Short Communication

Glassy carbon electrodes modified with CNT dispersed in chitosan: Analytical applications for sensing DNA–methylene blue interaction

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ABSTRACT

In this work we report the optimization of a chitosan (CHI) and carbon nanotubes (CNT) modified electrode, CNT-CHI, to study the dsDNA interaction with methylene blue (MB), a well-known DNA intercalator. The analytical signal was the reduction current of the MB associated with the dsDNA confined at CNT-CHI modified glassy carbon electrode. The selected CNT-CHI film stabilizing treatment was immersion for 2 s in a 0.20% GTA solution. The effect of ionic strength was also analyzed, concluding that high concentration of NaCl (90 mM) benefits the intercalative process. Below these conditions, a linear response between intensity current and MB concentration was obtained. In conclusion, modifying the initial conditions of the assembling GCE/CHI-CNT/dsDNA electrode the intercalative process was favored opening a fast and cheap possibility to evaluate the toxicity of new synthesized molecules.

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1. Introduction

DNA plays an important role in life processes because it contains heritage and synthesis of proteins and enzymes information. Studies about binding mechanism of small molecules with DNA have been identified as one important topic to understand the mechanism of action or toxicity of different pollutants and drugs [1,2].

There are several modes of interaction of different molecules with DNA, including intercalation, non-covalent groove binding, covalent binding/cross-linking, DNA cleaving, and nucleoside-analog incorporation [2–5]. As a result of the DNA–drug complex formation, there are changes in the thermodynamic stability and functional properties of DNA [6]. Chemical or physical agents can produce strand breaks, hydrolysis (enzymatic or acidic) of the phosphodiester bonds, release of bases after hydrolysis of the N-glycosidic bonds, changes in the physical–chemical properties or the structural conformation of nucleobases, formation of adducts between complex compounds and bulky side moieties [4,7,8].

Electrochemical methods have demonstrated to be highly successful, as alternative to other methods as the spectroscopic ones, in providing information about the mechanism of the interaction between nucleic acid-binding compounds and DNA and the conformation of DNA adducts [2,9–12]. Recent developments of DNA biosensors directed to clinical diagnostics, and forensic and biomedical applications have attracted substantial research efforts. Electrochemical DNA biosensors represent a new alternative to study DNA interactions [13] and DNA damage [7,8,14–18]. The immobilization of DNA at an electrode surface is a crucial aspect to develop DNA biosensors for monitoring drug interactions since it will be responsible for the accessibility of a given molecule in solution to the confined DNA and, hence, it will influence the affinity of drug binding. The key criterion for a successful DNA immobilization is to keep the DNA bases accessible to the binding of target molecules present in solution.

It is widely known that the organic 3,7-bis(dimethylamino)phenothiazine-5-ium-chloride dye (methylene blue, MB) intercalates with DNA [19]. Two possible mechanism of interaction have been established, by electrostatic interaction with negatively charged backbone phosphate groups or by intercalation between G–C base sequences. The spectroscopic information has confirmed the intercalation of the planar heterocyclic MB between neighboring base pairs through favorable stacking interactions [20]. Due to this interaction capability, MB has been extensively used as redox marker in electrochemical biosensors to detect the hybridization event [14,21–24]. A lower MB reduction current is observed upon DNA hybridization ascribed to the steric inhibition of the reducible groups of MB packed between the bulky double helix of the hybrid [25,26]. Several strategies have been employed to study the interaction between DNA and MB. In some cases thiolated ssDNA and dsDNA were immobilized at gold electrodes via self-assembly to

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obtain well-aligned DNA films, the conformation of DNA at surface was critical for MB electrochemistry. The oligonucleotide was covalently attached to the carboxylate residue coming from the thiolated layer immobilized at a gold surface previous activation with N-hydroxysuccinimide and carbodiimide [23]. Gold electrodes modified with multi-wall carbon nanotubes (MWCNT) produced by chemical vapor deposition technique with Ni as catalyst were also employed. The carbon nanotubes were activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and DNA oligonucleotides with amino terminal groups were covalently immobilized for 12 h [27]. MB was also used by Fang's group [24] for detecting the hybridization event at the surface of zirconium oxide modified gold electrode. All these strategies required chemically modified DNA sequences, e.g., tiol or amine modifications, highly specialized equipment and in general high adsorption times. MB was also employed as a DNA indicator in a biosensor based on graphite electrode modified with CHI doped with CNT fabricated to detect salmon sperm DNA. In this case the film was stabilized immersing the electrode in 0.1 M NaOH for 30 min [28]. High adsorption times of DNA and MB were employed and no direct DNA bases oxidation signal was analyzed.

