A novel bifunctional europium complex as a potential fluorescent label for DNA detection†

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A bifunctional europium complex of $Eu(TTA)_3(5\text{-}NH_2\text{-phen})$ using 2-thenoyltrifluoroacetonate (TTA) and 5-amino-1,10-phenanthroline (5-NH_2-phen) as ligand reagents was applied in DNA detection assays for the first time. The complex has a long fluorescence lifetime, high fluorescence quantum yield, and is easy to label oligonucleotides for time-resolved fluorescence bioanalysis. A two-probe tandem DNA hybridization assay including capture DNA_1, probe DNA_2, and target DNA_3 was employed to detect microbial pathogens. The DNA sequences in the assay were designed using software Primer Premier 5.0 based on published specific nucleotide sequences of *Staphylococcus aureus* and *Escherichia coli*. 3′-Amino-modified capture DNA_1 was covalently immobilized on the common glass slide surface and the 5′-amino-modified probe DNA_2 was combined with the functionalized $Eu(TTA)_3(5\text{-}NH_2\text{-phen})$ *via* glutaraldehyde. The detection was done by monitoring the fluorescence intensity from the glass surface after the hybridization reaction with complementary target DNA_3. The optimal concentration of capture DNA_1 of 1.0×10^{-6} mol l^{-1} dropped onto the glass slides and optimal hybridization temperatures of 48 °C and 39 °C respectively for *Staphylococcus aureus* and *Escherichia coli* were obtained. The proposed DNA detection system showed higher sensitivity than such a complex doped nanoparticle-based detection system in our previous study for the better uniformity and dispersity of monomolecular labels. The sensing system presented a short hybridization time of 2 h, satisfactory stability, and high selectivity. The results demonstrate that this complex might be a potentially excellent dye in area of biochemical analysis.

Introduction

Detection of microbial pathogens is of great importance for disease diagnosis, food safety, epidemic prevention, and environmental protection.[1] Among many detection techniques of microbial pathogens, DNA hybridization methods have been popularly developed for accurate specificity and high selectivity.[2] Fluorescence based detection, such as polymerase chain reaction (PCR) and fluorescence *in situ* hybridization (FISH) have become the standard techniques. PCR has disadvantages of a relatively long assay time on DNA purification and amplification, high assay cost, and an error-prone nature that occasionally leads to 'false-positive' signals.[3] The complicated fluorescence coloration system and relatively high background generated from biological tissues and substrates of the FISH technique limit its extensive application in practice.[4]

A fluorescent dye is usually utilized to signal the hybridization. Many fluorescent dyes are well known as fluorescent markers in biochemical analysis, such as fluorescein, rhodamine, cyanine dyes, and so on.[5–7] However, there is a main problem of how to eliminate background noises caused by autofluorescence from biological samples, the scattering light, and the luminescence from the optical components. For this purpose, more and more ultrasensitive bioanalytical assays based on time-resolved fluorescent techniques using lanthanide complexes as the fluorescence labels have been developed as described in many reviews.[8–10] Among them, quite a number of detection systems were based on the dissociative enhancement method of lanthanide ions, which is susceptible to interference.[11,12] Some of the assays were based on fluorescence resonance energy transfer (FRET) technology, which only occurs over a very small distance between the donor fluorophore and acceptor fluorophore.[13,14] Only a few examples of bifunctional lanthanide chelators directly used in bioanalysis have been reported in the literature.[15–17] The syntheses of these chelators were complicated and the luminescence capability was relatively weak. Thus, a time-resolved fluorescence based DNA direct detection system using a suitable indicator of a bifunctional lanthanide complex is still an exciting area of research.

In recent years, lanthanide complex doped silica nanoparticles as potential biolabels have been developed due to their advantages of signal enhancement, photostability, and surface modification for the immobilization of biomolecules.[18–20] More recently, two time-resolved fluorescence DNA detection assays using terbium complex doped nanoparticles and europium complex doped nanoparticles were described by Chen *et al.*[19] and our group.[18] The detection limits for target DNA are about 8.0×10^{-11} mol l^{-1} and 4.0×10^{-10} mol l^{-1}, respectively. The experimental process is somewhat tedious and the detection limit is not remarkably low for using nanotechnology.

