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A label-free photoelectrochemical cocaine aptasensor based on an electropolymerized ruthenium-intercalator complex

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ABSTRACT

A photoelectrode was designed by electrodeposition of a pyrrole monomer modified with a polypyridyl Ru(II) complex bearing benzo[i]dipyrido-[3,2-a:2′.3′-c]phenazine (dppn) ligand. Owing to the intercalating properties of these immobilized complexes towards DNA double helix, cocaine aptamer was immobilized on the modified electrodes thanks to its stem-loop configuration in order to design a photoelectrochemical cocaine aptasensor. Especially using a double-fragment aptamer strategy, the binding of cocaine and the formation of the aptamer/cocaine complex was successfully observed and modeled by a Langmuir-Freundlich isotherm, giving access to an apparent dissociation constant K_d of 3.8 mmol L^{-1}. The photoelectrochemical aptasensor exhibits a LOD of 10 nmol L^{-1} and linear range of 1 10^{-8}–5 10^{-4} mol L^{-1}.

1. Introduction

DNA-based biosensors benefit from the flexible synthesis, modification and use of oligonucleotides, in order to target a wide range of biomolecules-oligonucleotides [1], drugs [2], or proteins [2]-depending on the natural or artificial interactions involved in the transduction process. In this field, electrochemical sensors represent a simple, cheap and portable technique [1,3–5]. Among these electrochemical DNA sensors, optobioelectronic or photobioelectrochemical systems have been recently envisioned in DNA sensor design [6,7]. Photoelectrochemical biosensors use visible-light-irradiated photosensitive units, such as ruthenium complexes or quantum dots, to trigger transducing events in order to detect antibody-antigen [8,9], aptamer-molecule [9,10] and hybridization [6,11,12] recognition events. In label-free systems (without any additional DNA labeling step [5]), these biosensors rely on the concomitant immobilization of oligonucleotides and the photosensitive transducer. While most of DNA biosensors are based on complementary DNA hybridization detection, the development of aptamers has driven the design of novel DNA-based sensors. Aptamers are artificial nucleic acid ligands which are selected for their binding affinity towards targeted molecules via the combinatorial selection process SELEX (Systematic Evolution of Ligands by Exponential Enrichment) [13–15] Thanks to the ease of synthesis and modification of oligonucleotides as well as a high stability, aptamers offer many advantages in the design of biosensors, as compared to enzymes or antibodies.

The few examples of photoelectrochemical aptasensors which have been described are based on covalent attachment of oligonucleotides on surfaces associated with a labeling step with the photosensitive unit [10]. In a previous work we reported the intercalating properties of electrodes modified with an electrogenerated **[Ru(bpy-pyrrole)$_2$(dppn)]$^{2+}$** (bpy-pyrrole = 4-methyl-4′-butylpyrrole-2,2′-bipyridine, dppn = benzo[i]dipyrido-[3,2-a:2′.3′-c]phenazine) polymer and its use for the immobilization of DNA duplex [16]. It should be noted that this type of complexes has been investigated in solution for hybridized DNA labeling and DNA photocleavage [17–26]. We report herein an easy, fast and versatile procedure of aptasensor fabrication exploiting the photoelectrochemical and intercalating properties of this polypyrrole-ruthenium complex electrogenerated on an electrode surface. In addition, we describe an innovative transduction step based on the fact that the formation of the aptamer-small molecule complex may induce large steric hindrances drastically affecting the photochemical properties of the underlying polymer. In this work, we have investigated the immobilization of partially-hybridized cocaine aptamer as a single unit and as two subunits and investigated the binding of cocaine and its photoelectrochemical detection on these poly-**[Ru(bpy-pyrrole)$_2$(dppn)]$^{2+}$** DNA photoelectrodes.

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2. Experimental

2.1. Methods and Instrumentation

All reagents and chemicals were purchased from Aldrich. Acetonitrile was purchased from Rathburn (HPLC grade). Reagents and chemicals were used without further purification until it was mentioned. *bis (4-methyl-4′-butylpyrrole-2,2′-bipyridine)* (benzo[i] dipyrido-[3,2-a:2′.3′-c]phenazine) *ruthenium (II) hexafluorophosphate* **[Ru(bpy-pyrrole)$_2$(dppn)][PF$_6$)$_2$** was synthesized according to previously described procedures [16].

2.2. Preparation of the electrodes

For the polymerization of poly**[Ru(bpy-pyrrole)$_2$(dppn)]$^{2+}$** films and their characterization, the electrochemical experiments were carried out in the three-electrode electrochemical cell under dry argon atmosphere in a glove box using an Autolab PGSTAT 100 potentiostat. A Pt wire placed in a separated compartment was used as counter electrode, the Ag/$AgNO_3$ (0.01 mol L^{-1} in CH_3CN + 0.1 mol L^{-1} tetrabutylammonium perchlorate (TBAP) electrode served as reference and a Pt electrode (diameter 5 mm) as working electrode. All potentials given in this work are referred to a (Ag/$AgNO_3$) reference electrode in organic solutions and a saturated Ag/AgCl/KCl electrode in aqueous solutions.

Electropolymerization of 0.5×10^{-3} mol L^{-1} **[Ru(bpy-pyrrole)$_2$(dppn)]$^{2+}$** in CH_3CN was performed by chronocoulometry at 0.75 V *vs.* Ag/$AgNO_3$ (Q = 2.5 mC cm^{-2}); The resulting modified electrodes were then rinsed with CH_3CN.

