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A label-free ultrasensitive assay of 8-hydroxy-2′-deoxyguanosine in human serum and urine samples via polyaniline deposition and tetrahedral DNA nanostructure

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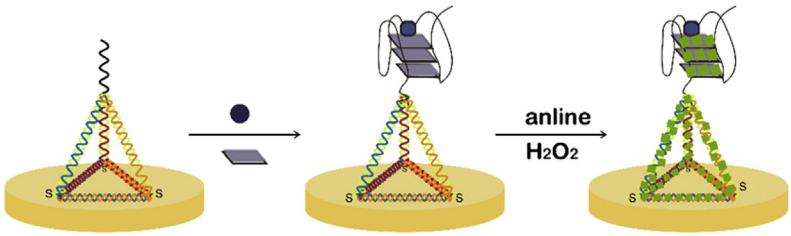
HIGHLIGHTS

- The method for detection of 8-OHdG is label free.
- High activity of hemin/G-quadruplex was induced by 8-OHdG.
- Both TDN and hemin/G-quadruplex were good template for PANI deposition.
- The sensitivity was improved to almost 300-fold compared with reported EC methods.
- The method has been applied in urine and serum samples with satisfactory results.

GRAPHICAL ABSTRACT



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ABSTRACT

Oxidative damage is an important factor in causing various human disease and injury. As an oxidative DNA damage product, 8-hydroxy-2′-deoxyguanosine (8-OHdG) is a key marker, which is widely used to study oxidative damage mechanism in diseases. Most reported electrochemical methods were based on oxidation current of 8-OHdG. In this work, a simple electrochemical biosensor for ultrasensitive detection of 8-OHdG was proposed based on it triggered polyaniline (PANI) deposition on tetrahedral DNA nanostructure (TDN). TDN was immobilized onto a gold electrode surface based on self-assembly between three thiolated nucleotide sequences. 8-OHdG-aptamer on the top of TDN formed a hemin/G-quadruplex structure in the presence of 8-OHdG and hemin, which have high catalytic activity to trigger PANI deposition. Numerous negative charges on the duplex DNAs contained in hemin/G-quadruplex and TDN supplied exquisite environment for PANI deposition, which improved the detection sensitivity greatly by increasing the DPV current to10-fold (~3 μA) compared to our previously reported method without TDN. The response signals correlated linearly with the concentration of 8-OHdG ranging from 10 pM to 2 nM, with a detection limit of 1 pM (S/N = 3). The sensitivity was improved to almost 300-fold when compared with most of previously reported electrochemical methods. The method was also simple and reliable, avoiding complex, expensive label procedures and nanomaterial

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synthesized procedures. The method had been successfully applied to quantify 8-OHdG in urine and human serum samples with satisfactory results.

1. Introduction

Oxidative damage brought by oxidative stress is an important factor in causing various human disease and injury [1]. As an oxidative DNA damage product, 8-hydroxy-2′-deoxyguanosine (8-OHdG) is a key biomarker, which is widely used to study oxidative damage mechanism in diseases [2]. 8-OHdG can be found in urine, multifarious lesion tissue or exposed cells.

The common detection methods that be used to measure 8-OHdG including high performance liquid chromatography with electrochemical detection (HPLC-EC) [3] and enzyme-linked immunosorbent assay (ELISA) [4]. In recent years, more methods have been developed: high performance liquid chromatography-mass spectrometry-mass spectrometry (HPLC-MS-MS) [5,6], high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) [6], high performance liquid chromatography-solid phase extraction (SPE-HPLC) [7], and carbon fiber microelectrode [8]. However, these methods require sophisticated instruments or tedious procedures. Therefore, the development of simple, highly sensitive and cost-efficient sensor for the determination of 8-OHdG is imminent.

Most electrochemical methods based on the oxidation current of 8-OHdG were developed due to its rapid and simplicity. These works focused on how to obtain good sensitivity and selectivity by modifying the electrode. For example, Galicia reported a method that studies the electrochemical behavior of 8-OHdG on electrodes modified with carbon nanotubes dispersed in polyethylenimine [9]. The technique of DPV enables quantification of 8-OHdG with the detection limit of 1×10^{-7} M. Wang proposed an electrochemical biosensor for the detection of 8-OHdG based on the ss-DNA functionalized graphene nanosheets. The biosensor showed high electro catalytic activity toward the oxidation of 8-OHdG with a detection limit down to 0.875 nM [10].

