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Electrochemical DNA sensor based on methylene blue functionalized polythiophene as a hybridization indicator

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ABSTRACT

A polythiophene functionalized with methylene blue (PMT-MB) was synthesized and used as an indicator for electrochemical oligonucleotides (ODNs) hybridization detection. After hybridization with complementary ODNs, the current signal of PMT-MB increased, which illustrated that PMT-MB can effectively recognize complementary ODN targets as an indicator. Compared to MB, PMT-MB showed much better resistance to the concentration change of buffer solution. In all buffer solutions tested, the hybridization always resulted in the increased current signal of PMT-MB due to the electrostatic interaction. While, when MB was used as an indicator, the inconsistent current response was obtained after the hybridization. When high concentration of buffer solution was used for accumulation, the hybridization resulted in the decreased current signal, while at the low concentrations, the current signal increased. The interaction between PMT-MB and dsODNs was also studied by UV–vis spectroscopy.

1. Introduction

DNA analysis plays an ever-increasing role in a number of areas related to human health such as diagnosis of infectious diseases, genetic mutations, drug discovery, forensics and food technology. A number of novel approaches that seek faster, sensitive and label-free gene detection, have been suggested using new detection techniques based on optical [1–3], acoustic [4] and electrochemical [5–7] interactions. Among these approaches, electrochemical DNA sensors are regarded as particularly suitable for direct and fast biosensing since they can convert the biorecognition event into a direct electrical signal [8–10]. Electrochemical DNA sensing approaches include the intrinsic electroactivity of DNA [11–14], electrochemistry of DNA-specific redox reporters [15,16], electrochemistry of colloidal gold nanoparticles and nanocrystals [17–19] and the electrochemistry of intrinsically conducting polymers [8,20,21].

Among the electrochemical approaches, great efforts have been made to search compounds which can be used as electrochemical indicators for simply, selective and sensitive DNA detection. Numerous metal compounds were reported to such applications [22]. For example, $Co(bpy)_3^{3+}$ can bind electrostatically in the minor groove of the DNA helix. Based on this principle, Millan and Mikkelson detected the DNA hybridization voltammetrically by using $Co(bpy)_3^{3+}$ [23]. Pozo et al. described the quantitative detection of a sequence of *Helicobacter pylori* by using 1,10-phenantroline-5,6-dione osmium complex as an indicator [24]. The detection limit reached 6 pmol. However, in terms of polymers as electrochemical indicators for the DNA hybridization detection, just a few works have been reported [25,26]. Gibbs et al. [26] synthesized several block copolymers containing oligonucleotide and ferrocenyl side chains and used them in the detection of DNA as electrochemical probes. Le Floch et al. [25] reported a ferrocene-functionalized cationic polythiophene and its use as an indicator for DNA hybridization detection by square wave voltammetry. In the suggested approach, neutral peptide nucleic acid (PNA) was used as a capture probe. After hybridization with complementary target, the resulted negatively charged duplex interacted with the ferrence-functionalized cationic polythiophene through an attractive electrostatic interaction. The detection limit reached 0.5 nM.

Methylene blue (MB), an organic dye which belongs to the phenothiazine family, has been widely used in DNA hybridization detection as an electrochemical indicator [27–33]. Spectroscopic studies have demonstrated that there are at least three different interactions between MB and DNA: (i) specific binding between MB and guanine bases, (ii) intercalation of MB in the DNA double helix, (iii) electrostatic interaction between cationic MB and anionic DNA [34,35]. Probably due to such complicated interactions, different results were obtained from MB-based DNA sensing

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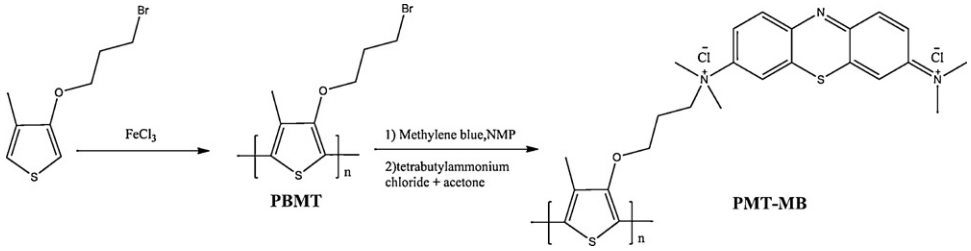

Scheme 1. Synthesis procedure of PMT-MB.

strategies reported in literatures. Ozsoz and co-workers reported that a decrease in the peak current of MB was observed upon hybridization of the probe e to the target [29,32,36,37]. The authors attributed the decrease in current signal to the inaccessibility of guanine bases in dsDNA which resulted in less MB bonded to dsDNA. In contrast, apparent increase of MB redox signals after DNA hybridization was also reported [38–40]. In this work, we presented the application of a polythiophene functionalized with methylene blue as an indicator for the electrochemical DNA hybridization detection and its performance was compared with that of MB.