In a previous work we have reported the analytical performance of glassy carbon electrodes (GCE) modified with MWCNT dispersed in chitosan (CHIT) (GCE/CHIT-CNT) for the quantification of DNA (dsDNA, ssDNA and oligo(dG)$_{15}$) [29]. The objective was determined the DNA accumulated at these electrodes from the electroactivity inherent to the nucleic acid itself, without any redox indicator. To stabilize the film we employed 2.0% glutaraldehyde which permits to work in acidic solution adequate for DNA oxidation. In the present paper we evaluate the usefulness of GCE/CHIT-CNT/dsDNA as platform to quantify the interaction of MB with DNA. In this case, the known electroactive intercalating mediator MB is used to illustrate the ability of the proposed platform to determine DNA–molecules interaction. The optimization of the GCE/CHIT-CNT/dsDNA electrode to obtain a reproducible and sensitive signal for this redox marker is discussed in the following sections.

2. Experimental

2.1. Reagents

Multi-wall carbon nanotubes (CNT) of 1–5 μm long and 30 ± 15 nm diameter were obtained from NanoLab (USA). CNTs were oxidized by chemical treatment with a mixture of concentrated sulfuric and nitric acids (1:1) by refluxing for 3 h. After that, the suspension was filtered and washed with water until neutral pH.

Glutaraldehyde (GTA) (25% v/v aqueous solution) was purchased from Baker. Methylene blue and sodium chloride were obtained from Merck. Ferrocene methanol (FcOH) and Chitosan of medium molecular weight (Cat. No. 44887-7) were obtained from Aldrich®.

Double stranded calf thymus DNA (dsDNA) (activated and lyophilized, Cat. No. 4522) was from Sigma. Stock solutions of dsDNA was prepared with tris–EDTA (TE) buffer (1× concentrate, 20 mM Tris–HCl, 1 mM EDTA, pH 8.0).

All solutions were prepared with ultrapure water (ρ = 18 MΩ cm) from a Millipore-Milli-Q system. A 0.20 M formate solution pH 5.00 was employed as supporting electrolyte. All chemicals were used as received.

2.2. Apparatus

Differential Pulse Voltammetry (DPV) was performed with a CHI 440 setup (CH Instruments Inc., USA). A platinum wire and Ag/AgCl, 3 M NaCl (BAS, Model RE-5B) were used as counter and reference electrodes, respectively. All potentials are referred to that reference electrode. A magnetic stirrer provided the convective transport when necessary.

2.3. Preparation of the working electrode

Prior to surface modification, the GCE was cleaned by polishing with 0.05 μm alumina slurrie for 1 min and sonicated in water for 20 s. One mg of oxidized CNT were dispersed in a 1.0% w/v of chitosan solution prepared in 1.0% v/v acetic acid solution by sonication for 30 min. The immobilization of CNT was performed by casting the GCE with 10 μL of the CHI-CNT dispersion and allowed to dry for 15 min at 50 °C. The resulting electrodes are called GCE/CHIT-CNT. Once the CHI/CNT layer was dried, it was allowed to react with GTA solution for 2 s (GCE/CHIT-CNT/GTA$_{x\%}$). Finally, the electrode was washed by immersion in ultrapure water for 10 s.

2.4. Procedure

2.4.1. DNA detection

The GCE/CHIT-CNT/GTA$_{x\%}$ electrodes were immersed in a stirred supporting electrolyte solution containing dsDNA and the accumulation was performed at open circuit potential for a given time. The GCE/CHIT-CNT/GTA$_{x\%}$/dsDNA electrodes containing the adsorbed DNA layer were washed for 10 s with the buffer solution. The electrochemical transduction was performed in clean buffer solution by DPV under the following conditions: potential increment 0.004 V, pulse amplitude 0.05 V, pulse width 0.017 s, and pulse period 0.2 s. The anodic current at around 1.0 V, corresponding to the guanine oxidation, was used as analytical signal.

