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Short communication

Ultrasensitive flow injection chemiluminescence detection of DNA hybridization using nanoCuS tags

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Abstract

A novel and sensitive biosensor for the determination of short sequence of DNA based on flow injection (FI)-chemiluminescence (CL) system of luminol–H_2O_2–Cu^{2+} was developed in the present work. The DNA probe labeled with copper sulfide nanoparticles (CuS NPs) could hybridize with target DNA immobilized on glass–carbon electrode (GCE). The hybridization events were monitored by the CL intensity of luminol–H_2O_2–Cu^{2+} after the cupric ions was dissolved from the hybrids. A preconcentration process of cupric ions was performed by anodic stripping voltammetry (ASV) technology to improve the sensitivity of the biosensor. Under the optimum conditions, the CL intensity was proportional to the concentration of target DNA in the range of 2.0×10^{-12}–1.0×10^{-10} M. A detection limit of 5.5×10^{-13} M of target DNA was achieved. The CL intensity of two-base mismatched sequences and noncomplementary sequences were also detected. The experiments indicated that two-base mismatched sequences showed weaker CL intensity and noncomplementary sequences gave no response at all.

Keywords: Flow injection chemiluminescence; Preconcentration; DNA biosensor; CuS nanoparticles

1. Introduction

Chemiluminescence (CL) strategy, along with fluorescence imaging (Jayne et al., 1985; Benoit et al., 2001), electrochemical methods (Hoang et al., 2005; Zhu et al., 2006), micro-gravimetrical techniques (Gasparac et al., 2004), bioluminescence, and electrogenerated chemiluminescence (ECL) techniques (Su et al., 2004), are all the most important methodologies for the detection of short sequence DNA in the fields of gene mutation, disease diagnosis, drug screening, and forensic activation analysis (Miao and Bard, 2003; Leslie et al., 2007; Rebeca et al., 2007; Wang, 2000; Shinsuke et al., 2005).

CL DNA biosensors are generally based on measuring the specific luminescence activity of the labels linked to the capture DNA probe for the detection of target DNA (Chen et al., 1998; Fumiko et al., 2005; Masaaki et al., 1999). Enzymes are often employed as biocatalytic labels for the amplified detection of DNA-sensing events. Willner and co-workers (Xiao et al., 2004a,b; Pavlov et al., 2004; Niazov et al., 2004) had done many works to create more sensitive nucleic acid biosensors by DNA-labels hybrid systems. They employed both hemin/quadruplex (G-quadruplex) DNAzyme and functionalized Au-NPs as carriers of the DNAzyme units for the amplified detection of DNA via the biocatalytic process. These methods enabled the analysis of the DNA with detection limits corresponding to 1.0×10^{-9}–1.0×10^{-10} M. Cao et al. (2006) reported on the use of a catalytic nucleic acid label (DNA-hemin) for the determination of DNA with a detection limit of 1.0×10^{-10} M. Chen et al. (2000) designed oligonucleotide probe labeled directly by alkaline phosphatase (AP) and about 4 pg target DNA could be detected.

Although large signal amplification can be achieved, the detection limit of DNAzyme-label method for the determination of DNA is not low enough, and once prepared, the activity of the conjugate must be periodically controlled owing to the inherent poor stability of the enzymes. Thus, use of nanomaterial as a label for the signal amplification attracts a great deal of attention. Mirkin and co-workers (Taton et al., 2000) found oligonucleotide-labeled gold nanoparticle substantially altered the melting profiles of the targets from an array substrate. They

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also developed gold nanoparticle-based electrical DNA detection with catalytically deposited silver as an enhancing element (Park et al., 2002). A sensitivity of 100 times higher than an analogous fluorescence system could be offered with a signal amplification method based on nanoparticle-promoted reduction of silver(I). Wang et al. (2003a,b) proposed a new strategy for amplifying a hybridization signal using an electroactive microsphere with redox marker confined. Metal sulfide nanoparticles were also used as labels for the detection of DNA targets using electrochemical stripping methods (Zhu et al., 2004; Wang et al., 2003a,b; Hansen et al., 2006). But up to now, few metal nanoparticles used as label in CL DNA sensor has been reported.

