Journal of Photochemistry and Photobiology B: Biology 133 (2014) 65–72

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Redox heme-proteins mediated fluorescence of CdSe/ZnS quantum dots

Lixia Qin, Luwei He, Congcong Ji, Xiangqing Li, Shi-Zhao Kang, Jin Mu *

School of Chemical and Environmental Engineering, Shanghai Institute of Technology, 100 Haiquan Road, Shanghai 201418, China

ARTICLE INFO

Article history:
Received 3 December 2013
Received in revised form 18 February 2014
Accepted 24 February 2014
Available online 19 March 2014

Keywords:
Redox proteins
Quantum dots
Electrostatic interactions
Spectroelectrochemistry
Fluorescence imaging

ABSTRACT

The redox properties of cytochrome *c* (Cyt *c*), hemoglobin (Hb) and myoglobin (Mb) were studied based on electrostatic interactions between Thioglycolic acid (TGA) capped CdSe/ZnS quantum dots (QDs) and proteins. Results indicated that only Cyt *c* quenched the fluorescence of the QDs at pH > 8.0. Under the optimized conditions, a significant fluorescence recovery of the QDs' system was observed when the reduced form of Cyt *c* incubated with TGA capped QDs, however, the reduced state of Hb and Mb resulted in a more fluorescence quenching on the same size of QDs. Interestingly, the fluorescence changes of QDs-proteins could be switched by modulating the redox potentials of proteins-attached QDs. Moreover, only the oxidized Cyt *c* form was reduced by the generated $O_2^{\cdot-}$ that significantly enhanced the fluorescence of the QDs' system, which was also demonstrated by fluorescence imaging in HeLa cells.

1. Introduction

Heme proteins, such as cytochrome *c* (Cyt *c*), hemoglobin (Hb) and myoglobin (Mb) are the key members of the cellular respiration chain and play a very important role in the pathology, pharmacology and mitochondrial changes in the process of apoptosis. Biological systems also rely on them to carry out versatile biological functions essential for their survival, ranging from electron transfer, ligand binding, catalysis, oxygen transport and storage, signal transduction, and control of gene expression [1–5]. It is well known that Cyt *c*, Mb and Hb have similar electrochemical and spectral behavior in that they have the same porphyrin complex of iron (II)-hemein or iron (III)-hemein. Although many methods have been employed to study the properties of three heme-containing proteins, there is a great demand for systematic studying the redox properties of the three heme-containing proteins.

Semiconductor quantum dots (QDs) have become a well-established photoluminescent platform for biological applications [6–9]. They are advantageous due to their strong and easily tunable luminescence, the flexibility in excitation wavelength, and their commercial availability or easily accessible synthetic routes [10]. The key to successful implementation of new uses depends on the ability to add functionality on the surface of nanoparticles and to stabilize their emission [11]. Many synthetic ways and chemical surface modifications with different capping ligands provide the possibility of effective coupling of QDs surface to biomolecules and the development of hybrid systems [12–14]. As the photoluminescence property of QDs is strongly dependent upon the nature of the surface [15], the interactions between given chemical species and the surface of QDs would have effect on QDs' fluorescence intensity. So far, the ligand-protected QDs have been increasingly explored as optical labels for various sensing biological species, such as cells [16], proteins [17], and DNA [18].

QDs are also highly sensitive to charge transfer, which can alter their optical properties [8,19], thus generating interest in charge-transfer-based biosensing [20]. Recently, the conjugation of QDs to biological molecules has been examined as a means of modulating the behavior of QDs and improving their ability to act as fluorescent probes. For example, QD–dopamine bioconjugates could be used to stain dopamine-receptor-expressing cells for exposing redox-sensitive patterns and confirming the redox interactions of quinones with QDs [21]. Dopamine can behave as an electron donor that can quench or sensitize QDs through different reactive oxygen mechanisms [21,22]. Recently we have demonstrated that coupling system of QDs with redox Cyt *c* is capable of fluorescnece imaging of a superoxide radical with high specificity [23]. Ubiquinone-coupled QDs could also be used for the quantitative detection of Reactive Oxygen Species (ROS) in living cells [24]. Cumulatively, these results confirm a role for redox molecules in redox interactions with QDs. Moreover, the coupling of QDs with redox-active species exhibits the following advantages. First, the redox species involved in the process of electron transfer from the QDs could modulate the photoluminescence of the QDs [25,26]. Second, the redox state of redox-active ligands could be tuned by application of external potential or by introduction of oxidizing/reducing reagent [27]. Third, the photogenerated charges in the QDs could take part in reduction/oxidation reactions with the species present

