Optimization of the production of active cytochrome c peroxidase

Caroline Proulx, Dr. Ann English, Department of Chemistry and Biochemistry, Concordia University, Montreal.

Cytochrome c peroxidase (CCP) is a heme enzyme that catalyses the reduction of hydrogen peroxide to water by ferrocytochrome c. The iron found in the porphyrin ring of the enzyme is crucial to its functioning, as it acts as the electron donor in the oxido-reduction reaction.

Cytochrome c peroxidase was overexpressed as a fusion protein in *Escherichia coli* DH5α bacteria cells. Different concentrations of δ-amunolevulinic acid (ALA), a precursor of heme, were added to the culture media while the cells were growing. Results show that very little (maximum 0.26 mg) active CCP was produced using 0.05, 0.1, 0.15, 1.0 and 1.5 mM ALA. To produce more active CCP, different concentrations of heme itself were added to the growing cells in the hope that CCP would incorporate the free heme as it was expressed. An estimated 26-35 mg of active CCP was produced when 0.8 – 1.0 mg of heme was added to 50 mL of 2xYTA culture media containing *Escherichia coli* cells. Comparative results obtained from SDS page electrophoresis show that production of CCP is optimized when IPTG is added at a cell density (OD_{600}) of 2.0, and that a three hour waiting period after IPTG induction is sufficient to maximize CCP expression.