Electrochemically driven enzyme catalysis with peroxidases

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Funded by: AiF
Period: 01.11.2004 – 30.06.2007

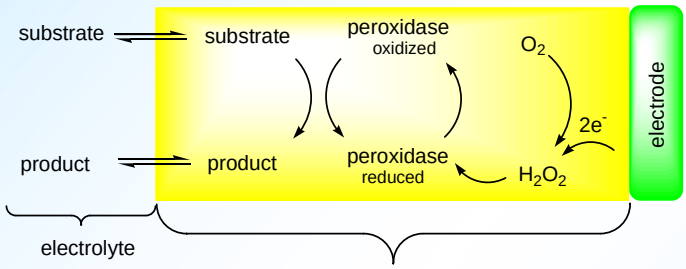
Aim

The aim of this project is to develop new electro-enzymatic processes with peroxidases. These peroxidases utilize hydrogen peroxide for catalysis but are concomitantly inactivated by this cosubstrate. In order to avoid this irreversible inactivation in our system hydrogen peroxide is generated electrochemically on a low, but sufficient level for catalytic activity.

First the immobilization of the peroxidases was investigated. Here we present two methods for the enzyme immobilization in polypyrrole:

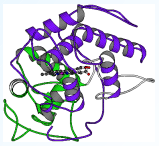
- incorporation of suspended enzyme during electropolymerization of pyrrole;
- potential dependent adsorption on polypyrrole coated electrodes.

Then the electrochemical oxygen reduction to produce H_2O_2 followed by the enzymatic conversion of hexenol to hexenal is demonstrated.



CPO – Chloroperoxidase

Chloroperoxidase from *Caldariomyces fumago* (CPO; EC 1.11.1.10) is a unique enzyme with respect to its broad substrate specificity and catalytic features including halogenations, halohydrin formations, oxygen insertions, alcohol and heteroatom oxidations. CPO catalyses the halogenation of a number of aliphatic substrates in the reaction (1):



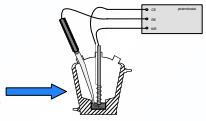
$AH + X^- + H^+ + H_2O_2 \rightarrow AX + 2\ H_2O$ (1)
(AH = substrate, X = Cl, Br, I, but not F).

Although the primary biological function of CPO seems to be chlorination, CPO also exhibits peroxidase (2), catalase (3) and monooxygenase (4) activities:

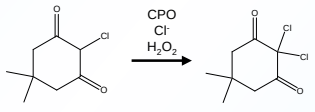
$2\ AH_2 + H_2O_2 \rightarrow HA\text{-}AH + 2\ H_2O$ (2)
$2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$ (3)
$AH + H_2O_2 \rightarrow AOH + H_2O$ (4)

Incorporation of enzyme during electropolymerization of pyrrole

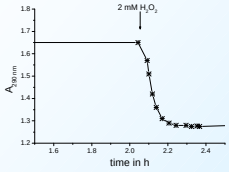
225 U CPO
100 mM NaCl
100 mM Pyrrole



Potentiodynamic deposition
Pt working electrode, A = 0,16 cm²
50 cycles -600 mV - 900 mV vs. Ag/AgCl
100 mV/s



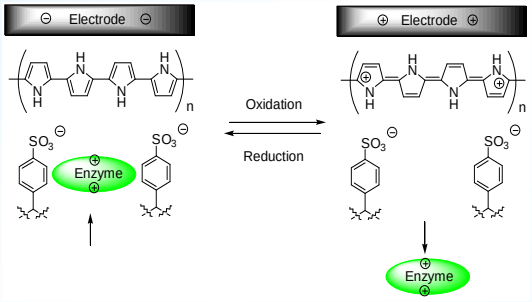
Monochlorodimedone (MCD) Dichlorodimedone



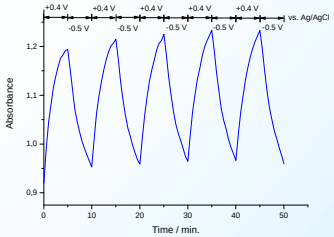
- Enzyme stable in the electropolymerization process
- Activity of the CPO in conducting polymer
- Small amount of immobilized enzyme

Potential dependent adsorption on polypyrrole coated electrodes

- Polypyrrole (PPy) with polystyrene sulfonate (PSS) counterion is a cation exchanger
- CPO – isoelectric point 4.0
- pH of the buffer solution 2.8
- Leads to a positive net-charge of CPO



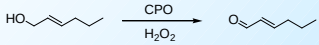
- Spectroelectrochemical cell
- Periodic change of the electrode potential
- Absorbance at 400 nm in the solution



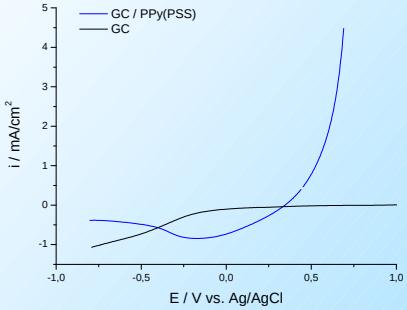
- Nearly reversible immobilization
- Activity of the immobilized CPO proofed by MCD-Assay

Electrochemically driven enzymatic reaction of hexenol to hexenal

Reaction:



Rotating disc electrode: PPy/PSS/CPO layer on glassy carbon (GC) electrode; the oxygen reduction current is overlapped by the strong current of PPy reduction.



Levich plot of the oxygen reduction at a GC/PPy/PSS/CPO rotating disc electrode; the enzymatic formation of *trans*-2-hexen-1-al was detected by gas chromatography.