

Design of Electron-Transfer Pathways Between Quinohemoprotein Alcoholdehydrogenase and Electrodes as a Basis for Reagentless Alcohol Biosensors

Thomas Erichsen¹, Mihaela Niculescu², Dima Kisel¹, Florin Turcu¹, Marcus Mosbach¹, Elisabeth Csöregi², Valdas Laurinavicius³, Wolfgang Schuhmann^{1*}

Anal. Chem. – Elektroanalytik & Sensorik; Ruhr-Universität Bochum;
D-44780 Bochum, Germany

² Department of Biotechnology, University of Lund, S-22100 Lund, Sweden

³ Institute of Biochemistry Vilnius, Mosklininku 12, Lt-2600 Vilnius, Lithuania

The detection principle of amperometric biosensors is based on electron-transfer processes between the active site of an immobilised enzyme and an electrode surface which is poised to a defined working potential. A direct electrochemical recycling of the enzyme's prosthetic group at the electrode surface leading to a corresponding current signal is rarely encountered because in most oxidoreductases the prosthetic group is buried deeply within the protein shell. Thus, the distance for a direct electron transfer (ET) is - according to Marcus theory [1,2] - too long. The ET kinetics between two redox species are exponentially dependent on the distance between both redox sites, the driving force of the reaction (i.e. the potential difference) and the reorganisation energy (which qualitatively reflects the structural rigidity of the redox species). Thus, reagentless biosensors (with all sensor components fixed on the transducer surface) imply a direct communication between enzyme and electrode surface or a pre-defined electron-transfer cascade channeling the electrons between the enzyme's active site and the electrode surface.

Based on previous results which showed that quino-protein alcohol dehydrogenase (QH-ADH) entrapped within polypyrrole is able to directly transfer electrons via the conducting polymer to the electrode surface [3], the electron-transfer properties of this multi-cofactor enzyme has been investigated in different electrode architectures:

First, the QH-ADH has been adsorbed and covalently-bound to self-assembled thiol monolayers. While the dissolved enzyme is able to transfer electrons to the electrode via heme *c*

as well as via the more deeply buried PQQ (fast adsorption - chemical reaction - desorption mechanism), an orientation of adsorbed QH-ADH on hydrophobic electrode surfaces, as well as of adsorbed and covalently bound QH-ADH on negatively-charged thiol monolayers could be observed. In these cases the heme *c* units are pointing towards the electrode surfaces result in an optimised direct ET rate.

Second, the enzyme has been integrated into a Os-complex based redox-polymer film by cross-linking with poly(ethylene glycol)diglycidyl ether. The redox hydrogel-based biosensor was evaluated in term of stability and was integrated as detector in an on-line automated analyzer, and used for continuous monitoring of ethanol during wine fermentation.

Third, the enzyme has been integrated within a polymer film which has been selectively deposited by means of an electrochemically-induced pH-shift. Using the Os-complex containing hydrogel pre-adsorbed on the electrode surface a novel type of reagentless biosensor could be obtained.

1 R.A. Marcus and N. Sutin, *Biochim. Biophys. Acta*, 1985, **811**, 265.

2 R.A. Marcus, *Angew. Chem.* 1993, **105**, 1161.

3 A. Ramanavicius, K. Habermüller, E. Csöregi, V. Laurinavicius, W. Schuhmann, *Anal. Chem.* **71** (1999) 3581-3586.

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