2.4.2. Methylene blue detection

After dsDNA adsorption, the GCE/CHIT-CNT/GTA$_{x\%}$/dsDNA, electrode was immersed in a stirred solution containing MB, and the accumulation was performed at open circuit potential for a given time. The electrode containing the adsorbed MB was washed for 10 s with buffer solution and the transduction was performed in buffer solution by DPV between +0.30 V and −0.50 V with a potential increment of 0.004 V, a pulse amplitude of 0.05 V, a pulse width of 0.017 s, and a pulse period of 0.2 s. The cathodic current at around −0.15 V, due to the reduction of MB, was used as analytical signal. The experiments were done under nitrogen atmosphere.

3. Results and discussion

3.1. Interaction of methylene blue with dsDNA using GCE/CHIT-CNT/GTA$_{x\%}$

Previous work has demonstrated that the chemical treatment of CNT-CHIT immobilized at GCE strongly influences the adsorption and electrooxidation of DNA, being the cross-linking with 3.0% v/v glutaraldehyde (GCE/CHIT-CNT/GTA$_{3\%}$) the best one [29].

The response of MB after interaction with the dsDNA adsorbed at GCE/CHIT-CNT/GTA$_{3\%}$ was evaluated by DPV in a formate buffer solution. Fig. 1 displays the dependence of the peak current for MB reduction with the interaction time. In all cases the signals obtained after the interaction were small and noisy. The calculated RSD were 20.3%, 18.3%, 32.2% and 23.5% for 1, 6, 12 and 18 min of interaction, respectively. These results indicate that, even when the GCE/CHI-CNT/GTA$_{3\%}$ is the best alternative to detect dsDNA, under these conditions, the system is not suitable to evaluate the interaction of MB with dsDNA. One possible explanation, as it was previously reported [30], is that some free GTA present within the CHIT-CNT/GTA$_{3\%}$ hydrogel interacts with the adsorbed dsDNA
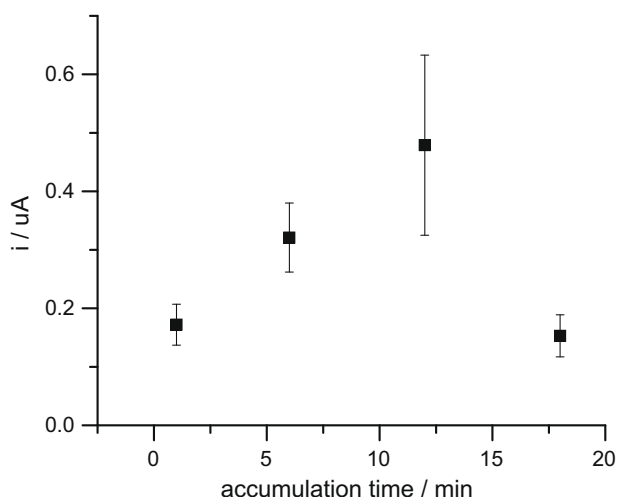
Fig. 1. Differential Pulse Voltammetry reduction peak currents obtained in supporting electrolyte after accumulation of MB at GCE/CHI-CNT/GTA$_{3\%}$/dsDNA at open circuit potential for different times. DNA adsorption: for 3 min from a 45 ppm dsDNA solution. Supporting electrolyte and stripping solution: 0.20 M formate pH 5.00. DPV conditions increment of potential: 4 mV, pulse amplitude: 50 mV; pulse period 0.2 s and pulse width: 0.05 s.

producing a decrease in the accessibility of DNA bases for further interaction. Therefore, the effect of GTA concentration on the reduction signal of MB was evaluated in order to find the best conditions for the intercalation of MB within the dsDNA structure confined at the electrode surface.

