Electrochemistry Communications 34 (2013) 327–330

Contents lists available at ScienceDirect

Electrochemistry Communications

journal homepage: www.elsevier.com/locate/elecom

Short communication

# Stabilizing redox polymer films by electrochemically induced crosslinking

Sascha Pöller, Dominique Koster, Wolfgang Schuhmann *

*Analytische Chemie — Elektroanalytik & Sensorik, Ruhr-Universität Bochum, Universitätsstr. 150, D-44780 Bochum, Germany*

ARTICLE INFO

*Article history:*
Received 1 July 2013
Received in revised form 21 July 2013
Accepted 23 July 2013
Available online 31 July 2013

*Keywords:*
Redox polymer
Crosslinking
Electrodeposition polymer
Electrochemically induced pH modulation
Biosensor
Biofuel cell

ABSTRACT

Electrochemically induced crosslinking is suggested to stabilize electrodeposition polymer/enzyme films selectively on an electrode surface. 4 different protected diamine or dithiol based bi-functional crosslinkers have been synthesized, which can be activated by a pH-shift invoked by electrochemical water oxidation or proton reduction. Deprotection occurs either simultaneously or sequentially to the deposition of specifically designed redox electrodeposition polymers. The stability of the resulting polymer films was substantially enhanced as evaluated using continuous potentiodynamic cycling alternated by difference pulse voltammetry. Electrochemically induced crosslinking is compatible with biological recognition elements using *Trametes hirsuta* laccase or glucose oxidase entrapped within specifically adapted Os-complex modified or phenothiazine-modified redox polymers.



## 1. Introduction

One crucial drawback in the development of amperometric biosensor architectures or biofuel cell electrodes is the often limited long-term stability. This may be due to deterioration of individual compounds composing the bioelectrochemical device or by a poor immobilization strategy leading to a loss of at least one of the crucial components with time. Immobilization is often performed by rather simple manual procedures such as drop or dip coating of liquid enzyme preparations containing suitable crosslinkers or precursors for the formation of sol–gel matrices [1–3]. In order to specifically address the electrode surface as site for the immobilization of the sensor components electrochemically induced procedures were proposed including the deposition of conducting polymer films [4,5]. Due to highly reactive radical intermediates exclusion of oxygen is required and comparatively high potentials have to be applied. Alternatively, the formation of intermediate radicals can be circumvented by electrochemically induced modulation of the pH value in front of the electrode surface. This strategy was increasingly used for depositing so-called electrodeposition polymers including electrodeposition polymers modified with redox mediators [6]. The local pH modulation in the diffusion zone in front of the electrode changes the solubility of the polymer chains which are precipitating on the electrode surface while simultaneously entrapping a biological recognition element present in the electrolyte solution. Evidently, the addition of bifunctional crosslinkers to the deposition solution would lead to crosslinking of dissolved polymer chains and/or the enzyme already in bulk solution. Electrochemical activation of crosslinkers like the electro-induced ligand exchange reaction between ruthenium complexes and chitosan was proposed [7]. While this is an elegant approach as long as the polymer backbone carries only one type of coordination site, it cannot be used for copolymers with multiple potential coordination sites causing the formation of multiple redox species. Recently we have introduced a new class of electrodeposition polymers which contain epoxide groups [6], which facilitate further crosslinking of preformed redox polymers. To prevent crosslinking in bulk solution, the crosslinker has to remain inactive while it is dissolved in bulk solution and it should be activated exclusively in vicinity of the electrode. To achieve this, we propose the synthesis of protected bifunctional crosslinkers with specifically designed protection groups which can be cleaved using pH changes. By this, the crosslinker is activated during the electrochemically induced precipitation of the electrodeposition polymer in the established pH gradient. The protected bifunctional crosslinker is activated inside the precipitated polymer film and thus selectively stabilizes the already precipitated polymer layer.

## 2. Experimental procedures

### 2.1. Chemicals and solutions

All not specifically mentioned chemicals were obtained from regular commercial sources and were of analytical grade. *N*-boc-2,2′-(ethylenedioxy)diethylamine was synthesized following [8], however, using 3 eq of diamine and EtOH/$NH_4OH$ (30% w/w) = 100/5 for flash chromatography. All aqueous solutions were prepared using water purified and deionized with a Milli-Q-system. The activity of glucose oxidase (GOx) from *Aspergillus niger* was 7815 U/g

* Corresponding author. Tel.: +49 234 3226200; fax: +49 234 3214683.
*E-mail address:* wolfgang.schuhmann@rub.de (W. Schuhmann).

