Electrochemistry Communications 36 (2013) 92–95

Contents lists available at ScienceDirect

Electrochemistry Communications

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

Short communication

On/off-switchable electrochemical folic acid sensor based on molecularly imprinted polymer electrode

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ARTICLE INFO

Article history:
Received 31 July 2013
Received in revised form 2 September 2013
Accepted 16 September 2013
Available online 25 September 2013

Keywords:
Molecularly imprinted polymer
Smart polymers
Temperature switchable electrode
Folic acid electrochemical sensor

ABSTRACT

The combination of smart polymers with molecular imprinting offers a powerful tool to design more effective sensors and medical devices. In this study, a temperature sensitive amine-terminated poly(*N*-isopropylacrylamide) block with (*N,N'*-methylenebisacrylamide) cross-linker along with *o*-phenylenediamine was electropolymerised on a gold electrode in the presence of folic acid (FA) as template to produce an on/off-switchable molecularly imprinted polymer (MIP) affinity sensor for folic acid. Differential pulse voltammetry and cyclic voltammetry were used to characterise the FA-imprinted layer. Incubation of the MIP-modified electrode with FA resulted in a suppression of the ferro/ferricyanide redox process. The highest sensitivity of this temperature gated on/off-switchable folic acid sensor was achieved at 22 °C. Such switchable affinity materials offer considerable potential for the design of highly selective and controllable biosensors and immunoassays.

1. Introduction

Over the past decade, much attention has been focused on the development of controlled switchable surfaces, also known as "smart surfaces", which switch their physicochemical properties in response to external stimuli [1,2]. Switching of a surface based on temperature can be realised using thermo-sensitive polymers, which undergo a phase transition at the lower critical solution temperature (LCST), where their behavior switches between hydrophobic and hydrophilic [3]. LCST modulation can be achieved by copolymerisation with other monomers in order to produce a LCST close to physiological temperature. Thus, it could be useful in controllable, temperature-responsive bio-switches for biomedical and biotechnology applications [4–6].

Furthermore, the field of molecularly imprinted polymers (MIPs) has grown exponentially, with numerous papers describing their use as biomimetic receptors in sensors and other devices [7–9]. Combining the properties of a thermo-sensitive polymer with molecular imprinting techniques furnishes a promising strategy for ensuring that the system responds more rapidly to an external temperature change. Folic acid (FA) is a well-known biomarker for cancers [3]. It specifically binds with a folate receptor that is significantly overexpressed on the surface of human cancer cells. Due to this region, detection of FA chosen as a model for this study. MIP technology offers one alternative [10,11], but since FA is sensitive to temperature, UV radiation, and other extreme conditions [12], imprinting of FA using bulk polymerisation is problematic and electropolymerisation is more promising. To the best of our knowledge, no study has been published so far reporting the fabrication of FA-imprinted by electropolymerisation using thermo-sensitive materials. Thus, here we report the preparation of temperature switchable plastic folic acid sensor using co-polymerisation of poly(*N*-isopropylacrylamide) (PNIPAAm) with a cross-linker (*N,N'*-methylenebisacrylamide) (MBA) and additional monomer (*o*-phenylenediamine (*o*-PD)), in the presence of folic acid as template (Fig. 1). The analytical performance of the sensor was evaluated by electrochemical methods.

2. Experimental

2.1. Materials and apparatus

o-Phenylenediamine (*o*-PD, ≥98%), poly(*N*-isopropylacrylamide), amine terminated (PNIPAAm, average M_n = 2500), folic acid (≥97%), *N,N'*-Methylenebisacrylamide (MBA ≥98%) and potassium chloride were purchased from Sigma–Aldrich and used as received. Potassium ferrocyanide and potassium ferricyanide were obtained from Merck. All other reagents were of analytical grade and solutions were prepared using Milli-Q water (18.2 MΩ/cm^2). Electrochemical measurements were performed using an Iviumstat potentiostat (Ivium, The Netherlands) controlled by software supplied by the manufacturer. A standard three-electrode configuration was used. A gold disk (2.0 mm diameter), a platinum wire and an Ag|AgCl|KCl (3 M) electrode were used as working electrodes, counter and reference electrodes respectively.

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http://dx.doi.org/10.1016/j.elecom.2013.09.014