In order to monitor the photogenerated current, the modified electrodes were then potentiostated at 0 V *vs.* Ag/AgCl in deaerated Tris-HCl buffer (20 mmol L^{-1}, pH 7.4) containing NaCl (140 mmol L^{-1}), $MgCl_2$ (2 mmol L^{-1}) and sodium ascorbate (10^{-2} mol L^{-1}) as oxidative quencher. An electrochemical cell with a quartz window was used instead of a classic electrochemical cell. The working electrode was placed in front of the quartz window and irradiated with a 200 W Hg lamp using UV and IR cut-off filters below 420 nm and 630 nm with a surface light intensity of 8.5 μW cm^{-2}.

2.3. Aptamer synthesis

Two types of aptamer systems were used: the full-length cocaine aptamer (**FL**: $^{5'}$GGG AGA CAA GGA AAA TCC TTC AAT GAA GTG GGT CGA CA$^{3'}$) [27,28] and the double-fragment aptamer [29,30] in which the full-length is split in two fragments (aptamer α: $^{5'}$GTT CTT CAA TGA AGT GTG GGA CGA CA$^{3'}$ and aptamer β: $^{5'}$GGG AGT CAA GAA C$^{3'}$).

Briefly, aptamer sequences were prepared as previously described [16] by automated DNA synthesis using of β-cyanoethylphosphoramidite chemistry at 1 μmol scale. Purifications were performed either by denaturing polyacrylamide gel electrophoresis for full length aptamer or by reversed phase HPLC for aptamer sequences α and β.

Cocaine and aptamers were solubilized in the buffer (20 mmol L^{-1} Tris-HCl pH 7.4, 140 mmol L^{-1} NaCl and 2 mmol L^{-1} $MgCl_2$).

3. Results and discussion

Metallopolymer-modified photoelectrodes were obtained by electropolymerization of a previously-described monomer [16] (Fig. 1).

Fig. 1B displays the electroactivity of the modified electrode obtained after electropolymerization performed by chronocoulometry at E_p = 0.75 V in CH_3CN. A charge of 2.5 mC cm^{-2} is applied, affording a Ru(II)-complex surface coverage comprised between 3 and 4 10^{-10} mol cm^{-2}. This is estimated from the integration of the charge under the Ru(III)/Ru(II) redox system observed at $E_{1/2}$ = +0.90 V. The second redox system at $E_{1/2}$ = +1.13 V is attributed to the reversible oxidation of the phenazine motif. In order to maximize photocurrent densities, the formation of a thin metallopolymer layer at the surface of the planar electrode was privileged to avoid intermetallic quenching, and to favor diffusion of the sacrificial donor, *i.e.* sodium ascorbate. Photocurrent measurements were measured in Tris-HCl buffer (20 mmol L^{-1}, pH 7.4) containing NaCl (140 mmol L^{-1}), $MgCl_2$ (2 mmol L^{-1}) and 10 mmol L^{-1} sodium ascorbate (Fig. 1C). This particular medium was chosen because it favors the proper conformation of the anti-cocaine aptamer in the presence of cocaine[28]. "On-Off" cycles

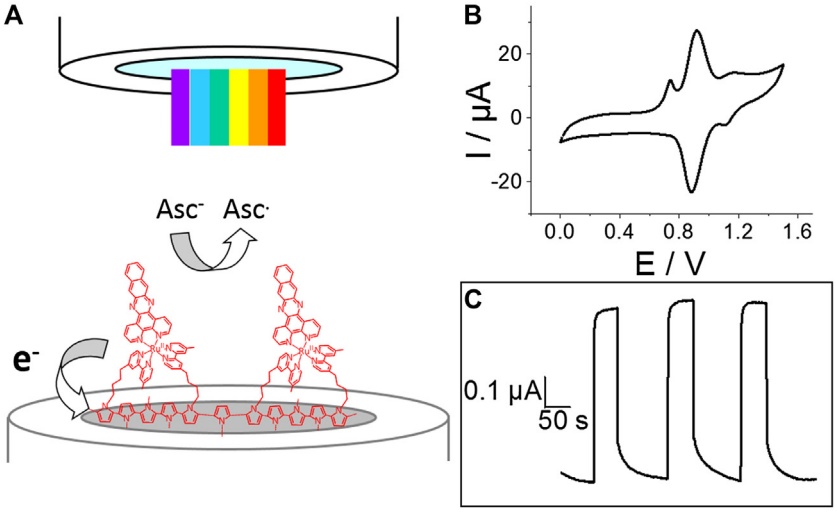

Fig. 1. Schematic representation of poly-[Ru(bpy-pyrrole)$_2$(dppn)]$^{2+}$ photoelectrode; (B) cyclic voltammogram of poly-[Ru(bpy-pyrrole)2(dppn)]2+-modified electrode in CH_3CN + 0.1 mol L^{-1} TBAP at v = 0.1 V s^{-1} after electropolymerization performed by chronocoulometry at 0.75 V (Q = 2.5 mC cm^{-2}); (C) Photocurrent measurement of poly-[Ru(bpy-pyrrole)$_2$(dppn)]$^{2+}$-+-modified electrode at 0 V vs SCE in Tris-HCl buffer (20 mmol.L^{-1}, pH 7.4) containing NaCl (140 mmol L^{-1}), $MgCl_2$ (2 mmol L^{-1}) and sodium ascorbate (10^{-2} mol L^{-1}).