Investigating Degradation Enzymes for Pentachlorophenols (PCPs)

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Introduction

Pentachlorophenol (PCP) is an extremely toxic compound that has been introduced into our environment through its use as a wood preservative, herbicide, and defoliant. As a wood preservative it was used to coat many telephone poles, which still remain today soaked with PCP. PCP is resistant to microbial degradation because of chloride substitution and therefore remains around much longer than most compounds. The pathway from *Sphingobium chlorophenolicum* that breaks down PCP has been studied extensively but a complete picture of how the enzymes work is still lacking. This study aims to solve the crystal structure of PcpC, which is an enzyme in the pathway that catalyzes two dechlorinations. Understanding its structure will give insight on its mechanism, which will lead to a better understanding of this pathway so that methods to safely degrade PCP can be developed.

PCP Degradation pathway

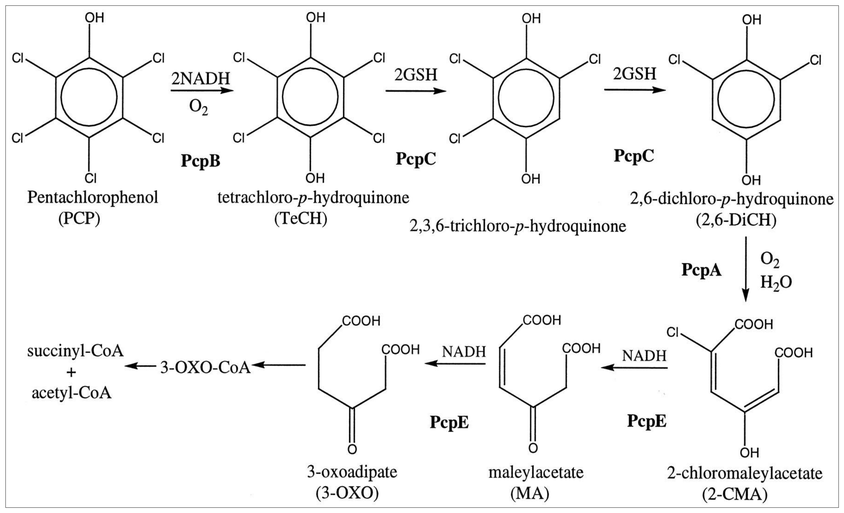
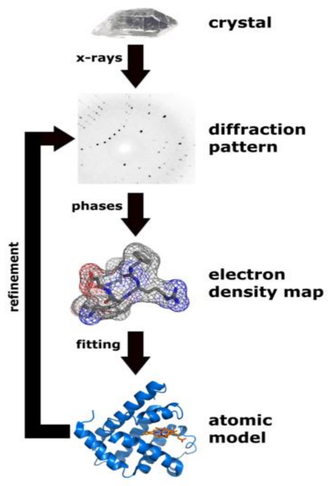


FIG. 1. PCP degradation pathway in *S. chlorophenolicum* strain ATCC 39723. PcpB, PCP 4-monooxygenase; PcpC, TeCH reductive dehalogenase; PcpA, 2,6-DiCH 1,2-dioxygenase; PcpE, MA reductase; CoA, coenzyme A.

Cai, M. et al. 2002. J. Bacteriol. 184(17):4672-4680

The Diffraction process

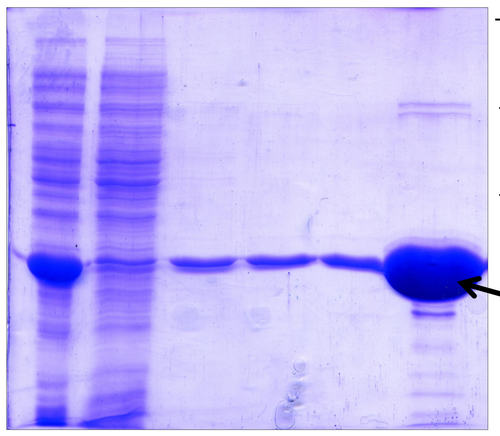


- After the crystal is grown, it will be shot with X-rays to collect a 2-D diffraction pattern
-Using a Fourier transformation, the 2-D diffraction pattern can be turned into a 3-D electron density map that gives the tertiary structure of the protein
-Using the primary structure amino acid sequence of the protein, each amino acid can be correctly fitted into the electron density map with the help of computer programs
- From this a model of the correctly folded functional protein can be established.

