Electrochemical Detection of Single A-G Mismatch Using Biosensing Surface Based on Gold Nanoparticles

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The study of small drug molecules interacting with nucleic acids is an area of intense research that has particular relevance in our understanding of relative mechanism in chemotherapeutic applications and the association between genetics (including sequence variation) and drug response. In this contribution, we demonstrate how the sequence-specific binding of an anticancer drug Dacarbazine (DTIC) to single base (A-G) mismatch could be sensitively detected by combining electrochemical detection with biosensing surface based on gold nanoparticles.

Key words: single base mismatch, DTIC, electrochemistry, gold nanoparticles, biosensor

Introduction

DNA recognition and its relative biosensor study has continuously attracted much attention from various research areas due to its importance to pave the way to rapidly diagnose or monitor the gene targeted illnesses like cancer, which is critical to disease prevention and clinical treatments. Particularly, since DNA sequence alterations on the relative helix are the cause of many gene related diseases (for example, many genetic disorders are caused by a point mutation or mismatch), a rapid and reliable tool for detecting mutated or mismatched base pairs would be valuable to offer some answers as to how genetic alteration or drug exposure, and the relationship between sequence variations (such as single nucleotide polymorphisms) and disease risk (*1*). It is already known that nanoparticles may present a versatile scaffold for biomolecular recognition, where nanoparticles could be fabricated to create some specific functional surface with a wide range of versatility for possible biosensing probes and/or diagnostic agents, which may afford a unique and advantageous platform for biomolecular recognition with a remarkable increase in the affinity and selectivity of the recognition process (*2–16*).

In the meantime, electrochemical DNA detection methods have received considerable attention due to the advantage of electrochemical devices such as high sensitivity, low cost, simplicity (easy handling), rapidness, and compatibility with microfabrication technology. A number of studies have shown that electroactive intercalators can detect selective hybridization of DNA because the presence of mutations or mismatches in the helix considerably affected the electrochemical signal of these intercalator probes. However, few non-intercalating binding probes were exploited to distinguish the specific targeted locations at fully matched base pairs and/or mismatched base pairs. In considering of all these and combining with the good biocompatibility of gold nanoparticles, we have utilized a DNA non-intercalating binding probe, an anticancer drug Dacarbazine (DTIC), as an electrochemical probe to detect single-base mismatches by introducing a perfectly matched sequence to the gold nanoparticle-tagged probe and comparing the two different target sequences that differ by a single base. As we know, different base mismatches in DNA helix have different influences to the stability of the DNA duplex, and it was already known that G-T and A-G mismatches may slightly destabilize a duplex, while A-A, T-T, C-T, and C-A mismatches significantly destabilize it (*17*). Many nuclear magnetic resonance (NMR) studies have been exploited to illustrate mismatched base pairs' structure properties (*18–20*) and their lifetimes (*21*) in oligonucleotides. Some electrochemical studies illustrate that the DNA helix containing A-G mismatches has the similar property to the fully matched sequence (*22*), therefore, it is difficult and particularly challenging to detect the single A-G mismatches in genomic DNA by the relative diagnostic technology. On the basis of these observations, in this study, the detection of sequence-specific binding of DTIC to single A-G mismatch has been ex-

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ploited by combining electrochemical detection with biosensing surface based on gold nanoparticles.

In this system, we have designed three 15-mer oligonucleotides S_1, S_2 and S_3 as follows. The 5′ end of S_1 was linked with a functional group of -$(CH_2)_6$-SH so that it could be covalently attached on 15-nm-diameter gold nanoparticle surface through Au-S bond. Then S_2 and S_3 were covalently immobilized through chitosan on two identical glassy carbon electrodes (GCEs), respectively. Furthermore, the 15-nm-diameter gold nanoparticle functionalized with 15-mer DNA S_1 was hybridized to target 15-mer DNA S_2 and S_3 in phosphate buffer at room temperature, respectively (Figure 1). Afterwards, DTIC was used as an electrochemical probe to detect the single A-G mismatch in the middle of the 15-mer oligonucleotides.

