Published in final edited form as:

J Electroanal Chem (Lausanne Switz). 2014 October 1; 731: 139–144. doi:10.1016/j.jelechem.2014.07.022.

DNA electrochemical biosensor for metallic drugs at physiological conditions

Angel J. Santiago-Lopez¹, José L. Vera², and Enrique Meléndez^{2,*}

¹University of Puerto Rico, Department of Chemical Engineering, PO Box 9019 Mayagüez, PR 00681

²University of Puerto Rico, Department of Chemistry, PO Box 9019 Mayagüez, PR 00681

Abstract

Entrapment of dsSS-DNA into the polypyrrole-polyvinyl sulphonate (dsSS-DNA-PPy-PVS) film over indium-tin-oxide (ITO) coated glass has been designed to detect titanium and platinum drugs, titanocene dichloride and cisplatin. The disposable dsSS-DNA-PPy-PVS/ITO biosensor was characterized by cyclic voltammetry, attenuated total reflectance Infrared spectroscopy and atomic force microscopy. Amperometric studies by cyclic voltammetry using, dsSS-DNA-PPy PVS/ITO biosensor, demonstrated the ability of this biosensor to detect these metallic drugs in millimolar concentration by monitoring the decrease of the guanine oxidation signal as a result of the DNA damage. The concentration range detected for titanocene dichloride is 0.25 to 1.5 mM and for cisplatin is 0.06 to 1.0 mM.

Keywords

DNA biosensor; electrochemical detection; electrochemical entrapment; titanocene dichloride; cisplatin

1. Introduction

Titanium is a metal with numerous uses and application. Its applications range from catalysis, alloys, biomaterials, pigments and nanomaterials among others [1–5]. Our interest in titanium resides on the biological activity of titanocene dichloride and its derivatives as promising anticancer agents in a wide range of tumor cells. *In vivo* studies suggest that these species have potential clinical applications [6–14]. Mechanistic studies on metallocenes indicate that DNA is the target place for titanocene dichloride [6–14]. While working in this research area, we decided to develop a simple device that can detect and measure titanium in buffer solutions. Taking advantage of the affinity of titanocene dichloride and cisplatin to

© 2014 Elsevier B.V. All rights reserved.

Corresponding author: (787)832-4040 ext. 2524, Fax (787)265-3847, enrique.melendez@upr.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

DNA, we decided to develop an electrochemical biosensor for titanium and platinum using a double stranded salmon sperm DNA as bioprobe.

A biosensor is an analytical device that provides specific quantitative or semi-quantitative information, in terms of the presence and concentration of a specific substance, using a biological recognition element which is in contact with a transduction element [15]. The biological recognition elements, or bioreceptors typically are enzymes, antibody/antigen, protein receptors, proteins and cells and the transduction methods (measurement techniques) are optical, electrical and magnetic, thermal, electrochemical and piezoelectric [15].

There is an increasing interest in the fabrication of DNA-based biosensors since they have applications in agriculture, environmental, medical device (diagnosis of diseases) and chemotherapy [16–19]. The efficacy and sensitivity of DNA-based biosensor is dependent on the selection of an immobilization procedure and the matrix composition [20]. The immobilization procedures typically involve adsorption, entrapment, cross-linking and covalent bonding. In terms of matrix composition, conducting polymers provide good signal transduction, sensitivity, selectivity, biocompatibility and flexibility to immobilize biomolecules [21, 22]. These polymers can be generated (synthesized) electrochemically and can entrap biomolecules within the process of electropolymerization. In this regard, polypyrrole-polyvinyl sulfonate (PPy-PVS) provides an excellent matrix to entrap biomolecules such as DNA [23]. Thus, we have entrapped double stranded salmon sperm-DNA into the PPy-PVS film to detect titanium and platinum under physiological conditions. Our single use biosensor is simple to prepare, inexpensive, easy to use and with good sensitivity. Herein we report our findings on single use biosensor for titanocene dichloride and cisplatin.

