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Colorimetric detection of biothiols based on aggregation of chitosan-stabilized silver nanoparticles

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ABSTRACT

We have described a simple and reliable colorimetric method for the sensing of biothiols such as cysteine, homocysteine, and glutathione in biological samples. The selective binding of chitosan capped silver nanoparticles to biothiols induced aggregation of the chitosan–Ag NPs. But the other amino acids that do not have thiol group cannot aggregate the chitosan–Ag NPs. Aggregation of chitosan–Ag NPs has been confirmed with UV–vis absorption spectra, zeta potential and transmission electron microscopy images. Under optimum conditions, good linear relationships existed between the absorption ratios (at A_{500}/A_{415}) and the concentrations of cysteine, homocysteine, and glutathione in the range of 0.1–10.0 μM with detection limits of 15.0, 84.6 and 40.0 nM, respectively. This probe was successfully applied to detect these biothiols in biological samples (urine and plasma).

1. Introduction

The biological aminothiols, including cysteine, homocysteine, and glutathione, play the important roles in maintaining redox balance of various physiological processes [1]. Their abnormal levels in biological systems have been linked to specific pathological conditions and closely associated with several human diseases [2]. Cysteine, a thiol-containing amino acid is one of the 20 amino acids found in proteins which play critical roles in protein synthesis, detoxification and metabolism. The cysteine deficiency could be involved in many syndromes, such as hair depigmentation, slower growth in children, edema, skin lesions, liver damage, lethargy, loss of muscle and fat, and weakness [3]. The normal level of homocysteine in the serum is in the range of 9–13 μM in healthy adults [4]. An elevated plasma and urine homocysteine levels are associated with many cardiovascular, metabolic, and neurodegenerative disorders [5]. Abnormally high levels (more than 15 μM) of homocysteine in the serum result in hyperhomocysteinemia [4]. Hyperhomocysteinemia has also been linked to increased risk of Alzheimer's disease, neural tube defects, complications during pregnancy, inflammatory bowel disease, and osteoporosis [6]. Abnormal levels of cellular glutathione is closely related to a number of diseases, such as leukocyte loss, psoriasis, liver damage, cancer, aging, heart problems, and other ailments [7]. Because of the vital roles of biothiols in biological systems, it is highly desired to develop sensitive and selective determination of these biothiols.

Up to date, lots of strategies for biothiols detection have been developed, including high performance liquid chromatography [8], mass-spectrometry [9], chemiluminescence [10], electrochemistry [11], fluorescence spectroscopy [12,13] and capillary zone electrophoresis [14]. Although these methods provided good sensitivity for detection of biothiols, some inherent issues still cannot be avoided, such as time-consuming, expensive instrumentation, and complicated operation procedures. As a simple, selective and inexpensive method, colorimetric assay has gained increasing attention for biothiols sensing [15–17].

In the past few decades, considerable scientific interest has been focused on the fabrication and application of metal nanoparticles (NPs), because they have important applications in a wide range of fields including catalysis, electronics, photonics, chemical sensors and biosensors. In particular, the development of highly selective colorimetric probes based on gold and silver nanoparticles are of great interest due to their easy functionalization, biostability, and their unique optical and electronic properties [18–20]. These unique properties are mainly due to the oscillation of electrons in the conduction band of metals upon excitation with incident radiation known as the localized surface plasmon resonance (LSPR). The LSPR frequency is mainly related to nanoparticle size, shape, composition, interparticle distance and dielectric constant (refractive index) of the surrounding medium. For gold and silver nanoparticles, LSPR corresponds to photon energies in the visible wavelength region, giving rise to significant interest in their optical properties. These optical characteristics include strong plasmon absorption, resonant Rayleigh scattering, and localized electromagnetic fields at the nanoparticle surface [21].

Colorimetric sensors has emerged as a promising technique for biochemical analysis because systems of this type offer several advantages

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such as a simple readout (often visible with the naked eye), as well as being highly sensitive and selective and cost effectiveness. Silver nanoparticles (Ag NPs) have attracted much interest because they have a high extinction coefficient at a low cost [22,23]. It is well known that the analytical applications, sensitivity and selectivity of these nanoparticles are directly related to the surface physicochemical properties of them. In this sense, the surface functionalization plays a crucial role in improving the stability and analytical applicability of Ag NPs. Appropriate functionalization of NPs can improve their properties and increase their selectivity, consequently enlarging their applications [24]. Recently, stable functionalized Ag NPs with appropriate ligands, such as biological molecules (glutathione [25,26], dopamine [27]), organic small molecules (p-sulfonatocalix [4] arene [28], 2,2′-thiodiacetic acid [29], cysteine [30], thioglycolic acid [31]) or complexes (β-cyclodextrin inclusion complex [32], cyclen dithiocarbamate host–guest chemistry [33]) have been synthesized and applied as sensitive and selective colorimetric probes for the detection of Co^{2+} [25], As^{3+} [26], melamine [27,32], histidine [28,30], creatinine [29], alkaline earth metal ions (Mg(II), Ca(II), Sr(II) and Ba(II)) [31], thiram and paraquat [33].

