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Detection of short oligonucleotide sequences using an electrochemical DNA hybridization biosensor

Short Communication

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Abstract: An electrochemical DNA hybridization biosensor was developed for the detection of DNA hybridization using MDB and proflavine as electrochemical labels. The biosensor was based on the interaction of 7-dimethyl-amino-1,2-benzophenoxazi-nium Meldola's Blue (MDB) and proflavine with double stranded DNA (dsDNA) The electrochemical behaviour of MDB and proflavine as well as its interaction with double stranded (dsDNA) were investigated by cyclic (CV) and square wave voltammetry (SWV) and screen printed electrodes (ScPE). Furthermore, DNA-hybridization biosensors were developed for the detection of hybridization between oligonucleotides, which was detected by studying changes in the voltammetric peaks of MDB (reduction peak at -0.251 V) and proflavine (reduction peak at 0.075 V). MDB and proflavine were found to intercalate between the base pairs of dsDNA and oligonucleotides. Several factors affecting the dsDNA or oligonucleotides immobilization, hybridization and indicator preconcentration and interaction time, were investigated. As a result of the interaction of MDB with dsDNA and hybridized oligonucleotides, the voltammetric signals of MDB increased. Furthermore, guanine's oxidation peak (at 0.901 V) was decreased as MDB's concentration was increased. As a result of the interaction of proflavine with dsDNA and hybridized oligonucleotides, the voltammetric signals of proflavine decreased. These results were similar for carbon paste and screen printed electrodes. A comparison of the performance between CPE and ScPE was done. Our results showed that lower concentrations of MDB and proflavine were detected using screen printed electrodes. Moreover, reproducibility was better using screen printed electrodes and the detection was faster (regarding the experimental steps), but they are more cost effective.

Keywords: *Meldola's blue • Proflavine • DNA hybridization • Electrochemical DNA biosensor • Screen printed electrode*

1. Introduction

The development of DNA hybridization biosensors holds great promise for obtaining sequence-specific information in connection with clinical, environmental or forensic investigations. Nucleic acid hybridization is a process in which inconsonant nucleic acid strands with specific organization of nucleotide bases exhibiting complementary pairing with each other under specific given reaction conditions, thus forms a stable duplex molecule. This phenomenon is possible because of the biochemical property of base-pairing, which allows fragments of known sequences to find complementary matching sequences in an unknown DNA sample [1]. DNA hybridization biosensors can be employed for determining early and precise diagnosis of infectious agents in various environments [2,3] and these devices can be exploited for monitoring sequence-specific hybridization events directly [4,5] or by DNA intercalators (metal coordination complexes, antibiotics, *etc.*) which form complexes with the nitrogenous bases of DNA [6-12]. Electrochemical transducers offer a very attractive route for converting the hybridization event into a useful analytical signal [13-16].

The interaction between DNA and other molecules is an important fundamental issue in life sciences because it is related to the mutation of genes, action mechanisms of some DNA-targeted drugs, origins of some diseases and action mechanisms of some synthetic chemical nucleases, *etc*. The mutations responsible for numerous inherited human disorders are now known and this knowledge is steadily increasing as the sequencing of the human genome continues. Pathogens responsible for disease states, bacteria and viruses, are also detectable via their unique nucleic acid sequences. Such recent advances in molecular biology and biotechnology have set the stage for exciting

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possibilities for DNA-based electrochemical biosensors. Unlike enzymes or antibodies, nucleic acid recognition layers are very stable, and can be readily synthesized or regenerated for repeated use. Such recognition layers add new and unique dimensions of specificity to our arsenal of electrochemical biosensors, and should play a major role in the future point-of-care analysis.

The detection of specific DNA sequences provides the basis for detecting a wide variety of microbial and viral pathogens. Traditional methods for DNA sequencing, based on the coupling of electrophoretic separations and radio isotopic (^{32}P) detection, are labour intensive and time consuming, and are thus not well suited for routine and rapid medical analysis, particularly for point-of-care tasks. Electrochemical hybridization biosensors for the detection of DNA sequences may greatly reduce the assay time and simplify its protocol by eliminating electrophoretic separations which uses carcinogenic ethidium bromide and radioisotopic detection. Such fast on-site monitoring schemes are required for quick preventive action and early diagnosis.

