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COMMUNICATION

Electrochemiluminescence signal amplification combining with conformation-switched hairpin DNA probe for determining methylation level and position in the Hsp53 tumor suppressor gene

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We report a new strategy for detection of methylation level and position in the Hsp53 tumor suppressor gene based on the electrochemiluminescence signal amplification combining with a conformation-switched hairpin DNA probe for improving selectivity.

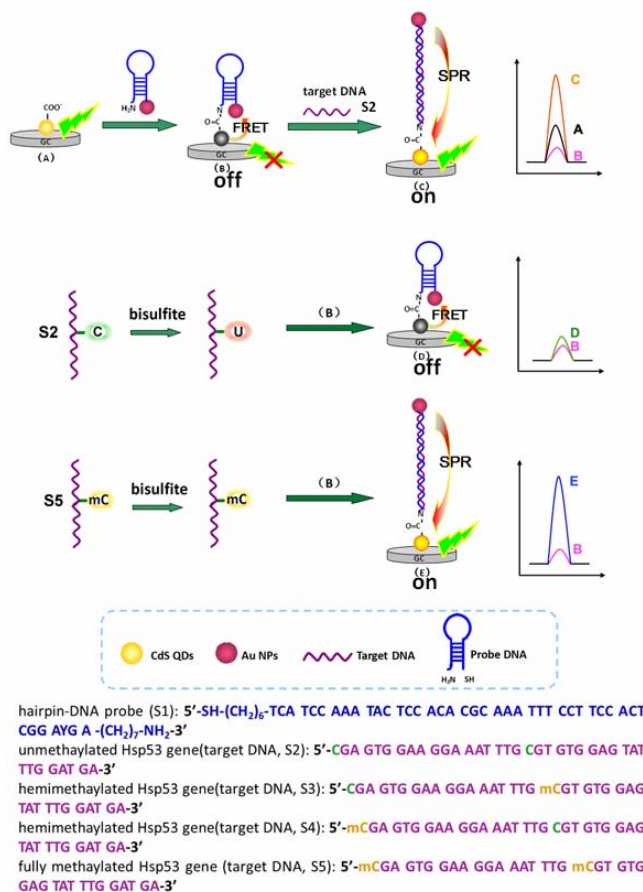
DNA methylation, which refers to the addition of a methyl group to adenine or cytosine residues in the specific DNA sequence, is one of the most important epigenetic events, and plays an important role in the regulation of gene transcription, eukaryotes development, and cellular differentiation as well as the pathogenesis of vital human diseases.¹ Therefore, the quantitative and selective determination of DNA methylation is very important for biological and clinical analysis. A variety of methods have been developed for the determination of DNA methylation, including fluorescence,² surface plasmon resonance,³ colorimetric assay,⁴ capillary electrophoresis,⁵ high performance liquid chromatography (HPLC),⁶ electrochemical⁷ and electrochemiluminescence (ECL) methods.⁸ Among these methods, ECL method has attracted significant attention and has become a promising assay for specific detection of DNA methylation, because it combines the advantages of luminescent analysis with electrochemical assay such as low cost and rapid response etc. In addition, the separated source for excitation and detection enhances the sensitivity of the ECL with low background signals.⁹ However, few works have been reported on the detection of DNA methylation using ECL method.⁸ Those reported works are based on the discriminative capability of methyl-cytosine insensitive restriction enzymes,^{8a,8b} or use an antimethyl cytosine antibody-labeled enzymes to recognize and determine methylated cytosine in a DNA sequence.^{8c}

