Analytica Chimica Acta 967 (2017) 85–92

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Determination of nanomolar levels of reactive oxygen species in microorganisms and aquatic environments using a single nanoparticle-based optical sensor

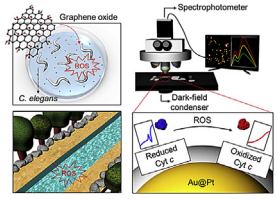
Yura Kim [a], Youngho Kim [b], Jinhee Choi [b], Taewook Kang [c, **], Inhee Choi [a, *]

[a] Department of Life Science, University of Seoul, Seoul 130-743, Republic of Korea
[b] School of Environmental Engineering, Graduate School of Energy and Environmental System Engineering, University of Seoul, Seoul 130-743, Republic of Korea
[c] Department of Chemical and Biomolecular Engineering, Sogang University, Seoul 121-742, Republic of Korea

HIGHLIGHTS

- Single nanoparticle-based optical sensor for monitoring reactive oxygen species (ROS).
- More sensitive monitoring and wider detection range than commercially available method.
- Limit of detection and quantification were determined from 8.3 to 12.8 nM and from 27.6 to 42.7 nM, respectively.
- Selectivity coefficients for major interfering solutes were much lower than 0.1.
- Determination of nanomolar level ROS in biological and environmental samples.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 October 2016
Received in revised form
8 March 2017
Accepted 9 March 2017
Available online 21 March 2017

Keywords:
Reactive oxygen species
Plasmonic nanoparticles
Plasmon resonance energy transfer
Cytochrome *c*
Hydrogen peroxide
Optical sensor

ABSTRACT

Reactive oxygen species (ROS) are strong oxidants, and have attracted considerable attention in both biological and environmental fields. Although various methods for ROS detection, including optical and electrochemical techniques, have been developed, they still face challenging issues in terms of poor sensitivity, reproducibility, stability, and *in vivo* applicability. Here, we present a sensitive and selective optical sensor for ROS detection, based on single plasmonic nanoprobes and redox-active cytochrome *c* (Cyt *c*)-mediated plasmon resonance energy transfer. By measuring the spectral changes of plasmonic nanoprobes, derived from the unique molecular absorption of Cyt *c* in accordance with the redox state, calibration curves for H_2O_2, a representative ROS, in various media were obtained over a wide concentration range from 100 mM to 1 nM. Limit of detection and limit of quantification in accordance with the used medium were determined from 8.3 to 12.8 nM and from 27.6 to 42.7 nM, respectively. Selectivity coefficients for major interfering solutes were much lower than 0.1 indicating a good selectivity for ROS. From the dynamic spectral changes, we sensitively monitored ROS generated in *Caenorhabditis elegans* (*C. elegans*) exposed to graphene oxide. Based on the calibration curves, we also determined ROS levels in various aquatic environments, such as river streams and a small pond, as a way of environmental

* Corresponding author.
** Corresponding author.
E-mail addresses: twkang@sogang.ac.kr (T. Kang), inheechoi1@uos.ac.kr (I. Choi).

http://dx.doi.org/10.1016/j.aca.2017.03.012

monitoring. We believe that our approach could provide an avenue for achieving dynamic and sensitive monitoring of ROS in toxicological, biological, and environmental fields in the future.

1. Introduction

Reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl radical ($\cdot OH$) are involved with disease-associated processes and environmental system maintenance. Biologically, ROS plays an important role in regulating cellular signaling pathways [1], however the generation of excessive ROS induces cell death by apoptosis or necrosis [2]. Since cellular lipids, proteins and DNA can be damaged by ROS [3–5], it has been frequently reported that changes in the cellular environment caused by ROS elicit disease, including degenerative diseases [6–11] and cancers [12,13]. In general, exposure of organisms to toxic substances induces the biological generation of ROS, which results in oxidative stress. In this regard, the generation of ROS has usually been considered as a nanotoxicity indicator [14–18]. Moreover, ROS can be generated by abiotic photolysis (*e.g.,* photo-oxidation) in various aquatic environments [19,20], as well as in underwater organisms [21], that may provide harsh environments for other living organisms. Due to these harmful aspects of ROS, generated within organisms or externally, precise monitoring of ROS generation is a very important issue in biological and environmental fields. Therefore, a variety of ROS detection methods, including optical techniques [22–24] and electrochemical techniques with [25,26] or without enzymes [27,28], have been developed to effectively detect ROS. For example, H. Lee et al. reported that intracellular ROS was detected in the range of 0.5–10 μM by using fluorescein labeled gold nanoparticle [23]. S.F. Wang et al. showed that an electrocatalytic method using carbon nanotube immobilized electrodes permitted to detect ROS between 1.07 μM and 48.4 μM [25]. J.C. Yuan and A.M. Shiller demonstrated a flow injection chemiluminescent method with the nanomolar level accuracy of 0.42 nM [24]. Also, M.G. Li et al. presented that detection limit was 0.11 μM by graphene-modified electrode [26]. Although these technologies are quite functional and sensitive, it is difficult in selectively detecting ROS when ROS are mixed with other materials (such as metal ions) [29,30] and many of the developed sensors work only in the specific concentration range [22,23,31]. In order to overcome these limitations of current technologies, development of effective methods for ROS detection is required.