Meldola's Blue (7-dimethyl-amino-1,2-benzophenoxazinium) is a member of the family of phenoxazines which are structurally similar with phenothialines [17]. It has been widely used in the development of enzymatic biosensors serving as redox mediator of NADH oxidation. Moreover, MDB is a well known DNA intercalator thus serving as a label in DNA hybridization biosensors.

K. Kerman, D. Ozkan, M.Ozsoz *et al*. [17] developed a method for the interaction study between dsDNA, ssDNA and oligonucleotides using (HMDE) (CPE) and (GCE). K. Kerman *et al.* [18] studied DNA hybridization using magnetic beads.

Proflavine belongs to the family of aminoacridines and interacts with DNA with a very complex mechanism. The most potent interaction proved to be the intercalation of the chromophore of proflavine between DNA base pairs.

Dasgupta *et al.* [19] used spectrophotometric methods to study the Profl/DNA interaction. Vanickova, Labuda *et al.* [20] used differential pulse voltammetry and carbon paste electrodes in order to study the behavior of acridine derivatives with DNA. They also compared their results with fluorimetry. Aslanoglu [21] studied DNA proflavine interaction using voltammetry and UV-Vis spectrometry and viscometry

In our laboratory, [22] voltammetry along with carbon paste and hanging mercury drop electrodes was used for the electroanalytical determination of proflavine. In the present work we compare the interaction of proflavine and Meldola's blue with DNA and oligonucleotides in order to apply proflavine and meldola's blue as DNA hybridization labels.

2. Experimental Procedure

2.1. Reagents

Double-stranded calf thymus DNA (Catalog No D-1501, highly polymerised, 41.9% G:C) (3,6 diaminoacridine hydrochloride, 131105 Sigma-Aldrich) proflavine and Meldola's blue were purchased from Sigma (www.sigmaaldrich.com).

Synthetic oligonucleotides were obtained as lyophilized powders (Sigma-Aldrich) purity desalted:

Oligo 1: 5'-AAT-GTG-CTC-CCC-CAA-CTC-CTC (MW: 6262)

Oligo 2: 5'-GAG-GAG-TTG-GGG-GAG-CAC-ATT (MW: 6591)

Oligo 3: 5'-AAT-GTG-GTC-CCC-CAA-CTC-CTC (MW: 6302)

Oligo 4: 5'-AAC-GTG-TGA-ATG-ACC-CAG-TACT (MW: 6439)

Oligo 2 (probe), oligo 1 complementary, target, oligo 3 one base mismatch, oligo 4, non complementary.

The stock solution of dsDNA (1 g L^{-1}) was prepared in a solution of 10 mM Tris–HCl and 1 mM EDTA at pH 8.0. Stock solutions of proflavine (10^{-1} M) were prepared in doubly distilled water and then diluted accordingly just before use. All other reagents used were of analytical grade.

2.2. Apparatus

Differential pulse and alternating current voltammetric measurements were performed using a PalmSens potentiostat purchased from IVIUM Technologies (The Netherlands, www.ivium.nl) and PalmSensPC software.

The screen printed electrodes (ScPE) were prepared using a DEK 248 screen printer (Weymouth, UK) using different inks obtained from Gwent Electronic. A graphite-based ink, C200802P2 a silver ink, C61003P7 and an insulating ink, D2070423D5. The substrate was a polyester flexible film obtained from Mc Dermid.

