Talanta 174 (2017) 148–155

Contents lists available at ScienceDirect

Talanta

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

Silver triangular nanoplates as an high efficiently FRET donor-acceptor of upconversion nanoparticles for ultrasensitive "Turn on-off" protamine and trypsin sensor

Hongyu Chen, Aijin Fang, Youyu Zhang*, Shouzhuo Yao

Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research (Ministry of Education), College of Chemistry and Chemical Engineering, Hunan Normal University, Changsha 410081, PR China

ARTICLE INFO

Keywords:
Upconversion nanoparticles
Silver triangular nanoplates
Fluorescence resonance energy transfer
Protamine
Trypsin

ABSTRACT

Silver triangular nanoplates (STNPs) as a high efficient fluorescence quenching reagent of upconversion nanoparticles (UCNPs) was used to constract a novel label-free fluorescence nanosensor for ultrasensitive detection of protamine and trypsin based on fluorescence resonance energy transfer (FRET) between STNPs and UCNPs. In this assay, the negatively charged STNPs can bind with positively charged UCNPs through electrostatic interaction, and then quenched the fluorescence of UCNPs. When protamine was added to the mixture of UCNPs-STNPs, the STNPs interacted with protamine and then detached from the surface of UCNPs and aggregated, which result in the recovery of the fluorescence of UCNPs. Trypsin could catalyze the hydrolysis of protamine and effectively quench the fluorescence recovered by protamine. By measuring the changes of the fluorescence of UCNPs, the concentrations of protamine and trypsin were determined. Under the optimized conditions, the linear response range was obtained from 10 to 500 ng/mL, 5–80 ng/mL and with the low detection limit of 3.1 ng/mL and 1.8 ng/mL for protamine and trypsin, respectively. Meanwhile, the nanosensor shows good selectivity, sensitivity and can be successfully applied to detection of protamine and trypsin in serum samples.

1. Introduction

Proteases, also known as proteolytic enzymes, are enzymes that catalyze the breakdown of proteins via hydrolyzing peptide bonds [1]. They can regulate many physiological processes such as protein digestion and turnover [2], blood clotting [3], cell growth, differentiation and apoptosis [4]. As such a protease, trypsin is formed in the pancreatic acinar cells, and responsible for cleaving peptides mainly at the C-terminal side of arginine or lysine residues [5,6]. It plays an irreplaceable role in numerous significant physiological processes such as cell differentiation and growth, immunological defense, and apoptosis [7]. The unbalanced quantity and quality of trypsin leads to some pancreatic diseases, such as pancreatic cancer, acute pancreatitis, cystic fibrosis, and so on [8,9]. Protamine is a low molecular weight protein [10]. It is a highly cationic peptide and has 20 positive charge in physiological condition [11]. Protamine is mainly isolated from the mature sperm cells of fish. it is widely used in regular clinic as an excipient in insulin formulations and plays a biological role in binding DNA and providing a highly compact configuration of chromatin in the nucleus of the sperm [12–14]. However, the protamine imbalance may cause sudden fall in blood pressure, bradycardia, pulmonary hypertension or dyspnea [15]. Therefore, it is vital to develop a simple, sensitive, and cost-effective method for the quantification of both trypsin and protamine.

To date, a series of approaches have been developed for trypsin or protamine detection, including enzyme linked immunosorbent assay (ELISA) [16], gel electrophoresis [17], liquid chromatography [18], and a number of electrochemical [19,20], colorimetric [21,22], and fluorometric methods [23–25]. Although the above-mentioned techniques are routine methods for the detection of trypsin or protamine and possess their respective advantages, many of them involved laborious synthetic procedures, tedious sample pretreatment, time-consume immobilizing processes and expensive instrumentation. Optical methods, especially fluorescence methods have attracted great interests due to simple instruments and easy operations. But they are still limited because of the difficulty to eliminate the background interference in complex detection system. Therefore, it is imperative to develop simple and efficient fluorescent method for the rapid detection of trypsin and protamine without autofluorescence interference and photobleaching.

In recent years, lanthanide-doped upconversion nanoparticles

* Corresponding author.
E-mail address: zhangyy@hunnu.edu.cn (Y. Zhang).

http://dx.doi.org/10.1016/j.talanta.2017.06.006
Received 23 March 2017; Received in revised form 31 May 2017; Accepted 2 June 2017
Available online 03 June 2017
0039-9140/

