A DNA biosensor based on a morpholino oligomer coated indium-tin oxide electrode and a cationic redox polymer

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A simple and ultrasensitive electrochemical biosensor employing a morpholino oligomer as capture probe and a cationic redox polymer as signal generator for direct detection of DNA is presented in this report. It is based on the immobilization of the morpholino oligomer on an indium-tin oxide (ITO) electrode and amperometric detection of target DNA by forming a DNA/cationic redox polymer bilayer on the ITO electrode. After hybridizing the morpholino capture probe (MCP) to the target DNA, the cationic redox polymer was introduced to the ITO electrode *via* electrostatic interaction with the hybridized DNA. The deposited redox polymer exhibited excellent electrocatalytic activity towards the oxidation of ascorbic acid (AA), allowing for direct voltammetric and amperometric detection of DNA. Under optimized experimental conditions, a detection limit of 1.0 pM and linear current–concentration relationship up to 500 pM were obtained in amperometry. The resulting biosensors offered much better mismatch discrimination against mismatch sequences than their DNA counterparts.

Introduction

In recent years considerable research efforts have been focused on the development of ultrasensitive nucleic acid biosensors following the completion of the human genome project. These biosensors have a wide variety of potential applications that range from genotyping to molecular diagnostics.[1–4] Technologies for nucleic acid detection most commonly use DNA capture probes to hybridize to target nucleic acids in a microarray format. To achieve required sensitivity, the use of PCR to amplify target nucleic acids has remained standard practice in many labs. However, microarray assays typically rely on fluorescence detection, which requires time-consuming chemical labeling, reverse transcription, PCR, and sophisticated and expensive optical instrumentation for data collection.[1–4] Consequently, microarray assays tend to be performed at centralized clinical laboratories rather than at the point-of-care. Direct detection methods that eliminate the requirement for a PCR step, on the other hand, could afford faster and simpler devices that can be deployed in field applications.[5]

To overcome the limitations of natural nucleic acids, intensive research efforts have also been directed to nucleic acids analogs. Replacing DNA probes with a class of DNA analogs, such as peptide nucleic acid (PNA), can significantly improve the performance of the nucleic acid detection devices. There are numerous advantages to using PNA instead of DNA probes in hybridization assays, including complete resistance to degradation by enzymes, increased sequence specificity to complementary DNA, and higher stability when bound with complementary DNA.[6–8] Despite these advantages, the use of PNA in DNA detection systems has not replaced the use of DNA probes. It is believed that one reason PNA is not affordably available is due to its limited use in antisense applications, leading to the high cost of PNA synthesis.[9] To circumvent cost problems associated with DNA analogs, Summerton devised the morpholino structural type, another member of the DNA analog group.[10] Morpholino oligomers are nucleic acid analogs in which the sugar phosphate backbone of natural nucleic acid has been replaced with morpholine rings and linked through phosphorodiamidate groups, resulting in uncharged mimics (Fig. 1). It is chemically stable and resistant to hydrolytic (enzymatic) cleavage and thus not expected to be degraded inside a living cell. Morpholino oligomers are capable of sequence-specific recognition of DNA and RNA obeying the Watson–Crick hydrogen bonding scheme, and the resulting heteroduplexes exhibit extraordinary thermal stability and unique ionic strength effects.[10] Moreover, morpholinos are cost-effective because they are synthesized by starting with much less expensive ribonucleosides to which an amine is introduced *via* a relatively simple ribose-to-morpholine transformation. The morpholino subunits can be assembled into oligomers *via* simple and efficient coupling to the morpholine nitrogen without the expensive catalysts and post-coupling oxidation steps required in the production of most of the nucleic acid analogs.[11] A number of morpholino derivatives have since been synthesized. Among them the standard non-ionic phosphorodiamidate-linked morpholinos appear to be the most promising. Substituting uracils with thymines in the standard morpholinos results in much higher affinity and stability than their DNA counterparts.[6] In addition, as compared to PNA, the standard morpholinos are highly soluble in water because they exhibit excellent base stacking, even better than that in DNA.[12] When nucleobases in an aqueous solution are well-stacked, the oligomer has good water solubility because of the ease of

