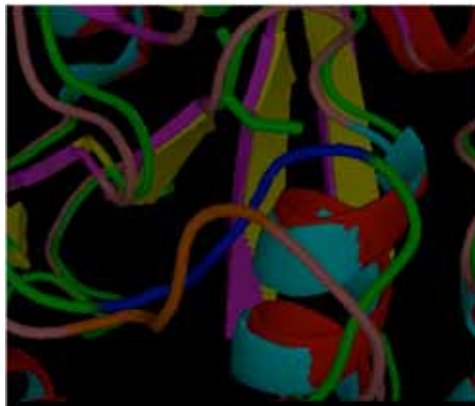


Figure 3. (F4) showing the change in the WPD loop as a result of allosteric inhibition. Blue (apo), orange (allosteric inhibited)

The effects of redox regulation of PTP1B were also investigated. When PTP1B is oxidized, a sulphenic intermediate is produced which is rapidly converted to a sulphenyl amide species. In this reaction, the sulfur atom of the catalytic cysteine is covalently

linked to the main chain nitrogen of serine216. The reaction mechanism can be seen in Figure 4. As a result of the sulphenyl-amide formation the catalytic site undergoes conformational changes (change can be seen in the PTP loop), which inhibits substrate binding protecting the cysteine residue from irreversible oxidation to sulphonic acid. Figure 5b shows the sulphenyl-amide species and also shows that the PTP loop is changed compared to the Apo form of PTP1B.

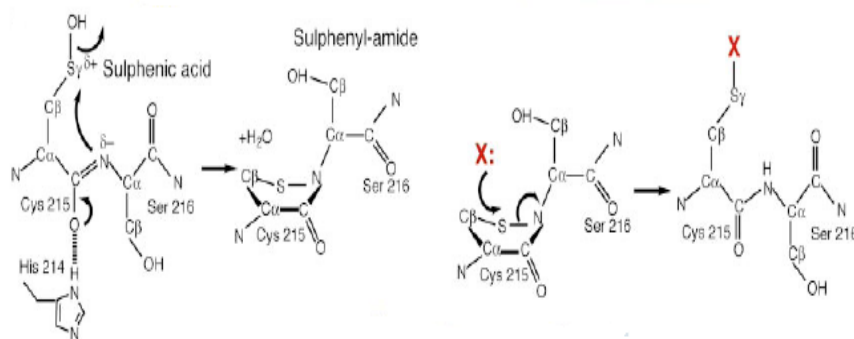


Figure 4. Mechanism for generating the sulphenyl-amide bond

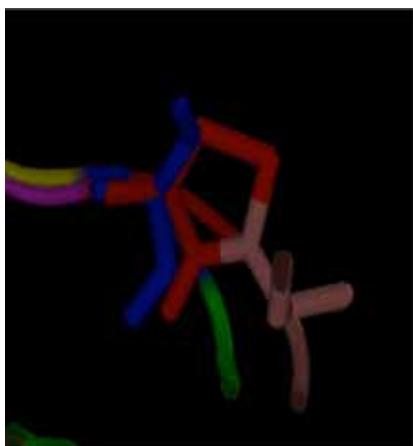


Figure 5a. (F1) Overlay of Apo active site and active site in the presence of the sulphenyl-amide showing the conformational change in the active

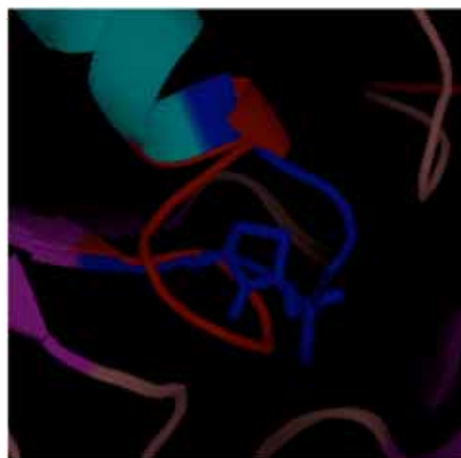


Figure 5b. (F3) Change in conformation of the PTP loop in the presence of the sulphenyl-amide.

The structure was colored by b-factor (shown in Figure 6) showing its mobility. The active site lies in a pocket, which shows blue meaning that the region is rigid.

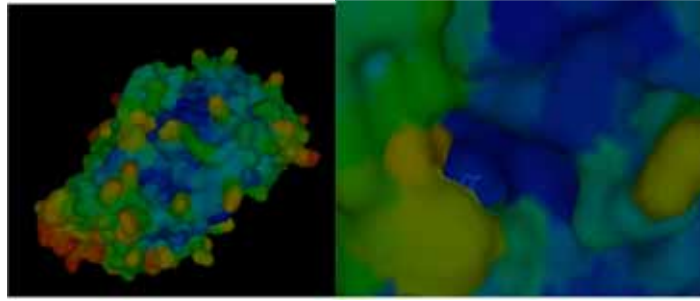


Figure 6. (F8) Colored by b-factor.

Figure 7 shows the concave regions of PTP1B. The protein has a lot of concave regions in which the active site lays.

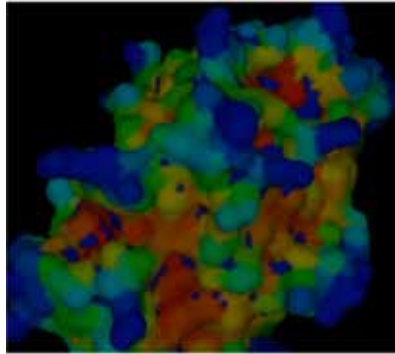


Figure 7 (F10). Showing the protein's concavity.

Figure 8 shows that the hydrophilic regions in the protein vary. This figure also shows that the active site is in a pocket.

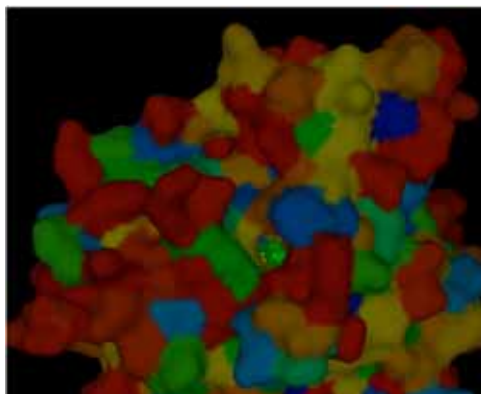


Figure 8. (F11) Showing the hydrophilic surfaces of PTP1B.

The structure of PTP1B has a lot of interesting attributes when placed in allosteric and oxidation environments. If PTP1B is inhibited then many people who develop diabetes later in life can be rid of the disease.

References

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- 2) Pedersen, A.K, Peters, G.H. et al. Water Molecule Network and Active site Flexibility of Apo Protein Tyrosine Phosphatase 1B. *ActaCrystallography, Sect D*. 60pp. 1527 (2004)
- 3) Salmeen, A., Andersen, J., Tonks, N., et al., Redox Regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. *Nature*, 23, June 12, 2003.