Polyanilne (PANI) that has many advantages such as good conductivity and redox property, environmental stability, simple procedures and mild polymerized conditions became more and more popular electrochemical probes that be used in electrochemical sensors [11,12]. DNA template which have numerous negative charges is an intriguing template for fabrication of PANI [13,14]. Horseradish peroxidase (HRP) mimicking DNAzyme, such as hemin/G-quadruplex has been extensively used as efficiently catalyst for the polymerization of anilines in the presence of H_2O_2 due to its several advantages: easy synthesize, low cost, superior stability and robust peroxidase activity [15–17].

The tetrahedral DNA nanostructure (TDN) that has many advantages including well-controlled density, orientation of DNA bioprobes, minimized nonspecific absorption, a superior environment for the bio-recognition process, has been used widely for immobilization of bio-molecules [18]. Fan group done many excellent works: they reported TDN electrochemical sensor for target DNA [19], thrombin [20], H7N9 virus [21], microRNA [22], and prostate-specific antigen [23] detection with satisfactory results. In addition, when hemin/G-quadruplex is grafted to TDN, the scaffold does enhance the catalytic activity of the DNAzyme [24]. Our group [25] reported a novel strategy for ultrasensitive detection of telomerase activity based on it triggered PANI deposition. Compared to single-strand telomerase substrate primer, the sensitivity for telomerase detection was enhanced ~20 times by using optimized TDN as good template for PANI deposition.

Aptamers refer to oligonucleotide fragments which was selected via the screening technique systematic evolution of ligands by exponential enrichment (SELEX) in vivo [26]. They recognize with target molecules specifically owing to their spatial structure and have several characteristics: wide target molecules, high affinity, strong specificity and easy modification [27]. Therefore, they were widely used as a new class of reporters for disease diagnosis and biological analysis [28,29]. 8-OHdG aptamer was filtered in 2009 [30] and have been showed their important roles for 8-OHdG detection in biosamples.

Inspired by high selectivity of aptamer, high catalytic efficiency of hemin/G-quadruplexes induced by 8-OHdG, good property for immobilization of bio-molecules of TDN and excellent template for PANI deposition supplied by hemin/G-quadruplexes and TDN, a simple, label-free electrochemical biosensor for ultrasensitive detection of 8-OHdG was proposed. Compared with previously reported electrochemical methods based on the CV current of 8-OHdG, the electrochemical signal obtained in this manuscript came from the reduction of PANI that polymerized under the catalysis of hemin/G-quadruplexes. DPV currents increased with the increasing 8-OHdG concentration because the amount of generated PANI was dependent strongly on the number of hemin/G-quadruplexes that induced by 8-OHdG. The response signals correlated linearly with the concentration of 8-OHdG ranging from 10 pM to 2 nM with a detection limit of 1 pM (S/N = 3). The detection sensitivity was greatly improved by increasing the electrochemical signal to 10-fold (~3 μA) compared to our previously reported method for methyltransferase detection [31], where the deposited PANI was also used as signal probe but without TDN served as template. The sensitivity was improved to almost 300-fold when compared with most of the previously reported electrochemical methods.

2. Experiment section

2.1. Chemicals and materials

All oligonucleotides synthesized and modified by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China). The sequences are shown in Table 1. 8-OHdG, tris-(2-carboxyehtyl)-phosphine hydrochloride (TCEP), ethylenediaminetetraacetic acid (EDTA), and aniline (99.5%) were obtained from Sigma-Aldrich Co. Ltd. (St. Louis, MO). Hemin was purchased from Porphyrin Products (Logan, UT, USA) and used without further purification.

The buffer solutions employed in this study were as follows: 1 × PBS (pH 7.2–7.4, 136.89 mM NaCl, 2.67 mM KCl, 8.24 mM Na_2HPO_4, 1.76 mM NaH_2PO_4), TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA), TM buffer (20 mM Tris, pH 8.0, 50 mM $MgCl_2$), Deposition buffer (100 mM acetic acid-sodium acetate (HAc-NaAc), pH 4.3, 100 mM aniline, 100 mM H_2O_2, prepared daily), Electrolyte (100 mM HAc-NaAc buffer, pH 4.3), Electrochemical impedance spectroscopy (EIS) buffer (1 × PBS, 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1), 100 mM KCl). Milli-Q water (18.2 MΩ cm at 25 °C, Barnstead, Thermo Scientific, USA) was used throughout the experiments.

