**Investigation of effects of WPD/IPD-loop mutations in specific protein tyrosine phosphatases**

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Abstract:
Phosphorylation is an important mechanism that cells utilize to activate or deactivate receptors, regulators, and enzymes. A few protein tyrosine phosphatases (PTPs) were studied in this project; one of which is PTP1B, a down-regulator for the human insulin receptor. Another enzyme studied is YopH, a virulence agent in Yersinia pestis, the bacteria responsible for the bubonic plague. YopH is the fastest phosphatase with over 1,000 turnovers per second. The last enzyme focused on in this project is Sso, a PTP found in the thermophilic bacteria Sulfolobus solfataricus. Although the active site of each of these enzymes is highly superimposable, their rates of reaction are dramatically different. A mobile protein loop called the WPD loop at the active site in PTP1B and YopH is thought to have a large impact upon their reaction rates. This project has measured the effects of the WPD mutation and the IPD mutation present in the corresponding position in the Sso IDO loop, and the mutation of I to W in the Sso enzyme. Successful mutagenesis has been confirmed for PTP1B and YopH. Rates of reaction have been reduced in both enzymes, as hypothesized to result from the change of the aromatic amino acid side chain from a planar aromatic group to a branched hydrocarbon. However, the catalytic rate of the YopH WPD loop was reduced much more dramatically than the PTP1B mutant, by three orders of magnitude for YopH. The effects of the I to W mutation on Sso catalysis have not yet been assessed.

**Hypothesis:**
The amino acid in the W or I position affects mobility of the loop bearing the aromatic (D) needed for catalysis. This project will determine the effect of interchanging the tryptophan (W) residue on PTP1B and YopH and the isoleucine (I) residue present on Sso/PTP.

**Obtaining W179I mutants**
- Design primer for plasmid DNA to mutate tryptophan codon to isoleucine codon
- Perform PCR and digestion according to established protocol
- Transform into DH5α ultra-competent cells to replicate mutant DNA
- Lyse cells and extract mutant DNA

![Steps of substitution mutagenesis](image)

**Figure 1:** Steps of substitution mutagenesis

**Characteristics of PTPs:**
- Same mechanism seen in all PTPs (Figure 1)
  - Acid protonation, cysteine attack, and arginine stabilization
  - Water attack and acid protonation
  - Open active site
- Some members of the family have mobile loops whereas other do not, it is hypothesized that loop mobility increases catalysis (Table 2)

**Table 1:** Comparisons of $k\text{cat}$ between Wild-type enzymes and experimentally obtained mutant $k\text{cat}$

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$k\text{cat}$</th>
<th>$k\text{cat}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTP1B WT</td>
<td>PTP1B W179I</td>
<td>YopH WT</td>
</tr>
<tr>
<td>$K\text{cat}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>0.060196</td>
<td>721</td>
</tr>
</tbody>
</table>

**Figure 4:** pH rate profile for PTP1B W179I indicating retained acid catalysis

**Results and Discussion**
- Experimental data confirms that PTP1B is more resilient to mutation in the W position of the WPD loop than YopH (Table 1)
- pH rate profile shows retained general acid catalysis
- If general acid were not retained, catalytic rate at higher pH when acid is deprotonated would be no different than rate at optimum pH (approximately 5.5-6.5)
- Retained general acid catalysis indicates that change in catalytic rate is due to factors other than complete loss of acid catalysis, this can be illustrated with crystal structures

![pH rate profile for PTP1B W179I](image)

**Table 3:** Rates of wild-type PTPs and their associated loop mobility

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$k\text{cat}$ (s$^{-1}$)</th>
<th>WPD/IPD-loop</th>
<th>Loop mobility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sso-PTP</td>
<td>3.1</td>
<td>WPD</td>
<td>Rigid</td>
</tr>
<tr>
<td>PTP1B</td>
<td>57</td>
<td>WPD</td>
<td>Mobile</td>
</tr>
<tr>
<td>YopH</td>
<td>721</td>
<td>WPD</td>
<td>Mobile</td>
</tr>
</tbody>
</table>

**Figure 6:** SDS-PAGE gel of PTP1B W179I purification

**Purification of PTP1B W179I**
- Purify mutant using Fast Protein Liquid Chromatography (FPLC)
- Begin with published wild-type purification protocol and adjust as needed
- Run SDS-PAGE gel to confirm protein
- Concentrate identified mutant protein for kinetics or crystal trays

**Future Goals & Learning Objectives Completed**
- Acquire crystal structure for PTP1B W179I to illustrate effect of mutation
- Obtain SsoPTP I67W mutant and perform kinetic analysis
- Complete kinetic data for YopH W354I
- The catalytic function of PTPs and their role in biology
- Application of knowledge obtained in the classroom

**Acknowledgements & References**
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