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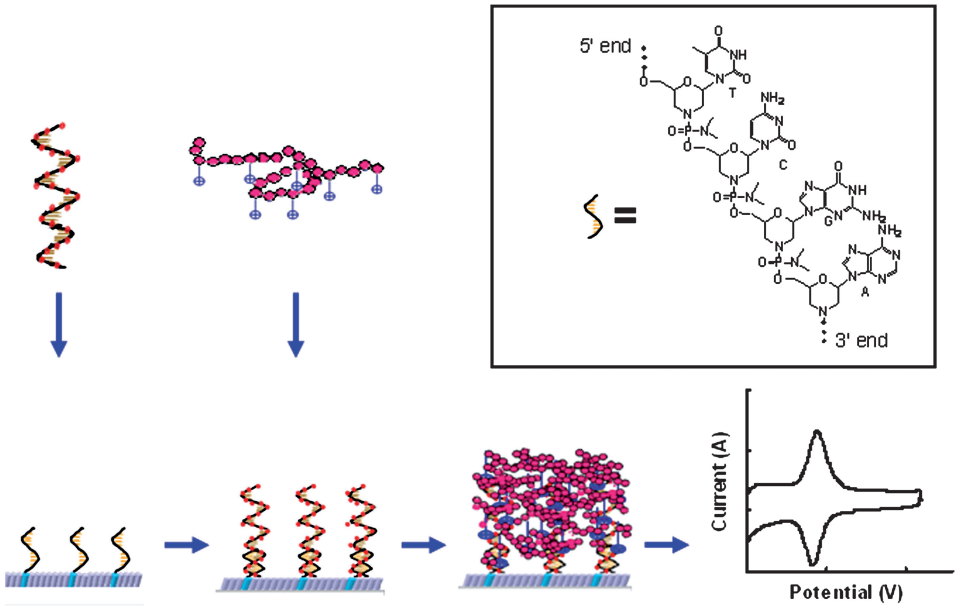

Fig. 1 Schematic illustration of direct DNA detection using a morpholino oligomer and a cationic redox polymer.

inserting the stacked bases into an aqueous environment. For example, a morpholino oligomer, having more rigid carbamate linkages, which prevent stacking of the bases, is several hundred fold less water soluble than a corresponding morpholino oligomer containing more flexible phosphoroamidate linkages.[12] In molecular biology, morpholinos are used primarily for classical antisense applications as they provide specific, reliable tools for blocking the expression of any selected gene throughout the course of embryogenesis in model organisms.[13] They have become the premier knockdown tool in developmental biology due to their cytosolic delivery in the embryos by microinjection.

In this report, we present a simple and ultrasensitive electrochemical procedure employing the morpholino oligomer as capture probe and the cationic redox polymer as signal generator for the direct detection of DNA, taking advantage of the electrostatic interaction between the cationic redox polymer and the negative backbone of DNA. The highly reversible electron-exchange process and excellent electrocatalytic activity of the redox moieties in the redox polymer towards the oxidation of ascorbic acid (AA) make it possible to conduct DNA detection. To our knowledge, these experiments demonstrate for the first time the potential of utilizing the morpholino oligomer for ultrasensitive detection of DNA. The proposed electrochemical procedure has a number of advantages: (i) simplicity, (ii) high sensitivity, (iii) high selectivity, and (iv) high compatibility with microfabrication and multiplexing.

Experimental

Materials and reagents

Unless otherwise stated, chemicals were obtained from Sigma-Aldrich (St Louis, MO) and used without further purification. The redox polymer used in this study was poly(4-vinylimidazole-*co*-acrylamide) (PVIA) partially imidazole-complexed with $Os(dmbpy)_2Cl^{+/2+}$ (PVIA-Os) (where dmbpy = 4,4′-dimethyl-2,2′-bipyridine). Synthesis of the redox polymer was described elsewhere.[14] Amino-terminated morpholino capture probes (MCPs): 5′-TGA TTG TAG TAT GTA TTG ATA AAG-3′-NH_2 (MCP1) and 5′-GAT TTG TAT TGA TTG AGA TTA AAG-3′-NH_2 (MCP2) used in this work were custom-made by Gene Tools (Philomath, OR) and all other DNAs were from 1st Base Pte Ltd (Singapore). The sequences of sample DNA used in this work are as follows: 5′-CTT TAT CAA TAC ATA CTA CAA TCA (fully complementary to MCP1), 5′-CTT TAA TCT CAA TCA ATA CAA ATC-3′ (fully complementary to MCP2) and 5′-CAA AAC AAA GAT CTA CAT GGA TGA-3′ (control). Indium-tin oxide (ITO) coated glass was from Delta Technologies Limited (Stillwater, MN). A pH 8.5 10 mM Tris-HCl–1.0 mM EDTA–0.10 M NaCl (TE) buffer solution was used as the hybridization and detection buffer. Phosphate-buffered saline (PBS, pH 8.0) consisted of 0.15 M NaCl and 20 mM phosphate buffer, and was used as the supporting electrolyte.