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Table 1
Sequences of oligonucleotides employed in this work.

Name	Sequence (from 5′ to 3′)
A-apt	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTATTTTTGCGGGCGATCGGCGGGGGGTGCGTGCGCTCTGTGCCAGGGGG TGGGACAGATCATATGGGGGTGCT
B-SH	SH-$(CH_2)_6$-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGG GTCCAATAC
C-SH	SH-$(CH_2)_6$-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGG CGGCTCTTC
D-SH	SH-$(CH_2)_6$-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGA CCCTCGCAT

2.2. Characterization and measurements

All cyclic voltammetry (CV), and differential pulse voltammetry (DPV) spectra were obtained with a CHI 660D electrochemical analyzer (Chenhua Instruments, Shanghai, China). The electrochemical system consisted of three electrodes: gold electrode or modified gold electrode as the working electrode, saturated calomel electrode (SCE) as the reference electrode and platinum wire as the auxiliary electrode. Surface plasmon resonance (SPR, DyneChem HiTech Ltd., China), scanning electron microscopy (SEM) system (JEM-2100, JEOL, Japan). Electrochemical Impedance Spectroscopy (EIS) was performed on a VersaSTAT 3 workstation (Princeton Applied Research, USA).

2.3. Synthesis of TDN and the mobilization of them on Au electrode

According to the procedures reported in the literature [32], the gold electrode was soaked in piranha solution (7:3 ratio of H_2SO_4/ 30% H_2O_2 (v/v)) for 2 h. Then, the gold electrode was polished with 0.3 and 0.05 μm gamma alumina powder successively. Finally, the gold electrode was rinsed with Milli-Q water and dried by nitrogen.

The TDN was fabricated according to previously reported methods [33]. Briefly, equimolar quantities of four strands (showed in Table 1) were dissolved in TE buffer to a final concentration of 50 μM, respectively. Then, four strands (1 μL of each strand) were mixed with 1 μL of TCEP (500 mM) and 45 μL of TM buffer, followed by heating to 95 °C for 5 min and then cooling to 4 °C for 30 min. In this manner, TDN with aptamer at one vertex and three thiol groups at the other three vertices was constructed on the electrode with Au–S bond. The resulting modified electrodes were rinsed with 1 × PBS and dried gently with nitrogen before next modification.

2.4. Formation of hemin/G-quadruplexes and PANI

Various concentrations of 8-OHdG were pipetted to the surface of modified gold electrode and incubated at room temperature for 60 min. Then hemin were dropped onto the gold electrode for 60 min. Followed by thoroughly washing with binding buffer to remove unbound 8-OHdG. The HRP-mimicking DNAzyme was formed. For the deposition of PANI, the electrode was incubated in deposition buffer for 90 min at room temperature. The samples were characterized by SPR, EIS, and SEM.

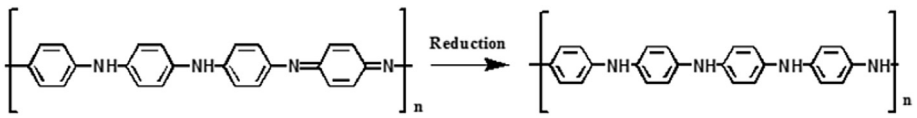
3. Results and discussion

3.1. Design strategy and sensing mechanism

The 3D DNA nanostructure-based electrochemical probe system.