Fig. 2 shows the influence of GTA concentration between 0.20 and 3.00% v/v on the guanine oxidation and MB reduction signals after 6 min interaction on GCE/CHIT-CNT/GTA$_{x\%}$/dsDNA (obtained from separate experiments). As GTA concentration is reduced from 3.00% to 0.20%, the guanine oxidation signal decreases from 3.02 ± 0.09 to 1.9 ± 0.1 μA. In the case of MB, the reduction current increases from 0.7 ± 0.2 μA to 1.20 ± 0.04 μA, while the RSD becomes significantly smaller (from 20.1% to 3.2%). Thus, the decrease in the concentration of GTA allows not only an important
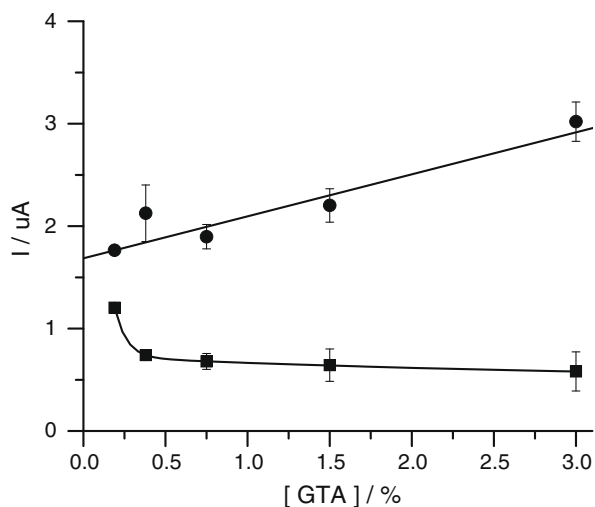
enhancement in the reduction signal of MB, but also a significant improvement in the reproducibility.

Fig. 3 shows voltammograms obtained for MB reduction at GCE/CHIT-CNT/GTA$_{0.2\%}$ (solid line) and GCE/CHIT-CNT/GTA$_{0.2\%}$/dsDNA (dashed line). The presence of dsDNA produces an increase in the MB reduction current from 0.64 to 1.19 μA, indicating that the DNA confined at the electrode surface produces the accumulation of MB as a result of the intercalation process. The effect of dsDNA accumulation time at GCE/CHIT-CNT/GTA$_{0.2\%}$ on the reduction current of MB was also evaluated (not shown). The MB reduction current at GCE/CHIT-CNT/GTA$_{0.2\%}$ increases up to 2 min dsDNA accumulation, to remain almost constant thereafter.

The response of the sensor using other strategies to stabilize the CHIT/CNT layer, were also investigated. Sodium hydroxide was used as ionic film stabilizer (by immersion in NaOH 0.1 M for 30 min, as it was previously reported by Li et al. [28]. Under these conditions, the MB signals was very small and the stability of the film was poor, i.e., CNT fell off from the GCE. Similar results were obtained when the determination of MB was performed using the electrode without stabilizing treatment. In consequence, the use of GTA 0.20% is the best choice to study the interaction of MB with dsDNA confined to the electrode surface.

3.2. Effect of NaCl on the response of GCE/CHIT-CNT/GTA$_{0.2\%}$

The effect of ionic strength (IS) on the intercalation of MB within the dsDNA is a very controversial aspect. Some authors found that the decrease in the ionic strength makes the intercalation more favorable, while others found a mixed behavior at high ionic strength. Song et al. [26] have reported that is also necessary to consider the effect that the surface conformational structure exerts on the electrochemistry of MB under the different conditions.

Fig. 4 shows the effect of IS on the electrochemical response of MB evaluated at different accumulation times in the absence and in the presence of NaCl. The results reveal that the peak currents for MB reduction were higher at more elevated IS. The ratio between the currents obtained with and without NaCl in the reaction medium gave the following values, 1.71, 1.71, 1.91, 1.69 and 1.43 for 6, 12, 18, 24 and 30 min, respectively. Over 24 min of accumulation time, the ratio between both currents decreases, indicating a faster saturation of the dsDNA to intercalate MB in the presence of NaCl.
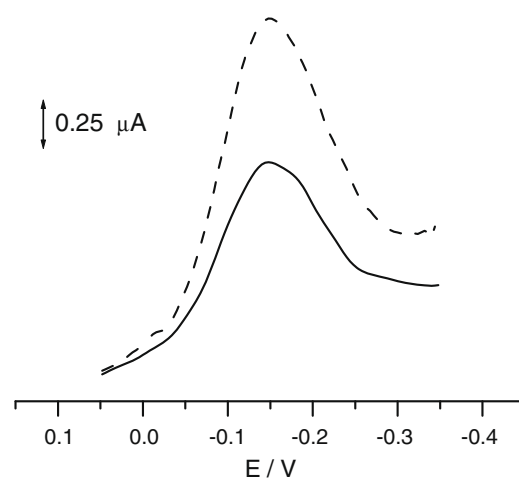

Fig. 2. Effect of GTA concentration employed during the stabilizing treatment of CNT-CHI film on guanine oxidation (●) and MB reduction (■) currents obtained at GCE/CHIT-CNT/GTA$_{x\%}$/dsDNA in supporting electrolyte conditions after the interaction with 20 μM MB solution for 6 min at open circuit potential. Other conditions as in Fig. 1.

