Biosensors and Bioelectronics 52 (2014) 367–373

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A distance-dependent metal-enhanced fluorescence sensing platform based on molecular beacon design

Zhenpeng Zhou [a], Hongduan Huang [a], Yang Chen [a], Feng Liu [a], Cheng Zhi Huang [b,*], Na Li [a,*]

[a] Beijing National Laboratory for Molecular Sciences (BNLMS), Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, Institute of Analytical Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China
[b] Education Ministry Key Laboratory on Luminescence and Real-Time Analysis, College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, China

ARTICLE INFO

Article history:
Received 12 August 2013
Received in revised form
4 September 2013
Accepted 6 September 2013
Available online 19 September 2013

Keywords:
Metal-enhanced fluorescence
Molecular beacon
Distance-dependence
Mercuric ion

ABSTRACT

A new metal-enhanced fluorescence (MEF) based platform was developed on the basis of distance-dependent fluorescence quenching-enhancement effect, which combined the easiness of Ag-thiol chemistry with the MEF property of noble-metal structures as well as the molecular beacon design. For the given sized AgNPs, the fluorescence enhancement factor was found to increase with a d^6 dependency in agreement with fluorescence resonance energy transfer mechanism at shorter distance and decrease with a d^{-3} dependency in agreement with plasmonic enhancement mechanism at longer distance between the fluorophore and the AgNP surface. As a proof of concept, the platform was demonstrated by a sensitive detection of mercuric ions, using thymine-containing molecular beacon to tune silver nanoparticle (AgNP)-enhanced fluorescence. Mercuric ions were detected via formation of a thymine–mercuric–thymine structure to open the hairpin, facilitating fluorescence recovery and AgNP enhancement to yield a limit of detection of 1 nM, which is well below the U.S. Environmental Protection Agency regulation of the Maximum Contaminant Level Goal (10 nM) in drinking water. Since the AgNP functioned as not only a quencher to reduce the reagent blank signal but also an enhancement substrate to increase fluorescence of the open hairpin when target mercuric ions were present, the quenching-enhancement strategy can greatly improve the detection sensitivity and can in principle be a universal approach for various targets when combined with molecular beacon design.

1. Introduction

Fluorescence spectroscopy is now a dominant methodology that has been used extensively in biotechnology, bioimaging, medical diagnostics and biosensing among others, and high sensitivity of fluorescence-related methods is most desirable (Lakowicz, 2006; Li et al., 2007; Sato et al., 2010). Metal-enhanced fluorescence (MEF), occurring between the intense plasmon-induced electric field and the excited-state of fluorophores located in close proximity to noble-metal nanostructures, is used to achieve sensitivity improvement in fluorescence measurements (Geddes and Lakowicz, 2002; Lakowicz, 2006; Rosa et al., 2011; Wilson and Nicolau, 2011). According to the mechanism of MEF, the enhancement is closely related to the localized electric field intensity of the metal, which is strongly dependent on the distance between the fluorophore and the metal surface (Geddes and Lakowicz, 2002). There have been many attempts to measure the dependence of the fluorescence enhancement factor on the distance from a metal surface in a macrosystem (Dragan et al., 2012) and a single molecule system (Anger et al., 2006; Kühn et al., 2006). However, it is still necessary to tune and predict the actual fluorescence enhancement factor especially on silver nanoparticles (AgNPs) for better analytical chemistry applications. Furthermore, current MEF researches, to the best of our knowledge, mainly focused on the elucidation of mechanisms and physicochemical aspects of nanostructures used for MEF (Choudhury et al., 2012; Chowdhury et al., 2007; Kelly et al., 2003), and there are still limited number of publications found for analytical purposes (Aslan et al., 2006; Doria et al., 2012; Geddes et al., 2003; Goldys et al., 2007; Gryczynski et al., 2012; Li et al., 2012, 2011b; Shtoyko et al., 2008; Touahir et al., 2010; Wang et al., 2012). In such a case, studying the distance dependence of the enhancement factor can not only help verifying and in-depth understanding the enhancement mechanism, but also facilitate significant applications in analytical chemistry.

