Electrochemical methods in investigation of methylene blue interaction with DNA using screen printed graphite sensor

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Abstract: - Cyclic voltammetry, differential pulse voltammetry, and square wave voltammetry are used to investigate pre and post electrochemical signals of DNA or methylene blue (MB) interactions at screen printed graphite sensor. Strong interactions between MB and guanine are evidenced. Very week or no interactions between MB and adenine are observed. Electrochemical signals of MB suggest its mediating role in guanine oxidation reaction.

Key-Words: - dsDNA Biosensor, Methylene blue, DNA, Screen printed graphite sensor, Cyclic voltammetry, Differential pulse voltammetry, Square wave voltammetry, Guanine, Adenine.

1 Introduction

Many molecules show high affinity to nucleic acids as they interact with DNA by several mechanisms [1]. They can join nucleic acids and inhibit basic functions of the living cells such as: replication, transcription and translation. They can block interactions of DNA with specific, required proteins, or can prevent the DNA from adopting conformations required for biological functions. To analytical chemist, nucleic acids offer a powerful tool in recognition and monitoring of many important compounds, e.g. toxic molecules and anticancer agents [2, 3]. The DNA biosensor technologies are currently under intensive investigation owing to their great applicability in rapid and low-cost detection of specific DNA interactions. Electrochemical techniques are frequently used for the detection. Observing pre and post electrochemical signals of DNA or ligand molecule interaction provides good evidence for the interaction mechanism to be elucidated.

2 Problem Formulation

Methylene blue (MB) is a water soluble phenothiazine dye, and is known as a redox-active molecule interacting with double stranded DNA (dsDNA) in several ways. The DNA – MB interactions include: electrostatic interactions between positively charged MB and negatively charged sugar-phosphate DNA backbone, intercalation, especially in guanine – cytosine rich DNA regions, and interactions in DNA grooves, especially in guanine rich ones.

The goal of this work is to investigate the interactions of MB with dsDNA using home made screen printed three electrode system (Fig. 1) and various electrochemical techniques: cyclic voltammetry, differential pulse voltammetry, and square wave voltammetry.

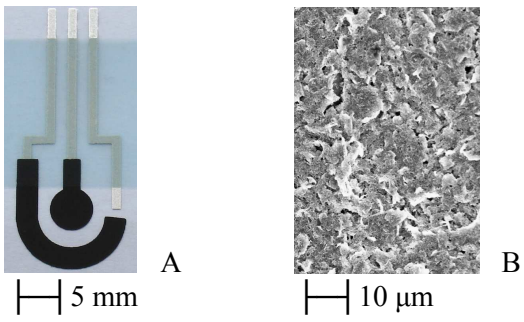

Fig.1. (A): Screen printed three electrode system with round graphite working electrode, 5mm in diameter, graphite counter-electrode and silver reference electrode. (B): Electron microscope image of working electrode surface.

3 Problem Solution

The explanation of the mechanism of interaction between MB and dsDNA is based on the electrochemical behavior of DNA in the absence and in the presence of MB as well as on the electrochemical behavior of MB in the absence and in the presence of DNA. The DNA presence is detected as signals due to oxidation of guanine and adenine and MB presence is detected as signals arising from oxidation or reduction of MB interacting with the DNA. The immobilization of DNA onto an electrode surface is in many ways the crucial aspect of the development of DNA biosensors for monitoring ligand interactions. It will dictate the accessibility of the DNA to the ligand in the solution and therefore will influence the affinity of ligand binding. In our case, dsDNA is immobilized on the surface of the electrode by electrostatic attraction enabling very effective electron transfer between the nucleic acid bases and the electrode. Due to the extended surface area, the screen printed graphite electrode is especially suitable to electrostatic adsorption (Fig. 1. (B)). After dsDNA immobilization, the electrode is rinsed with deionized water and then immersed into 10 mmol/L KCl in a pH 4.75 acetic acid buffer and electrochemical signals of oxidation of guanine and adenine are measured (blank). Alternatively, after rinsing, the electrode is immersed into a MB solution of given concentration in 10 mmol/L KCl and acetic acid buffer of pH 4.75, and after rinsing electrochemical signals of oxidation of guanine and adenine in buffered KCl solution are measured (sample). The signals obtained using these two procedures are compared and the difference detected proves the presence of MB interactions with DNA. Electrochemical signals of oxidation and reduction of MB intercalated into DNA are measured and compared with measurements of electrode modified only by MB adsorption. The difference detected proves the presence of MB interactions with DNA.