Scheme 1 depicts the functional sensing platform based on TDN and hemin/G-quadruplexes. TDN structure that contained the 8-OHdG aptamer on one vertice and modified with sulfhydryl on other three vertices, were immobilized on gold electrode surface via three gold-sulfur bond. Then, the 8-OHdG aptamer was on the top vertice of the TDN as showed in Scheme 1a. In the presence of 8-OHdG, hemin as cofactor, hemin/G-quadruplexes were formed on the electrode surface (Scheme 1b). Both TDN structure and hemin/G-quadruplexes were good template for PANI deposition because they have plentiful negative charges to absorb aniline [13]. Thus, these multiple HRP-mimcking DNAzymes that had high catalytic activity initiate the PANI deposition not only on the TDN structures but also on the hemin/G-quadruplexes in the presence of aniline and H_2O_2, (Scheme 1c). Strong current was observed when DPV was used due to the reduction of PANI. The possible reduction mechanism of the PANI was listed as follows:

The DPV current increased with the increasing 8-OHdG concentration because the amount of generated PANI was dependent strongly on the number of hemin/G-quadruplexes, and, the number of hemin/G-quadruplexes was closely related to the content of 8-OHdG. Thus, a label-free ultrasensitive electrochemical 8-OHdG detection method via polyaniline deposition was proposed.



The assembly of the TDN apt-sensor, formation of hemin/G-quadruplex/TDN apt-sensor and deposition of PANI were illustrated by SPR (Fig. 1A), electrochemical impedance spectroscopy (EIS) (Fig. 1B), cyclic voltammetry (CV) (inset in Fig. 1C) and differential pulse voltammetry (DPV) (Fig. 1C). Fig. 1A gave the changes of SPR signal as a function of scanning angle on SPR chips with different modifications. The resonance angles of the bare chip (Fig. 1A, curve a), TDN modified chip (Fig. 1A, curve b), hemin/aptamer/TDN modified chip (Fig. 1A, curve c) were 63.78°, 64.32° and 65.37°, respectively. The increase of the resonance angle was ascertained to the multiple coupling process in which the mass coated to the metal surface are positively shifted. Deposition of PANI led to the resonance angle increased to 66.41° (Fig. 1A, curve d), indicating polyaniline successfully polymerized on the chip

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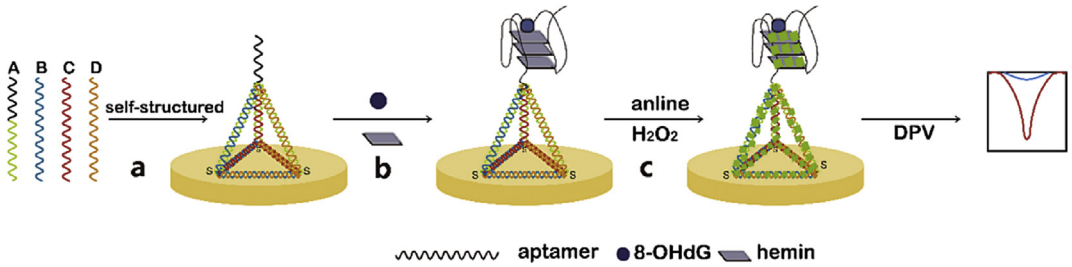

Scheme 1. Mechanism for ultrasensitive detection of 8-OHdG via PANI deposition and tetrahedral DNA nanostructure.

surface.

The bare electrode has no observable semicircle region. Electron-transfer resistance (R_{et}) values increased stepwise with the TDN, 8-OHdG, hemin modified to the gold electrode step wisely because the negative charges of DNA prevent repelling electrons from approaching the electrode surface (Fig. 1B, curve a, b, c). After the polymerization of PANI onto the electrode, a large increasing R_{et} was observed (Fig. 1B, curve d), suggesting the formation of PANI further inhibited the interfacial electron transfer. On the basis of these results, we could conclude that the self-assembly of DNA, formation of hemin/G-quadruplex and the deposition of PANI process were successfully achieved on the gold electrode surface.