2. Experimental

2.1. Materials

Methylene blue (3,7-bis(dimethylamino)-phenothiazin-5-ium chloride) with a laboratory grade was obtained from B.D.H. Laboratory Chemicals Division and used without further purification. Phosphate buffered saline pellets (PBS, pH 7.4) was obtained from Aldrich. Oligonucleotides (ODNs) were purchased from Alpha DNA including a probe with a loop structure: 5′-SH-C6-GCGATCACCGGCAAAGAGGTTCATTCAGATCGC-3′, complementary target: 5′-TGAATGAACCTCTTTGCCGGTGAT-3′, and non-complementary target 5′-CGCTGGGTTTAGTGGCTTGACGTC-3′. All aqueous solutions were prepared using Milli-Q water (18.2 MΩ cm). 5 mM of phosphate buffer solution containing 1.3 mM of KCl and 68 mM of NaCl was used for the experiments. Please note that all other phosphate buffer solutions used in this work were prepared by diluting this buffer solution.

2.2. Synthesis of polythiophene functionalized with MB

The synthesis procedure of polythiophene functionalized with MB (PMT-MB) was shown in Scheme 1. 3-(3-Bromopropoxy)-4-methylthiophene (BMT) was prepared according to the reported procedure [41]. After polymerization by using $FeCl_3$ as an oxidant, the obtained polymer PBMT was reacted with methylene blue in N-methylpyrrolidone (NMP) for 5 days in the dark at room temperature. After reaction, PMT-MB was precipitated by addition of a saturated acetone solution of tetrabutylammonium chloride. The crude product of PMT-MB was purified by acetone/Soxhlet extraction.

2.3. Preparation of ODN modified gold electrode

Before immobilization of ODN probes, gold electrodes (1.6 mm diameter from BAS) were treated by Piranha's solution (H_2SO_4/H_2O_2 70/30) for 20 min and washed with Milli-Q water, followed by potential scanning from 0 V to −1.0 V in 0.05 M sulfuric acid at a scan rate of 100 mV/s until a reproducible cyclic voltammogram was obtained. After washed with Milli-Q water, the electrodes were dried.

20 μL of thiol-ended ODN probe solution (0.1 μM) was dropped on the surface of cleaned gold electrode which was placed upright in a glass vial with a neighbouring vial with Milli-Q water. This was covered with a large clean beaker to keep certain humidity and left for an hour. After immobilization, the gold electrode modified with ODN probes was washed successfully with phosphate buffer to remove any unimmobilized ODN probes.

2.4. Hybridization

Hybridization was carried out by incubating ODN modified gold electrode with target ODN solution for 30 min at 37 °C. After hybridization the electrode was thoroughly rinsed with phosphate buffer to remove any non-hybridized target ODN.

2.5. PMT-MB accumulation and electrochemical measurements

The accumulation of PMT-MB was carried out by immersing modified gold electrode into phosphate buffer solution containing PMT-MB (0.032 mg/mL) for 25 min. Then the electrode was washed with phosphate buffer solution.

All the electrochemical measurements were carried out by using a BAS 100B instrument with a conventional three electrode system including the modified gold working electrode, an Ag/AgCl (3 M NaCl) reference electrode and a platinum wire counter electrode. The differential pulse voltammograms were collected with an amplitude of 50 mV at 20 mV/s scan rate in phosphate buffer solution under nitrogen atmosphere.

2.6. UV–vis spectroscopic measurement

For the investigation of the interaction between PMT-MB and double strands oligonucleotides (dsODNs), 05 mL of PMT-MB solutions (0.01 mg/mL) containing different concentrations of dsODNs were prepared (note: dsODNs stock solution was prepared by hybridizing equal mole of probe and complementary ODN). UV–vis spectroscopic measurements were performed on a Shimadzu UV1700 UV–vis spectrophotometer by using a quartz cell with a volume of 0.7 mL and a light path of 2 mm.