Apparatus

Electrochemical experiments were carried out using a CH Instruments model 660A electrochemical workstation (CH Instruments, Austin, TX). A conventional three-electrode system, consisting of the 2.0 mm ITO working electrode, a non-leak Ag/AgCl (3.0 M NaCl) reference electrode (Cypress Systems, Lawrence, KS) and a platinum wire counter electrode, was used in all electrochemical measurements. To avoid the spreading of the sample droplet beyond the 2.0 mm diameter working area, a patterned hydrophobic film was applied to the ITO electrode after the immobilization of the MCP. All potentials reported in this work were referred to the Ag/AgCl electrode.

Immobilization of CP on ITO electrode

The silanization of the ITO electrode was performed according to a previously described method after cleaning.[15] A patterned 1 mm thick adhesive spacing/insulating layer was assembled on the top of the ITO, to form an array of electrodes. The diameter of the individual electrodes was 2.0 mm. Amine-terminated MCP was denatured for 10 min at 90 °C and diluted to a concentration of 5.0 μM in pH 6.0 0.10 M acetate buffer. 5 μl aliquots of MCP solution were dispensed onto the ITO and incubated for 4 hours at 20 °C in an environmental chamber. After incubation, the electrode was rinsed successively with 0.10% SDS and water. The unreacted aldehyde moieties were blocked by 1,6-hexanediamine in a 1.0 mM 1,6-hexanediamine solution in the acetate buffer. The reduction of the imine was carried out by a 5 min incubation of the electrode in a 2.5 mg/ml sodium borohydride solution made of phosphate-buffered saline (PBS, pH 7.4)–ethanol (3 : 1). The electrode was then soaked in vigorously stirred hot water (90–95 °C) for 2 min, copiously rinsed with water, and blown dry with a stream of nitrogen.

Hybridization and detection

The hybridization of a target DNA and its electrochemical detection were carried out in three steps, as depicted in Fig. 1. First, the MCP coated ITO electrode was placed in a moisture-saturated environmental chamber maintained at 60 °C. A 5.0 μl aliquot of hybridization solution containing the target DNA was uniformly spread onto the electrode. It was then rinsed thoroughly with a blank hybridization solution at 60 °C after 30 min of hybridization and incubated at 25 °C for 10 min with a 5.0 μl aliquot of 5.0 mg/ml of PVIA-Os in pH 6.0 PBS. PVIA-Os was immobilized onto the hybridized target DNA *via* electrostatic interaction, forming a DNA/PVIA-Os bilayer. It was then thoroughly rinsed with NaCl-saturated pH 6.0 0.10 M phosphate buffer. Amperometric detection was performed at 0.15 V in the pH 8.0 PBS containing 5.0 mM AA.

