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Ultrasensitive Fluorescence Immunoassay for Detection of Bisphenol A in Milk Products Using Functionalized Gold Nanoparticles as Probe

Wei Ji[1] · **Lingyun Du**[1] · **Yuanfu Zhang**[1] · **Guofu Liu**[2] · **Shuhao Wang**[1]

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Abstract An ultrasensitive fluorescence immunoassay was developed for detection of bisphenol A (BPA). Herein, thiol-modified single-strand DNA (SH-ssDNAs) and anti-BPA antibodies were simultaneously conjugated with gold nanoparticles (AuNPs), and the fluorescein isothiocyanate-labeled single-strand DNA was hybridized with SH-ssDNAs to form a dual-codified probe. In the presence of BPA, a competitive immunoreaction was conducted between BPA and BPA coating antigens immobilized on microplate for the anti-BPA antibodies of dual-codified probe. Then, in order to avoid the fluorescent signals quenched by AuNPs, restriction enzyme BamH I was employed to cut the fluorescent labels from immunocomplex, resulting in an increase of fluorescence emission. Under optimized conditions, the linear range for detection of BPA is from 1.0×10^{-2} to 1.0×10^{3} ng/L with a low limit of detection of 3.4×10^{-3} ng/L. This method is of good sensibility, stability, and specificity, and it can be used to detect BPA in actual milk products and its migration from packaging materials into milk.

Keywords Fluorescence immunoassay · Functionalized gold nanoparticles · Restriction enzyme · Bisphenol A

✉ Shuhao Wang
shuhaowang@sohu.com

[1] Shandong Provincial Key Laboratory of Chemical Energy Storage and Novel Cell Technology, College of Chemistry and Chemical Engineering, Liaocheng University, Liaocheng 252059, China

[2] College of Life Science, Liaocheng University, Liaocheng 252059, China

Introduction

Bisphenol A (BPA, 2,2-bis (4-hydroxyphenyl) propane), an indispensable organic raw material of elasticizer, antioxidant, hot stabilizer, and coating waterproofing material is widely used in cosmetics, paper products, food packaging, and water bottles (Staples et al. 1998; Vandenberg et al. 2007; Liao and Kannan 2011). However, numerous reports revealed that BPA possess certain estrogenic properties, constant exposure of BPA can induce the proliferation of cells and cause a series of potential pathological alteration of reproductive system even at extremely low exposure levels (Howdeshell et al. 1999; Hu et al. 2002; Takayanagi et al. 2006). And, BPA cannot participate in the process of normal metabolism, so a low content of compounds may bring discomfort. BPA in the organism mainly origins is from the intake of food (Schecter et al. 2010). Even though BPA was hydrophobic, BPA still can readily migrate into the food in various ways such as sustained exposure of food to BPA package or high temperatures process with BPA container or contact liposoluble components with BPA (Errico et al. 2014; Munguía-López et al. 2005; Cao and Corriveau 2008; Nam et al. 2010). Marsha K detected that the preschool children's urine samples contained BPA (Marsha et al. 2011). Therefore, for safety consideration, it is urgent to establish a simple and effective method for the analysis of BPA in food products. Recently, many detection methods of BPA have been established (Fontana et al. 2011; Zhou et al. 2014; Mei et al. 2013; Hegnerová et al. 2010; Maiolini et al. 2014; Huang et al. 2011). Among those, fluorescence immunoassay exhibited attractive attention based on its high sensitivity of fluorescence detection with the high specificity of immunoassay, which has been widely used in clinical

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medicine, biological sciences, and environments for several decades (Wang et al. 2014; Xu et al. 2011). Although traditional fluorescence immunoassay has the advantages of low background and wide dynamic range, the labeling procedure are relatively complicated and usually low temperature is extremely required. Therefore, searching for the stable, easy-to-prepare, low-cost, and highly biocompatible labels is of critical importance for the fluorescence immunoassay.

With simple preparation process, gold nanoparticles (AuNPs) have been widely employed as biological labels for bioanalyses for their unique physical properties and good biological compatibility (Thaxton et al. 2009; Gao et al. 2013; Zhou et al. 2013), which overcomes the safety problems, poor sensitivity, and poor stability associated with the radioisotope, fluorescent, and enzyme labels. In fluorescence immunoassay, AuNPs have already been applied as labels to detect estrogens (Wang et al. 2013) or DNA (Xia et al. 2010), biotoxin (Liu et al. 2013), and metal ion (Date et al. 2012).

We have reported a sensitive method for determination of BPA in the previous work (Du et al. 2015). It was based on gold nanoparticles and a europium(III)-labeled streptavidin tracer as probe of TRFIA. Although the sensitivity of this method is excellent, the preparation of probe is tedious, the lanthanide chelate was quite pricy, and the fluorescence quantum yield of was fairly low. Thus, the development of high sensitive fluorescence immunoassay has become even more attractive.