1388-2481/$ – see front matter 
http://dx.doi.org/10.1016/j.elecom.2013.07.033

(Sigma-Aldrich). Laccase from the basidiomycete *Trametes hirsuta* (*Th*L) has been kindly provided by Prof. Alexander I. Yaropolov.

### 2.2. Synthesis of the redox polymers

The synthesis of polymers P024, P029, dimethyldioxyrane, Os-complex P91 as well as the synthesis of the redox polymers were carried following procedures described in [6].

### 2.3. Synthesis of the crosslinkers

#### 2.3.1. 2,2′-(Ethylenedioxy)-bis-(S-acetylethanthiol) (crosslinker 1)

2,2′-(Ethylenedioxy)diethanethiol (7.29 g) and triethylamine (11.6 mL) were dissolved in $CH_2Cl_2$ (250 mL) and cooled to 4 °C. Acetyl chloride (6.28 g) was added and the reaction mixture was stirred for 2 h at 4 °C followed by 14 h at room temperature. The organic phase was washed with $NaHCO_3$ (sat., aq., 4 × 50 mL), dried over $Na_2SO_4$ and the solvent was evaporated *in vacuo* to obtain the product as yellow oil (10.2 g).

$^1$H NMR (400 MHz, $CDCl_3$, 300 K): δ (ppm) = 3.61–3.58 (m, 8H); 3.09 (t, 4H); and 2.33 (s, 6H). FAB-MS: calculated for $C_{10}H_{18}O_4S_2H$ ($[M + H]^+$): 267.1; found: 267.0.

#### 2.3.2. 2,2′-(Ethylenedioxy)-bis-(N-triflouroacetethanamide) (crosslinker 2)

2,2′-(Ethylenedioxy)diethylamine (1.48 g) was dissolved in diethylether (5.0 mL) and cooled to 4 °C. Trifluoroacetic acid anhydride (4.55 g) dissolved in diethylether (5.0 mL) was added and the reaction mixture was stirred for 1 h at 4 °C. The organic phase was washed with $NaHCO_3$ (sat., aq., 2 × 10 mL), dried over $Na_2SO_4$ and the solvent was evaporated *in vacuo* to obtain the product as pale yellow solid (3.27 g).

$^1$H NMR (400 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.16 (br, 2H); 3.67–3.65 (m, 8H); and 3.58–3.56 (m, 4H). FAB-MS: calculated for $C_{10}H_{14}F_6N_2O_4H$ ($[M + H]^+$): 341.1; found: 341.0.

#### 2.3.3. 1,14-Dinitro-2,13-diphenyl-6,9-dioxa-3, 12-dithiatetradecane (crosslinker 3)

2,2′-(Ethylenedioxy)diethanethiol (3.03 g), *trans*-β-nitrosytrene (5.00 g) and *N*-methylmorpholine (3.4 mL) were successively dissolved in $CH_2Cl_2$ (35 mL) and stirred for 3 h at room temperature. The organic phase was washed with $NaHCO_3$ (sat., aq., 3 × 15 mL), dried over $Na_2SO_4$ and the solvent was evaporated *in vacuo*, followed by further drying in high vacuum to obtain the product as dark yellow oil (7.77 g).

$^1$H NMR (400 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.35–7.27 (m, 10H); 4.89–4.69 (m, 6H); 3.68–3.58 (m, 8H); and 2.59 (t, 4H). FAB-MS: calculated for $C_{22}H_{28}N_2O_6S_2H$ ($[M + H]^+$): 481.1; found: 481.0.

#### 2.3.4. 2,2′-(Ethylenedioxy)-bis-(N-tritylethanamine) (crosslinker 4)

Trityl chloride (22.3 g) and triethylamine (11.6 mL) were dissolved in diethylether (250 mL) and cooled to 4 °C. 2,2′-(Ethylenedioxy) diethylamine (5.93 g) was added and the reaction mixture was stirred for 13 h at room temperature. The organic phase was washed with $H_2O$ (2 × 70 mL), dried over $Na_2SO_4$, and the solvent was evaporated *in vacuo* to obtain the product as white solid (23.9 g).

$^1$H NMR (400 MHz, $CDCl_3$, 300 K): δ (ppm) = 7.47 (m, 12H); 7.25 (t, 12H); 7.17 (t, 6H); 3.59 (t, 4H); 3.48 (s, 4H); 2.34 (t, 4H); and 2.05 (s, 2H). FAB-MS: calculated for $C_{44}H_{44}N_2O_2H$ ($[M + H]^+$): 633.3; found: 633.2.