Results and discussion

Feasibility study of sequence-specific hybridization of MCP to target DNA

To demonstrate the successful immobilization of MCP and the sequence-specific hybridization to target DNAs, a mixture of an equal molar ratio of two complementary target DNAs labeled with Cy3 and FAM at their 3′ ends were applied to two ITO electrodes coated with their complementary MCPs, respectively. The ITO electrodes were then characterized under epi-fluorescence microscopy. Strong fluorescent signals on the ITO electrodes were observed, and the pure red (Cy3) and green (FAM) colors at the two electrodes clearly indicated that only complementary target DNAs are able to hybridize with their corresponding MCP coated electrodes since non-sequence related uptake of the target DNAs will greatly compromise the purity of the two colors. On the contrary, no detectable fluorescent signals could be seen at either of the two electrodes when the non-complementary target DNA label with Cy 3 was employed. These results reveal that the complementary target DNAs were completely hybridized to their MCPs as expected and confirmed the specificity of binding between the target DNAs and the MCPs. In a subsequent test the hybridized ITO electrodes and the control electrode were soaked in the 5.0 mg/ml PVIA-Os solution for 10 min. Typical cyclic voltammograms of the ITO electrodes after the PVIA-Os treatment are shown in Fig. 2A, traces 2 and 3. For comparison, a voltammogram of an MCP coated electrode treated by the control DNA is shown in Fig. 2A, trace 1. As seen in Fig. 2A, considerably higher peak currents were observed for both oxidation and reduction processes at the hybridized ITO electrode indicating that a larger amount of redox polymer is brought to the electrode surface, due to the captured DNA molecules which bring a layer of negative charge to the electrode surface. In other words, treating the biosensor in the redox polymer solution results in the formation of the DNA/PVIA-Os bilayer. This bilayer has very rapid electron-exchange processes, as indicated by the voltammetric responses at different potential scan rates (Fig. 2A, traces 2 and 3). At scan rates up to 500 mV/s, the separation of the current peaks of the voltammetric electroreduction and electrooxidation waves was generally less than 25 mV. Extensive washing and potential cycling produced no noticeable changes, showing that the redox polymer is robustly bound at the electrode surface through the formation of the electrostatic bilayer with some degree of overlapping of the two layers. Trace 4 in Fig. 2A is the voltammogram of the ITO electrode in PBS containing 5.0 mM AA. An obvious catalytic current was observed due to the presence of $Os(dmbpy)_2$ redox moieties in the bilayer. The oxidation current prior to the peak rose rapidly and the peak current was greatly enhanced. The oxidation potential is similar to that reported for such a process at electrodes coated with thin $Os(dmbpy)_2$-based redox polymer films.[16] The increase in peak current and the decrease in the oxidation potential demonstrated an efficient electrocatalysis of AA, which is attributed to the improvement in the reversibility of the electron transfer process of AA.[17] The $Os(dmbpy)^{2+/3+}$ sites of the redox polymer effectively act as a catalyst in the electrochemical oxidation of AA. Moreover, at the applied potential of >0.10 V, the thus reduced redox polymer is instantly oxidized electrochemically, forming a substrate-recycling mechanism in the bilayer.

Redox polymers consisting of $[Os(dmbpy)_2]$ and three different polymer backbones, namely, PVIA (cationic), poly-(vinylimidazole) (neutral), poly(vinylimidazole-*co*-acrylamido-2-methyl-1-propanesulfonic acid) and poly(vinylimidazole-*co*-acrylic acid) (anionic) were tested for their capabilities of forming stable bilayers. It was observed that among the three kinds of polymers tested PVIA is the best in terms of stability of the bilayer and the amount of redox polymer being immobilized on the electrode surface. Due to partial protonation of acrylamide moieties at pH 7.4, some stability reinforcement is brought to the bilayer which brings the osmium redox centers to close proximity of the electrode surface. Various Os–bpy complex containing redox polymers were then tested for their capabilities in bilayer formation and electrocatalysis. Although many $Os(bpy)_2$-bearing redox polymers could form a bilayer with the hybridized DNA and showed some electrocatalytic activities toward the oxidation of AA, $Os(dmbpy)_2$ showed the lowest redox potential at which the electrocatalysis of AA occurs which offers excellent opportunity in biosensor applications, since one of the challenges in the development of amperometric nucleic

