



Short communication

Electrochemically induced deposition of poly(benzoxazine) precursors as immobilization matrix for enzymes

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ABSTRACT

Water-soluble benzoxazine oligomers were synthesized by reacting bisphenol A, tetraethylenepentamine and formaldehyde. The pre-formed benzoxazine oligomers can be further electropolymerized from aqueous suspensions under formation of poly(benzoxazine)-based films on electrode surfaces. Integration of glucose oxidase in a poly(benzoxazine) film led to highly reproducible and stable biosensors. Poly(benzoxazines) are proposed as a new family of stable polymers for the design of enzyme electrodes.

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1. Introduction

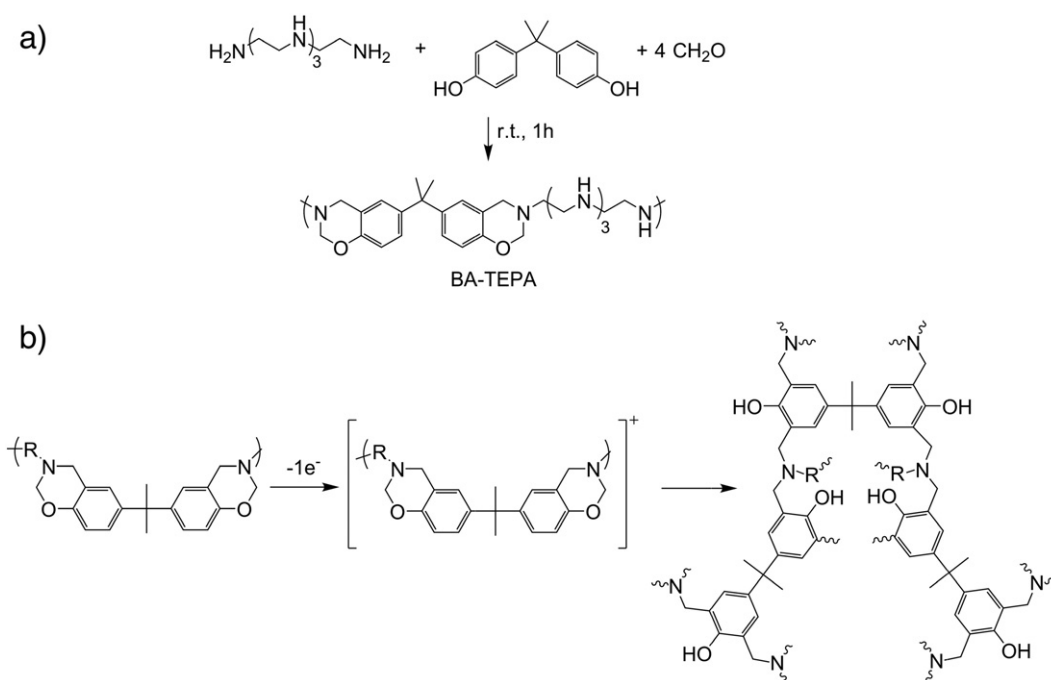
Stable immobilization of enzymes on electrode surfaces is crucial for the development of bioelectronic devices. Entrapment of biological recognition elements within a polymer matrix in front of the electrode is commonly applied using an insulating ramified network of polymer chains, a conducting polymer or a redox polymer [1,2]. A large variety of different polymers was investigated using mainly manual deposition such as drop-coating [3,4]. The specific deposition exclusively on the surface of even microelectrodes leading to a stable immobilization of polymer/enzyme films remains difficult. Electrochemically induced formation of polymer chains in front of the electrode surface e.g. for the deposition of conducting polymers involves the formation of highly reactive and low-molecular radical intermediates making exclusion of oxygen necessary. Hence, mainly preformed polymers or redox polymers were used [5], however using manual deposition techniques. We have earlier proposed the electrochemically induced deposition of polymer films invoked by an electrochemically triggered pH modulation [6] preventing the formation of reactive intermediates while simultaneously providing non-manual control over the polymer deposition process. Functionalization of the polymer backbones and the electrode surface as well as electrochemically induced crosslinking were used to increase the stability of such polymer films [7]. However, the stability of these polymer films is often not sufficient for the anticipated application.

Evidently, new classes of polymers with intrinsically higher stability but accessible to a controlled and electrochemically induced polymer film formation process are needed. In addition, the polymers should be accessible for modifications e.g. with redox relays and/or their hydrophobic/hydrophilic balance as well as their swelling properties in aqueous electrolytes. For this, a highly variable synthesis strategy is indispensable.

Poly(benzoxazines) are a class of phenolic resins exhibiting high thermal stability, near zero shrinkage volume upon curing and very low water absorption. The design flexibility of the monomer allows tailoring poly(benzoxazine) properties [8]. They found applications mainly as polymer matrix for composite materials in electronics and aerospace industries [9]. Poly(benzoxazines) are obtained by heating the monomer in the absence or presence of a catalyst. Poly(benzoxazines) can be directly synthesized at electrode surfaces by electropolymerization of the monomers in acetonitrile/alkali aqueous solution [10]. Due to hydrophobic starting materials and the need of organic solvents immobilization of enzymes was impossible. Water soluble poly(benzoxazine) precursors were obtained by introducing long polyamines as amine component [11]. The pre-formed benzoxazine derivative is supposed to be deposited upon electrochemical oxidation and further polymerization on the electrode surface. Hence, co-immobilization of enzymes becomes possible.

