Sensors and Actuators B 264 (2018) 45–51

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Dual-signal detection of trypsin using controlled aggregation of conjugated polymer dots and magnetic nanoparticles

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ARTICLE INFO

Article history:
Received 23 December 2017
Received in revised form 14 February 2018
Accepted 15 February 2018
Available online 18 February 2018

Keywords:
Conjugated polymer dots
Magnetic nanoparticles
Trypsin
Fluorescence
MRI

ABSTRACT

We developed a new ensemble for trypsin sensing by manipulating an aggregation of conjugated polymer dots (CPdots) and magnetic nanoparticles (MNPs) that generates dual signals of fluorescence and spin-spin relaxation (T_2)-based magnetic resonance imaging (MRI). Upon addition of trypsin, the dual signals were induced by the aggregation of CPdots with MNPs, generating MNPs-induced fluorescence quenching (or fluorescence color change), as well as a change in MRI relaxivity because of the increase in the size of aggregated MNPs. The MNPs played a role in the quencher for the fluorescence-related ensemble and in the MRI-signal generator for the magnetic property-related ensemble. The presence of polypeptide on the surface of CPdots was essential for the formation and destruction of the aggregates of CPdots and MNPs. Different fluorescence colors could be obtained by suitable selection of CPdots and polypeptide in terms of "turn-off" and "fluorescence color-changeable" modes.

1. Introduction

The development of new detection methods and techniques for biological molecules, including proteins and nucleic acids, is attractive because of the increasing interest in human health, including in the diagnosis and treatment of disease. Among biomolecules, proteases are responsible for a variety of physiological diseases [1–4]. Abnormal protease levels render humans unhealthy and are even considered life-threatening: therefore the detection of proteases is important [5,6]. Among proteases, trypsin is known as a digestive enzyme that selectively hydrolyzes polypeptide chains of lysine or arginine residues and participates in the invasion and metastasis of pancreatic cancer by promoting the degradation of the extracellular matrix. In addition, trypsin can activate the protease-activated receptor 2 (PAR-2) to stimulate pancreatic cancer cell proliferation and adhesion. Therefore, pancreatic trypsin in serum can be used as a marker for pancreatic cancer [7–13], and as a result, is overexpressed in pathological conditions such as cancer and inflammation [14–16].

New, sensitive, and noninvasive methods for the detection of serum trypsin are needed to monitor the trypsin levels of high-risk individuals. Many techniques for the determination of protease activity and related inhibitor screening have been reported, but most of these require specialized equipment and complicated laboratory procedures [17–22]. Thus, the development of a simple, inexpensive, highly sensitive and selective method for the detection of protease activity is urgently needed. Among the reported techniques, fluorescence–based assays are attractive because of their high sensitivity, easy operation, and rapid response. The probes of specifically-labeled peptide sequences have been generally designed and used as enzyme substrates in most fluorescence–based protocols [20–22]. Fluorescent assays for trypsin have been developed based on conjugated materials for label-free methods and photonic nanoparticles (NPs) [23–27]. However, chemical modification of peptide molecules may lower the affinity of the probe to protease, thereby reducing detection sensitivity. In addition, the synthesis and modification of a specific peptide substrate are more tedious and expensive. Hence, development of a simple, sensitive, fluorescent method for sensing protease activity using an affordable substrate with a label–free mode is highly desirable. As fluorescent materials, CPdots are fabricated from NPs of fluorescent conjugated polymers that have much potential as functional materials with high luminescence for light-emitting displays and stable probes for fluorescent sensing. CPdots exhibit desirable characteristics for biological sensing and imaging, including good quantum efficiency, high photostability, small-sized spherical shapes and easy modification of the surface for various functionalities [28–36].