In this work, a novel and sensitive method for the determination of short sequence DNA based on FI-CL system of luminol–H_2O_2–Cu^{2+} was described. Cupric ions were dissolved from hybrids tagged with copper sulfide nanoparticles (CuS NPs), and the concentration of target DNA could be determined based upon the concentration of dissolved cupric ions by the CL intensity of luminol–H_2O_2–Cu^{2+}. To increase the sensitivity of the DNA biosensor, the copper(II) was retained temporarily by electrochemical preconcentration on a platinum plat electrode placed in an anodic stripping voltammetric cell. From the analytical chemistry point of view, this protocol will be quite promising for numerous applications in DNA hybridization and immunoassay.

2. Experimental

2.1. Reagents

Eighteen-base synthetic oligonucleotides were purchased from SBS Genetech Co. Ltd. (China). Their base sequences are as below: probe sequence, 5′-NH_2-CGA CAG TGG TCC CAA AGA-3′; target sequence, 5′-TCT TTG GGA CCA CTG TCG-3′; two-base mismatched sequence, 5′-TCT TCG GGA CCG CTG TCG-3′; noncomplementary sequence, 5′-CGA CAG TGG TCC CAA AGA-3′ (the same as the probe sequence without the amino-group attachment at the 5′ end).

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was purchased from Sigma (US). Luminol was purchased from ABCR GmbH&Co. (Germany). Imidazole was obtained from Guoyao Chemical Company (China). Mercaptoacetic acid was obtained from Yuanhang Chemical Company (China). All reagents were of analytical grade and used without further purification.

Luminol stock solution of 1.5×10^{-2} M was prepared by dissolving 2.6574 g of luminol in 100 mL of 0.5 M NaOH solution. Chitosan solution (1 wt%) was prepared by ultrasonically dissolving chitosan powder in 1% acetic acid. NaOH–$NaHCO_3$ buffer (pH 11.0), PBS buffer (pH 7.4), TE buffer (pH 8.0), and phosphate buffer containing SDS (pH 7.0) were prepared by general methods. Doubly distilled and deionized water was used throughout.

2.2. Apparatus

A FI-CL instrument (IFFM-E, Remex Analytical Instrument Co. Ltd., Xian, China) consists of two peristaltic pumps (P1, P2), two mixture valves, a switching valve, which was connected with an ASV cell and a CL detector (IFFS-A, Remex Analytical Instrument Co. Ltd., Xian, China) as shown in Fig. 1A. All components in the flow system were connected by polytetrafluoroethylene (PTFE) tubing (0.8 mm i.d.).

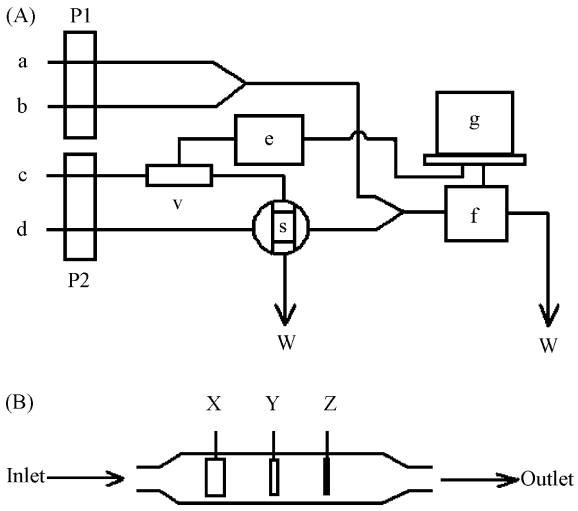

Fig. 1. Schematic diagram of the FI-CL system (A) and the anodic stripping voltammetric cell with electrodes (B). (a) Luminol, (b) H_2O_2, (c) sample, (d) water carrier, (p1 and p2) peristaltic pumps, (s) switching valve, (v) anodic stripping voltammetric cell, (e) electrochemical analyzer, (f) flow cell and detector, (g) computer, (w) waste, (x) working electrode, (y) reference electrode, (z) counter electrode.