This work reports an ECL approach for high sensitive determination of methylation in human p53 tumor suppressor gene (Hsp53). This approach is based on metallic nanoparticles (NPs)-semiconductor nanocrystals (NCs)-distance-dependent Förster resonance energy transfer (FRET) quenching and the surface plasmons resonance (SPR) enhancement for ECL. The Hsp53 gene is one of the most intensely investigated genes due to its dysregulation in the majority of human cancers.¹⁰ Aberrantly methylation of the CpG islands in Hsp53 gene can result in silencing of the gene, which is regarded as a hall mark of cancers.¹⁰ Thus, the

development of rapid and sensitive methods for identification and quantification of methylation status of Hsp53 is highly desirable in basic biomedical research and may provide an applicable approach for the early diagnosis of the related cancers. In this case, CdS NCs was selected as ECL emitter, Au NPs was used as ECL quencher and enhancer, and a hairpin-structured DNA with a rigid structure was used to control the distance between Au NPs and CdS NCs (The details of the synthesis and device fabrication are depicted in ESI). To achieve the assay, we first modified the glassy carbon electrode (GCE) with CdS NCs (~5 nm), then linked them with a hairpin-structured DNA probes (via forming the amide between -COOH at CdS NCs and -NH₂ moiety at DNA probes), which were labeled with Au NPs (~5 nm, Scheme 1). In the absence of the target DNA (Hsp53), a very weak ECL signal can be observed due to the quenching caused by FRET between the proximal CdS NCs and Au NPs (curve B in Scheme 1).¹¹ Upon the hybridization with Hsp53, the stem of the hairpin DNA is opened and the CdS NCs and Au NPs are separated by a certain distance, an ECL enhancement can be observed owing to the energy transfer of ECL excited by SPR from Au NPs to CdS NCs (curve C in Scheme 1).¹¹ However, when Hsp53 was pretreated by bisulfite, the C in the gene sequence was deaminated and converted to uracil (U), the pretreated Hsp53 cannot be hybridized with the hairpin-structured DNA probe, and ECL enhancement cannot be observed (curve D in Scheme 1). If the Hsp53 is methylated (converting C to mC) before treated by bisulfite, mC cannot be converted to U,¹² and therefore remains hybridization ability with probe DNA,¹² and ECL enhancement can still be observed (curve E in Scheme 1). As a consequence, the recognition of Hsp53 methylation can be realized based on the ECL enhancement. In comparison with those previously reported ECL approaches to determine DNA methylation, a notable advantage of our developed assay is the scission- and enzyme-free, and therefore the results are not easily affected by many ambient factors such as pH, temperature and humidity (enzymes are easily affected by these ambient factors). Moreover, this assay is based on ECL signal amplification, which can ensure the highly sensitive detection of DNA methylation.

In order to achieve high energy transfer efficiency, the FRET-SPR pair must ensure maximum spectral overlap between the donor emission spectrum and the acceptor excitation spectrum.¹¹ As shown in curve a, Fig. 1, Au NPs displayed a strong absorption band around

507 nm. After conjugated with S1, Au/S1 exhibited the maximum absorption at ~ 515 nm (curve b), having a slight red shift (8 nm) in comparison with that of the Au NPs. This red-shift probably resulted from the changes of the surface charges of Au NPs caused by conjugation with oligonucleotide (S1, the sequences of various DNA are listed in Scheme 1). The ECL emission of CdS NCs has a considerable overlap with SP absorption of Au NPs because the ECL emission of CdS NCs was at ~ 511 nm (curve c, Fig. 1). Therefore, the hybridization of S1 with target DNA (S2) led to a significant enhancement of ECL signals (curve d, Fig. 1).



Scheme 1. Schematic representation of the recognition of Hsp53 gene methylation based on enhancement of ECL emission from CdS NCs film by Au NPs.

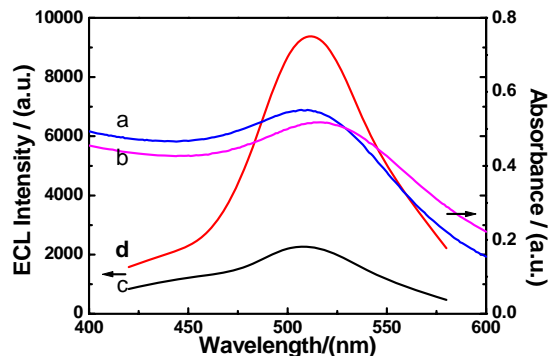


Fig. 1 The UV-vis absorption spectrum of Au NPs (a) and Au/S1 (b), the ECL spectrum of CdS NCs film on GCE (c) and Au/S1 functionalized NCs film on GCE hybridization with S2 (d).