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The authors tried to prepare a DNA probe directly labeled with a bifunctional europium complex in this study and better results were obtained. Three kinds of europium complexes using 2-thenoyltrifluoroacetone (TTA), dibenzoylmethane (DBM), 1,10-phenanthroline (phen), and 5-amino-1,10-phenanthroline (5-NH_2-phen) as ligand reagents were synthesized. They are tris(2-thenoyltrifluoroacetonate)mono(1,10-phenanthroline)europium(III), $Eu(TTA)_3$phen; tris(dibenzoylmethane)mono(1,10-phenanthroline)europium(III), $Eu(DBM)_3$phen; and tris(2-thenoyltrifluoroacetonate)mono(5-amino-1,10-phenanthroline)europium(III), $Eu(TTA)_3$(5-NH_2-phen). Ultra-intense fluorescence intensity of the complex of $Eu(TTA)_3$(5-NH_2-phen) was observed over the other two. It gave an intense and long-lived signal which enables the efficient elimination of background signal. A long lifetime of 0.688 ms and a very high fluorescence quantum yield of 0.62 were obtained. To the best of author's knowledge, assays using such a complex of $Eu(TTA)_3$(5-NH_2-phen) have not yet been reported.

A two-probe tandem DNA hybridization assay was employed to detect the DNA of *Staphylococcus aureus* and *Escherichia coli* genes using $Eu(TTA)_3$(5-NH_2-phen). The sequences of these DNA strands were designed using software Primer Premier 5.0, and commercially synthesized. The complex presented satisfactory stability in DNA analysis. The detection system showed high sensitivity, and the detection limits could be less than 4.0×10^{-11} mol l^{-1}. The authors believe that the complex must have a wide application in biochemical analysis.

Experimental

Instrumentation

All fluorescence measurements were conducted on a Perkin-Elmer LS-55 spectrofluorimeter with both excitation and emission slits set at 10 nm and controlled by a personal computer data processing unit. The light source was a pulsed Xe lamp. A modified home-made poly(tetrafluoroethylene) cell[21] and two arms of the bifurcated optical fiber were fixed in the detecting chamber of the spectrofluorimeter to carry the excitation and emission light. The excitation light was carried outside the spectrofluorimeter to the sensitive system fixed in the cell through one arm of the fiber and the emission light was collected inside the spectrofluorimeter through the other. A foursquare common glass plate (side length 12 mm, thickness 1.5 mm) covered with the product after hybridization was fixed on the top of the chamber by the mounting screw nut. Solution pH was measured by use of a pHS-3B pH meter (Shanghai Precision & Scientific Instrument Co. Ltd., Shanghai, China). All measurements were performed at room temperature and atmospheric pressure.

Materials and reagents

Europium oxide was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Ligand reagents of 2-thenoyltrifluoroacetone (TTA) and dibenzoylmethane (DBM), were obtained from J&K chemical Ltd. 3-Aminopropyltrimethoxysilane, a silanization reagent, was purchased from Acros organics. 1,10-Phenanthroline (phen), ammonium hydroxide (25–28 wt%), hydrazine hydrate (80 wt%), and all other chemicals of analytical reagent grade, were commercially obtained. The oligonucleotides were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China). PBS buffer (pH 7.2) was used during the oligonucleotide labeling and immobilization. Double-distilled water was used for the preparation of all aqueous solutions.

Synthesis of europium ternary complexes

In this paper, a europium ternary complex $Eu(TTA)_3$(5-NH_2-phen) using TTA and 5-NH_2-phen as the ligands was synthesized. The synthetic procedure of 5-NH_2-phen was according to the literature with some modifications.[22]

5.0 g of phen was dissolved in 30 ml of concentrated sulfuric acid. To this stirred solution 15 ml of fuming nitric acid was added when the temperature was about 165 °C. The mixture was stirred at 160–170 °C for three hours, and then poured into 300 g of ice water. The pH of this aqueous solution was adjusted to 5.0 by adding a sodium hydroxide solution. The precipitate of 5-NO_2-phen was collected by filtration, washed with distilled water, dried, and recrystallized in ethanol to eliminate by-products. The yellow solid was obtained with a yield of 72.3%, mp 192–193 °C.