3.1 Cyclic voltammetry

The cyclic voltammetry technique was not sensitive enough to observe the oxidation of guanine and adenine bases. However, the observation of the electrochemical behavior of MB was possible. The dependence of peak current of MB oxidation and reduction on the concentration of MB is shown on Figs. 2 and 3, respectively. It can be seen that the MB signals increase with the MB concentration up to ca. 10 μM and then remain constant.

3.2 Differential pulse voltammetry

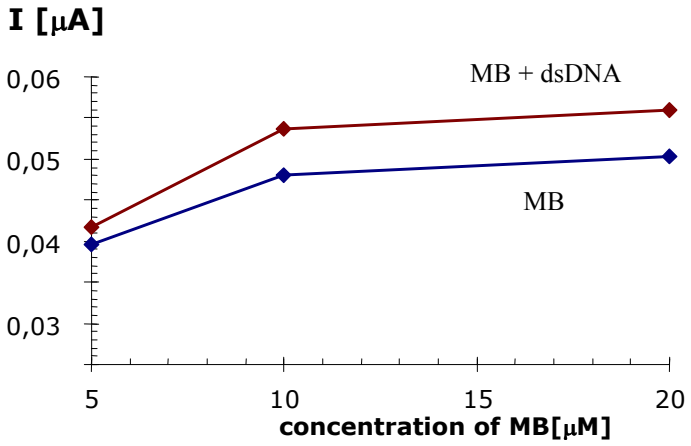

Fig. 2. The dependence of CV peak current of MB oxidation on the concentration of MB.

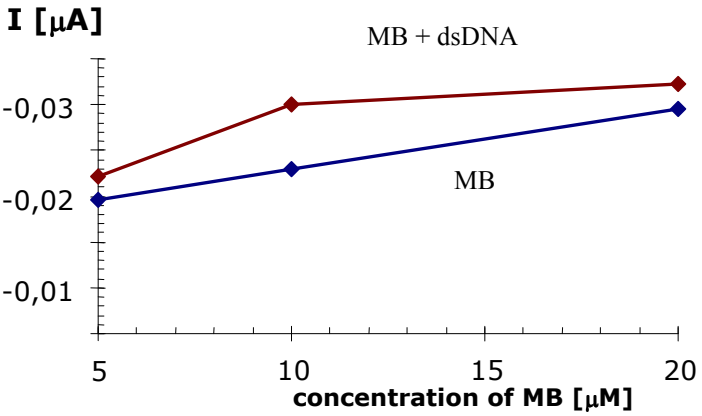

Fig. 3. The dependence of CV peak current of MB reduction on the concentration of MB.

The differential pulse voltammetry allowed for the observation of the DNA - MB interactions when using both procedures, i.e. detection of DNA and MB signals. The percentage changes of the oxidation of guanine and adenine bases signals after immersing in buffered MB solution in comparison with blank solution can be given according the following equation:

$$S_{G/A} = (S^{sample}{}_{G/A} / S^{blank}{}_{G/A}) \cdot 100\% \quad (1)$$

where: $S^{sample}{}_{G/A}$ and $S^{blank}{}_{G/A}$ are values of peak current of guanine or adenine oxidation in sample and blank, respectively. These changes with MB concentration changes in the sample solution are presented in Fig. 4.

The dependence of peak current of MB oxidation and reduction on the concentration of MB is shown in Fig. 5. Both methods of detection of DNA – MB interactions give comparable results, showing an increase of the signals at least to ca. 10 μM of MB.