### 2.4. Electrodes and electrochemical measurements

If not stated otherwise all potentials refer to a Ag/AgCl/3 M KCl reference electrode (RE). A Pt-wire was used as counter electrode (CE). Either glassy carbon (GC; 3 mm) or graphite (C; 3 mm) electrodes were used as working electrodes (WE). GC electrodes were polished using $Al_2O_3$ suspension of 3.0 μm/1.0 μm/0.3 μm (Leco). GC electrodes were pre-modified with an amine linker following a procedure as in [8] using a Luggin–Haber-RE (Ag/$AgClO_4$10 mM/TBATFB 0.15 M). C electrodes were modified with Pt nanoparticles by means of chronoamperometry (0.0 V/1.0 s; −1.0 V/0.2 s; 0.0 V/5.0 s; 50 repetitions) from a 0.4 mM $H_2PtCl_6$ solution. All electrochemical experiments were recorded with a PGSTAT12 potentiostat (Autolab) controlled by the NOVA 1.7 software (Metrohm), with the exception of the long term chronoamperometric measurements for which a PED 300 bipotentiostat (Biometra) was used.

### 2.5. Electrodeposition procedure

#### 2.5.1. Preparation of GC/P024-P91 and GC/P024-P91/ThL

The modification of amino-terminated pre-modified GC electrodes with redox polymer P024-P91 by electrodeposition and concomitant stabilization by electrochemically induced crosslinking (EICL) was performed using a suspension of P024-P91 (10 mg/mL in $H_2O$, 200 μL) containing the respective crosslinker (saturated in DMSO, 30 μL) and *Th*L (15 mg/mL in 0.05 M phosphate buffer (PB), pH 6.5, 20 μL) by applying a potential pulse sequence: (−2.5 V/0.2 s; −0.2 V/1.0 s; 0.0 V/5.0 s; 25 repetitions) for crosslinkers *1,2,3* or (+2.5 V/0.2 s; −0.2 V/1.0 s; 25 repetitions) followed by (−2.5 V/0.2 s; −0.2 V/1.0 s; 0.0 V/5.0 s; 25 repetitions) for crosslinker *4*.

#### 2.5.2. Preparation of C/P029-TB/GOx

The modification of C electrodes with redox polymer P029-TB and GOx by electrodeposition and stabilization by EICL was performed from a suspension of P029-TB (30 mg/mL in $H_2O$, 50 μL) with crosslinker *2* (0.01 M in DMSO, 10 μL) and GOx (5 mg/mL in 0.05 M PB, pH 7.3, 200 μL) by applying a potential pulse sequence: (−2.5 V/0.2 s; −0.2 V/1.0 s; 0.0 V/5.0 s; 25 repetitions).

## 3. Results and discussion

The non-manual and controlled electrochemically induced deposition selectively addressing the electrode surface is seen as the major advantage of electrodeposition polymers or redox electrodeposition polymers as a basis for the fabrication of reagentless biosensors or membrane-free biofuel cells [9]. Evidently, during the electrochemically induced deposition process it is impossible to mix the formed polymer film with suitable bifunctional crosslinkers without risking crosslinking already in bulk solution. Hence, we aim on extending the electrochemically induced formation of the polymer layer by an electrochemically induced activation of protected crosslinkers by an electrochemically induced pH shift occurring simultaneously within the polymer film. For this, we synthesized a variety of bifunctional crosslinkers which are protected with different pH-sensitive protective groups (Fig. 1). In combination with different epoxide-groups modified redox electrodeposition polymer (Fig. 1) model bioelectrodes based on entrapped laccase or GOx were prepared and their stability after electrochemically induced crosslinking was evaluated.

Using previously described electrodeposition polymers [10] containing epoxide groups generated from allylic double bonds [6] two different redox electrodeposition polymers were synthesized (see Fig. 1). The polymer P024-P91 contains an Os-complex in the side chain with a redox potential specifically adapted to the T1 site of laccase while the polymer P029-TB uses toluidine blue as a redox species to communicate with the FAD-centers in oxidases. The polymer integrated epoxide functionalities are not only the target for crosslinking by an epoxide opening reaction with nucleophilic crosslinkers but also allow covalent binding to e.g. amino group modified electrode surfaces thus additionally stabilizing the precipitated polymer film. The local pH modulation in front of the electrode surface is initiated by water oxidation or reduction. To facilitate this, the used graphite electrodes were modified with Pt nanoparticles by means of an electrochemical pulse deposition procedure. For activation of the suggested crosslinkers a