ORIGINAL ARTICLE

GRAPHENE OXIDE MODIFIED APTASENSOR FOR THE MUCIN 1 DETECTION

BIANCA CIUI[1], MIHAELA TERTIŞ[1,2], DIANA PEIA[1], CECILIA CRISTEA[1], ROBERT SĂNDULESCU[1]*

[1]"Iuliu Haţieganu" University of Medicine and Pharmacy, Faculty of Pharmacy, Analytical Chemistry Department, 4 Pasteur Street, Cluj-Napoca, Romania
[2]"Ion Chiricuţă" Oncology Institute, 34-36 Republicii Street, Cluj-Napoca, Romania

*corresponding author: rsandulescu@umfcluj.ro

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Abstract

An electrochemical aptasensor based on screen printed electrodes modified with graphene oxide was developed for the indirect detection of Mucin 1 which is a tumour biomarker. The signal was obtained after the methylene blue electrochemical reduction. The nanostructured platform was obtained *via* layer-by-layer deposition of graphene oxide onto the electrode. After the complete optimization and characterization, the platform was incubated with the specific aptamer (5′-GCAGTTGATCTTTGGATACCCTGGTTTTTTTTTTTTTTT-3′) with NH_2 terminal group, and then the methylene blue was used as redox probe in order to obtain the electrochemical signal. After the incubation with Mucin1, the aptasensor was successfully tested using electrochemical impedance spectroscopy and differential pulse voltammetry.

Rezumat

A fost elaborat un aptasenzor electrochimic pe bază de electrozi planari imprimaţi modificaţi cu oxid de grafenă pentru detecţia indirectă a biomarkerului tumoral Mucină 1. Semnalul electrochimic înregistrează reducerea electrochimică a moleculei de albastru de metilen. Platforma nanostructurată a fost obţinută în urma depunerii strat-cu-strat a oxidului de grafenă pe suprafaţa electrodului. După întregul proces de optimizare şi caracterizare, platforma a fost incubată cu aptamerul specific (5′-GCAGTTGATCTTTGGATACCCTGGTTTTTTTTTTTTTTT-3′) cu grupare NH_2 terminală, apoi cu albastru de metilen cu rol de sondă redox pentru obţinerea semnalului electrochimic. Aptasenzorul astfel obţinut a fost testat cu succes după incubarea cu proteina Mucină 1 cu ajutorul spectroscopiei de impedanţă electrochimică şi voltametriei puls diferenţiale.

Keywords: Mucin 1, aptasensor, methylene blue, graphene oxide, electrochemical methods, screen-printed electrode

Introduction

As a result of the increasing number of breast cancer related deaths in women population [1, 14], it is imperious to develop fast and accurate devices in order to detect and monitor this type of cancer in early stages of its evolution [11, 26]. Mucins are glycoproteins that play an important role in mucosal protection and communication with the external environment [5, 24]. Due to the fact that Mucin 1 (MUC 1, CA-15-3) was found to be over-expressed in breast cancer it becomes one of the most common tumour markers for the diagnosis and therapy monitoring purposes [4, 12].

Aptamers are artificially synthesized and selected nucleic acid (DNA or RNA) ligands obtained with systematic evolution of ligands by exponential enrichment method (SELEX) [5, 16]. Aptamers based systems present improved chemical and thermal stability, high affinity, are more specific, selective and flexible than the antibody based systems [20, 23]. Considering this, there have been reported numerous electrochemical aptasensors based on nanomaterials such as quantum dots [3], gold nanoparticles [2, 9], carbon nanotubes [1, 15] and graphene [13, 25].

Screen printed electrodes (SPEs) are disposable devices which allow a single use analysis. The employment of these electrodes is mandatory for the present study, due to the irreversibility of the performed modification of the surface.

Graphene oxide (GO) is an oxidized form of graphene, but unlike graphene, it has an increased water dispersibility [7, 18, 19]. Another advantage of the GO platform is the capacity to exhibit a high affinity in anchoring the bio-component molecules (including aptamers) *via* amidic bonds. These strong bonds formed between the GO sheets activated carboxylic groups and the terminal amino groups from the aptamer chain were exploited.

Methylene blue (MB) is a redox indicator that has been widely used as an electron transfer mediator [5, 6, 22]. MB is a simple and cost-effective molecule that proves not to affect the external structure of the biomolecules [5]. It presents direct and specific

interactions with the G bases from MUC1 aptamer [21, 22], this being the reason to use MB as redox probe.

