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Highly selective manganese-doped zinc sulfide quantum dots based label free phosphorescent sensor for phosphopeptides in presence of zirconium (IV)

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ABSTRACT

We report a room-temperature phosphorescence (RTP) sensor for phosphopeptides based on zirconium (IV)-modulated mercaptopropionic acid (MPA)-capped Mn-doped ZnS quantum dots (QDs). This sensor incorporates the advantages of the well-known Zr^{4+}-phosphopeptide affinity pair and the RTP properties of doped QDs. The RTP of Mn-doped ZnS QDs capped with MPA can be effectively quenched by Zr^{4+}. The high affinity of phosphopeptides to Zr^{4+} enables the dissociation of the ion from the surface of MPA-capped ZnS QDs, thereby forming a stable complex with phosphopeptides in the solution, and recovering the RTP of the QDs. The Zr^{4+}-induced RTP quenching and subsequent phosphopeptide-induced RTP recovery for MPA-capped ZnS QDs provide a solid basis for the present RTP sensor based on QDs for the detection of phosphopeptides. The detection limit for phosphopeptides is 0.9 ng mL^{-1}, the relative standard deviations is 2.5%, and the recovery of urine and serum samples with phosphopeptides addition rangs from 96% to 105% at optimal conditions. The proposed method was successfully applied to biological fluids and obtained satisfactory results.

1. Introduction

Sensors based on quantum dots (QDs) for chemical and biological detections have gained considerable attention over the past decade (Gill et al., 2008; Katz and Willner, 2004; Rosi and Mirkin, 2005; Yue et al., 2013) because of the unique properties exhibited by a variety of nanomaterials in conjunction with natural or artificial molecular recognition units (Ren and Chen, 2014; Song et al., 2014; Zhang et al., 2012). In comparision with bulk materials or traditional organic dyes, QDs exhibit excellent optical properties, such as high quantum yield, tunable size-dependent emission, resistance to photobleaching, and narrow emission peaks, which have gained significant interest in fundamental research and technical applications (Wu and Yan, 2010). The modification of QDs with biomolecules (e.g. enzyme, DNA, antibody/antigen, aptamer, and avidin/biotin) (Alivisatos, 2003; Costa-Fernández et al., 2006) and metal ions (Han et al., 2009; Shang et al., 2009) has emerged as an important field in sensor/biosensor applications (He et al., 2008; Wang et al., 2009a; Wu and Fan, 2012). Compared to the fluorescence, the phosphorescence of doped-QDs demonstrates longer average life, allowing an appropriate delay time to prevent fluorescence emission and light scattering (Wang et al., 2009a).

The chemistry of metal (IV) and phosphate/phosphonate has been studied over the past three decades. The coordination of the phosphate/phosphonate group to metal (IV), particularly Zr^{4+}, has now been extensively illustrated in the literature (Zhou et al., 2013). The high affinity of Zr^{4+} to phosphopeptides has been recognized and well explored in metal ion affinity chromatography for the separation and purification of phosphopeptides (Hsu et al., 2009; Li et al., 2007; Zhou et al., 2013). Zirconia-coated magnetic nanoparticles (Lo et al., 2007), zirconium dioxide microtips (Kweon and Håkansson, 2006), Zr^{4+} ions immobilized on the surface of Fe_3O_4 magnetic nanoparticles can effectively enrich phosphopeptides from complex samples. The unique coordination of Zr^{4+} with the phosphate/phosphonate group implies the potential application of the Zr^{4+}-phosphopeptide affinity pair in modern nanobiotechnology. We hypothesized that Zr^{4+} ions adsorbed on the surface of ZnS QDs exhibit similar capability to selectively bind to phosphopeptides; this binding can affect the room-temperature phosphorescence (RTP) intensity of the QDs.

Protein phosphorylation is important in regulating many cellular processes in eukaryotes and prokaryotes (Hunter, 1995). The deregulated signaling of phosphorylated protein/peptide is a hallmark of cancer and neurodegenerative diseases (Johnson and Lewis, 2001). Hence, the detection of phosphorylated protein is

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clinically important (Macek et al., 2009). Research on protein phosphorylation level is mostly based on detection of phosphopeptides (Kerman et al., 2007). The most commonly used methods include mass spectrometry (Garcia et al., 2005), fluorescent-labeled (Kawai et al., 2004; Li et al., 2015), matrix-assisted laser desorption/ionization mass spectrometry (Ho et al., 2014), radiolabeled methods (Houseman et al., 2002; Tuerk et al., 2009), surface plasmon resonance (SPR) (Chen and Chen, 2011; Lin et al., 2006; Stenlund et al., 2006) and fluorescence resonance energy transfer-based systems (Allen et al., 2006; Chen et al., 2004). However, these methods mainly focus on the enrichment and sequence indentification of phosphopeptides, and require expensive equipment and complicated procedures to preconcentrate phosphopeptides from the samples. Few studies have focused on the direct detection of phosphopeptides in biological fluids despite its clinical significance. Several fluorescent phosphopeptide sensors have been reported. Nevertheless, utilization of the Zr^{4+}-phosphopeptide affinity pair in conjunction with optically active nanomaterials has not been reported for phosphopeptide sensor designs.

