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Intrinsically fluorescent and highly functionalized polymer nanoparticles as probes for the detection of zinc and pyrophosphate ions in rabbit serum samples

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ABSTRACT

Intrinsically fluorescent polymer nanoparticles (F-PNPs) were synthetized from 2-hydroxy-5-methylisophthaladehyde and melamine by solvothermal method. F-PNPs can emit strong yellow green fluorescence at 542 nm without the conjugation to any external fluorescent agent and surface modification. Owing to the abundant amino and hydroxyl groups on their surface, the F-PNPs possess multiple binding sites, good biocompatibility and excellent water-solubility. Addition of Zn^{2+} to the F-PNPs solution resulted in a blue shift ($\Delta\lambda = 40$ nm) with obvious enhancement in the fluorescence intensity at 502 nm; while there was negligible change in the presence of other metal ions. The subsequent treatment with pyrophosphate (PPi) can cause fluorescence recovery of F-PNPs by pulling the Zn^{2+} out of the coordination cavity of F-PNPs-Zn^{2+} nanocomposites. No interference was observed from other anions and nucleotides, making the F-PNPs-Zn^{2+} ensembles highly sensitive and selective nanoprobes for PPi. The detection limit is 2.75×10^{-8} M/L and 7.63×10^{-8} M/L for Zn^{2+} and PPi, respectively. The proposed nanoprobes were then used for detecting the recovery of Zn^{2+} and PPi in rabbit serum samples, which were found to be 99.4–104.2% and 98.6–104.7%, respectively. The present strategy for the fabrication of nanoparticles may offer a new sight for the preparation of polymer nanostructures. The F-FNPs based probes can provide an accurate method for the detection of Zn^{2+} and PPi in serum samples.

1. Introduction

Fluorescent probes possess such advantages as high sensitivity, specificity, fast response and technical simplicity, resulting in an enormous demand in many areas including clinical analysis, environmental monitoring, waste management, industrial processing and biomedical technology [1,2]. As biologically related and very important ions, Zn^{2+} and PPi play vital role in many metabolic processes in human health. Zinc ion is involved as a cardinal cofactor in many biological processes such as brain function and pathology, gene transcription, immuno-function and reproduction [3–5]. The disorder of zinc metabolism is linked with many severe diseases such as epilepsy, hypoxia ischemia and Alzheimer's diseases [6–8]. Thus the need to characterize the roles of Zn^{2+} in disease related processes has made this field an interested research area. As one the by-product of ATP hydrolysis, pyrophosphate (PPi) can be employed as a potential biomarker for the clinic diagnosis and therapy of familial chondrocalcinosis or calcium pyrophosphate crystal deposition disease [9–12]. Therefore, there is an urgent need to develop technically simple yet effective methods for real-time PPi monitoring.

To date, enormous efforts have been devoted to develop optical sensing platforms for the detection of Zn^{2+} and PPi. In the early period, optical probes for Zn^{2+} and PPi were mainly focused on small organic dye-based sensing platforms [13–16]. However, the small organic fluorophores suffer from several inherent shortcomings, including photobleaching, limited brightness, and low aqueous solubility [17,18]. In the last decade, fluorescent nanomaterials, including semiconductor quantum dots (QDs), noble metal nanoclusters (NCs), fluorescent carbon dots (CDs), and other fluorescent nanostructures have garnered tremendous research attention and have been used in the design of Zn^{2+} and PPi-probes owing to their outstanding electronic and photonic properties [19–22]. Unfortunately, some of these optical nanoprobes have some inherent drawbacks such as relatively complicated and costly synthesis, or even harmful systems, which hamper their practical applications.

Recently, fluorescent polymer nanoparticles (F-PNPs) have attracted increasing interest in chemical, environmental and biological applications because of their intrinsic advantages including low toxicity, good

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biocompatibility and flexibility of synthesis [23–25]. Most reported F-PNPs were usually created by entrapping fluorescent agents in polymer matrixes, such as chitosan, polyamide and polystyrene, or covalently linking fluorophors with polymers [26,27]. But such fabrication methods generally involve in sophisticated synthetic pathways in organic solvents. Additionally, if the F-PNPs are derived from physical entrapment, there is a risk that the fluorescent dyes may leak out of the as-prepared nanoparticles in practical applications [28,29]. Thus, an ideal F-PNPs-based probe should be intrinsically fluorescent without the need of incorporating an external fluorochrome. In this respect, conjugated polymeric nanoparticles (CPNPs) can meet this requirement because of their large π-conjugated backbones and delocalized electronic structure [30,31]. Furthermore, when CPNPs are used as fluorescent nanoprobes, the excitation energy along the whole backbone of the polymers can transfer to a lower energy electron or energy acceptor fleetly, resulting in the amplification of fluorescent signal and giving the enhanced sensitivity [32–34]. However, most CPNPs are commonly composed of hydrophobic linear conjugated polymers carrying no functional groups, and the CPNP-based nanoprobes are mainly fabricated by blending the CPNPs with sensing substrates [35–37]. Therefore, the fabrication of F-PNPs with tailored recognition moieties, high quantum yield, good water solubility and favorable biocompatibility as well as with high selectivity towards the analytes in complex biological samples still constitutes a great challenge.

In this work, a facile and effective approach to synthesize intrinsically fluorescent polymer nanoparticles (F-PNPs) was developed using 2-hydroxy-5-methylisophthalaldehyde and melamine as reactants. Similar to conjugated polymer nanoparticles, F-PNPs can emit strong intrinsic fluorescence without introduction of any external fluorescent agents and surface modification. F-PNPs were found to display selective fluorescent response toward Zn^{2+} in neutral aqueous media and can form F-PNPs-Zn^{2+} complexes. These F-PNPs-Zn^{2+} ensembles were further employed for PPi sensing by metal displacement approach.