In this study, we present nanomolar level determination of ROS via an optical method using a single nanoparticle-based sensor. The sensing principle is based on the plasmon resonance energy transfer (PRET) phenomenon between a nanoplasmonic particle and redox active cytochrome *c* (Cyt *c*) proteins, characterized by a vertical spectral quenching dip on the Rayleigh scattering spectrum of the nanoparticle. Owing to the unique molecular absorption characteristics of Cyt *c* according to its redox state, it is possible to continuously monitor ROS generation through dynamic changes in the spectral quenching dip. Using this method, we successfully monitored ROS generation in *Caenorhabditis elegans* (*C. elegans*) exposed to graphene oxide (GO) to evaluate nanotoxicity. Furthermore, we applied the method to determine ROS levels in various aquatic environments as a way of environmental monitoring.

2. Experiment section

2.1. Materials

Sulfuric acid (H_2SO_4, 95%) and hydrogen peroxide (H_2O_2, 34.5%) were purchased from Samchun Pure Chemical Co. Ltd., (Gyeonggi, Korea). Ethanol (99.9%) was purchased from JT Baker Chemical Company (New Jersey, USA). Chloroplatinic acid hexahydrate ($H_2PtCl_6 \bullet 6H_2O$, 37.5%), 3-aminopropyltriethoxysilane (APTES, 99%), L-ascorbic acid (AA), cytochrome *c* (Cyt *c*) from bovine heart (≥95%), phosphate buffered saline (PBS), sodium nitrite (99.9%), sodium hypochlorite solution (5%), iron(II) perchlorate hydrate (98%), and pyrogallol (≥98%) were purchased from Sigma-Aldrich (Missouri, USA). A polydimethylsiloxane (PDMS) elastomer kit (Sylgard 184) was purchased from Dow Corning Corporation (Michigan, USA). 40 nm Au nanoparticles were purchased from BBI solutions (Cardiff, UK). Dulbecco's modified Eagle medium (DMEM) was purchased from Lonza (Basel, Switzerland). Amplex® Red hydrogen peroxide/peroxidase assay kit was purchased from Thermo Fisher Scientific (California, USA). Sodium dodecyl sulfate was purchased from Duchfa Biochemie (Haarlem, Netherlands) Graphene oxide (GO) was purchased from Graphene Laboratories Inc (New York, USA).

2.2. Au@Pt nanoparticle synthesis

The Au@Pt core@shell nanoparticle was synthesized by decorating small Pt nanoparticles onto a 40 nm Au spherical nanoparticle. Briefly, 10 mL of the 40 nm Au nanoparticle was mixed with 10 mL of deionized water. This mixture was then heated to 100 °C with vigorous stirring. As a reducing agent for Pt ions, AA was added to the above solution based on the desired Pt to Au molar ratio. The molar ratio of AA to H_2PtCl_6 was kept at 5:1 to ensure the complete reduction of Pt ions. A Pt nanoshell on Au cores was then formed by adding Pt precursor solution to make the Au:Pt = 1:1 (molar ratio). The heating was continued for 1 h, and then the mixture was allowed to cool to room temperature.

2.3. Sensor chip fabrication

The glass slides were cleaned in piranha solution (sulfuric acid:hydrogen peroxide = 7:3 v/v) for 60 min and then rinsed with ethanol. The cleaned glass slide was modified with 1 mM APTES, incubated in ethanol for 5 h. The glass was then rinsed with ethanol and dried with N_2 gas. In order to immobilize nanoparticles on the glass, a small aliquot of the nanoparticle solution (~10 μL) was dropped onto the APTES-modified glass slide sensor chip. To confine the reaction solution on the sensor chip, a PDMS well was fabricated. A 10:1 (by weight) mixture of PDMS base/curing agent was poured into a square dish, and degassed under vacuum for 1 h. After curing at 70 °C for 1 h, the solidified PDMS film was cut into a rectangular well. The prepared PDMS well was placed on the nanoparticle-immobilized glass slide, before measurement of dark-field nanospectroscopy.