Materials and Methods

Mucin 1 specific aptamer with NH_2 terminal group (5′-GCAGTTGATCTTTGGATACCCTGGTTTT-TTTTTTTTTTT-3′-NH_2) was purchased from AlphaDNA (Canada) and Mucin1 protein was purchased from Novus Biological (USA). Graphene oxide, methylene blue and sodium chloride were purchased from Sigma Aldrich (Taufkirchen Germany). Sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), potassium ferricyanide ($K_3[Fe(CN)_6]$), ferrocyanide ($K_4[Fe(CN)_6]$) and hydroxylmethylaminomethane (TRIS) were purchased from Merck (Whitehouse Station, NJ, USA). 1-Ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride (EDAC) was purchased from Calbiochem (Canada) and N-hydroxysuccinimide (NHS) from Alfa Aesar (Kalsruhe, Germany).

The solutions were prepared in ultrapure water and the supporting electrolytes were: 0.02 M phosphate buffer saline (PBS) solution with NaCl (pH 7.4) and 10 mM TRIS buffer (pH 7.2) saline solution (with 100 mM NaCl, 100 mM KCl and 5 mM $MgCl_2$).

Graphite based SPEs (DRP110) were purchased from Dropsens (Spain). An Autolab PGSTAT 100 potentiostat with electrochemical impedance spectroscopy (EIS) module and Nova 1.10.4 software (Metrohm, Eco Chemie Netherlands) was used in order to perform electrochemical experiments.

EIS experiments were performed at open circuit potential for a frequency range from 10 mHz to 100 KHz, by using ($[Fe(CN)_6]^{3-/4-}$) as redox probe.

Differential pulse voltammetry (DPV) measurements were performed in PBS solution after the incubation with 5 mM MB solution prepared in TRIS buffer (scan rate of 10 mV/s; from 0.2 to -0.8 V; step potential -0.005 V; modulation amplitude -0.025 V; modulation time 0.05 s).

The graphene oxide film deposited on the electrode was characterized by Fourier transformed infrared spectroscopy (FTIR), Raman Spectroscopy and optical microscopy [17].

Graphene oxide activated with 400 µM EDAC and 100 µM NHS and electrode modification were performed according to Tertiş *et al.* [17]. The graphene based nanostructured platform was used for the development of MUC1 aptasensor by its deposition on the graphite based SPEs.

Elaboration of MUC1 aptasensor

The modified electrode obtained after the deposition of 10 successive drops of 7.5 µL containing 0.5 mg mL^{-1} activated graphene oxide suspension (each one dried at 45ºC) was incubated with 7.5 µL aptamer solution (prepared in TRIS buffer) for 30 minutes, in order to form amidic bonds between graphene activated carboxyl groups and aptamer NH_2 groups. The aptamer solution was first kept at 80ºC for 5 minutes in a bath water [12] in order to reach an unfold configuration (signal-off mode) [8]. Further on, MB was used as marker for indirect electrochemical detection of MUC1 by the mean of DPV signal recorded in the reduction domain of potential. For this purpose, 7.5 µL of 5 mM MB was deposited onto the electrode and incubated for 30 minutes, followed by the incubation with different concentrations of MUC1 (prepared in PBS buffer) for 30 minutes. The incubation with aptamer, MB and MUC1 solutions were performed at 4ºC and humid environment in order to avoid the solvents evaporation and the change of solutions concentration. The aptasensor elaboration is presented in Figure 1.

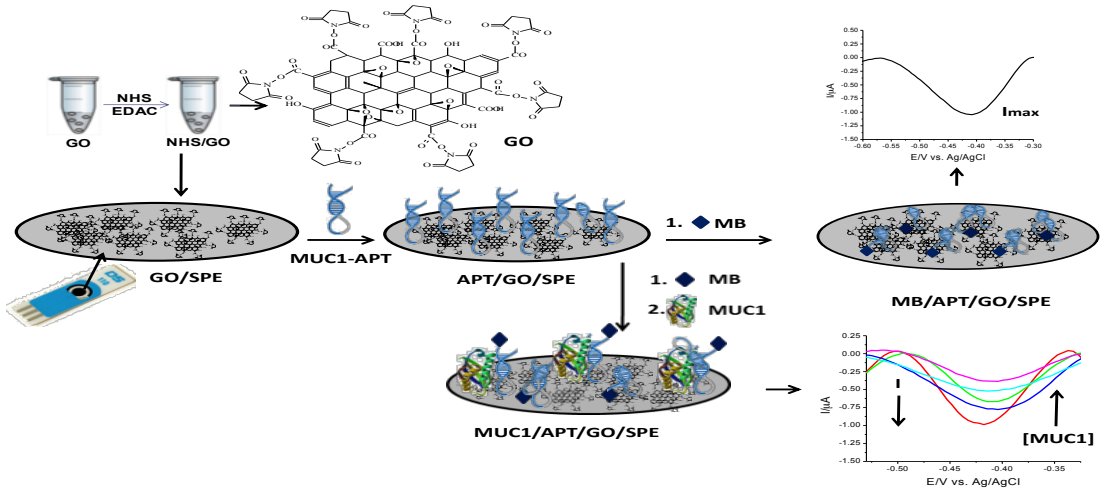

Figure 1.
Aptasensor elaboration and detection mode (APT = MUC1 specific aptamer)

