

Supplemental Data

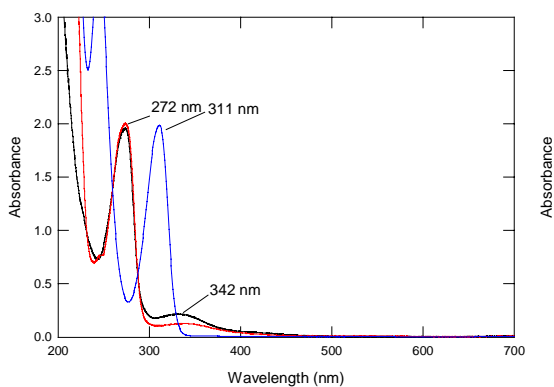


Figure 1a. Electronic absorption spectral changes observed for the CCPO (0.1 μM), TCP (100 μM) and H_2O_2 (400 μM) reaction mixture in 100 mM potassium phosphate solution, pH 3.0 (black). For comparison spectra of 2,6-dichloro-1,4-benzoquinone (red) (Aldrich) and 2,4,6-trichlorophenol (blue) (Aldrich) standards are also shown. This figure shows that the product formed (black) has an electronic absorption spectrum that matches an authentic sample of the quinone product (red) and is clearly different from the starting phenol (blue).

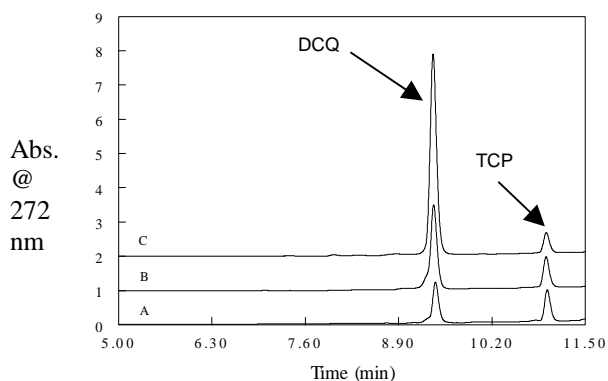


Figure 1b. HPLC monitored at 272 nm obtained for the CCPO (0.1 μM), TCP (500 μM) and H_2O_2 **A**) 1 mM **B**) 2 mM **C**) 4 mM all at 30° C for 1.5 hr in 100 mM potassium phosphate buffer, pH 5.0. This figure shows that a single product is formed in the CCPO-catalyzed dehalogenation of TCP. Similar results were also seen at pH 3.0.

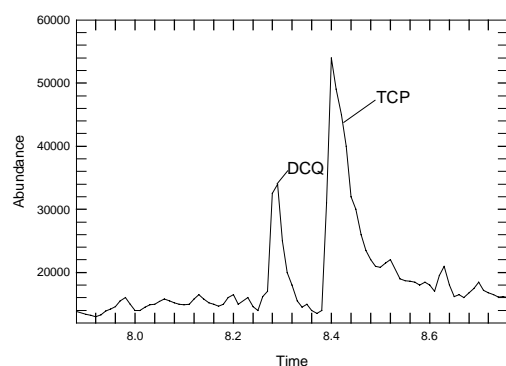
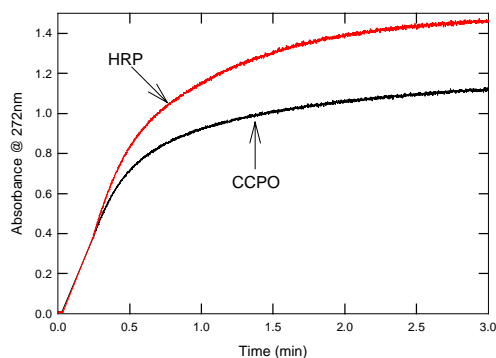


Figure 1c. Gas chromatogram observed for the CCPO (0.1 μM), TCP (500 μM) and H_2O_2 (1 mM) reaction mixture in 100 mM potassium phosphate solution, pH 3.0; products extracted in ethyl acetate.



