

Proc. Eurosensors XXVI, September 9-12, 2012, Kraków, Poland

Surface Imprinting approach on screen printed electrodes coated with carboxylated PVC for Myoglobin detection with Electrochemical Transduction

Felismina T.C. Moreira^{1,3,4}, Rosa A. F. Dutra², João P. C. Noronha⁴, M. Goreti F. Sales^{1*}

¹BIOMARK/ISEP, Instituto Superior de Engenharia do Porto, Porto, Portugal.

²PROCAPE, LAPED, Universidade Federal de Pernambuco, Recife, Brazil

³UOSE, INESC-Porto, Faculdade Ciências/Universidade Porto, Porto, Portugal.

⁴REQUIMTE/FCT-UNL, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Portugal

Abstract

A novel surface molecularly-imprinted (MI) material to detect myoglobin (Myo) using gold screen printed electrodes (SPE) was developed. The sensitive detection was carry out by introducing a carboxylic polyvinyl chloride (PVC-COOH) layer on gold SPE surface. Myo was attached to the surface of gold SPE/PVC-COOH and the vacant spaces around it were filled by polymerizing acrylamide and *N,N*-methylenebisacrylamide (cross-linker). This polymerization was initiated by ammonium persulphate. After removing the template, the obtained material was able to rebind Myo and discriminate it among other interfering species. Various characterization techniques including electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) confirmed the surface modification. This sensor seemed a promising tool for screening Myo in point-of-care.

Keywords: Biosensor; Surface molecular imprint; Screen-printed electrodes; Sensor; Myoglobin; Biosensor

1. Introduction

A biosensor contains a biorecognition element that is capable of transforming its interaction with a specific analyte into an electrical or optical-based signal. Recent advances in nanotechnology emerged in a wide range of novel biorecognition elements assembled in small platforms, such as molecular imprinting polymers (MIP) [1-5].

MIPs are polymeric structures designed to resemble natural antibodies. They combine the high specificity and affinity of the corresponding natural materials with the robustness, low cost, long term stability, and reusability of the synthetic ones. For a successful MIP design, several variable parameters need to be optimized, such as the selection of the monomers, solvents, temperature, reaction time, etc. The selection of suitable monomers is a critical step for the good performance of the imprint approach in

terms of selectivity and specificity. The specificity depends of the exposed surface area of the sensor, its functionality and charge. This concept could be successfully applied to a biomarker of clinical significance.

Myo is the cardiac biomarker of protein nature that changes more rapidly after cardiac injury. Different strategies for Myo detection and quantification based on surface imprinting were described in the literature, including sol-gel methods and self assembled monolayer's with potentiometric transduction [5,6] surface coating [7] thin-film and micro-contact imprinting mass with spectrometry-based profiling system [8]. New alternative methods of quick and low-cost response are still desired.

This research paper describes a new sensitive MI material assembled on carboxylic polyvinyl chloride matrix casted on the gold surface of a SPE for Myo detection in ischemic episodes. All modification steps on polymer surface were investigated by cyclic voltammetry (CV) and impedance spectroscopy (IS).

2. Experimental Section

The electrochemical measurements were carried out using a Metrohm-Autolab potentiostat/galvanostat Autolab PGSTAT302N interfaced to a computer. SPEs were purchased from DROPSSENS (DRP-C220AT). Working and counter electrode were made of gold whereas reference electrode and electric contacts were made of silver. Working electrode size was 4 mm respectively.

CV measurements were conducted in HEPES buffer pH 5, with 5.0 mmol/L $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ with KCl 0.1 mol /L. The potential was scanned between -0.2 and $+0.4$ V, at 30 mV s^{-1} . Impedimetric analysis was conducted in the same solution, using a standard potential of 0.225V, with a number of frequencies equal to 50 and of amplitude 0.01 V. The frequency range was 0.1–100 KHz. Data were fitted to a Randles equivalent circuit using the implemented ANOVA software.

3. Results and Discussion

Novel MIP materials tailored on screen printed electrodes (SPE) where the gold surface was coated with a PVC-COOH membrane are designed. These materials display the ability of interacting mostly with Myo. Their set-up may be seen in Figure 1.

