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Selective detection of cysteine/cystine using silver nanoparticles

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ABSTRACT

The selective detection of cysteine and cystine amino acids over other standard amino acids was possible with the naked eye using silver nanoparticles (AgNPs) in a simple procedure at room temperature. The change in color and the aggregation of NPs were studied using UV–vis spectroscopy and transmission electron microscopy, respectively. It was observed that when the derivative and substructures of cysteine were employed as analytes the detection was possible only when there is free SH or S–S group present in the analytes. The method was extended to a dipeptide 'cys-gly' as a model peptide where the detection was successful due to the presence of SH moiety.

Amino acids are organic compounds that are needed by the body to perform normal functions. Methionine is an essential amino acid that is involved in the metabolic cycle and methionine metabolism is involved in the production of the antioxidant, cysteine. The susceptible oxidation of the cysteine (–S–H) bond leads to the formation of the disulfide cystine (–S–S–), which plays a crucial role in protein structure and folding.[1] The amino acids methionine (**1**), cysteine (**2**), and cystine (**3**) are interlinked through bio-chemical processes and are involved in a variety of cellular functions.[1,2] The analysis of sulfur-containing amino acids is thus important to our understanding of problems in living systems. Few instruments, such as HPLC,[3] mass spectrometry,[4] and electrochemical systems,[5] are used for detecting sulfur-containing amino acids. The colorimetric detection method is a convenient technique because the detection can be performed without the aid of advanced instruments. Some common chromogenic[6] and fluorogenic[7] compounds have been employed for detecting these amino acids.

The use of nanoparticles for the detection of analytes is a topic of interest that is being actively explored for many applications.[8] Metal nanoparticles with the size range of 10–100 nm exhibit bright color and high extinction coefficients much greater than those of traditional organic chromophores.[9] Noble metal nanoparticles of gold (AuNPs) and silver (AgNPs) exhibit a characteristic surface plasmon absorption in the visible region that can be tuned depending upon the environment.[10] Conventional AuNPs,[11] tagged with the DNA/fluorescing entity,[12] and triangular silver nanoprisms[13] are employed for detecting sulfur-containing amino acids using the thiophilic nature of these metals.[14] The detection of the sulfur-containing amino acids is shown in the color change that occurs upon the interaction of the NPs with the analytes. This can be favored by suitably adjusting the temperature, pH, or by the addition of another metal salt in the presence of NPs.[11–13] This visible color change allows the sensitive detection of small amounts of analytes.

In this study, we employed AgNPs for detecting sulfur-containing amino acids. AgNPs can be synthesized by the reduction of a silver salt in the presence of a stabilizer. Synthesis of AgNPs becomes easier when the stabilizer performs the dual function of reduction and stabilization.[15] Amines, such as triethylamine, triethylenetetramine, and amine terminated dendrimers, are thus exploited; however these species require longer reaction times and an external reducing agent for the synthesis.[16] We have established[15] the dual function of a hexaazamacrocycle, **L1**, in the synthesis of AgNPs. **L1** was synthesized easily (Fig. 1b) by the simple condensation of terephthaldehyde with diethylenetriamine, followed by reduction using $NaBH_4$.

Facile synthesis of the AgNPs was accomplished in this work following our recent protocol[15] by the reduction of 1 mM of $AgNO_3$ in the presence of 0.5 mM **L1** in different media, such as H_2O, MeOH, and DMSO, at 90 °C. The synthesis was usually completed within 30 min, producing a yellow-colored solution of AgNPs. The prepared nanoparticles were stable and exhibited a characteristic surface plasmon peak at approximately 420 nm (Fig. 1a). Their size falls in the range of 5–20 nm, as found by TEM (Fig. 2a).

