Original Paper

Redox-amplified biosensors based on selective modification of nanopore electrode structures with enzymes entrapped within electrodeposition paints

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Abstract. An electrode structure consisting of two separately addressable electrodes with 200 nm inter-electrode spacing in a layered nanoporous electrode/insulator/electrode configuration was used as transducer for redox-amplified biosensors. Nanopores were selectively modified with two different enzymes, namely glucose oxidase and alkaline phosphatase by means of electrochemically induced precipitation of electrodeposition paints. Both commercially available and specifically synthesized redox-active electrodeposition paints were used. Selectivity of modification was proven by electrochemical methods and atomic force microscopy. The principle feasibility to amplify the measured current in a model sensor based on alkaline phosphatase could be demonstrated. Signal amplification by a factor of two with respect to the non-amplified response was achieved.

Keywords: Nanopore electrodes; redox amplification; electrodeposition paint; immobilization; redox cycling

Electrochemical detection schemes are attractive read-out strategies in sensing devices based on microarrays [1, 2] due to their ability to combine high sensitivity and selectivity with inexpensive equipment and the potential for miniaturization. This high potential for miniaturization makes electrochemical equipment an ideal candidate for the use in small portable sensing devices with integrated sensors or microsensor arrays.

The demand for highly sensitive analysis of compounds in complex matrices often calls for an amplification of the initial sensor response. Electrochemical sensors allow for an amplification of the sensor signal by means of redox cycling [3]. Redox cycling requires a specific electrode layout that typically features two closely spaced electrodes such as interdigitated electrodes [4, 5]. The detection of the analyte occurs at one of the electrodes while the other electrode serves as a recycling electrode that regenerates the analyte. Obviously, this method is restricted to analytes or mediators that undergo a reversible electrochemistry to allow multiple redox cycles that lead to the amplification of the sensor signal. Often, labeling steps are introduced into the detection scheme to introduce a reversible redox couple as terminal component in the signal transduction chain. An often-used redox mediator is p-aminophenyl phosphate (p-APP) that is cleaved to p-aminophenol (p-AP) by the enzyme alkaline phosphatase (AP). While p-aminophenyl phosphate is not electrochemically active, p-aminophenol can be reversibly oxidized to p-quinone imine.
ferent enzymes within the nanopore volumes of the have been employed as immobilization matrix for dif-

Another issue that is crucial in the design of new biosensors but not restricted to electrochemical sen-
sors is the immobilization of a biological recognition element onto a transducer surface. When a micraarrayed format is desired, immobilization has to be confined within small spaces to avoid crosstalk between indi-

selective modification of micro-

and nanoscaled objects can be listed as one of the key steps in microarrayed (bio)sensor technology. As a matter of fact, electrochemically induced proce-
dures are addressing selectively the electrode surface and the diffusion zone in front of the electrode and are hence well suited for the individual and controlled modification of electrodes in a microarray. Previously, we have developed methods based on so called ele-

trodeposition paints (EDP) as a means to securely im-

mobilize biological recognition element on electrode surfaces [12]. Electrodeposition of paints is originally an industrial technique mainly used for color painting and corrosion protection [13, 14]. The electrochemi-

cally induced precipitation of suitable EDP is invoked by a local modulation of the pH-value within the dif-

fusion zone in front of an electrode surface leading to protonation or deprotonation of charged sidechains in the polymer backbone which is in turns causing a change in the solubility of these paints. Co-immobilization of a biological recognition element leads to a selective bio-modification of the electrode surface at

which the pH modulation was invoked by applying potential pulses for the electrolysis of water. Both commercially available [12, 15] and specifically syn-

thesized [16–18] EDP have been successfully em-

ployed as immobilization matrices in biosensors.

In this communication, we present results on the selective modification of a nanopore electrode structure that features electrodes with an inter-electrode spacing of 100 nm [19–21]. The small inter-electrode spacing and a fabrication process that avoids costly photolithographic steps makes the electrode structure a potential candidate for the cost-effective construction of sensors with high signal amplification by means of redox cycling. Both commercially available and specifically synthesized electrodeposition paints have been employed as immobilization matrix for dif-

ferent enzymes within the nanopore volumes of the

electrode structures to demonstrate the feasibility of a selective modification of the nanopores with biologi-

cal recognition elements. A model sensor with signal amplification by redox cycling demonstrates the prin-

ciple potential of the nanopore electrode platform in combination with selective immobilization strate-
gies for the design of highly sensitive electrochemical biosensors.

**Experiments**

**Chemicals and materials**

Tri-distilled water prepared by a Destamat (Heraeus, Wehrheim, Germany, www.heraeus.com) was used for preparation of all solu-

tions unless otherwise stated. HPLC-grade water was from J.T. Baker (Deventer, The Netherlands, www.jtbaker.com). Glucose oxi-

dase from *aspergillus niger* (EC 1.1.3.4) and alkaline phosphatase from bovine intestinal mucosa (EC 3.1.3.1) were from Sigma (Steinheim, Germany, www.sigmaaldrich.com). NaH2PO4·2H2O and KOH were from Riedel-de-Haën (Seelze, Germany, www. riedeldehaen.de), Na2PO4·2H2O was from J.T. Baker (Deventer, The Netherlands, www.jtbaker.com). Tris-hydroxymethyl-amin-

methane (TRIS) was from Biomol (Hamburg, Germany, www. biomol.com), D(+)-glucose monohydrat was from AppliChem (Darmstadt, Germany, www.applichem.de), sodium p-aminophenyl phosphate monohydrat was from Universal Sensors (Kinsale-

Sandycove, Ireland, intel.ucc.ie/sensors/universal/). As commer-

cial EDP, Resydrol® AY498w/35WA (Solutia, Wemdon, Austria, www.solutia.com) and Cathodip® (BASF, Ludwigshafen, Germany, www.basf.com) were used.

**Instrumentation**

For electrochemical measurements, a PalmSens bipotentiotast (Palm Instruments, Houten, The Netherlands, www.palmssens.com) and a multichannel electrochemical analyzer 1030 (CH Instruments, Austin, USA, www.chinstruments.com) were used. All potentials were measured with respect to a home-made Ag/AgCl micro refer-

eence electrode with 3 M KCl as internal electrolyte and a coiled platinum wire (Goodfellow, Huntington, UK, www.goodfellow.com) as counter electrode if not otherwise stated.

Atomic force microscopy (AFM) was conducted using an Explorer AFM (Topometrix, Santa Clara, USA, www.veeco.com) in contact-mode.

**Fabrication of nanopore electrode structures**

Fabrication of nanopore electrode structures was described in detail elsewhere [19–21]. The electrodes consist of a layered gold/ insulator/gold structure with the top gold layer and the insulating layer (Si3N4) being nanostructured by means of nanoparticle litho-

graphy [19]. In brief, nanoparticles are decorated on top of the insulating layer before sputtering of the second gold layer. Hence, the top layer is of porous nature after removal of the nanoparticles. The top gold layer then serves as shadow mask for the etching of the insulating layer. Thus, an array of nanopores is formed having a combined bottom electrode which is separated by the insulator from a combined top electrode. The thickness of the insulating layer defines the interelectrode spacing between top and bottom electrodes. Pore density and pore diameter can be influenced by concen-