Talanta 147 (2016) 63–68

Contents lists available at ScienceDirect

Talanta

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

Gold nanoclusters-based chemiluminescence resonance energy transfer method for sensitive and label-free detection of trypsin

Xiaoying You [a], Yinhuan Li [a,*], Baoping Li [a], Jie Ma [b]

[a] Department of Chemistry, School of Science, Xi'an Jiaotong University, Xi'an 710049, China
[b] Department of Genetics and Molecular Biology, School of Medicine, Xi'an Jiaotong University, Xi'an 710049, China

ARTICLE INFO

Article history:
Received 1 August 2015
Received in revised form
9 September 2015
Accepted 12 September 2015
Available online 16 September 2015

Keywords:
Trypsin
Chemiluminescence
Resonance energy transfer
Gold nanoclusters

ABSTRACT

A chemiluminescence resonance energy transfer (CRET) platform was developed for sensitive and label-free detection of protease by using trypsin as a model analyte. In this CRET platform, bis(2,4,6-trichlorophenyl)oxalate–hydrogen peroxide chemiluminescence (CL) reaction was utilized as an energy donor and bovine serum albumin (BSA)-stabilized gold nanoclusters (Au NCs) as an energy acceptor. The BSA-stabilized Au NCs triggered the CRET phenomenon by accepting the energy from TCPO-H_2O_2 CL reaction, thus producing intense CL. In the presence of trypsin, the protein template of BSA-stabilized Au NCs was digested, which frustrated the energy transfer efficiency between the CL donor and the BSA-stabilized Au NCs, leading to a significant decrease in the CL signal. The decreased CL signal was proportional to the logarithm of trypsin concentration in the range of 0.01–50.0 μg mL^{-1}. The detection limit for trypsin was 9 ng mL^{-1} and the relative standard deviations were lesser than 3% ($n=11$). This Au NCs-based CRET platform was successfully applied to the determination of trypsin in human urine samples, demonstrating its potential application in clinical diagnosis.

1. Introduction

Proteases, also known as proteolytic enzymes, are enzymes that catalyze the breakdown of proteins via hydrolyzing peptide bonds [1]. They are involved in controlling a variety of indispensable physiological and pathological processes, including protein digestion and turnover, blood clotting, hormone activation, cell growth, differentiation and apoptosis [2–4]. The ill-regulated protease activities are closely associated with many diseases, e.g. inflammation, cancer, neurodegeneration, cardio vascular disorder, senile dementia, and diabetes [3–5]. It is of highly desirable to detect protease activity in consideration of proteomic research, disease diagnosis, and drug development.

Trypsin is a pancreatic serine protease with substrate specificity on positively charged lysine and arginine side chains through cleaving proteins on the α-terminal side of lysine and arginine residues [6]. As a biomarker for pancreatitis, trypsin plays a critical role in regulating pancreatic exocrine function. This self-regulating process can be adversely disturbed by pathologies, such as pancreatitis, which results in the damage of organ and release of enzyme into the blood [6]. The level of trypsin in biological fluids was reported to increase with some types of pancreatic diseases such as acute pancreatitis, cystic fibrosis, and pancreas transplant patients [7,8]. Thus, it is of great importance to develop simple and sensitive methods for the detection of trypsin in biological fluids.

Liquid chromatography [9], electrophoresis [10,11], and enzyme-linked immunosorbent assay [12] are the conventional methods for trypsin detection. These methods are usually laborious and/or require sophisticated instrumentation. Metal nanoparticles-based colorimetric methods were reported for detection of trypsin, based upon trypsin regulating the dispersed and aggregated states of nanoparticles [13,14]. Since the stability of metal nanoparticles is susceptible to external environments, such as pH and ionic strength, careful management on experimental variables is required for such methods. Electrochemical [15,16] and fluorometric [17–19] methods were also reported for the determination of trypsin. These methods usually need to design/synthesis labeled protease substrates or special molecular probes. The complicated labeling as well as high cost impedes wide applications of such methods. Therefore, it still remains a challenge to develop simple, label-free, cost-effective, easy-to-use, and sensitive methods for the detection of trypsin.

The analytical applications of chemiluminescence (CL) method have received considerable attention in recent years for its characteristics of simplicity, low detection limit, wide calibration graph, and inexpensive instrumentation [20–23]. Few CL methods are available for the determination of trypsin [24,25]. Yu et al. [24] reported a CL method for the detection of trypsin, based upon the

* Corresponding author. Fax: +86 029 82663914.
E-mail address: liyh@mail.xjtu.edu.cn (Y. Li).

http://dx.doi.org/10.1016/j.talanta.2015.09.033
0039-9140/

digestion of cytochrome c with trypsin to release heme, which catalyzed luminol–H_2O_2 CL reaction. A biotinylated peptide containing an arginine and a terminal cysteine was immobilized on the surface of streptavidin-modified magnetic beads by Fan group [25]. Upon the addition of trypsin, the peptide at C-terminus of arginine was hydrolyzed to release free cysteine-containing residues and caused an enhancement on luminol-$NaIO_4$ CL reaction [25]. Luminol-based CL assay had high sensitivity for trypsin, however they encountered with the incompatibility of strong basic condition of luminol CL reaction (pH > 11.5) and weak basic medium of protease hydrolysis (pH 8.0).