2. Experimental

2.1 Materials

Pyrrole (Py) 98%, polyvinyl sulfonic acid (PVS), indium tin oxide (ITO) glass slides (15–25 Ω /sq), bis(cyclopentadienyl) titanium dichloride (titanocene dichloride) 97%, *cis*-diaminedichloroplatinum(II) (cisplatin) 99.9% and sodium perchlorate hydrate (NaClO_4) were obtained from Sigma-Aldrich. Double stranded salmon sperm Deoxyribonucleic Acid (dsSS-DNA) was obtained from ICN Biomedicals and used as received. Deionized water (18.2 M Ω) was used through all experiments.

2.2 Equipment

CP-II VEECO Atomic Force Microscopy (AFM) with scanning probe microscopy and lateral force microscopy and nanomanipulation capabilities with 100 $\times$ 100 μm scanning area capacity. The IR spectral data was obtained from Perkin Elmer Spectrum Two spectrophotometer using attenuated total reflectance (ATR) as a sampling technique. Electrochemical experiments were carried on an Epsilon Potentiostat/Galvanostat (Bioanalytical Systems, Inc.) using a specially designed three-electrode electrochemical cell having ITO glass as working, a platinum wire as counter (auxiliary) and Ag/AgCl paste covered wire as reference electrodes. Most of the current electrochemical cells on the market are designed for disk-type electrodes to be placed at a certain angle, a configuration not

suitable when using flat plates such as ITO glass plates. The polyvinyl chloride cell (Figure 1) with removable cap makes use of the regular triangular electrode geometry and employs an alligator clip addition, which holds steady and vertically the ITO plate. This simple modification optimizes the ease of handling of electrodes with non-traditional geometries at electrochemical procedures while reducing the amount of working solution needed.

2.3 Preparation of DNA-biosensor (DNA entrapment)

ITO electrodes were treated with water, acetone and ethanol in a sequential solvent sonication cleaning process for 3 min and dried under a gentle stream of nitrogen. Prior to the electrochemical polymerization synthesis, a monomer solution of 0.3 M freshly distilled Py, 0.1 M PVS and $10 \mu\text{g mL}^{-1}$ dsSS-DNA dissolved in buffer-saline media (50 mM Tris and 0.1 M NaClO_4 , pH 7.4) was prepared to a total volume of 10 mL and degassed with N_2 5 min before use. The polypyrrole-polyvinyl sulfonate (PPy-PVS) polymeric matrix was synthesized using chronopotentiometry by applying a constant current of 400 μA for a time interval of 10 min to 5 mL of the monomer solution, reaching an optimum potential of 0.51 V after 3 mins (Figure 2). The presence of dsSS-DNA during the polymer growth facilitated its physical entrapment over the ITO surface. The dsSS-DNA-PPy-PVS film covered an approximate of 0.7 cm^2 of ITO glass surface area after the immobilization process. The biosensors were stored under dry conditions at 5 °C when not in use and showed to be stable within a time frame of 2 months. Biosensor stability was also evaluated by the multiple measurements (each one a newly fabricated sensor) of the DNA oxidation signal without previous exposure to the analyte (See supporting info). Scheme 1 depicts a conceptual view of the modified DNA biosensor and entrapment procedure.

2.4 Determination of titanium

Titanocene dichloride solutions were prepared in a concentration range from 0.25 to 1.50 mM by dissolving titanocene dichloride first in 750 μL of DMSO followed by addition of 50 mM Tris/100 mM NaClO_4 buffer solution at pH 7.4 to make a final solution with a content of 5% DMSO. Solutions were prepared and used within a time frame of 10 min due to the rapid hydrolysis of titanocene dichloride and therefore loss of its DNA-binding capacity. Similarly, cisplatin solutions (0.06 – 1.00 mM) were prepared dissolving cisplatin in 50 mM Tris/100 mM NaClO_4 buffer solution at pH 7.4. For the DNA-drug interaction, the biosensor was exposed to the titanium or platinum drug solution for 3 min in a dip-in fashion. After the interaction with the analyte, the bioelectrodes were rinsed with DI and cyclic voltammetry (CV) scans were recorded from -0.8 V to 2.25 V at an optimum rate of 20 mV s^{-1} in 5 mL of Tris- NaClO_4 buffer media. This bioelectrode is a single use biosensor. Thus, each measurement was performed on a new fabricated biosensor. The measurements were performed in triplicates, showing high reproducibility (90%). The overall steps for the fabrication and detection principle of the disposable dsSS-DNA-PPy-PVS biosensor are illustrated in Scheme 2.

