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Electrochemical investigation of interactions between quinone derivatives and single stranded DNA

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ABSTRACT

The interactions of 5-hydroxy-1,4-naphthoquinone with three nucleobases (thymine, cytosine, adenine) and one nucleoside (guanosine), investigated by cyclic voltammetry in aprotic solvent, showed significant change in the redox behavior of the quinone group. Then, the interactions between 5-hydroxy-1,4-naphthoquinone and single-stranded oligonucleotides were studied in phosphate buffer saline solution using a random sequence and homo-oligonucleotides (polyA$_{20}$, polyT$_{20}$, polyC$_{20}$ and polyG$_{20}$). Finally, the interactions of 1,4-benzoquinone and 1,4-naphthoquinone were studied to compare with 5-hydroxy-1,4-naphthoquinone and to propose different interaction modes. The results help to elucidate the transduction mechanism involved in label-free quinone-based electrochemical DNA sensors.

1. Introduction

Quinones, present in several living organisms, participate to various biological processes. For instance, their redox system allows them to work as electron carriers to transport electrons between macromolecular complexes embedded in the membrane of the mitochondria in eukaryotic cells. Quinones can also interact with DNA, which has been demonstrated in many works. Some quinones can interact with nucleobases from DNA and inhibit replication [1], or bind to double-stranded DNA (dsDNA) by intercalation [2,3] or groove binding [4]. These characteristics make quinoid compounds attractive for anti-cancer drugs [5,6]. Besides, redox compounds which can interact with single or double stranded DNA can be used as electrochemical hybridization indicators in DNA sensors [7]. Such sensors take advantage of interactions between the target in solution, the recognition layer and a redox indicator, whether present in solution or immobilized on the sensor surface. Among these redox indicators, quinone derivatives have been investigated, mostly anthraquinones [8–11]. In our group, a quinone derivative, 5-hydroxy-1,4-naphthoquinone (commonly called juglone), has been advantageously used as transducer for direct and label-free electrochemical DNA detection [12–15]. In some cases [14,15], the transduction step was achieved by monitoring the change in interactions between oligonucleotides (ODN) and juglone. In these models, ODN probes and juglone were grafted together within a thin organic layer. The conformational change of ODN probes upon hybridization led to variation of interactions with juglone, which is finally expressed by change in juglone electroactivity. ODN/juglone interactions were assumed to be either hydrogen bonding (for DNA, the capability of hydrogen bonding comes mainly from nucleobases) or local pH changes (ODN have acidic character). In the literature, the interaction mechanisms between nucleobases and quinone derivatives have been already studied by physical [16–18] and electrochemical methods [7,19,20]. However, no work relative to the influence of ODN strand on juglone electroactivity in aqueous medium has been done yet, which can help to elucidate the transduction mechanisms involved in such juglone-based sensors. This is the aim of this work.

We have first studied the interactions between nucleobases and juglone in aprotic solvent (DMF), using cyclic voltammetry. Significant variations of juglone electroactivity were observed in the presence of nucleobases. It is also shown that these changes depend on the type of nucleobase (G, C, T or A). Then the interactions between juglone and a single-stranded ODN (ssODN) have been studied in phosphate buffer saline solution (PBS). Obvious changes of both peak current (I_p) and peak potential (E_p) of juglone were observed and interpreted. Finally, we investigated the interactions between ssODN and two other quinone derivatives (1,4-benzoquinone and 1,4-naphthoquinone) in PBS, in order to compare with juglone.

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2. Materials and methods

2.1. Chemicals

Dimethylformamide (DMF) and phosphate buffer saline (PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4) were purchased from Sigma. Tetrabutylammonium fluoroborate ($TBABF_4$), 5-hydroxy-1,4-naphthoquinone (juglone), thymine (T), cytosine (C), adenine (A) and guanosine (G) were purchased from Aldrich, as well as bovine serum albumin (BSA). All aqueous solutions were made with MilliQ (18 MΩ cm) water.

All ODN fragments were purchased from Eurogentec, the sequences of which are: $ssODN_{27}$, 5′-TCG CAC CCA TCT CTC TCC TTC TAG CCT-3′ (this sequence corresponds to the GEM91 sequence from the GAG protein of the HIV virus); $polyT_{20}$, 3′-TT TTT TTT TTT TTT TTT TTT-5′; $polyA_{20}$, 3′-AA AAA AAA AAA AAA AAA AAA-5′; $polyC_{20}$, 3′-CC CCC CCC CCC CCC CCC CCC-5′; $polyG_{20}$, 3′-GG GGG GGG GGG GGG GGG GGG-5′.

2.2. Electrochemical apparatus and methods

Glassy carbon (GC) disk electrodes (area 0.07 cm^2) were purchased from BAS Inc. For all electrochemical experiments, a conventional one-compartment, three-electrode cell was used with a glassy carbon disk as working electrode, a platinum grid as counter electrode and a commercial saturated calomel electrode (SCE) as reference electrode. Cyclic voltammetry was performed with an Autolab (PGSTAT 30) controlled by GPES software (no resistive compensation was applied). In order to avoid non-specific adsorption of ODN strands on the electrode surface, each electrode was pre-treated by dipping in a bovine serum albumin (BSA, 66.5 kDa, $pK_i = 4.7$) solution (5 mg mL^{-1} in PBS) for 10 min; this has been shown to prevent ODN physisorption on the electrode material (mainly because BSA is negatively charged at neutral pH), without blocking electron transfer from electroactive species in solution.