(UCNPs) emitting higher-energy visible light via a two-photon or multiphoton mechanism with large anti-stokes shift have recently aroused considerable attention [26–28]. Owing to their specific near-infrared (NIR) excitation at 980 nm, UCNPs offer excellent penetration depth in biosystems, and it can eliminate the autofluorescence from backgrounds. In addition, rare-earth doped nanocrystals also show superior chemical and optical properties, including low toxicity, narrow emission band width, long fluorescence lifetimes and high resistance to photobleaching [29–31]. Because of these advantages, UCNPs donor based fluorescence resonance energy transfer (FRET) by constructing a donor-acceptor nanoplatform in complex system is becoming increasingly favourable for the medical diagnosis and disease surveillance [32–34]. However, the fluorescence of UCNPs is hardly quenched by small molecule quenching agent, which limits the FRET efficiency and reduces the sensitivity of the FRET-based analytical system. Gold nanoparticles are usually considered to be very good quenching reagents because of their unique advantages, including high fluorescence quenching efficiency, tunable quenching property. Compared with gold nanoparticles, silver nanoparticles exhibit certain advantages. First, silver is much cheaper than gold. Second, silver is better form medicinal application due to their antimicrobial, antibiofilm, anti-inflammatory, and anticancer properties [35]. Furthermore, silver nanoparticles with strong plasmon absorption property appear to be better energy acceptors because of higher extinction coefficient [36]. However, most of the plasmon absorption peaks of silver nanoparticles are located at the near ultraviolet or purple window [37–39], which separates far away from most UCNPs' emission window. Thus, silver nanoparticles did not been chosen as a fluorescent donor-acceptor of upconversion nanoparticles. It will be very useful for instructing the nanosensor if the plasma absorption peaks of silver nanomaterials can be adjusted to overlap the emission peak of UCNPs. In fact, silver nanostructures with different size and shape such as nanorods, nanowires, nanoprisms, nanodisks/nanoplates, nanocubes, silver triangular nanoplates (STNPs) possess unique and tunable optical properties. In these silve nanoparitcles, STNPs contain three special sharp "corners" or "tips" that contribute significantly to their optical, electronic and chemical properties. For instance, they have maximum electromagnetic-field enhancement and an extreme degree of anisotropy due to the larger lateral dimensions than the thickness [40–42]. Furthermore, STNPs possess high extinction coefficient, optical and chemical stability and could display surface plasmon resonance absorption (SPR) in the visible to near infrared spectral region [43,44]. Such attractive features make it an excellent candidate as FRET donor-acceptor of upconversion nanoparticles for constructing nanosensor. However, to the best of our knowledge, there is no study associated with enzyme-catalyzed events with optical sensor by using STNPs as acceptor and UCNPs as donor.

Herein, we established a new optical nanosensor by means of an assembly of UCNPs and STNPs for ultrasensitive detection of trypsin and protamine. The proposed strategy is depicted in Scheme 1. An efficient FRET would occur between UCNPs and STNPs, with UCNPs acting as the donors and STNPs as the acceptor. So the fluorescence of UCNPs can be effectively quenched by the STNPs. With the existence of protamine, it could compete with UCNPs and STNPs can be induced the aggregation, which resulted in the detaching of the adsorbed STNPs from the surface of UCNPs and the restore fluorescence of UCNPs. Trypsin, which could cleave exclusively C-terminal to arginine and lysine residues, can easily hydrolyze protamine, resulting in the de-aggregation of STNPs and leading to the quenching of the fluorescence recovered by protamine. The concentration of protamine and trypsin were proportional to the changes of the fluorescence of UCNPs. Thus, a rapid, highly selective and highly sensitive sensing method has been developed for the detection of protamine and trypsin in biological samples.

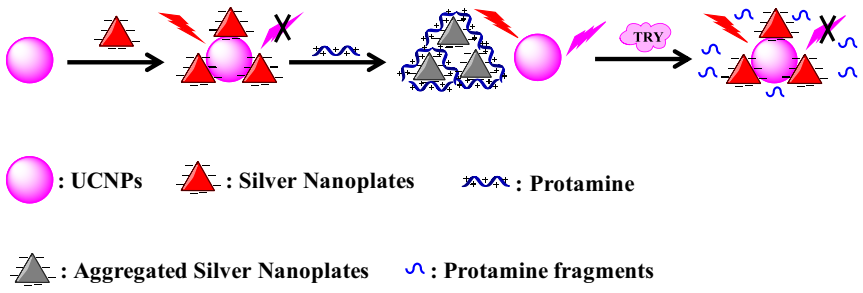
2. Experimental section

2.1. Materials

Yttrium oxide (Y_2O_3, 99.99%), ytterbium oxide (Yb_2O_3, 99.99%) and thulium oxide (Tm_2O_3, 99.99%), were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The solution was prepared by dissolving them in hot nitric acid, then diluted with deionized water to final concentrations of 0.4 mol/L, 0.2 mol/L and 0.05 mol/L, respectively. Trypsin was obtained from Beijing Dingguo Biotechnology Co., Ltd. (Beijing, China). Alkaline phosphatase (ALP) was from TaKaRa Biotechnology Co., Ltd. (Dalian, China). Bovine serum albumin (BSA), lysozyme, lactate oxidase, pepsin and glucose oxidase (GOx) were purchased from Sigma-Aldrich (Shanghai, China). Heparinase I (10,000 U/L) was purchased from Si Qing Yuan Biotechnology (Beijing). Protamine sulfate salt, heparin sodium salt, hexadecyl trimethyl ammonium bromide (CTAB) and other chemicals were obtained from Sigma (Shanghai, China). The buffer solutions with different pH were prepared by 0.01 mol/L NaH_2PO_4-Na_2HPO_4. All the chemicals were of analytical grade and used as received without further purification. Ultrapure water (18.2 MΩ cm) was purified by a Millipore-Q system. Clinical serum samples were made available by Hospital of Hunan Normal University, China.

2.2. Apparatus

The size and morphology of UCNPs and STNPs were characterized by transmission electron microscopy (TEM) images using a JEOL-1230 TEM (JEOL, Japan). Fourier transform infrared (FT-IR) spectra were obtained on an FT-IR spectrophotometer (Nicolet Instrument Co., USA). UV–vis absorption spectra of STNPs were collected on an UV-245 spectrophotometer (Shimadzu Co., Japan) and fluorescence spectra of UCNPs were measured using an F-4500 fluorescence spectrophotometer (Hitachi Ltd., Japan), where an extern laser at 980 nm continuous-wave (CW) laser (Hi-Tech Optoelectronic Co.,Ltd. China) replaced the xenon lamp as the excitation source. The crystalline phases of UCNPs were characterized using a Rigaku 2500 (Japan) Xray diffractometer (XRD). A Nano-ZS Zetzsozer ZEN3600 (Malvern Instruments Ltd., U.K.) was used to measure the Zeta potential of UCNPs and STNPs.



Scheme 1. Schematic illustration of the UCNPs-STNPs fluorescence assay for the detection of protamine and trypsin.