Insidious Intermediates

How novel detoxifying microbes avoid poisoning themselves

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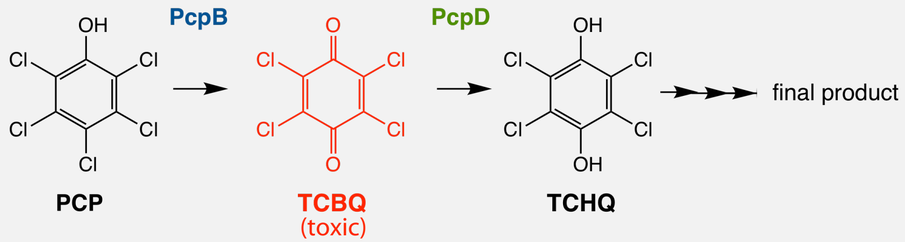
- Anthropogenic chemicals are ubiquitous in nature and often have negative effects on human health and the environment
- Microorganisms have been shown to evolve the ability to detoxify/degrade many different anthropogenic compounds
- Novel degradative biochemical pathways can have adverse effects on the cell, for instance through the production of a toxic intermediate
- Cells must evolve strategies to cope with novel toxic intermediates

Pentachlorophenol (PCP) is an acutely toxic herbicide and fungicide that was banned by the EPA when it was found to damage human health and the environment. It is still used in US forestry. PCP can be degraded by the bacterium *Sphingobium chlorophenolicum,* which has evolved a pathway to degrade this anthropogenic compound[1].

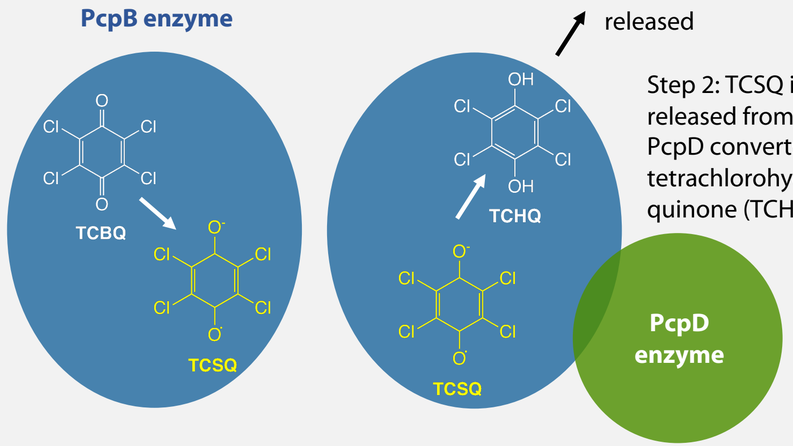
The problem

PCP degradation produces an intermediate, tetrachlorobenzoquinone (TCBQ), that is toxic to the cell

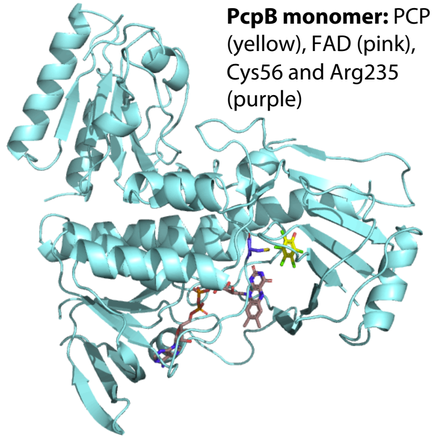


The solution

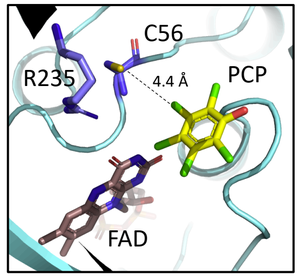
Step 1: PcpB converts PCP to TCBQ. TCBQ reacts with the enzyme to form tetrachlorosemiquinone (TCSQ), which is **trapped** in the enzyme[2-3]



Step 2: TCSQ is not released from PcpB until PcpD converts it to tetrachlorohydro-quinone (TCHQ).