5-NO_2-phen was reduced to the corresponding amino derivative of 5-NH_2-phen. A solution of hydrazine hydrate (1 ml) in ethanol was added dropwise to a stirred solution of 5-NO_2-phen (1.50 g) and 5% Pd/C (300 mg) in ethanol. The mixture was refluxed for five hours. After removal of most of the ethanol, water was added until formation of a green-yellow precipitate. Upon crystallization from ethanol, the product was obtained with a yield of 56.8%, mp 251–252 °C, M^+ *m/z* 195.

The ternary europium complex was prepared by stirring an ethanol solution of the $EuCl_3$ (176.0 mg europium oxide were dissolved in 0.5 ml hydrochloric acid to form europium chloride) and 666.5 mg TTA for 40 min (pH was adjusted to 6 by adding a sodium hydroxide solution). Subsequently, another ethanol solution of 5-NH_2-phen was added, and the mixture was stirred

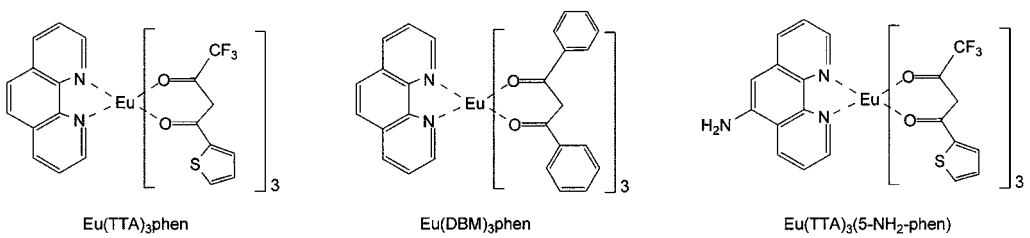

Fig. 1 Structures of complexes $Eu(TTA)_3$(5-NH_2-phen), $Eu(DBM)_3$phen, and $Eu(TTA)_3$phen.

for 5 h at 55–60 °C. A yellow precipitate formed upon cooling was filtered and recrystallized from a mixed solvent of methanol and chloroform (1:1, v/v), $M^+ + H_2O$ 1032, ^1H NMR (acetone) δ (ppm): 2.83 (6 H, –CO–CH_2–), 6.02–7.28 (9 H, –thiophene), 7.40 (2 H, –NH_2), 8.01–11.11 (7 H, –phenanthrene). The ternary complex was further characterized on the basis of IR spectra and elemental analysis. The ν_{max}/cm^{-1} stretching vibration in the infrared spectrum at 1637 (CO) of TTA shifts to 1626 (CO) in the spectrum of Eu(TTA)$_3$(5-NH_2-phen), and the ν_{max}/cm^{-1} stretching vibration at 1564 (CN) of NH_2-phen shifts to 1550 (CN) of Eu(TTA)$_3$(5-NH_2-phen). Found: Eu, 15.13; C, 42.58; H, 2.31; N, 4.19. Calcd. for $EuC_{36}H_{24}N_3F_9S_3O_6$: Eu, 14.99; C, 42.62; H, 2.37; N, 4.14%. The synthesis and characteristics of Eu(DBM)$_3$phen and Eu(TTA)$_3$phen were similar to the complex of Eu(TTA)$_3$(5-NH_2-phen), the structures of the complexes are shown in Fig. 1.