Fluorescein isothiocyanate (FITC) can directly couple with DNA to produce a brilliant probe with high fluorescence intensity which is excessively suitable to fluorescence immunoassay. A novel, rapid, and ultrasensitive fluorescence immunoassay for the detection of BPA was developed based on dual-codified AuNPs as probe in present paper. In the study, AuNPs was simultaneously modified by thiol-modified single-strand DNA (SH-ssDNAs) and anti-BPA antibodies. Then, FITC-labeled ssDNA was hybridized with SH-ssDNA to form a novel probe. To optimize the condition, restrict enzyme BamH I, for its mild biological and nontoxic properties, was used as a dissociative solvent to cut off the double-helix structure to release the FITC-labeled ssDNA from the AuNPs. These operations can effectively prevent fluorescence quenching from AuNPs, subsequently, resulting in an increase of fluorescence emission. The contents of BPA in several of milk product samples were screened to assess the reliability and sensitivity of the present method; the results indicated that the method was relatively reliable with excellent specificity for distinguishing BPA from analogues. And, ultrasensitive with the detection limit of the method for BPA is up to 3.4×10^{-3} ng/L.

Materials and Methods

Material and Reagents

N-hydroxysuccinimide (NHS, ≥97.0 %), *N*,*N*′-dicyclohexylcarbodiimide (DCC, ≥99.0 %), 4,4-bis(4-hydroxyphenyl) valeric acid (BHPVA, 95 %), bisphenol A (BPA, ≥99.0 %), 4,4′-(1-phenylethylidene)bisphenol (BP-AP, 99.0 %), 4,4′-sulfonyldiphenol (BPS, 98 %), 2,2-bis(4-hydroxy-3-methylphenyl)propane (BPC, 97.0 %), bis(4-hydroxyphenyl)methane (BPF, 98.0 %), diethylstilbestrol (DES, ≥99.0 %), dienestrol vetranal (DI, 98.1 %), Coomassie brilliant blue G 250 (CBB G 250), Tween-20 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Albumin bovine V, albumin egg, tris(hydroxymethyl)-aminomethane (Tris), tetrachloroauric acid trihydrate ($HAuCl_4\cdot3H_2O$) were supplied by J&K Scientific Ltd. (Beijing, China). Restriction enzyme BamH I was produced by Takara Biotechnology Co, Ltd. (Dalian, China). Polyclonal rabbit antibodies against BPA were obtained from our own laboratory. The modified oligonucleotide were synthesized and purified by Sangon. Biotech Co., Ltd. (Shanghai China). The sequence of DNA will be shown as follows: S_1: 5′HS-$(CH_2)_6$—AGC TCT TCC CAT ACC GGA TCC TGA CAC AG, S_2: 5′FITC—TAC TGC TGT GTC AGG ATC C GG TAT GGG AAG AG which was dissolved in Tris-HCl buffer solution (50 mM, pH 7.4 50 mM NaCl, 1 mM EDTA) and stored at 4 °C. The concentration of DNA was quantified by UV–Vis absorption spectroscopy with the following extinction coefficients (ε_{260nm}, M^{-1} cm^{-1}): A=15,400, G=11,500, C=7400, and T=8700. Otherwise, all other reagents and solvents were purchased in their highest available purity and used without further purification. Millipore Milli-Q water (≥18 MΩ cm) was used in all experiments.

Apparatus

In this study, black 96-well polystyrene microplate (Costar, Corning, USA) were used as solid phase carrier. The signal probes purified from suspensions by Himac CR 22G high-speed refrigerated centrifuge (Hitachi Co., Japan). All fluoroimmunoassay testing were measured by Infinite M200 automatic multifunction microplate reader (Tecan, Switzerland). The representation of signal properties was monitored by UV-2550 UV-Vis spectrometer (Shimadzu, Japan) and JEM-2100 (Japan Electronics Co., Ltd).

Preparation of Coating Antigen

The coating antigen was synthesized according to the literature with slight modification (Feng et al. 2009). Briefly, BHPVA, NHS, and DCC (molar ratio 5:6:7) added into 2 mL of DMF. Then, the solution was stirred at room

temperature overnight. The mixture was added dropwised into 8 mL of 10.25 mg/mL BSA solution (sodium bicarbonate buffer, pH 7.0) under vigorous stirring. The final solution was dialyzed against phosphate buffer (pH 7.4) and H_2O at 4 °C for 2 days, respectively. All steps were operated in the dark, and the mixture showed turbid state in whole processes. The dialysate was lyophilized and stored at −20 °C. The coupling ratio of coating antigen was calculated by the method of Coomassie brilliant blue (G 250) staining (Sohl and Splittgerber 1991), and the results showed that the coupling ratio of BHPVA and BSA was 13.3:1.

Preparation of Dual-Codified AuNP Probes

The AuNPs were prepared according to the literature method (Turkevich et al. 1951). One hundred milliliter of 0.01 % $HAuCl_4$ (*w*/*v*) solution was boiled and stirred vigorously. Then, 4 mL of 1 % (*w*/*v*) trisodium citrate was added rapidly into boiling solution. After boiling for 10 min and followed stirring for 15 min, the claret red solution was cooled to room temperature. The solution was stocked at 4 °C for further use.

