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A facile label-free colorimetric method for highly sensitive glutathione detection by using manganese dioxide nanosheets

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ABSTRACT

Glutathione (GSH) provides essential functions towards metabolism process in biological systems and is related to many diseases such as human immunodeficiency virus, Parkinson, cardiovascular diseases, etc. In this work, a very simple, fast and label-free colorimetric method for highly sensitive GSH quantification is proposed. This strategy is based on the color change of MnO_2 nanosheets solution from yellowish-brown to colorless. Without GSH, MnO_2 nanosheets exhibit a strong ultraviolet–visible (UV–vis) absorption peak at 376 nm with the color showing as yellowish-brown. Once GSH is introduced, MnO_2 nanosheets are reduced to Mn^{2+} ions rapidly, which leads to a yellowish-brown color fading and a remarkable decrease of the absorbance at 376 nm. Based on the distinct color change, a method for highly sensitive and selective detection of GSH was realized with bare eyes and UV–vis spectroscopy, respectively. This method has a wide dynamic range from 0.5 μM to 100 μM and shows a good linear relationship from 0.5 μM to 10 μM with a detection limit of 0.1 μM. More importantly, this procedure is very simple and the whole assay can be accomplished quickly within 5 min. Additionally, this developed assay has also been successfully applied to the detection of GSH in biological fluids with satisfactory results.

1. Introduction

Glutathione (GSH) is an important predominant non-protein thiol widely distributed in mammalian and eukaryotic cells. GSH serves several essential functions in biological systems, such as reduces intracellular free radicals, maintains the metabolism procedure, and prevents organism from toxins [1–6]. The abnormal level of GSH has been reported to be closely related to aging and many human diseases, such as canner, liver damage, heart problems, Parkinson and Alzheimer, etc [7–9]. Accordingly, sensitive detection of GSH with high simplicity is of sustained interest to satisfy clinical and medical requirements. To date, many approaches have been developed for the detection of GSH including high performance liquid chromatography (HPLC) [10–12], electrochemical analysis [13–15], capillary electrophoresis [16], fluorescence spectroscopy [17–21], magnetic resonance imaging (MRI) [22] and colorimetry [23]. Among them, colorimetric method offers promising advantages due to its high sensitivity, simplicity and low cost. Moreover, the detection results of colorimetric method can be easily readout with bare eyes through a visual color change in reaction media.

Over the past several years, a number of colorimetric sensing mechanisms have been employed for GSH detection. The gold nanoparticles (AuNPs)-based colorimetric sensor has been developed for GSH detection based on the color changing of AuNPs in the turnover process of dispersion to aggregation state [24]. Liu et al. [25] reported a simple colorimetric method to determine GSH by utilizing BSA–MnO_2 nanoparticles as mimic. BSA-MnO_2 nanoparticles possess oxidase-like activity that can catalyze the oxidization of 3,3′,5,5′-tetramethylbenzidine (TMB), and the introduce of GSH will cause reduction of oxidized TMB along with a visual color variation. Recently, Ni et al. [23] established an assay for quantitative detection of GSH based on the color change of TMB that was oxidized by silver ion. Although the reported colorimetric methods have shown sensitive and rapid properties, these strategies still suffer from some disadvantages such as time-consuming laborious synthesis to obtain probes and participation of high-cost noble metals (gold, silver). Additionally, the activity of mimic materials is highly dependent on some environmental conditions such as salt concentration and incubation time, which may bring the effect of

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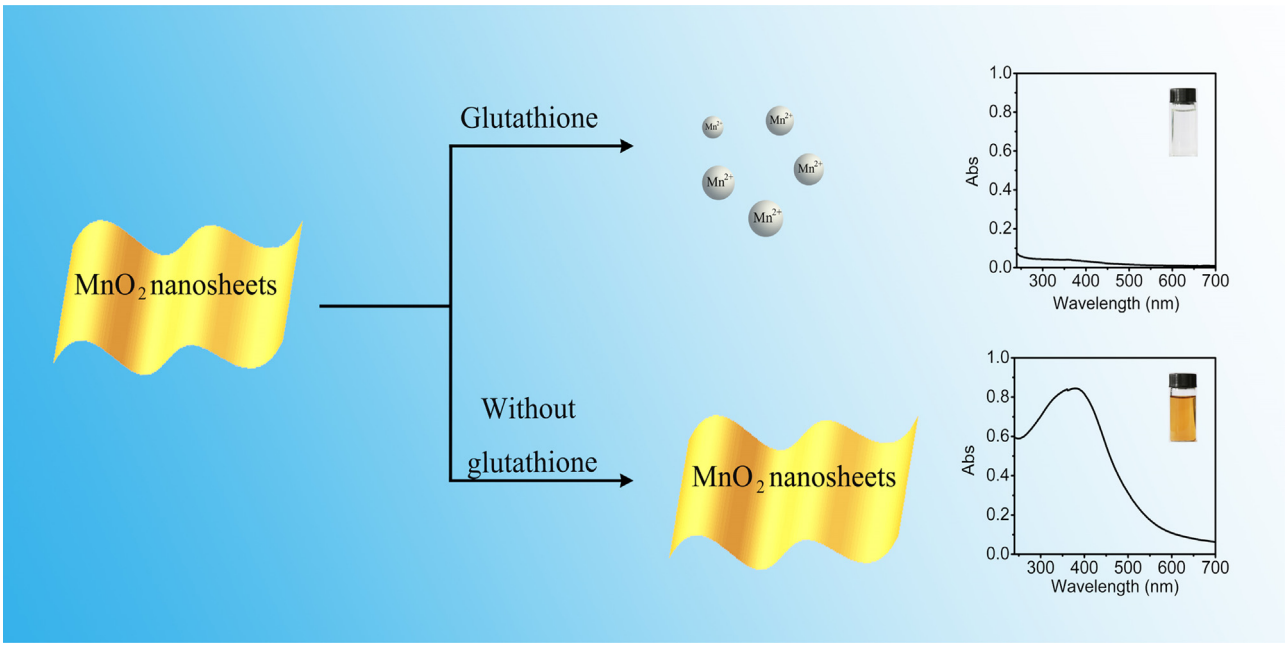