3. Results and discussion

3.1. Characteristics of PMT-MB

Fig. 1 presents the FTIR spectra of MB, PBMT and PMT-MB. The vibration bands of MB have been well assigned by Yu et al. [42]. The characteristic bands appears at 1592 cm^{-1} (C=N central ring stretching), 1488 cm^{-1} (C=C side ring stretching), 1386 cm^{-1}(multiple ring stretching), and 1327 cm^{-1} (C_{Ar}–N stretching), respectively (Fig. 1, curve a). After binding to the polymer PBMT, the relative intensities of bands in the range of 700–1490 cm^{-1} increased, as shown in the curve c of Fig. 1. The band at 544 cm^{-1} corresponding to the vibration of C–Br in the FTIR spectrum of PBMT (Fig. 1, curve b) disappeared, indicating the attachment of MB to PBMT. The optical property of PMT-MB was investigated by UV–vis spectroscopy. After attached to

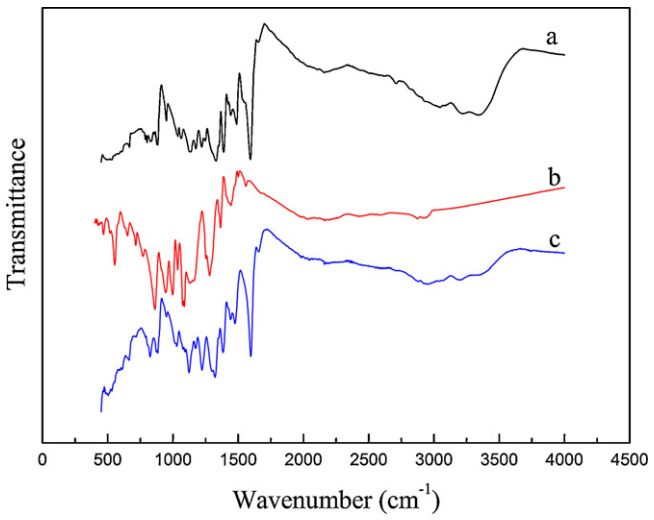

Fig. 1. FTIR spectra of MB (a), PBMT(b) and PMT-MB (c).

polythiophene, the visible absorption band of MB blue shifted 17–644 nm (data not shown) because of increased positive charge. The electrochemical property of PMT-MB was characterized by cyclic voltammetry, as shown in Fig. 2. In phosphate buffer, PMT-MB shows a couple of well-defined redox peaks in the applied potential window, which are attributed to oxidation and reduction of MB part in PMT-MB. The reduction and oxidation peak potentials (E_p) are −0.27 V and −0.19 V, respectively. Compared to the cyclic voltammogram of MB (Fig. 2, curve a), ΔE_p of PMT-MB (80 mV) is almost two times larger than that of MB (48 mV, which is very close to the predicted 45 mV for a $2e^-$ Nerstian system), which indicates the attachment of MB to the polymer makes its electrochemical reversibility worse.

3.2. PMT-MB as an indicator for electrochemical ODN hybridization detection

To test the efficiency of PMT-MB as an electrochemical indicator for the hybridization detection, ODN probe modified gold electrode was incubated with target ODN solutions. The recognition of different target ODN was carried out by collecting DPV signals of PMT-MB accumulated on the modified electrode in the blank phosphate buffer solution. Fig. 3A shows the DPV curves of PMT-MB accumulated on the ODN probe modified electrode before and

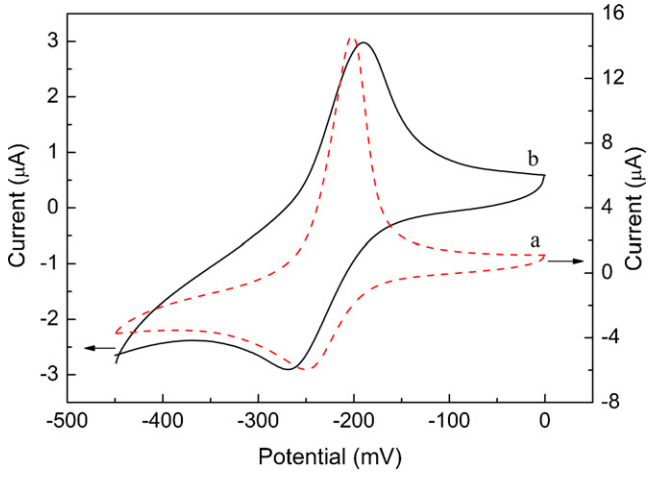

Fig. 2. Cyclic voltammograms of (a) MB and (b) PMT-MB in 5 mM of phosphate buffer solution at a bare gold electrode. Scan rate: 100 mV/s.