We used our AgNPs to investigate the detection of the sulfur-containing amino acids methionine (**1**) and cysteine (**2**) over other standard amino acids (50 ppm final concentration) at room temperature in three different solvents, DMSO, MeOH, and H_2O at

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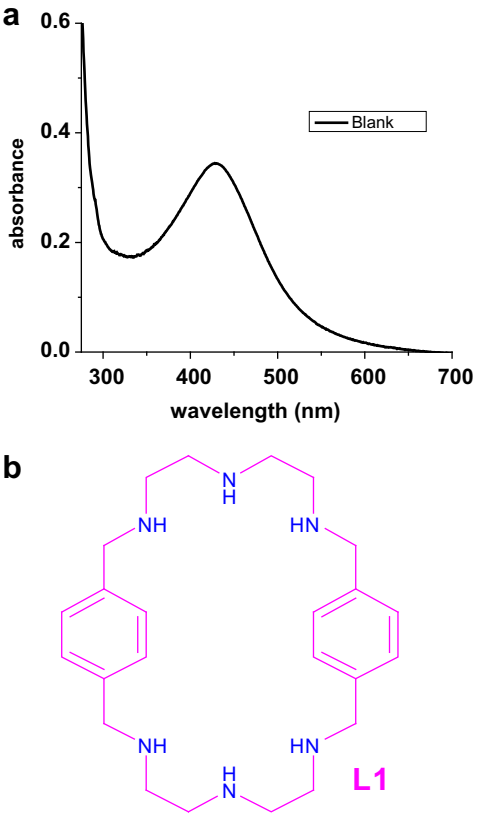

Figure 1. (a) Representative UV–vis spectrum of AgNPs obtained from the reduction of 1 mM $AgNO_3$ in the presence of 0.5 mM **L1** in DMSO at 90 °C within 30 min, and (b) the structure of **L1**.

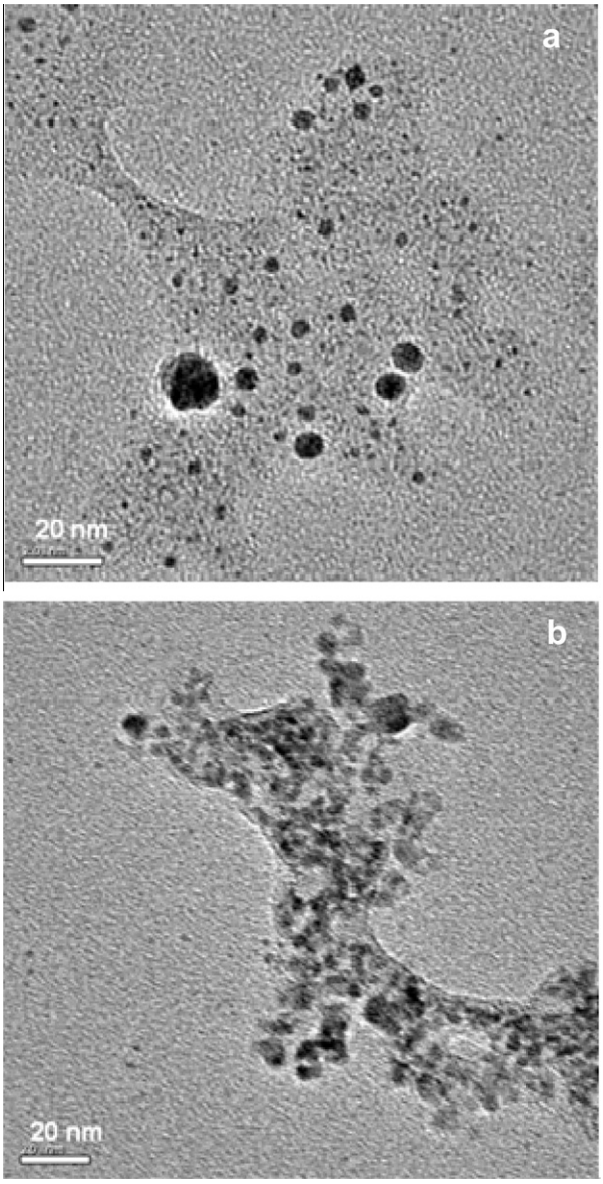

Figure 2. TEM image of the **L1** stabilized AgNPs (a) before and (b) after the addition of cysteine. (0.1 mL of 1 mM aqueous solution of cysteine was added to 2 mL of AgNPs in DMSO and allowed to stand for 12 h).

