Chemical Engineering Journal 342 (2018) 331–338

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Mussel chemistry assembly of a novel biosensing nanoplatform based on polydopamine fluorescent dot and its photophysical features

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HIGHLIGHTS

- Glutathione (GSH) was employed to assemble polydopamine.
- Its photoluminescence was excitation wavelength-dependent.
- The intracellular detection of Cu^{2+} or Fe^{3+} has been realized.

ARTICLE INFO

Keywords:
Fluorescent polydopamine
Sensor
Cell imaging
pH dependence

ABSTRACT

The study of fluorescent polydopamine nanoparticles (F-pDA NPs) for the purpose of sensing and cell-staining has been investigated over the past decade. However, most of general synthesis strategies include potentially dangerous process or need to use cytotoxic chemicals that restrict the application range. Inspired by the adhesive structures in mussel chemistry, we employ glutathione (GSH) as a stabilizing agent to assemble the fluorescent GSH-integrated polydopamine nanoparticles (F-GSH-pDA NPs) via a straightforward one-pot oxidation method. In contrast to common methods that rely on H_2O_2 or specific catalyst, this novel way has been carried out through a single step based on only adjusting the pH value of GSH/Dopamine solution. The photoluminescence results demonstrated the possibility of controlling the emission wavelength from 480 to 540 nm by simply changing the excitation wavelength (from 320 to 450 nm). In particular, the emission profile shifted towards the longer wavelength region (from 493 to 525 nm) in the presence of acidic environment (pH 6 to 1). This blue-green luminescence could be switched *off* by the addition of Cu^{2+} or Fe^{3+} and the detection limits were determined to be 0.73 μM and 0.66 μM respectively. Further discrimination between Cu^{2+} and Fe^{3+} could be realized by absorption spectra changes. For the first time, the intracellular delivery of the functional nanoplatform has been reported and Cu^{2+} or Fe^{3+} recognition could be performed in two adherent cell lines (U937 and HeLa cells).

1. Introduction

Physical adhesion to biotic surfaces will be essential in living organisms and the immobilization strategy has been exemplified by mussel chemistry. In imitation of mussel protein adhesion, the pioneer work was focused on the development of surface modification method established on oxidative polymerization of dopamine [1]. The merit of such melanin-like building block has been attributed to its versatility and this biopolymer has been extensively studied in the range of surface coating, oil/water separation and molecular imprinting [2–5]. The detailed adhesion mechanism of mussel-inspired chemistry and the extension to different application fields have been fully summarized [6,7].

Apart from its biological significance, polydopamine was found to be photoluminescent and the labeling feature is far from being explored. As for the design of optical-active nanoplatforms, diverse

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https://doi.org/10.1016/j.cej.2018.02.099
Received 19 December 2017; Received in revised form 7 February 2018; Accepted 23 February 2018
Available online 24 February 2018
1385-8947/

fluorescent inorganic nanoparticles including semiconductor quantum dots, silica dots, metal nanoclusters and lanthanide-incorporated nanomaterials were assembled and investigated for the purposes of imaging and sensors. In particular, carbon-related quantum dots (including carbon dots and graphene quantum dots) and transitional metal dichalcogenides were extensively explored for the potential uses in clinical diagnosis [8–11]. Very recently, fluorescent organic nanoparticles have also emerged as the potential alternatives for functional imaging of biological systems due to their diffusion capability, intrinsic toxicity and biocompatibility [12–14]. Nevertheless, the low stability of the self-assembled organic structure in aqueous environment delayed its application under physiological conditions. To circumvent such problem, dopamine has been selected as a powerful molecular motif and its catechol groups would be oxidized to form polymerized dopamine under a weak alkaline condition.

From the application point of view, the examples of control the relevant photophysical properties in polydopamine (pDA) that regulate the emissions are very limited. In recent years, a few biocompatible fluorescent nanoparticles with extraordinary optical features were prepared based on hydroxyl radical-induced degradation, sacrificial template or the copolymerization with polyethyleneimine [15–19]. However, the luminescence signals of the particles could be quenched by aggregation with each other after they were purified from the mixture of H_2O_2 or peroxidase-mimicking nanozyme. Additionally, the intrinsic cytotoxicity of polyethyleneimine has severely restricted its utilization in biology [20,21]. Inspired by the above process during the synthesis stage, we desire to fabricate a whole new fluorescent pDA through using an endogenous molecule that possesses low cytotoxicity and good biocompatibility. As a result of the efficient assembly, a striking blue-green emission occurs immediately after excitation based on fluorescent glutathione-capped pDA nanoparticles (FDA). The synthesis steps have been carried out by a facile one-pot treatment approach at room temperature by simply adjusting the pH of GSH/DA mixture (Fig. 1). Glutathione (GSH) is a tripeptide (glutamic acid, cysteine, and glycine) found in many organisms. It bears –SH, –COOH, $–NH_2$ functional groups that are widely used as protection ligands in constructing fluorescent metal clusters [22,23]. GSH may also serve as an effective stabilizing and reactivity agent during the synthesis of FDA that –SH can react with the pDA by Michael addition. To our knowledge, the employment of glutathione as the linkage unit based on mussel inspired chemistry has not been reported. This effective approach offers several distinct merits. At first, the polydopamine particles by the grafting of GSH would lead to improved water permeability and were achieved to be regular or monodisperse. Secondly, the nanostructure could be fabricated by using a single-step based on polymerization reaction, avoiding the complicated synthesis processes in conventional methods. Thirdly, the typical recognition experiments of fluorescent organic nanoparticle only remained to be effective in aqueous solution. But in our research, confocal microscopy images of two cell lines (U937 and Hela cells) treated with pDA nanoparticles demonstrated bright-blue luminescence. Cell viability results gave negligible cytotoxic effects after the incubation at a concentration of 0.5 g/L. In particular, we report for the first time that the intracellular Cu^{2+} or Fe^{3+} responsive behavior could be verified clearly by the fluorescence changes. Lastly, this quantum-sized material was found to be sensitive to the low pH (from 6 to 1) and insensitive to the high pH conditions (from 7 to 14).