It has been proved that gold/silver-thiol chemistry is very effective in tuning the distance between the fluorophore and the metal surface since the thiol binding can provide a facile approach to attach fluorescently labeled DNA with different length to the noble-metal surface (Li et al., 2010; Peng et al., 2009; Qiao et al., 2011). The distance between the fluorophore and the nanostructure surface thus can be simply adjusted by the length of the DNA, so the distance effect on the enhancement factor can be easily

* Corresponding authors.
E-mail address: lina@pku.edu.cn (N. Li).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.09.013

formulized. Consequently, analytical methods based on functionalized DNA can be integrated with the MEF strategy, facilitating more sensitive functional nucleic acid-based analytical applications (Conde et al., 2013; Farjami et al., 2011; Jayagopal et al., 2010; Ma et al., 2012; Rosa et al., 2012). Even so, analytical applications of MEF based on functionalized DNA as the recognition moiety are still limited. With this respect, great efforts are most desirable to best combine the easiness of Au/Ag-thiol chemistry and the MEF property of noble-metal structures for sensitive fluorescence sensing applications.

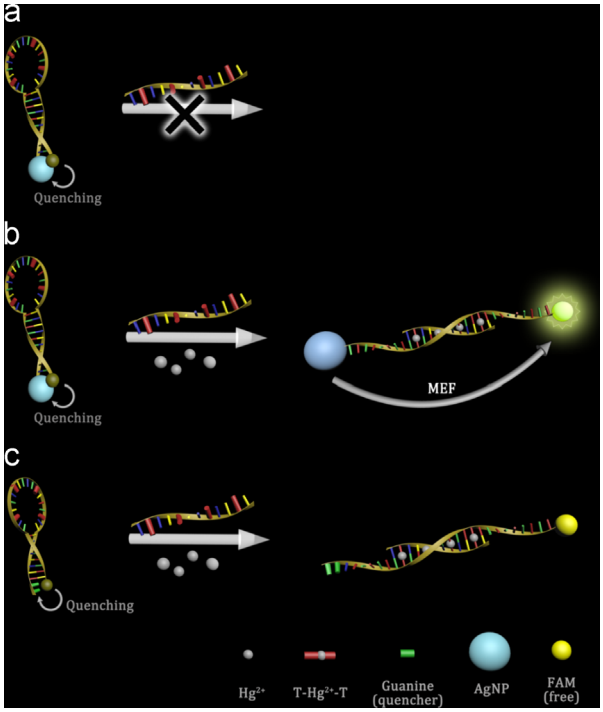
It is known that mercury, which has severe health threats to human beings, is one of the most poisonous metal ions in water and the environment in general (Harris et al., 2003). Optical sensors using enzymes (Cano-Raya et al., 2006) DNA (Ono, 2004), and organic small molecules (Zhao et al., 2006) have been proposed as a convenient, rapid, simple and cost-effective approach to determine Hg^{2+} with the detection limit in the range from sub-nM to several dozens of nanomolar (Chang et al., 2011; Huang et al., 2013; Li et al., 2011a; Lin and Chang, 2011; Liu et al., 2009a, 2009b; Wang et al., 2008, 2009). In recent years, an interesting strategy using the thymine (T)-containing molecular beacon (MB) design with which formation of T–Hg^{2+}–T complex has been used as the on/off fluorescence switch to open or close the hairpin structure. Although selective determination of mercuric ions was achieved with the T–Hg^{2+}–T structure, the detection limit of around 20 nM (Stobiecka et al., 2012; Wang et al., 2009; Xu and Hepel, 2011; Xu et al., 2011) with most methods is difficult to meet the 2 ppb (10 nM) criteria of the U.S. EPA's regulation (EPA), thus further sensitivity improvement is still needed.

