SUBMISSION

Comparison of cytochrome *c* with conventional biocatalysts in the degradation of environmental toxicants

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Abstract

Peroxidases and laccases are amongst the most attractive enzymes for the degradation of concerning pollutants like polycyclic aromatic hydrocarbons (PAHs) and organic dyes. In spite of the availability of high activity enzymes, their applicability is hindered by specificity and stability limitations in real environmental conditions, so more suitable biocatalysts are demanded. Cytochrome c (Cc) is best known as a protein electron carrier at mitochondria, but it also displays (pseudo-)peroxidase activity. In this work, we aimed to evaluate the potential of Cc as biocatalyst of PAHs' and azo dyes' degradation and compare it with two more conventional enzymes - plant peroxidases and fungi laccases.

The studies were carried out with Cc from horse heart, horseradish peroxidase (HRP) and laccase from Trametes versicolor. The enzymes were tested with two major PAHs, anthracene and benzo[a]pyrene (BaP), and with methyl orange (MO) as a model azo dye. The enzyme-catalyzed oxidation of PAHs was determined by HPLC, and MO decolorization was followed in spectrophotometric kinetic assays. Several degradation studies were performed to assess the catalytic capacity at different pH and the effect of the redox mediator ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)). Peroxidase reactions of HRP and Cc were initiated with 100 μM H_2O_2 (in media containing 100 μM DTPA as metal chelator).

In pH 5 acetate buffer, the specific ABTS-oxidizing activity of Cc was much lower than that of laccase and HRP. However, these two enzymes lost almost all activity at pH 7, while Cc exhibited catalytic activity even at pH 8 (phosphate buffer). Decolorization assays showed laccase alone to be a weak catalyst of MO degradation, although addition of ABTS to the reaction media greatly accelerated the transformation. On the contrary, both HRP and Cc directly catalyzed MO decolorization, including at neutral pH, and the redox mediator offered no advantage. As for the PAHs, HRP and laccase catalysis benefited from the presence of ABTS in the media, but again Cc oxidized anthracene and BaP directly. Indeed, 24h incubation of BaP (1 mg/L) with Cc (0.1 mg/mL) resulted in the transformation of 70±4% of the PAH and generation of major reaction products different of the BaP quinones produced by laccase- and HRP-ABTS systems.

This work disclosed catalytic properties of Cc different from laccase and HRP that are relevant for the design of new enzymatic remediation processes.