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Synthesis and characterization of pyrene modified polyethylenimine as a novel fluorescent self-reporter for gene condensation

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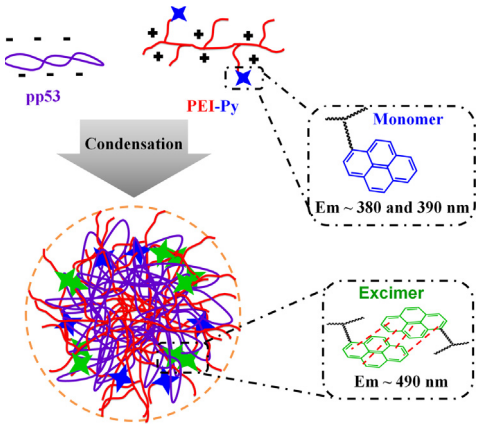
HIGHLIGHTS

- PEI-Py was used as a fluorescent self-reporter to understand the gene condensation situation.
- The fluorescence analysis of PEI-Py/pp53 could detect the gene condensing process.
- PEI-Py could be a novel fluorescent self-reporter to improving the optimal gene condensation.

GRAPHICAL ABSTRACT



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ABSTRACT

The appropriate composite ratio of cationic polymer and gene is fundamental but paramount determinant for efficient gene condensation. In this study, we proposed to construct a novel strategy to comprehensively understand gene condensation situation by using the pyrene (Py) modified polyethylenimine as fluorescent self-reporter, owing to the unique monomer and excimer fluorescence of Py groups highly depending on their intermolecular interval. Constant with the traditional measurements for polymer-gene complex including gel retardation analysis, DLS, ζ-potential, and TEM, the typical curve of fluorescence intensity ratio at I_{380}/I_{490} was verified to easily have an insight into the gene condensing process, and further recommend the appropriate range of N/P ratio (the ratios of moles of the amine groups of cationic polymers to those of the phosphate ones of DNA) for enhancing gene delivery. Thus, the facilely fabricated fluorescigenic cationic polymer based on an unique excimer-monomer switch could be used as an novel fluorescent self-reporter for comprehensively discerning the gene

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condensation, which may provide a feasible way to improving the optimal gene encapsulation for efficient and safe gene delivery.

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1. Introduction

Gene therapy has attracted a great number of attentions in preventing and treating a wide range of genetic diseases [1–4]. To date, cationic polymers, such as polyethylenimine (PEI), is widely exploited as one of the efficient non-viral gene carrier, which is capable of condensing the therapy gene as nano-complex for enhancing gene delivery [5,6]. However, many challenges do hinder its potential application in clinic, especially the premature gene release before arriving in the lesion location. Actually, polymer-gene complex would suffer an extreme dilution in bloodstream, which may lead to the loaded gene loss when nano-complex is lack of sufficient stability in case of inadequate cationic polymer to provide enough electrostatic attraction with gene. On the other hand, the over-packed nano-complex in case of the excessive cationic polymer would result in undesirable toxicity and relatively low gene loading efficacy. Thus, it is crucial to discern the composite process of gene and cationic polymer for optimizing the gene delivery. Currently, DNA condensation has been extensively studied both experimentally and theoretically [7–11]. In these studies, fluorescence correlation spectroscopy (FCS) and fluorescence resonance energy transfer (FRET) are commonly used method to detecting DNA condensation. However, FCS is more complex which need to fit the experimental autocorrelation curve to a theoretical diffusion model. FRET between a donor fluorophore on the DNA and an acceptor fluorophore on the carrier (or vice versa) could be a better alternative to study the complexation. Indeed, FRET only occurs when the donor and acceptor fluorophores are in close proximity, in other words when the DNA is associated to its carrier. However, FRET can not detect the gene condensing process and needs a proper positioning of the donor and acceptor molecules, which is hard to obtain in DNA complexes as they arise through (spontaneous) self-assembling of the DNA and the carrier molecules. In our study, we just utilize the typical curve of fluorescence intensity ratio at I_{380}/I_{490} to easily have an insight into the gene condensing process. Meanwhile, to the best of our knowledge, utilizing an excimer-monomer switching mechanism of Py groups which grafted on cationic polymers to discern the condensation process of gene and cationic polymer was not reported. For proof of concept, a cationic polymer, 1-pyrenebutyric acid grafted polyethylenimine (PEI-Py), as a novel fluorescence self-reporter, was synthesized to condense gene. Owing to the unique excimer formation of Py pairs highly depending on their intermolecular interval [5,12–15], the condensation process of polymer-gene nano-complex was easy to be prospected using the fluorometer. Hence, PEI-Py could be a feasible fluorescent self-reporter to conveniently discern the gene condensation for improving its safety and efficacy in gene delivery.

