DOI: 10.1002/chem.200902479

A Circular Dichroism Probe for L-Cysteine Based on the Self-Assembly of Chiral Complex Nanoparticles

Jing Nan and Xiu-Ping Yan*[a]

Cysteine is a naturally occurring, sulfur-containing amino acid that is involved in a variety of important cellular functions, including protein synthesis, detoxication, and metabolic processes.[1] The level of cysteine in physiological fluids, such as plasma and urine, has been recognized as an important indicator for a number of clinical disorders.[2] Therefore, it is highly desirable to develop sensitive and selective methods for detecting cysteine in biological fluids.

To date, various approaches have been used for the detection of cysteine, in particular, optical methods, such as colorimetry, spectrofluorimetry, and plasmon resonance light scattering spectrometry, have received great interest owing to their apparent advantages over other methods in virtue of sensitivity and convenience.[3] For the optical detection of cysteine, various optical probes have been designed and synthesized, including a functionalized dye probe,[3a] a heavy-metal-complex-based chemosensor,[4a] a semiconductor nanoparticle probe,[4b] and a metal nanoparticle probe.[4c]

Herein, we report a novel circular dichroism (CD) method to probe L-cysteine based on the self-assembly of chiral complex nanoparticles from Ag^I and L-cysteine. The CD method possesses high selectivity and sensitivity for detecting L-cysteine without the need for any design and synthesis of a probe.

We observed that the addition of Ag^I to a solution of L-cysteine induced a significant change in the CD characteristic of L-cysteine. Without the addition of Ag^I, L-cysteine shows a single weak chiral conformation peak at 200 nm (Figure 1a), and both the position and shape of its CD peak are similar to those of most other α-amino acids. However, upon the addition of Ag^I (50 μM) to the solution of L-cysteine (50 μM), the solution immediately became slightly turbid and a new CD signal appeared with two positive peaks at 210 and 300 nm, and three negative peaks at 250, 280, and 360 nm (Figure 1a). The CD signal continuously varied with reaction time (Figure 1b); meanwhile the solution became more turbid. After evolution for 100 h, the CD spectrum became stable and the original positive peak of L-cysteine at 200 nm disappeared, while an S-shaped signal with a negative peak at 290 nm and a positive peak at 254 nm was produced. This observed change in the CD peak effectively avoided the interference from the natural CD peaks of α-amino acids. Although the intensity of the CD peak increased by a factor of 30 after equilibrium was reached, the CD signal evolved too slowly for practical detection.

To speed up the reaction between Ag^I and L-cysteine, the mixture of Ag^I and L-cysteine was ultrasonicated and heated in a water bath. As a result, the time for the reaction to reach equilibrium was reduced from 100 h to 30 min because of the synergistic effects of temperature and ultrasonication (Figure S1 in the Supporting Information). Besides, under ultrasonication (120 W) in a water bath at 37 °C for 30 min, the addition of Ag^I resulted in a 150-fold improvement in the sensitivity in comparison with direct CD detection of L-cysteine in the absence of Ag^I (compare Figure S2b in the Supporting Information with Figure 1a).

To confirm that the CD spectra observed above, after treatment of L-cysteine with Ag^I, originated from the differential absorption of left- and right-circularly polarized light rather than the effect of light scattering on small particles in solution, we replaced L-cysteine with D-cysteine. The CD spectra were measured either immediately after mixing D-cysteine (50 μM) and Ag^I (50 μM), or after ultrasonication (120 W) of a mixture of D-cysteine (50 μM) and Ag^I (50 μM) in a water bath at 37 °C for 30 min. The results show that replacement of L-cysteine with D-cysteine produced an opposite CD signal, and demonstrate that the observed behavior did result from the differential absorption of left- and right-circularly polarized light (Figure S2 in the Supporting Information).