Scheme 1. Schematic representation of glutathione detection by using MnO_2 nanosheets as colorimetric probe.

accuracy reduce. Thus, there is still an increasing demand for developing simple, low-cost and rapid GSH assay in biological and clinical occasions.

Recently, our group [21] has developed a fluorescence method to detect GSH by using carbon dots–MnO_2 nanocomposites. In this assay, fluorescence of carbon dots can be quenched by MnO_2 nanosheets and will be recovered quickly when GSH is introduced, which causes the rapid reduction of MnO_2 nanosheets to Mn^{2+}. Afterwards, we realized that MnO_2 nanosheets could also be used as colorimetric probes for the determination of GSH without any other nanomaterials. Very simply, detection of GSH can be accomplished through directly visualizing the color variation of MnO_2 nanosheets by bare eyes or UV–vis spectroscopy. Herein in this work, we report a simple, label-free and low-cost method for GSH detection using MnO_2 nanosheets as colorimetric probe. Scheme 1 illustrates the mechanism of this proposed method for GSH detection. In the presence of GSH, MnO_2 nanosheets will be reduced to Mn^{2+} ions with yellowish-brown color fading of the solution. As a result, the UV–vis absorption of MnO_2 nanosheets solution is dependent upon the concentration of GSH, which allows its colorimetric detection. This assay is very fast and the whole process can be accomplished in 5 min with a low detection limit of 0.1 μM. More importantly, the analytical potential of this proposed assay in practical applications was also demonstrated successfully for analyzing GSH in human serum samples.

2. Experimental section

2.1. Reagents and apparatus

Glutathione (reduced form) and Tetramethylammonium hydroxide were obtained from J&K Scientific Ltd. (Beijing, China). L-Glycine (Gly), L-leucine (Leu), L-isoleucine (Ile), L-proline (Pro), L-serine (Ser), L-valine (Val), glucose, sucrose and BSA were purchased from Sigma-Aldrich reagent Co. Ltd. (St. Louis, MO, USA). NaCl, KCl, Na_2SO_4, $MnCl_2{\cdot}4H_2O$ (99.99%), H_2O_2 (ω = 30%) and other salts were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). GSH assay kit was purchased from Beyotime biological technology Co. Ltd. (Shanghai, China). All of these reagents were used as received without any further purification. All solutions were prepared using ultrapure water generated by a Millipore Milli-Q water purification system (Billerica, MA, USA) with an electric resistance ≥18.2 MΩ.

Ultraviolet–visible (UV–vis) absorption spectra were recorded on TU-1810 ultraviolet and visible spectrophotometer (Beijing Persee Co., Ltd., China). Morphology images of MnO_2 nanosheets were photographed by a transmission electron microscope (FEI-Tecnai G2, USA) at an accelerating voltage of 200 kV. The crystal form characterization was measured with X-ray diffractometer (PANalytical, Netherlands).

2.2. Preparation of MnO_2 nanosheets

MnO_2 nanosheets were prepared according to the previously reported method [26] with minor modification. In a typical synthesis process, 8 mL tetramethylammonium (TMA·OH, 1.0 M) and 1.5 mL H_2O_2 were mixed and then diluted to 15 mL with ultrapure water. After that, the solution was mixed with 0.415 g $MnCl_2{\cdot}4H_2O$ within 30 s. The mixture was stirred vigorously overnight and MnO_2 nanosheets were obtained from the suspension by centrifugation at 10,000 rpm for 10 min, followed by washing three times with methanol. Then, MnO_2 nanosheets were washed three times with deionized water. To obtain homogeneous MnO_2 nanosheets solution, the as-prepared MnO_2 samples were further treated by a SCIENTZ-IID ultrasonic homogenizer (Ningbo Scientz Biotechnology Co., Ltd, China).

2.3. GSH detection

In a typical GSH assay, 600 μL MnO_2 nanosheets solution (35 μg/mL) was added into a centrifuge tube containing 2.37 mL ultrapure water, and then mixed with 30 μL GSH with the concentration in a range of 0–10 mM. After 5 min, UV–vis absorption spectra were measured by spectrophotometer. Meanwhile, photographs were captured by a digital camera (Canon, EOS 6D, Japan).