Designing of oligonucleotide sequences

All the sequences were designed using Primer Premier 5.0 software, on the basis of the published specific nucleotide sequences of *S. aureus* genes of 5′ACAAT ACACA TGAAC AACAT TTAAG AAAAA GTGAA GCACA AGCGA AAAAA GAGAA AATTA AATAT TTGGA GCGAA GACAA CGCTG ATTCA GGTC 3′ and *E. coli* of 5′ TGTGG AGTAT TGCCA ACGAA CCGGA TACCC GTCCG CAAGG TGCAC GGGAA TATTT CGCGC CACTG GCGGA AGCAA CGCGT AAACT CGACC CGACG CGTCC GATCA CCTGC GTCAA TGTAA TGTTC TGCGA CGCTC ACACC GATAC CATCA G 3′.[23] The capture, probe, and target sequences for *S. aureus* were: DNA_1, CAC TTT TTC TTA AAT GTT GTT C(A)$_{10}$-NH_2 (T_m, melting temperature = 52.30 °C); DNA_2, NH_2-(A)$_{10}$ ATT TTC TCT TTT TTC GCT T (T_m = 54.36 °C); DNA_3, GAA CAA CAT TTA AGA AAA AGT GAA GCA CAA GCG AAA AAA GAG AAA AT. For *E. coli* were: DNA_1, ACA TTG ACG CAG GTG ATC GGA CG(A)$_{10}$-NH_2 (T_m = 63.30 °C); DNA_2, NH_2-(A)$_{10}$ GTA TCG GTG TGA GCG TCG CAG AA (T_m = 63.30 °C); DNA_3: CGT CCG ATC ACC TGC GTC AAT GTA ATG TTC TGC GAC GCT CAC ACC GAT AC.

Glass slide surface modification with capture DNA_1

To show the excellent utility of the complex in sensitive DNA analysis, the authors employed a two-probe tandem DNA hybridization assay,[24] shown in Fig. 2. The glass slide surface was modified as described in the literature.[25] The glass slides were immersed in 25.0% ammonium hydroxide overnight then washed with double-distilled water. A solution of 2.0% 3-aminopropyltrimethoxysilane in 95% ethanol was prepared and adjusted to the desired pH of 4.5 with glacial acetic acid. The glass slides were soaked in this solution for 30 min then ultrasonically washed by ethanol and double-distilled water. Reactive amino groups were then introduced at the glass surface. The amine-modified glass slides were then immersed in the solution of 2.5% glutaraldehyde in PBS buffer stirring for 3 h, and washed with PBS buffer and double-distilled water (1 min time^{-1}), respectively, then dried at normal temperature.

Capture DNA_1 was immobilized on the glass surface by incubating DNA_1 in PBS buffer for 12 h at room temperature. The DNA_1 modified glass slides were then incubated in a 20 mM glycine solution for 1 h to block any remaining glutaraldehyde groups. The final substrate was dried at room temperature after washing with 0.2% SDS solution (sodium dodecyl sulfate) and double-distilled water.

Labeling of probe DNA_2 with Eu(TTA)$_3$(5-NH_2-phen)

The probe DNA_2 was combined with the functionalized Eu(TTA)$_3$(5-NH_2-phen) *via* cross-linking by glutaraldehyde. The complex was first dispersed in the solution of 2.5% glutaraldehyde in PBS buffer, and stirring for 3 h at normal temperature. After centrifuging and washing with PBS buffer and double-distilled water, a certain amount of probe DNA_2 diluted in double-distilled water was added to the solution of aldehyde