[a] J. Nan, Prof. Dr. X.-P. Yan
Research Center for Analytical Sciences
College of Chemistry, Nankai University
94 Weijin Road, Tianjin 300071 (China)
Fax: (+86) 22-23506075
E-mail: xpyan@nankai.edu.cn

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200902479.

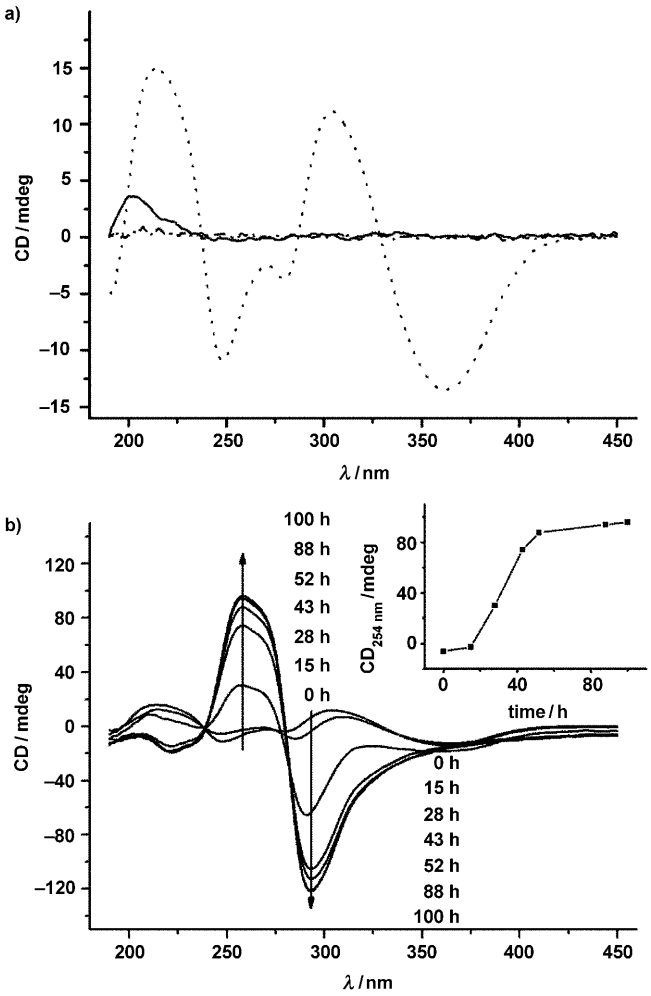


Figure 1. a) CD spectra of L-cysteine (——, 50 μM), Ag^I (–•–•–, 50 μM), and a mixture (•••••) of L-cysteine (50 μM) and Ag^I (50 μM). The CD measurement was taken immediately after mixing. b) CD spectral evolution of L-cysteine (50 μM) upon addition of Ag^I (50 μM) at room temperature without ultrasonication. The inset shows the time-dependent intensity of the CD peak at 254 nm.

