Evolution of War, Evolution of Science – In search for an antidote for nerve agent poisoning

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Abstract

The dark side of human progress is it's ability to develop and perfect killing methods. During the 20th century there was an acceleration in the development of chemical and biological weapons. For example, the Iran-Iraq war demonstrated the dangers of nerve agent poisoning. The fear from exposure to organophosphorus compounds made us search for a good protection from nerve agent poisoning.

Background

Nerve agents are organophosphorus compounds which were discovered in 1937.
The symptoms of nerve agent poisoning are:

- Intensified saliva production
- Convulsions
- Death by Asphyxia

PON1 is an enzyme that can be considered as an effective cure for nerve agent poisoning because it accelerates the hydrolysis of organophosphorus compounds. Human PON1 can be produced in small quantities, but it tends to sink as non-soluble clusters because of it's hydrophobic traits. That's why we made better versions of human PON1 using the Directed Evolution technique.

Objectives

The aim of the project was to check the potential of PON1 as a cure for nerve agent poisoning. My research question was:

> How would the concentration of the competitive inhibitor 2HQ* affect the activity of the enzyme PON1 in the W.T mutation

The purpose of my research was to check the mutation's resistance to enzyme inhibitors.

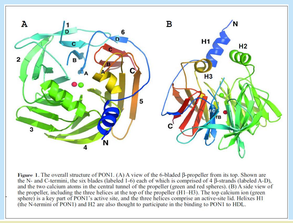


Figure 1. The overall structure of PON1. (A) A view of the 6-bladed β-propeller from its top. Shown are the N- and C-termini, the six blades (labeled 1–6) each of which is comprised of 4 β-strands (labeled A–D), and the two calcium atoms in the central tunnel of the propeller (green and red spheres). (B) A side view of the propeller, including the three helices at the top of the propeller (H1–H3). The top calcium ion (green sphere) is a key part of PON1's active site, and the three helices comprise an active site lid. Helices H1 (the N-terminus of PON1) and H2 are also thought to participate in the binding to PON1 to HDL.

*2HQ = 2 HydroxyQuinoline

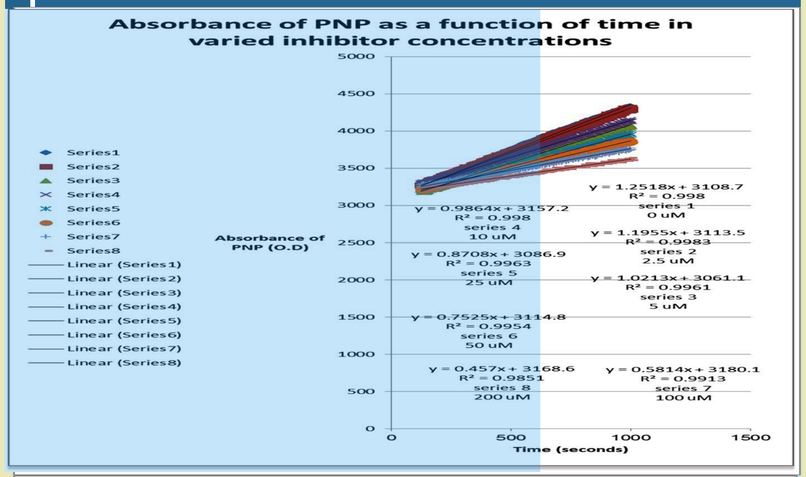
Methods

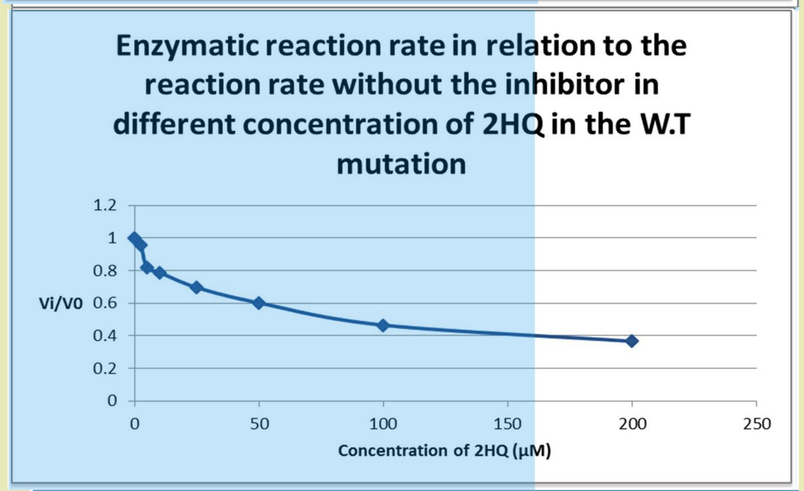
- Transformation of the recombinant plasmid into E.Coli bacteria
- Inoculate the bacteria on culture medium that contains antibiotics (AMP) to get transgenic bacteria.
- Growth of bacteria until the mid of the log phase.
- Addition of IPTG to induce creation of PON1 under the control of lac operon.
- Explosion of the bacteria in order to get a mixture of cell parts and different molecules.
- Centrifugalization in order to get a soluble material that contains PON1 (and other molecules).
- Using affinity chromatography to separate PON1 from other molecules (contains nickel beads that have affinity to the Histidine ring in PON1).
- Checking the absorbance of PON1 in a spectrophotometer using the Bradford Assay in order to calculate the enzyme's concentration.
- Determining the activity of PON1 by testing the absorbance of the enzymatic product PNP as a function of time in the presence of varied concentrations of 2HQ. We use the ELISA machine.

Results

Absorbance of PNP in presence of inhibitor concentration 200 uM	Absorbance of PNP in presence of inhibitor concentration 100 uM	Absorbance of PNP in presence of inhibitor concentration 50 uM	Absorbance of PNP in presence of inhibitor concentration 25 uM	Absorbance of PNP in presence of inhibitor concentration 10 uM	Absorbance of PNP in presence of inhibitor concentration 5 uM	Absorbance of PNP in presence of inhibitor concentration 2.5 uM	Absorbance of PNP in presence of inhibitor concentration 0 uM	Time (seconds)
3420	3430	3380	3390	3330	3290	3310	3240	5
3360	3380	3330	3340	3290	3230	3260	3180	10
3370	3400	3330	3340	3320	3250	3260	3200	15
3310	3350	3280	3300	3280	3190	3210	3160	20
3310	3360	3280	3290	3290	3200	3210	3190	25

Results





Conclusions

From the graph "Enzymatic reaction rate in relation to the reaction rate without the inhibitor" we can see that PON1 in the W.T variant is very much affected by enzyme inhibitors.

As for the future of the research, I recommend:

- Finding new mutations that are not affected at all from enzyme inhibitors
- Develop PON1 so that it could hydrolyze many kinds of nerve agents
- Find mutations that have a higher enzyme activity (so that it could cure poisoning faster)

Overall, PON1 is a very promising candidate for an effective cure against nerve agent poisoning.