After AuNP solution was adjusted to pH value 9 with 0.1 M K_2CO_3 solution, 20 μL of 0.5 mg/mL anti-BPA antibodies and 1.7 μL of 100 μM SH-ssDNAs (S_1) were added into 1 mL AuNPs. The mixture was stirred for 2 h; subsequently, 20 μL of 2 M NaCl (5 mM Tris-HCl buffer, pH 7.0) was added into the above solution. And then, the mixture was centrifugated at 14,000 rpm for 20 min, the supernate was removed, and the red sediment was dispersed in buffer solution (50 mM Tris-HCl, 5 mM EDTA, 50 mM NaCl, pH 7.4). Afterward, the FITC-ssDNAs (S_2) were added and hybridized with the thiolated ssDNA sequences (S_1) from AuNPs for 24 h at room temperature. After that, the precipitate of dual-codified AuNP probes (Ab-AuNP-DNA probes) was collected by repeated centrifugation and resuspended in buffer (50 mM Tris-HCl, 5 mM EDTA, 50 mM NaCl, pH 7.4) and stored at 4 °C for further use.

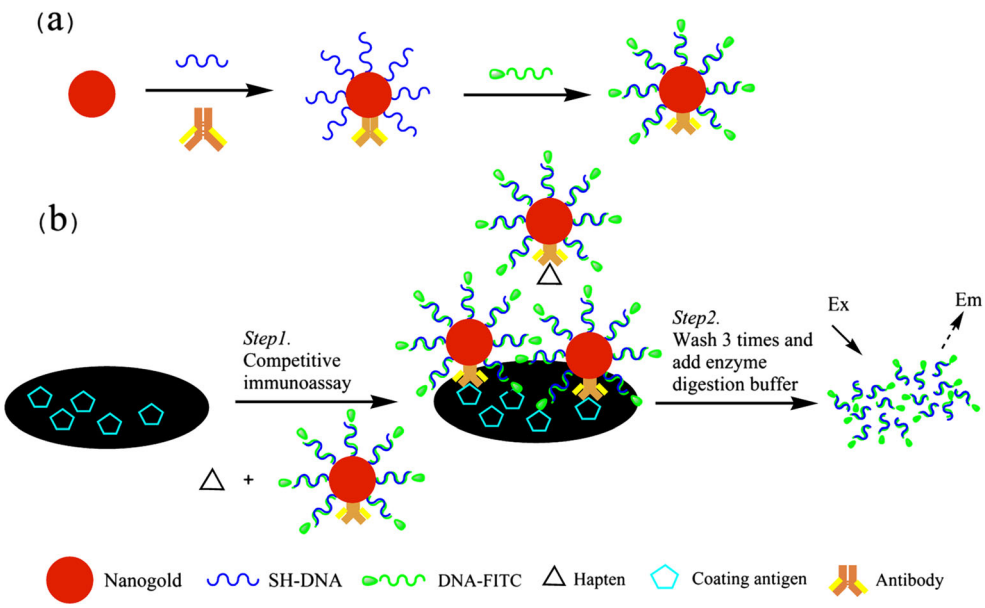
Competitive Fluorescence Immunoassay

The microplate were coated with 100 μL of BPA coating antigen at 4 °C overnight and rinsed three times with washing buffer (50 mM Tris-HCl, 50 mM NaCl, 0.1 % Tween-20, pH 8.0) to remove the unbonded antigen. Then, 150 μL of 0.15 % OVA was added to the microplate and incubated for 40 min with strong shaking at 37 °C and washed three times. Afterwards, 50 μL of BPA sample solution and 50 μL of Ab-AuNP-DNA probes were added and incubated 60 min at 37 °C and washed thoroughly to remove the unbound biomolecules. After that, the 100 μL of restriction enzyme (BamH I) (10 mM Tris-HCl, 7 mM $MgCl_2$, 100 mM NaCl, 2 mM 2-mercaptoethanol, 0.01 % BSA, pH 8.0) was added into microplates and incubated at 37 °C for 80 min. The fluorescence intensity of the incubated solution was measured at 524 nm at excitation wavelength of 490 nm.

Sample Collection and Preparation

The samples purchased from supermarkets including pure milk, yoghourt, milk beverage, and its packaging materials. All these samples in the quality guarantee period were stored at 2~6 °C.