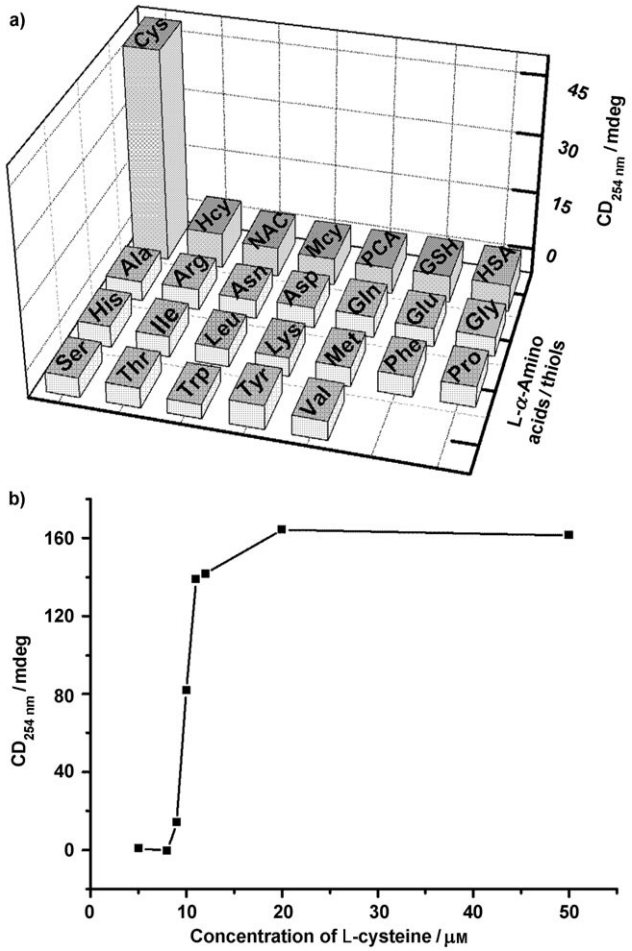
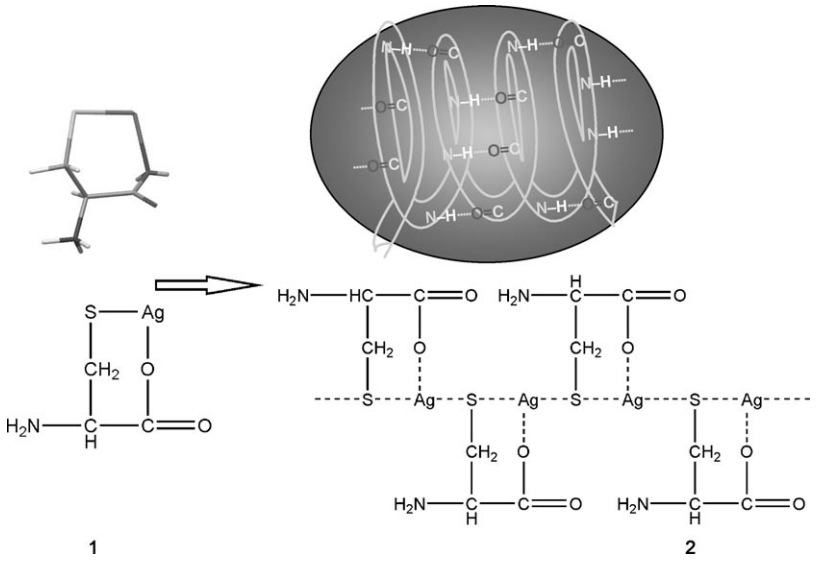


Figure 2. a) A 3D plot showing the selectivity of Ag^I as a CD probe for L-cysteine (5 μM), with respect to the other L-α-amino acids and structurally related chiral thiol molecules (500 μM) under ultrasonication (120 W) in a water bath at 37 °C for 30 min. b) Dependence of CD intensity (254 nm) on the concentration of L-cysteine in the presence of Ag^I (10 μM) under ultrasonication (120 W) in a water bath at 37 °C for 30 min.

To demonstrate the selectivity of Ag^I as a CD probe for L-cysteine, the CD response of Ag^I (5 μM) to L-cysteine (5 μM), the other nineteen α-amino acids, and six structurally related thiols (500 μM) was investigated. Among the tested analytes, only L-cysteine caused an S-shaped CD signal in the range of 230–350 nm, even though its concentration was only 1% that of the other analytes (Figure S3 in the Supporting Information). The results show that Ag^I possesses high selectivity for L-cysteine (Figure 2a). It is worth noting that the six thiols are structurally related of biological or pharmaceutical molecules. Reduced L-glutathione (GSH) and human serum albumin (HSA) are examples of proteins and oligopeptides, respectively, both of which contain the L-cysteine residue. *N*-Acetyl-L-cysteine (NAC) and L-mecysteine (MCy) are both derivatives of L-cysteine. L-Homocysteine (HCy) and D-penicillamine (PCA) are homologues of L-cysteine. The structures and functional groups of the six thiols are very similar to that of L-cysteine. Therefore, Ag^I offers great selectivity as a CD probe for the recognition of L-cysteine.