Results and Discussion

The above mentioned electrochemical methods (see Materials and Methods chapter) were used in order evaluate the modifications during the aptasensor development, to optimize the experimental conditions and to test the performance of the MUC1 aptasensor.

The aptasensor optimization was achieved by the assessment of the MB reduction peak variation recorded by using DPV experiments. A solution of ($[Fe(CN)_6]^{3-/4-}$) was used as a redox probe in the EIS experiments performed during the optimization steps, without the need of any label molecule.

The MB reduction peak intensity is 1.5 times higher at GO modified SPE (GO/SPE, Figure 2, curve (c)) comparing the DPV signal obtained after the incubation with 5 mM MB for 30 minutes, on bare graphite SPE (Figure 2, curve (a)). This is due to the increase of the electrode active surface determined by the presence of the GO which allows more MB molecules to be reduced on the modified electrode in the same experimental conditions. After the incubation of the GO modified SPE with 5 μM aptamer solution (aptamer/GO/SPE, Figure 2, curve (b)), the MB electrochemical signal is decreasing, which demonstrates the aptamer bonding on the GO. A further decrease of 30 % of MB signal was observed after the incubation with 15 ng μL^{-1} MUC1 protein (MUC1 protein/ aptamer/GO/SPE, Figure 2 curve (d)).

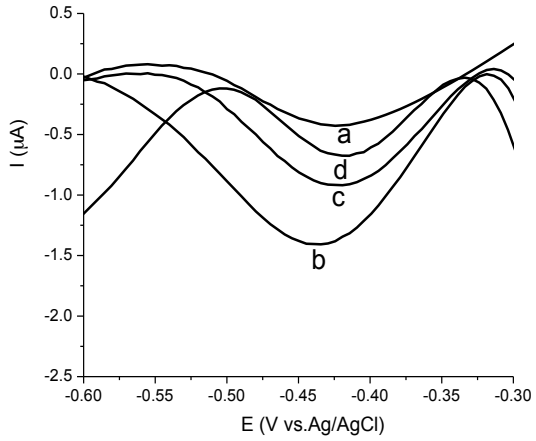

Figure 2.

DPVs after the incubation with 5 mM MB solution for 30 min registered for: graphite based SPE (a); GO/SPE (b); aptamer/GO/SPE (c) and MUC1 protein/ aptamer/GO/SPE (d) (in 0.02 M PBS, pH 7.4)

As was demonstrated before, the MB molecules are bonded with G bases form aptamers [10, 21], thus it was assumed that seven MB molecules were bonded on each of the seven G bases of the aptamer molecule (5´-GCAGTTGATCTTTGGATACCCTGGTTTT-TTTTTTTTTTT-3´). When considering the aptamer unfolding all the MB molecules are situated far from the electrode surface, which hinders their reduction and explains the decreasing of the electrochemical signal after the incubation with the aptamer.

The electrochemical impedance spectroscopy experiments were performed in order to prove the modification of the electrode surface. A 10 mM $[Fe(CN)_6]^{3-/4-}$ solution prepared in 0.02 M PBS (pH 7.4) was used as redox probe for EIS measurements. The modifications of electrode surface were followed by Nyquist plot of EIS which represents the correlation between the theoretic and real coefficients of impedance expressed as complex number.

The interpretation of the Nyquist representation was done based on the variation of charge transfer resistance (R_{ct}). The R_{ct} value was evaluated during all the electrode modification steps, as depicted in Figure 3. In the case of graphite based SPE the R_{ct} value was 300.14 Ω, while after the deposition of graphene oxide film it increased reaching the value of 323.86 Ω, due to the fact that graphene oxide has insulating properties and their presence at the surface of the electrode hinder the electron transfer. A further increase of R_{ct} value to 372.74 Ω was observed after the incubation with aptamer solution. The aptamer being a large molecule, it makes the access of the redox species to the electrode more difficult, slowing down the electrochemical process. After the incubation with MUC 1, it was observed a decrease of R_{ct} value to 309.72 Ω, probably due to the partial removal of the graphene oxide sheets during the succession of numerous incubation and washing steps.

