SYNTHESIS AND APPLICATION OF A BIFUNCTIONAL REDOX POLYMER FOR THE DEVELOPMENT OF A HIGHLY SENSITIVE ENZYME SENSOR

Charles Merlin Tientcheu, Patrick Broeker, Axel Warsinke

University of Potsdam, Institute of Biochemistry and Biology, iPOC Group, c/o FhIBMT Am Muehlenberg 13, 14476 Potsdam, Germany
E-mail: tientche@uni-potsdam.de

For enzyme sensors based on mediated electron transfer, it is mandatory that enzyme, mediator and electrode surface are in close contact to permit an electron transfer from the catalytic center of the enzyme to the electrode surface. As enzyme sensors based on adsorbed polymer and adsorbed enzymes are lacking long term stability due to possible protein denaturation and fast desorption, it was interesting to investigate another immobilisation approach. A bifunctional poly-N-isopropylacrylamide (PNIPAM)-ferrocene polymer carrying several epoxy groups was synthesized [1] and used for both covalent attachment to a cysteamine-modified gold electrode and for immobilisation of a pyrroloquinoline quinone (PQQ) dependent glucose dehydrogenase (PQQ-GDH) as a model redox enzyme.

The presence of ferrocene in the redox polymer enabled electrical communication between the cofactor PQQ and the electrode for sensitive detection of glucose. When PNIPAMFoxy was immobilised on the cysteamine-modified gold surface the cyclic voltammogram recorded the oxidation and the reduction peak at 0.3V and 0.22V respectively. This corresponds to the published values for ferrocene derivatives.

To investigate if the PQQ-GDH is only adsorbed to the polymer structure or as expected covalently attached to the epoxy groups several control experiments were carried out.

It was shown that if the epoxy groups were inactivated by using alkaline buffer and/or ethanolamine before the PQQ-GDH was applied; the chronoamperometrical current after glucose addition was nearly zero. In comparison if the epoxy groups were not inactivated a much higher current was measured.

The sensitivity of the developed enzyme sensor for glucose was extremely high. The reasons are the very high catalytic activity of the PQQ-GDH, the good electron transfer via the ferrocenyl groups of the redox polymer and the nearly unlimited diffusion of the glucose to the catalytic center of the enzyme. With the developed sensor it was possible to determine glucose concentrations within the nanomolar range. The approach of using bifunctional redox polymers in combination with redox enzymes will help to develop new enzyme sensors for analytes present in very low concentrations.

Reference