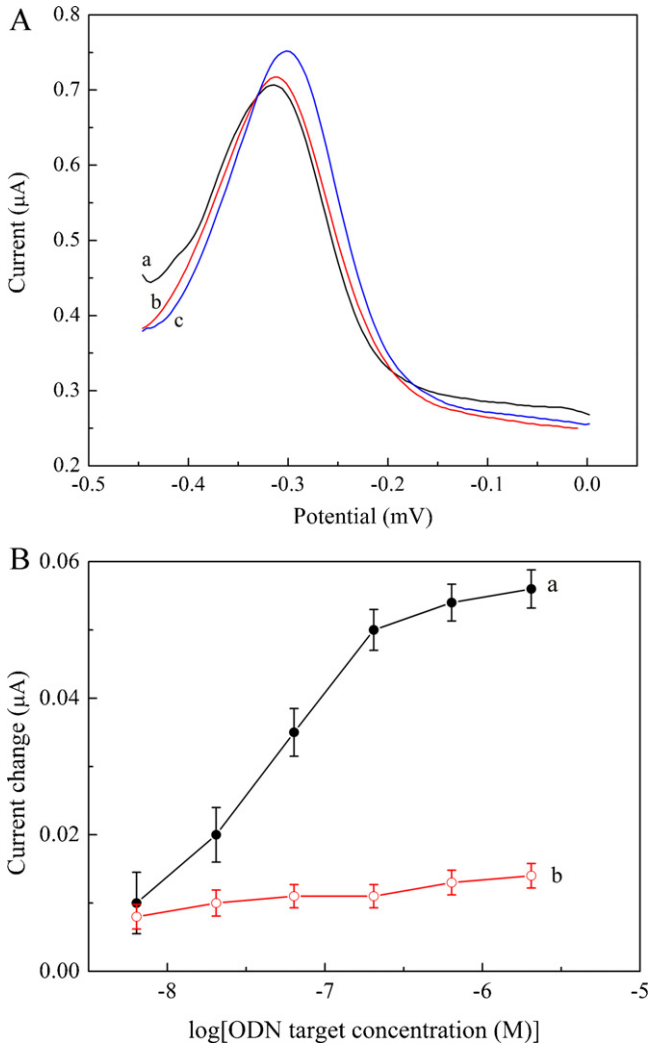

Fig. 3. (A) Differential pulse voltammograms of accumulated PMT-MB on modified gold electrodes. (a): ssODN modified; (b): after incubation with 0.204 μM of noncomplementary ODN target; (c) after incubation with 0.204 μM of complementary ODN target. (B) The current change in DPV response of PMT-MB with the concentration of complementary ODN target (a) and non-complementary ODN (b). PMT-MB was accumulated for 25 min in 5 mM phosphate buffer solution containing 0.032 mg/mL of PMT-MB. DPV was measured in blank phosphate buffer.

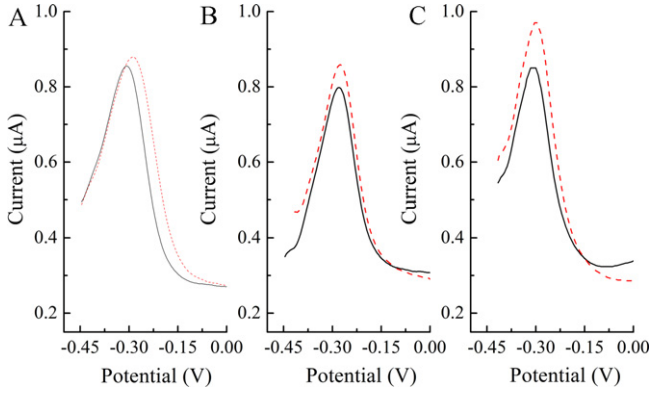

Fig. 4. Differential pulse voltammograms of accumulated PMT-MB on ssODN modified gold electrodes (solid line) and after incubation with 0.204 μM of complementary ODN target (dash line). The accumulation of PMT-MB was carried out in different concentrations of phosphate buffer solutions containing PMT-MB (0.032 mg/mL) for 25 min. Concentrations of phosphate buffer: (A) 1 mM; (B) 0.1 mM; (C) 0.02 mM. DPV was measured in PMT-MB free phosphate buffer solution.