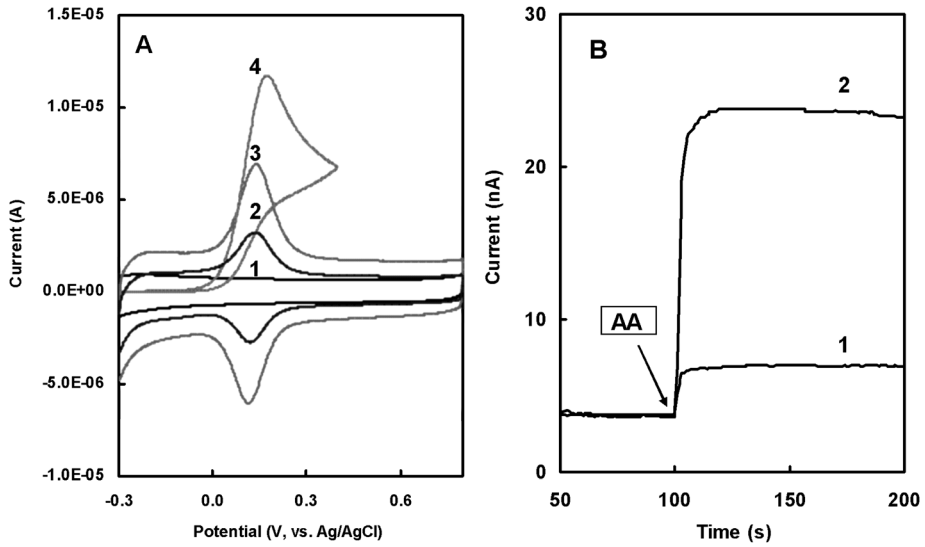

Fig. 2 (A) Cyclic voltammograms at 100 mV/s of 5.0 mM AA at an MCP coated ITO electrode after hybridization to its control (1) and complementary 20 nM DNA (4), and cyclic voltammograms of the hybridized electrode in blank PBS at 100 and (3) 200 mV/s (1 and 2, resp.). For clarity, (1), (2) and (3) were scaled up 10 times. Hybridization was carried out in TE buffer, supporting electrolyte PBS. (B) Amperometric responses of control (1) and 5.0 pM complementary DNA (2) at 0.15 V in 5.0 mM AA.

acids biosensors is the electrochemically catalyzed reaction of a substrate at the lowest possible potential, close to the thermodynamic potential of the respective substrate. This feature is significant for biosensors in order to eliminate non-specific redox processes under an applied potential. On the basis of the above voltammetric investigations, better analytical characteristics can be expected when employing PVIA-Os as the electrocatalytic layer. Indeed, under identical experimental conditions, the voltammetric catalytic current generated at the biosensor employing PVIA-Os was the highest among the redox polymers studied. To attain the highest current sensitivity, PVIA-Os was used in all subsequent experiments. The $Os(dmbpy)_2$ redox moieties in the redox polymer can be used an indicator for the direct voltammetric detection of DNA. A detection limit of 0.50 nM and a dynamic range up to 200 nM were obtained. On the other hand, to test for possible oxidation through direct electron-exchange with the substrate electrode, a hybridized electrode without applying the redox polymer was fabricated and its voltammogram was measured in PBS containing AA. Comparison of the voltammogram with that of the control electrode showed no measurable difference. Furthermore, while AA was catalytically oxidized at a potential as low as 0.10 V on the hybridized electrode with the redox polymer overcoating, electrooxidation of AA was not observed at both the control and the hybridized electrode without the redox polymer at potentials <0.60 V AA due to the high overpotential of AA oxidation at the ITO electrode and in the presence of MCP and DNA which further impede electron-exchange between the underlying ITO electrode and AA. The above results rule out the possibility that the oxidation of AA is a direct electron-exchange with the substrate electrode and confirm that the electrocatalytic effect of the redox polymer towards the oxidation of AA is genuine.

In view of the low potential at which electrocatalytic oxidation of AA takes place, it seems highly likely that better analytical characteristics can be achieved in amperometry. Amperometric detection at significantly low operating potentials minimizes potential interference and reduces the background signal, yielding an improved signal-to-noise ratio and a lower detection limit. As shown in Fig. 2B, upon addition of 5.0 mM AA to PBS the oxidation current in amperometry increased to 24 nA at 0.15 V at the ITO electrode hybridized to 5.0 pM complementary target DNA. In a control experiment in which the non-complementary target DNA was used, only a 6.8 nA increment in AA oxidation current was observed.

In the case of the electrocatalytic oxidation of AA by $Os(dmbpy)_2$, the rate-determining step(s) must be one of the following: (i) mass-transport process of AA in solution, (ii) catalytic process at the $Os(dmbpy)_2$–solution interface, and (iii) electron-transfer at the electrode–$Os(dmbpy)_2$ interface.[18] Under well-controlled conditions, when both the electron transfer and the mass-transport are much faster than the catalytic process, the limiting current is then solely controlled by the catalytic process, which in turn, by the total amount of $Os(dmbpy)_2$ at the electrode surface. This sole $Os(dmbpy)_2$-controlled AA oxidation can be achieved by 'speeding up' the mass-transport and electron-transfer rate. The mass-transport rate of AA is directly proportional to its concentration, obeying Cottrell equation.[17] Higher mass-transport rates are obtainable when working with higher concentrations of AA. It was observed that the amperometric signal is strongly dependent on the concentration of AA. The oxidation current increased with increasing AA concentration and plateaued beyond 5.0 mM (Fig. 3A), suggesting that the oxidation current of AA is now controlled by the amount of $Os(dmbpy)_2$ at the electrode surface. Higher catalytic currents were observable with higher AA concentrations, but with very little improvement in analytical performance owing to a simultaneous increase in background signal. Subsequent experiments were therefore conducted in 5.0 mM AA solution. It was found that pH has a profound effect on the catalytic oxidation of AA. The pH of the electrolyte solution was adjusted by adding