The dependence of CD intensity (254 nm) on the concentration of L-cysteine shows a special characteristic (Figure 2b). When the molar ratio of L-cysteine to Ag^I was increased to approach the jump point, a clear S-shaped CD signal appeared abruptly and the CD signal increased significantly. In a concentration range of 2–100 μM Ag^I, the jump point was found to be within a 10% range of a 1:1 molar ratio of Ag^I to L-cysteine. However, outside a 10% range of the jump point, an increase in the concentration of L-cysteine led to an insignificant change in the intensity of CD signal (Figure 2b, Figure S4).

CD titration experiments were carried out for the quantification of L-cysteine. The CD titration curve was obtained by plotting the CD intensity at 254 nm of a solution of L-cysteine and Ag^I against the concentration of Ag^I (Figure S5 in the Supporting Information). Thus, we can determine the



Scheme 1. Possible structural change in L-cysteine upon the addition of Ag^I.

concentration of L-cysteine at μM levels in a sample by means of CD titration with a series of solutions of Ag^I at various concentrations.

The mechanism for the action of Ag^I as a selective CD probe for the recognition of L-cysteine could be attributed to the self-assembly of chiral complex nanoparticles. Previous studies on the thiolate–Ag^I complexes and their antimicrobial activity suggested a possible structure, **1** (Scheme 1), resulting from the coordination of Ag^I through the sulfur atom, and the likely presence of weak bonding through O atoms in the ligand.[5] The formation of S–Ag coordination was indirectly confirmed by the absence of the band for the S−H group stretching vibration at 2552 cm^{-1} in the FT–Raman spectrum (Figure S6 in the Supporting Information). The formation of O−Ag bonding was also indirectly confirmed by the absence of the band for the O−H bending vibration (1425 cm^{-1}), C−O stretching vibration (1295 cm^{-1}), and C−O−H combining vibration (1348 cm^{-1}) in the FTIR spectrum (Figure S7 in the Supporting Information). Our results, in combination with previous studies,[5,6] enabled us to assume a possible oligomeric structure **2** with multiple N−H and C=O bonds formed through self-complementary hydrogen-bond interactions (N−H···O=C) (Scheme 1). The formation of hydrogen bonds was confirmed by the simultaneous enhancement of the 1677 and 1566 cm^{-1} bands in the FTIR spectra of **2** (Figure S7 in the Supporting Information), which was observed in helical conformation proteins.[7] It is well known that the hydrogen-bond interactions (N−H···O=C) play an essential role for maintaining helical secondary structure in proteins. The helical conformation of **2** can be used to interpret the appearance of the strong S-shaped CD signal.[8] Meanwhile, the high selectivity of Ag^I toward L-cysteine can also be attributed to the structure and conformation of **2**, because any change of functional group or structure probably inhibits the formation of a helical conformation, especially because the formation of the hydrogen bond is easily affected by steric hindrance.

Unlike the macrostates of conventional metal–organic coordination complexes, both **1** and **2** are neither true solution nor bulk solid, but colloids with good dispersibility. The TEM images (Figure 3) show that the colloids of **1** are small nanoparticles with various morphologies because of the orderless stacking of precipitate **1**. However, the nanoparticles of **2** are much bigger than those of **1**, and the morphology appears to be ellipsoidal with short diameters of 20–40 nm and long diameters of 20–80 nm. The results from AFM confirmed the