* Corresponding author. Tel./fax: +86 21 60873061.
E-mail address: mujin@sit.edu.cn (J. Mu).

http://dx.doi.org/10.1016/j.jphotobiol.2014.02.017
1011-1344/

in the nanoparticle shell [28]. Although the possibility of such charge transfer has been established with QDs [29–31], it is still a challenge to investigate the redox properties of QDs-protein bioconjugates by spectroscopic methods.

In this paper, the fluorescence enhancement/quenching of QDs-proteins will be modulated on QDs surface by employing a variety of spectroscopic methods (Scheme 1). Results indicate that a significant fluorescence recovery of the QDs' system was observed when reduced Cyt *c* was incubated with TGA capped QDs; whereas the reduced Hb and reduced Mb resulted in a more fluorescence quenching on the same size of QDs. Interestingly, for three proteins, only the oxidized state Cyt *c* is sensitive to the concentration of $O_2^{\cdot-}$ concentration from 0 to 1.2×10^{-6} mol L^{-1} and an obvious recovery in the fluorescence intensity is observed, which are demonstrated by fluorescence imaging in HeLa cells upon Phorbol Myristate Acetate (PMA) stimulation for the same time.

2. Experimental section

2.1. Reagents

All reagents were of analytical grade, and doubly distilled water was used throughout. Thioglycolic acid (TGA), 2-(dimethylamino) ethanethiol hydrochloride (DMAET), KBH_4 (96%) and selenium powder (99.999%) were purchased from Sigma–Aldrich. Zinc sulfate ($ZnSO_4$, 99%), sodium sulfide (Na_2S, 99%), cadmium chloride hemi (pentahydrate) ($CdCl_2{\cdot}2.5H_2O$, 99%), sodium phosphate monobasic (NaH_2PO_4, 99%), sodium phosphate dibasic (Na_2HPO_4, 99%), sodium hydroxide (NaOH, 99%), Sodium dithionite (80%) and ethanol (99%) were obtained from Aldrich (Milwaukee, WI) and used without purification. N_2 (99.998%, pre-purified) was obtained from Cryogenic Gases (Detroit, MI). Cytochrome *c* (from horse heart), Hemoglobin, Myoglobin and Phorbol Myristate Acetate (PMA) were from Sigma–Aldrich.

2.2. Apparatus

Fluorescence spectra were recorded with a Shimadzu Cary Eclipse (Varian). The pH value of a solution was measured by a PHS-3C (Switzerland Mettler Toledo Delta 320 pH meter). Desktop multi-function centrifugal ultrafilters were used as received (Eppendorf-5430, St. Co. Germany). A CHI660D electrochemical workstation (Shanghai Chenhua Co., Ltd., China) equipped with a stirring machine (CH Instruments Inc.), the three-electrode system consisting of a glassy carbon working electrode, a saturated calomel electrode (SCE) as a reference electrode, and a platinum counter electrode was used for all electrochemical measurements, unless noted otherwise. Ultraviolet–visible absorption spectra were recorded on a Shimadzu UV-3600PC UV–vis–NIR spectrophotometer (Japan). Atomic force microscopy (AFM) images were acquired by using Nanoscope IIIa Multimode AFM with an extender electronics module (Veeco, Santa Barbara, CA). All fluorescence images of cellular QDs were acquired with the same parameters using wide-field inverted fluorescence microscopy (Nikon-Ti, Co. Ltd., Japan) using a 60 × 1.2 NA objective and a matched electron multiplying charge-coupled device (EMCCD) (Roper).

