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New naphthalimide modified polyethylenimine nanoparticles as fluorescent probe for DNA detection

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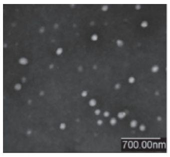
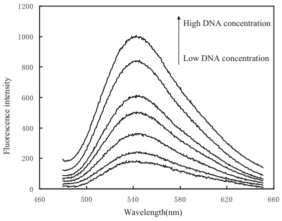
HIGHLIGHTS

- Naphthalimide modified PEI nanoparticles was developed as new DNA fluorescent probe.
- DNA condensing property and water-solubility of PEI were utilized in probe design.
- Fluorescence-enhanced DNA detection was achieved at 545 nm with exciting at 460 nm.
- The detection limits for fsDNA and ctDNA were 1.6 and 2.0 ng/mL, respectively.

GRAPHICAL ABSTRACT

Nanoparticles resulted from the self-assembly of naphthalimide modified polyethylenimine. Fluorescence of the nanoparticles was enhanced by increasing the DNA concentration.





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ABSTRACT

A new naphthalimide modified polyethylenimine (PEI) nanoparticles (called NPEI-NPs) was synthesized and applied as fluorescent probe for rapid, selective and sensitive fluorometric detection of trace DNA. The synthesis involved the covalent modification of PEI with 4-butylamino-N-carboxymethyl-1,8-naphthalimide(BACMN) for getting amphiphilic polymer. Then the amphiphilic polymer was self-assembled in water to give the NPEI-NPs. NPEI-NPs was soluble in water and emitted fluorescence at 545 nm with exciting at 460 nm. The fluorescence spectra resulting from the interaction between NPEI-NPs and DNA indicated that the fluorescence of NPEI-NPs increased in the present of DNA. Therefore, a fluorescence enhancement method was developed for the determination of trace fish sperm DNA (fsDNA) and calf thymus DNA (ctDNA). Under the optimal conditions, the calibration curves were linear over the concentration ranges of 0.05–2.8 μg/mL for fsDNA and 0.08–3.0 μg/mL for ctDNA. The detection limits for fsDNA and ctDNA were 1.6 and 2.0 ng/mL, respectively. The proposed method has been employed to quantify DNA in synthetic samples with the satisfactory results.

Introduction

The quantitative analysis of DNA plays an important role in biomedical and other aspects. For the detection of DNA, fluorescent probes were usually employed to increase the sensitivity and selectivity. Small molecular compounds including ethidium bromide [1], cyanine dyes [2–3] and nile blue [4] have been used as DNA fluorescent probes, but they were likely to suffer from photobleaching, carcinogen or high cost. In recent years, fluorescent nanomaterials have opened up new horizons for the development of DNA probes due to their unique physical and chemical properties. Various inorganic nanoparticles have been applied for exploiting DNA probes such as metal nanoparticles [5], silica nanoparticles [6] and luminescent quantum dots(QDs) [7–8]. Since they

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were usually hydrophobic, chemical surface modifications with organic molecules were needed to improve their water-solubility for the applications. In addition, the materials for getting inorganic nanoparticles have limitations and the preparations were difficult. Compared with the surface-modified inorganic nanoparticles, organic nanoparticles were comparatively readily available and tuned their properties by choosing the initial materials. Thus, the exploitation of organic nanoparticles in DNA testing has also gained research attention. For example, pyrene/polyacrylic acid (PAA) nanoparticles [9], 1-pyrenebutyric acid (PBAC)/β-cyclodextrin(β-CD) complex nanoparticles [10] and cetyltrimethylammonium bromide(CTAB) coated perylene colloids [11] have already been developed as fluorescent probes to detect DNA by fluorescence quenching method. However, the detections by pyrene/PAA and PBAC/β-CD complex nanoparticles were carried out at short emission wavelength of ~400 nm with exciting at ~340 nm and likely affected by background matrix. CTAB coated perylene colloids were used to detect DNA at a longer wavelength of 565 nm with exciting at ~400 nm, while such dye-coated nanoparticles was apt to meet the problem of dye leakage. Nowdays, nanoparticles-based DNA testing is still in its infancy. There is a long way to go in the development of DNA nanoprobes with the advantages of simple preparation method, water solubility, high sensitivity and selectivity. Because of the diversity of organic molecules, it is significant to extend the research on organic DNA nanoprobes.

Polyethylenimine (PEI), a water-soluble aliphatic polyamine, has been used in the paper industry as wet-strength additives or in water treatment as flocculants for a long time. Since PEI contains primary, secondary, and tertiary amino groups and exhibits a high positive charge density in aqueous solution, it can easily bind to the negatively charged outer surface of cells, as well as establish electrostatic interactions with DNA. During the past decades the applications of PEI in biological and medical fields have received increasing concerns [12–14]. Up to date extensive studies on PEI-based nanoparticles were made in cellular transfection and gene vector fields [15–17], but little attention was paid to the bioanalytical applications of PEI.