Peroxyoxalate CL reaction is one of the most popular CL systems, in which the oxidation of an oxalate ester with H_2O_2 generates CL emission in the presence of a suitable fluorophore [26,27]. Compared with other CL systems, peroxyoxalate CL reaction has none or low background emission, high luminescence efficiency, long luminescence life and can perform at near-neutral pH (pH 7.0-8.0), which cater for most enzyme-mediating biological reactions. Noble metal nanoclusters (NCs), especially Au NCs, have gained considerable attention in recent years owing to their attractive features of lesser light bleaching than organic fluorophore, lower toxicity than quantum dots, good biocompatibility, and easy surface functionalization [28]. Our group as well as Lu's [29] found that Au NCs could be used as an energy acceptor in bis(2,4,6-trichlorophenyl) oxalate (TCPO)–H_2O_2 CL reaction. In the work of Lu's, they reported the aggregation-induced CL emission of glutathione-protected Au NCs in TCPO–H_2O_2 reaction [29]. In our present work, we attempted to develop a sensitive and label-free CRET platform for the detection of protease by marring TCPO–H_2O_2 CL reaction with bovine serum albumin (BSA)-stabilized Au NCs. As illustrating in Scheme 1, the reaction between TCPO and H_2O_2 generates energy-rich intermediate 1,2-dioxetanedione, which transfers the energy to BSA-stabilized Au NCs and produces intense CL signal. BSA molecule can be cleft by trypsin [30,31]. When BSA molecule was degraded, the molecule structure of Au NCs was destroyed. Consequently, the energy transfer efficiency between the CL donor and BSA-stabilized Au NCs was frustrated. As a result, an evident decrease in the CL signal was observed.

2. Experimental

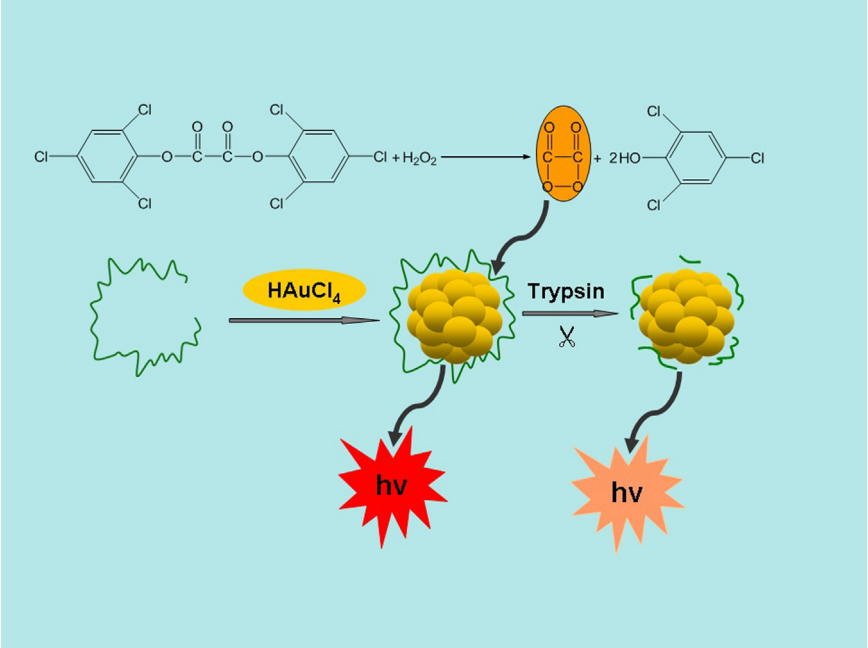
2.1. Apparatus

CL measurements were carried out on an IFFS-A multifunction CL detector (Xi'an Remex Analytic Instrument Co., Ltd, China) using a batch method. The CL signal produced was monitored by a CR 105 photomultiplier tube (Hamamatsu Photonics (China) Co., Ltd) with an operating voltage biased at 600 V. CL data was acquired with IFFM-D data processing software (Xi'an Remex Analytic Instrument Co., Ltd, China). CL spectra were obtained by means of a series of interference filters (Institute of Biophysics of Chinese Academy of Science, China) by setting the filter between the reaction cell and the window of the photomultiplier tube. Fluorescence spectra were taken on an F-2700 spectrofluorometer (Hitachi, Japan) with excitation and emission slits both at 10 nm. Transmission electron microscopy (TEM) image of Au NCs was measured on a JEM-2100 transmission electron microscope (Japan Electronic Company, Japan) at an accelerating voltage of 200 kV. The X-ray photoelectron spectrum (XPS) was measured on an X-ray photoelectron spectrometer (UltraDLD, Kratos, Britain) using Al-Kα as the exciting source (1486.6 eV) and binding energy calibration was based on C 1s at 284.8 eV.

2.2. Reagents and solutions

All chemicals used were of analytical grade. Ultrapure water (resistivity 18.2 kΩ) was produced from an AXLC1805 ultrapure water purification system (Beijing ASTK Technology Development Co., Ltd). Trypsin was purchased from Sigma, St. Louis, MO. Pepsin, lysozyme, and ribonucleases were purchased from Shanghai BlueGene Biotech Co. Ltd., China. Bis(2,4,6-trichlorophenyl)oxalate (TCPO) was purchased from Tokyo Chemical Industry Co. Ltd., Japan. Chloroauric acid was obtained from Shanghai Reagent Company, China. Bovine serum albumin (BSA) was purchased from Beijing Xinjingke Biotechnology Company, China. Hydrogen peroxide (H_2O_2, 30%) and other reagents were obtained from Xi'an Chemical Reagent Company, China.

A 1.0×10^{-3} g mL^{-1} trypsin stock solution was daily prepared by dissolving 0.1000 g trypsin in 100 mL of 0.05 mol L^{-1} Tris–HCl



Scheme 1. Schematic illustration of CL detection of trypsin with BSA-stabilized Au NCs amplifying TCPO–H_2O_2 CL system.