room temperature. The use of H_2O or MeOH as the solvent system was not selective for the detection of our analytes. DMSO alone showed the selective detection of cysteine over other standard amino acids and, more importantly, the sulfur containing-methionine (Supplementary Fig. S1). This reveals that the selectivity of detection is controlled by the solvent, DMSO. Thus our investigation was continued further in DMSO.

Experiments were performed with natural amino acids by adding 0.1 mL of 1 mM aqueous solution of amino acids to 2 mL of AgNPs in DMSO. Due to poor solubility of cystine (**3**), a 1 mM suspension of cystine was prepared and the insoluble part was allowed to settle. The saturated solution of cystine was used for the study. Among the samples, cysteine/cystine showed an immediate change in color from yellow to pink, and a broadening of the absorption peak in the UV–vis spectra was also observed (Fig. 3a). As seen, the sample solution containing cysteine and cystine showed a change in color from yellow to pale pink which further intensified over a period of time (Fig. 3b). Even at lower concentration, the sample containing cystine showed an intense color change. It showed that the increase of absorption ratio (A_{450}/A_{423}) could quantitatively reflect the amount of cysteine added (Supplementary Fig. S2). A linear correlation exists between (A_{450}/A_{423}) and the logarithm of the cysteine concentration C over the range of 25–250 μM. The linear equation is as follows: $Y = 1.06851 + (0.1414 \times \log C)$. Detection limit was calculated and obtained as 2.5 ppm (Supplementary Fig. S2). A good linear relationship with the relative coefficient of 0.991 is obtained in the range of 25–250 μM concentration of cysteine.

It is noteworthy to mention that DMSO is used as a mild oxidizing agent for alcohols, thiols, and other organic compounds.[17] Cysteine (–S–H) can be easily oxidized to cystine (–S–S–) under ambient conditions by DMSO.[18] Therefore, we continued our investigations using DMSO as a dispersant and tested the detection of cystine (**3**) under the same conditions over other amino acids. The result of oxidation and sequential change in the environment can be visually sensed by AgNPs. The reaction was performed by adding a higher concentration of cysteine to AgNPs. At room temperature, mixing of AgNPs with cysteine (**2**) yielded a white precipitate with time. ^{13}C NMR spectrum of the white solid was obtained by dissolving the solid in 3% NaOD–D_2O solution that showed a shift compared to cysteine. This reveals the formation of cystine in the presence of AgNPs (Supplementary Fig. S3).

We tested the influence of pH on the color of AgNPs-cysteine systems in three different solvents, that is, DMSO, H_2O, and MeOH. To 2 mL of as prepared AgNPs, 0.1 mL of 1 mM aqueous cysteine was mixed where the pH was ~7. The solution was monitored by recording UV–vis spectra. The pH of the solution was adjusted by adding 0.1 N HCl and NaOH. The solution was adjusted at pH 3, 7, and 11. It was observed that, at lower pH a white precipitate was formed due to protonation of the ligand **L1** while at higher

