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A highly sensitive upconverting phosphors-based off–on probe for the detection of glutathione

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ABSTRACT

At present, glutathione (GSH) is a very important biomarker in vivo. However, the sensitive and selective assay for the detection of GSH in biological matrix still keeps challenging due to the high complexity of samples. Lanthanide-doped upconverting phosphors (UCPs) which exhibit unique near-infrared (NIR) excitation nature are able to overcome interferences from complex samples. In this study, a novel probe based on the linkage of UCPs and dopamine-quinone through hydrogen bonding and electrostatic interaction has been designed for rapid, economic, sensitive and selective detection of GSH in aqueous solution and human blood serum. Here, dopamine-quinone served as an efficient quencher for upconverting fluorescence, while GSH as a strong reducing agent chemically reduced the dopamine-quinone turning on the fluorescence. The fluorescence recovery was found to be proportional to GSH concentration within the range from 1 to 75 μM. The detection limit of this sensor was 0.29 μM, which was quite competitive for GSH detection. The simple, sensitive and selective fluorescence method took full advantages of UCPs properties of low interferences and broadened the application scope of UCPs in complex biological detection.

1. Introduction

Glutathione, a thiol-containing tripeptide (γ-Glu-Cys-Gly), is known as the primary cellular thiol and has a pivotal role in maintaining redox homeostasis. It acts as an antioxidant, keeping the cysteine thiol group in proteins in the reduced state and protecting cells from free radicals and reactive oxygen species [1]. Besides, GSH levels are implicated in many diseases, such as leukocyte loss, psoriasis, liver damage, cancer and HIV [2–4]. Thus, it is of great importance to detect GSH qualitatively. Up-to-date, many strategies have been documented for the quantification of GSH in the literatures including colorimetric method [5], electrochemistry [6,7], enzymatic method [8], spectrofluorimetry [9,10], and newly developed high performance liquid chromatography (HPLC) [11]. However, due to the rather low level and high complexity of the clinical samples such as serum, plasma, or even the whole blood, it was still imperative to develop analytical methods with high sensitivity, great simplicity and potential ability to circumvent the interference arising from the complex biological sample matrixes.

The last few years have seen the emergence and rapid development of a new class of nanoparticles termed upconverting phosphors (UCPs) which are excitable with NIR lights [12]. The NIR-excitation nature endows UCPs with remarkable merits of eliminating interferences from auto-fluorescence and scattered light, enabling bioassays with low background fluorescence signals thus enhancing the signal-to-noise ratio and sensitivity. UCPs are lanthanide-doped inorganic phosphors which exhibit unique luminescent properties compared with quantum-dots and organic dyes, including the ability to convert longer wavelength radiation to shorter wavelength fluorescence via a two-photon or multi-photon mechanism, a large anti-stokes shift, narrow emission peaks, good chemical stability and photostability, and low toxicity [13–19]. In addition, even optically challenging sample materials, such as blood serum, are compatible with the UCPs technology due to the possibility to exploit far-red and NIR wavelength (>650 nm) where even the red-colored blood is practically transparent [20,21]. These notable advantages have hence led to increasing applications of UCPs in bioassays. Wang and coworkers have constructed several upconversion-FRET biosensors for the determination of various biomolecules in clinical samples [22–24]. Considering the above advantages of UCPs, upconverting nanocrystals show promising prospects in biosensing and bioassays.

Hence, UCPs were used to detect GSH in human blood serum here. To detect GSH selectively and sensitively, GSH should react with the probe directly, rapidly and stoichiometrically. So, apparent, if not dramatic, spectroscopic property changes should be produced for easy monitoring [9]. Rightly, the off/on sensing systems matched the conditions well in which fluorescence was

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firstly quenched and then restored when target substances were appeared. The back-ground signal was expected to be minimal due to the off/on mechanism that a larger quenching efficiency will lead to higher detection sensitivity in quantitative assays. Based on this mechanism, Ai and coworkers have wisely developed a quantum dot-based nanosensor to detect GSH [25]. Inspired by these pioneers and combined the features of UCPs, in this study, we developed a new off/on probe for the detection of GSH selectively and sensitively. The probe was designed with dopamine (DA), UCPs and GSH. Dopamine-quinone was linked to the UCPs surface via hydrogen bonding and electrostatic interaction resulting in UCPs quenching (off state) through electron transfer process. When GSH was introduced, it reduced dopamine-quinone and restored UCPs fluorescence (on state) by inhibiting electron transfer. This probe connected inorganic nanoparticles and bioassays together, which took full advantages of UCPs properties broadening the application scope of UCPs in complex biological detection.

2. Experimental

2.1. Chemicals

All reagents were of analytical grade and used as received without further purification. Polyacrylic acid (PAA, Mw = 800) was from Tianjin Kemiou Co. Ltd. (Tianjin, China). Dopamine hydrochloride was the product of Adamas Reagents Co. Ltd. L-Glutathione (reduced) was obtained from Aladdin. The rest reagents were purchased from Chengdu Huatao Chemical Reagent Co. Ltd. (Chengdu, China). The human serum samples were collected from Huaxi Hospital (Chengdu, China). Pure water (18.2 MΩ cm^{-1}) from a water purification system (ULUPURE, Chengdu, China) was used throughout this work.