CV (inset in Fig. 1C) and DPV (Fig. 1C) were also employed to characterize the feasibility of the strategy. No peak was observed on the CVs obtained from bare gold electrode (inset in Fig. 1C, curve a). Weak peaks were observed for TDN modified gold electrode (inset in Fig. 1C, curve b) and hemin/aptamer/TDN modified electrode (inset in Fig. 1C, curve c) due to the weak catalysis of H_2O_2 on the polymerization of aniline. However, a pair of well-defined redox peaks was observed for the TDN-aptamer modified gold electrode in the presence of 8-OHdG, H_2O_2 and aniline with the anodic and cathodic potential located at −0.06 and −0.10 V, respectively (inset in Fig. 1C, curve d). Obviously, this new couple of peaks was due to the redox of PANI. The reason is that 8-OHdG induced aptamer to form G-quadruplex structure that have high catalytic activity for PANI polymerization. DPV results were in well agreement with CV. No obvious peak was observed from bare gold electrode (Fig. 1C, curve a), TDN modified gold electrode (Fig. 1C, curve b), hemin/aptamer/TDN modified electrode (Fig. 1C, curve c). An obvious peak at −0.06 V was obtained after added 8-OHdG (Fig. 1C, curve d). These observations suggested that the electrochemical response owed to 8-OHdG induced high catalytic hemin/G-quadruplex, which resulted in the deposition of PANI not only on TDN structure but also on hemin/G-quadruplex themselves. The detection sensitivity was improved greatly by increasing the DPV current to 10-fold (~3 μA) compared to our previously reported method for detection of methyltransferase using PANI as signal probe but without TDN as template.

The surface morphology of formative PANI on the gold electrode surface was also characterized by SEM (Fig. 1D). The separation of

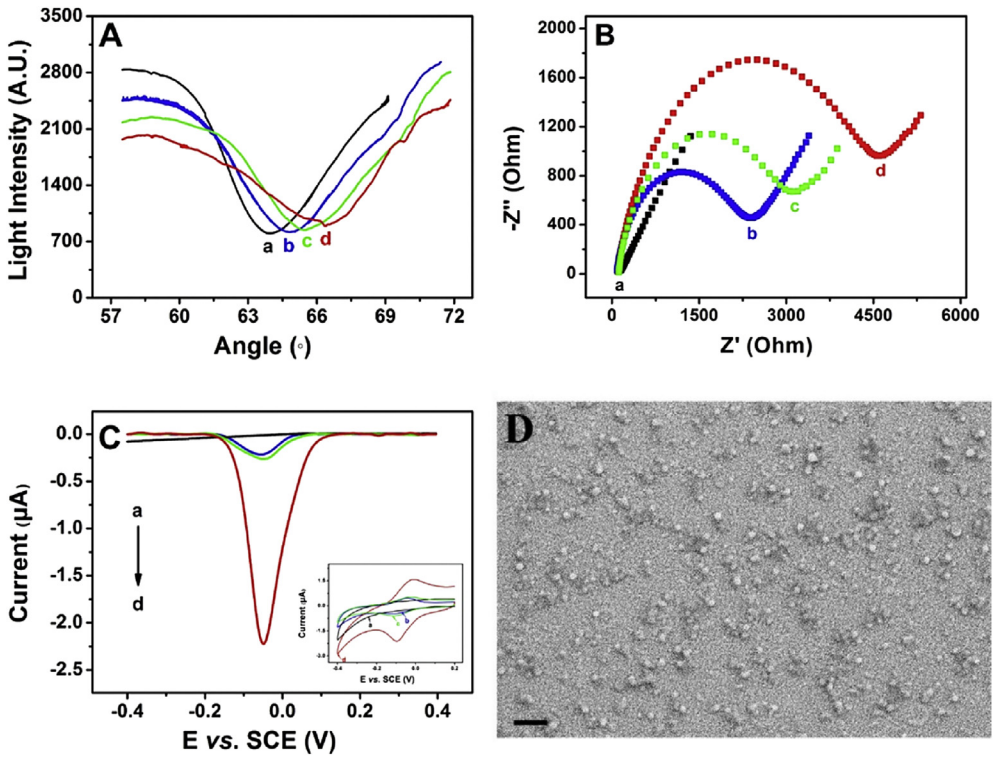

Fig. 1. (A) SPR of bare chip (a), TDN modified chip (b), hemin/aptamer/TDN modified chip (c), and hemin/G-quadruplex/TDN on chip (d). (B) EIS, (C) DPV and CV (Inset in C) of the bare gold electrode (a), TDN modified gold electrode (b), hemin/G-quadruplex/TDN modified gold electrode (c), and hemin/G-quadruplex/TDN gold electrode (d). (D) SEM image of PANI deposition on the gold substrate with TDN. The scale bar indicates 200 nm.

