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Nucleic acid probe and stain based on water-soluble tetraphenylethene derivatives modified with different kinds of amino binding groups: The role of hydrogen bond

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ABSTRACT

A series of tetraphenylethene (TPE) derivatives modified with different kinds of amino groups were designed and synthesized in order to search the best binding groups for nucleic acid detection. NTPE, TPE1, TPE2, and TPE3 have similar frames with amino, ethylamino, diethylamino, and triethylammonium groups at the end of side arms, respectively. Both the *cis* and *trans* configurations of these dyes were separated and tested, respectively. With the number of the substitutional ethyl on *N* atoms increased from *Z*-NTPE to *Z*-TPE3 or *E*-NTPE to *E*-TPE3, the probability of hydrogen bond interactions between probes and nucleic acids would increase, and the fluorescent enhancement of the dyes upon binding with nucleic acid decreased gradually. *Z*-NTPE can stain 10 ng of X20 with 20 nt, and 1 ng of dsDNA with 300 bp in polyacrylamide gel electrophoresis. Such high sensitivity is attributed to its *cis* configuration and strong hydrogen bond interactions with nucleic acid. This work will be instructive for the further design of nucleic acid probes with high sensitivity.

1. Introduction

As one of the most valuable techniques in life sciences, polyacrylamide gel electrophoresis (PAGE) has been widely used for the separation of low rang DNA and assessment of DNA purity [1,2]. Thus, the ability to analyze and identify DNA [3,4] in gel matrix with high sensitivity is particularly important. There are various DNA staining materials, including organic fluorescence dyes, visible dyes, silver stain, and zinc-imidazole [5–8]. The most popular methods is staining with fluorescent agents [9,10], such as ethidium bromide (EB) and SYBR stains, which offer superb sensitivity, relatively rapid and easy operation approach [11,12]. These commercial dyes have been developed for specific targeting of double-strand DNA by a binding mode of intercalation, thus many of them do not show significant fluorescence response to single-strand DNA without secondary structures [13–15]. Besides, the most commonly used stain EB is reported to be a toxic and mutagenic agent because of its intercalating property [16].

Tetraphenylethene (TPE) derivatives have been widely explored as new fluorescence materials for identifying important biological macromolecules (e.g., DNA) [17,18]. Unlike most of conventional fluorescent dyes that self-quenched themselves when aggregated in large quantities, tetraphenylethene is non-emissive when molecularly dispersed in solution, but becomes highly emissive when aggregated due to the restriction of intramolecular rotation (RIR) that prohibits energy dissipation *via* nonradiative channels [19]. Based on the aggregation-induced emission (AIE) characteristic, a series of TPE derivatives with tetraalkylammonium cation as "light up" probes for DNA detection by use of electrostatic interaction were designed [20,21]. These TPE-based probes are particularly preferred as stain without background in electrophoresis gels, which have been used for DNA visualization with a simple and sensitive operation approach. Recently, by introducing amino group to strengthen the interaction between TPE dye and nucleic acid through hydrogen bond, we developed a simple, universal and highly sensitive method for the detection of dsDNA and oligonucleotides [22]. The newly developed material provided a detection limit down to 1 ng of oligonucleotide, followed with 30 min staining and 2–3 min washing [23].

In this article, aiming to systematically study the relationship between molecular structures and the binding ability with nucleic acids and consequent sensitivity for the detection, we designed and synthesized a series of tetraphenylethene (TPE) derivatives modified with different kinds of amino groups. As Scheme 1 pre-

