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Controlled grafting of molecularly imprinted films on gold microelectrodes using a self-assembled thiol iniferter

Deiene García-Mutio, Alberto Gómez-Caballero*, Amaia Gotiandia, Iñigo Larrauri, M. Aránzazu Goicolea, Ramón J. Barrio

Department of Analytical Chemistry, Faculty of Pharmacy, University of the Basque Country UPV/EHU, 01006, Vitoria-Gasteiz, Álava, Spain

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ABSTRACT

Herein, a straightforward approach for the controlled coating of gold microelectrodes with molecularly imprinted polymer (MIP) thin films is presented. To this end, a new iniferter has been synthesised, 3-mercaptopropyl diethylcarbamodithioate, which contains terminal thiol groups capable of forming self-assembled monolayers (SAM) on gold substrates. The iniferter SAM was activated under UV radiation after the immersion of the microelectrode in the polymerisation solution, containing 4-ethylphenol (4EP) as template and 4-vinylpyridine and ethylene dimethacrylate as functional monomer and cross-linker respectively. The voltammetric behaviour of the resulting MIP-coated microsensor was assessed next, finding that electrochemical response was linear to the concentration of the target analyte (4EP) in a range comprised between 10^{-6} and 10^{-3} mol L^{-1}, showing a sensitivity of 1.02 ± 0.02 (nA L μmol^{-1}) (R^2: 0.9983). Measurement repeatability was also evaluated performing a series of measurements with the same sensor at two concentrations levels of 4EP, 5×10^{-6} mol L^{-1} and 10^{-4} mol L^{-1}, obtaining relative standard deviations (RSD) of 3.41% and 1.92% respectively. Similarly, sensor-to-sensor repeatability was also determined obtaining RSD values of 10.02% and 16.97% respectively. Sensor selectivity was finally studied comparing the peak current intensity of 4EP with current intensities registered with structural analogues. It was found that sensor was capable of discriminating the target compound from structural analogues presenting remarkable selectivity, even for almost identical compounds such as 4-vinylphenol.

1. Introduction

Electrochemical sensors based on molecularly imprinted polymers (MIP) have gained considerable attention in recent years [1], partly, due to their potential benefits over sensors constructed using biological elements [2] such as enzymes [3], antibodies [4], aptamers [5,6] or peptides [4]. MIPs mimic the recognition event that happens biologically and present substantial advantages such as high chemical and mechanical stability, simplicity and low cost of fabrication [7]. Oppositely, deficiencies such as heterogeneous binding site distribution, responsible for nonspecific binding, and low binding capacity are also attributable to these materials [8]. Recognition elements or target receptors [9] like MIPs provide electrochemical sensors with the capability to discriminate target analytes from structural analogues [10], which may present similar electrochemical behaviour having close oxidation/reduction potentials. This happens thanks to the existence of imprinted sites in the polymer network, which are complementary in shape, size and functionality to a particular target molecule. These three-dimensional cavities are specific recognition sites generated during the synthesis of the polymer, due to the presence of that target molecule, referred to as the template, in the polymerisation medium. As a result, molecular imprints are created in the resulting polymer network, which will later bind the target compound with higher affinity than structural analogues.

The integration of the recognition element on the transduction system is critical for sensor construction. In this light, MIPs have been implemented on electrochemical sensors in different formats [4] including, but not limited to, membranes for potentiometric sensing [11–13], electropolymerised [14] or *in situ* polymerised composite films [15,16] and grafted [17] or self-assembled micro- [18] or nanosized imprinted particles [19]. Among all implementation approaches, electropolymerisation [20] seems to be the most employed one, which is usually carried out under

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* Corresponding author. Tel.: +34 945013857.
E-mail address: a.gomez@ehu.eus (A. Gómez-Caballero).

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potentiodynamic conditions [14,21]. One of the principal benefits of electropolymerisation is the possibility of controlling the thickness of the resulting polymer, selecting proper current density and applied voltage [10]. However, the use of this technique narrows the range of monomers that may be used for MIP synthesis and makes it necessary the use of a conductive substrate. Furthermore, in most cases, electropolymerisation is conducted in aqueous media, what may result in weaker monomer-template prepolymerisation adducts and, therefore, higher heterogeneity in binding site distribution.

The use of MIP films based on acrylic or vinyl monomers as recognition elements has been a more complicated approach for developing electrochemical MIP sensors. Thus, most of MIP sensors developed by free radical polymerisation using acrylic or vinyl monomers have been oriented to optical or gravimetric transduction [10,21]. This may be explained by the fact that acrylic or vinyl layers are electrically insulating, thereby hindering proper charge transfer from imprinted sites to the electrode transducer [14,20]. To avoid this shortcoming, acrylic MIP coatings on the electrode transducer need to be extremely thin, in the nm scale [22], instead, MIP coatings could also be combined with conducting materials such as gold [23] or nickel [24] nanoparticles.

