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Short Communication

A Recent Approach for Monitoring Interactions Between Methylene Blue and DNA Using Carbon Fiber Based DNA Biosensor

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This paper reports a systematic and detailed investigation on the interactions between methylene blue (MB) and DNA immobilized onto carbon fiber microelectrode (CFME). In this study, designed CFME based DNA biosensor was successfully carried out considering three different hybridization mechanisms to obtain more specific and selective results. The consistency of the results was tested with the various probe configurations (unlabeled and amino-labeled). The voltammetric signals of MB were measured single stranded DNA (ssDNA)-modified CFME and double stranded DNA (dsDNA)-modified CFME by means of square wave voltammetry (SWV). The electrochemical parameters for MB on binding to DNA onto single CFME in the solution and at the electrode surface were described. This study shows that before or after hybridization, MB accumulation is related with probe configuration. It provides very useful information for developing carbon fiber based sequence-specific electrochemical DNA sensors.

Keywords: DNA hybridization mechanism; carbon fiber microelectrode; methylene blue.

1. INTRODUCTION

The detection of specific DNA sequences has been of great interest for a long time due to its significance in many areas including clinical, food, biological warfare agent and environmental analysis. Binding mechanism of redox-active molecules with DNA have been detected as one important topic to understand the mechanism of action or toxicity of different pollutants and drugs [1-3].

Electrochemical redox-active molecules capable of binding with different affinity to ssDNA and dsDNA are of particular interest for electrochemical analysis of DNA sequences [4,5]. It is widely

known that MB, an aromatic heterocycle molecule, is often utilized as an electrochemical redox indicator toward selective discrimination of ssDNA and dsDNA [3,5-15]. Previous studies have indicated that MB binds to DNA through at least three different interactions; electrostatic interaction between cationic MB and anionic DNA structure, intercalation of MB in the DNA double helix and preferential binding between MB and guanine bases. Ozsoz et al. have reported ssDNA modified carbon electrodes produced large electrochemical signals for MB while hybridization led to considerable signal reduction [16].

DNA immobilization on the transducer has an important role in the performance of the DNA biosensors. CFMEs can experience much higher current densities during electrochemical pretreatment that enhances electron transfer reactivity [17]. Because of its simple fabrication and high sensitivity, the CFME is widely used during the in vivo experiments [18]. Despite their many advantages for monitoring DNA and RNA in microliter samples, the use of carbon fibers for the analysis of nucleic acids has not yet been sufficiently reported.

In the literature, only after hybridization mechanism (probe+cDNA+MB) has been applied in the DNA biosensor studies containing MB as hybridization indicator [5-15]. However, different mechanisms are also possible and need to be investigated.

This paper focuses on a recent approach for monitoring interactions between MB and DNA. Various probe configurations (labeled and unlabeled) were designed. Relationship between MB binding mechanism and designed probes was analyzed in detail. Three different hybridization mechanisms were developed between MB and DNA to obtain more specific and selective results. For this, MB was accumulated on ssDNA and dsDNA in three different ways: i) just before hybridization, ii) before and after hybridization, iii) only after the hybridization. The consistency of the results was tested with the different designed probes.

2. EXPERIMENTAL

2.1. Materials

The synthetic oligonucleotides were obtained from Avetra Bioscience (Mountain View, CA, USA); their base sequences were:

- Amino-labeled ssDNA, probe1 (18-base sequence): 5'-NH_2-GCTGCCTCCCGTAGGAGT-3'
- Complementary target (18-base sequence): 5'-ACTCCTACGGGAGGCAGC-3'
- Noncomplementary target (18-base sequence): 5'-CTCTCTCTCTCTCTCTCT-3'
- Unlabeled ssDNA, probe2 (18-base sequence): 5'-GCTGCCTCCCGTAGGAGT-3'
- Complementary target (18-base sequence): 5'-ACTCCTACGGGAGGCAGC-3'
- Noncomplementary target (18-base sequence): 5'-CTCTCTCTCTCTCTCTCT-3'
- Amino-labeled ssDNA, probe3 (15-base sequence): 5'-NH_2-TTGAACCATCCACCA-3'
- Complementary target (20-base sequence): 5'-TGGTGGATGGTTCAATCATG-3'
- Noncomplementary target (18-base sequence): 5'-CTCTCTCTCTCTCTCTCT-3'

All oligonucleotides, dsDNA and ssDNA stock solutions (100 ppm) were prepared with TE solution (20 mM Tris-HCl, 100 mM EDTA, pH 7.4) and kept frozen.