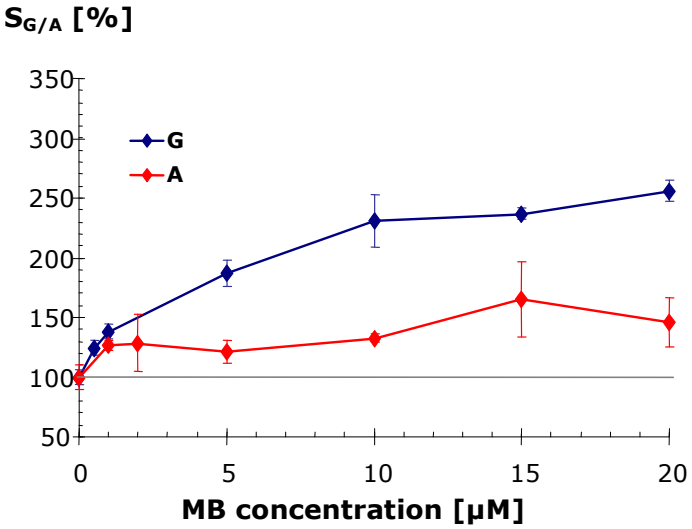

Fig. 4. Changes of guanine and adenine oxidation DPV peak current in dependence on MB concentration in the sample solution.

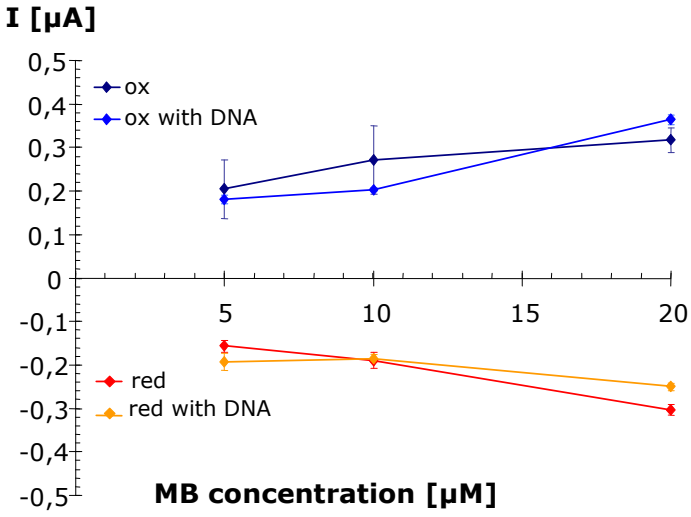

Fig. 5.The dependence of DPV peak current of MB oxidation and reduction on the concentration of MB.

3.3 Square wave voltammetry

When using the square wave voltammetry both nucleic acid bases electrochemical signals are available. The dependence of current on potential applied in guanine oxidation region is shown in Fig. 6. and $S_{G/A}$ values are plotted vs. MB concentration in Fig. 7. The S_G signals increase with MB concentration increasing.

The dependence of SWV peak current of MB oxidation and reduction on the concentration of MB is shown in Fig. 8. Again, the signals increase.

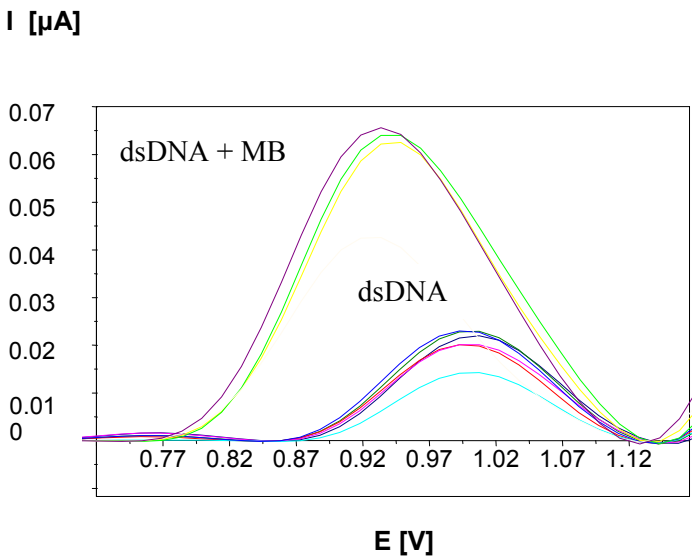

Fig. 6. The dependence of current in SWV on potential applied. The peaks of guanine oxidation without and with MB are visible.