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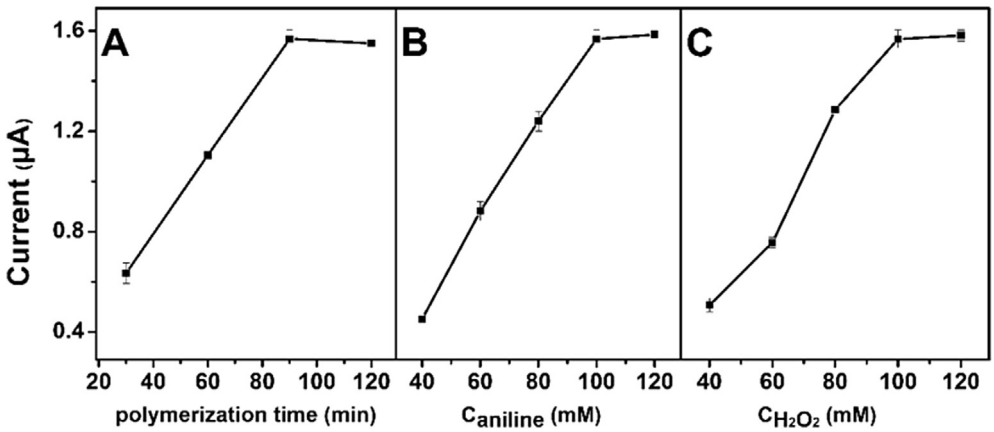

Fig. 2. The impact of (A) polymerization time: 30, 60, 90, 120 min, (B) H_2O_2 concentration: 40, 60, 80, 100, 120 mM, and (C) aniline concentration: 40, 60, 80, 100, 120 mM respectively. Error bars showed the standard deviation of three experiments.

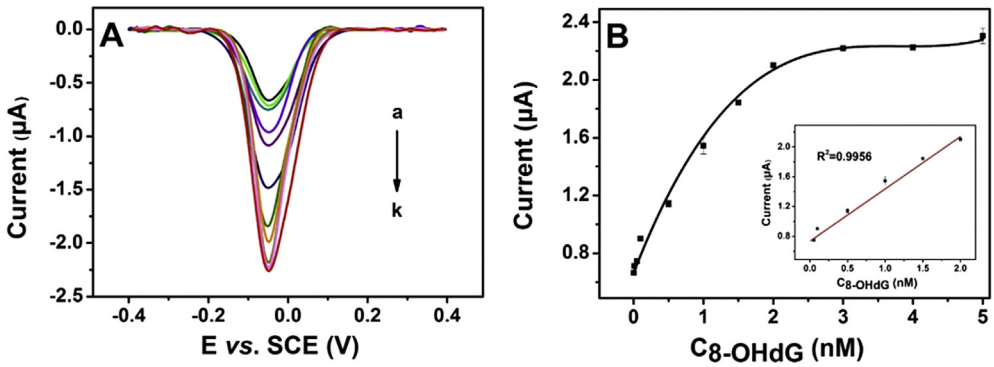

Fig. 3. (A) DPV corresponded to the different concentrations of 8-OHdG. From a to k:0, 0.01, 0.05, 0.1, 0.5, 1, 2, 3, 4, 5 nM of 8-OHdG. (B) The scatter plot for the relationship of DPV current versus 8-OHdG concentration ranging from 10 pM to 5 nM. Inset showed the linear response at low concentrations from 0.01 nM to 2 nM. Error bars showed the standard deviation of three experiments.

TDN anchored aptamer led to the intermittent PANI deposition on the gold chip surface, which allowed us to observe the morphology of nanoparticles or their accumulation intermittently coating on substrate.

3.2. Optimization for 8-OHdG detection

It is expected that the performance of the biosensor would mainly be affected by polymerization time, H_2O_2 concentration, and aniline concentration. Thus the effect of PANI deposition time was first investigated. As shown in Fig. 2A, the peak current raised with increasing time from 30 min to 90 min, and then leveled off when the time extended further. Therefore, 90 min was selected to ensure the best performance of the biosensor. Fig. 2B and C showed the relationship between the current and H_2O_2, aniline concentration. The optimization of H_2O_2 and anilne concentration was carried out in the range of 40–100 mM similarly. The current increased with concentration of H_2O_2 and aniline in the range of 40–100 mM, then reached a plateau. Thus, 100 mM H_2O_2, and 100 mM aniline were used in the subsequent research.