Turnover # $\sim (20 \text{ mol product})/(\text{mol enzyme} \cdot \text{second})$

Figure 2. Electronic absorption spectra monitored at 272 nm vs. time showing essentially identical turnover number (~ 20 cycles/s) for the CCPO (0.1 μM), TCP (100 μM) and H_2O_2 (400 μM) reaction (black) compared to the HRP/TCP/ H_2O_2 reaction (red) carried out in 100 mM potassium phosphate solution, pH 3.0.

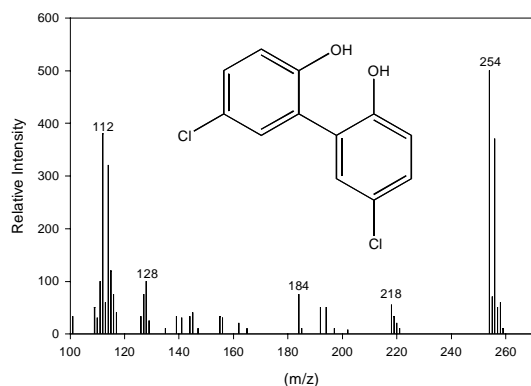


Figure 3. Mass spectrum of *p*-chlorophenol dimer extracted with ethyl acetate from the CCPO (0.1 μM), 4-chlorophenol (0.5 mM), H_2O_2 (1 mM) reaction in 100 mM potassium phosphate solution, pH 3.0. The structure of the dimer shown is that anticipated based on the proposed radical intermediate.

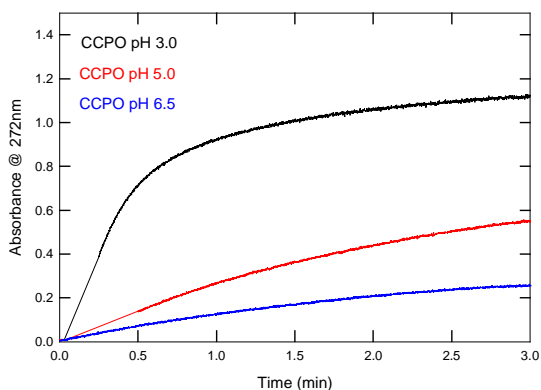


Figure 4. Electronic absorption spectra monitored at 272 nm vs. time for the CCPO (0.1 μ M), TCP (100 μ M) and H₂O₂ (400 μ M) reaction at pH 3.0 (black), pH 5.0 (red) and pH 6.5 (blue).

Additional Information

Chloride quantification. The formation of Cl⁻ (HCl) was measured indirectly by following a pH drop at low buffer capacity during the reaction. Efforts to quantify Cl⁻ by addition of silver nitrate resulted in a cloudy solution, but even doubling the concentration of our original conditions (1 mM 2,4,6-trichlorophenol) did not result in sufficient AgCl for quantification. Nonetheless, the cloudiness of the reaction mixture is indicative of AgCl precipitation.

Chloride oxidation. Two different experimental approaches were taken to probe the potential role of Cl⁻ oxidation. First, the dehalogenation of 2,4,6-trichlorophenol was carried out in the presence of excess KCl, and the same product distribution was observed in the presence and absence of excess Cl⁻. We tried this reaction in the presence of the usual CCPO halogenation substrate, monochlorodimedone, but did not observe any measurable absorbance at 292 nm for dichlorodimedone. These results suggest that the reduction of CCPO compound I to CCPO compound II that we propose is the first step in dehalogenation (after formation of CCPO compound I) occurs at a faster rate than compound X or HOCl formation.

Catalase activity. The formation of dioxygen was not monitored, however, considering the fact that CCPO has considerable catalase activity and the conditions of our experiment involve the use of excess hydrogen peroxide, the generation of dioxygen is very likely. Additionally, small bubbles were observed in the reaction mixture.