Development of a Biomimetic Sensor for Tamoxifen with MIP-Based Specificity

1. Introduction

In order to mimic the active sites of proteins, synthetic polymers are imprinted by the analyte during the polymerisation as conceptualised by Wulff, Shea and Mosbach.

Molecular imprinting is a methodology used to create recognition sites in synthetic polymers by copolymerising a functional and a cross-linking monomer in the presence of the target analyte. This imprint molecule acts as a molecular template by the interaction with the complementary groups of the functional monomer and arranges the cross-linker to resemble the molecular shape. In the pre-polymerisation mixture, the dissolved target interacts by covalent (pre-organised approach) or non-covalent (self-assembly approach) binding with the functional monomer responsible for localising the chemically active moieties of the target molecules during copolymerisation. After polymerisation the template molecules are removed, providing binding sites ideally complementary in size, shape and functionality to the template so that the template preferentially rebinds to the cavity (Yarman A., 2012; Yarman et al., in press).

In this report molecularly imprinted polymers were used as recognition element for developing biomimetic sensor for tamoxifen (TAM) which is a nonsteroidal anti-estrogen used in the therapy of human breast cancer (Fig.1). Among the different formats for the preparation of molecularly imprinted polymers (MIPs) bulk polymerisation is most frequently used. The disadvantage of this method is that it is time consuming, and that slow binding kinetics are obtained. To overcome these drawbacks, other methods for the preparation of MIPs have been introduced (Yarman A., 2012). This study is based on the electropolymerisation of o-phenylenediamine and resorcinol mixture in the presence of template molecule tamoxifen. In order to increase the imprinted sites graphene was integrated.

Fig.1 Structure of tamoxifen.
2. Materials and Methods

2.1. Chemicals

o-Phenylenediamine dihydrochloride (o-PD) and Resorcinol (Res) were purchased from Sigma (Steinheim, Germany), tamoxifen from Molekula (Germany). Sulfonated graphene was supported from the group of Prof. Turner (Linköping University). All reagents were of analytical grade and used without further purification.

2.2 Preparation of electrodes

Glassy carbon disk electrodes (3 mm in diameter) were used for the voltammetric and amperometric measurements. Prior to electropolymerisation, electrodes were cleaned with ethanol and treated with 60% nitric acid for 15 minutes. After this, mechanical cleaning was performed with 1.0, 0.3 and 0.05 μm Al₂O₃, respectively and electrodes were rinsed with Millipore water by sonication.

TAM-imprinted GCEs were prepared either in the presence or in the absence of graphene. Electropolymerisation was performed in 5 mM o-PD:5mM resorcinol mixture (in 100 mM acetate buffer, pH 5.2) containing 0.1-0.4 mM TAM (Stock solution was 2 mM in methanol) by cyclic voltammetry sweeping between 0 V and 0.8 V (20 scans) at a scan rate of 50 mV/s. Non-imprinted electrodes were prepared in a similar way in the absence of template. Template molecules were removed by the treatment with the mixture of methanol:water:1 M NaOH (2:1:1, volume ratio) at 70°C for 1 h shaking at thriller with a speed of 300 rpm.

2.3. Apparatus and electrochemical measurements

Electrochemical measurements were performed in a stirred electrochemical cell with a three-electrode configuration. A glassy carbon disk electrode (3 mm in diameter) or gold electrode was used as the working electrode, an Ag/AgCl (in 1 M KCl solution) electrode was the reference electrode, and a platinum wire served as the counter electrode. Cyclic voltammetry (CV) was performed using PalmSENS potentiostate (Netherlands). Amperometric measurements were performed under aerobic conditions in 85 mM phosphate acetate buffer containing 15% methanol (v/v) at pH 5.2. A working potential of 1.1 V was applied. After baseline stabilisation had occurred, the current was recorded after TAM addition into the reaction chamber as a function of time. All the experiments were carried out at room temperature.
3. Results and Discussion

Fig. 2 shows CVs of o-PD:Res electropolymerisation on glassy carbon electrode in the presence of graphene and 0.4 mM TAM. Similar CVs were obtained in the absence of TAM, graphene or both (data not shown). In the first scan irreversible peak between 400-450 mV was obtained. Current decreased with the following sweeps and approached to zero showing the formation of a non-conducting film on the electrode surface.

Fig. 2 CVs of EP on glassy carbon electrode: MIP: 0.4 mM TAM in 5 mM o-PD:Res+ 10 \(\mu\)L Graphene (Stock: 0.1 mg/mL) in 100 mM acetate buffer, pH 5.2, 20 scans, 0-0.8 V 50 mV/s.

In order to characterise the permeability after EP, template removal and rebinding, ferricyanide was used as a redox probe. Figure 3 shows the cyclic voltammograms of these steps. Bare GCE gave the highest response (Not shown). On the other hand, MIP modified electrode gave a markedly increased ferricyanide signal after template removal. This signal is again suppressed after rebinding as expected for filling the cavities by target binding. However, ferricyanide signal could not be suppressed by rebinding to the MIP which was prepared in the presence of graphene.
Fig. 3 Overlay of CVs of MIP electrode a) after electropolymerisation, b) after TAM removal, and c) after TAM rebinding in 10 mM ferricyanide (in 100 mM KCl) at a scan rate of 50 mV/s.

The amperometric responses on stepwise addition of TAM of MIP electrodes containing different amounts of graphene and bare GCE (in the absence of graphene) are shown in the Fig.4. Since TAM is electroactive, the anodic currents indicate that TAM can permeate through pores of the electopolymer. However, in all cases similar responses and linearity were observed.

Fig. 4 Amperometric response on stepwise addition of 1x0.1 µM, 1x0.2 µM, 5x1 µM, 3x10 µM TAM on different electrodes in 85 mM Acetate buffer containing 15% Methanol at pH 5.2 at 1.1 V.
Furthermore, cross-reactivity studies were performed in the absence of graphene by using different concentrations of template TAM (0.1-0.4) during electropolymerisation. It was observed that efficiency for re-binding of the template TAM was almost 2.3 higher as compared to its metabolite 4-Hydroxytamoxifen for TAM-imprinted electrode (0.1 mM in electropolymerisation solution). This showed that TAM imprinted electrode to some extend preferentially recognises the template molecule itself.

4. Outlook

The results obtained so far showed that graphene could not increase the amount of imprinted sites which can allow the extension of linear measuring range. Further studies will be done with different amounts of graphene. However, TAM-imprinted electrode in the absence of graphene showed some preferential recognition of the template molecule itself as compared to its metabolite 4-Hydroxytamoxifen.

Furthermore, the results should be included in a further joint publication with the host institution. A further stay via COST is planned.

References