S_1: SH-5′-CGA TGA A**A**A TAT AAC-3′
S_2: 5′-GTT ATA T**T**T TCA TCG-3′
S_3: 5′-GTT ATA T**G**T TCA TCG-3′

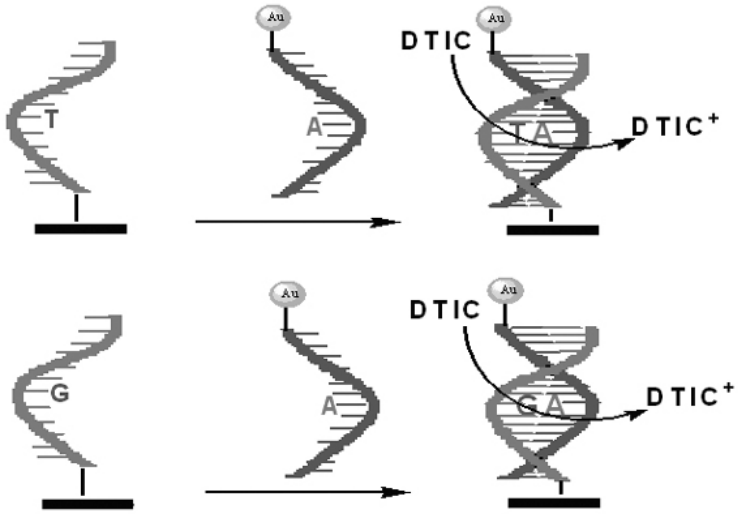


Fig. 1 Schematic illustration of electrochemical detection of single A-G mismatch using biosensing surface based on gold nanoparticles.

Results and Discussion

In the relative study, we have investigated the binding behaviour of DTIC to DNA and DNA bases by using electrochemical methods and atomic force microscopy (AFM), and our results illustrated that DTIC binds to DNA with a non-intercalating mode and the binding affinity of DTIC to purine bases is stronger than that with pyrimidines (*23*). Besides, our AFM study clearly indicated that the interaction of DNA with DTIC will lead to the appearance of some different sizes of particles on some specific sites of DNA chains, suggesting that DTIC could recognize some specific sites of DNA sequence so that the binding of DTIC to the relative specific sequence may result in the bending or kinking of the DNA chains. In addition, our recent work also illustrated that DTIC can distinguish single base change in a single-stranded oligonucleotides. Furthermore, our study on the binding of DTIC to DNA bases in the absence and presence of gold nanoparticles indicated that the presence of gold nanoparticles could facilitate the specific interaction between DTIC and DNA bases (*23*).

Based on the above study, DTIC has been utilized to probe specific DNA sequence by incorporation of DNA binding moieties on the gold nanoparticle surface (Figure 1). Figure 2 shows the cyclic voltammetry (CV) of DTIC on glassy carbon electrode with or without modification of the single-stranded oligonucleotides. The CV study indicated that the coverage of the oligonucleotides on GCE was 95% and the peak potential positively shifted about 50 mV (from 0.77 V on bare GCE to 0.82 V with modified GCE) after the attachment of the single stranded oligonucleotides to the electrode, accompanying with the considerable decrease of the peak current. Furthermore, after hybridization with the gold-nanoparticle-tagged oligonucleotide probe, this non-intercalating electrochemical probe can sensitively recognize the single base mismatch from the two different target sequences that differ by a single base. Figure 3 illustrates the CV study of DTIC to detect its sequence-specific binding

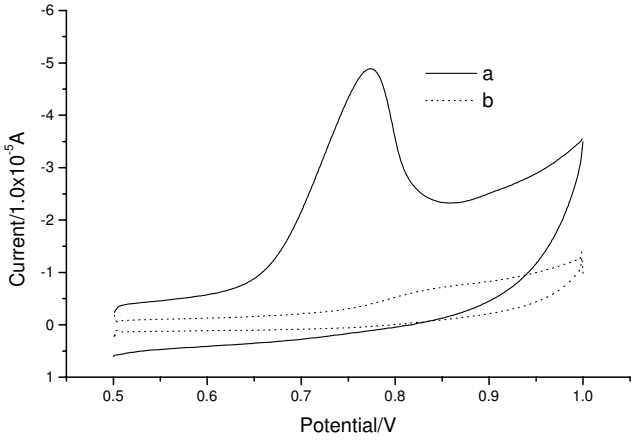


Fig. 2 Cyclic voltammetry of DTIC on bare GCE (a) and ssDNA modified GCE (b), scan rate: 50 mV/s.

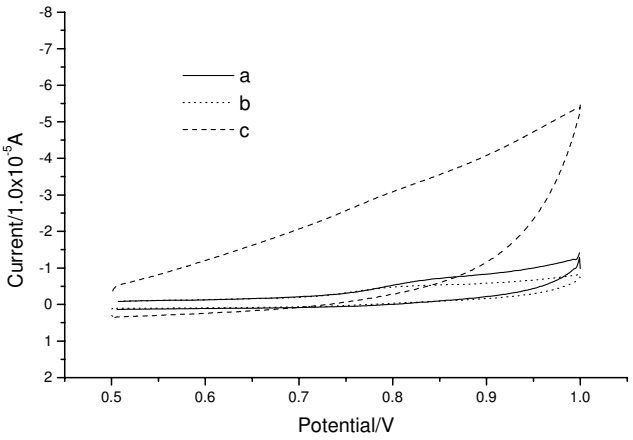


Fig. 3 Cyclic voltammetry of DTIC on ssDNA modified GCE (a), after hybridization with fully matched sequence (b), and single A-G mismatched sequence (c), scan rate: 50 mV/s.

and recognize the single A-G mismatch from a perfectly matched sequence. It can be observed that the specific binding of DTIC to the perfectly matched or single base mismatched sequences at targeted locations lead to the distinguished changes of its electrochemical behaviour, which indicates that DTIC could sensitively recognize the single A-G mismatch, and the binding affinity of DTIC to this mismatched sequence is stronger than that of the perfectly matched sequence. This is consistent with our previous study that the binding affinity of DTIC to G is relatively stronger than that with T.