1-[3-(dimethyl amino) propyl]-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysulfo succinimide sodium salt (NHS), methylene blue (MB), tris(hidroksimetil)aminometan and gold (III) choloride trihydrate were purchased from Sigma-Aldrich and reagents were all of analytical reagent grade. All H_2O used in the preparation of buffers and for rinse solutions had a resistivity of 18.2 mΩ, as producted by Millipore Elix 5 UV and Milli-Q Gradient ultra-pure water system. The stock solution of 5 mM EDC and 8 mM NHS was prepared in 50 mM phosphate buffer solution (PBS), pH 7.40. Hybridization is carried out in the DNA hybridization buffer containing 50 mM Tris-EDTA-HCl and 100 mM NaCl, (pH 7.4, 25 $^{\circ}$C). All the buffer solutions contained 20 mM NaCl.

High Strength (HS) carbon fibers C320000A (CA) (Sigri Carbon, Meitingen, Germany) containing 320,000 single filaments carbon were used to fabricate carbon fiber microelectrodes.

2.2. Instrumentation

Electrochemical measurement is performed on the electrochemical workstation (CHI 842B, CHI Instruments Inc., USA) in a typical three-electrode system with an Ag/AgCl electrode (saturated with KCl) as the reference electrode, a platinum wire as the counter electrode and a carbon fiber microelectrode (CFME) as the working electrode.

2.3. Experimental procedures

In this study, three different hybridization mechanisms were tested. MB was accumulated on ssDNA and dsDNA in three different ways; 1) before hybridization (probe+MB+cDNA), 2) before and after hybridization (probe+MB+cDNA+MB) and 3) after hybridization (probe+ cDNA+ MB) (Fig. 1). The most discriminative MB signals were investigated among the mechanisms. The consistency of the results was tested with the different designed probes in two approach. First, amino-labeled and unlabeled ssDNA probes (probe1 and probe2) containing the same base sequence were used to determine label effect on the mechanism. Then, different base sequence containing ssDNA probes (probe1 and probe3) with the same label was used to determine base sequence effect on the mechanism.

2.3.1. Preparation of CFMEs

All of the CFMEs were prepared by using single CFME (diameter ~7μm) attached to a copper wire with a Teflon tape. A half centimeter of the CFME was immersed into the solution to keep the electrode area constant (~ 0.0011 cm^2) and the rest of the electrode was covered with a Teflon tape [19].

2.3.2. Pretreatment of carbon fiber surfaces

The fabricated CFMEs were first consecutively sonicated in acetone, 3 M HNO_3, 1.0 M KOH, and distilled water each for 3 min. Then, the electrodes were subjected to electrochemical activation, first with potential-controlled amperometry at +2.0 V for 60 s, at -1.0 V for 20 s, and then with cyclic voltammetry in 0.5 M H_2SO_4 within a potential range from 0 to 1.0 V at a scan rate of 0.1 V s^{-1} until a steady cyclic voltammogram was obtained [20].

2.3.3. Immobilization of ssDNA on modified CFME

CFME was immersed in a solution containing 8 mM sulfo-NHS and 5 mM EDC in 50 mM PBS (pH 7.40) 48 h. After air-drying of this solution, the electrode was rinsed with distilled water. After rinsing with distilled water, the electrode was transferred into 100 mM PBS containing 10 ppm (µg/mL) ssDNA probe1, probe2 and probe3 for 30 min. with gentle shaking at room temperature. Then, the electrode was rinsed with distilled water.