PcpB monomer: PCP (yellow), FAD (pink), Cys56 and Arg235 (purple)



Preliminary structure. **2.3 Å** Dr. Klara Hlouchova and Dr. Johannes Rudolph

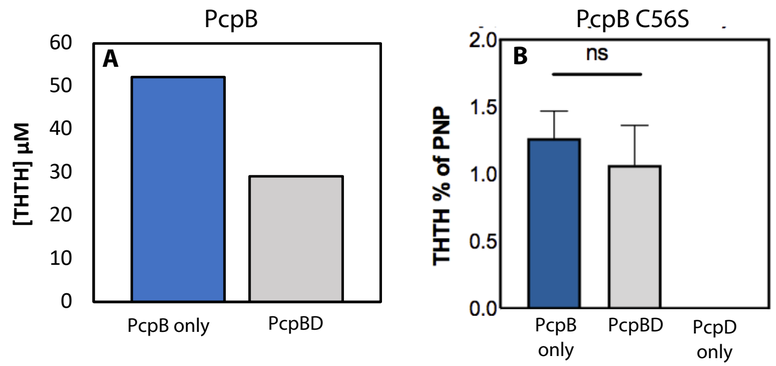
How does sequestration occur?

The structure of PcpB shows the active site has a cysteine side chain (Cys56) close enough to the substrate, PCP, to react and form TCSQ.
If mutation of this position (substitute to serine: C56S) affects sequestration, then we can confirm it is involved in the transfer of electrons by PcpD to TCBQ in the PcpB active site

Is Cys56 involved in electron transfer?

We can determine whether toxic intermediate TCBQ is released from the enzyme by chemically trapping it as THTH. If sequestration occurs, we expect less THTH to form when PcpB AND PcpD are present.

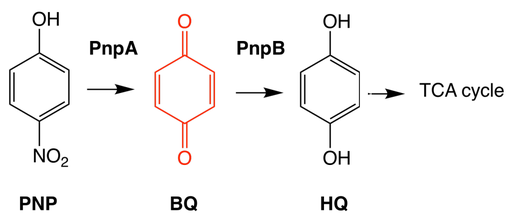
These data (right) confirm that **Cys56 is involved in e- transfer**, as there is no sequestration detected when it is mutated.



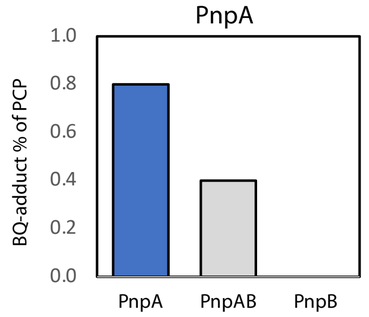
Chemical trapping of TCBQ by βME to form THTH (2,3,5,6-tetrakis[(2-hydroxyethyl)- thio]-1,4-hydroquinone). **A)** Unmutated PcpB with PcpD, THTH detected by spectrometry. **B)** PcpB C56S and PcpD, THTH detected by HPLC. Normalised to internal standard, PNP. Error SEM of 3 reps.

Is the PcpB strategy to cope with its toxic intermediate conserved in related degradative enzymes?

***p*-nitrophenol** (PNP) is a precursor for herbicides and other chemicals and is an EPA Priority Pollutant. It is degraded by a range of different environmental bacteria, including *Pseudomonas* WBC-3[4]. The first step in PNP degradation is catalysed by PnpA, which is related to PcpB and generates toxic intermediate benzoquinone (BQ).



Preliminary chemical trapping data with βME (right) support that **BQ is sequestered by PnpA** in the presence of PnpB (which is unrelated to PcpD).



Sequestration of toxic benzoquinone intermediates is a conserved trait.

1. McCarthy et al., *Appl. Environ. Microbiol.,* 1997; 2. Yadid et al., *PNAS,* 2013; 3. Rudolph et al., *Biochemistry*, 2014; 4. Zhang et al., *J. Bacteriol.,* 2009.

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