2. Experimental

2.1. Materials

1-pyrenebutyric acid (Py, 98%), branched polyethylenimine (PEI, molecular weight 10 kDa, 99%), *N*-Hydroxysuccinimide (NHS, 98%), and Dicyclohexylcarbodiimide (DCC, 98%) were obtained from Aladdin Biological Technology (Xi'an, China). Dimethyl sulfoxide (DMSO) was purchased from Bodi Chemical Holding (Tianjin, China) and used after dehydration. The p53-encoding plasmid (pp53, pCMV3-TP53-t1) was purchased from Sino Biological (Beijing, China). All other reagents without further instruction were used as received. Ultrapure water with a resistivity of 18.2 M was used throughout.

2.2. Synthesis and characterization of PEI-Py

PEI-Py was synthesized by a one-step chemical modification (Fig. 1a). Briefly, NHS (13 mg, 0.11 mmol), DCC (21 mg, 0.11 mmol) and Py (21 mg, 0.075 mmol) were dissolved in DMSO (3 mL). The mixture was stirred at room temperature (RT) for 18 h. Then, PEI (200 mg, ~1.8 mmol -NH_2) dissolved in 2 mL DMSO was added into the mixture, and stirred at RT for additional 24 h. After filtering off the white solid, the filtrate was dialyzed against ultra-pure water for 2 days, and freeze-dried to obtain the pure PEI-Py. The chemical structure of PEI-Py was detected using proton nuclear magnetic resonance (^{1}H NMR) to confirm its successful modification.

2.3. Preparation and characterization of PEI-Py/pp53 nano-complex

PEI-Py/pp53 nano-complex with different N/P ratios were prepared under gentle vortex, and further incubated at RT for 30 min. Gel retardation analysis was used to measure the pp53 retardation. Samples were respectively loaded into 2% agarose gel with ethidium bromide (0.1 μg/mL), ran at RT in Tris–acetate (TAE) buffer at 100 V for 40 min, and irradiated with UV light (254 nm) for visually imaging. The equal amount of pp53 was used as the control group. Hydrodynamic diameter and ζ-potential of PEI-Py/pp53 nano-complexes were measured using dynamic light scattering (DLS, Zetasizer Nano ZS 90, Malvern, UK) with a helium-neon laser (λ = 633 nm) at a scattering angle of 90°, respectively. In addition, the morphologies of PEI-Py/pp53 nano-complexes were photographed using transmission electron microscopy (TEM, H-600, Hitachi, Japan) at an accelerating voltage of 75 kV. Fluorescence emission spectra of PEI-Py/pp53 nano-complexes were obtained by exciting at 345 nm using spectrofluorometer (LS 55, Perkin-Elmer, UK).

3. Results and discussion

According to the typical ^{1}H NMR spectrum of PEI-Py in D_2O (Fig. 1b), the several broad peaks located at 8.0–8.5 ppm [16] and 2.3–3.2 ppm [17] were ascribed to the proton resonance of Py and

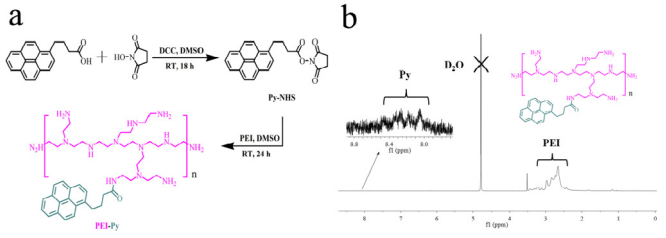

Fig. 1. Synthesis route (a) and ^{1}H NMR spectrum (b) of PEI-Py.

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