2. Experimental section

2.1. Materials and instruments

Melamine and 2-hydroxy-5-methylisophthalaldehyde were purchased from Acros Organics Co. Ltd. (Beijing, China). All other reagents and chemicals were purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China) and were used as received. Rabbit serum samples were provided by College of Chemistry and Biologic Engineering, Hunan University of Science and Engineering (Yongzhou, China).

Transmission electron microscopic images (TEM) were recorded with a PHILIPS TECNAI 10 TEM instrument (Philips, Netherlands). Raman spectra were carried out on a battery-powered Raman spectrometer (Model Inspector Raman, diode laser, excitation wavelength λ_{ex} = 785 nm) in the range of 100–2000 cm^{-1} (DeltaNu, USA). Fluorescence spectra were recorded with an F-7000 fluorescence spectrometer (SHIMADZU, Japan). UV–Vis absorption spectra were acquired by the UV–Vis 2600 spectrophotometer (Shimadzu, Japan). The absorbance of the cell viability by CCK-8 was tested using a microplate reader (Thermo Scientific Multiskan GO, Finland).

2.2. Synthesis of F-PNPs

To obtain intrinsically fluorescent and highly functionalized nanoprobes for Zn^{2+}, polymer nanoparticles (PNPs) were first synthesized using a facile hydrothermal method. Briefly, melamine (12.6 g, 100 mmol), 2-hydroxy-5-methylisophthalaldehyde (13.0 g, 100 mmol) and DMSO (120 mL) were added to a 250 mL three-neck bottom. After stirring for 20 min to dissolve these chemicals, the mixture was then heated gradually to 180 °C in an oil bath and stirred for 3 h under nitrogen at this temperature. After removal of the solvents, a brownish black precipitate appeared. Subsequently, this solid was dissolved in water by ultrasound and the large dots were abandoned by centrifugalization at 12,000 rpm for 20 min, affording a ploymer nanoparticle solution. Finally, the resulting solution was dialyzed against ultrapure water through a dialysis membrane (3500 kD) for 48 h. After vacuum-freeze drying, the cinnamon-colored solid residue was obtained.

2.3. Preparation of probe and test solutions

The nanoprobes F-PNPs were dissolved in a HEPES buffer (10 mM, pH 7.4) for a stock solution (1.0 mM). The stock solution containing F-PNPs-Zn^{2+} nanocomposites were prepared by adding 10 μL Zn^{2+}(0.01 mol/L) to 1.0 mL of F-PNPs solution (1.0 mM). All ion and molecule stock solutions were prepared at 2.0×10^{-2} M/L by dissolving appropriate amounts of their compounds in water. These nanoprobe stock solutions were used for different spectroscopic determinations after appropriate dilution. Test solutions were prepared by displacing 100 μL of the stock solution and an appropriate aliquot of each testing species solution to a 10 mL volumetric flask, and the solution was diluted to 10 mL with a HEPES buffer(10 mM, pH 7.4). The titrations were carried out by successive incremental addition of ion solutions to a fixed volume of F-PNPs solution in a 10 mL volumetric flask.

2.4. Absorption and fluorescence spectroscopies

All fluorescence and absorbance spectra were recorded after a 5 min-reaction at room temperature. The fluorescence quantum yields of F-PNPs and F-PNPs-Zn^{2+} nanocomposites were measured by the steady-state comparative method using quinine sulfate as the standard ($\Phi = 0.54$). The fluorescence intensity was recorded at 502 nm for Zn^{2+} and 542 nm for PPi with the excitation wavelength of 445 nm, and the slit widths of emission and excitation were fixed at 5 nm. The detection approaches for other interferences including different anions, nucleotides and metal ions were similar to that of Zn^{2+} or PPi.

2.5. Determination of Zn^{2+} and PPi in rabbit serum samples

The treatment of rabbit serum samples were conducted as according to the following procedures. a 2.0 mL-solution containing 1.5 mL of perchloric acid (1.0 mol/L) and 0.5 mL ammomium molybdate (0.1 mol/L) was added in to 7.0 mL of serum samples, and after thoroughly mixing, 1.0 mL of 0.1 mol/L triethylamine hydrochloride (pH 5.0) was added. The resulting solution was centrifuged at 5000 rpm for 15 min, and the clear supernatant solution was poured into another tube and centrifuged at 12,000 rpm for 20 min. The resulting supernatant solution was then used for the determination of Zn^{2+}. After dilution of rabbit serum with a HEPES buffer(10 mM, pH 7.4), the solutions were directly employed for the detection of PPi.

3. Results and discussion

3.1. Synthesis and characterizations of F-PNPs

In this study, intrinsically fluorescent polymer nanoparticles (F-PNPs) were synthesized by the solvothermal method using 2-hydroxy-5-methylisophthalaldehyde and melamine as reactants. As illustrated in Scheme 1a, the fluorescent polyazomethines were achieved by refluxing the mixture of 2-hydroxy-5-methylisophthalaldehyde and melamine in dimethyl sulfoxide (DMSO). After subsequent treatment, including removal of solvent, ultrasonic dissolution and dialysis in water, these polyazomethines self-assembled into the fluorescent and multifunctional polymer nanostuctures F-PNPs with desired properties.

As shown in TEM images in Fig. 1a, the products F-PNPs formed and show that they consist of nanoparticles well separated from each other