Two milliliter of liquid samples were placed into a centrifuge tube, and 20 mL of acetonitrile (0.05 g/mL NaCl) was added and mixed with ultrasonic for 20 min to denature and precipitate protein in the samples. The mixture was



Scheme 1 **a** Preparation procedure of dual-codified gold nanoparticles with polyclonal antibody and dsDNAs—FITC. **b** A schematic illustration of the amplifying fluorescence immunoassay for detection of BPA

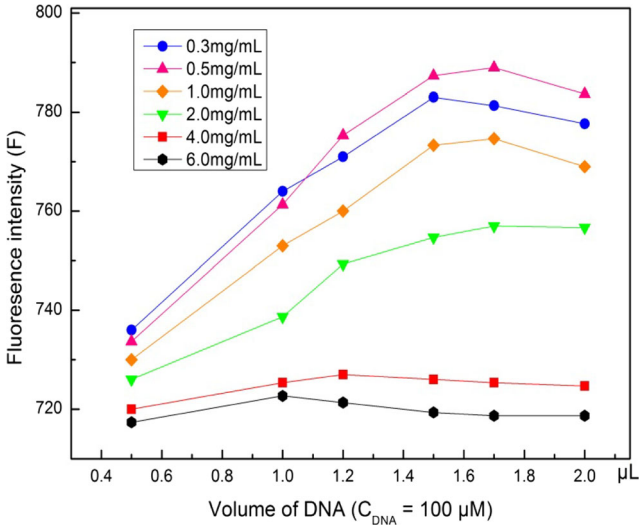


Fig. 1 Effects of DNA concentration at different concentrations of antibodies ranging from 0.3 to 6.0 mg/mL

centrifugated at 4000 rpm for 5 min. The supernatants were further repurified three times. The final solution was concentrated by rotary evaporation under vacuum and dissolved in 20 % (*w*/*v*) ethanol aqueous solution.

The packaging material was fragmented to 0.5 g (each) and immersed in 50 mL of 65 % (*w*/*v*) ethanol aqueous solution and stirred for 6 h at 80 °C, and then, the solution was collected and concentrated by rotary evaporation under vacuum.

Results and Discussion

Design of Sensing Strategy

The highlights in this design based on the following merits: (1) AuNPs with ultrahigh surface area are especially suitable for loading multiple molecules as carrier; (2) restriction enzyme was chosen, which can specifically cut double-stranded DNA (ds-DNA) to release the fluorophore into solution to heighten the fluorescence intensity; and (3) the ds-DNA bind fluorophore and AuNPs as linker. In this study, we use the indirect determination of competitive immunoassay mode. The typical analytical process for BPA was depicted, as shown in Scheme 1. Firstly, Ab-AuNP-DNA signal probes were prepared by conjugating SH-ssDNAs and anti-BPA antibodies to AuNPs simultaneously, and the FITC-ssDNAs was added to

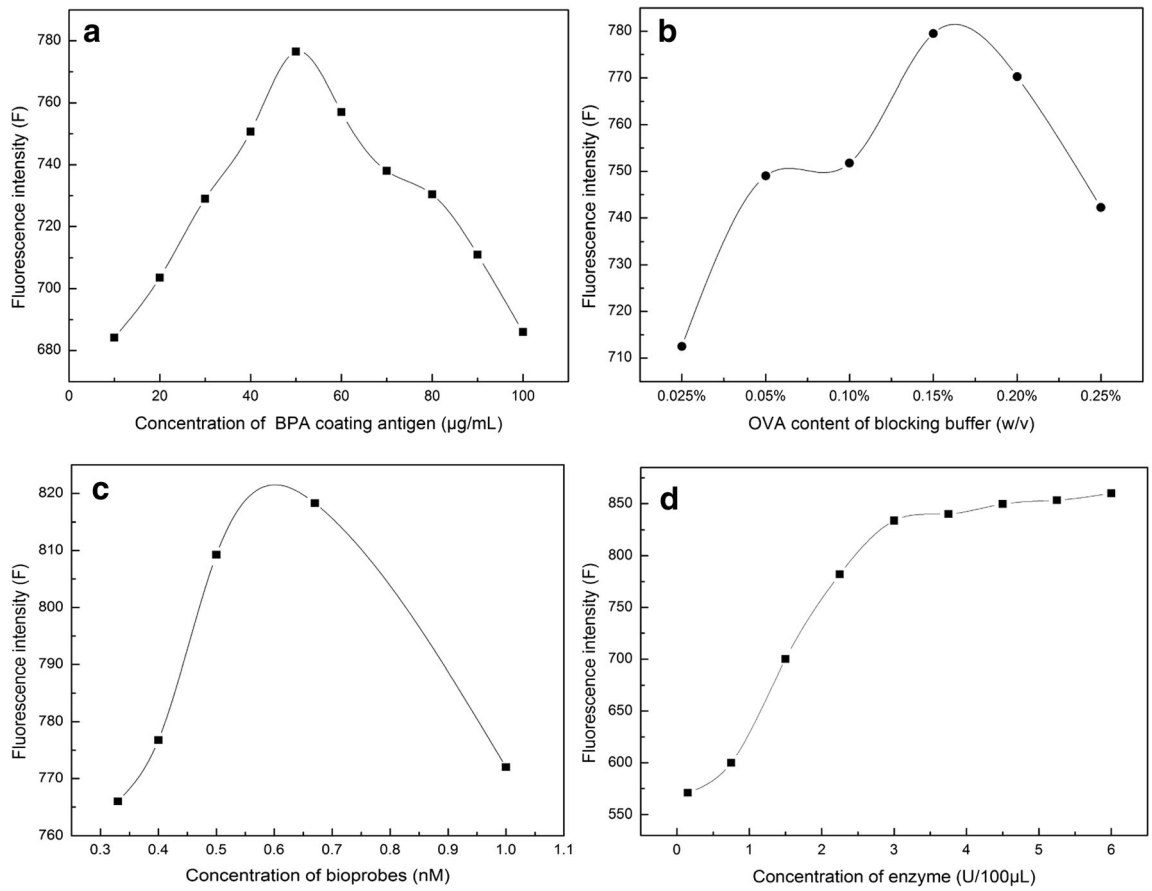


Fig. 2 Effects of the reagents for detection: **a** concentration of coating antigen, **b** OVA content of blocking buffer, **c** concentration of bioprobes, and **d** minimum of enzyme concentration (U/100 μL)

