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Upconversion ratiometric fluorescence and colorimetric dual-readout assay for uric acid

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ABSTRACT

A new upconversion colorimetric and ratiometric fluorescence detection method for uric acid (UA) has been designed. Yb^{3+}, Er^{3+} and Tm^{3+} co-doped $NaYF_4$ nanoparticles (UCNPs) was synthesized. The co-doped $NaYF_4$ nanoparticles, emit upconversion fluorescence with four typical emission peaks centered at 490 nm, 557 nm, 670 nm and 705 nm under the 980 nm near-infrared (NIR) irradiation. The $ZnFe_2O_4$ magnetic nanoparticles (MNPs) possessing excellent peroxidase-like activity was prepared and used to catalyze oxidation the coupling of N-ethyl-N-(3-sulfopropyl)-3-methylaniline sodium salt (TOPS) and 4-amino-antipyrine (4-AAP) in the presence of H_2O_2 to form purple products (compound 1) which has a characteristic absorption peak located at 550 nm. The upconversion fluorescence at 557 nm was quenched by the compound 1 while the upconversion emission at 705 nm was essentially unchanged, the fluorescence ratio $((I_{557}/I_{705})_0/(I_{557}/I_{705}))$ is positively proportional to UA concentration in existence of uricase. More importantly, colorimetric signal can be easily observed and applied to directly distinguish the concentration of UA by the naked eye. Under the optimized conditions, the linear range of colorimetric and ratiometric fluorescence sensing towards UA was 0.01–1 mM, the detection limits were as low as 5.79 μM and 2.86 μM (S/N=3), respectively. The proposed method has been successfully applied to the analysis of UA in human serum. These results indicate that the colorimetric and ratiometric fluorescence dual-readout assay method has great potential for applications in physiological and pathological diagnosis.

1. Introduction

Uric acid (UA) is an end product of purine metabolism (Álvarez-Lario and Macarrón-Vicente, 2010) and it is a very important biological molecule in body fluids, which can be used as an indicator in diagnosis including leukemia and pneumonia (Luo et al., 2006). The uric acid concentration of abnormal in serum is an indication for patients experiencing hypertension, proteinuria, gout, hyperuricemia or Lesch-Nyan syndrome (Wang et al., 2011). So an sensitive, selective, and quantitative analytical method is urgently needed for low concentrations of UA detection. Higher concentration of UA (> 0.4 mM) is closely related to severe pre-eclampsia (Buhimschi et al., 2005). Hence, it is an urgent requirement of monitoring UA in human serum for disease diagnosis. A variety of analytical methods for detecting UA in human serum and urine have been reported, such as optical methods (Huang et al., 2004; Kashkarov et al., 2015; Petra and Wolfbeis, 2008), electroanalysis (Roohollah et al., 2006), high performance liquid chromatographic (HLPC) (Wang et al., 1987). Among most of the mentioned methods, optical method possesses a series of advantages, such as low cost, time-saving, facile operation, good reproducibility, and therefore it is considered to be an ideal method for UA detection.

There are mainly two types of optical analytical methods for UA detection, including colorimetric/UV–*vis* absorption method and fluorescence (FL) method. The color-change based colorimetric detection observed by the naked eye has been attracted considerable attention because of the simplicity, convenience, practicality, low cost (Vladimir et al., 2012; Wang et al., 2009). Because both qualitative and semiquantitative assessment can be performed in real time without using any complicated and expensive instruments, the colorimetric detection is particularly important in the field of point-of-care test. To this end, the horseradish peroxidase (HRP) has been used as an important colorimetric analysis for the detection of biomolecules (Gao et al., 2011; Liu et al., 2015;

