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Cy5 labeled single-stranded DNA-polydopamine nanoparticle conjugate-based FRET assay for reactive oxygen species detection

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ABSTRACT

This work reports on a simple and feasible fluorescence resonance energy transfer (FRET) assay for detecting reactive oxygen species (ROS) both in solution and living cell using polydopamine nanoparticle (PDA NP) as energy acceptor and Cy5 labeled single-stranded DNA (Cy5-ssDNA) as energy donor. The Cy5-ssDNA and PDA NPs form self-assembled conjugates (Cy5-ssDNA-PDA NP conjugates) via π-stacking interactions. In the presence of ROS, the PDA NP adsorbed Cy5-ssDNAs can be effectively cleaved, resulting in the release of Cy5 molecules into solution and recovery of fluorescence emission of Cy5. In order to obtain ROS solution, the glucose oxidase-catalyzed oxidation reaction of glucose with O_2 is employed to generate hydrogen peroxide for Fenton-like reaction. The formation of ROS in Fenton-like reaction can be detected as low as glucose oxidase-catalyzed oxidation of 100 pM glucose by the Cy5-ssDNA-PDA NP conjugate-based FRET assay. The recovery ratio of Cy5 fluorescence intensity is increased linearly with logarithm of glucose concentration from 100 pM to 1 μM, demonstrating that the FRET assay has wide dynamic range. In particular, intracellular ROS has been successfully detected in chemical stimulated HepG-2 cells by the Cy5-ssDNA-PDA NP conjugate-based FRET assay with a fluorescence microscopy, indicating that this approach has great potential to monitor ROS in living cells.

1. Introduction

As biochemistry mediators of cellular pathology, reactive oxygen species (ROS) including superoxide (O_2^-), singlet oxygen (O_2^1), hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), hypochlorite (ClO^-) and peroxynitrite ($ONOO^-$) play key roles in metabolic processes [8]. In normal aerobic cells, ROS level is normally adjusted by biochemical antioxidants [5]. Overaccumulation of ROS can induce oxidative stress, which is related to many diseases such as diabetes, cancer and molecular neurodegenerative disorders [2,10,24]. Several techniques/assays have been used to determinate ROS including electron spin resonance (ESR) spectroscopy, fluorescence spectroscopy, chemiluminescence assay and electrochemical biosensors [1,16,17,28,3]. Among of these methods, fluorescence-based assays can be used for real-time monitoring ROS level in cells and tissues [4,11,9].

Recently, due to the unique physicochemical properties, excellent biocompatibility and biodegradability, polydopamine nanoparticles (PDA NPs) have attracted much attention and been extensively investigated for various applications including surface modification, bio-inspired hydrogel generation, biosensor fabrication, metal deposition and drug delivery [21,18,20,31,13,32,30,26,22,14]. Especially, it was found that the fluorescence quenching capacity of PDA NPs was equivalent to that of graphene oxide [23]. For instance, PDA NP-based FRET assay has been used to detect DNA and thrombin [18,20,23]. The PDA NP-based FRET assay is fast, simple and homogeneous, and could be used for *in vivo* fluorescence imaging.

In this paper, we developed a "light on" assay for detecting ROS through FRET between PDA NP (acceptor) and Cy5 labeled ssDNA (Cy5-ssDNA, donor). The Cy5-ssDNA and PDA NPs form self-assembled conjugates (Cy5-ssDNA-PDA NP conjugates) via π-stacking interactions, leading to significantly quenching of fluorescence of Cy5. The fluorescence of Cy5 can be restored by the decomposition of Cy5-ssDNA-PDA NP conjugates through the ROS cleavage of ssDNA. The capacity of the Cy5-ssDNA-PDA NP conjugate-based FRET assay has been demonstrated by detection of Fenton-like reaction generated ROS in solution and intracellular ROS of chemical stimulated HepG-2 cells. To the best of our knowledge, this is the first example of using fluorescence quenching property of PDA NPs for monitoring intracellular ROS level.

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2. Experimental section

2.1. Materials and reagents

Dopamine hydrochloride and ammonia aqueous ($NH_4{\cdot}H_2O$, 28–30%) solution were purchased from Alfa Aesar (USA). Synthetic oligonucleotide 5′-TTTTTAGATACTATAT-Cy5-3′ was supplied by Sangon Ltd. (Shanghai, China). The concentration of DNA was determined by measurement of UV absorbance at 260 nm. Glucose, glucose oxidase (GOD) and Iron (II) sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$) were purchased from Sigma–Aldrich (USA). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were purchased from Aladdin Industrial Corporation (Shanghai, China). HepG-2 cell line was obtained from Shanghai cell bank of Chinese Academy of Science (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from HyClone (USA). Phorbol-12-Myristate-13-Acetate (PMA) was purchased from Beyotime Institute of Biotechnology (Jiangsu, China). All other chemicals were analytical grade. Milli-Q water (18.2 MΩ cm) was used in all experiments.