Table 2
Comparison of analytical performance of various methods.

Method	System	Detection range	Detection limit	Reference
Fluorescence	K^+-stabilized G-quadruplex	3.96–211 nM	1.19 nM	[34]
RLS	Conformational switching of aptamer	0.09–14.1 nM	27 pM	[35]
ECL	CQDs coated Au/SiO_2 core-shell NPs	0.7–70 nM	0.3 nM	[36]
UV–vis	G-quadruplex/hemin DNAzyme	0.466–247 nM	141 pM	[37]
CD	Chiroplasmonic assemblies of AuNPs	0.05–2 nM	33 pM	[38]
FSCV	Carbon fiber microelectrode	3–1000 nM	–	[2]
DPASV	CNT/PEI	0.5–30 μM	0.1 μM	[9]
CV	ss-DNA/GNs/GCE	0.056–36.55 μM	0.875 nM	[10]
DPV	G-rich/gold electrode	1.75–3675 nM	0.315 nM	[39]
DPV	PICA/CHI/GCE	0.353–35300 nM	105.9 pM	[40]
CE-ECD	MIP monolithic capillary column	0.01–1.5 μM	2.6 nM	[41]
CV	MWCNTs/GCE	0.0563–16.4 μM	18.8 nM	[42]
EIS	MIP	3.5–3500pM	2.6 pM	[43]
DPV	Hemin/G-quadruplex/gold structure	0.01–2 nM	1 pM	this work

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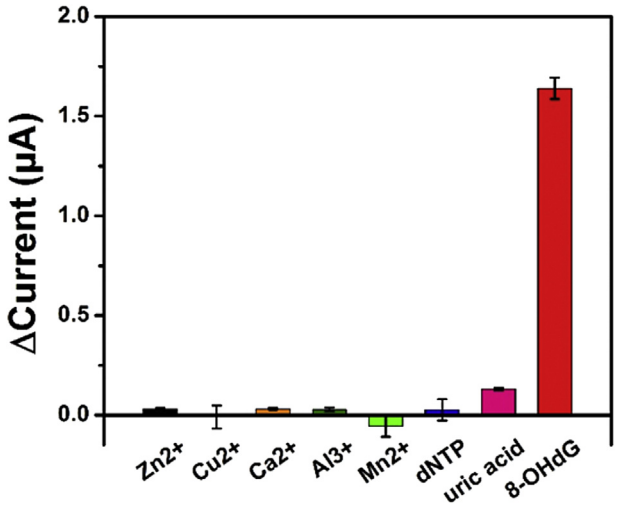

Fig. 4. Selectivity of the sensing system in the presence of 8-OHdG (2 nM), Zn^{2+}, Cu^{2+}, Ca^{2+}, and Al^{3+}, dNTPs, and uric acid (200 nM). Error bars showed the standard deviation of three experiments.

3.3. Detection performance of 8-OHdG

The electrochemical response of PANI toward different concentrations of 8-OHdG were measured by DPV under the optimal conditions. As shown in Fig. 3A, the DPV intensity gradually increased with the increment of 8-OHdG concentration in the range of 10 pM to 5 nM. The reason is that more hemin/G-quadruplex was formed and more PANI were polymerized on the TDN and hemin/G-quadruplex with the increasing concentration of 8-OHdG. Hemin/G-quadruplex, induced by 8-OHdG, not only served as catalyst but also served as template for PANI deposition. Compared with our previously reported method for detection of methyltransferase activity by using modified hemin/G-quadruplex as catalyst and deposited PANI on a signal probe, DPV current increased from 0.28 μA to 2.8 μA. The 10-fold current improved the detection sensitivity greatly. The calibration curve was constructed by plotting the variation of DPV current with different 8-OHdG concentrations (Fig. 3B). The method could be used to detect 8-OHdG in the linear range of 10 pM to 2 nM, with the linear equation of $y = 0.735 + 0.702x$, correlation coefficient of 0.9956, detection limit of 1 pM (S/N = 3). The comparison between the proposed method and some of the previously reported methods was shown in Table 2. The sensitivity was improved to almost 300-fold when compared with most of the previously reported electrochemical methods [9,10,36,39–41].