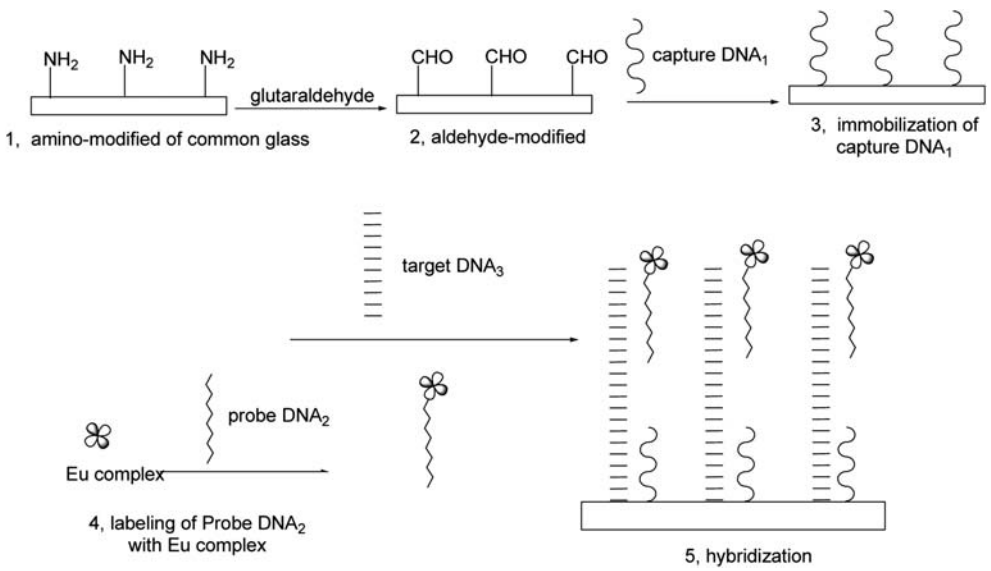

Fig. 2 Schematic diagram of DNA hybridization based on a two-probe tandem DNA hybridization assay.

combined complex, and stirring was continued for 3 h at 37 °C. $Eu(TTA)_3(5\text{-}NH_2\text{-}phen)$ modified DNA_2 were then treated with a 20 mM glycine solution for an additional 1 h. The final product was washed, resuspended in PBS buffer for future usage.

Hybridization with target DNA_3

The target DNA_3 was first diluted to the desired concentration in double-distilled water. The above $Eu(TTA)_3(5\text{-}NH_2\text{-}phen)$ labeled DNA_2 (15 μl, 4.0×10^{-7} mol l^{-1}) and target DNA_3 (5 μl) were then added to the capture DNA_1 modified glass slides surface for hybridization under the condition of hybridization solution containing 5.0 × SSC (NaCl, sodium citrate) and 0.1% SDS. Following hybridization, the glass slides were washed with 1.0 × SSC (NaCl, sodium citrate) + 0.03% SDS, 0.2 × SSC, 0.05 × SSC, and double-distilled water at room temperature in order to remove non-hybridized oligonucleotides. The detection of the DNA_3 was done by monitoring the fluorescence signals of the DNA_2 labeled $Eu(TTA)_3(5\text{-}NH_2\text{-}phen)$ conjugates left on the glass slides surface with three repetitive measurements.

Results and discussion

Luminescent properties of the ternary europium complexes

Time-resolved fluorescence emission spectra of the complexes (λ_{ex} = 401 nm, 379 nm, 365 nm for $Eu(TTA)_3phen$, $Eu(DBM)_3phen$, and $Eu(TTA)_3(5\text{-}NH_2\text{-}phen)$, respectively) were characterized at a same concentration of 4.0×10^{-5} mol l^{-1} in acetone. As shown in Fig. 3, $Eu(TTA)_3(5\text{-}NH_2\text{-}phen)$ showed quite remarkable luminescent properties (906.9, fluorescence intensity), which was at least 4 times higher than the signal of $Eu(TTA)_3phen$ (225.1) and more than 10^3 times than $Eu(DBM)_3phen$ (< 1). Attaching an amino-group on the phenanthroline may improve the luminous performance of the complex. It was also found that the fluorescence intensity of complex using TTA as a ligand was much higher than using DBM. The fluorescence quantum yield value of the complex is 0.62, calculated in acetone by the comparative method using quinine as standard.

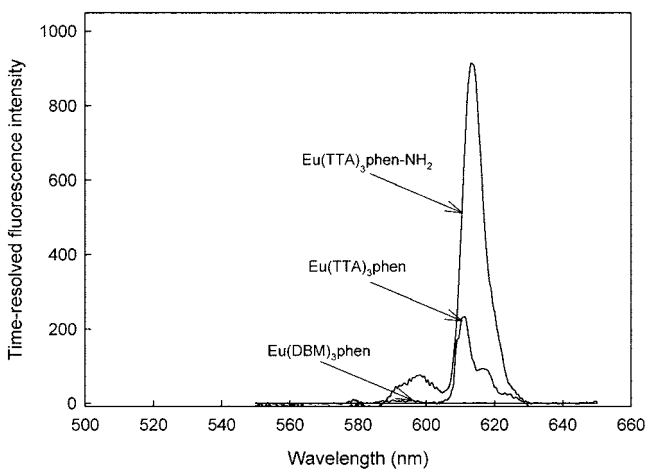

Fig. 3 Time-resolved fluorescence emission spectra of three complexes at a same concentration of 4.0×10^{-5} mol l^{-1} in acetone, delay time, 0.5 ms, gate time, 1.0 ms.