2.2. Instrumentation

Fluorescent emission spectra were recorded on a QE65 Pro fiber optic spectrometer (Ocean Optics, USA). Laser scanning confocal fluorescence microscope (ZEISS LSM 780, Germany) was used for living cell imaging. Transmission electron microscopy (TEM) micrographs were obtained on a Hitachi H-600 transmission electron microscope (Hitachi, Japan) with an accelerating voltage of 100 kV. Scanning electron microscopy (SEM) measurements were made on a XL30 ESEM FEG scanning electron microscope at an accelerating voltage of 20 kV (FEI, USA). UV–visible spectra were obtained by Mini 1240 UV–visible spectrophotometer (Shimadzu, Japan). The Fourier transform infrared (FTIR) spectra were recorded on a Bruker Vertex 70 spectrometer (Bruker, Germany). Raman spectra were recorded on a J-Y T64000 Raman spectrometer with 514.5 nm wavelength incident laser (J-Y, France). X-ray photoelectron spectra (XPS) measurements were conducted with a VG ESCALLAB MKII spectrometer (VG, UK). Dynamic light scattering (DLS) experiments were carried out on a Malvern Nano-ZS Zetasizer (Malvern, UK).

2.3. Preparation of PDA NPs

The PDA NPs were synthesized according to a previous report with slight modification [19]. Briefly, 0.5 mL $NH_4{\cdot}H_2O$ was mixed with 10 mL ethanol and 22.5 mL H_2O under mild stirring at 30 °C for 30 min. Then, a total of 0.125 g dopamine hydrochloride was dissolved in 2.5 mL H_2O and immediately injected into reaction mixture. The reaction mixture was stirred at 30 °C for another 24 h. The as-prepared PDA NPs were washed with 25 mL H_2O (3 times) and collected by centrifugation (9000 rpm).

In order to obtain pure Cy5-ssDNA-PDA NP conjugates for intracellular ROS detection, 14 μL Cy5-ssDNA (1.44 μM) with 2 μL PDA NPs (2 mg/mL) were mixed in 184 μL PB (10 mM, pH 7.0) and incubated at 30 °C for 1 h. Subsequently Cy5-ssDNA-PDA NP conjugates were purified by centrifugation (9000 rpm, 3 times) and redispersed in 200 μL PB.

2.4. Sensing and inhibition assay of ROS

Detection of Fenton-like reaction generated ROS. Typically, 3 μL of 2 mg/mL PDA NPs solution was incubated with 20 μL of 1.44 μM Cy5-ssDNA in 147 μL PB at 30 °C for 1 h. Subsequently, varying concentrations of glucose solution, 6.3 U GOD and 30 μL of 30 μM $FeSO_4$ solution were added into the reaction mixture, respectively. The total volume of the reaction mixture was adjusted to 300 μL by PB and the solution was incubated under O_2 atmosphere at 30 °C for another 5 min. Finally, the fluorescence spectra of the mixture were recorded on a QE65 Pro fiber optic spectrometer at the excitation wavelength of 633 nm.

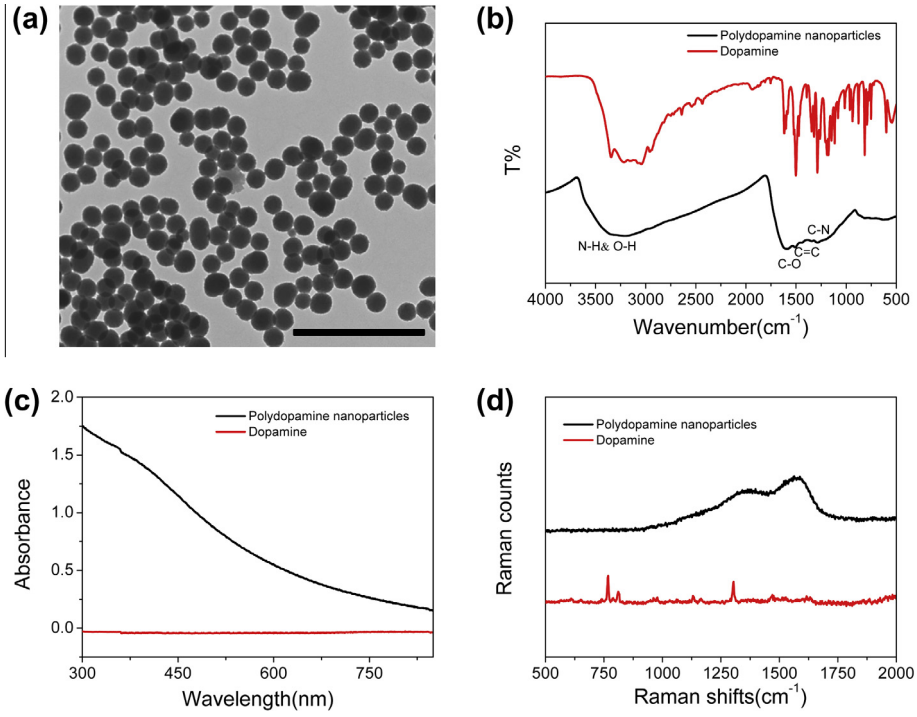

Fig. 1. (a) TEM micrograph, (b) FTIR spectrum, (c) UV–visible spectrum and (d) Raman spectrum of as-prepared PDA NPs. For comparison, the spectra of dopamine monomer have also been shown in the figure, respectively. The scale bar of (a) is 1 μm.