Herein we present a straightforward approach for developing electrochemical MIP sensors based on living polymerisation mediated by an iniferter, which is an initiator that induces radical polymerisation involving initiation, chain transfer and termination steps [25]. Living polymerisation, namely reversible-deactivation radical polymerisation (RDRP), allows for the control over the polymer architecture, including molecular weight, polidispersity or tacticity, thereby reducing binding site distribution heterogeneity and improving template recognition and its diffusion across the polymer [26]. The controllability of polymerisation with these compounds resides on the fact that they can be separated into an active and a dormant species establishing a dynamic equilibrium between them [18]. A new iniferter has been synthesised here containing thiol groups, thereby being able to direct self-assemble to gold substrates leaving exposed the functional groups that will initiate polymerisation. Thiol groups form a coordinate bond with the gold surface with its subsequent deprotonation forming a thyil radical that will produce a gold-thiolate covalent bond [27]. Having the iniferter immobilised on gold, polymer synthesis takes place at the very surface of the substrate by the so-called grafting from technique [28]. Conventional initiators such as free radical initiators [29] or initiators with triplet photosensitizers [30] containing thiol end groups have already been used to form self-assembled monolayers (SAM) on gold surfaces [28]. However, these initiators do not allow for the control of the polymerisation process. In this regard, the principal aim of the present work was to explore the possibility of establishing a new simple approach to develop MIP sensors in a controlled manner, using a single compound having a dual character. Being, on the one hand, capable to self-assemble to gold substrates giving stable SAMs and, on the other hand, capable of initiating living or RDRP polymerisations. The thiol iniferter presented here was used to develop a voltammetric sensor for the electroactive compound 4-ethylphenol, employed as model, but the methodology presented here may be easily extrapolated to other electroactive targets for the straightforward and controlled synthesis of MIP thin layers on gold substrates for electrochemical sensing.

2. Experimental

2.1. Reagents and materials

4-ethylphenol (4EP), 4-ethylguaiacol (4EG), paracetamol hydrochloride and dopamine hydrochloride were acquired from Sigma-Aldrich (Madrid, Spain), coumaric acid ethyl ester (CAEE) was purchased from LGC Standards (Barcelona, Spain) and 4-vinylphenol (4VPh) (10% in ethylene glycol) from Cymit Química (Barcelona, Spain). Ethylene dimethacrylate (EDMA), 4-vinylpyridine (4VPy), 3-chloro-propanethiol (98%) and sodium diethyldithiocarbamate trihydrate were acquired from Sigma-Aldrich. Prior to use, 4VPy was purified by distillation under vacuum obtaining a colourless liquid which was stored below −20 °C, in the dark and under nitrogen atmosphere.

HPLC grade Acetonitrile (ACN) and isopropanol were purchased from Scharlab (Barcelona, Spain) and dry acetone was purchased from Panreac (Barcelona, Spain). Every buffer solution was prepared with ultra-pure water obtained from Elix20 reverse osmosis and Milli-Q water purification systems. All other reagents were analytical grade and they were used as received.

2.2. Synthesis and characterisation of the thiol iniferter

The synthesis of the thiol iniferter 3-mercaptopropyl diethylcarbamodithioate was carried out following a procedure adapted from Bossi et al. [31], who reported the synthesis of a silane iniferter. First, sodium diethyl dithiocarbamate trihydrate (1.5×10^{-2} mol) was ground to obtain a fine powder and it was then lyophilised to remove water of hydration. Next, the salt was dissolved in 50 mL of dry acetone in a 100 mL round bottom flask, fitted with a stirrer and a condenser. Finally, 3-chloro-1-propanethiol (1.8×10^{-2} mol) was added dropwise and the mixture was kept stirring for 15 h at 50 °C. The resulting product was filtered to remove the insoluble salts and the excess of solvent was evaporated by a rotary evaporator, obtaining a yellowish oily product, which was stored at 4 °C under inert atmosphere until use. The synthesised iniferter was characterised by Fourier-Transform Infrared Spectroscopy (FT-IR), nuclear magnetic resonance (^{1}H NMR) and quadrupole-time of flight mass spectrometry (QTOF-MS). FT-IR measurements were performed using a FT-IR spectrometer, model 6300 type A, from Jasco (Madrid, Spain). NMR spectra were recorded on a Bruker Avance 400 instrument (at 400 MHz for ^{1}H, and 100 MHz for ^{13}C). MS-TOF measurements were acquired using an Agilent 6530 hybrid quadrupole-time of flight mass spectrometer with an ESI (electrospray ionisation) Agilent Jet Stream source (Agilent Technologies, Waldbronn, Germany). A 50:50 mixture of water and ACN containing 0.1% formic acid was used as mobile phase. Ionisation gas temperature 325 °C, drying gas flow 5 L min^{-1}, nebuliser pressure 40 psig, shelt gas flow and temperature 11 L min^{-1} and 375 °C, capillary voltage 3500 V.

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.02 (q, $^3J_{HH} = 7.1$ Hz, 2H, NCH_2), 3.74 (q, $^3J_{HH} = 7.2$ Hz, 2H, NCH_2), 3.41 (t, $^3J_{HH} = 7.1$ Hz, 2H, CH_2S), 2.64 (q, $^3J_{HH} = 6.6$ Hz, 2H, $SHCH_2$), 2.02 (m, 2H, $SHCH_2CH_2$), 1.44 (br, 1H, SH), 1.28 (m, 6H, $N(CH_2CH_3)_2$). ^{13}C {^{1}H} NMR (100 MHz, $CDCl_3$): δ 195.3 (C_{quat}, C=S), 49.6 (NCH_2), 46.8 (NCH_2), 35.4 (SCH_2), 33.1 (SCH_2), 23.7 (CH_2CH_2), 12.6 (CH_3), 11.7 (CH_3). Chemical shifts (δ) are reported in ppm relative to residual $CHCl_3$ ($\delta = 7.26$ ppm for ^{1}H and $\delta = 77.2$ ppm for ^{13}C NMR). Coupling constants *(J)* are reported in Hertz. Data for ^{1}H NMR spectra are reported as follows: chemical shift, multiplicity, coupling constant, integration). Multiplicity abbreviations are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad). ^{13}C NMR peak assignments were supported by Distortionless Enhanced Polarization Transfer (DEPT).

High resolution mass spectra for the iniferter (Fig. 1) having a chemical formula $C_8H_{17}NS_3$ were obtained by positive-ion electrospray ionisation. Calculated mass *m/z*: 223.0523, found: 223.0517.