DPV tests were performed after the labelling step with MB. The maximum current intensity was obtained when no MUC1 protein was added on the surface of the aptasensor. This is probably due to the fact that the majority of the aptamer molecules have regained the stable (initial) configuration [8]. The MB molecules bonded with the aptamers are in the proximity of the electrode surface, being thus easily electrochemical by reduced (Figure 4 A, curve 1). Also, Figure 4 A presents the MB reduction peak intensity decreasing with the increase of MUC1 concentration. Figure 4 B presents the linear correlation between the electrochemical signal of MB and the MUC1 concentration in the range from 2 to 20 ng μL^{-1}. The limit of detection (LOD, estimated on the signal to noise ratio S/N=3) of 0.6 ng μL^{-1} and 0.62 $\mu A\ \mu L\ ng^{-1}\ cm^{-2}$ sensitivity were obtained, with a 0.96 correlation coefficient and 3.29 % RSD.

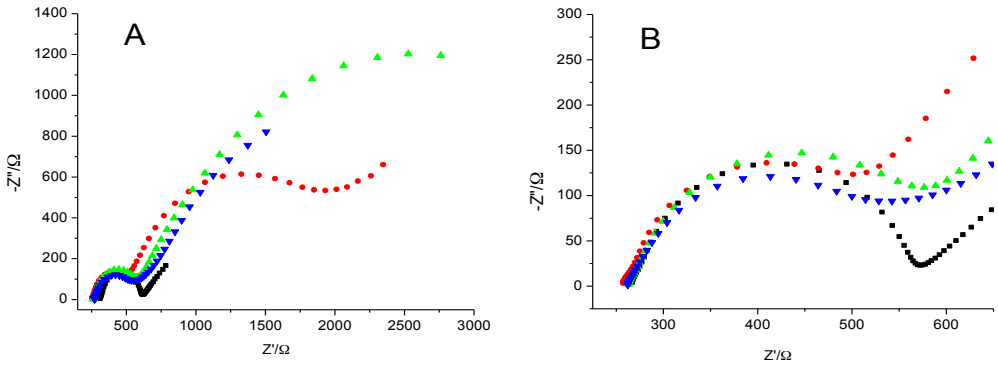

Figure 3.

(A) The Nyquist spectra of 10 mM $[Fe(CN)6]^{3-/4-}$ in 0.02 M PBS (pH 7.4) recorded for: graphite based SPE (black); GO/SPE(red); aptamer/GO/SPE (green) and MUC1 protein/aptamer/GO/SPE (blue). (B) Details in high frequencies domain

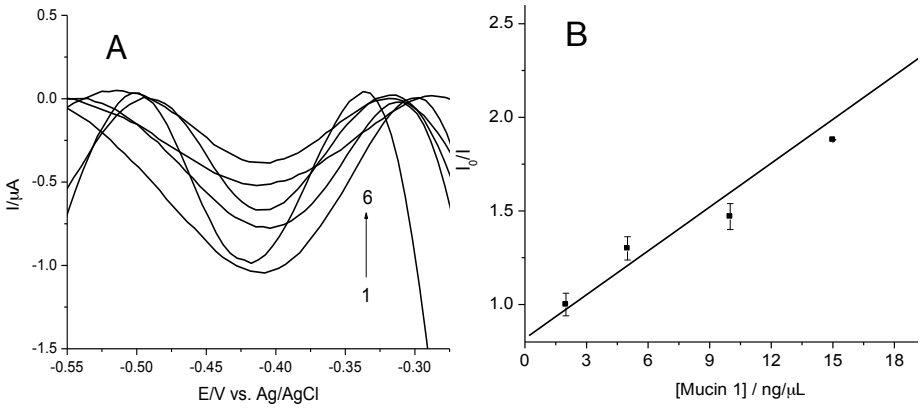

Figure 4.

(A) DPVs after the incubation with 5 mM MB solution for 30 min for: 0 ng μL^{-1} (1); 2 ng μL^{-1}(2); 5 ng μL^{-1}(3); 10 ng μL^{-1}(4); 15 ng μL^{-1}(5) and 20 ng μL^{-1}(6) MUC1 protein registered on aptamer/GO/SPE (in 0.02 M PBS, pH 7.4); (B) The corresponding calibration curve

Conclusions

A novel label-free aptasensor for the cancer biomarker Mucin 1 was developed. Different strategies were used in order to immobilize the functionalized graphene oxide at the surface of graphite based screen-printed electrodes. The optimized platform was successfully used for the elaboration of Mucin 1 aptasensor with a LOD of 0.6 ng $\mu L^{-1}$and a sensitivity of 0.62 μA μL ng^{-1} cm^{-2}.

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