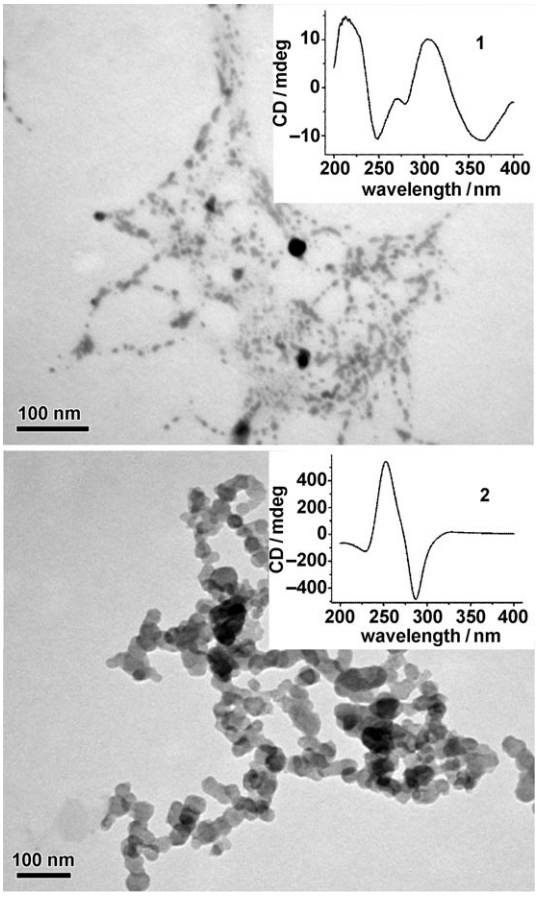


Figure 3. TEM images of **1** and **2**. Insets show their corresponding CD spectra. The sample of **1** was prepared immediately after mixing L-cysteine (50 μM) and Ag^I (50 μM), whereas the sample of **2** was prepared from the mixture of L-cysteine (50 μM) and Ag^I (50 μM) after ultrasonication (120 W) in a water bath at 37 °C for 30 min.

morphological difference between **1** and **2** (Figure S8 in the Supporting Information) by showing their vertical distance. The two microcosmic states contributed to different CD signals, which implies that the nanoparticles **2** come from the self-assembly of chiral nanoparticles **1**. XRD (Figure S9 in the Supporting Information) shows that both nanoparticles of **1** and **2** are crystalline, but silver nanoparticles passivated by L-cysteine are not. Moreover, the XRD spectra of **1** and **2** are almost identical, which also suggests that **2** is formed by supramolecular self-assembly of **1**. Further evidence to support the mechanism is that the CD signal of **2** diminished on heating (Figure S10 in the Supporting Information), which is considered to be a method to demonstrate the formation of nanoscale chiral aggregates.[9] From the above results a new class of noble-metal–organic hybrid chiral nanoparticle with high optical activity can be gained through the self-assembly of chiral nanoparticles.

To show the potential of the CD method for the analysis of real biological samples, we applied it to the quantitative determination of L-cysteine in human urine samples from volunteers. An appropriate dilution of urine (20-fold) without the need for any other pretreatments was found sufficient to obtain a quantitative recovery of spiked L-cysteine by means of CD titration. Titration curves of L-cysteine in urine samples are shown in Figure 4. The concentrations determined and the recovery of the L-cysteine from the urine samples are summarized in Table 1. The quantitative recovery of L-cysteine from 98 to 107% indicate that no significant interference from urine matrixes was encountered after 20-fold dilution of urine samples. The above results demonstrate that the CD method developed possesses great potential for detecting L-cysteine in biological fluids.

In summary, we have demonstrated a novel CD method for probing L-cysteine in biological fluids on the basis of chiral nanoparticle self-assembly from Ag^I and L-cysteine. The method enables highly selective and sensitive detection of L-cysteine at the μM level without the need for any special synthesis or any sample pretreatment procedures except an appropriate dilution.

Table 1. Analytical results for the determination of L-cysteine concentration in human urine samples.