Electrochemical preconcentration performed in the ASV cell was carried out on a CHI 832B electrochemical analyzer (CH Instrument Company, USA) with a three-electrode system consisting of a platinum wire that served as an auxiliary electrode, an Ag/AgCl electrode as reference electrode, and a platinum plat electrode as working electrode with an area of 2.5 mm × 8 mm. The electrodes were fabricated in the preconcentration cell as shown in Fig. 1B.

UV–vis spectra were taken by using a Cary 50 UV-Vis-NIR spectrophotometer (Varian). Transmission electron microscopy (TEM) image was taken with a JEOL JSM-6700F instrument (HITACHI).

All glassware used was cleaned in a bath of freshly prepared HCl-HNO_3 (3:1) solution, rinsed thoroughly in doubly deionized water and dried in air.

2.3. Modification of DNA probe with CuS NPs

CuS NPs were prepared according to the literature (Milica et al., 1990) by using mercaptoacetic acid as the stabilizer. Briefly, 9.22 μL mercaptoacetic acid was added to 50 mL of 0.4 M $Cu(NO_3)_2$ solution, and the pH of the mixture was adjusted to 7.0 with 0.5 M NaOH solution. After being bubbled with N_2 for 30 min, 1.34 M Na_2S solution was added dropwise to the mixture to keep the molar ratio of Na_2S to $Cu(NO_3)_2$ at approximately 2.5. The reaction was carried out for 24 h under N_2 bubbled, and a brown colloid was formed gradually. The CuS NPs have an average diameter of 20 nm.

Two hundred microlitres of 0.1 M imidazole solution (pH 6.8) was added to the 1.2×10^{-6} M of 5′-amino group capped DNA probe solution. After 30 min, 100 μL of 0.1 M EDC and 5.0 μL of CuS colloid were added to the mixture, and incubated at room temperature for 12 h with stirring. Finally the DNA probe tagged with CuS NPs was collected by centrifugation for 30 min at 10,000 rpm. The precipitate was washed and then resuspended in water. The solution of DNA probe–nanoCuS conjugates was stored at −5 °C for the hybridizations.

2.4. Immobilization and hybridization of DNA

The GCE was polished with 1.0, 0.3 and 0.05 μm alumina, respectively, before immobilization of the target DNA. The electrode was thoroughly washed with water, sonicated in ethanol, and finally dried with N_2.

The target DNA was immobilized on GCE according to the reference (Zhu et al., 2004). Briefly, 2.0 μL of 1% chitosan solution was dropped uniformly on the surface of a freshly smoothed GCE. After being dried naturally, the electrode was immersed in 1.0 mL of TE buffer containing a certain concentration of target DNA at room temperature for 1 h with stirring. The electrode was then taken out and rinsed for three times with phosphate buffer.

Hybridization reaction was performed by immersing the target DNA modified GCE in the prepared solution of DNA probe modified with CuS NPs, and incubated at room temperature for 1 h with stirring. And then the electrode modified with hybrids–nanoCuS conjugates was washed for three times with 0.1% SDS-phosphate buffer to remove the unhybridized DNA probe.

The same protocol as above was also applied for the immobilization and hybridization of two-base mismatched sequences or noncomplementary sequences with DNA probe modified with CuS NPs.

2.5. FI-CL detection

The GCE modified with hybrids–nanoCuS conjugates was immersed into a colorimetric tube containing 200 μL of 0.2 M nitric acid solution for 5 min. The CuS NPs anchored on the hybrids were dissolved immediately. The volume of the solution was adjusted to 1 mL with doubly distilled and deionized water.