As a proof-of-concept in this work, we for the first time proposed a metal-enhanced fluorescence strategy on the basis of optimizing the distance-dependent silver-thiol chemistry for highly sensitive detection of mercuric ions by using T-containing molecular beacon as the recognition moiety. In our design, fluorescently labeled molecular beacon was attached to silver nanoparticles via silver-thiol chemistry (Scheme 1), wherein AgNPs, in addition to acting as a quencher to reduce the reagent blank signal, provided superimposed electric field over incident light to enhance the fluorescence intensity as the closed hairpin was opened upon binding with mercuric ions. In an effort to provide an effective approach for the better method development, we evaluated in detail the fluorophore-metal distance effect as well as the AgNP size effect on the fluorescence enhancement, in hope that this investigation could help an in-depth understanding of the analytical chemistry aspects of metal-enhanced fluorescence for further extended applications.

2. Experimental

2.1. Chemicals and instrumentation

All nucleotides were synthesized and HPLC-purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequence information as indicated in the specific experiment has been provided in the Supporting information. Silver nitrate (AR), trisodium citrate (AR), sodium chloride (AR), sodium dihydrogen phosphate (AR), disodium hydrogen phosphate (AR), and disodium tetraborate (AR) were purchased from Beijing Chemical Plant (Beijing, China). Sodium borohydride (AR) was from Tianjin Xuan'ang Co. Ltd. (Tianjing, China), and ascorbic acid (AR) was from Shanghai Chemical Reagent Plant (Shanghai, China). Tris(2-carboxyethyl) phosphine (TCEP) ($\geq$99%) was from Sangon Biotechnology Co., Ltd. (Shanghai, China). All other chemicals, unless mentioned otherwise, were of analytical grade (AR). Wahaha® purified water was used throughout the study.



Scheme 1. A schematic illustration of the principle of the proposed method.

Phosphate buffer (pH 7.5, 200 mM) was prepared by mixing 84.0 mL of 0.2 M Na_2HPO_4 with 16.0 mL of 0.2 M NaH_2PO_4.

Fluorescence spectra were recorded with a Hitachi F-4500 spectrofluorometer (Tokyo, Japan). Extinction spectra of AgNPs were obtained with a Hitachi U-3010 spectrophotometer (Tokyo, Japan). Transmission Electron Microscopic (TEM) images were acquired with a JEOL-200CX transmission electron microscope (Tokyo, Japan). For the TEM measurement, a 5-μL silver nanoparticle sample was dropped on carbon-coated copper grids (PELCO, USA), air-dried, and then examined with TEM.

Time-resolved measurements were made with an Olympus BX51 fluorescence microscope at the 377-nm excitation using a pulsed laser diode (Horica DeltaDiode™). The instrument response function (IRF) was measured without sample plate. A bandpass filter of 520 BP was used in the collection path to eliminate the scattered excitation light and collect the fluorescence. The fluorescence lifetime was fitted by mono-exponential decay model using Origin 9.0 (OriginLab).

Finite-difference time-domain (FDTD) simulation was performed with FDTD Solutions 8.5 (Lumerical Solutions, Inc.). A near field plane wave at 495 nm was used as incident light.

2.2. Preparation of AgNPs

AgNPs were prepared according to the reported procedure with slight modification (Kvitek et al., 2005; Prucek et al., 2011; Sharma et al., 2009). The detailed procedure has been provided in Supporting information. In this study, AgNPs were prepared with diameter distribution of 36 ± 2 nm ($n=50$), 89 ± 2 nm ($n=50$), and 199 ± 4 nm ($n=50$), respectively, as obtained from TEM images (Fig. S1).

2.3. Hybridization of DNA

Lyophilized DNA sample was first suspended in 200 μL of 20 mM pH 7.0 phosphate buffer, and the two complementary DNA strands were mixed and heated for 10 min at 85 °C, then