CdS NCs generated an ECL emission at the potential of ~ -1.2 V (curve a, Fig. 2). In this cathodic process, CdS NCs immobilized on the electrode were reduced to CdS⁻, while the co-reactant S₂O₈²⁻ ion was reduced to the strong oxidant SO₄^{-•}.^{13c} The generated CdS⁻ could react with SO₄^{-•} through electron transfer, and produce the excited state (CdS*) to emit light. After linked with S1-labeled Au NPs, the ECL intensity of CdS NCs had an obvious descent (curve b, Fig. 2), implying that ECL emission of CdS NCs was effectively quenched by the proximal Au NPs. However, the ECL signals showed a significant enhancement (~ 3 times of that for CdS NCs and ~ 10 times of that for Au/S1 functionalized NCs) after the immobilized DNA probe (S1) was hybridized with target DNA (S2) (curve c, Fig. 2). This signal enhancement was resulted from the effective amplification of the SPR of Au NPs toward ECL signal of CdS NCs due to the hybridization-induced separation between the Au NPs and CdS NCs.¹¹ If S2 was pretreated with bisulfite (C in the sequence was converted to U, therefore, S2 was converted to 5'-UGA GTG GAA GGA AAT TTG UGT GTG GAG TAT TTG GAT GA-3') before hybridized with S1, the ECL intensity remained almost unchanged (curve e, Fig. 2), indicating the bisulfite-treated S2 cannot be hybridized with S1. However, ECL intensity remained almost invariant compared with curve c after DNA probes hybridized with S5 (curve d. Note that S5 is the methylated S2), indicating the cytosine methylation modification does not affect the DNA hybridization. For S5, after the bisulfite treatment, mC remained unchanged, and can be complete hybridized with S1, showing the enhanced ECL intensity (curve f). These results demonstrated that the methylation of Hsp53 can be detected sensitively from the ECL signals resulted from energy transfer between the Au NPs and CdS NCs.

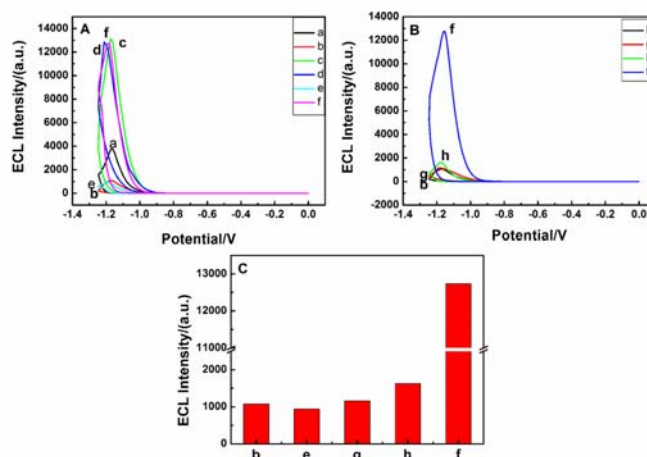


Fig. 2 (A) Cyclic ECL curves of CdS NCs film on GCE (a); Au/S1 functionalized NCs film on GCE (b); Au/S1 functionalized NCs film on GCE hybridization with S2 (c), S5 (d), S2 pretreated with bisulfite (e) and S5 pretreated with bisulfite (f). (B) Cyclic ECL curves of Au/S1 functionalized NCs film on GCE before (b) and after hybridization with pretreated target DNA S4 (g), S3 (h) and S5 (f). (C) Corresponding histograms of the ECL peak height.