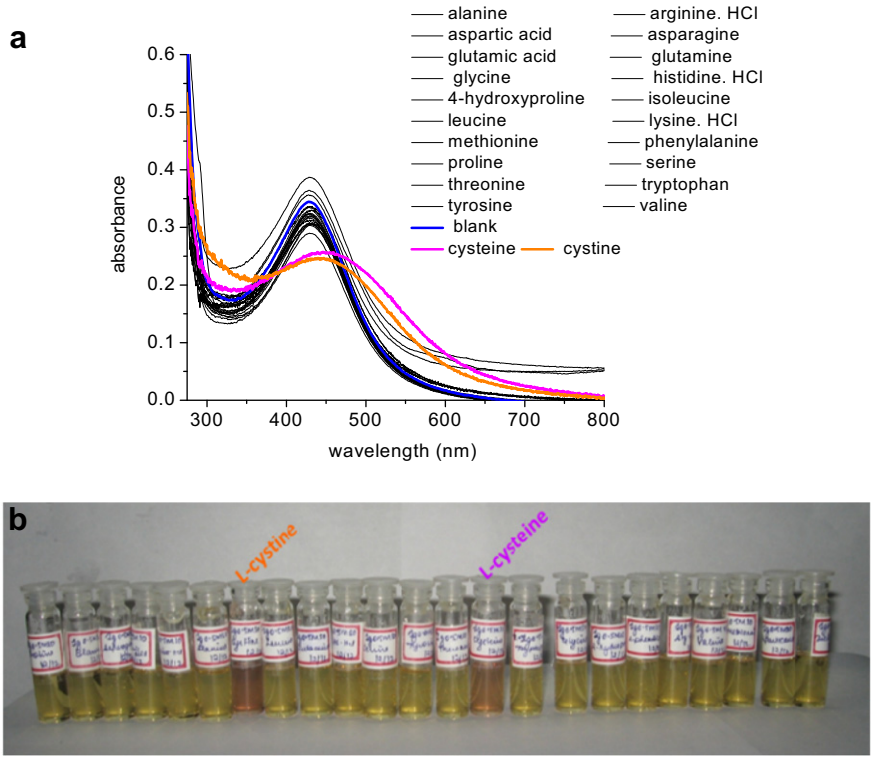

Figure 3. (a) UV–vis spectra of **L1** stabilized AgNPs obtained after the addition of 0.1 mL of a 1 mM aqueous solution of amino acids to 2 mL of 1 mM AgNPs in DMSO and then allowed to stand for 3 h. (b) Photograph of the solutions.

pH an intense color change was observed possibly favoring easy oxidation and represented in UV–vis graphs (Supplementary Figs. S4, S5, S6 and S7).

Among the samples, cysteine/cystine showed an immediate change in color from yellow to pink, and a broadening of the absorption peak in the UV–vis spectra was also observed (Fig. 3a). We believe that this broadening was likely due to the aggregation of AgNPs. This assessment was supported by the TEM study wherein aggregation of the AgNPs was shown in the TEM image (Fig. 2b) recorded within 12 h. No interaction of AgNPs with other amino acids, including methionine, showed a color change or a broadening of the surface plasmon peak in the UV–vis spectra, even after 10 days. Therefore, a simple method of visualizing the presence of cysteine/cystine selectively over other amino acids, and more importantly over methionine, at room temperature is established.

To further probe our findings we performed experiments to test the color changes of the AgNPs upon addition of cysteine substructures and derivatives (Fig. 4), such as glycine (**4**) (see previous), cysteamine (**5**), and mercaptopropionic acid (**6**), S-protected

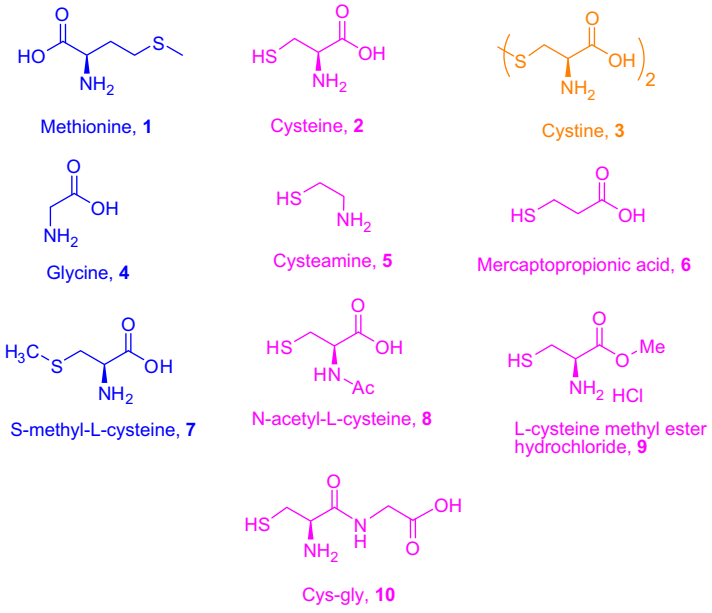

Figure 4. List of substructures and derivatives of cysteine.