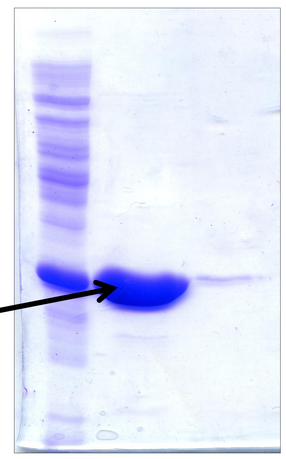
Procedure

Two separate BL21 (DE3) *E. coli* strains were transformed with His tagged PcpC 14S and PcpC 157S, which are homologs of PcpC, which have mutations at the number 14 Cysteine and number 157 Cysteine respectively. The cysteines have been replaced with Serine so that the sulfur in cysteines won't interfere with crystal formation. The cultures were grown at 37°C, induced with IPTG and harvested through centrifugation and sonication. The lysate was then purified using an Ni-NTA column and were concentrated to approximately 11mg/mL(14S) and 8mg/mL (157S) to be set up on crystal plates. They were set up under 192 different conditions, 96 on the Hampton Research Crystal screen HT screen and 96 on the Hampton Research Index HT screen. They were then stored at 4°C in order to induce crystal formation. The crystals that form will be placed on an X-ray defractometer and the diffraction pattern will be used to help solve the structure of PcpC.

SDS-PAGE gels of protein after purification by Ni-NTA

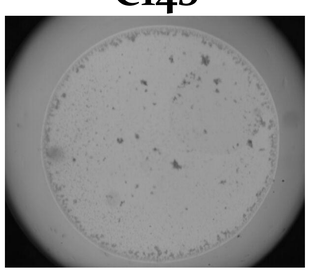


- The protein was run over a Nickel (Ni-NTA) Column three times and washed with a buffer four times (W1-W4)
- A high imidazole buffer was then run over the column four times (E1-E4) which was collected and run on a gel
- The first Elution of both C14S and C157S contained the most protein and was pure enough to set up on crystal plates

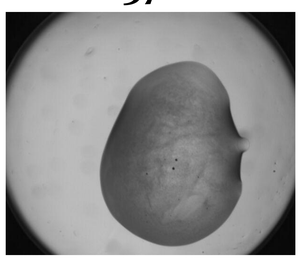


Crystal Images

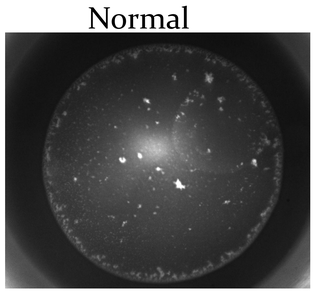
-Once set up, the plates are stored at 4 degrees C and imaged under both regular light and ultraviolet light regularly to check for crystal formation
-Larger crystals can be seen eith regular light, but smaller crystals are much easier to see under UV light, as they show up very bright
-In this case, no diffractable crystals were found in any of the 192 different conditions that were set up on plates
-However, the UV images show that for both C14S and C157S there is at least one condition that produced crystal-like formations. These are shown on the right, with the normal Image on top and UV on bottom.



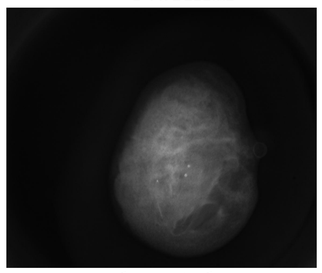
C14S Normal



C157S Normal



Ultraviolet



Ultraviolet

Future Research

-The conditions that produced crystals will be recreated by hand and set up on new plates with numerous drops that will likely produce larger, more useful crystals
-These crystals will then be diffracted using a rotating anode x-ray diffraction machine to collect the diffraction pattern and resolve the structure

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