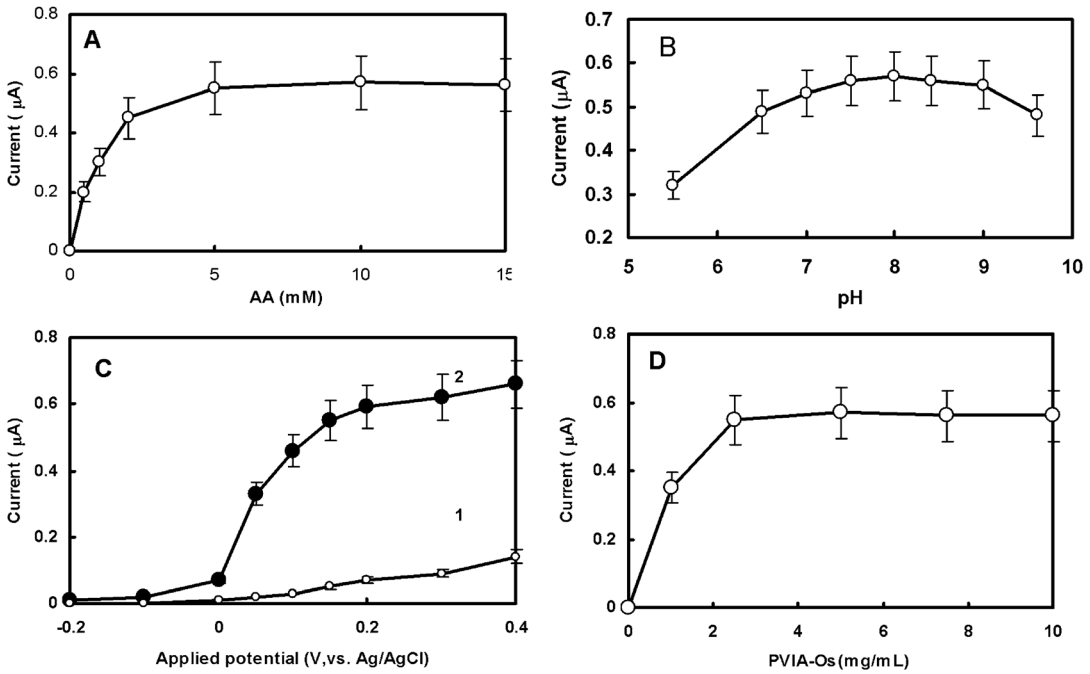

Fig. 3 Effect of (A) AA concentration, (B) pH, (C) applied potential and (D) PVIA-Os concentration on the amperometric responses of 50 pM complementary DNA.

a 0.10 M Na_3PO_4 or a 0.10 M H_3PO_4 solution. As illustrated in Fig. 3B, when the pH values were changed from 5.5 to 7.5, the oxidation current increased gradually, whereas, the oxidation potential remained unchanged. Moreover, stable voltammetric response was observed throughout the pH range studied. The highest sensitivity was obtained in the pH range of 7.5 to 9.0. Meanwhile, higher electron-transfer rates are achievable by working with more positive applied potentials,[17] which provides a convenient means for manipulating the electron transfer rate. The threshold potential of electrocatalysis was found to be 0.0 V and the electrocatalytic oxidation current of AA increased rapidly beyond the threshold potential. (Fig. 3C, trace 2). Higher catalytic currents were observable with higher applied oxidation potential, but with very little improvement in analytical performance owing to a simultaneous increase in the background signal (Fig. 3C, trace 1). The best balance between higher sensitivity and better signal-to-noise ratio was found when measurements were conducted in 5.0 mM AA at 0.15 V. Fig. 3D illustrates the dependence of the AA oxidation current on the concentration of the PVIA-Os. In all cases, an immediate increase in the AA oxidation current in amperometry was observed at the working electrode (Fig. 3D). As the PVIA-Os concentration increased, the signal rose rapidly and almost linearly at first, and then leveled off until a maximum sensitivity was reached between 2.5 and 10 mg/ml.