The lifetime and stability of $Eu(TTA)_3(5\text{-}NH_2\text{-}phen)$

Different from organic fluorescent dyes, lanthanide complexes have the fluorescence properties of long fluorescence lifetime, large Stokes shift, and sharp emission profile, which make them favorable to be used as the fluorescent labeling reagents for biological analysis. The lifetime of $Eu(TTA)_3(5\text{-}NH_2\text{-}phen)$ was calculated with the spectrofluorimeter. There is satisfactory linearity between the logarithm of the fluorescence intensity and the delay time ($\ln F = -1.4536t + 9.2042$, $R = 0.9993$), and the lifetime is the negative reciprocal of the slope of 0.688 ms. The complex gave an intense and long-lived signal which enables efficient elimination of the background signal, and all measurements were carried out at a delay time of 0.1 ms. The time-resolved fluorescence intensity at the emission wavelength 611 nm of $Eu(TTA)_3(5\text{-}NH_2\text{-}phen)$ ultrasonically dispersed in aqueous solution was recorded over a period of 10 h. A mean value of 536.8 ($n = 20$) and a relative standard deviation of 0.42% was obtained. The complex exhibited extreme photostability.

DNA hybridization detection

It is desirable to use time-resolved fluorometry of lanthanide chelates in the area of nucleic acid hybridization assays as a means of improving the signal-to-background ratio by decreasing the background associated with conventional fluorescence measurements. Time-resolved fluorescence spectra of different concentrations (4.0×10^{-7} mol l^{-1}, 4.0×10^{-8} mol l^{-1}, 4.0×10^{-9} mol l^{-1}, 4.0×10^{-10} mol l^{-1}, 4.0×10^{-11} mol l^{-1}, and 0 mol l^{-1}) of target DNA_3 of *S. aureus* detected from glass surfaces were recorded. By observing Fig. 4, one can easily notice that the time-resolved fluorescence intensity was increased with the increasing concentration of DNA_3 (from 12.7 ± 5.24 of blank to 244.5 ± 8.16 of 4.0×10^{-7} mol l^{-1}). The complementary target

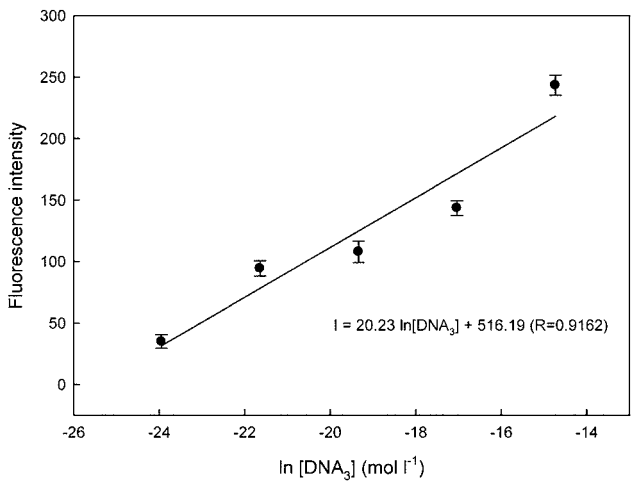

Fig. 4 Time-resolved fluorescence signals are plotted *vs.* different concentrations of target DNA_3 of *S. aureus* (from left to right: 0 mol l^{-1}, 4.0×10^{-11} mol l^{-1}, 4.0×10^{-10} mol l^{-1}, 4.0×10^{-9} mol l^{-1}, 4.0×10^{-8} mol l^{-1}, 4.0×10^{-7} mol l^{-1}), for time resolved fluorescence: λ_{ex} = 365 nm, λ_{em} = 611 nm, delay time, 0.1 ms, gate time, 1.0 ms.