3. Results and discussion

3.1 Biosensor Characterization

Atomic Force Microscopy Studies—Figure 3 presents the AFM pictures of the (a) PPy-PVS/ITO and (b) dsSS-DNA-PPy-PVS/ITO bioelectrodes. The roughness values for PPy-PVS/ITO and dsSS-DNA-PPy-PVS/ITO bioelectrodes are 43.25 and 20.46 nm, respectively. This change in roughness value indicates the surface of the PPy-PVS/ITO has been modified and become smoother as a result of the electrochemical entrapment of DNA into the polymeric matrix/surface. Thus, it is clear that the inclusion of DNA modifies the surface of the polymeric matrix.

Attenuated Total Reflectance (ATR) IR Studies—The ATR IR spectra of PPy-PVS and ds-SSDNA-PPy-PVS matrixes are reported in Figure 4. The previously published IR spectrum of ds-SSDNA showed broad bands from 1000–1700 cm^{-1} but some particular peaks in the 1400–1750 cm^{-1} region can be recognized and helped us to analyze our spectral data [24]. The peaks 1713, 1695, 1659, 1653, 1645, 1633, 1623, 1539 and 1504 cm^{-1} observed in the dsSS-DNA-PPy-PVS and not in PPy-PVS corresponds to the DNA bases. The peaks in the 100–1300 cm^{-1} region (1029, 1082, 1163, 1270 cm^{-1}) are characteristic of the polypyrrole (data not presented) [25].

Cyclic voltammetric studies—The cyclic voltammograms of PPy-PVS/ITO and dsSS-DNA-PPy-PVS/ITO films, in 50 mM Tris/100 mM NaClO_4 buffer solution, showed oxidation peaks at 1.35 V and 1.81 V, respectively (Figure 5). The oxidation peak of PPy-PVS/ITO corresponds to the PPy-PVS matrix, while for dsSS-DNA-PPy-PVS/ITO corresponds to the irreversible guanine oxidation of the entrapped DNA molecules at the surface and inside the PPy-PVS matrix. This electrochemical behavior is characteristic of DNA and has been demonstrated in various DNA-based electrochemical systems [20, 26, 27]. The presence of metal ions such as Mg^{2+} , Ca^{2+} , Zn^{2+} and Cu^{2+} has no effect on the guanine oxidation signals but the sensor is not intended to be used on complex solution matrix such as plasma. [20, 31, 32].

3.2 Biosensor response principle

In a cyclic voltammogram, the guanine peak is used as the signal transduction for the recognition (interaction) of DNA to titanocene dichloride and cisplatin drugs. The interaction of dsSS-DNA--PPy-PVS/ITO to titanium or platinum yields a decrease in the guanine peak as detected by cyclic voltammetry. The amount of modified DNA (measured by the guanine peak), which is related to the amount of Ti or Pt in solution, is measured with the value of the percentage of guanine peak height change ($S\%$). $S\%$ is the ratio of the guanine peak height after the interaction with a sample (S_s), and the guanine peak height after the interaction with the buffer solution (S_b), equation 1. The modification (perturbation) of DNA measured by the guanine peak corresponds to either covalent binding to the grooves of the double helix, intercalation, electrostatic interactions, phosphate bonding, hydrogen bonds or van der Waals interactions. These interactions will be reflected in the % of guanine peak height change.

$$S\% = \left(\frac{S_s}{S_b} \right) \times 100 \quad \text{eq. 1}$$

The sensitivity of the bioelectrode depends on the metal nature. For instance, platinum can be detected at lower concentrations than titanium. As presented in Figure 6, at 0.5 mM the guanine signal intensity is very small for platinum whereas for titanium the guanine signal remains about 50% height of the original signal. This behavior clearly shows that platinum and titanium bind to DNA in a different manner. Evidently, platinum binds more strongly and promotes major DNA damage than titanium as explained in the next section [28, 29].