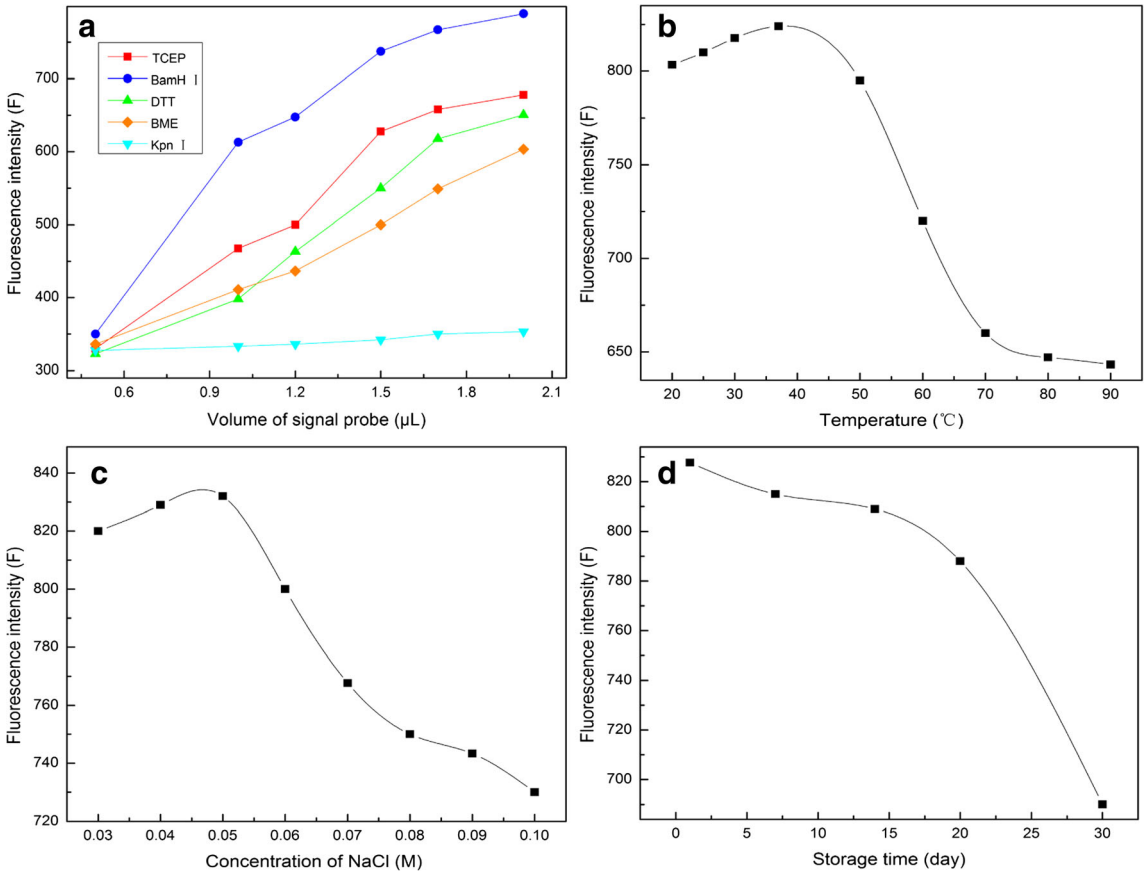


Fig. 3 Effects of various conditions for bioprobes: **a** different dissociation reagents, **b** temperatures, **c** graded salinity, and **d** storage time for the stability of probes

hybridize SH-ssDNAs to form a dual-codified probe. Secondly, the determinand containing analyte (BPA) competed with BPA coating antigen conjugate to the anti-BPA antibodies. Signal probes which combined with coated antigens were strongly immobilized to microplates. After washing steps, the unfixed probes were separated from the plates. Thirdly, restriction enzyme (Bam I) were added to cut off the specific recognition sites of DNA to release the fluorophore. Therefore, the addition of restriction enzyme (Bam I) caused hundreds of FITC-labeled DNA strands release into the solution which resulted in amplifying the fluorescence signal. We detected the fluorescence intensity of fluorophores from fixed probes which was inversely to the actual samples.