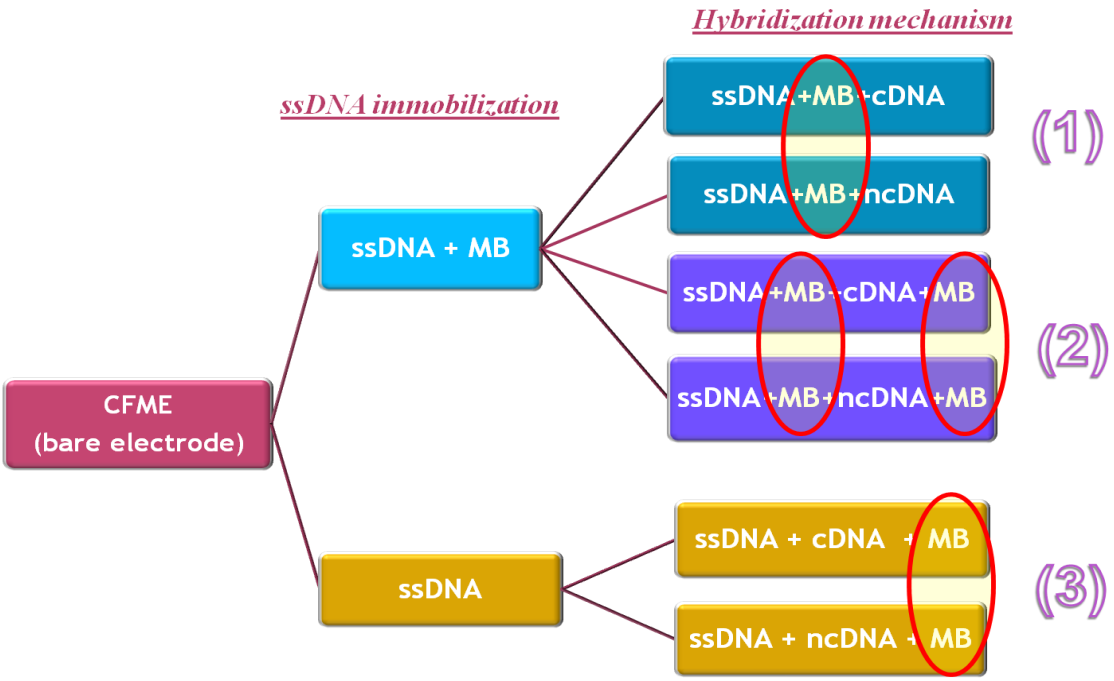


Figure 1. Process diagram for DNA biosensor according to the proposed hybridization mechanisms. MB was accumulated on ssDNA and dsDNA in three different ways; 1) before hybridization (probe+MB+cDNA), 2) before and after hybridization (probe+MB+cDNA+MB) and 3) after hybridization (probe+cDNA+MB).

2.3.4. Hybridization

For the DNA hybridization, ssDNA modified CFMEs were incubated with 10 ppm target DNA (complementary and noncomplementary) in the hybridization buffer at 47 ˚C, 120 min.

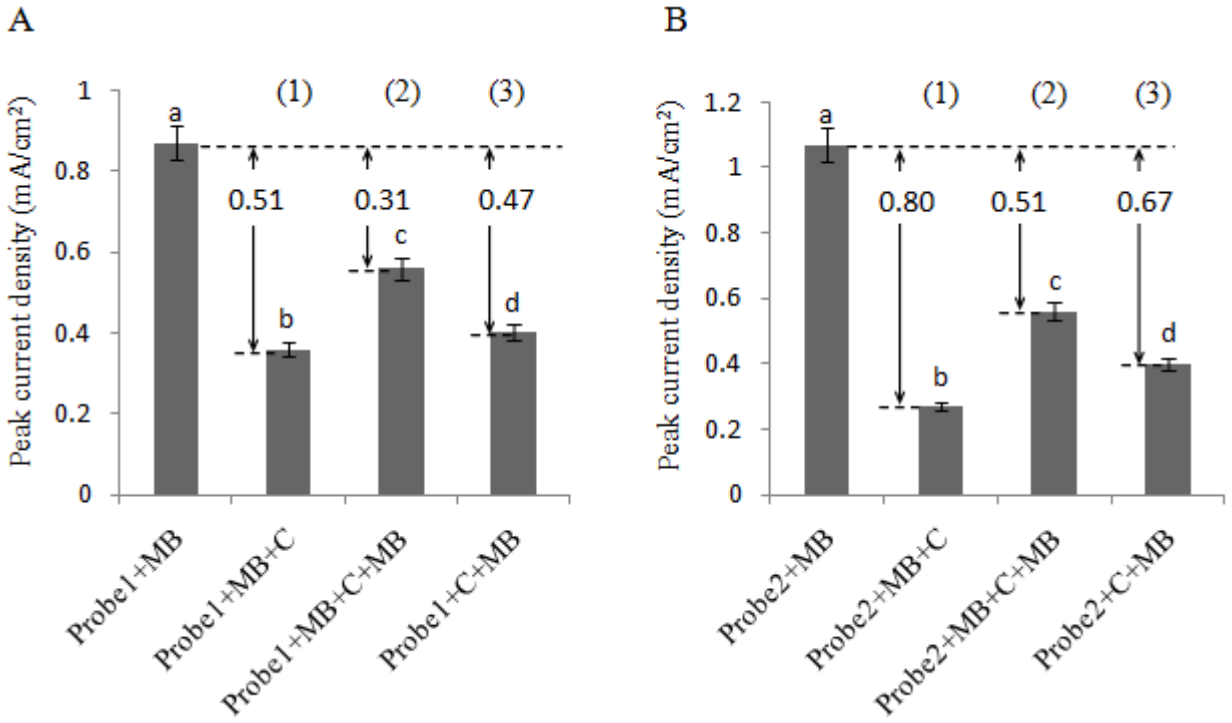
2.3.5. MB accumulation and voltammetric transduction