2. Experimental

2.1. Reagents and apparatus

Dopamine hydrochloride (DA, 99.9%) and Glutathione (GSH, reduced, 99%) were purchased from Aladdin Chemistry Co. Ltd. Dulbecco's modified medium (DMEM), RPMI1640 medium, Fetal bovine serum (FBS), antibiotic solution (penicillin and streptomycin) and 0.25% trypsin-EDTA were obtained from Sigma-Aldrich. The CellTiter 96 AQueous One Solution Reagent (MTS kit) was obtained from Promega. Annexin V-APC/Propidium Iodide (PI) kit was purchased from BD Biosciences (USA). All the other chemicals were analytical reagents and used without further purification.

UV-vis absorption spectra were collected by using a UV 2500 spectrophotometer with 10 mm quartz cells. ^1H NMR spectra were measured by Bruker ultrashield 400. Infrared spectra were recorded by a Nicolet-Magna 550 FT-IR instrument (resolution: 1 cm^{-1}; range 4000–400 cm^{-1}) with the KBr pellet technique. Fluorescence spectra were measured on a Hitachi F-4600 fluorescence spectrophotometer with the xenon lamp as the light source. The excitation slit width was 5 nm and the emission slit width was 5 nm (Scan speed: 1200 nm/min; voltage: 700 V). Transmission electron microscopic images and energy dispersive X-ray analysis (EDX) were measured by JEOL JEM-2100 UHR microscope. Zeiss confocal laser scanning microscope (LSM710) equipped with a laser at 405 nm was used to investigate the cellular images. MTS was measured at 490 nm using a Polarstar microplate

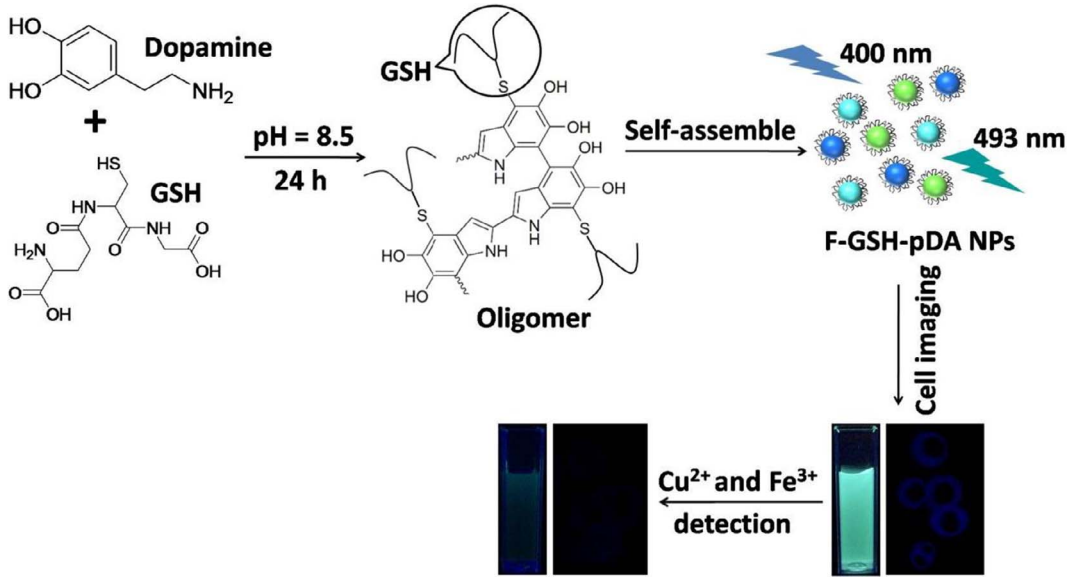

Fig. 1. Preparation of F-GSH-pDA NPs by one-pot oxidation of polydopamine and the subsequent utilization of the F-GSH-pDA NPs for cell imaging and metal ions detection.