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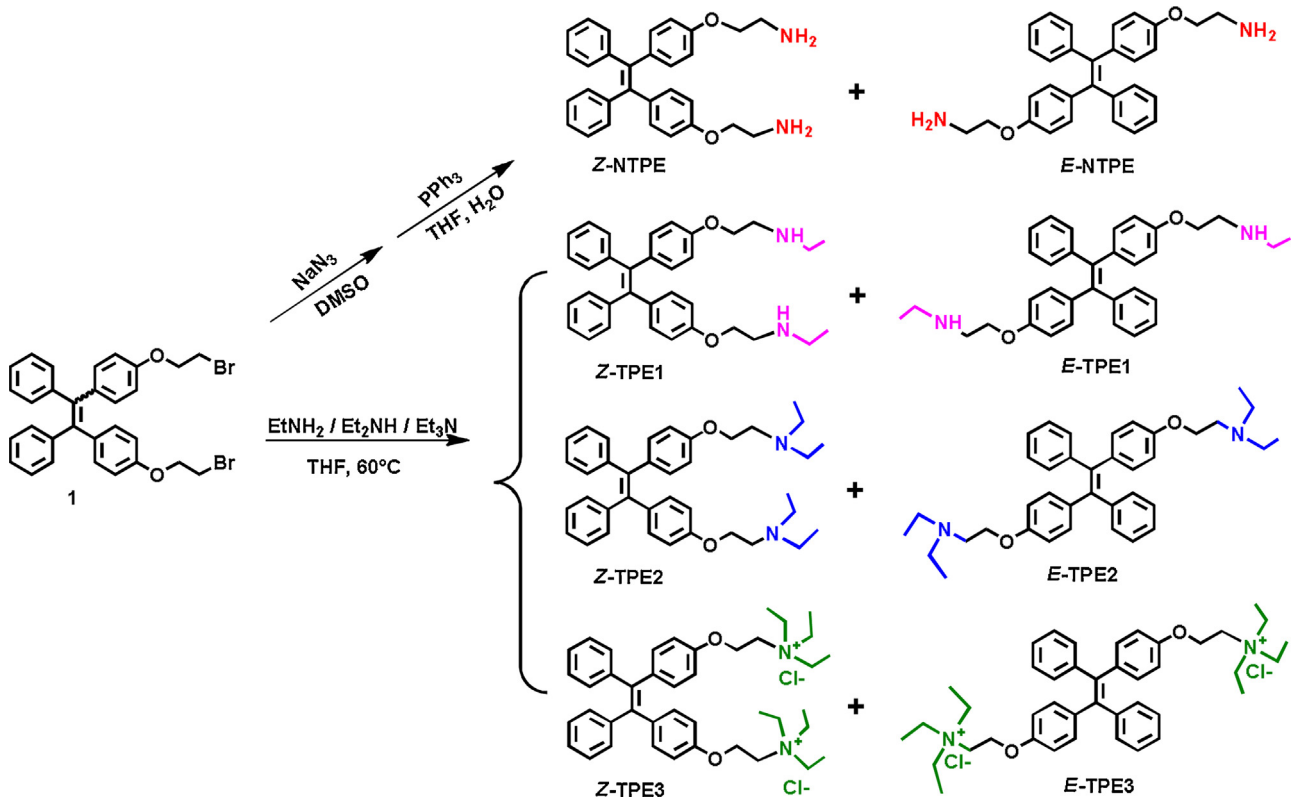

Scheme 1. The synthetic routes of tetraphenylethene derivatives.

sented, the new compounds of NTPE, TPE1, TPE2, and TPE3 have similar frames with amino, ethylamino, diethylamino, and triethylammonium groups at the end of side arms, respectively. We anticipated that with the number of the substitutional ethyl on *N* atoms decreased, the probability of hydrogen bond interactions between probes and nucleic acids would increase, consequently the binding affinity of these TPE derivatives with nucleic acids would get stronger gradually, thus exhibiting higher sensitivity.

2. Materials and methods

2.1. Materials

Oligonucleotides (X10, X20, X30) were purchased from Sangon Biotech (Shanghai) Co., Ltd. The sequence of DNA was shown in Table S1. Gene Ruler Ultra Low Range DNA Ladder was purchased from Thermo Scientific. All the other reagents were commercially available and used without further purification.

2.2. Instrumentation

^{1}H NMR and ^{13}C NMR spectra were measured on a MECUYRVX300 using $CDCl_3$ or CD_3OD as solvent. Mass spectra were measured on a Micromass-ZQ mass-spectrometer. Fluorescence spectra were recorded on a Hitachi F-4600 fluorescence spectrophotometer. Ultrapure filtered water (resistivity: 18.2 MΩ) was used in all experiments. Water was purified using a Millipore filtration system. The gels were scanned by BioDoc-it imaging system with LMS-26E Transilluminator.

2.3. Fluorescence measurement

TPE dyes were dissolved in DMSO to get a 10 mM solution. When carrying out the fluorescence titration experiment, 1 μL of 10 mM DMSO solution was added to the fluorometer quartz cuvette with 1 mL of deionized water or buffer solution, then followed the addition of DNA. The mixtures were vortex mixed and stood for 8 min prior to the measurements. All the titration experiments were carried out three times for calculating error bars.

2.4. Electrophoresis and gel conditions

DNA was electrophoresed in 1.0 mm thick 18% polyacrylamide gels in 1*TBE buffer (89 mM Tris, 89 mM boricacid, 2 mM EDTA, pH 8.3) at 158 V/cm for 1.2 h. To determine sensitivity limits for oligonucleotides, dilutions containing 40 ng to 10 ng random-sequence oligonucleotide size markers (a mixture of equal mass oligonucleotides with 10, 20, and 30 based in length) in formamide loading buffer were electrophoresed in lane 1–3. To determine sensitivity limits for dsDNA in polyacrylamide gels, dilutions containing 133.6 ng to 16.7 ng per lane Ultra Low Range DNA ladder were used in lane 4–8, which means 12 ng to 1 ng per band at 300 bp and 42 ng to 3.5 ng per band at 50 bp.

TPE stains were dissolved in DMSO to get a 10 mM solution, and then it was diluted by deionized water according to 1:1000 ratio to afford a 10 μM solution at last. The gels were incubated in 10 μM dye-containing solutions for 30 min, then photographed using a 300-nm UV transillumination. No special destaining was performed for any of these dyes, but briefly washed two times with water. For sensitivity comparisons, the photographic conditions were the same.

2.5. Synthesis of TPE molecules

1 and **NTPE** were synthesized according to the reported literatures [16].

TPE1. 1 (1 g, 1.73 mmol) was dissolved in 30 mL of THF, and then 2 mL of $EtNH_2$ were added. The mixture was stirred for 2 d at