Calibration curve for DNA

As mentioned above, utilizing $Os(dmbpy)_2$ redox moieties in the DNA/PVIA-Os bilayer as the catalyst and the electrocatalytic oxidation of AA as the signal amplification for the detection of DNA, the current in amperometry is primarily dependent on the amount of the redox polymer deposited on the electrode surface when the substrate (AA) concentration is sufficiently high. The more the target DNA molecules captured, the more the redox polymer deposited, thus the higher the oxidation current. Fig. 4A shows representative amperometric data obtained from the electrodes treated with solutions of increasing concentrations of DNA. As the concentration of DNA was increased, the AA oxidation current in amperometry increased accordingly. Under optimal experimental conditions, a dynamic range was found to be from 2.0 to 500 pM with a relative standard derivation (RSD) of <18% and a detection limit of 1.0 pM, estimated from 3-fold of noise. To evaluate the capability of the proposed method in discriminating mismatches, three target DNAs, namely, complementary, one-base-mismatched and two-base-mismatched, were tested at 50 pM. As shown in Fig. 4B, the normalized amperometric responses (100% for the full complementary target) dropped by ~89% when one-base-mismatched target DNA was tested and to background level with two-base-mismatched target DNA. This mismatch sequence discrimination is much better than those of similar electrochemical biosensors where oligonucleotides were used as capture probes,[19,20] probably because of their uncharged and flexible phosphoroamidate backbone. The amperometric data agreed well with the voltammetric results obtained earlier and confirmed that the target DNA is successfully detected with high specificity and sensitivity.

Comparison of morpholino oligomer and PNA

Since PNA and morpholino oligomers share a number of key properties, such as uncharged backbones, whose structures differ drastically from those of natural nucleic acids, resistance to enzymatic cleavage, high affinity for complementary nucleic acids, and low salt dependence of base-pairing. It is therefore

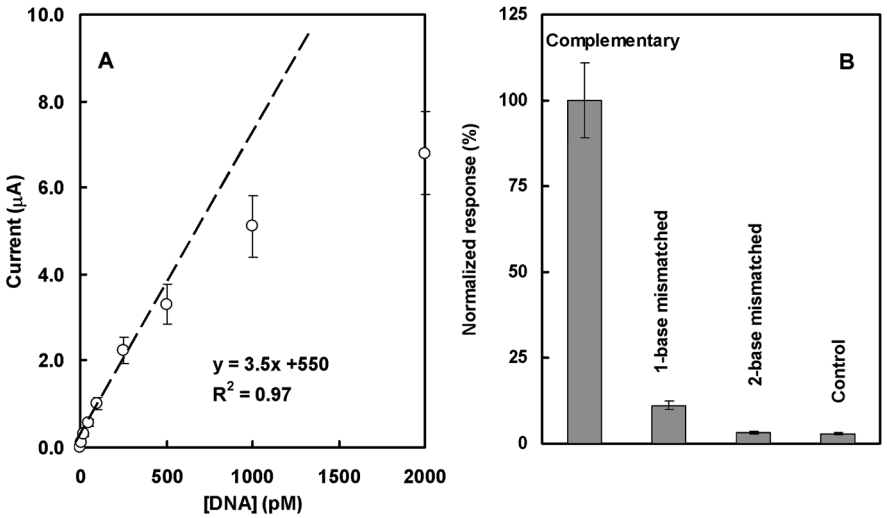

Fig. 4 (A) Calibration curve; (B) normalized amperometric responses of the MCP coated ITO electrode hybridized to the complementary and mismatched target DNAs at 50 pM. Hybridization was carried out in TE buffer, amperometry was conducted at 0.15 V in 5.0 mM AA in PBS.