2.3. Procedures

2.3.1. Interaction of surface-confined DNA/oligos with MDB / proflavine

The procedure consists of DNA/oligos immobilization, interaction of MDB/ proflavine with immobilized DNA/oligos and transduction by transfer voltammetry with differential pulse mode. Prior to each medium exchange, the electrode was rinsed carefully with supporting electrolyte for 5 s. After the pre-treatment of the screen printed electrode, as previously described, the nucleic acid was subsequently immobilized onto the activated electrode surface by adsorptive accumulation for 5 min at +0.5 V. The DNA/oligos-coated electrode

was transferred to the stirred sample solution (analyte plus supporting electrolyte) for the optimal interaction time. The transduction was carried out in blank solution (only supporting electrolyte) with differential pulse voltammetry and the following conditions: E_{begin} = 0.1 V, E_{end} = 1.5 V, E_{step} = 0.005 V, E_{pulse} = 0.025 V, scan rate = 0.01 V s^{-1} and t_{pulse} = 0.07 s. The interaction between immobilized DNA/oligos and increasing concentrations of MDB/proflavine in solution was studied.

3. Results and Discussion

3.1. Redox study of meldola's blue

The redox behaviour of MDB was studied at the screen printed electrode surface, in PBS 0,05 M (+ 20 mM NaCl), pH 7, at a concentration level of 10^{-4} M. A cyclic voltammogram is shown in Fig. 1. MDB gives an oxidation peak at -0.3 V and a reduction peak at -0.18 V.

3.2. Interaction study between dsDNA and MDB

The oxidation and the reduction peak of MDB increases after its interaction with dsDNA. The increase in the voltammetric signal of MDB showed that hybridization occurred and the planar phenoxazine ring of MDB could easily intercalate into the double strands of hybrid. When the aromatic ring intercalated into DNA, the electrochemically active centre of MDB was not enveloped by the bulky DNA molecule and was available for redox activity [23].

Regarding the oxidation peak of guanine a decrease was observed, while adenine oxidation peak increased, after its interaction with DNA, a fact which is probably due to their specific interaction characteristics with DNA.

3.3. Interaction study between oligos and MDB

A decrease in the reduction peak of MDB, Fig. 3, was observed in the range:

Oligo (2+1, complementary) > Oligo (2+3, one base mismatch) > MDB > Oligo(2+4, non complementary) > Oligo(2, probe)

MDB can give valuable information as it proves to possess a behaviour similar to that of a hybridization label. The hybrid formed in the case of complementary oligo sequences is comparable with that of dsDNA with MDB. The case of the single-base mismatch containing oligonucleotide indicates that complete hybridization could not be accomplished. Moreover, a relatively higher peak than the signal of probe was observed following the exposure of the probe-modified ScPE to the noncomplementary oligonucleotide. This behaviour could be explained by the partial hybrid formation of the noncomplementary oligonucleotide on the surface of the electrode [24].

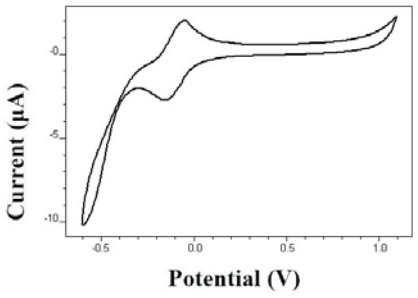

Figure 1. Cyclic voltammogram of MDB.

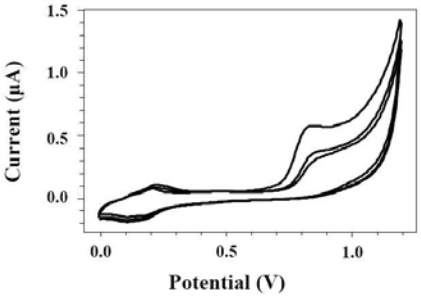

Figure 2. Cyclic voltammogram of proflavine.

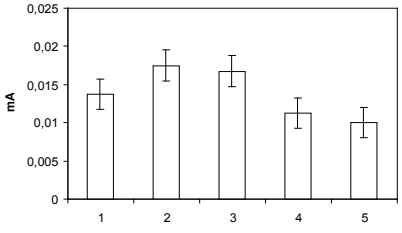

Figure 3. Dependence of the reduction peak of MDB after its interaction with oligos 1- MDB; 2 - Oligo (2+1, complementary); 3 - Oligo (2+3, one base mismatch); 4 - Oligo(2+4, non complementary); 5 - Oligo(2, probe).