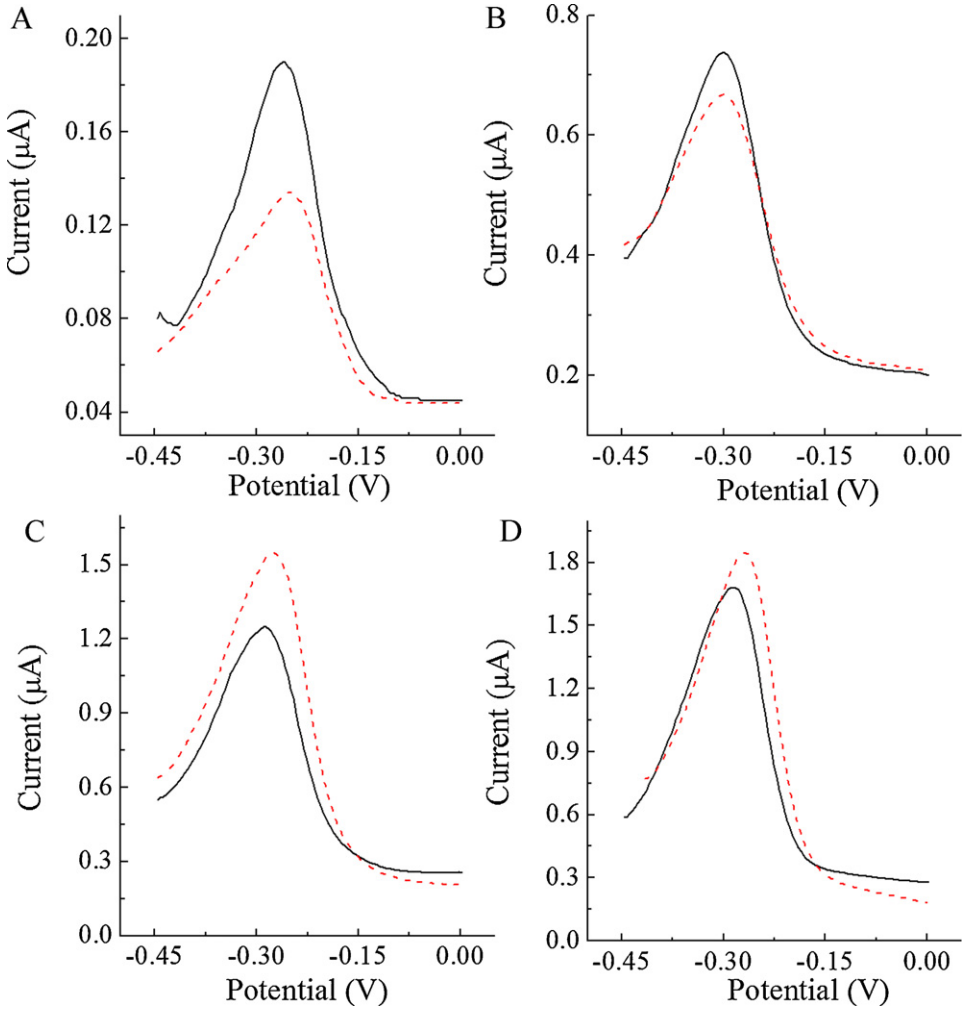

Fig. 5. Differential pulse voltammograms of accumulated MB on ssODN modified gold electrodes (solid line) and after incubation with 0.204 μM of complementary ODN target (dash line). The accumulation of MB was carried out in different concentrations of phosphate buffer solutions containing 20 μM of MB for 25 min. Concentrations of phosphate buffer: (A) 5 mM, (B) 1 mM; (C) 0.1 mM; (D) 0.02 mM. DPV was measured in MB free phosphate buffer solution.

after incubation with ODN target solutions. After incubation with 0.204 μM of noncomplementary ODN target, a small increase in the peak current was discovered (Fig. 3A, curve b), which was probably due to the non-specifically adsorbed noncomplementary ODN target, resulting in the increased amount of PMT-MB accumulated on the electrode surface. While after incubation with complementary ODN target, the peak current largely increased (Fig. 3A, curve c), which illustrated more PMT-MB were accumulated on the electrode surface due to the formation of ODN duplex. This result clearly demonstrated that PMT-MB can be used as an effective indicator for electrochemical ODN hybridization detection.

