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 mutation of tryptophan (W) of the WPD loop in PTP1B and YopH to the isoleucine (I) present in the corresponding position in the Sso IPD 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 amino acid side chain from a planar aromatic group to a branched hydrocarbon. However, the catalytic rate of the YopH mutant was reduced much more dramatically than the PTP1B mutant, by three orders of magnitude for YopH and 100-fold for PTP1B. 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 aspartic acid (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 SsoPTP.

Results and Discussion

Table 1: Comparisons of $K_{cat}$ between Wild-type enzymes and experimentally obtained mutant $K_{cat}$

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$K_{cat}$ (s$^{-1}$)</th>
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<tr>
<td>PTP1B WT</td>
<td>57</td>
</tr>
<tr>
<td>PTP1B W179I</td>
<td>0.60196</td>
</tr>
<tr>
<td>YopH WT</td>
<td>271</td>
</tr>
<tr>
<td>YopH W354I</td>
<td>0.01154</td>
</tr>
</tbody>
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*pH rate profile shows retained general acid catalysis*

- 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, rates of reaction would be different than 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

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

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