Available online at www.sciencedirect.com
ScienceDirect
Journal of Colloid and Interface Science 311 (2007) 400–406
JOURNAL OF Colloid and Interface Science
www.elsevier.com/locate/jcis

Spectroscopic studies on the interaction between human hemoglobin and CdS quantum dots

Xing-Can Shen *, Xin-Yan Liou, Li-Ping Ye, Hong Liang *, Zuo-Yuan Wang

College of Chemistry and Chemical Engineering, Key Laboratory of Medicinal Chemical Resources and Molecular Engineering, Guangxi Normal University, 15 YuCai Road, Guilin 541004, People's Republic of China

Received 17 December 2006; accepted 1 March 2007

Available online 11 April 2007

Abstract

The interaction between human adult hemoglobin (Hb) and bare CdS quantum dots (QDs) was investigated by fluorescence, synchronous fluorescence, circular dichroism (CD), and Raman spectroscopic techniques under physiological pH 7.43. The intrinsic fluorescence of Hb is statically quenched by CdS QDs. The quenching obeys the Stern–Volmer equation, with an order of magnitude of binding constant (K) of 10^7. The electrostatic adsorption of Hb on the cationic CdS QDs surface is energetically favorable ($\Delta S^0 = 70.22\ \mathrm{J\,mol^{-1}\,K^{-1}}$, $\Delta H^0 = -23.11\ \mathrm{kJ\,mol^{-1}}$). The red shift of synchronous fluorescence spectra revealed that the microenvironments of tryptophan and tyrosine residues at the $\alpha_1\beta_2$ interface of Hb are disturbed by CdS QDs, which are induced from hydrophobic cavities to a more exposed or hydrophilic surrounding. The secondary structure of the adsorbed Hb has a loose or extended conformation for which the content of α-helix has decreased from 72.5 to 60.8%. Moreover, Raman spectra results indicated that the sulfur atoms of the cysteine residues form direct chemical bonds on the surface of the CdS QDs. The binding does not significantly affect the spin state of the heme iron, and deoxidation is not expected to take place on the coated oxyhemoglobin. The change of orientation of heme vinyl groups was also detected.

Keywords: Hemoglobin; CdS quantum dots; Binding constant; Conformational change; Thermodynamic parameters; Fluorescence spectroscopy; CD spectroscopy; Raman spectroscopy

1. Introduction

Fluorescent semiconductor nanocrystals, also known as quantum dots (QDs), offer significant advantages over conventional organic fluorophores in terms of high luminescence, continuous excitation spectrum, stability against photobleaching, and controllable and narrow emission bands [1]. Over the past few years, QDs have been tested as luminescent probes for labeling of cells and tissues [1–3], targeting the image of surface proteins [4], immunostaining of membrane proteins [5], hybridization on chromosomes [6], and detecting single DNA molecules [7]. These studies have shown the potential biological applications of QDs; however, there are questions about their practical applications as well as their effectiveness in biological systems. The physiological effect of QDs remains a major concern to human health [8,9], and one of the unanswered questions centers on the mechanism of interaction between QDs and biomacromolecules.

Cadmium sulfide (CdS) QDs are well known as typical fluorescence semiconductor materials used in biological labeling experiments [1]. The biocompatibility of CdS QDs must be critically examined for their inherent chemical composition, as well as the unknown physiological effect due to their size. A basic study of the interaction between biomacromolecules such as DNA and proteins and CdS QDs could contribute to the elucidation of the mechanisms involved in the biocompatibility of fluorescent QDs; however, the studies have been rather limited [10–12].

Human adult hemoglobin (Hb), the major protein component in erythrocytes, exists as a tetramer of globin chains that is composed of two α and two β subunits; the relationship between the structure and the function of Hb is well established [13–15]. Research on its interaction with other molecules

* Corresponding authors. Fax: +86 773 583 2294.
E-mail address: xcshen@mailbox.gxnu.edu.cn (X.-C. Shen).

0021-9797/$ – see front matter
doi:10.1016/j.jcis.2007.03.006

is almost limited to a few small molecules, such as dimethyl sulfoxide [16], anionic amphiphiles [17], Ln^{3+} ions [18], and hematoporphyrin [19]. Since Hb is an important functional protein for reversible oxygen carrying and storage, as well as a mode protein with high α-helical content, the potential changes of conformation and function for Hb after binding of nanoparticles have been a focus of study. The adsorption of Hb on ultrafine inorganic particles, such as silica, zirconia, and titania, has been reported [20,21]. Our earlier work has examined the interaction between bovine Hb and Ag nanoparticles [22]; this is now extended to the interaction between Hb and CdS QDs. The fluorescence quenching mechanism, binding constant, thermodynamics parameters, and conformational changes of Hb during the binding process are reported in the present paper.