MB was firstly accumulated onto the ssDNA and the DNA hybrid coated electrode surfaces by immersing the electrode into the stirred 50 mM TRIS-HCl containing 20 μM MB for 5 min. The electrode was then transferred into the blank 50 mM Tris-EDTA HCl buffer solution (pH 7.4, 25 ˚C) with 100 mM NaCl for the voltammetric measurement. MB is a redox indicator with the formal potential in the range of 0 to -0.6 V. After accumulation of MB, electrochemical measurements were performed [21]. Triplicate measurements were carried out by renewing the surface and repeating the above assay preparation procedure.

3. RESULTS AND DISCUSSION

3.1. Investigation of MB signal between ssDNA and dsDNA to determine mechanism of MB binding

For amino-labeled ssDNA (probe1), the peak current density differences (ΔIp) were 0.51 mA/cm^2, 0.31 mA/cm^2, 0.47 mA/cm^2 before hybridization (probe+MB+cDNA), before and after hybridization (probe+MB+cDNA+MB) and after hybridization (probe+cDNA+MB), respectively (Fig. 2A). For unlabeled ssDNA (probe2), the peak current density differences (ΔIp) were 0.80 mA/cm^2, 0.51 mA/cm^2, 0.67 mA/cm^2 for the three hybridization mechanisms, respectively (Fig. 2B). For amino-labeled ssDNA (probe3), the peak current density difference (ΔIp) was 0.68 mA/cm^2 before hybridization 0.48 mA/cm^2 before and after hybridization and 0.55 mA/cm^2 after hybridization (Fig. 2C).