DNA_3 was successfully hybridized with capture DNA_1 and probe DNA_2 based on the base-pairing reaction. The higher concentration of the target DNA_3 was, the stronger signal of the dye $Eu(TTA)_3(5\text{-}NH_2\text{-phen})$ that was detected. The complex $Eu(TTA)_3(5\text{-}NH_2\text{-phen})$ could be a super-excellent labeling reagent for DNA detection with high sensitivity. The fitting spectra of *E. coli* detection were similar to that of *S. aureus*. The detection limit for *S. aureus* and *E. coli* was about 4.0×10^{-11} mol l^{-1}, which is good compared with the reported literatures.[18,19]

Capability and selectivity of the prepared detection system

The experiments were carried out by recording the time-resolved fluorescence intensity of hybridization with different sequences of target DNA_3 and a mixture where other DNA species existed. Take *S. aureus* for example. In order to investigate the availability of this assay, the authors employed three random target sequences: s_1, 5′ GAA GAA CAT TTA AGA AAA AGT GAA GCA CAA GCG AAA AAA GAG AAA AT 3′; s_2, 5′ AAG CCA TGA AGC GGC TTA TGA TTC TTA CCG CCC ACT TGA CGA AAG CG 3′; s_3, 5′ AAG GGA CGA TGA AAG CCG ATG AAG CCT CCT TTA CCT GGA CCG ATA AC 3′. Four samples of different target DNA in a same concentration including pure target DNA_3 of *S. aureus*, s_1, s_2, and s_3 were prepared. A mixture sample of the pure target DNA_3 with s_1, s_2, and s_3 was also employed. The results showed that relative fluorescence intensities of the five samples were 101.5 ± 4.80, 22.2 ± 5.88, 14.2 ± 3.82, 17.9 ± 3.04, and 119.3 ± 5.40, respectively, showing little non-specific binding of random DNA sequences.

Optimal concentration of capture DNA_1 dropped onto the glass slides

As reported in the literature, the amount of capture DNA placed on the surface of the glass slides strongly affects the hybridization efficiency.[26] If the density of the capture DNA is too high, entanglement could occur, resulting in reduction of the hybridization efficiency. Hence, for high hybridization efficiency, the concentration of capture DNA_1 dropped onto the glass slide surface acts as a very important factor. The authors investigated the hybridization fluorescence intensity of *S. aureus* as a function of capture DNA_1 concentration and this varies from 6.0×10^{-7} to 1.6×10^{-6} mol l^{-1} (6.0×10^{-7}, 8.0×10^{-7}, 1.0×10^{-6}, 1.2×10^{-6}, 1.4×10^{-6}, and 1.6×10^{-6}) dropped onto the glass slide surface. As expected (Fig. 5), the fluorescence intensity was sharply increased when the concentration was below 1.0×10^{-6} mol l^{-1}, but mildly reduced when the concentration was bigger than 1.0×10^{-6} mol l^{-1}. Hence, all of the experiments were carried out at a capture DNA_1 concentration of 1.0×10^{-6} mol l^{-1} dropped onto every glass slide surface.

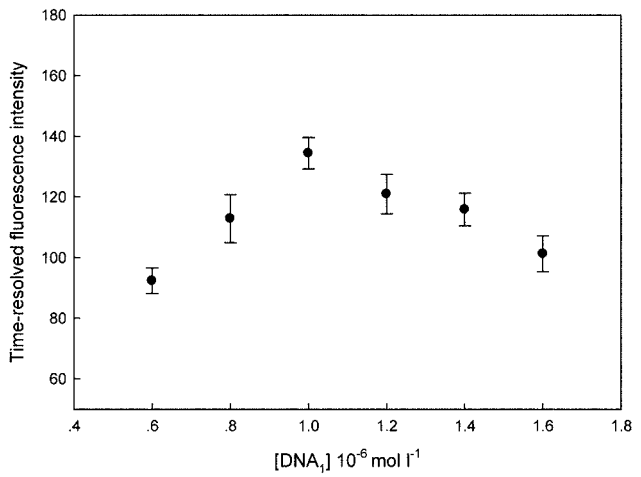

Fig. 5 Optimization of the concentration of capture DNA_1 dropped onto glass slides (from left to right: 6.0×10^{-7} mol l^{-1}, 8.0×10^{-7} mol l^{-1}, 1.0×10^{-6} mol l^{-1}, 1.2×10^{-6} mol l^{-1}, 1.4×10^{-6} mol l^{-1}, and 1.6×10^{-6} mol l^{-1}.