Fig. 3B shows the current change of PMT-MB for increasing levels of complementary ODN target (curve a) and non-complementary ODN (curve b). The current changes due to hybridization with the complementary ODN target increased with target concentration up to 0.64 μM and then tended to remain constant, which indicates all immobilized probes were hybridized and PMT-MB bound reached maximum. There is a linear relationship between the current change and the logarithmic value of the ODN target concentration ranging from 6.37 nM to 0.204 μM. When non-complementary ODN was used for hybridization, the current change is much smaller, as shown by the curve b of Fig. 3A. Furthermore, the current change shows less dependence on the concentration of non-complementary ODN. These results illustrates that the proposed system has a good selectivity.

3.3. Comparison of the performances of PMT-MB and MB as indicators in the different concentrations of buffer solutions

The effects of buffer solution concentration on the performances of PMT-MB and MB as indicators were investigated, respectively. Fig. 4 gives the results of PMT-MB. Under all tested buffer solution concentrations, the peak current of PMT-MB increased after hybridization. While for MB, the peak current decreased after hybridization when the accumulation was carried out in the buffer solution with high strength, as shown in Fig. 5A and B. This decrease in the current clearly demonstrates less MB were accumulated on the electrode surface, due to the decrease in the accessibility of the guanine bases, for MB binding, in the double strands oligonucleotides (dsODNs) with helix structure [37]. In the lower concentrations of phosphate buffers, the peak current of MB increased after hybridization, as shown in Fig. 5C and D. This indicates that the electronic attraction between the negatively charged phosphate backbone of ODNs and positively charged MB overcame the specific binding of MB to guanine bases at these low concentrations. Thus a higher signal of MB was found at low concentration of buffer solution.

In the case of PMT-MB, because current responses are always increased, it is reasonable to draw a conclusion that the electronic attraction between PMT-MB and ODN is the dominated interaction under the tested concentrations of the buffer solution. This result

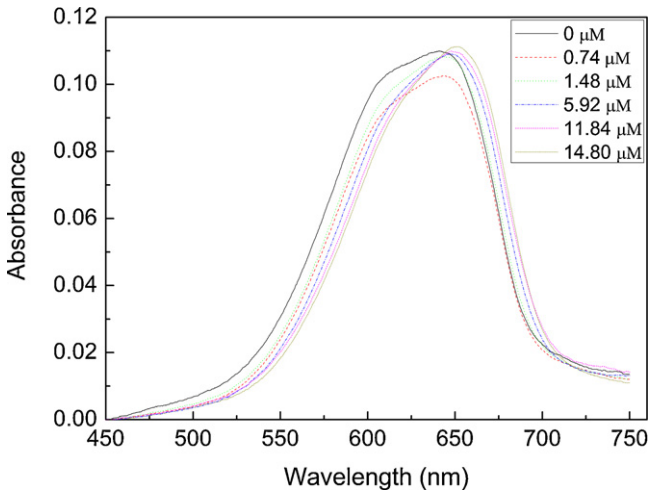

Fig. 6. Absorption spectra of PMT-MB in different concentration of dsODN solutions. The concentrations of dsODNs were shown in the inset.

also illustrates PMT-MB shows higher electronic affinity to negatively charged ODN than MB, which is attributed to the positively charged polymer chains. It also can be seen from Fig. 4 that the current changes after hybridization increased with the decrease of ionic strength, because the electronic screen effect was greatly weakened at the low ionic strength condition, more PMT-MB was associated with dsODNs.

The peak potential of PMT-MB accumulated on dsODNs modified electrode was also investigated. This was carried out by the accumulation of PMT-MB in 1.0 mM phosphate buffer solution with additional added NaCl to change the ionic strength. When the concentration of additional added NaCl is less than 20 mM, the peak potential positively shifted with the increase of NaCl concentration. Then the value of peak potential kept almost constant with the increase of NaCl, which implies that PMT-MB was no longer attached to dsODNs electrostatically.