Table 1 Amperometric responses of MCP and PNA coated ITO electrodes (50 pM target DNA, hybridization was carried out in TE buffer, amperometry was conducted at 0.15 V in 5.0 mM AA in PBS)

	Complementary (nA)	1-Base-mismatched (nA)	2-Base-mismatched (nA)	3-Base-mismatched (nA)	Control (nA)
PNA	530 ± 70	100 ± 20	42 ± 8	20 ± 4	16 ± 3
MCP	510 ± 75	115 ± 20	40 ± 8	19 ± 4	14 ± 3

essential to compare the analytical performance of PNA and MCP coated biosensors under identical experimental conditions. As tabulated in Table 1, both biosensors displayed excellent and, in many aspects, similar analytical characteristics. Nonetheless, the PNA coated biosensor showed better single-base-mismatch discrimination.

Conclusions

In summary, the present work has demonstrated the possibility of using the morpholino oligomer and the redox polymer for the ultrasensitive detection of DNA. The dramatically improved signal-to-noise characteristics and high sensitivity are attributed to the effective catalytic oxidation of AA at 0.15 V and the rapid electron-exchange rate of the $Os(dmbpy)_2$ redox moieties. It is likely that the morpholino oligomer can also be employed in other types of nucleic acid biosensors where anionic oligonucleotide capture probes are unwanted. The proposed biosensor promises to improve the practicability of electrochemical detection approaches due to the use of a very simple bilayer configuration. The combination of the electrostatic formation of bilayer and electrocatalysis provides a simple, direct, and highly sensitive non-labeling method for DNA quantification. We believe that this method could have wide applicability to ultrasensitive DNA assays. The flexibility of non-labeling allows for the fast and easy operation of the biosensor/biosensor array.

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References

1 J. J. Rautio, R. Satokari, P. Vehmaan-Kreula, E. Serkkola and H. Soderlund, *Expert. Rev. Mol. Diagn.*, 2008, **8**, 279–285.
2 L. J. van 't Veer and R. Bernards, *Nature*, 2008, **452**, 564–570.
3 D. Gresham, M. J. Dunham and D. Botstein, *Nat. Rev. Genet.*, 2008, **9**, 291–302.
4 A. Sassolas, B. D. Leca-Bouvier and L. J. Blum, *Chem. Rev.*, 2008, **108**, 109–139.
5 C. S. Thaxton, D. G. Georganopoulou and C. A. Mirkin, *Clin. Chim. Acta*, 2006, **363**, 120–126.
6 K. Shantanu and B. Deepak, *Appl. Microbiol. Biotechnol.*, 2006, **71**, 575–586.
7 O. Brandt and J. D. Hoheisel, *Trends Biotechnol.*, 2004, **22**, 617–622.
8 P. Heather, X. W. Yao, J. M. Coull, M. Fuchs and M. Egholm, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 14670–14675.
9 A. Porcheddu and G. Giacomelli, *Curr. Med. Chem.*, 2005, **12**, 2561–2599.
10 J. Summerton, in *Discoveries in antisense nucleic acids*, ed. C. Brakel, Portfolio, Woodlands, TX, 1989, pp. 71–80.
11 J. Summerton and D. Weller, *Antisense Nuc. Acid Drug Dev.*, 1997, **7**, 187–195.
12 H. Kang, P. J. Chou, W. C. Johnson, D. Weller, S. B. Huang and J. E. Summerton, *Biopolymers*, 1992, **32**, 1351–1363.
13 S. C. Ekker and J. D. Larson, *Genesis*, 2001, **30**, 89–93.
14 C. N. Campbell, D. Gal, N. Cristler, C. Banditrat and A. Heller, *Anal. Chem.*, 2002, **74**, 158–162.
15 G. F. Zheng, F. Patolsky, Y. Cui, W. U. Wang and C. M. Lieber, *Nat. Biotechnol.*, 2005, **23**, 1294–1301.
16 J. Fei, L. Luo, S. Hu and Z. Gao, *Electroanalysis*, 2004, **16**, 319–323.
17 A. J. Bard and L. R. Faulkner, *Electrochemical Methods*, John Wiley & Sons, New York, 2001.
18 C. P. Andrieux, J. M. Dumas-Bouchiat and M. Saveant, *J. Electroanal. Chem.*, 1982, **131**, 1–20.
19 H. Xie, C. Zhang and Z. Gao, *Anal. Chem.*, 2004, **76**, 1611–1617.
20 Y. Zhang, H. H. Kim and A. Heller, *Anal. Chem.*, 2003, **75**, 3267–3269.