In the current study, the chitosan molecule was a favorable choice for functionalize silver nanoparticle surface [34] and biomedical applications because of nontoxicity, biodegradability, biocompatibility, and its high capacity to adsorb dyes, proteins and heavy metal ions including Ag^{+} [35]. The aggregation of the chitosan capped silver nanoparticles could be induced by cysteine, homocysteine and glutathione but not by other amino acids and biomolecules. Biothiols have several binding sites (—SH, —NH_2 and —COOH) which may interact with functionalized silver nanoparticles through silver-thiol (Ag—S) bond, hydrogen bonding and electrostatic interaction. The interaction results in visual color change of the Ag NPs solution from yellow to orange which, in turn, is due to the variation of the localized surface plasmon resonance (SPR) absorption. The chitosan–Ag NPs have been successfully applied to determine the cysteine, homocysteine and glutathione content in human serum and urine samples with satisfactory results.

2. Experimental

2.1. Materials and Reagents

All commercial grade chemicals and solvents were purchased and were used without further purification. $AgNO_3$, $NaBH_4$, cysteine, chitosan, hydrochloric acid and sodium hydroxide were purchased from Merck Company, Darmstadt, Germany. Also, all the chemicals used to investigate of interference effect were obtained from Merck Company. Homocysteine and glutathione were purchased from sigma Aldrich. All solutions were prepared with deionized water. Chitosan stock solution was prepared by dissolving suitable amount of chitosan in 0.05% acetic acid solution for use. Working solutions of cysteine, homocysteine and glutathione were prepared daily by suitable dilution of the stock solution with deionized water.

2.2. Instruments

All absorption spectra of Ag NPs were recorded on a UV–visible spectrophotometer (analytic jena specord 210, Germany) with a variable wavelength between 300 and 700 nm using a glass cuvette with 1.0 cm optical path. The sizes, shape and their distribution of silver nanoparticles were confirmed through transmission electron microscope (TEM) images using a JEOL 2000 FXII microscope at 200 kV, 0.28 nm point to point spatial resolution (Japan). The morphology of the Ag NPs in the presence of homocysteine and glutathione were studied on Zeiss-EM10C (Germany) operated at 80 keV. All pH adjustments were carried out by a digital pH meter BANTE instrument 922 (China) with a combined glass electrode. A 100 μL Hamilton syringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) was used for injection of μL volume of solution.

2.3. Preparation of Chitosan-capped Silver NPs

Chitosan-Ag NPs were synthesized using a facile approach by $NaBH_4$ reduction of $AgNO_3$ according to the previous reported method with slight modification [36]. Briefly, chitosan solution (0.5 mL, 0.1% (w/v)) and $AgNO_3$ solution (0.5 mL, 0.01 mol L^{-1}) were added to 100 mL water in turn at room temperature. After stirring for 10 min in a dark place, 8.8 mg of $NaBH_4$ was added into the reaction solution immediately, and then continued stirring for 2 h.

2.4. Procedure for Cysteine Detection

For the cysteine, homocysteine and glutathione determination using chitosan-Ag NPs, 20 μL of the solution of target compound with a given concentration was added to 2.0 mL of the chitosan–Ag NPs (pH 8.2), separately and the mixture was maintained at room temperature for 20 min. Depending on the concentration of soluble cysteine, homocysteine and glutathione, the color was changed from yellow to orange. Then, the absorbance spectra were recorded with 1 cm path-length cells. The calibration graph was obtained by plotting the absorption ratio A_{500}/A_{415} against the concentration of biothiols in the range of 0.1 to 10.0 μM.

The application of present method for determination of biothiols was tested in human serum and urine samples. The human serum and urine samples were obtained from clinical laboratory (Sanandaj, Iran). For this purpose, 0.1 mL of the human serum and urine of healthy volunteers were separately diluted into a 500.0 mL volumetric flask. Then, the solution was centrifuged at 3000 rpm for 3 min [37], and the recovery of biothiols was determined using standard addition method. The standard addition method was performed by spiking three different levels of biothiols concentration into serum and urine samples and quantified by the aforesaid procedure.

3. Results and Discussion

3.1. Reason for the Color Change of Chitosan-Ag NPs with Biothiols

The chitosan–Ag NPs are synthesized by the reduction of $AgNO_3$ with $NaBH_4$ and stabilized with chitosan which is a polysaccharide biopolymer with excellent dispersive properties and stability in aqueous media. Silver nanoparticles were modified by chitosan because of the presence of –NH_2 and –OH groups in the chitosan. The existence of these functional groups in chitosan can help in possible adsorption interactions between chitosan and heavy metal ions including Ag^{+}, dyes and proteins [34]. Cysteine, homocysteine and glutathione contain the binding sites of —COOH, —SH and —NH_2, and exist in different species depending on solution pH (Fig. S1 (in Electronic Supplementary materials)). Our approach was based on the hypothesis that biothiols is probably bound to the silver nanoparticles by their thiol (—SH) group [23]. After biothiols adsorption on the silver nanoparticles, the biothiol molecules still have free functional groups to form bonds between nanoparticles. The negative carboxylate group of biothiols is capable to form hydrogen bond with —OH and —NH_2 groups from chitosan adsorbed on silver nanoparticles. In addition to the hydrogen bond between biothiols and chitosan, electrostatic interactions between amino acid groups of biothiol molecules bound to different silver nanoparticles are also possible (Fig. 1). The aggregation of chitosan-Ag NPs, resulting in a visible color change that was used as a simple and selective method for determination of biothiols. Fig. 1 shows the synthesis procedure and the possible mechanism of colorimetric detection of biothiols with chitosan-Ag NPs.

3.2. Characterization of Chitosan-Ag NPs

Chitosan-Ag NPs were characterized by transmission electron microscopy (TEM), zeta potential and UV visible spectrophotometry. The