In our work, a new nanoprobe, naphthalimide modified PEI nanoparticles (called NPEI-NPs) was developed for sensitive DNA detection. In the design of NPEI-NPs, PEI was adopted owing to its water-solubility and DNA-bonding property. Besides, considering the good fluorescence properties of naphthalimide fluorophore, a naphthalimide derivative, 4-butylamino-N-carboxy-methyl 1,8-naphthalimide (BACMN), was synthesized and conjugated as hydrophobic chain with PEI resulting in a amphiphilic polymer. Then the amphiphilic polymer was self-assembled in water to give core/shell NPEI-NPs. It was found that the interaction between NPEI-NPs and DNA could enhance the fluorescence of NPEI-NPs, thus a method for fluorometric detection of trace fish sperm DNA (fsDNA) and calf thymus DNA (ctDNA) was established. According to our knowledge, this is the first time for bioanalytical application of PEI in fluorescence detection of DNA.

Experimental

Apparatus and reagents

^{1}HNMR spectra were obtained on an INOVA400 (Varian Inc., USA). Fourier transform infrared spectra (FTIR) were tested with a Nicolet 7199 spectrometer (Nicolet Co., USA). Transmission electron micrograph (TEM) images were taken with a JEM-100CX transmission electron microscope (JEOL Ltd., Japan). All fluorescence measurements were made by a LS 55 fluorescence spectrometer (PerkinElmer Inc., USA) equipped with a 1 cm quartz cell. The UV–vis measurements were carried out on a UV1601 spectrophotometer (Shimadzu Co., Japan) by using a 1 cm quartz cell. All pH measurements were made with a Delta 320 pH-meter (Mettler–Toledo Inc., Switzerland).

4-Bromo-1,8-naphthalic anhydride ($\geqslant$98.0%) was from Huifeng Chemicals Co. (Anshan, China) and PEI (Mw 10 kD) was purchased from Aladdin Reagent Co. (Shanghai, China). All other reagents were of analytical-reagent grade and used without further purification. Millipore-Q water was used to prepare the solutions.

Commercially available fsDNA and ctDNA from Sigma were respectively dissolved in water to the concentration of 1.0 mg/mL and stored at 4 °C. The working solutions were prepared from the stock solutions by appropriate dilution with water just before use. The phosphate buffer solutions were obtained by mixing 20.0 mmol/L Na_2HPO_4 solution with 0.5 mol/L HCl solution to the required pH value.

Synthesis of 4-butylamino-N-carboxymethyl-1,8-naphthalimide (BACMN)

4-Bromo-1,8-naphthalic anhydride (18 mmol) reacted with glycine (27 mmol) at 100–110 °C in the mixed solvents of dimethylformamide (DMF) and acetic acid, monitoring the reaction end-point with thin layer chromatography (TLC). The hot reaction mixture was filtered quickly at the end of the reaction. The filtration was allowed to cool slowly, followed by adding water to precipitate the product of 4-bromo-N-carboxymethyl-1,8-naphthalimide. The obtained crude product was purified by recrystallization with ethylene glycol monomethylether and ethanol. This product (5 mmol) was dissolved in 30.0 ml ethylene glycol monomethylether and n-butylamine (15 mmol) was slowly added. The reaction mixture was refluxed and the reaction progress was followed by TLC. Once the reaction was complete, the solvent was removed and water was added to the residue for separating the expected product of BACMN. The crude product was purified by recrystallization with ethanol. Yield: 67%. ^{1}H NMR(DMSO-d6, 400 MHz), δ(ppm): 0.91 (3H, CH_3); 1.21~1.44 (4H, $(CH_2)_2$); 3.34(2H, CH_2); 4.35 (2H, CH_2); 6.75 (1H, Ar–H), 7.65(1H, Ar–H), 8.22 (1H, Ar–H),8.55 (1H, Ar–H), 8.70 (1H, Ar–H); 7.74 (1H, NH).

Synthesis and characteristics of NPEI-NPs

To a solution of 2.5 mmol of BACMN in DMF, 1.0 mL of $SOCl_2$ was added dropwise at ~30 °C with stirring. The acid chloride reaction was allowed to continue for 2.5 h at 30–40 °C, and then 1.0 g PEI dissolved in 15.0 mL of DMF was added. After the reaction proceeded at 40–50 °C for 6 h, the reaction solution was extensively dialyzed against water using dialysis tube (molecular weight cut off = 3500 Da). Dialysate was replaced every 2 h for the first 8 h of the dialysis, next at least two times a day. The fluorescence intensity of the dialysate at 545 nm with exciting at 460 nm was measured to assess whether the dialysis was completed or otherwise. The final dialyzed mixture free from BACMN was lyophilized and further characterized by FTIR and UV–vis. The particle size and morphology were investigated by TEM after staining with tungstophosphoric acid.

According to the UV–vis absorbencies of NPEI-NPs and BACMN at 453 nm, the degree of substitution (DS) of naphthalimide fluorophore in the particle was estimated by using a standard calibration curve obtained experimentally with solutions of BACMN.

Measurement of fluorescence spectra

1.0 mg/mL of BACMN and 1.0 mg/mL of NPEI-NPs stock solutions were prepared in DMF and water, respectively. They were diluted to appropriate concentrations with water for fluorescence determination.