Characteristic of AuNPs and Ab-AuNP-DNA Probes

The TEM image showed that AuNPs is dispersive uniformly and the average diameter is 12.6±0.1 nm. The AuNPs has a surface plasma resonance absorption peak at about 520 nm and appears claret red. According to Lambert-Beer law (Jin et al. 2003), the concentration of colloidal gold is about 3.7 nM ($\varepsilon_{520}=2.7\times10^8\ M^{-1}\ cm^{-1}$). To investigate conjugation effect of Ab and DNA with AuNP complex on photophysical property, the resulting Ab-AuNP-DNA conjugates was conducted with spectroscopy. A red shift absorption peak indicated a novel dual-codified probe was formed from covalent complex of antibodies and ssDNAs linked with AuNPs. Meanwhile, the result showed that the concentration of bioprobes was about 2.0 nM.

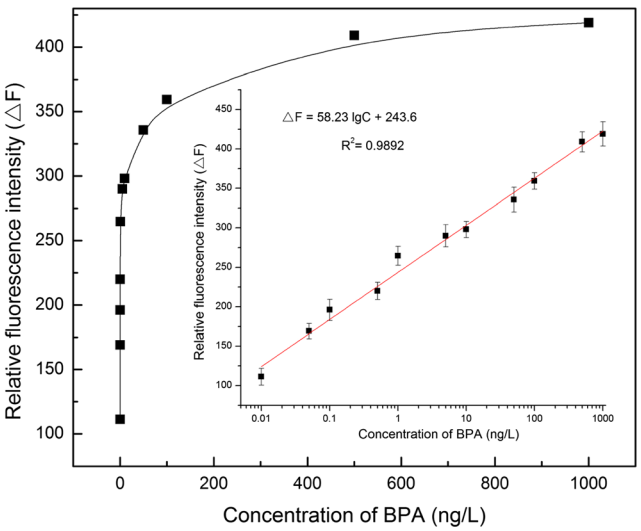


Fig. 4 Changes of the relative fluorescence intensity with various concentration of BPA. *Inset*: Liner relationship between the logarithm of BPA concentration and relative fluorescence intensity

Table 1 Comparison of the proposed method for BPA detection with other methods

Method	Calibration curves range (ng/L)	LOD (ng/L)	Reference
GC-MS	1.0×10^{2}–1.25×10^{3}	38	Fontana et al. 2011
HPLC	1.0×10^{2}–1.0×10^{3}	1.0×10^{2}	Zhou et al. 2014
LFS	50–5.0×10^{3}	76	Mei et al. 2013
SPR	50–1.0×10^{6}	80	Hegnerová et al. 2010
CL-ELISA	1.0–1.0×10^{6}	20	Maiolini et al. 2014
LC-MS/MS	2.0×10^{3}–5×10^{5}	5.80×10^{3}	Maiolini et al. 2014
EC	21.5–1.56×10^{6}	0.687	Huang et al. 2011
The present method	1.0×10^{-2}–1.0×10^{3}	3.4×10^{-3}	

GC-MS gas chromatography-mass spectrometry, *HPLC* high-performance liquid chromatography, *LFS* lateral flow strip, *SPR* surface plasmon resonance biosensor, *CL-ELISA* chemiluminescence enzyme-linked immunosorbent assay, *LC-MS/MS* liquid chromatography-tandem mass spectrometry, *EC* electrochemical method

In addition, the appropriate ratio between DNAs and antibodies on dual-codified probe is crucial. As shown in Fig. 1, the fluorescence intensity increased with the increasing of DNA, under the certain antibody concentrations. In the presence of 1.7 μL of 100 μM DNA and 0.5 mg/mL antibodies, the fluorescence intensity reached the summit. Based on above experimental results, the quantity of DNA combined on AuNPs was calculated from fluorescence spectrometry. The result indicated that the surface coverage of SH-dsDNA-FITC was about 33±1 branches (SH-dsDNA-FITC/AuNPs). Meanwhile, the content of antibody was also measured by globulin G. The abundance of antibodies coated of the AuNPs was about 10±1 (antibody/AuNPs); therefore, the binding ratio of antibodies to SH-dsDNA-FITC is about 10:33.

Optimization of Detection Conditions

The assay condition parameters including the content of coating antigens, the probes' dilution ratio, the OVA content of blocking buffer, the content of restriction enzyme (BamH I), and the biological activity of probes played important roles in the developed BPA assay. The effects of different parameters were thoroughly investigated in this study.