2. Experimental procedures

2.1. Reagents

Human adult Hb (Sigma Chemical Co.) was used without further purification, and was dissolved in doubly deionized water that had been adjusted to a pH of 7.43 by the addition of 0.01 M phosphate buffer (KH_2PO_4–K_2HPO_4). The concentrations of Hb solutions were determined on the basis of the molar extinction coefficient $\varepsilon_{406} = 41{,}000\ M^{-1}\,cm^{-1}$ [15]. All other chemicals used were of analytical reagent grade.

2.2. Preparation and characterization of CdS QDs

Water-soluble CdS QDs were synthesized according to the literature [23]; 10 mL of 0.1 M $CdCl_2$, 10 mL of 0.1 M thioacetamine (TAA), and 10 mL of 0.1 M sodium hexametaphosphate were dissolved in 180 mL H_2O. The pH of the mixture was adjusted to 10.4 by 0.1 M NaOH, under N_2. The mixture was kept at room temperature for 35 min for the growth of CdS QDs. The resulting yellow colloid was diluted to a concentration of $[Cd] = 1.0 \times 10^{-4}$ M (the concentration of CdS QDs was approximately 4.99×10^{-7} M; supplementary material), and was stored at 4 °C.

Transmission electron microscopy (TEM) images of CdS QDs were measured on a Tecnai G^2 20 transmission electron microscope (FEI, Netherlands). The images showed that the average diameter of the CdS QDs was about 9.1 ± 0.5 nm (Fig. 1). The absorption and luminescence spectra of CdS QDs were recorded on Cary-100 UV (Varian, USA) and RF-5301 (Shimadzu, Japan) spectrometers, respectively. The spectra showed characteristic peaks at 410 nm, and a comparatively strong and sharp band-edge emission peak about 450 nm, with a quite weak and a flat trap-state emission peak around 570 nm (supplementary material). The X-ray powder diffraction (XRD) pattern was obtained on a D/Max–RC X-ray diffractometer (Rigaku, Japan), and it showed zinc blende phase (supplementary material). The ζ potential of CdS QDs was measured by the electrophoretic light-scattering method by using a Nano-ZS90 ζ potentiometer (Malvern, the UK). The ζ potential of CdS QDs was 0.01 mV, which indicated the existence of slightly excessive Cd^{2+} ions on the surface of the bare CdS QDs.

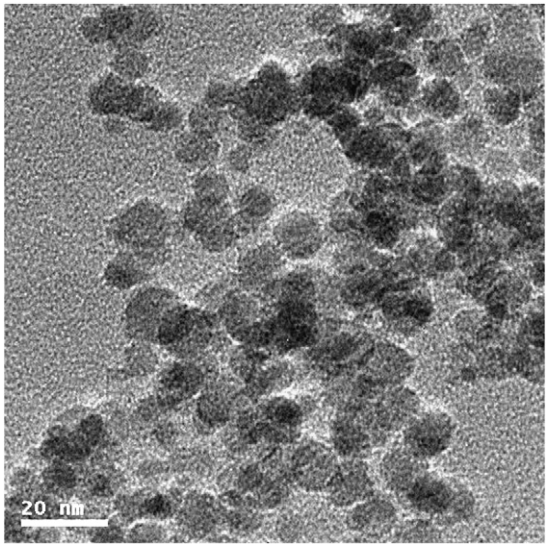

Fig. 1. TEM image of CdS QDs.

2.3. Methods

In spectroscopic experiments, the Hb solution was introduced by the CdS QDs colloid at a certain concentration. The incubation time was about 2 h in order to attain binding equilibrium.

2.3.1. Fluorescence spectra

The fluorescence spectra were recorded on a RF-5301 spectrofluorometer (Shimadzu, Japan) with a 150 W xenon lamp and 1-cm quartz cells. The slits for excitation and emission widths were both 5 nm. The intrinsic fluorescence spectra of Hb were recorded at 300–400 nm at an excitation wavelength of 280 nm [24]; 3 mL of the 9.77×10^{-5} M Hb solution and an appropriate volume of the CdS QDs colloid solution were placed into a 10-mL volumetric flask. The mixture was diluted to 10 mL with phosphate buffer. All measurements were performed at 295, 300, 305, and 310 K.

2.3.2. Synchronous fluorescence spectra

The synchronous fluorescence spectra were recorded by scanning simultaneously the excitation and emission wavelength at 80- or 30-nm intervals [25].

2.3.3. Far-UV CD spectra

The far-UV CD spectra were measured by a Jasco J-810 sepectropolarimeter using a 0.1-mm quartz cell. The CD spectra of Hb solutions containing CdS QDs were recorded from 185 to 250 nm. Corresponding absorbance contributions to CdS QDs colloid and buffer were also measured and were subtracted with the same instrumental parameters.

2.3.4. Raman spectra

The Raman sample was a mixture of Hb and CdS QDs, and the Hb of the same concentration in the absence of CdS QDs was used for comparison. The Raman spectra were measured with an inVia Raman microscope (Renishow, UK), equipped