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https://doi.org/10.1016/j.snb.2018.02.118
0925-4005/

Herein, we attempt to develop a dual signal-generating sensor (fluorescence and MRI) for trypsin detection based on the manipulation of the electrostatic interaction between two types of particles (CPdots and MNPs). The CPdots are used as fluorescent probes; and MNPs are used as both MRI agents and fluorescence quencher. MNPs have been used in various applications, including biomedical imaging, medical diagnostics and MRI contrast agents [37,38]. One of the advantages of MNPs made from iron oxide is that they can be easily functionalized on their surface to bind organic or biological molecules, which enhance the selectivity and binding affinity to target molecules. Electrostatic interaction between the CPdots and MNPs were manipulated with the aid of poly(L-arginine) (pArg), which was introduced on the surface of the CPdots and could be degraded to the simple amino acids (Arg) by trypsin, leading to the aggregation of two particles (CPdots and MNPs) [7,24]. Such controlled interaction was the basis of trypsin sensing via: i) MNP-induced fluorescence quenching or a fluorescence color-changeable system by aggregation between MNPs (quencher) and CPdots (fluorophore), or ii) changes in MNP relaxivity owing to changes in the size of the aggregates of MNPs and CPdots. Thus, a suitable polypeptide should be introduced on the surface of CPdots for manipulation of the interaction of CPdots and MNPs. The dual-sensing ensemble has been successfully applied to trypsin sensing, demonstrating its potential application in biologically-related fields. Moreover, because this technique is based on the interaction of two kinds of particles, the "turn-off" and "fluorescence color-changeable" modes can be attained easily by choosing a polypeptide to be coated on the surface of CPdots.

2. Experimental

2.1. Materials and instrumentation

2,7-Dibromo-9,9-dioctyl-9H-fluorene (1), 9,9-dioctylfluorene-2,7-bis(trimethylene borate) (2), 2,1,3-benzothiadiazole, 2-Thienylboronic acid, N-bromosuccinimide (NBS), and tetrakis(triphenylphosphine)palladium(0) were purchased from Sigma-Aldrich and used without further purification. Polypeptides including poly(L-lysine hydrobromide) (pLys; mol wt = 4000−15000), poly(L-arginine hydrochloride) (pArg; mol wt = 5000−15000), poly(L-histidine hydrochloride) (pHis; mol wt ≥ 5000), and trypsin (23,300 g/mol) were purchased from Sigma-Aldrich and used without further purification. 4,7-Dibromo-2,1,3-benzothiadiazole (3) and 4,7-di(2′-bromothien-5′-yl)-2,1,3-benzothiadiazole (4) were synthesized according to previously published methods [24,36]. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker DRX-500 spectrometer at Korea Basic Science Institute. Elemental analysis was performed with a CE Instruments EA-1110 elemental analyzer. FT-IR spectra were recorded on a Tensor 27 FT-IR spectrometer (Bruker). Gel permeation chromatography (GPC) was used to determine the molecular weight of the polymer with polystyrene standards using tetrahydrofuran (THF) as the eluent. UV–vis absorption spectra were recorded on a PerkinElmer Lambda 35 spectrometer. Photoluminescence spectra were taken using a Varian Cary Eclipse spectrophotometer equipped with a xenon lamp excitation source. Particle size and zeta potential were measured using Malvern Zetasizer Nano ZS. Transmission electron microscope (TEM) images were obtained with a JEOL JEM-3010 operating at 200 or 300 kV accelerating voltage, using the images acquired with an ORIUS-SC 600 CCD camera. All MR imaging experiments were performed using a 4.7 T clinical MRI instrument (Bruker BioSpec 47/40) at Korea Basic Science Institute.