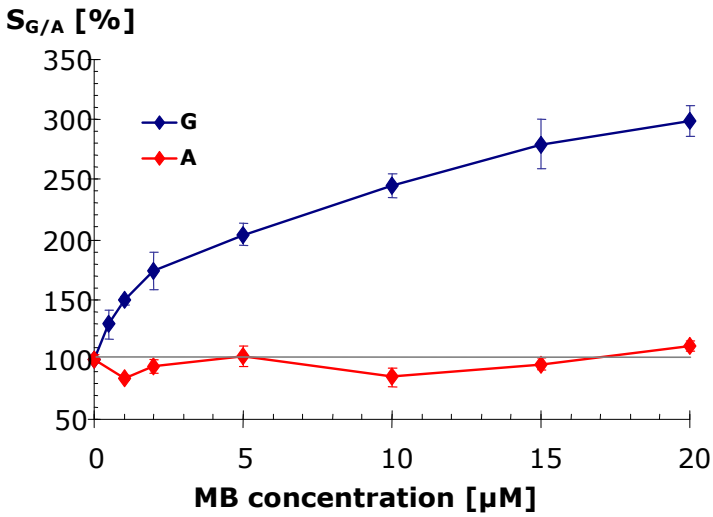

Fig. 7. Changes of guanine and adenine oxidation SWV peak current in dependence on MB concentration in the sample solution.

4 Conclusion

Various electrochemical techniques enrich our knowledge about the mechanism of interaction between

DNA and ligand. Cyclic voltammetry indicates the existence of the interactions between MB and dsDNA. The similar observations of MB oxidation and reduction current using DPV and SWV indicate some difference when either DNA is present or absent. These suggest that MB can play a role of mediator between DNA and electrode surface. The dependence of SWV peak current

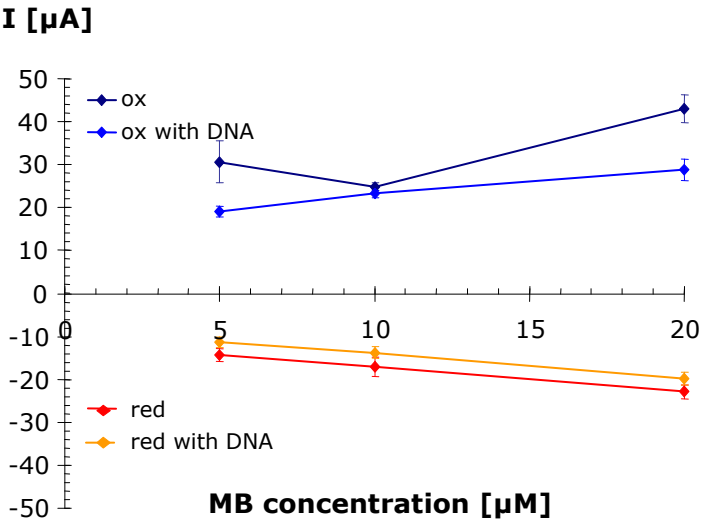

Fig. 8. The dependence of SWV peak current of MB oxidation and reduction on the concentration of MB.

and peak potential of the guanine oxidation on MB presence indicates strong interaction between this nucleic acid base and MB and kinetic changes in oxidation reaction of guanine. The dependence of peak current of nucleic bases oxidation in DPV and SWV on MB presence indicates strong interaction between guanine and MB facilitating the oxidation of guanine and week (DPV, SWV) interactions between adenine and MB, proving the DNA and MB interactions in guanine rich DNA regions.

References:

[1] S. Rauf, J.J. Gooding, K. Akhtar, M.A. Ghauri, M. Rahman, M.A. Anwar, A.M. Khalid, Electrochemical Approach of Anticacer Drugs-DNA Interaction, *Journal of Pharmaceutical and Biomedical Analysis,* Vol.37, No.2, 2005, pp. 205-217.

[2] D. Maciejewska, I. Szpakowska, I. Wolska, M. Niemyjska, M. Mascini, M. Maj-Zurawska, DNA-based Electrochemical Biosensors for Monitoring of *bis*-indoles as Potential Antitumoral Agents, Chemistry, X-ray Crystolagraphy, *Bioelectrochemistry*, Vol.69, No.1, 2006, pp.1-9.

[3] I. Szpakowska, B. Krassowska-Swiebocka, D. Maciejewska, P. Kazmierczak, W. Jemielita, M. Konrad, J. Trykowska, M. Maj-Zurawska, Electrochemical DNA Biosensor for Testing Pentamidine and Its Analogues as Potential Chemoterapeutics, *Electroanalysis*, Vol.18, No.13-14, 2006, 1422-1430.