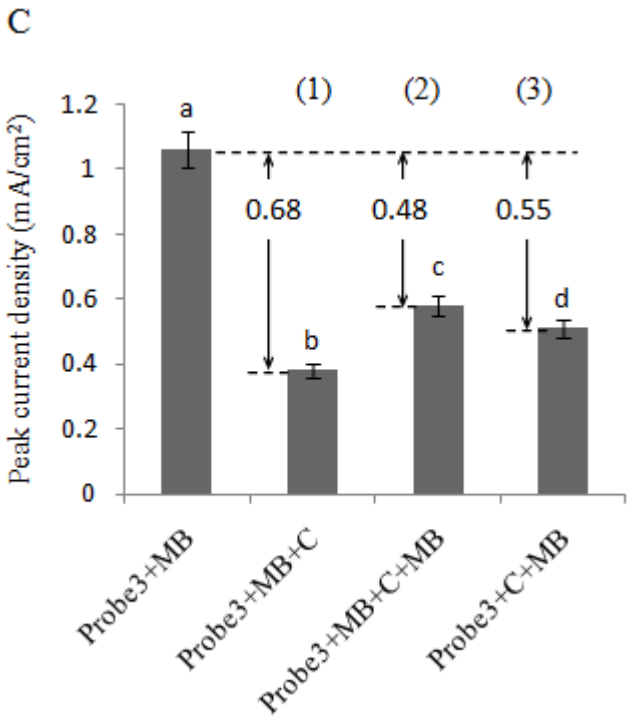


Figure 2. Histogram of SWV signals for (A) amino-labeled ssDNA probe1 (18-base sequence), (B) unlabeled ssDNA probe2 and (C) amino-labeled ssDNA probe3 (15-base sequence). For three different mechanisms MB accumulation at (a) probe DNA modified CFME, (b) before hybridization, (c) before and after hybridization, and (d) after hybridization. MB accumulation: 5 min in 20 mM Tris-HCl buffer (pH 7.4) including 20 μM MB. Measurement of accumulated MB in 50 mM Tris-HCl, 100 mM NaCl buffer (pH 7.4). Error bars show the standard deviation of three experiments.

For all probes, the first mechanism (probe+MB+cDNA) was the most dicriminative. It was found that the peak current densities of MB at ssDNA were considerably higher than at dsDNA. In addition, different DNA labels (amino-labeled and unlabeled) and DNA base sequences (18 base sequence and 15 base sequence) didn't change the MB binding mechanism to DNA.

3.2. Selectivity of the DNA biosensors

In this study, the selectivity of these DNA biosensors was also evaluated using noncomplementary and complementary DNA base sequences. Table 1 shows the peak current densities for probe DNA hybridized with its noncomplementary and complementary base sequence. The peak current density differences ($\Delta Ip = Ip_{ssDNA} - Ip_{dsDNA}$) were 0.05 mA/cm^2 and 0.51 mA/cm^2 for probe1, 0.06 mA/cm^2 and 0.80 mA/cm^2 for probe2, 0.03 mA/cm^2 and 0.68 mA/cm^2 for probe3, respectively. Because hybridization did not happen effectively due to the sequence mismatch between the modified ssDNA and the noncomplementary base sequence there was not considerable current density difference change for the ssDNA modified CFME and its hybridization with noncomplementary base sequence. This implies that the surface characteristics of the ssDNA modified CFME was not altered

after its interaction with noncomplementary base sequence. On the other hand, when the ssDNA modified CFME interacted with the complementary target sequence in solution, the peak current density for the MB reduction decreased remarkably. This decrease in current density obviously demonstrated that the ssDNA modified on the CFME effectively hybridized with its complementary target sequence, reducing the intercalation level of MB on the modified CFME because of the steric inhibition effect of MB packing. These results show that the fabricated DNA biosensor can distinguish noncomplementary and complementary target DNA.

Table 1. The peak current density difference (ΔIp) for different DNA sequences

types of probe		probe sequence	noncomplementary sequence (ncDNA)	complementary sequence (cDNA)
Probe1[a]	Ip (mA/cm^2)	0.87	0.82	0.36
	ΔIp*(mA/cm^2)		0.05	0.51
Probe2[b]	Ip (mA/cm^2)	1.07	1.01	0.27
	ΔIp*(mA/cm^2)		0.06	0.80
Probe3[c]	Ip (mA/cm^2)	1.06	1.03	0.38
	ΔIp*(mA/cm^2)		0.03	0.68

* $\Delta Ip = Ip_{ssDNA} - Ip_{dsDNA}$

[a] Amino-labeled ssDNA (18-base sequence)

[b] Unlabeled ssDNA (18-base sequence)

[c] Amino-labeled ssDNA (15-base sequence)

3.3. Analytical performances of the biosensors

Under the optimal conditions, the analytical performance of the fabricated DNA biosensors was investigated using the probe DNA to hybridize with the various concentrations of DNA sequences. Fig. 3 shows the SWVs of the probe modified electrode at different complementary target DNA concentrations. The optimum DNA target concentration was determined as 10 ppm for amino-labeled probe1 and probe3 in Fig. 3A,C since the minimum MB signal was seen while the probe-modified CFME was subjected to 10 ppm target-including solution. Therefore, it was thought that complete coverage of the electrode surface with the hybrid was formed with the complementary target. The best DNA target level was 15 ppm for unlabeled probe2 in Fig. 3B because the minimum MB signal was determined once the probe-modified CFME was subjected to 15 ppm target including solution.