3.2 Electrochemical detection – Amperometric response

Electrochemical detection of titanocene dichloride drug under physiological conditions was performed using cyclic voltammetry experiments, monitoring the amperometric response (Figure 7). It can be observed that the guanine oxidation peak height decreases as we increase the amount of titanocene dichloride in solution in a linear fashion. That is, there is a linear decrease in the guanine oxidation peak height as we increase the concentration of titanium in the solution within the concentration range of 0.25–1.50 mM with a correlation coefficient of 0.994 and follows the equation $y = -57.679 \times + 96.833$, see inset Figure 7. At concentration higher than 1.50 mM of titanocene dichloride, the guanine oxidation peak height is too low to provide reliable results and the detection limit is roughly 0.06 mM.

In a similar manner, interaction of the DNA bioelectrode was performed for cisplatin at similar concentration but after 0.13 mM the response levels off substantially and the response becomes non-linear. It has a detection limit of roughly 0.014 mM, Figure 8. It is known that the interaction between DNA and platinum is stronger than the interaction with titanocene dichloride. Given that we are monitoring guanine oxidation peak, since DNA is our recognition molecule, the interaction with cisplatin involves G-G intra- and inter-strand crosslinking causes a global bend of the helix axis toward the major groove by 20–40 degrees, making the guanine inaccessible for oxidation [28]. On the other hand, titanium-DNA interaction is weaker and does not involve crosslinking. It involves external phosphate coordination and produces less structural distortion on the DNA structure than cisplatin [29]. As a result the biosensor can detect higher concentration of titanium. The biosensor has lower sensitivity for titanium but higher dynamic range than platinum.

There are precedents of DNA biosensors for titanocene dichloride using ds-calf thymus DNA (dsCT-DNA) on a screen-printed electrode [30–32]. In their studies, the analysis of titanocene dichloride reached a maximum concentration of 0.5 mM apparently due to solubility constraints. Our DNA biosensor, combined with the appropriate sample preparation, was able to detect up to 1.5 mM with good reproducibility and linear behavior ($R^2 = 0.994$) and detection limit approaching 0.06 mM. Similar to their results, for platinum we observed non-linear behavior indicating the strength of the perturbation on DNA is significantly higher and different to titanium and that these differences can be monitored electrochemically.

3.3 Economic Viability

The benefits of disposable sensors have been discussed widely for over two decades, being the main advantage of these the avoidance of contamination or interference between samples [33–36]. For these types of sensors to be feasible from an economic standpoint, they must be engineered in a way where their main components (electrode material, immobilization matrix and biological entity) have low costs and be easily accessible. In this regard, ITO glass electrodes have risen as a viable platform for the development of disposable electrochemical biosensors for diverse applications [20, 36]. Clearly, ITO glass provides an advantage over other materials such as gold or platinum-covered electrodes that could prove unfeasible for the fabrication of a disposable-type biosensor. Hence, our selection of ITO glass as the electrode material. Another major component to take into consideration is the biological entity, in this case DNA. For instance, most DNA-based electrochemical sensors being developed nowadays for DNA-drug interaction analysis or DNA sensing employ the widely used, while extremely expensive, dsCT-DNA [37–39]. In this work, we opted to use dsSS-DNA instead, as it provides a comparable degree of sensitivity and stability demonstrated in our electrochemical studies, while reducing the fabrication costs substantially. Finally, taking into consideration our experimental set up and the relatively small quantities required for fabrication, we could argue that the device presented in this work is economically viable and represents an attractive alternative when compared to other electrode-based sensors.

4. Concluding remarks

In this work, we present the design of a simple, inexpensive and single use bioelectrode (biosensor) to detect titanocene dichloride and cisplatin drugs. This biosensor is simple to prepare, has constant sensitivity, requires minimum sample preparation and as single use electrode, avoidance of contamination between samples is eliminated. Also, it has been demonstrated that dsSS-DNA can be electrochemically entrapped into the PPy-PVS matrix. This disposable DNA biosensor is able to detect titanocene dichloride and cisplatin in micromolar quantities. The concentration range for Ti detection is 0.25 to 1.5 mM with significant accuracy while for Pt is 0.06 to 1.0 mM in a non-linear behavior. Here it is demonstrated the potential of this biosensor to determine metallic drugs using a sensitive, rapid and easy procedure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

EM is grateful to Dr. Oscar Perales and the Nanomaterials Processing Laboratory for the access to the AFM instrument. EM thanks the financial support of NIH-RISE 2 Best program (NIH-R25GM088023) for the research assistantships of Angel J. Santiago-Lopez and José L. Vera.