Above solution was introduced into the flow line by P2 at a constant speed of 0.75 mL min^{-1} when the direction of the switching valve was in the load position. At the same time, deposition of copper was performed on the flat platinum electrode at −0.8 V for 80 s. During the next step of 6 s, 400 μL of nitric acid solution (pH 5.0) was drawn into the ASV cell. During the third step, copper(II) was stripped out from the platinum plat electrode surface at the potential of +0.3 V. In the following step of 8 s, the direction of the switching valve was changed to the detection position. P1, P2 and the switching valve started to work simultaneously, the cupric ions preconcentrated were pumped by P2, the solution of luminol and H_2O_2 were pumped by P1 into the flow lines, respectively. During the last step of 15 s, the direction of the switching valve was changed to the original position, cupric ions was reacted with the mixture of luminol and H_2O_2 in the flow cell to produce CL signal. The concentration of target DNA was quantified based upon the concentration of dissolved cupric ions which was quantified by the CL intensity. The analytical frequency is about 3 min. The detection system is reproduced by cleaning the whole flow system with the dilute nitric acid solution (pH 5.0) for30 s.

3. Results and discussion

3.1. Preparation of DNA probe–nanoCuS conjugates

In the presence of EDC, the 18-base of 5′-amino group capped DNA probe was labeled with CuS NPs functionalized with carboxyl group. The UV–vis spectra of the CuS colloid, unmodified DNA probe and DNA probe labeled with CuS NPs were recorded by the spectrophotometer. Compared with the CuS colloid, a new band at about 235 nm appeared, which was similar to the unmodified DNA probe but a certain shift to the section of short wavelength. This result indicated that the target DNA was successfully labeled with the CuS NPs.

3.2. Optimization of detection conditions

The concentrations of luminol and H_2O_2 were examined, and 1.5×10^{-3} M of luminol solution and 0.4 M of H_2O_2 solution were chosen as the optimal concentrations.

The CL intensity was found to be greatly affected by the acid of the solution employed for the elution of cupric ions from the electrode surface. The low CL intensities at high pH values might be ascribed to the hydrolysis of cupric ions, which affects the deposition of cupric ions onto the platinum plat electrode. However, the low pH may destroy the optimal CL condition of luminol–H_2O_2. So the pH of 5.0 for the elution solution was selected for higher CL intensity and better reproducibility. The result of the experiment showed that maximum CL intensity could be obtained when the deposition potential was held at the Pt electrode between −0.6 V and −1.0 V. An optimum potential of −0.8 V was finally employed. Considering a compromise between analysis time and sensitivity, a deposition time of 80 s was chosen, and the anodic stripping potential of +0.3 V was selected (Qin et al., 1998). The flowing rate was 0.75 mL min^{-1}.

3.3. Kinetic curve of CL system

The CL kinetic curve of luminol–H_2O_2–Cu^{2+} system is shown in Fig. 2. It can be seen that the luminescence reaction was rapid. The CL signal could be observed after 5 s from injection of the solutions and the CL intensity reached a maximum at 7.5 s. Then the CL intensity decreased with elapsed time and almost disappeared at 12.5 s.

3.4. Sensitivity of the DNA biosensor

The target DNA was detected and quantified according to the constructed protocols. The CL signal of luminol–H_2O_2–Cu^{2+} increased with the increase of the concentration of the tar-

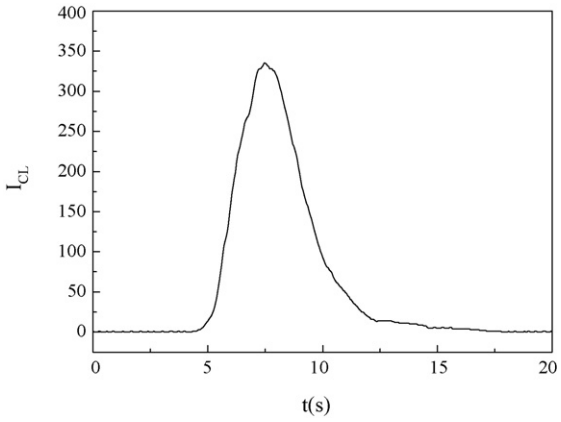

Fig. 2. CL kinetic curve of luminol–H_2O_2–Cu^{2+}, the concentration of target DNA was 1.0×10^{-10} M, the concentrations of luminol and H_2O_2 were 1.5×10^{-3} M and 0.4 M, respectively, the pH value was 5.0.