2.3. Synthesis of QDs

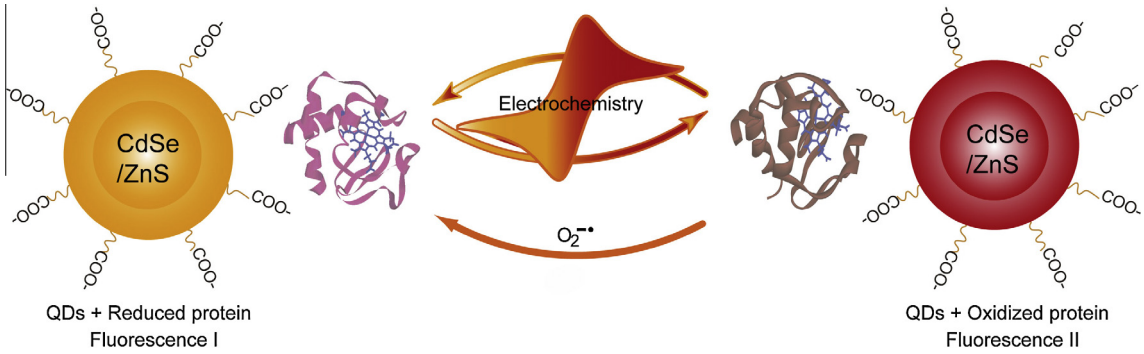
Negatively capped CdSe/ZnS QDs were performed according to the method reported previously [32]. Specifically, in a three necked flask (250 mL) equipped with a reflux condenser, septa, and valves, $CdCl_2{\cdot}2.5H_2O$ (0.2987 g, 1.31×10^{-3} mol) was dissolved in 98.0 mL degassed water. Thioglycolic acid (TGA) (0.118 mL, 1.7×10^{-3} mol) was added; the solution was adjusted to pH 11.2 with aqueous NaOH solution (1 mol L^{-1}), and stirred under argon bubbling at room temperature for 30 min. Then, the clear supernatant NaHSe solution was added under argon and the molar ratio of Cd/TGA/Se was set as 1.2:1.3:0.54, with an initial Cd^{2+} concentration of 13.05×10^{-3} mol L^{-1}. The mixture of the precursor materials turned from colorless to dark orange and was then refluxed to allow the growth of quantum dots. The solution composed of 40 mL of TGA and $ZnSO_4$ (1.25×10^{-3} mol L^{-1}) was quickly injected into 15 mL of prepared CdSe solution, and Na_2S was simultaneously added under vigorous stirring, causing an immediate nucleation and growth of nanoparticles. The pH was then adjusted to 11.2 by l mol L^{-1} NaOH ($Zn^{2+}/Cd^{2+}/S^{2-}$/TGA = 1:0.2:0.4:2.4). The precipitates were centrifuged, washed with water and acetone in sequence, and then dried with nitrogen gas. For the nanocrystal thin-film preparation, the resulting powders were ultrasonically dispersed in ethanol and then filtered to obtain a colloidal solution of CdSe/ZnS nanoparticles, which was kept in a refrigerator at 4 °C.

2.4. Reduction of Cyt c, Hb and Mb

Stock solutions of 1.08×10^{-4} mol L^{-1} oxidized Cyt *c*, Hb and Mb were incubated with sodium dithionite for 5 min at room temperature (1 g of the salt permmolprotein). The excess salt was removed using a NAP 25 column (Amersham Biosciences). The concentrations of reduced Cyt *c*, Hb and Mb were determined by evaluating the absorption at 550 in UV–vis spectra nm prior to use. The solution was stored for a maximum of 24 h at 4 °C.

2.5. Fluorescence spectra of CdSe/ZnS QDs-protein bioconjugates

A set of oxidized and reduced Cyt *c*, Hb and Mb with the constant concentration of 9.82×10^{-6} mol L^{-1} in the presence of TGA or DMAET modified CdSe/ZnS QDs were dissolved in 2 mL of



Scheme 1. Schematic diagram of the reduced and oxidized proteins by spectroelectrochemistry modulation and fluorescence change of the CdSe/ZnS QDs-oxidized protein system in the presence of $O_2^{\cdot-}$.