3.4. Spectroscopic investigation the interaction with dsODNs

In order to further understand the interaction of PMT-MB with dsODNs, the absorption spectra of PMT-MB were measured in the presence of dsODNs. The results are presented in Fig. 6. It can be seen that the absorbance of PMT-MB decreased with a small red shift of peak wavelength in the presence of 0.74 μM of dsODNs. With the further increase of the concentration of dsODNs, the absorbance started to increase and an obvious wavelength red shift was observed. The results suggest that more than one binding mode exists between PMT-MB and dsODNs and different binding modes have different effects on the absorption spectra. At low concentration of dsODNs, the major binding mode of PMT-MB with dsODNs was intercalation due to the hypochromism and red shift of absorption wavelength observed which are common characteristics of small molecules intercalating to the double helix of DNA [43,44]. At high concentration of dsODNs, the major binding mode is no longer the intercalation because the absorbance of PMT-MB increased with the increase of dsODNs concentration which means cationic PMT-MB was attached to the negatively charged phosphate backbone of ODN through electrostatic interaction.

4. Conclusions

In this work, a polythiophene functionalized with MB (PMT-MB) was synthesized and its possibility as an indicator for electrochemical DNA hybridization detection was investigated. The current change of PMT-MB to complementary ODN target is four times more than that of non-complementary ODN with the same concentration. There is a linear relationship between the current change and the logarithmic value of the ODN target concentration ranging from 6.37 nM to 0.204 μM. The current response of PMT-MB to the non-complementary ODN illustrates the good selectivity of proposed detection system. Furthermore, PMT-MB as an electrochemical indicator shows a good resistance to the concentration change of the buffer solution (namely, ionic strength change). At all concentrations of buffer solution tested, the hybridization always resulted in the increased current signal of PMT-MB due to the electrostatic interaction between PMT-MB and dsODNs. While, when MB was used as a control, the hybridization resulted in the decreased current signal at the high concentration of buffer solution used and increased current signal at the low concentration due to the change of dominated interaction. The interaction between PMT-MB and dsODNs was further studied by UV–vis spectroscopy. In the presence of low concentration of dsODNs, the major binding mode of PMT-MB with dsODNs was intercalation and at high concentration of dsODNs, the major binding mode is electrostatic interaction. Our preliminary results illustrate that PMT-MB can effectively and selectively recognize complementary ODN targets as an electrochemical indicator and has a great potential application in the life science and medical diagnostics.

Acknowledgements

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References

[1] C. Peter, M. Meusel, F. Grawe, A. Katerkamp, K. Cammann, T. Boerchers, Fresenius. J. Anal. Chem. 371 (2001) 120–127.
[2] J. Liu, S. Tian, L. Tiefenauer, P.E. Nielsen, W. Knoll, Anal. Chem. 77 (2005) 2756–2761.
[3] H. Peng, L. Zhang, T.H.M. Kjaellman, C. Soeller, J. Travas-Sejdic, J. Am. Chem. Soc. 129 (2007) 3048–3049.
[4] Y. Okahata, K. Niikura, Denki Kagaku oyobi Kogyo Butsuri Kagaku 66 (1998) 7–13.
[5] E. Katz, Y. Weizmann, I. Willner, J. Am. Chem. Soc. 127 (2005) 9191–9200.
[6] L. Alfonta, A.K. Singh, I. Willner, Anal. Chem. 73 (2001) 91–102.
[7] J. Wang, Anal. Chim. Acta 469 (2002) 63–71.
[8] H. Peng, C. Soeller, N. Vigar, A. Kilmartin Paul, B. Cannell Mark, A. Bowmaker Graham, P. Cooney Ralph, J. Travas-Sejdic, Bioelectron 20 (2005) 1821–1828.
[9] E. Katz, I. Willner, Electroanalysis 15 (2003) 913–947.
[10] J. Wang, Chem. Eur. J. 5 (1999) 1681–1685.
[11] H. Karadeniz, B. Gulmez, F. Sahinci, A. Erdem, G.I. Kaya, N. Unver, B. Kivcak, M. Ozsoz, J. Pharmaceut. Biomed. Anal. 33 (2003) 295–302.
[12] J. Wang, G. Rivas, J.R. Fernandes, J.L. Lopez Paz, M. Jiang, R. Waymire, Anal. Chim. Acta 375 (1998) 197–203.
[13] K. Kerman, Y. Morita, Y. Takamura, E. Tamiya, Electrochem. Commun. 5 (2003) 887–891.
[14] P. Singhal, W.G. Kuhr, Anal. Chem. 69 (1997) 4828–4832.
[15] K. Millan, S.R. Mikkelson, Anal. Chem. 65 (1993) 2317.
[16] K. Hashimoto, K. Ito, Y. Ishimor, Anal. Chem. 286 (1994) 219.
[17] H. Cai, Y. Wang, P. He, Y. Fang, Anal. Chim. Acta 469 (2002) 165–172.
[18] J. Wang, Anal. Chim. Acta 500 (2003) 247–257.
[19] H. Peng, C. Soeller, M.B. Cannell, G.A. Bowmaker, R.P. Cooney, J. Travas-Sejdic, Biosens. Bioelectron. 21 (2006) 1727–1736.
[20] F. Garnier, H. Korri-Youssoufi, P. Srivastava, B. Mandrand, T. Delair, Synthetic Met. 100 (1999) 89–94.
[21] H. Peng, C. Soeller, J. Travas-Sejdic, Macromolecules 40 (2007) 909–914.
[22] K. Mukumoto, T. Nojima, S. Takenaka, Nucleic Acids Symp. Ser. (2004) 251–252.
[23] K.M. Millan, S.R. Mikkelsen, Anal. Chem. 65 (1993) 2317–2323.
[24] M.V. Del Pozo, C. Alonso, F. Pariente, E. Lorenzo, Anal. Chem. 77 (2005) 2550–2557.
[25] F. Le Floch, H.-A. Ho, P. Harding-Lepage, M. Bedard, R. Neagu-Plesu, M. Leclerc, Adv. Mater. 17 (2005) 1251–1254.
[26] J.M. Gibbs, S.-J. Park, D.R. Anderson, K.J. Watson, C.A. Mirkin, S.T. Nguyen, J. Am. Chem. Soc. 127 (2005) 1170–1178.
[27] E.M. Boon, J.K. Barton, Bioconjugate Chem. 14 (2003) 1140–1147.