3.1. Assembly of the MIP material

First, the carboxylic acid groups on the PVC-COOH layer were first activated by 1-(ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) and *N*-hidroxysuccinimide (NHS). The reaction of -COOH with EDAC formed a highly reactive O-acylisourea unstable intermediate that reacted later with NHS to produce a more stable active ester. This ester underwent nucleophilic substitution reaction with any amine group on the protein, resulting in the formation of an amide bond between PVC-COOH and Myo. This layer was washed several times with PBS pH 7 in order to remove unbound protein from the surface. Subsequent nonspecific reactions between dangling activated groups and Myo were avoided by deactivating these after their reaction with Tris.

The vacant space around the protein was filled with a thin-layer of an amide-based polymer. This was a polar non-charged material, enabling multiple weak interactions (dipole-dipole and hydrogen bonds), which would allow any subsequent protein rebinding to this polymer structure. The polymerization was carry out using acrylamide (AAM, monomer) and *N,N*-methylenebisacrylamide (NNMBA, cross-linker). These specific materials were selected for their biocompatibility with protein structures and for their successful polymerization under mild aqueous conditions [9]. Five hours were given to monomer and cross-linker orientation and then the polymerization was initiated by ammonium persulphate (APS).

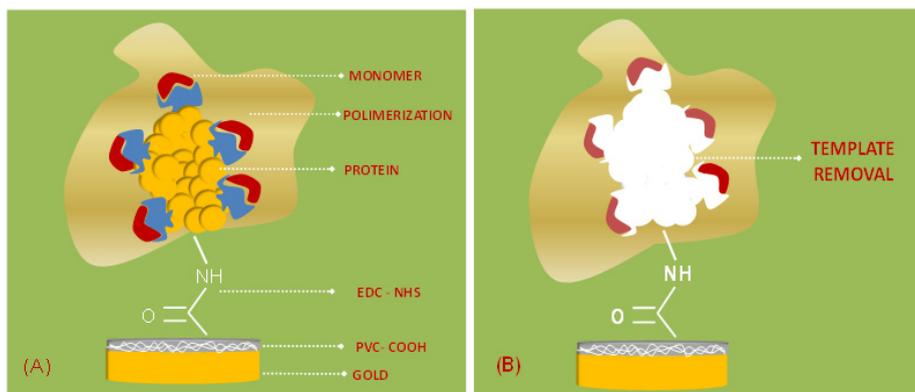


Fig. 1 – Several stages of the synthesis of the MIP material (A) and the vacant sites (B) created after template removal.

The binding sites were created after protein removal. This was done by incubating the modified layer in oxalic acid for 12h. Several washing steps were performed subsequently to remove the released peptide fractions from the surface of the modified polymer. Finally, the pH was increased from 1.2 to 8.0 with phosphate buffer, to enhance the efficiency of peptide/amino acid removal. The sensor was washed several times with ultrapure water.

3.2. Characterization of the immobilization steps

All immobilization steps were followed by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). These techniques allowed investigating the integrity and the electrical properties of immobilized organic films on the polymer surface and interfacing surface. For this purpose, the changes in the resistance of the modified surface were measured by monitoring the electrical variations in the electron transfer of a redox system $[\text{Fe}(\text{CN})_6]^{4-}/[\text{Fe}(\text{CN})_6]^{3-}$, both by EIS and CV.

The EIS data were fitted to a Randles equivalent circuit, composed by the resistance of the solution phase (R_s), the capacitance of the double layer (C_{dl}), the charge transfer resistance (R_{ct}) and the Warburg diffusion element (W) from the bulk solution to the electrode interface. The obtained data are the Nyquist plots shown in Figure 2A. In general, the semicircle region in these plots implies a charge-transfer controlled process. The diameter of this semicircle equals the R_{ct} , and this resistance controls the electron transfer kinetics of the redox-probe at the electrode interface [10]. The gold SPE coated with a PVC-COOH membrane displayed a high electron transfer resistance. The linkage of Myo followed by the polymerization reaction produced an additional barrier for the redox probe access to the Au-SPE modified electrode. This resulted in an extra increase in the electron transfer resistance, reflected by further substantial increase in R_{ct} .