This study demonstrates a RTP sensor based on Zr^{4+}-quenched RTP of MPA-capped Mn-doped ZnS QDs for the selective detection of phosphopeptides. Zr^{4+} ions exhibit a strong binding ability to the surface of MPA-capped Mn-doped ZnS QDs and a quenching effect on the RTP of the QDs because of electrostatic adsorption (Costa-Fernández et al., 2006). The high affinity of phosphopeptides to Zr^{4+} ions can selectively separate the ions from the surface of the QDs and recover the RTP of the QDs. The RTP of the QDs is first quenched by Zr^{4+} ions and then selectively recovered by phosphopeptides. The possible scheme was illustrated in Scheme 1. The developed QDs-based RTP sensor functions in a turn-on mode and provides high selectivity for phosphopeptides. The proposed method was successfully applied to determine phosphopeptides in biological fluids and obtained satisfactory results. This method is selective and facile, and exhibits high sensitivity.

2. Materials and methods

2.1. Materials and chemicals

MPA, $ZnSO_4 \cdot 7H_2O$, $Na_2S \cdot 9H_2O$, and $MnCl_2$ (Tianjing Kermel Chemical Reagent Co., China) were used to prepare MPA-capped Mn-doped ZnS QDs. Tris(hydroxymethyl)aminomethane (Tris) was purchased from Tianjing Kermel Chemical Reagent Co., China. All chemicals used were of analytical reagent grade. Ultrapure water (18.2 MΩ cm) was obtained using a Water Pro water purification system (Labconco Corporation, Kansas City, MO). Phosphopeptides were provided by GL Biochemistry Ltd., Shanghai, China.

2.2. Instrumentation

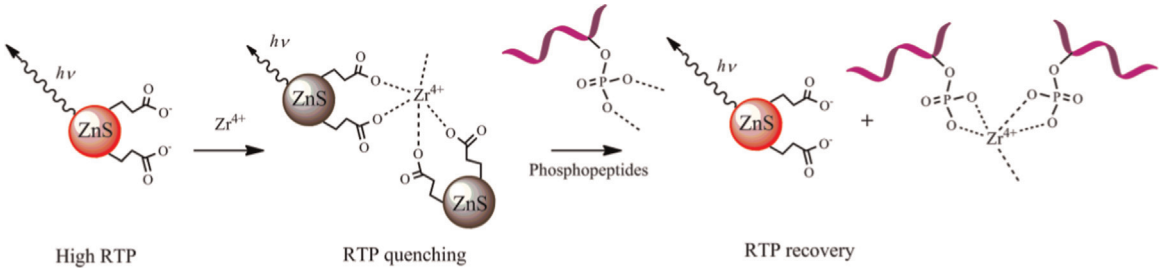
A Cary Eclipse phosphorescence spectrophotometer with an excitation wavelength at 295 nm (Varian, American) and equipped with a plotter unit and a quartz cell (1.0 cm × 1.0 cm) was used in this study. The slit widths of excitation and emission were 10 and 20 nm for phosphorescence mode with an excitation wavelength of 295 nm and emission wavelength of 590 nm. UV–visible absorption spectra were acquired using a UV-4100 spectrophotometer (Shimadzu, Japan). The QDs were characterized with a JEM-2100 (JEOL, Japan) transmission electron microscope (TEM) and a D8 Advance (Bruker, Germany) X-ray diffractometer (CuKα). The samples for TEM were obtained by drying the sample droplets from water dispersion onto a 100 mesh Cu grid coated with a lacey carbon film. The grid was then dried prior to imaging. The Zr^{4+}-induced aggregation of the QDs was characterized using a JSM-7500F (JEOL, Japan) scanning electron microscope (SEM). Resonance light scattering (RLS) spectra were recorded using the same spectrofluorometer by simultaneously scanning the excitation and emission monochromators ($\Delta\lambda=0$) from 200 nm to 700 nm.

2.3. Synthesis of aqueous MPA-capped Mn-doped ZnS QDs

MPA-capped Mn-doped ZnS QDs were prepared in our laboratory via previously described procedures (Gong and Fan, 2014; Wu et al., 2010; Zhuang et al., 2003) with minor modifications. The whole process was carried out at deoxygenated condition in an argon atmosphere. Briefly, 100 mL of 0.04 M MPA, 10 mL of 0.1 M $ZnSO_4$, and 4 mL of 0.01 M $MnCl_2$ were sequentially added into a 250 mL three-necked flask, and pH was adjusted to 11 with 1 M NaOH. After stirring at room temperature for 40 min, 10 mL of 0.1 M Na_2S was rapidly injected into the solution with deoxygenation to allow the nucleation of the nanoparticles. The mixture was stirred for another 30 min, and the solution was aged at 50 °C under air for 3 h to form MPA-capped Mn-doped ZnS QDs. The QDs were precipitated with the same volume of ethanol, centrifuged at 4000 rpm for 10 min, washed with ethanol three times to remove unreacted MPA, and dried in a vacuum. The prepared QD powder is highly soluble in water.

2.4. Analytical procedures

To determine the quenching effect of Zr^{4+} on the RTP of MPA-capped Mn-doped ZnS QDs, we sequentially added 500 μL of Tris–HCl buffer solution (pH 5.0, 0.02 M), 50 μL of MPA-capped Mn-doped ZnS QDs (2 mg mL^{-1} dissolved in ultrapure water), and a series of different volumes of Zr^{4+} solution (0.5 μg mL^{-1} dissolved in ultrapure water) to a 10 mL calibrated test tube. The mixture was diluted to 10 mL with ultrapure water. RTP intensity was recorded every minute for a total of 10 min to observe the time course of RTP quenching with the following steps. To detect the phosphopeptides, we mixed solutions containing 500 μL of Tris–



Scheme 1. Schematic illustration of the developed RTP sensor for phosphopeptides based on MPA-capped Mn-doped ZnS QDs modulated with Zr^{4+}.