Reagentless sensors based on wired laccase

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We have developed over the past several years reagentless voltammetric enzyme sensors for the detection of enzyme activity[1-5]. These sensors can measure redox enzyme activity of oxygenase enzymes without the need for a reagent addition step, once the sensors are constructed. Construction of the sensors is achieved by co-immobilization of oxygenase enzymes, such as tyrosinase, ceruloplasmins or laccases, with an osmium-based redox polymer as an electron transfer mediator, onto solid electrode surfaces using a simple one-pot procedure. The activity of the immobilized enzymes is addressed upon electrochemical reduction of the osmium redox centre at the electrode surface. The reduced form of the osmium mediator is re-oxidized by the enzyme, which is in turn re-oxidized by molecular oxygen dissolved in the electrolyte solution. Thus the only reagent consumed in the measurement of the enzyme activity is molecular oxygen, as depicted in the scheme. Constant-potential amperometric monitoring of the steady-state reduction currents for these sensors can be modulated upon introduction of modulators of the enzyme and/or the redox polymer into the electrochemical cell. Toxic pollutants and poisons such as azide, cyanide, catechols and phenols, chlorophenols, thiols, and organic solvents can all modulate the steady-state reduction currents.

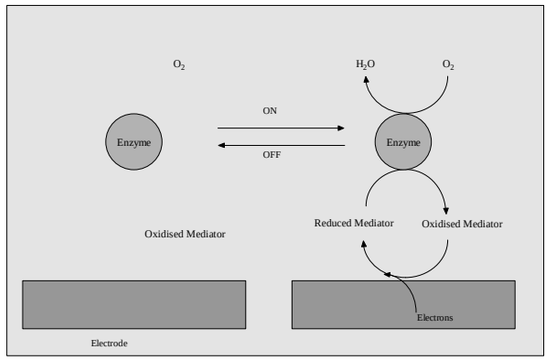
Indeed the use of the catalytic recycling of phenolic substrates of laccase, such as the catecholamines, by the osmium redox polymer can lead to very low detection limits (nM) for these important biomolecules. We are currently investigating the use of such sensing systems in receptor-ligand binding assays for high-throughput screening of antagonists to the dopamine receptor, for example.

The broad specificity of the sensors can allow them be applied as early warning systems for the presence of pollutants in, for example, waste waters or water systems.

To this end we have recently been involved in an EU-funded collaborative project, INTELLISENS, with an objective to create a methodology for fast monitoring of pollution levels in wastewater and pollution incidents, based on a biosensor array-pattern recognition system.

An amperometric reagentless biosensor for detection of phenols is proposed, with the enzyme laccase immobilized in a redox hydrogel onto screen-printed electrodes. The behavior of the biosensor in a flow-injection system was investigated in terms of sensitivity, reproducibility of response, applied potential, flow rate and stability of response.

The other part of this work concerns the building of data libraries on the chemical composition of wastewater from municipal origin, pharmaceutical-, pulp and paper industry. The samples have been analyzed and characterized using GC-MS technique. A sequential solid phase extraction approach based on different sorbents in series and in parallel was applied to the preconcentration of the samples.



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