Poly(benzoxazines) were to the best of our knowledge not used as immobilization matrix for biological recognition elements and for the development of bioelectronic devices. In this communication, we want to propose electrochemically controlled deposition of water-soluble poly(benzoxazines) in the presence of an enzyme by electropolymerization of preformed benzoxazine oligomers.

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Scheme 1. a) Synthesis of the water-soluble poly(benzoxazine) precursor. b) Electrochemically induced crosslinking of poly(benzoxazine) precursors on the electrode surface.

2. Experimental

2.1. Chemicals and materials

All chemicals were of analytical grade. Bisphenole A (BA), tetraethylenepentaamine (TEPA), hexachloroplatinic acid (solid, hexahydrate), potassium hexacyanoferrate(II) trihydrate, glucose oxidase (GOx; 11,440 U/g) and dimethylaminomethylferrocene were from Sigma-Aldrich (Steinheim, D). Formaldehyde 37% stabilized with 10% methanol was from Merck (Darmstadt, D). 1,4-Dioxane, sodium carbonate, acetic acid, citric acid, and disodium hydrogen phosphate were from J.T. Baker (Deventer, NL). Potassium ferricyanide was from Riedel-de-Haen (Seelze, D) and β -D-glucose was from AppliChem (Gatersleben, D).

2.2. Polymer synthesis

The polybenzoxazine precursor was synthesized following a previously described procedure [12] using BA, TEPA and formaldehyde with the molar ratio of 1:1:4. 4.00 g (0.0175 mol) of BA was dissolved in 10 mL 1,4-dioxane and the temperature was kept below 5 °C using an ice bath. 5.69 g (0.07 mol) formaldehyde solution (37% w/w in H₂O) was added and subsequently 3.49 g (0.0175 mol) TEPA was added dropwise under stirring. The reaction mixture was stirred for 1 h at room temperature. The obtained polymer (BA-TEPA) was precipitated using 250 mL 0.1 M NaHCO₃ solution and washed with 250 mL H₂O. BA-TEPA was dried under vacuum at room temperature.

Electrochemical experiments were carried out using a μ Autolab III (Autolab, Utrecht, NL), equipped with a FRA 2 module and controlled by the NOVA 1.9 software in a 10 mM solution of BA-TEPA in 1.0% v/v CH₃COOH in H₂O.

2.3. Electropolymerization

Electrochemical polymerization of BA-TEPA was performed using cyclic voltammetry (potential range 0.0 to 1.8 V; scan rate 50 mV/s; 5 cycles) in a three electrode cell containing a Pt net cylinder counter electrode (CE), a glassy carbon electrode (GCE) working electrode

(WE) and a Ag/AgCl/3 M KCl reference electrode (RE). Potential pulse deposition was performed in 5.0 mL of a 10 mM BA-TEPA solution containing K₄[Fe(CN)₆] and K₃[Fe(CN)₆] (25 mM each) by applying a pulse profile of 2.0 V/0.01 s; 0.0 V/0.5 s (3 repetitions) followed by a cyclic voltammogram (from -0.1 to +0.6 V; 50 mV/s). This sequence was repeated 60 times until the electrode surface was completely blocked.

2.4. Immobilization of BA-TEPA and glucose oxidase on glassy carbon electrodes

A polished glassy carbon electrode (GCE) was modified with Pt nanoparticles followed by different mixtures of BA-TEPA and glucose oxidase (1:1, 1:2.5 and 1:3 w/w). For the deposition of Pt nanoparticles an electrochemical cell containing a Ag/AgCl/3 M KCl RE, a Pt net cylinder CE and a GCE (3 mm) WE was used. The GCE was polished for 3 min using an 800 μ m corundum powder (Naxos-Schmiergelwerk Carl Wester, D). Pt nanoparticle deposition was done by pulse deposition (potential pulse sequence: -1.0 V/0.2 s; 0.0 V/1 s, 200 repetitions) using 0.4 mM hexachloroplatinic acid in water. Deposition of poly(benzoxazine)/glucose oxidase films was realized by pulse deposition in a gold 96 well plate (potential pulse sequence: 1.7 V/0.05 s; 0.0 V/0.5 s, 5 repetitions). Glucose determination was performed using an electrochemical robotic system [13] equipped with a three electrode bundle consisting of the GCE WE, a Pt wire CE and a Ag/AgCl/0.1 M KCl RE. Electrolyte solutions containing anomeric glucose at different concentrations in citric acid/Na₂HPO₄ buffer (pH 7.4) were placed in the wells of the microtiter plate. The electrode bundle was placed in the wells using the micropositioning system. The working potential (0.6 V vs. Ag/AgCl/0.1 M KCl) was applied and glucose was determined chronoamperometrically. Between the wells the electrode bundle was automatically rinsed in pure buffer.

3. Results and discussion

A water-soluble benzoxazine derivative was synthesized by reaction of bisphenol A (BA), tetraethylenepentaamine (TEPA) and formaldehyde as shown in Scheme 1a. To investigate the polymer

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