Conclusion

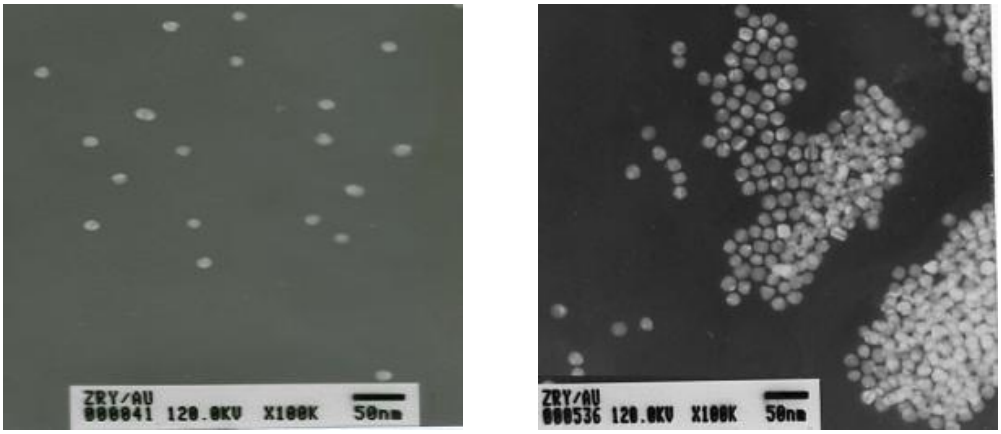
In summary, we have utilized a DNA non-intercalating binding probe, an anticancer drug DTIC, as an electrochemical probe to sensitively detect its sequence-specific binding and recognize single A-G mismatch by using biosensing surface based on gold nanoparticles. Some other sequences have been studied in our laboratory by using the same method. This approach will offer a new way to detect single base mismatch by non-intercalating probe and to illustrate the interaction mechanism of anticancer drugs with DNA sequence variations.

Materials and Methods

Gold nanoparticle synthesis was performed according to the method reported by Gearheart *et al* (*24*). Then the synthesized gold nanoparticles were characterized by transmission electron microscopy (TEM) and UV-Vis spectroscopy. An absorption peak at 520 nm was detected for the colloidal Au by UV-Vis spectroscopy, and TEM showed that the diameter of gold nanoparticle was 14±1 nm.

During the relative experiments, a colloidal solution containing gold nanoparticles with a diameter of about 15 nm was synthesized in our lab following the procedure reported by Gearheart *et al* (*24*). The sequence S_1 was functionalized on the gold nanoparticles according to the method described by Cai *et al* (*25*). After the incubation of 10 nM S_1 in gold colloidal solution for 16 h at room temperature, the solution was adjusted to the pH and ionic strength of the 0.1 M PBS (0.1 M NaCl, 0.01 M sodium phosphate buffer, pH 7.0) and allowed to stand for 40 h, followed by centrifugation for at least 30 min at 14,000 rpm to remove excess oligonucleotides. The nanoparticles were washed with PBS buffer and redispersed in a fresh PBS buffer, recentrifuged, and then suspended again. This procedure helped to afford the nanoparticle oligonucleotide conjugates free from the excess sulfurized oligonucleotides employed in the loading process. Figure 4 shows the TEM images of gold nanoparticles before and after modification with oligonucleotides.

Electrochemical measurements were carried out under atmosphere of nitrogen at ambient temperature (20±2℃) in a three-compartment electrochemical cell consisting of a glassy carbon electrode (with or without oligonucleotides) as the working electrode, a saturated calomel electrode as the reference electrode,



A **B**

Fig. 4 TEM images of gold nanoparticles (A) and oligonucleotide-modified gold nanoparticles (B).

and a Pt counter electrode. Cell and voltammetric flasks were protected from light by means of aluminum foil to avoid DTIC photodecomposition. Every experiment has been repeated for at least three times, and the repeated experiments gave the identical results.