2.3. Study of the interactions between nucleobases and juglone in DMF

Cyclic voltammetry was performed from 0 V to −1.4 V (vs SCE) at 100 mV s^{-1} in 1 mmol L^{-1} juglone + 0.1 mol L^{-1} $TBABF_4$ DMF solution, containing various concentrations of one type of nucleobase (either T, C, A or G), from 0.01 mmol L^{-1} to 3 mmol L^{-1}. Due to the extremely low solubility of guanine in DMF, the study for this nucleobase was done by using its nucleoside, consisting of a guanine bound to a deoxyribose sugar to form guanosine, soluble in DMF in acceptable quantity.

2.4. Study of the interactions between ssODN and juglone in PBS

Cyclic voltammetry was performed from 0 V to −0.6 V (vs SCE) at 100 mV s^{-1} in PBS containing 0.05 mmol L^{-1} juglone and $ssODN_{27}$ for concentrations between 0 and 30 μmol L^{-1}. In order to study interactions as a function of the nucleobases, 4 different homo-oligonucleotides were used, containing 20 similar bases (either A, T, C or G, named $polyA_{20}$, $polyT_{20}$, $polyC_{20}$ and $polyG_{20}$, respectively). Cyclic voltammetry was performed from 0 V to −0.6 V (vs SCE) at 100 mV s^{-1} in PBS containing 0.02 mmol L^{-1} juglone and the homo-oligonucleotide at various concentrations from 0 to 13 μmol L^{-1}.

2.5. Study of the interactions between ssODN and two other quinones in PBS

1,4-Benzoquinone and 1,4-naphthoquinone were used to compare with juglone. Cyclic voltammetry was performed from 0 V to −0.6 V (vs SCE) at 100 mV s^{-1} in PBS containing 0.05 mmol L^{-1} of the quinone derivative, for concentrations of $ssODN_{27}$ from 0 to 30 μmol L^{-1}.

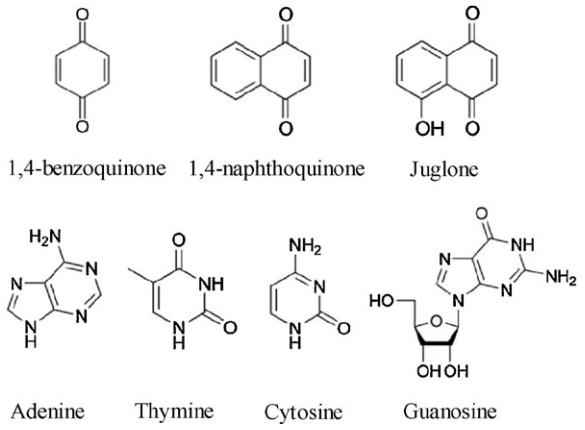

Fig. 1. Structure of the quinone derivatives, nucleobases and nucleoside used in this work.

3. Results and discussion

3.1. Influence of nucleobases on juglone electroactivity

Fig. 1 represents the chemical structures of all the molecules studied in this work. The voltammetric behavior of juglone in DMF is shown in Fig. 2a. Two reversible redox couples are seen, corresponding to the reduction through two successive one-electron transfers, which is typical for quinone in aprotic solvent. The first step corresponds to the quinone (Q) reduction into semiquinone ($Q^{\bullet -}$) at $E_{1/2\,1} = -0.39$ V (vs SCE) and the second step the reduction of $Q^{\bullet -}$ into quinone dianion (Q^{2-}) at $E_{1/2\,2} = -1.04$ V (vs SCE). When comparing the averaged peak potentials of juglone with those of 1,4-benzoquinone, situated at −0.385 V and −1.20 V (vs SCE) respectively [19], we find that the second redox couple of juglone is shifted toward more positive values, which indicates that the transition from $Q^{\bullet -}$ into Q^{2-} is easier for juglone than for 1,4-benzoquinone. This could be explained by the presence of intra-molecular hydrogen bonds in juglone, which can stabilize

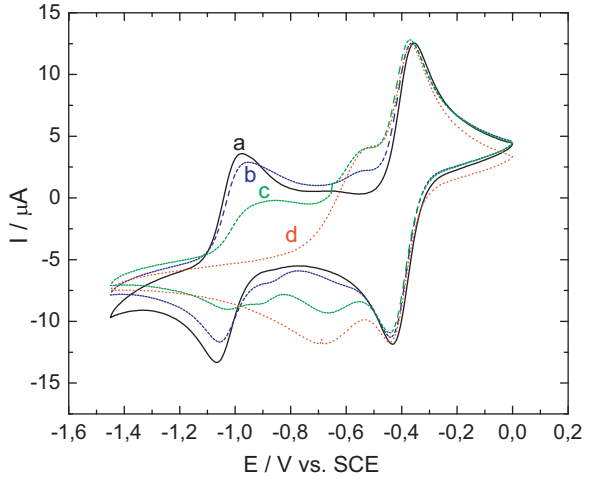

Fig. 2. Cyclic voltammetry of 1 mM juglone + 0.1 M $TBABF_4$ in DMF at 100 mV s^{-1} upon the presence of thymine. Concentrations of thymine were (a) 0 mM; (b) 0.1 mM; (c) 0.5 mM and (d) 1 mM. Cyclic voltammograms shown correspond to the second scan, starting from 0 V.