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Wang et al., 2014). The HRP could catalyze the oxidation of peroxidase substrates including 3,3′,5,5′-tetramethylbenzidine (TMB) (Gao et al., 2014; Goff et al., 2011; Su et al. 2007), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Mubarak et al., 2011) and *o*-phenylenediamine (OPD) (Aumiller Jr. et al., 2014) by H_2O_2 to produce typical color reaction. However, natural peroxidases as proteins suffer some disadvantages such as low stability due to easily being denatured in complex conditions, being digested by proteases, being expensive and difficult to prepare and purify (Lin et al., 2014a). Therefore, much attention has been focused on developing efficient peroxidase mimetics with good stability to overcome these drawbacks. Recently, Gao *et. al.* reported Fe_3O_4 nanoparticles (NPs) with an intrinsic enzyme mimetic activity, which is similar to that of natural peroxidases (Gao et al., 2007). Subsequently, a variety of inorganic nanomaterials with peroxidase-like activity came into sight, such as CeO_2 NPs (Talib et al., 2010), Co_3O_4 NPs (Mu et al., 2012), MoS_2 nanosheets (Lin et al., 2014b), AuNPs (Yun et al., 2010), carbon nitride nanosheets (Lu et al., 2015), and silicon nanomaterials (Chen et al., 2014). Compared with natural peroxidases, these peroxidase mimetics have the advantages of simple synthesis, low cost, improved stability, ease of storage and high catalytic activity. Recently, Su's group (Su et al., 2012) reported the $ZnFe_2O_4$ magnetic nanoparticles (MNPs) possessing an intrinsic enzyme activity similar to HRP. They limited to utilize $ZnFe_2O_4$ to design a colorimetric probe to glucose, however, the FL-based method for sensing has not been developed. In this work, $ZnFe_2O_4$ MNPs were synthesized and confirmed to possess an intrinsic enzyme activity similar to HRP. If the $ZnFe_2O_4$ MNPs serve as a substitute for HRP, it will be very meaningful in developing a dual-readout assay method.

Another optical method is fluorescence method, which exhibits high sensitivity and low background. Nur Ellina *et. al.* reported a simple and sensitive FL biosensor for the determination of UA using H_2O_2-sensitive quantum dots/dual enzymes (Kashkarov et al., 2015). Subsequently, Jin *et. al.* reported quantitative determination of UA using CdTe nanoparticles as FL probes (Jin et al., 2016). However, most of these reported fluorescence methods for detecting UA are based on the change in single fluorescence intensity of fluorophore, which may be susceptible to instrumental efficiency, operation and measurement condition. In contrast, ratiometric FL methods based on the ratio of the dual fluorescence intensities can alleviate most of the ambiguities by self-calibration of two or more different emission bands (Domaille et al., 2010; Goel et al., 2015; Haidekker et al., 2006; Shynkar et al., 2007; Wu et al., 2009). In addition, if the ratiometric sensor can provide multiple emissions with different color, the perceived color changes will be useful not only for the ratiometric sensing but also for rapid visual identification (Haidekker et al., 2006; Shynkar et al., 2007). Thus, it is still of high demand to design simple and efficient fluorescent nanoprobes for the biosensing with low fluorescence background and alleviating deviation. In recent years, lanthanide-doped upconversion nanoparticles (UCNPs) have received considerable attention for applications in sensing due to their several outstanding features such as low toxicity, greater tissue penetration, high chemical stability, and reducing excitation light scattering, all of which have obvious advantages over quantum dots and fluorescent dyes (Chatterjee et al., 2010; Fischer et al., 2011; Gnach et al., 2015; Li et al., 2012; Tang et al., 2013; Yang et al., 2012). More importantly, upconversion FL is an anti-Stokes' emission process, which generates shorter wavelength emission under longer wavelength excitation (typically 980 nm). The NIR excitation source is not be absorbed by biological samples in the detection process (Liu et al., 2011) and can prevent background autofluorescence, photobleaching, and photodamage (Wang et al., 2013; Zhou et al., 2014). If UCNPs can emit upconversion FL with multi typical emission peaks under excitation at 980 nm, it could be used to construct efficient ratiometric FL sensors for biomolecules, which will provide amplified signal-to-noise ratios and improved sensitivities. To the best of our knowledge, combining UCNPs and $ZnFe_2O_4$ MNPs has not been reported based on ratiometric fluorescence and colorimetric for detecting UA.