The calibration curve showed that the peak current density values decreased as the concentrations of the complementary target DNA increased, and it presented good linearity with the concentration of the complementary target DNA from 2 to 10 ppm, with a regression equation of $Ip(mA/cm^2) = -0.0396C(ppm) + 0.771$, $R^2 = 0.9949$. The detection limit for the target DNA was determined as 1.55 pm from S/N = 3 for amino-labeled probe1 in Fig. 3A. The peak current density de-

creased as the concentrations of the complementary target DNA increased, and it presented good linearity with the concentration of the complementary target DNA from 0.5 to 15 ppm, with a regression equation of Ip(mA/cm^2) = -0.0197C(ppm) + 0.972, R^2 = 0.9469. The detection limit for the target DNA was calculated as 3.11 ppm for unlabeled probe2 from S/N = 3, in Fig. 3B. The peak current density values decreased as the concentrations of the complementary target DNA

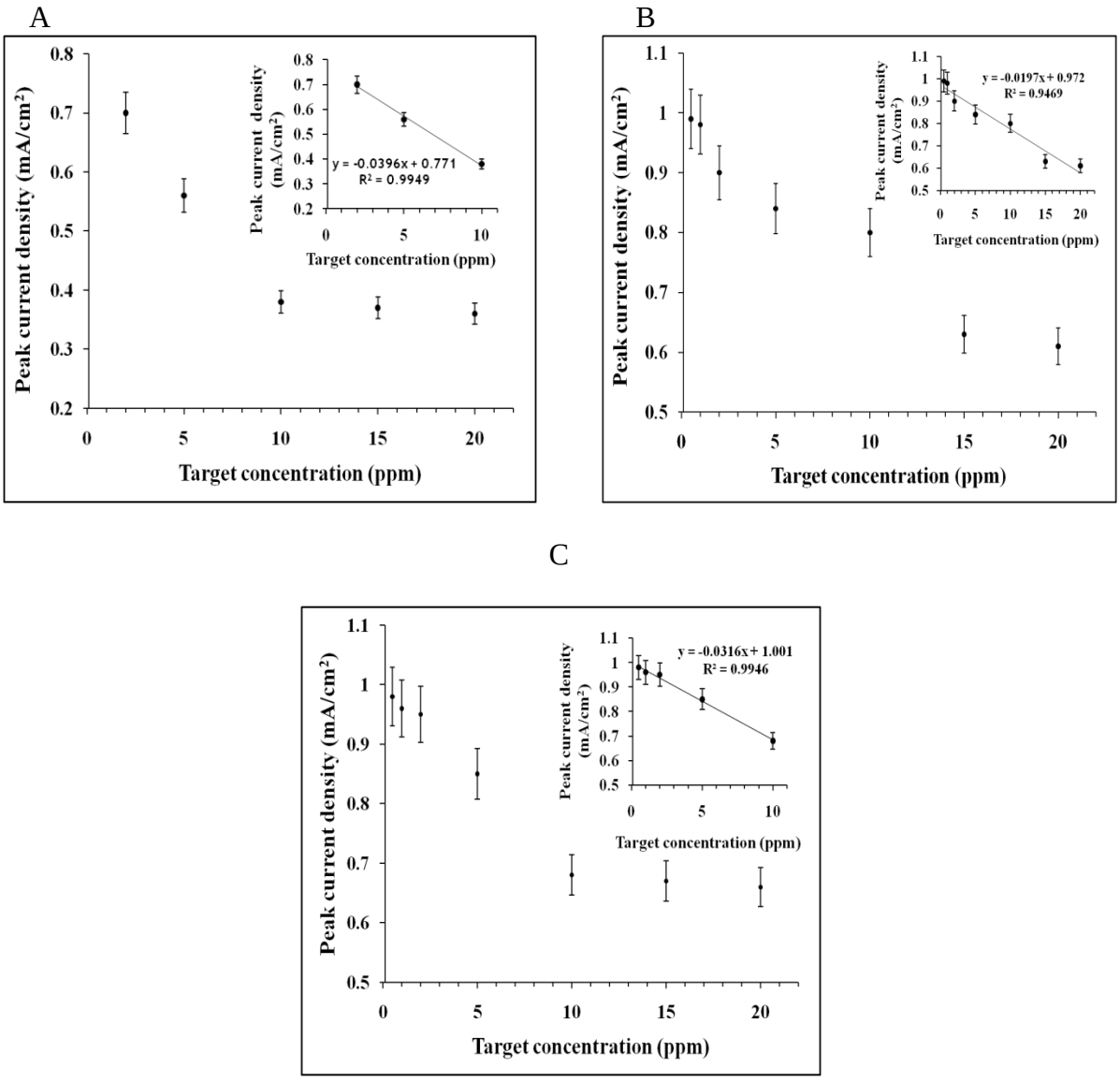


Figure 3. Calibration plots of MB peak current density against DNA target concentration for (A) amino-labeled ssDNA probe1(18-base sequence), (B) unlabeled ssDNA probe2 and (C) amino-labeled ssDNA probe3 (15-base sequence). MB accumulation: 5 min in 20 mM Tris-HCl buffer (pH 7.4) containing 20 μM MB. Measurement of accumulated MB in 50 mM Tris-HCl, 100 mM NaCl buffer (pH 7.4). Error bars show the standard deviation of three experiments.

increased, and it presented good linearity with the concentration of the complementary target DNA from 0.5 to 10 ppm, with a regression equation of Ip(mA/cm^2) = -0.0316C(ppm) + 1.001, R^2 = 0.9946. The detection limit for the target DNA, estimated from S/N = 3, correspond to 0.72 ppm for amino-labeled probe3 in Fig. 3C. A comparison between biosensors used in this study and previously reported

DNA biosensors based on different electrode types using MB as hybridization indicator was shown in Table 2. Although the results for biosensor performance seems to be comparable to the performance of the reported results by Wang et al. [22], in the recent researches some glassy carbon, screen printed carbon paste electrode configurations showed very high sensitivity, low detection limit and linear range [23-25]. The objective of the study was to investigate the potential performance and the design possibilities of a CFME based DNA biosensor by using MB as a hybridization indicator. The