References

1. Chen X, Mao SS. Chem. Rev. 2007; 107:2891–2959. [PubMed: 17590053]

2. Macyk W, Szacilowski K, Stochel G, Buchalska M, Kunciewicz J, Labuz P. *Coord. Chem.* 2010; 254:2687.
3. Geetha M, Singh AK, Asokamani R, Gogia AK. *Prog. Mater. Sci.* 2009; 54:397.
4. Jaouen, G.; Metzler-Nolte, N., editors. *Medicinal Organometallic Chemistry*. Dordrecht, London, NY: Springer-Verlag Berlin, Heidelberg; 2010. p. 81-117.
5. Jaouen, G., editor. *Bioorganometallics*. Germany: Wiley-VCH, Weinheim; 2006. p. 65-95.
6. Köpf H, Köpf-Maier P. *Angew. Chem. Int. Ed. Engl.* 1979; 18:477. [PubMed: 111586]
7. Köpf-Maier P. *Eur. J. Clin. Pharmacol.* 1994; 47:1. [PubMed: 7988618]
8. Köpf-Maier P, Köpf H. *Struct. Bond.* 1988; 70:103.
9. Köpf-Maier P, Köpf H. *Chem. Rev.* 1987; 87:137.
10. Köpf-Maier, P.; Köpf, H. *Metal Compounds in Cancer Therapy, Organometallic Titanium, Vanadium, Niobium, Molybdenum and Rhenium Complexes - Early Transition Metal Antitumor Drugs*. Fricker, SP., editor. London: Chapman and Hall; 1994. p. 109-146.
11. Köpf-Maier, P.; Keppler, BK., editors. *Metal complexes in cancer chemotherapy-Antitumor Bis(cyclopentadienyl)metal Complexes*. New York: VCH, Weinheim; 1993. p. 260-296.
12. Harding MM, Mokdsi G. *Curr. Med. Chem.* 2000; 7:1289. [PubMed: 11032972]
13. Meléndez E. *Crit. Rev. Oncol. Hematol.* 2002; 42:309. [PubMed: 12050022]
14. Caruso F, Rossi M. *Metal Ions Biol. Syst.* 2004; 42:353.
15. Theâvenot DR, Toth K, Durst RA, Wilson GS. *Pure Appl. Chem.* 1999; 71:2333.
16. Serna-Cock, L.; Perenguez-Verdugo, JG. *Biosensors Applications in Agri-food Industry, Environmental Biosensors*. Prof. Vernon, Somerset, editor. 2011. ISBN: 978-953-307-486-3.
17. Wang S, Wang R. *Biosensors and Cancer*. 2012:359.
18. Rana JS, Jindal J, Beniwal V, Chhokar V. *J. Am. Sc.* 2010; 6(9):353.
19. Liang H, Zhang XB, Lv Y, Gong L, Wang R, Zhu X, Yang R, Tan W. *Acc. Chem. Res.* 2014; 47:1891. [PubMed: 24780000]
20. Prabhakar N, Arora K, Sing SP, Sing H, Malhotra BD. *Anal. Biochem.* 2007; 366:71. [PubMed: 17445760]
21. Bidan G, Billon M, Livache T, Mathis, Roget GA, Rodríguez MT. *Synth. Metals.* 1999; 102:1363.
22. Wang D, Gong X, Heeger PS, Rininsland F, Bazan GC, Heeger AJ. *P.N.A.S.* 2002; 99:49. [PubMed: 11756675]
23. Ramanavi ius A, Ramanavi ienè A, Malinauskas A. *Electrochim. Acta.* 2006:6025.
24. Mello MLS, Vidal BC. *PLOS ONE.* 2012; 7(8):e43169. [PubMed: 22937023]
25. Kato H, Nisbikawa O, Matsui T, Honma S, Kokado HJ. *Phys. Chem.* 1991; 95:6014.
26. Yin H, Zhou Y, Ma Q, Ai S, Ju P, Zhu L, Lu L. *Process Biochem.* 2010; 45(10):1707.
27. Ravera M, Bagni G, Mascini M, Osella D. *Bioinorg. Chem. Appl.* 2007 Article ID 91078, 11 pages.
28. Fuertes MA, Castilla J, Alonso C, Prez JM. *Curr. Med. Chem.* 2003; 10(3):257. [PubMed: 12570712]
29. Guo M, Guo Z, Sadler P. J. *Biol. Inorg. Chem.* 2001; 6:698. [PubMed: 11681703]
30. Bagni G, Osella D, Sturchio E, Mascini M. *Anal. Chim. Acta.* 2006; 573-574:81. [PubMed: 17723509]
31. Mascini M, Bagni G, Di Pietro ML, Ravera M, Baracco S, Osella D. *BioMetals.* 2006; 19:409. [PubMed: 16841250]
32. Ravera M, Bagni G, Mascini M, Osella D. *Bioinorg. Chem. Appl.* 2007 Article ID 91078, 11 pages.
33. Bilitewski U, Chemnitiu GC, Rüger P, Schmid RD. *Sensor Actuator.* 1992; 7(1-3):351.
34. Quinto M, Losito I, Palmisano F, Zambonin CG. *Anal. Chim. Acta.* 2000; 420(7):9.
35. Alonso-Lomillo MA, Domínguez-Renedo O, Matos Patricia, Arcos-Martínez MA. *Anal. Chim. Acta.* 2010; 665(1):26. [PubMed: 20381686]
36. Wang J, Wang L, Di J, Tu Y. *Sensor Actuator.* 2008; 135(1):283.