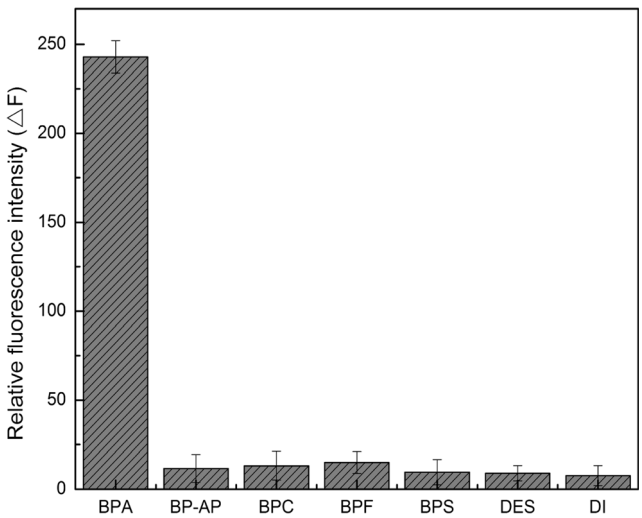


Fig. 5 The relative fluorescence intensity with different BPA or BPA congener (BPA 1.00 ng/L, other congener 1.00 ng/L)

Selection of Immunoassay Conditions and Optimal Dissociation Buffer

The coated coating antigens play the key role in capturing the modified probes. As shown in Fig. 2, the fluorescence intensity increased gradient until 50 μg/mL of BPA coating antigens. After that concentration, the adverse effect was induced due to excessive coating antigens. This phenomenon showed that low content cannot saturate the residual probe; on the contrary, excess of coating antigens restrain the combination of BPA with probes. To minimize the nonspecific adsorption, 0.15 % OVA content of blocking buffer and 0.67 nM (three times diluted original bioprobes) of the probes were chosen for BPA detection.

Restriction enzyme, being dissociation reagent, was screened after evaluation of BME, TCEP, Kpn I, and DTT; the restriction enzyme of BamH I was chosen to be the ideal candidate for whose outstanding biocompatibility and efficient recognition capability to DNA sequences (Figs. 3a and

Table 2 Assay of BPA in milk products (n=5)

Samples	Spiked (ng/L)	Found±SD (ng/L)	Recovery (%)
Pure Milk	0	ND	–
	1.0	1.053±0.023	105.3
	10.0	9.697±0.047	96.9
Yoghourt	0	ND	–
	1.0	0.932±0.036	93.2
	10.0	10.712±0.059	107.1
Milk beverage	0	ND	–
	1.0	1.08±0.021	108.6
	10.0	11.05±0.055	110.5

Table 3 Assay of BPA in packaging materials (n=5)

Samples	Spiked (10^3) (ng/L)	Found±SD (10^3) (ng/L)	Recovery (%)
Black PE	0	0.2204±0.019	–
	0.1	0.3230±0.020	102.6
	0.3	0.4991±0.029	92.9
White PE	0	0.1202±0.033	–
	0.1	0.2176±0.026	97.4
	0.3	0.4432±0.041	107.7
Carton	0	0.0778±0.023	–
	0.1	0.1699±0.025	92.1
	0.3	0.3637±0.036	95.3
PET bottle	0	0.2794±0.020	–
	0.1	0.3810±0.027	101.6
	0.3	0.5619±0.030	94.1

2d) and 3 U of enzyme in reaction system was determined according to the fluorescence intensity.

Effect of Temperature on the Biological Activity of Probes

A cozy environmental factor, which could efficiently regulate the reaction activity of system, is prerequisite to optimize the stability of constructed probe. It has been reported that temperature was one of the most important environmental factor; it tightly associated with energetic molecules and its spatial structure which depends on the functional specificity and stability of compounds. The impact of temperature on the bioactivity of probes was evaluated, and the result showed that the fluorescence declined slowly from about 40 °C (Fig. 3b); after the temperature was more than 50 °C, the fluorescence intensity sharply decreased which is more likely due to protein denaturation at high temperature. Moreover, high temperature induces fluorescence quenching (Liu et al. 2005). So, 37 °C is chosen to be the suitable temperature for this immunoreaction.

Influence of Ionic Strength

Ionic strength, another crucial environmental factor, usually affects the affinity of receptors and ligands in most biological events. In the work, we investigated whether ionic strength would have an impact on the preparation of bioprobes. It was difficult to hybridize for DNA for mutual repulsion from phosphate group with excessive negative charge at lower concentration of NaCl; however, the extremely high concentration of NaCl could induce the AuNP aggregation. As shown in Fig. 3c, the fluorescence increased with increasing of ionic strength. While the concentration of NaCl was 0.05 M, the fluorescence intensity reached the maximum. After the concentration of NaCl over 0.05 M, the fluorescence intensity decreased instead. So, 0.05 M of NaCl appeared to be the best concentration in the preparation of bioprobes for DNA hybridization.

Storage Time of Probes

The storage stability of Ab-AuNP-DNA probe was investigated. It was revealed that probe kept a good stability at 4 °C for 2 weeks in Fig. 3d. Various levels of coagulation events will occur after 20 days. Therefore, these probes showed the highest efficiency which can last for 20 days under temperature of 4 °C.