Fig. 3. DPV obtained in supporting electrolyte solution at GCE/CHI-CNT/GTA$_{0.2\%}$ (solid line) and GCE/CHI-CNT/GTA$_{0.2\%}$/dsDNA (dashed line) after the interaction with 20 μM MB solution for 6 min at open circuit potential. DNA adsorption: 1 min from a 45 ppm dsDNA solution. Other conditions as in Fig. 1.
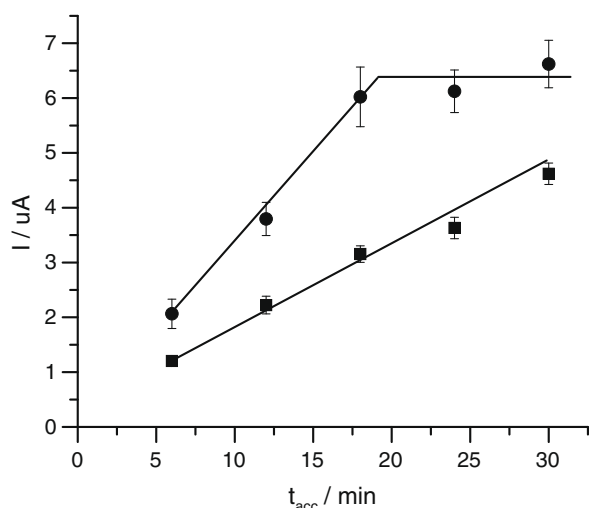
Fig. 4. DPV response in supporting electrolyte solution at GCE/CHI-CNT/GTA$_{0.2\%}$/dsDNA after the interaction with 20 µM MB solution for 6 min at open circuit potential in the absence (■) and presence (●) of 90 mM NaCl. Other conditions as in Fig. 1.
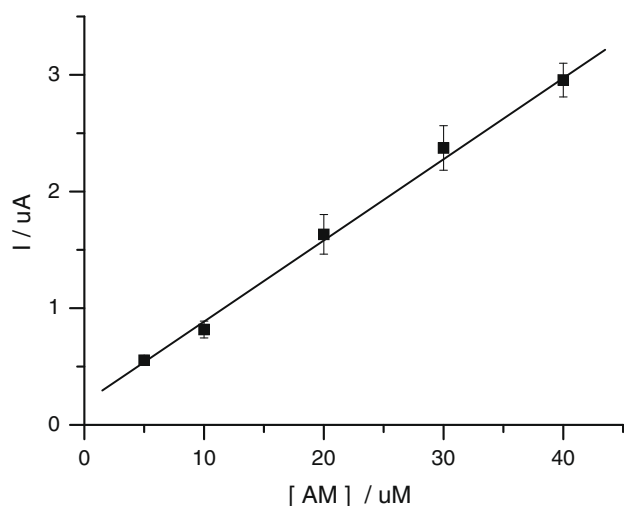

Fig. 5. Calibration plot for MB obtained after 6 min interaction with GCE/CHI-CNT/GTA$_{0.2\%}$/dsDNA. Other conditions as in Fig. 1.

3.3. Sensor response below optimal conditions

Once optimized the GTA concentration and determined the importance of the ionic strength, we evaluate the ability of the sensor to quantify MB from the interaction with the adsorbed dsDNA, as a model to evaluate the usefulness of the proposed sensor to determine dsDNA–molecule interaction. Fig. 5 displays the dependence of the MB reduction current as a function of MB concentration. A linear relationship was obtained between 5.0 and 40.0 µM MB with a sensitivity of 0.070 ± 0.003 µA L mol^{-1}, $r = 0.998$ with a reproducibility interelectrode of 8.0%.

4. Conclusions

The performance of the glassy carbon electrodes modified with CNT-CHIT was improved to study MB intercalative process modifying the experimental conditions for the film treatment. The optimal conditions were cross-linking with 0.2% v/v glutaraldehyde. The electrochemical signals for the reduction of the MB intercalated within GCE/CHIT-CNT/GTA$_{0.2\%}$/dsDNA, are highly reproducible and allow its highly sensitive quantification. This new electrode opens the doors to the development of biosensors to sense intercalative process with a simplest and fast methodology, exemplified in this case with MB. This strategy could allow evaluating the toxicity of new synthesized molecules.

Acknowledgments

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