2.2. Apparatus

The crystal phase of $NaYF_4$:Yb, Er nanoparticles were characterized by a Philips Analytical Netherlands X-ray diffractometer (XRD) with a 2θ range from 10° to 85° at a scanning rate of 4°/min, with Co Kα irradiation (κ = 1.78897 Å). The morphologies of PAA modified UCPs were identified by a scanning electron microscope (SEM, JSM-5900LV, Japan) with an acceleration voltage of 200 kV. Fourier transform infrared spectra (FT-IR) of PAA-UCPs were measured on a Nicolet IS10 FT-IR spectrometer (Thermo Inc., America) with the KBr pellet technique. Zeta potential measurements were conducted on a Nano-ZS analyzer (Malvern Instruments Ltd., UK). The UV–vis absorption spectra in the region of 200–800 nm were recorded on a U-2910 UV–vis spectrophotometer (Hitachi, Tokyo, Japan). A 980 nm diode continuous-wave (CW) laser (Beijing Viasho Technology Co., Ltd.) was used as the excitation source, with the power being set at 230 mW for all upconverting experiments. The upconversion fluorescence spectra and intensities were recorded on an F-7000 spectrophotometer (Hitachi, Tokyo, Japan) with the emission silt being set at 10 nm. Throughout this work, the samples were excited with a 980 nm CW laser and the emission intensity at 550 nm was taken for quantification.

2.3. Preparation of PAA modified UCPs

The water-soluble PAA-UCPs were synthesized with a one-pot hydrothermal method according to the literature [23,26]. In brief, to acquire $Ln(NO_3)_3$ (Ln = Y, Yb, Er), 0.25 mmol of lanthanide oxides Ln_2O_3 (Y/Yb/Er = 0.78:0.2:0.02, mole-to-mole ratio) was dissolved in hot nitric acid (65 °C) which was evaporated after 6 h reaction. The as-obtained nitrate salts were added to the solution in which 900 mg PAA was introduced and then another aqueous solution containing 0.21 g NaF was added dropwise under vigorous stirring. Both PAA and NaF were dissolved in a mixed solvent ($V_{ethanol}/V_{water}$ = 1.0). Then, the mixture was transferred into a 50 mL Teflon autoclave and heated at 180 °C for 24 h hydrothermal treatment. A precipitate was obtained after the naturally cooling of the Teflon autoclave to room temperature which was then centrifuged and washed with ethanol and water for several times, respectively. The product was dried under vacuum before use.

2.4. Detection of GSH in aqueous solutions and human serum

Dopamine was attached to UCPs surface with hydrogen bonding (such as N—H. . .O and O—H. . .O hydrogen bonds) and electrostatic interaction [27,28]. In brief, a mixture of dopamine (36.6 mM) and UCPs (0.8833 mg/mL) was vigorously stirred at room temperature for 3 h in alkaline solution (pH 7.0–8.0). The as-obtained black solution was washed with water for several times by centrifugation at 8000 rmp to remove excess dopamine. Thus, a black precipitation (UD) was formed by the linkage of dopamine-quinone to UCPs and then dissolved in water.

GSH assay was first performed in an acetate acid (0.1 M, pH 7.0) solution. In a typical measurement, UD containing 0.8833 mg/mL UCPs was added to GSH solutions with different concentrations. The mixtures were then incubated at 50 °C for 15 min before upconverting fluorescence measurements. For the sensing of GSH in human serum samples, a procedure identical to that in the aqueous solutions was employed except that GSH was prepared with 100-fold diluted (0.1 M, acetate acid, pH 7.0) human serum. Fluorescence emission of UD was recorded under excitation at 980 nm with a CW laser.

3. Results and discussion

3.1. Characterization of UCPs

The morphologies of the as-synthesized PAA-coated UCPs were characterized by SEM. The SEM image in Fig. 1A showed that the PAA modified UCPs were spherical in shape and possessed an average diameter of ca. 60 nm. The crystal phases of UCPs were revealed by the XRD pattern. As demonstrated in Fig. 1B the obtained UCPs were comprised of hexagonal and cubic phase. The peaks in the FT-IR spectrum in Fig. 1C at about 1718 cm^{-1} and 3422 cm^{-1} indicated the existence of C═O and the OH stretching mode. In a period of one month, no obvious fluorescence change was observed for the UCPs. The fluorescence of the obtained UD kept quenching from pH 7.0 to 12.0 as shown in Fig. 1D.

3.2. UCPs fluorescence quenching induced by dopamine

UCPs and dopamine were linked by hydrogen bonding and electrostatic interaction leading to the quenching of UCPs fluorescence. Fig. 2A displayed the typical fluorescence spectra as a function of the concentration of dopamine from 10.98 to 109.8 mM. With a fixed amount of UCPs (0.8833 mg/mL), the fluorescence intensity reduced gradually with increasing amounts of dopamine and a maximum overall quenching efficiency of 98% at 109.8 mM was achieved. According to Radu et al. [29], the isoelectric point of dopamine was 9.7, which meant the dopamine was positively charged under our experimental conditions. A significant increase of the zeta potential from −20.3 of UCPs to 9.01 of UD, as shown in Fig. 2B, could attribute to the addition of dopamine, with a predominant electrostatic interaction between UCPs and dopamine. The formation of UD can be confirmed by the UV spectra as shown in Fig. 2C. Compared with the spectra of UCPs, a new peak appeared at 280 nm which was the characteristic absorption peak of dopamine, sufficiently proving the successful linkage between UCPs and dopamine. On the other hand, there were —COOH on the