The ECL signal was also sensitive to the position of the methylated C in the Hsp53 gene. This feature can be used to recognize the different methylation status in the DNA sequence. For Hsp53 gene, there are two methylated position, therefore, three kinds of methylation status exist. One is fully methylated status (S5), and the other two are hemimethylation status (S3 and S4). As shown in Fig. 2B and 2C, the highest ECL signal was observed after DNA probe (S1) was hybridized with S5 (the fully methylated Hsp53 gene, f in Fig. 2B, 2C). However, if the DNA probe was hybridized with

pretreated S3 or S4 (both them are the hemimethylated Hsp53 gene), the ECL signal had a slight increase (curve g and h in Fig. 2B, 2C) in comparison with that of S2 (curve b in Fig. 2B, 2C). After carefully analyzing the ECL signal depicted in curve g and h (Fig. 2B, 2C), one could find that the ECL signal in curve h (for mC in the middle of the sequence, S3) is slightly higher than that in curve g (for mC in the head of the sequence, S4), implying that our developed method can recognize the methylation position in the Hsp53 tumor suppressor gene. Note that several experimental parameters need to be carefully selected and optimized before this method extends to analyze the methylation position and level in other DNA sequences.

We applied the developed method to quantitatively determine the concentration of the methylated DNA (use S5 as an example). The concentration-dependent ECL signal was depicted in Fig. 3A. With the increasing concentration of S5, the ECL signal is increased. The ECL increment ΔI ($\Delta I = I - I_0$, I_0 and I is ECL signal of the Au/S1-functionalized CdS NCs on GCE before (I_0) and after (I) hybridization with S5, respectively) was found to be logarithmically related to the concentration of S5 in the range from 20 pM to 10 nM ($R=0.999$, Fig. 3B). The limit of detection (LOD) was experimentally estimated to be 8 pM, which is superior to those previously report based on electrochemistry methods,^{7a} implying high sensitivity of the developed method. The high sensitivity is mainly attributed to the signal amplification of Au NPs to the CdS NCs. In addition, the reproducibility of this ECL assay was evaluated from the response of a pretreated S5 concentration of 100 pM at seven electrodes. A relative standard deviation (RSD) of ca.5.5% is obtained, suggesting high reproducibility of the developed methods.

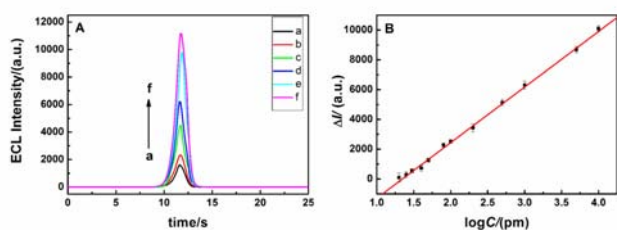


Fig. 3. (A) Cyclic ECL curves of Au/S1 functionalized NCs film on GCE hybridization with different concentrations of pretreated S5. The concentration of S5 is (a) 30 pM, (b) 50 pM, (c) 200 pM, (d) 500 pM, (e) 5 nM, and (f) 10 nM pm. (B) the liner relationship between relative ECL intensity (ΔI) and the logarithm of S5 concentration.

In summary, an ECL signal amplification method has been developed for detecting DNA methylation status of Hsp53 gene. This assay has the ability to recognize the methylation position in the Hsp53 gene and to detect the concentration of methylated Hsp53 gene as low as 8 pM. This method can extended to analyze the methylation position and level in other DNA sequences.

In contrast to those previously reported methods for DNA methylation assay, our method detects ECL signal enhancement resulted from the specific, hybridization-induced conformational change of the hairpin probe. Therefore, our approach should avoid false signals arising from the nonspecific adsorption of interferences. Therefore, our approach has the potential to become a new method for DNA-methylation-related disease diagnosis.

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Notes and references

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