In this work, we synthesized Yb^{3+}, Er^{3+}, and Tm^{3+} ions co-doped $NaYF_4$ UCNPs, which can be excited at 980 nm and emit upconversion FL with multi typical emission peaks centered at 490, 557, 670 and 705 nm. We prepared $ZnFe_2O_4$ MNPs possessing excellent intrinsic peroxidase-like catalytic ability and combined the distinct advantages of UCNPs to develop a new ratiometric FL and colorimetric sensing platform for UA. Based on the peroxidase mimetics of $ZnFe_2O_4$ MNPs, catalytic oxidation coupling of TOPS with 4-AAP in the presence of uricase and UA to form purple products (1-proanaminium,-N-[4-[(2,3-dihydro-1,5-dimethyl-3-oxo-2-phenyl- 1H-pyrazol-4-yl) imino]-2,5-cyclohexadien-1-ylidene]-N-ethyl-3-sulfo-, sodium salt; compound 1). The absorption band of the compound 1 was centered at 550 nm which fully covered the emission band of UCNPs at 557 nm and resulted in FL quenching of UCNPs. The compound 1 has no absorbance in 705 nm and the FL intensity at 705 nm of UCNPs still remains unchanged with the increasing concentration of UA. Therefore, the ratio between the FL intensity at 557 nm and that at 705 nm $((I_{557}/I_{705})_0/(I_{557}/I_{705}))$ can be used to quantitatively detect UA. On the other hand, colorimetric signal can be easily observed by the naked eye and applied to directly distinguish the concentration of UA. The designed double signal system has been successfully used in developing upconversion sensors for selective and sensitive detection of H_2O_2 and UA.

2. Experimental section

2.1. Materials and apparatus

Yttrium oxide (Y_2O_3, 99.99%), ytterbium oxide (Yb_2O_3, 99.99%), erbium oxide (Er_2O_3, 99.99%) and thulium oxide (Tm_2O_3, 99.99%), were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Hydrogen peroxide (H_2O_2, 30%), $ZnCl_2$, $FeCl_3 \cdot 6H_2O$, CH_3COOH (HAc) and CH_3COONa (NaAc) were purchased from Beijing Chemical Corp. N-ethyl-N-(3-sulfopropyl)-3-methylaniline sodium salt (TOPS, $\geq$ 98%), 4-amino-antipyrine (4-AAP, $\geq$ 99%), UA and uricase were purchased from Aladdin Reagent Company (Shanghai, China). Other reagents and chemicals were of analytical reagent grade and used without further purification. Solutions were prepared with water purified by a Milli-Q purification system (Millipore, USA). Transmission electron microscopy (TEM, JEOL, Japan) and scanning electron microscope (SEM) with a Hitachi S-4800 (Japan) were used for measuring the morphologies and sizes of materials. An X-ray diffraction (XRD) pattern of UCNPs was carried out with a Rigaku 2500 (Japan) X-ray diffractometer. Fourier transform infrared (FTIR) spectra were collected on an FTIR spectrometer (Nicolet Instrument Co., U.S.A.). UV–vis absorption spectra were recorded on an UV-2450 spectrophotometer (Shimadzu Co., Japan). Fluorescence spectra of UCNPs were measured using an F-4500 fluorescence spectrophotometer (Hitachi Ltd., Japan), where an extern laser at 980 nm continuous-wave (CW) laser (Hi-Tech Optoelectronic Co., Ltd. China) replaced the xenon lamp as the excitation source.

2.2. Ratiometric fluorescence detection for H_2O_2 and UA

Briefly, different volume of H_2O_2, 250 μL of 0.2 M HAc-NaAc buffer solution, 4-AAP, TOPS and $ZnFe_2O_4$ were mixed to reach a