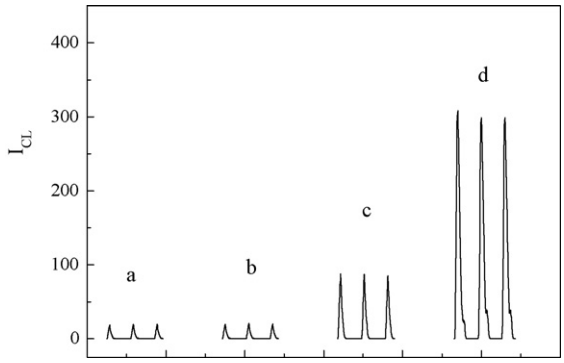

Fig. 3. CL signals of luminol–H_2O_2 catalyzed by cupric ions dissolved from DNA probe hybridized with different target DNA. (a) Blank solution, (b) noncomplementary sequences, (c) two-base mismatched sequences, (d) complementary sequences; all the concentrations of target DNA in (b), (c), and (d) were 1.0×10^{-10} M.

get DNA. The CL intensity revealed a linear relationship with the concentration of the target DNA ranging from 2.0×10^{-12} to 1.0×10^{-10} M. The regression equation could be expressed as $Y = 2.82X + 20.09$ (X was the concentration of target DNA for immobilization, 10^{-12} M; Y was the CL intensity) with a coefficient of 0.9937. The relative standard deviation (R.S.D.) was obtained to be 8.6% at a target DNA concentration of 2.0×10^{-12} M with 11 independent experiments. A detection limit of 5.5×10^{-13} M of target DNA could be further estimated using 3σ.

Wang et al. (2007) had investigated the application of luminescent NPs as probes for Affymetrix GeneChips detection. The low-abundance target DNA of 50 fM was detected without any amplification step. It is not the same kind of research with our work.

Li and co-workers (Liu et al., 2006) developed a new nanoparticle-based CL method for the detection of DNA hybridization with a detection limit of 5 fM. Compared with Li's work, there are three merits in our work: (1) The CL reaction of luminol–H_2O_2–Cu^{2+} is more simple and fast than the coupling CL reaction system of Ag^+–$K_2S_2O_8$–Mn^{2+}–H_3PO_4–luminol, which must be performed in the 90 °C water bath for 7 min. (2) Compared with 116 h being needed to prepare Ag NP-based oligonucleotides, it takes only 13 h to modify the DNA probe with CuS NPs. (3) CuS NPs which are cheaper than Ag NPs can be prepared easily at room temperature compared with Ag NPs prepared in ice bath.

3.5. Selectivity of the DNA biosensor

The selectivity of the present biosensor was investigated by using the DNA probe labeled with CuS NPs to hybridize with the complete complementary target DNA sequences (1.0×10^{-10} M), the two-base mismatched DNA sequences (1.0×10^{-10} M) and the noncomplementary DNA sequences (1.0×10^{-10} M), as shown in Fig. 3. A well-defined CL signal of luminol–H_2O_2–Cu^{2+} was obtained for the complementary sequences. The CL intensity of two-base mismatched sequences was significantly weaker than that of the complementary sequences, and the noncomplementary sequences showed no response. The results showed that an ultrasensitive DNA biosensor with high selectivity was obtained.

4. Conclusions

An ultrasensitive biosensor for rapid determination of the sequence of DNA was developed by detecting the CL intensity of luminol–H_2O_2–Cu^{2+}, where the cupric ions were dissolved from DNA probe hybridized with target DNA. The sensitivity of the sensor was improved with the preconcentration of the dissolved cupric ions using ASV process. The CL intensity demonstrated a wide linear relationship with the concentration of target DNA ranging from 2.0×10^{-12} to 1.0×10^{-10} M, and the detection limit of 5.5×10^{-13} M of target DNA was obtained. This CL biosensor with high sensitivity and selectivity might be a good alternative for other bioassays. The main drawback of the present CL method is the requirement of one or two steps more than other CL methods for the detection of DNA hybridization because the dissolution and preconcentration of CuS NPs are employed. We now envision finding more suitable nanoparticle tags and CL reactions to make the CL DNA assay more sensitive and simple.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2007.12.005.

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