performance of the biosensor representing by the biosensor parameters which are sensitivity, regression coefficient, linear range and detection limit clearly indicated that CFME was capable to use as a mediated electrode for DNA biosensor. From this respect, the obtained experimental parameters can be developed through the further investigations, and the performance of CFME based DNA biosensors can be increased.

Table 2. Comparison between proposed electrochemical DNA biosensors based on CFMEs and other previously reported electrochemical DNA biosensors based on different electrode types using MB as hybridization indicator

DNA biosensor method	Sensitivity, μA/M	Regression coefficient, R^2	Linear range, M	Detection limit, M	Ref.
Thiolated DNA immobilized gold electrode	nd[d]	nd[d]	2.0×10^{-8} to 2.0×10^{-6}	1.0×10^{-8}	[22]
Based on glassy carbon electrode modified with gold nanoparticles and graphene	10.79	0.997	1.0×10^{-12} to 1.0×10^{-7}	2.0×10^{-13}	[23]
Based on screen printed carbon paste electrode genetically modified DNA	nd[d]	0.989	2.0×10^{-12} to 2.0×10^{-7}	1.4×10^{-13}	[24]
Based on a glassy carbon electrode modified with electropolymerized Eriochrome Black T	0.38	0.994	5.0×10^{-15} to 5.0×10^{-12}	0.11×10^{-15}	[25]
Probe1[a]-cDNA*	19×10^{7}	0.995	8.0×10^{-8} to 1.6×10^{-6}	1.55×10^{-6}	This work
Probe2[b]-cDNA*	10×10^{7}	0.947	2.0×10^{-8} to 2.4×10^{-6}	0.24×10^{-6}	This work
Probe3[c]-cD-NA*	15×10^{7}	0.995	2.0×10^{-8} to 1.6×10^{-6}	0.06×10^{-6}	This work

[a] Amino-labeled ssDNA (18-base sequence)

[b] Unlabeled ssDNA (18-base sequence)

[c] Amino-labeled ssDNA (15-base sequence)

***** Complementary sequence

[a] nd: not determined

4. CONCLUSION

We have presented a recent approach for monitoring interactions between methylene blue and DNA using carbon fiber based DNA biosensor. Three different hybridization mechanisms were developed between MB and DNA to obtain more specific and selective results. The consistency of the results was tested with the different designed probes. These results demonstrated that difference of the MB signal in first proposed mechanism (Probe+MB+C) was the most discriminative for amino-labeled DNA probes and unlabeled DNA probe. This study shows that before or after hybridization, MB accumulation is related to probe configuration. In this study, carbon fiber microelectrodes was used to reveal opportunities of material science.