37. Primo EM, Oviedo MB, Sánchez CG, Rubianes MD, Rivas GA. *Bioelectrochemistry*. 2014; 99:8. [PubMed: 24951898]
38. Tsai WC, Lan YC. *Anal. Lett.* 2011; 44(4):717.
39. Shervedani RK, Pourbeyram S. *Bioelectrochemistry*. 2010; 77(2):100. [PubMed: 19716776]

Research Highlights

Design of single use, disposable biosensor for titanocene dichloride

Double stranded SS-DNA can be electrochemically entrapped into the PPy-PVS matrix

dsSS-DNA--PPy-PVS/ITO biosensor can detect titanocene in micromolar quantities

Reference: Ag/AgCl
Counter: Platinum (Pt) wire

Working:
Indium Tin
Oxide Glass

Figure 1.
Electrochemical cell with a modified cap using triangular electrode geometry.

Figure 2. PPy-PVS electroynthesis by chronopotentiometry in the presence of dsSS-DNA for its physical entrapment over the ITO electrode.

Figure 3.
Atomic force microscopy pictures of (a) PPy-PVS/ITO (b) dsSS-DNA-PPy-PVS/ITO films.

Figure 4.

ATR spectra of PPy-PVS and dsSS-DNA-PPy-PVS. Notable differences due to the presence of dsSS-DNA are circled.

Figure 5.
Cyclic voltammograms of PPy-PVS/ITO (red) and dsSS-DNA-PPy-PVS/ITO (black) films
in in 50 mM Tris/100 mM NaClO₄ buffer solution at a scan rate of 20 mV/s.

Figure 6.

The effect on the metal center on the current of the cyclic voltammetric experiment at the same concentration. Conditions: 50 mM Tris/100 mM NaClO_4 buffer solution at $\text{pH} = 7.4$.

Figure 7.

Cyclic voltammograms of the DNA biosensor upon addition of different concentrations of titanocene dichloride in 50 mM Tris/100 mM NaClO₄ buffer solution at pH = 7.4. Inset: Linear plot of percentage of peak reduction as function of titanocene dichloride concentration.

Figure 8.

Cyclic voltammograms of the DNA biosensor upon addition of different concentrations of cisplatin in 50 mM Tris/100 mM NaClO₄ buffer solution at pH = 7.4. Inset: Linear plot of percentage of peak reduction as function of cisplatin concentration.

Scheme 1.

Scheme 2.