Sensitivity and Selectivity

The quantitative behavior of the fluorescent assay was tested with different concentrations of BPA under the above optimized condition. As seen from the Fig. 4, it can be seen that the fluorescence intensity was enhanced with the decreasing concentration of BPA. A good positive linear regression manner of the increased relative fluorescence intensity against concentrations of BPA was found (ΔF, $\Delta F=F_0-F$, where F and F_0 are the fluorescence intensity of the system in the

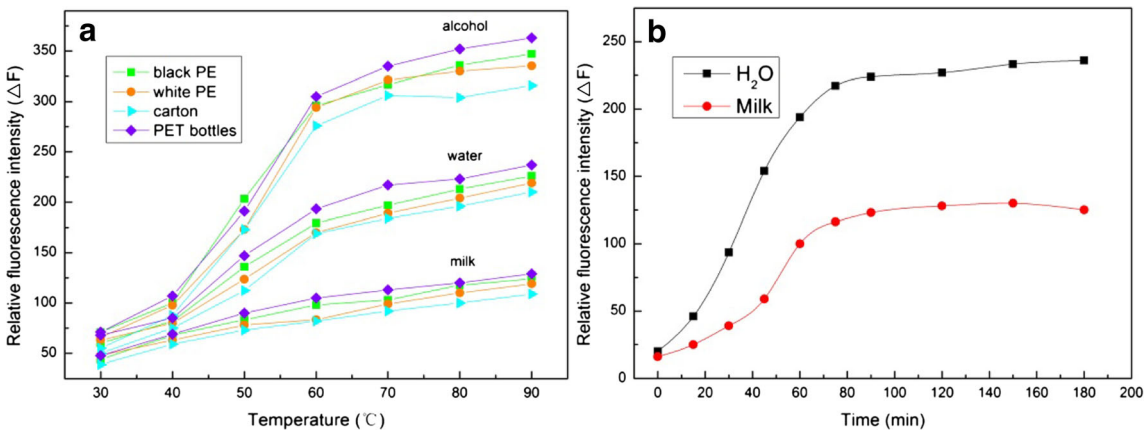


Fig. 6 The BPA migration with temperature and time: **a** temperature: each temperature continuous extraction for 30 min and **b** extraction duration to migration ratio of BPA at 50 °C

presence and absence of BPA, respectively) and regress equation is ΔF=243.6+58.23 lgC (ng/L) (C is the concentration of BPA, ng/L) in the range of 1.0×10^{-2}~1.0×10^{3} ng/L with a correlation coefficient (R^2) of 0.9892. The limit of detection (LOD) (taken to be three times the standard deviation in the blank solution) was 3.4×10^{-3} ng/L. A comparison of the analytical characteristics of this method and other methods for BPA are summarized in Table 1 which showed that the present method produced more two orders of magnitude sensitivity than other methods.

The selectivity of this method for BPA was also evaluated by distinguishing the response of the assay to the same concentration of analogues (bisphenol AP, bisphenol C, bisphenol F, bisphenol S, diethylstilbestrol, and dienestrol vetranal) (Fig. 5). The results showed that only BPA could significantly induce the change of fluorescence, and other analogues had negligible influence on the fluorescence indicating that anti-BPA antibodies specifically respond to BPA. The results reveal that the present fluorescence immunoassay method constructed for BPA detection is sensitive and effective.

Analysis of Real Samples

In order to evaluate the reliability of the present method, the concentration of BPA in various kinds of milk products and their packaging materials were analyzed. As shown in Tables 2 and 3, although, it was found that the concentration of BPA in all kinds of drink was low and kept constant unless they are heated. Notably, their packaging materials (black PE, white PE, carton, and PET bottle) contained different levels of BPA (Table 3). It was reported that BPA can permeate into drinks from materials under condition of thermal treatment or microwave heating (Fasano et al. 2012). In order to further verify the fact, we heated the container gradually to evaluate the dissolved BPA in three model beverages (water, milk, and alcohol) by the present method. In the test, we use the relative fluorescence intensity (ΔF) of detection to express the dissolved BPA. As shown in Fig. 6a, the dissolved BPA concentration increased slowly below 40 °C; however, the content of BPA migration was increased rapidly while temperature over 50 °C. Meanwhile, the impact of immersing time was also taken into account. It was shown in Fig. 6b, the ΔF increased with time at 50 °C and saturation appeared at the point of 70 min, the ΔF value reached the peak which is equivalent to 115 ng/L based on calculation and experiments. Therefore, it is necessary to take off the packaging for heating milk in daily life. Furthermore, the experiments showed that BPA was more efficiently transferred into alcohol than water or milk for its lipophilic characteristics. And, BPA also can obtain higher mobility in water than milk due to the intermolecular forces and hydrogen bonding.

Conclusions

In summary, a novel and sensitive strategy to convert the antibody-antigen recognition and restriction enzyme event into fluorescence immunoassay by coupling AuNPs as signaling probes is constructed. The assay exhibited excellent sensitivity with a limit of detection up to 3.4×10^{-3} ng/L. The proposed method was applied to measure the content of BPA in different kinds of milk. It infers that BPA migration from packaging will be sustained under heating condition. For safety consideration, strict manufacturing technique of packaging should be established, and the right disposal method of heating milk should be recommended to consumers.

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Conflict of Interest Shuhao Wang has no conflict of interest. Wei Ji has no conflict of interest. Lingyun Du has no conflict of interest. Yuanfu Zhang has no conflict of interest. Guofu Liu has no conflict of interest. This article does not contain any studies with human or animal subjects.

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