CV assays are shown in Figure 2B and supported the previous studies of EIS. The subsequent immobilization steps on the PVC-CCOOH layer reduced the overall area of the voltammograms accounting for the increased charge-transfer resistance on the working area. The cathodic and anodic peaks were not presented in the voltammograms attributing by this way an irreversible character to the reaction of the redox probe.

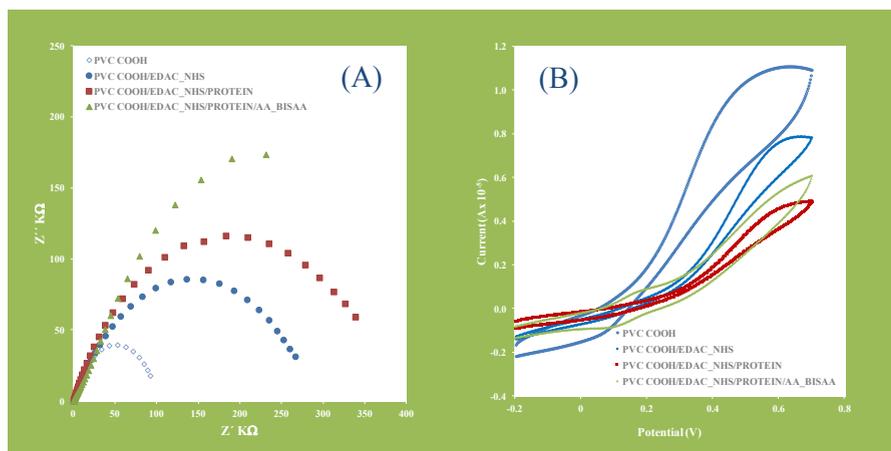


Fig. 2 - Electrical response of the several stages of sensor modification, obtained in 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-}$ /5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$ prepared in HEPES buffer pH 5, and traduced by Nyquist plots (A) or Cyclic voltammograms (B).

Acknowledgements

One of the authors (FTCM) gratefully acknowledges Fundação para a Ciência e Tecnologia for the financial support (PhD grant reference SFRH/BD/66735/2009).

References

- [1] Vestergaard M, Kerman K, Tamiya E (2007). An Overview of Label-free Electrochemical Protein Sensors. *Sensors* 7: 3442-3458.
- [2] Lopez MCB, Caston MJL, Miranda-Ordieres AJ, Blanco T (2004) Electrochemical Sensors Based on Molecularly Imprinted Polymers. *Trends Anal Chemistry* 23: 36-48.
- [3] Piletsky SA, Turner, APF (2002) Electrochemical Sensors Based on Molecularly. Imprinted Polymers. *Electroanalysis*, 14: 317-323.
- [4] Mayes AG, Whitcombe MJ (2005) Synthetic strategies for the generation of molecularly imprinted organic polymers. *Adv Drug Del Rev* 57: 1742-1778.
- [5] Moreira FT, Dutra RA, Noronha JP, Sales MG (2011) Myoglobin-biomimetic electroactive materials made by surface molecular imprinting on silica beads and their use as ionophores in polymeric membranes for potentiometric transduction, *Biosens Bioelectron* 26:4760-47666.
- [6] Wang Y, Zhou Y, Sokolov J, Rigas B, Levon K, Rafailovich M (2008). A potentiometric protein sensor built with surface molecular imprinting method, *Biosensors and Bioel* 24: 162–166.
- [7] Turan E, Caykara T (2012) Construction of myoglobin imprinted polymer films by grafting from silicon surface, *Ertan Yildirim, J Mater Chem*, 22: 636-642.
- [8] Liao PC, Tyan YC, Wang CY, Hsu JF, Chou TC, Lin HY (2009) Assessing the binding selectivity of molecularly imprinted polymer artificial antibodies by mass spectrometry-based profiling system, *J Biomed Mater Res A*, 91: 597-604.
- [9] Tong D, Heényi C, Bikádi Z, Gao J, Hjertén S (2001) Some studies of the chromatographic properties of gels ('Artificial antibodies/receptors') for selective adsorption of proteins, *Chromatographia*, 54:7-14.
- [10] Panagopoulou MA, Stergiou DV, Roussis IG, Pedromidis MI (2010) Impedimetric Biosensor for the Assessment of the Clotting Activity of Renet. *Anal Chem* 82: 8629-8636. An example appendix