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References

1. Schork, N.J., *et al.* 2000. Single nucleotide polymorphisms and the future of genetic epidemiology. *Clin. Genet.* 58: 250-264.
2. Boon, E.M., *et al.* 2000. Mutation detection by electrocatalysis at DNA-modified electrodes. *Nat. Biotechnol.* 18: 1096-1100.
3. Patolsky, F., *et al.* 2003. Magnetically amplified DNA assays (MADA): sensing of viral DNA and single-base mismatches by using nucleic acid modified magnetic particles. *Angew. Chem. Int. Ed. Engl.* 42: 2372-2376.
4. Weizmann, Y., *et al.* 2004. Magneto-mechanical detection of nucleic acids and telomerase activity in cancer cells. *J. Am. Chem. Soc.* 126: 1073-1080.
5. Park, S.J., *et al.* 2001. Directed assembly of periodic materials from protein and oligonucleotide-modified nanoparticle building blocks. *Angew. Chem. Int. Ed. Engl.* 40: 2909-2912.
6. Park, S.J., *et al.* 2002. Array-based electrical detection of DNA with nanoparticle probes. *Science* 295: 1503-1506.
7. Hutter, E. and Pileni, M.P. 2003. Detection of DNA hybridization by gold nanoparticle enhanced transmission surface plasmon resonance spectroscopy. *J. Phys. Chem. B* 107: 6497-6499.
8. Kerman, K., *et al.* 2004. Electrochemical coding of single-nucleotide polymorphisms by monobase-modified gold nanoparticles. *Anal. Chem.* 76: 1877-1884.
9. Verma, A., *et al.* 2004. Recognition and stabilization of peptide alpha-helices using templatable nanoparticle receptors. *J. Am. Chem. Soc.* 126: 10806-10807.
10. Lyon, L.A., *et al.* 1998. Colloidal Au-enhanced surface plasmon resonance immunosensing. *Anal. Chem.* 70: 5177-5183.
11. He, L., *et al.* 2000. Colloidal Au-enhanced surface plasmon resonance for ultrasensitive detection of DNA hybridization. *J. Am. Chem. Soc.* 122: 9071-9077.
12. Sato, K., *et al.* 2003. Rapid aggregation of gold nanoparticles induced by non-cross-linking DNA hybridization. *J. Am. Chem. Soc.* 125: 8102-8103.
13. Chen, Y., e*t al.* 2004. Gold and silver nanoparticles functionalized with known numbers of oligonucleotides per particle for DNA detection. *Chem. Commun.* (*Camb.*) 24: 2804-2805.
14. Caruana, D.J. and Heller, A. 1999. Enzyme-amplified amperometric detection of hybridization and of a single base pair mutation in an 18-base oligonucleotide on a 7-μm-diameter microelectrode. *J. Am. Chem. Soc.* 121: 769-774.
15. Marrazza, G., *et al.* 1999. Disposable DNA electrochemical sensor for hybridization detection. *Biosens. Bioelectron.* 14: 43-51.
16. Paleek, E. and Fojta, M. 2001. Detecting DNA hybridization and damage. *Anal. Chem.* 73: 74A-83A.
17. Ikuta, S., *et al.* 1987. Dissociation kinetics of 19 base paired oligonucleotide-DNA duplexes containing dif-

ferent single mismatched base pairs. *Nucleic Acids Res.* 15: 797-811.

18. Faibis, V., *et al.* 1996. Solution structure of two mismatches G.G and I.I in the K-*ras* gene context by nuclear magnetic resonance and molecular dynamics. *Biochemistry* 35: 14452-14464.
19. Borden, K.L., *et al.* 1992. Conformational properties of the G.G mismatch in d(CGCGAATTGGCG)2 determined by NMR. *Biochemistry* 31: 5411-5422.
20. Cognet, J.A., *et al.* 1991. Solution conformation of an oligonucleotide containing a G.G mismatch determined by nuclear magnetic resonance and molecular mechanics. *Nucleic Acids Res.* 19: 6771-6779.
21. Bhattacharya, P.K., *et al.* 2002. ^{1}H NMR determination of base-pair lifetimes in oligonucleotides containing single base mismatches. *Nucleic Acids Res.* 30: 4740-4750.
22. Kelley, S.O., *et al.* 1999. Single-base mismatch detection based on charge transduction through DNA. *Nucleic Acids Res.* 27: 4830-4837.
23. Zhang, R.Y., *et al.* 2004. A voltammetric study of the effect of gold nanoparticles on the binding of DTIC to DNA bases. *Electrochem. Solid-State Lett.* 7: J27-J28.
24. Gearheart, L.A., *et al.* 2001. Oligonucleotide adsorption to gold nanoparticles: a surface-enhanced Raman spectroscopy study of intrinsically bent DNA. *J. Phys. Chem. B* 105: 12609-12615.
25. Cai, H., *et al.* 2002. Electrochemical detection of DNA hybridization based on silver-enhanced gold nanoparticle label. *Analytica Chimica Acta* 469: 165-172.