2.2. Synthesis of blue-emitting conjugated polymer (B-CP)

1 (0.3 g, 0.547 mmol) and 2 (0.42 g, 0.656 mmol) were added to a 100 mL round-bottom flask charged with dry toluene (18 mL) and 2 M aqueous sodium carbonate solution (8 mL) under nitrogen. After the addition of tetrakis(triphenylphosphine) palladium(0) (5 mol%) as a catalyst, the mixture was heated up to 90 °C and stirred for 40 h. The reaction mixture was poured into acetone and the precipitate was washed with methanol, water, and acetone. The polymer was obtained after drying in a vacuum oven (yield: 0.21 g, 40%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.9–7.8 (m, 2H), 7.7–7.5 (m, 4H), 3.3–2.8 (t, 6H), 2.1 (m, 4H), 1.2 (m, 20H), 0.9–0.7 (m, 10H) ppm. ^{13}C NMR ($CDCl_3$, δ): 151.8, 140.5, 140.0, 121.5, 120.0, 55.4, 31.8, 30.1, 29.2, 22.6, 14.1 ppm. FT-IR (KBr, cm^{-1}): 1618 (C=C), 2939 (C—H), Anal. Calcd. for $C_{29}H_{44}$:C, 89.16%; H, 10.84%. Found: C, 89.74%; H, 10.58%.

2.3. Synthesis of green-emitting conjugated polymer (G-CP)

1 (0.2 g, 0.365 mmol), 2 (0.56 g, 0.876 mmol), and 3 (0.11 g, 0.365 mmol) were added to a 100 mL round-bottom flask charged with dry toluene (18 mL) and 2 M aqueous sodium carbonate solution (8 mL) under nitrogen. The reaction and work-up procedures were the same as those used for the synthesis of B-CP (yield: 0.19 g, 40%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.12–7.95 (m), 2.2–2.0 (m), 1.26–0.78 (m) ppm. ^{13}C NMR ($CDCl_3$, δ): 154.4, 151.8, 136.5, 133.6, 128.0, 120.1, 55.5, 31.9, 30.1, 29.3, 22.6, 14.1 ppm. FT-IR (KBr, cm^{-1}): 1151 (aryl C—N), 1618 (C=C), 2939 (C—H), Anal. Calcd. for $C_{46.5}H_{62}N_1S_{0.5}$:C, 85.9%; H, 9.5%; N, 2.2%; S, 2.47%. Found: C, 88.2%; H, 9.8%; N, 1.9%; S, 2.47%.

2.4. Synthesis of red-emitting conjugated polymer (R-CP)

1 (0.2 g, 0.365 mmol), 2 (0.35 g, 0.55 mmol), and 4 (0.042 g, 0.091 mmol) were added to a 100 mL round-bottom flask charged with dry toluene (18 mL) and 2 M aqueous sodium carbonate solution (8 mL) under nitrogen. The reaction and work-up procedures were the same as those used for the synthesis of B-CP (yield: 0.23 g, 40%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.85–7.59 (m), 7.36–7.34 (m), 6.86 (m), 2.11 (m), 1.25–1.14 (m), 0.88–0.79 (m) ppm. ^{13}C NMR ($CDCl_3$, δ): 151.8, 140.5, 140.1, 121.4, 117.3, 93.0, 55.5, 40.4, 31.8, 30.4, 30.1, 29.7, 29.2, 27.1, 22.6, 14.1 ppm. FT-IR (KBr, cm^{-1}): 1151 (aryl C—N), 1618 (C=C), 2939 (C—H), Anal. Calcd. for $C_{55}H_{73.2}N_{0.4}S_{0.6}$: C, 86.89%; H, 9.67%; N, 0.73%; S, 2.53%. Found: C, 89.74%; H, 10.58%; N, 0.81%; S, 2.53%.

2.5. Preparation of CPdots

A reprecipitation method was used to prepare CPdots for the investigation of fluorescent sensing and imaging. Each tetrahydrofuran (THF) solution of R-CP (0.5 wt%), G-CP (1 wt%), or B-CP (1 wt%) was quickly dispensed into deionized water (10 mL) under vigorous sonication. After removing the THF by gentle heating, large-sized aggregates were removed by syringe filtration (0.45 mm), and CPdots with desirable size were obtained. The resulting red-, green-, and blue-emitting CPdots were denoted as R-, G-, and B-CPdots, respectively.

2.6. Measurement of quantum yields (QYs) of CPdots

The relative QYs of CPdots were calculated using reference molecules, in which rhodamine B was used (QY 31%). The following equation was used to calculate the relative QYs of CPdots:

$$\Phi_x = \frac{A_R\ XD_x}{A_R\ XD_x}$$