References

1. I. Palchetti and M. Mascini, *Analyst* 133 (2008) 846.
2. S. Rauf, J. J. Gooding, K. Akhtar, M. A. Ghauri, M. Rahman, M. A. Anwar, A. M. Khalid and J. Pharmaceut, *J. Pharm. Biomed Anal.* 37, (2005) 205.
3. P. Arias, N. F. Ferreyra, G. A. Rivas and S. Bollo, *J. Electroanal. Chem.* 634 (2009) 123.
4. P. Santos-Álvarez, M. J. Lobo-Castanon, A. J. Miranda-Ordieres and P. Tunon-Blanco, *Anal. Bioanal. Chem.* 378 (2004) 104.
5. E. Farjami, L. Clima, K. V. Gothelf and E. E. Ferapontova, *Analyst* 135, (2010) 1443.
6. A. Erdem, K. Kerman, B. Meric, U. S. Akarca and M. Ozsoz, *Analytica Chimica Acta* 422 (2000) 139.
7. A. Tani and A. J. Thomson, J. N. Butt, *Analyst* 126 (2001) 1756.
8. E. M. Boon, D. M. Ceres, T. G. Drummond, M. G. Hill and J. K. Barton, *Nat. Biotechnol.* 18 (2000) 1096.
9. W. Yang, M. Ozsoz, D. B. Hibbert and J. J. Gooding, *Electroanalysis* 14 (2002) 1299.
10. K. Kerman, D. Ozkan, P. Kara, B. Meric, J. J. Gooding and M. Ozsoz, *Anal. Chim. Acta* 462 (2002) 39.
11. V. Ostatna, N. Dolinnaya, S. Andreev, T. Oretskaya, J. Wang and T. Hianik, *Bioelectrocliemistry* 67 (2005) 205.
12. Y. Jin, X. Yao, Q. Liu and J. H. Li, *Biosensors & Bioelectronics* 22 (2007) 1126.
13. L. Zhu, R. Zhao, K. Wang, H. Xiang, Z. Shang and W. Sun, *Sensors* 8 (2008) 5649.
14. J. Chen, J. Zhang, K. Wang, X. Lin, L. Huang and G. Chen, *Anal.Chem.* 80, (2008) 8028.
15. S. H. Zuo, L. F. Zhang, H. H. Yuan, M. B. Lan, G.A. Lawrance and G. Wei, *Bioelectrochemistry* 74 (2009) 223.
16. D. Ozkan, P. Kara, K. Kerman, B. Merica, A. Erdem, F. Jelen, P. E. Nielsen and M. Ozsoz, *Bioelectrochemistry* 58 (2002) 119.
17. B. Uslu and S. A. Ozkan, *Analytical Letters* 40 (2007) 817.
18. X. Jiang and X. Lin, *Analyst* 130 (2005) 391.
19. A. S. Sarac, E. Dogru, M. Ates and E.A. Parlak, *Turk J. Chem.* 30 (2006) 401.
20. M. Zhang, K. Liu, L. Xiang, Y. Lin, L. Su and L. Mao, *Anal. Chem.* 79 (2007) 6559.
21. X. Lin, P. Wu, W. Chen, Y. Zhang and X. Xia, *Talanta*, 72 (2007) 468.
22. J. Wang, F. Wang and S. Dong, *J. Electroanal. Chem.*, 626 (2009) 1.
23. H. Pan, H. Yu, N. Wang, Z. Zhang, G. Wan, H. Liu, X. Guan, D. Chang, *J. of Biotech.*, 214 (2015) 133.
24. A. Ulianas, L. Y. Heng, H. Y. Lau, Z. Ishak and T. L. Ling, *Anal. Methods*, 6 (2014) 6369.

25. L. Wang, X. Liao, Y. Ding, F. Gao, Q. Wang, *Microchim. Acta*, 181 (2014) 155.