3.4. Selectivity of 8-OHdG detection

The specificity of the biosensor was evaluated by assay of other metal ions, uric acid (possible coexisted materials in the real urine samples) and deoxy-ribonucleotide triphosphates includes dATP, dGTP, dTTP and dCTP (dNTPs, 8-OHdG analogues). Fig. 4 showed a dramatic increase of DPV intensity was clearly observed in the presence of 8-OHdG whereas there was no obvious DPV change with the addition of various metal ions and 8-OHdG analogues, including Zn^{2+}, Cu^{2+}, Ca^{2+}, and Al^{3+}, uric acid, dNTPs, even at 200 times higher concentration than that of 8-OHdG. Thus, this proposed strategy had high selectivity for detection of 8-OHdG.

3.5. Analytical application to urine and serum samples

Long-term accumulation of 8-OHdG in the body of DNA will increase spontaneous mutation rate, and correlate with the occurrence and development of cancer [5]. Therefore, determination of 8-OHdG expression levels is important for understanding the role of oxidative DNA damage in the process of tumor occurrence and development. According to this, Bladder cancer's urine samples provided by Nanjing General Hospital of Nanjing Military Command and health volunteer were detected. The obtained results were provided in Table 3. From the results obtained, the results of normal and bladder cancer were significantly different, which were consistent with the clinical diagnosis and the results obtained by ELISA method. Levels of 8-OHdG in serum samples were also measured. Human serum from volunteer were gathered. The human serum was diluted in 1:10 ratio with the DNA hybridization buffer. Then, the sample spiked with 0.02 and 0.05 nM 8-OHdG in healthy urine sample, 0.1, 0.2, 0.5, 1.0, and 1.5 nM 8-OHdG in both urine and serum samples were analyzed. Recoveries of 8-OHdG in urine and serum samples were from 91.3% to 115% and the relative standard derivations were in the range of 2.78%–6.46%, indicating that the method had good accuracy and

Table 3
Application of the detection strategy in urine and serum samples.

Sample	Patient ID	Clinical diagnosis	Added (nM)	Total found (nM)	Recovery (%)	RSD (%, n = 3)
Urine	–	Normal	0	–	–	3.31
			0.02	0.023	115	5.35
			0.05	0.047	94.0	4.89
			0.1	0.11	110	2.88
			0.5	0.49	99.4	6.13
			1.0	1.02	102	5.80
Urine	1005328808	Bladder cancer I[a]	0	0.21	–	3.55
			0.1	0.29	96.4	2.78
			0.5	0.78	109	3.97
			1.0	1.12	92.9	4.11
Urine	1005351971	Bladder cancer II[a]	0	0.35	–	5.22
			0.1	0.49	109	5.12
			0.5	0.88	103	4.18
			1.0	0.93	92.9	4.56
Serum	–	Normal	0	0.1353	–	6.46
			0.1	0.225	95.6	3.88
			0.2	0.306	91.3	5.07
			0.5	0.663	104.4	4.38
			1.0	1.103	97.2	4.25
			1.5	1.595	97.5	3.60

[a] 8-OHdG was 0.193 nM for bladder cancer I and 0.387 nM for bladder cancer II when they were detected by ELISA.

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high precision. Therefore, these results indicated that the strategy held great potential for clinical examinations evaluation of cancer development.

4. Conclusion

In summary, a label-free ultrasensitive sensing platform for 8-OHdG was proposed based on it induced hemin/G-quadruplexes from its aptamer. The high catalytic activity of hemin/G-quadruplexes not only triggered PANI deposition but also served as good deposition template together with TDN structure due to their ample duplex DNA structure. Taking advantages of high selectivity of aptamer, high catalytic activity of hemin/G-quadruplexes, exquisite environment for PANI deposition supplied by both TDN and hemin/G-quadruplex and remarkable conductivity of PANI, 8-OHdG could be detected in the linear range from 10 pM to 2 nM, with the detection limit of 1 pM. Compared with most previously reported electrochemical method that based on detection the oxidation current of 8-OHdG, the sensitivity of this method was improved to almost 300-fold. The method was also simple, selective and reliable. It avoided the complex label procedures and nanomaterial synthesize. In addition, it has been successfully applied in complex matrix of human urine and serum samples with good accuracy and precision, which indicated that the method has good applicability and huge potential to be applied in clinical examinations.

Notes

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