[28] S.O. Kelley, J.K. Barton, N.M. Jackson, M.G. Hill, Bioconjugate Chem. 8 (1997) 31–37.
[29] K. Kerman, D. Ozkan, P. Kara, B. Meric, J.J. Gooding, M. Ozsoz, Anal. Chim. Acta 462 (2002) 39–47.
[30] P. Kara, K. Kerman, D. Ozkan, B. Meric, A. Erdem, P.E. Nielsen, M. Ozsoz, Electroanalysis 14 (2002) 1685–1690.
[31] A. Erdem, K. Kerman, B. Meric, D. Ozkan, P. Kara, M. Ozsoz, Turk. J. Chem. 26 (2002) 851–862.
[32] A. Erdem, K. Kerman, B. Meric, U.S. Akarca, M. Ozsoz, Anal. Chim. Acta 422 (2000) 139–149.
[33] E.M. Boon, N.M. Jackson, M.D. Wightman, S.O. Kelley, M.G. Hill, J.K. Barton, J. Phys. Chem. B 107 (2003) 11805–11812.
[34] E. Tuite, B. Norden, J. Am. Chem. Soc. 116 (1994) 7548–7556.
[35] Y. Wang, A. Zhou, J. Photochem. Photobiol. A 190 (2007) 121–127.
[36] P. Kara, K. Kerman, D. Ozkan, B. Meric, A. Erdem, Z. Ozkan, M. Ozsoz, Electrochem. Commun. 4 (2002) 705–709.
[37] A. Erdem, K. Kerman, B. Meric, M. Ozsoz, Electroanalysis 13 (2001) 219–223.
[38] O.A. Loaiza, S. Campuzano, M. Pedrero, J.M. Pingarron, Talanta 73 (2007) 838–844.
[39] J. Gu, X. Lu, H. Ju, Electroanalysis 14 (2002) 949–954.
[40] O.A. Loaiza, S. Campuzano, M. Pedrero, J.M. Pingaron, Electroanalysis 20 (2008) 1397–1405.
[41] K. Faid, M. Leclerc, Chem. Commun. (1996) 2761–2762.
[42] Z. Yu, S.S.C. Chuang, J. Phys. Chem. C 111 (2007) 13813–13820.
[43] E.C. Long, J.K. Barton, Acc. Chem. Res. 23 (1990) 271–273.
[44] G. Dougherty, W.J. Pigram, CRC Crit. Rev. Biochem. 12 (1982) 103–132.