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Label-free DNA hybridization detection by various spectroscopy methods using triphenylmethane dyes as a probe

Jiaojiao Tu, Changqun Cai *, Ying Ma, Lin Luo, Chao Weng, Xiaoming Chen

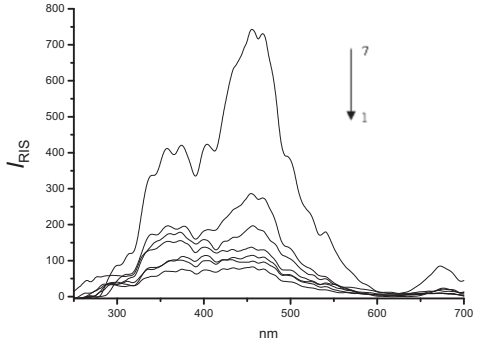
Key Laboratory of Environmentally Friendly Chemistry and Applications of Ministry of Education, College of Chemistry, Xiangtan University, Xiangtan, Hunan 411105, China

HIGHLIGHTS

- A new assay hybridization using trace of triphenylmethane dye as a probe.
- With the RLS measurement, labeling neither the target nor the probe DNA.
- The actual sample testing with satisfactory results.
- The technique is simple, robust, accurate, can be completed in less than 1 h.

GRAPHICAL ABSTRACT

When BG solution was added to P1 or T1 solution, negligible enhanced RLS signals could be observed. But when BG was added into the solution of P1 ≈ T1, greatly enhanced RLS signals could be observed with RLS peaks characterized at 371.3 nm, 454.9 nm and 674.1 nm, respectively.



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ABSTRACT

A new assay is developed for direct detection of DNA hybridization using triphenylmethane dye as a probe. It is based on various spectroscopic methods including resonance light scattering (RLS), circular dichroism (CD), ultraviolet spectra and fluorescence spectra, as well as atomic force microscopy (AFM), six triphenylmethane dyes interact with double strand DNA (dsDNA) and single strand DNA (ssDNA) were investigated, respectively. The interaction results in amplified resonance light scattering signals and enables the detection of hybridization without the need for labeling DNA. Mechanism investigations have shown that groove binding occurs between dsDNA and these triphenylmethane dyes, which depends on G–C sequences of dsDNA and the molecular volumes of triphenylmethane dyes. Our present approaches display the advantages of simple and fast, accurate and reliable, and the artificial samples were determined with satisfactory results.

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Introduction

DNA hybridization assays are of wide-ranging utility in molecular biology and forensic testing [1–3]. The most common DNA assay technique employs fluorescent labeling of the DNA molecules to be detected [4,5], because traditional fluorescence-based DNA detection has a number of attractive features such as broad availability and ease of use [6–8]. But such techniques are poor reproducibility, and expensive given that they require the use of fluorescent labels which result in high reagent costs. And the DNA hybridization also requires overnight incubation given that thousands of molecules must hybridize in order to produce enough optical signals to be readable by the fluorescent scanner. So, label-free strategies have begun to emerge as potential methods for detecting DNA hybridization with lower cost and at higher sensi-

* Corresponding author. Tel.: +86 7328298182.
E-mail address: cai_mao3@hotmail.com (C. Cai).

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tivity, for example, bioassays based on resonance light scattering (RLS) have long been anticipated [9,10].

Resonance light scattering, is a sensitive and selective technique for monitoring molecular assemblies. Pasternack and co-workers first established the RLS technique to study the biological macromolecules by means of an ordinary fluorescence spectrometer [11–14]. In recent years, the microarray RLS technique has been developed to detect DNA hybridization with high sensitivity, but the target DNA must be labeled [15].

Herein, we report a spectroscopic assay with the main readout principles, RLS, which can be applied for detection of DNA hybridization and polymorphisms detection. In the presence of Bromcresol Green (BG) probe, the groove binding between BG and the hybridization complexes P1 ≈ T1 (P1 ≈ T1, a kind of dsDNA, formed by P1 and T1, herein we use "≈" to indicate a double strand) occur, which induces the assembly of P1 ≈ T1–BG and leads to the formation of large particles with an increase of RLS intensity, and the further experiments demonstrated that the interaction between BG and P1 ≈ T1 depends on G–C sequences of dsDNA and the volumes of probes. Our approach is simple, robust, accurate, and can be completed in less than an hour using less than a milliliter of reagent, and eliminates the need of using labels or secondary reagents to monitor the oligonucleotide hybridization.

Experimental

Apparatus

RLS and fluorescence spectra were measured with RF5300 luminescence spectrometer (Shimadzu, Japan) with use of a 600-μL micro quartz fluorescence cell. Circular Dichroism (CD) spectra were conducted on a J-810 circular dichroism dichrograph (Jaseo, Japan). All absorption spectra were measured on a La 25 UV/vis spectrometer (PE, USA). All AFM experiments were conducted on Multimode NS 3d electron microscope (VEECO Inc., USA.) with a resonance frequency of 233 kHz. All pH measurements were made with a pHs-3C digital pH meter (Leici, Shanghai).