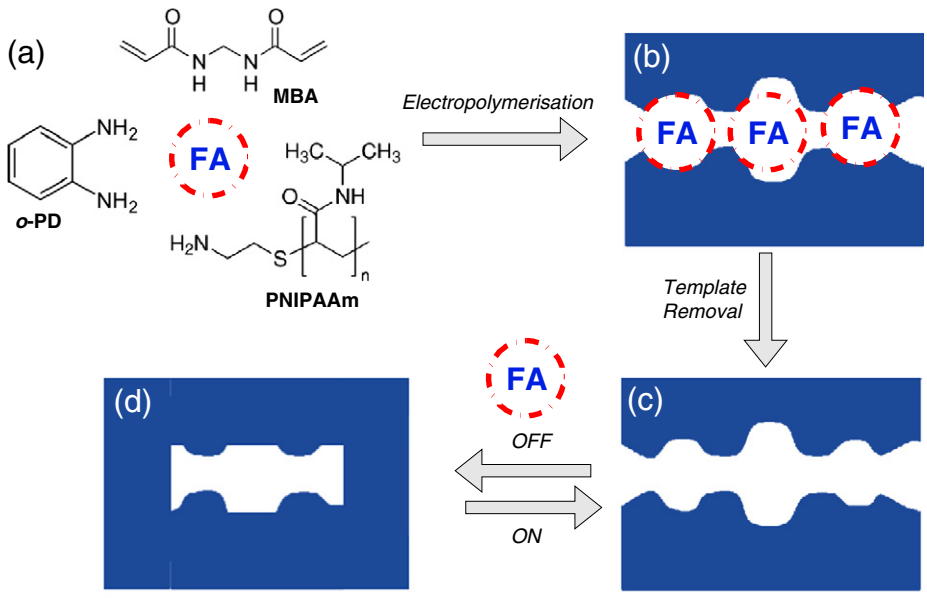

Fig. 1. Schematic representation of molecular imprinting and the on/off-recognition of folic acid (a) The folic acid (FA, template), amine terminated poly(*N*-isopropylacrylamide) (PNIPAAm), *o*-phenylenediamine (*o*-PD) and (*N,N'*-methylenebisacrylamide) (MBA, cross-linker) interact to form a complex during electropolymerisation, (b) removal of the FA template leaves rebinding cavities, (c) template rebinding, (d) On/Off-switchable behavior of MIP electrode.

2.2. Preparation of imprinted electrode

In this study, five molecularly imprinted polymer and corresponding non-imprinted polymer were prepared by electrosynthesis as shown in the Table 1. Prior to electropolymerisation, the surface of the gold electrode was polished with 1.0 and 0.05 μm wet alumina slurry followed by 1 min cleaning in distilled water. Then the electrode was subjected to cyclic potential sweeps between 0.2 and 1.5 V in 0.5 M H_2SO_4 until a stable cyclic voltammogram was obtained. For the preparation of the polymers, the components listed in Table 1 were dissolved in acetate buffer (0.5 M, pH 5.8). After deoxygenating the reaction solution by bubbling nitrogen gas for about 15 min, the electropolymerisation was performed on the surface of the gold electrode, by cyclic voltammetry (20 cycles) in the potential range of 0–1.1 V (versus Ag/AgCl) with a scan rate of 50 mV/s. Then, the polymer film was rinsed in methanol-acetic acid (9:1, v/v) solution for 20 min at 50 °C, followed by subsequent washing with methanol to remove the template entrapped in the polymeric matrix. A control electrode (non-imprinted polymer electrode, NIP electrode) was prepared in every case following the same procedure, but in the absence of template molecule.

2.3. FA sensing and temperature switching study

The interaction between FA and MIP film was evaluated by incubating the FA-MIP electrode in the solution containing appropriate concentrations of FA (phosphate buffer at pH 7.4), for 10 min with stirring. Electrochemical measurements to characterise the MIP film were carried out in the presence of 0.5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution containing 0.1 M KCl. Cyclic voltammograms (CVs) of the imprinted membranes were recorded in the potential range 0.0 to 0.6 V vs. Ag/AgCl, with a scan rate of 50 mV/s. Differential pulse voltammetry (DPV) runs for each concentration of test analyte were quantified over a potential range of 0.0 to 0.4 V at a scan rate of 50 mV/s and pulse amplitude of 25 mV. In order to verify the ability of the MIP to recognise the template molecule, the binding process was examined at different temperatures of 22 and 34 °C.

3. Results and discussion

3.1. Synthesis and characterisation of the MIP

Putative FA-imprinted polymers, consisting of different functional monomers; *o*-PD, PNIPAAm and also MBA cross-linker in the presence of folic acid as template molecule using composition listed in Table 1 were electrosynthesised. *o*-Phenylenediamine and PNIPAAm consist of $-NH_2$ groups which can be generated radical ions. The terminal double bonds of MBA might be cross linked to respected amine terminals during imprinting. Fig. 2a shows a typical cyclic voltammogram recorded during the electropolymerisation of *o*-PD in the presence of PNIPAAm, MBA and folic acid (MIP4) on a gold electrode in acetate buffer (pH 5.8) at room temperature (22 °C). A significant decrease in the anodic peak corresponded to irreversible monomer oxidation on the electrode surface during continuous cycling [13]. This illustrates the formation of non-conductive film on electrode surface, which suppresses the anodic oxidation of monomer and is confirmed by the disappearance of the redox peaks of ferro/ferricyanide probe (Fig. 2b). For characterisation of the polymeric films before and after template removal, differential pulse voltammetry (DPV) and cyclic voltammetry

Table 1
Polymer composition for preparation of different MIP electrodes.

Composition (mM)	MIP1	NIP1	MIP2	NIP2	MIP3	NIP3	MIP4	NIP4	MIP5	NIP5
Folic acid	0.2	–	0.2	–	0.2	–	0.2	–	0.2	–
o-PD	10	10	10	10	10	10	10	10	10	10
PNIPAAm	–	–	2.5	2.5	–	–	2.5	2.5	2.5	2.5
MBA	–	–	–	–	2.5	2.5	2.5	2.5	2.5	2.5
Polymerization temperature (°C)	22	22	22	22	22	22	22	22	34	34