3.4. Redox study of proflavine

Proflavine gives an oxidation peak at 0.775 V and a reduction peak at 0.075 V, which are concentration dependent (5 – 100×10^{-8} M). At higher concentrations the reduction peak splits into two. A cyclic voltammogram is shown in Fig. 2.

3.5. Interaction study between dsDNA and profl

The interaction study between the immobilized at the ScPE surface dsDNA and proflavine was evaluated on the basis of the changes of the peak height of redox peaks of proflavine as well as the oxidation peaks of adenine and guanine.

The reduction peak of proflavine decreased after its interaction with proflavine.

Similar results were obtained upon the oxidation peak of proflavine with dsDNA.

The decrease revealed that the structure of DNA has changed and guanine residues were not accessible to such an extent at the electrode surface [24].

3.6. Interaction study between oligos and profl

A decrease in the reduction peak of proflavine, Fig. 4, was observed in the range:

Profl > Oligo(2+3, one base mismatch) > Oligo(2+1, complementary) > Oligo(2+4, non complementary) > Oligo(2, probe)

Regarding the oxidation peak of proflavine, Fig. 5, a decrease was observed in the range:

Profl>Oligo(2+3, one base mismatch)>Oligo(2+1, complementary) > Oligo(2+4, non complementary) > Oligo(2, probe)

The relatively higher peak than the signal of probe was also observed following the exposure of the probe-modified ScPE to the noncomplementary oligonucleotide. This fact also means that the noncomplementary oligonucleotide can form a partial hybrid on the surface of the electrode.

As it can be concluded from this interaction study, profl cannot give adequate information regarding the DNA hybridization event.

As it can be concluded MDB proves to be most promising label in DNA hybridization biosensors, differentiating in a most efficient way the complementary compared to non complementary oligonucleotides.

Moreover, the subsequent experiments (6 in each experiment) for the detection of hybridization gave reproducible results as shown in the histograms of Figs. 3-5 with a RSD of 5.10, 5.30 and 5.70%, respectively.

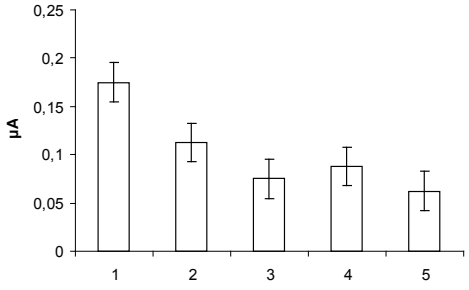

Figure 4. Dependence of the reduction peak of proflavine(profl) after its interaction with oligos 1 – Profl; 2 - Oligo(2+3, one base mismatch); 3 - Oligo(2+1, complementary); 4 - Oligo(2+4, non complementary); 5 - Oligo(2, probe).

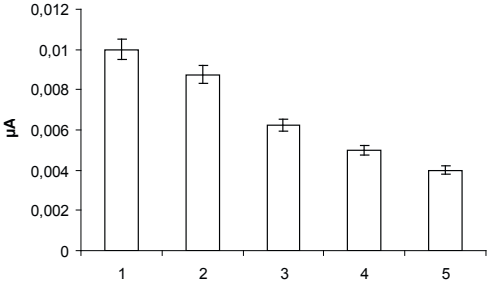

Figure 5. Dependence of the oxidation peak of profl after its interaction with oligos 1 – Profl; 2 - Oligo(2+3, one base mismatch); 3 - Oligo(2+1, complementary); 4 - Oligo(2+4, non complementary); 5 - Oligo(2, probe).

4. Conclusions

In the present study we describe a comparison between MDB and Profl as hybridization labels in the development of DNA hybridization biosensors at screen printed electrode surfaces. MDB proves to possess more informative characteristics regarding the hybridization event.

As it refers to the perspectives of the current study we could conclude by pointing out the following research trends: a) Development of a DNA hybridization biosensor, as a tool, for the detection of infectious and inherited diseases. b) Application of screen printed electrodes (low sample consumption, improved analytical characteristics regarding, accuracy and reproducibility) and c) Study of chemical compounds having affinity for DNA (toxic effects, potential application in therapeutic protocols).

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