Reagents

Oligonucleotide sequences were synthesized by Beijing Sunbiotechnology Co. (Beijing, China), and used without further purification. The used oligonucleotide probes (P) and targets (T), respectively, include P1, 5′-CTG AAC GGT AGC ATC TTG AC-3′, its complementary sequences T1, 5′-GTC AAG ATG CTA CCG TTC AG-3′, and its two-base mismatched sequences MT1, 5′-GTC AAG ATT TTA CCG TTC AG-3′; P2, 5′-ATA ATT TAT T-3′, and its complementary T2, 5′-AAT AAA TTA T-3′; and P3, 5′-CGC GCC CGC C-3′, and its complementary T3, 5′-GGC GGG CCG C-3′. Thermally denatured fish sperm DNA (fsDNA) was used for comparison, which was obtained by incubating fsDNA in boiling water bath for 10 min and cooling immediately in ice water. The concentration of fsDNA was determined according to the absorbance values at 260.0 nm by using ε_{DNA} 6600 mol L^{-1} cm^{-1}[16].

The 1.0×10^{-4} mol L^{-1} stock solutions of six triphenylmethane dyes, including Bromcresol Green (BG), Methyl Violet (MV), Chrome azurol S (CAS), Light Green (LG), Xylenol Orange (XO) and Fuchsin Basic (FB) (the Molecular structure displayed in Supplementary Fig. 1) (Shanghai Chemical Reagents Co. Shanghai, China) were prepared by dissolving the commercial products in water in 250 mL volumetric flask. DNA hybridization buffer contained 10 mmol L^{-1} Tris, 140 mmol L^{-1} NaCl, 80 mmol L^{-1} $MgCl_2$ (we tried a series of different concentrations of NaCl and $MgCl_2$, and found that when the concentration of NaCl and $MgCl_2$ are 140 mmol L^{-1} and 80 mmol L^{-1}, respectively, the RLS intensity increased mostly, the figures were shown in Supplementary Fig. 2) and was adjusted to pH 7.4 by adding 1.0 mol L^{-1} HCl (in order to close to the body's physiological environment, the optimum pH used in this assay is determined to be 7.4).

All chemicals were analytical reagents and were used without further purification. Milli-Q purified water (18.2 MΩ) was used for all sample preparations.

Experimental procedure

One-hundred microliters probe DNA (P1 5.0×10^{-6} mol L^{-1}, after diluted, the concentration is 1.0×10^{-7} mol L^{-1}, displayed in Supplementary Fig. 3) solution, 200.0 μL of hybridization buffer solution, and an appropriate volume of target DNA (T1 5.0×10^{-6} - mol L^{-1}, after diluted, the concentration is 1.0×10^{-7} mol L^{-1}, displayed in Supplementary Fig. 3) solution according to the desired concentration were added to a 1.00 mL microtube. After the mixture was incubated for 30 min for hybridization at 37 °C, 100.0 μL dyes (0.1 m mol L^{-1}) solution was added. The mixture was diluted to 500.0 μL with water, and then vortex-mixed thoroughly before the RLS measurements. All RLS spectra were obtained by scanning the excitation and emission monochnoromators simultaneously (namely $\Delta\lambda = 0$ nm) from 200.0 to 700.0 nm. The RLS intensity was measured at 454.9 nm with a slit width at 3.0 nm for the excitation and emission. The fluorescence intensity was measured with a slit width at 5.0 nm for the excitation and emission. All measurements were made at the room temperature.

AFM measurements

AFM specimens were prepared by dropping the 5 μg mL^{-1} solutions onto freshly cleaved mica (already glued on a steel disc if necessary) and incubated for 5–10 min in air.

Results and discussion

RLS spectral properties and the application

The RLS spectral properties of six dyes (including BG, MV, CAS, LG, XO and FB), P1 (a kind of ssDNA)-dyes (the mixture of P1 and dyes), and P1 ≈ T1–dyes (the mixture of P1 ≈ T1 and dyes, P1 ≈ T1, the hybridization complexes formed from P1 and T1, a kind of dsDNA, herein we use "≈" to indicate a double strand) were studied (shown in Supplementary Fig. 4). We tried a series of different concentrations of dyes and found that when the concentration is 1.0×10^{-4} mol L^{-1}, the RLS intensity increased mostly, the figures were shown in Supplementary Fig. 5, so 1.0×10^{-4} mol L^{-1} is selected for further research.

It can be seen that the RLS signals of BG, P1–BG, P1, and P1 ≈ T1 are weak over the range of 200.0–700.0 nm (shown in Fig. 1). When BG solution was added to P1 or T1 solution, negligible enhanced RLS signals could be observed. But when BG was added into the solution of P1 ≈ T1, greatly enhanced RLS signals could be observed with RLS peaks characterized at 371.3 nm, 454.9 nm and 674.1 nm, respectively. According to the RLS theory, the strong RLS will be obtained if the molecular form aggregates, but when the incident light and the scattered light absorbed at the same time, the weak RLS intensity will be obtained at the band of the maximum absorption [11,12,17], so the RLS peak at 371.3, 454.9 and 674.1 nm is ascribed to the trough of molecular absorption at 308.2 nm, 398.6 nm and 614.2 nm, respectively (shown in Fig. 2). Furthermore, the amplified extent (ΔI_{RLS}) of P1 ≈ T1 at 454.9 nm is in good proportion to the concentration of T1 at a gi-

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