Optimal hybridization temperatures

The hybridization efficiency and the selectivity of the sensing system can be successfully improved using a proper hybridization reaction temperature. The effects of different hybridization temperatures between 35 °C and 63 °C (35 °C, 48 °C, 53 °C, and 63 °C) for *S. aureus*, and between 25 °C and 54 °C (25 °C, 39 °C, 44 °C, and 54 °C) for *E. coli* on the hybridization ability were tested. As displayed in Table 1, the optimal hybridization temperatures of *S. aureus* and *E. coli* obtained were 48 °C (T_m = 63.30 °C, where 'T_m' is melting temperature, the temperature at which one half of the DNA duplex will dissociate to become single stranded and indicates the duplex stability) and 39 °C (T_m = 54.36 °C), respectively.

Optimal hybridization time

To have a more optimized condition for DNA hybridization, the effect of hybridization time (4.0×10^{-7} mol l^{-1} target DNA_3 of *S. aureus*) on the time-resolved fluorescence intensities was investigated. The results showed that, in the initial stage of hybridization time less than 2 h, the hybridization signal increased, and then reached a steady state at a hybridization time longer than 2 h. On the basis of this, all of the experiments were carried out for the hybridization time of 2 h.

Preliminary analytical application

Detections of DNA extracted from pure cultured *S. aureus* and *E. coli* were studied with the proposed hybridization assay under the optimal conditions. Strains of *S. aureus* and *E. coli* purchased from Guangdong Institute of Microbiology were cultured, and the double-stranded DNA were extracted use Genomic DNA extraction Kit (Generay Biotech Co., Ltd., Shanghai, China).

Table 1 The effects of different hybridization temperatures on the hybridization ability

Staphylococcus aureus		*Escherichia coli*	
Hybridization temperature (°C)	Fluorescence intensity	Hybridization temperature (°C)	Fluorescence intensity
35	105.2 ± 5.45	25	98.3 ± 5.56
48	133.1 ± 5.23	39	119.6 ± 4.30
53	118.0 ± 6.84	44	104.8 ± 4.50
63	117.0 ± 5.15	54	96.3 ± 6.62

Table 2 Results from preliminary analytical application

Staphylococcus aureus			*Escherichia coli*		
Pure cultured	Positive control (synthesized DNA_3 fragment)	Negative control (H_2O)	Pure cultured	Positive control (synthesized DNA_3 fragment)	Negative control (H_2O)
70.1 ± 5.70	75.7 ± 4.67	11.1 ± 5.24	65.9 ± 6.42	67.7 ± 4.06	11.1 ± 5.24

The double-stranded DNA fragments were denatured to single-stranded DNA by heating at 90 °C for 15 min before use. Targets DNA of *S. aureus* and *E. coli* in a same concentration of about 1.0×10^7 CFU l^{-1} were prepared. As shown in Table 2, satisfactory fluorescence intensities of 70.1 ± 5.70, 65.9 ± 6.42 were obtained and showed that the method might have potential value for pathogen detection. Moreover, further study on this system applied in *E. coli* strain detection has been done by our group.[27]

Conclusion

This work reported a novel bifunctional lanthanide europium complex of $Eu(TTA)_3(5\text{-}NH_2\text{-phen})$ as a potential fluorescent label for DNA detection. The complex exhibited long lifetime, high fluorescence quantum yield, and excellent signaling ability of a trace amount of target DNA. The hybridization conditions, selectivity, and capability of the prepared detection system were also investigated. The detection system using such a complex presented higher sensitivity compared with its nanoparticle-based detection systems. The method has the advantages of easy operation, short hybridization time, satisfactory selectivity, *etc*. The complex might be a new fluorescent label for biochemical analysis.

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