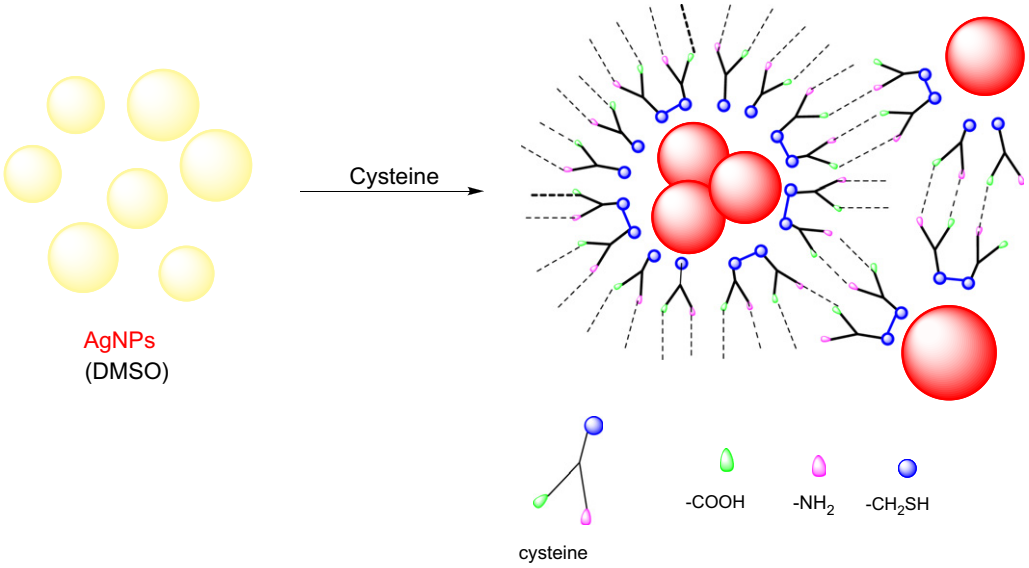

Figure 5. Proposed model of the aggregation of AgNPs over time after the addition of cysteine.

(*S*-methyl-L-cysteine (**7**), N-protected (*N*-acetylcysteine) (**8**), and O-protected (L-cysteine methylester hydrochloride) (**9**), and the dipeptide cys-gly (**10**). UV–vis spectra (Supplementary Fig. S8) were recorded for these solutions, and as expected the color change from yellow to pink (Supplementary Fig. S9) and peak broadening were observed for the analytes having free thiols (**2**, **5**, **6**, **8**, **9**, and **10**) and for the disulfide (**3**). No change could be seen after the addition of glycine (**4**) or S-protected analytes, such as methyl cysteine (**7**). This method is thus also effective for the selective detection of simple thiols and disulfides over sulfide. We hope that this method can be elaborated further to determine the presence of free thiols and disulfide moieties in proteins.

Our method involves a simple procedure that can be performed at room temperature for the detection described. The selectivity of the detection is controlled by the solvent, DMSO,[19] in which the probable disulfide formation is catalyzed by AgNPs (Fig. 5). The use of DMSO as a dispersant is also advantageous because DMSO is a mild oxidizing agent of thiols.

To conclude, selective detection of cysteine/cystine at a ppm level was successful by using AgNPs prepared in the presence of the hexaazamacrocycle **L1** in DMSO.

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Supplementary data

Supplementary data (Photograph and UV–vis spectra of the AgNPs showing the selective detection of cysteine/cystine in DMSO) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.11.050.

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