Samples	Spiked L-cysteine [μM]	Measured [μM] (mean $\pm s$, $n=3$)	Recovery [%] (mean $\pm s$, $n=3$)
urine 1	0	83.3 ± 3.1	–
	80	158.0 ± 3.5	97.5 ± 4.4
urine 2	0	64.7 ± 3.1	–
	60	119.3 ± 1.2	98.9 ± 1.9
urine 3	0	74.7 ± 4.2	–
	70	144.7 ± 3.1	106.7 ± 4.1

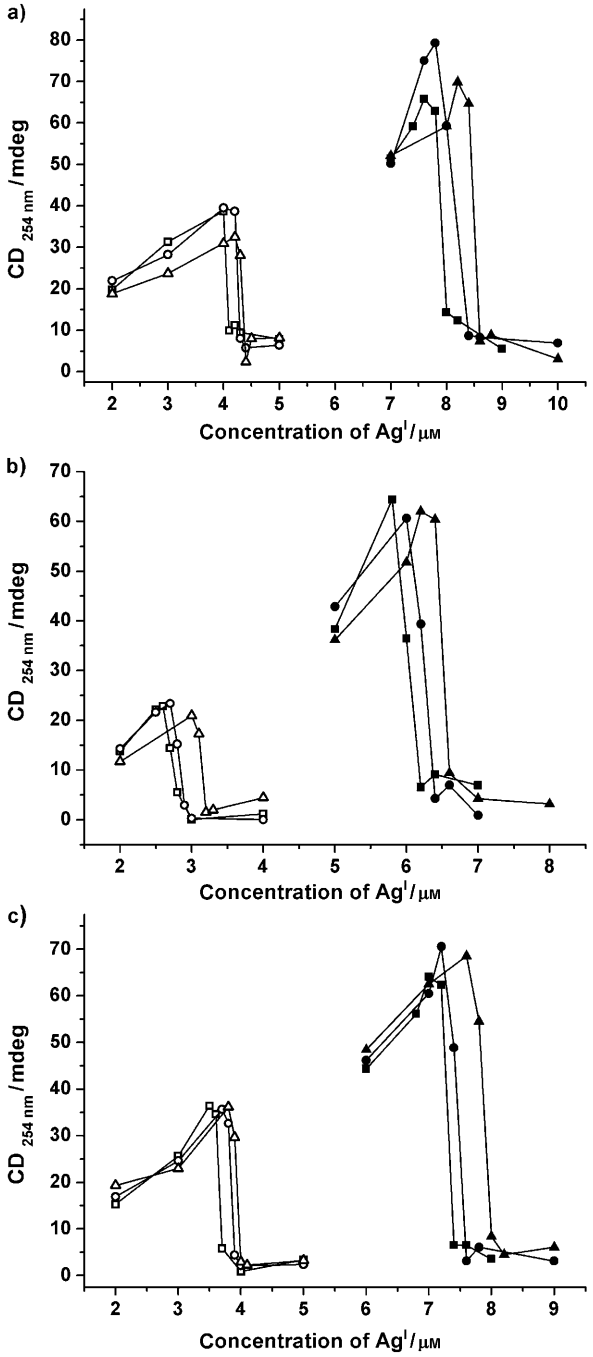


Figure 4. CD titration curves for the determination of L-cysteine concentration in human urine samples: a) urine 1, b) urine 2, c) urine 3. (□, ○, Δ), before spiking; (■, ●, ▲), after spiking with 80, 60, and 70 μM of L-cysteine in urines 1, 2, and 3, respectively. The titrations were repeated three times after 20-fold dilution of urine samples.

Experimental Section

Materials and chemicals: All chemicals used were of at least analytical grade. Ultrapure water (18.2 MΩ cm) obtained from a WaterPro water purification system (Labconco Corporation, Kansas City, MO, USA) was used throughout this work. All of the amino acids and reduced GSH were from Beijing Newprobe Biotechnology (Beijing, China). L-Homocysteine and D-cysteine were from International Laboratory Limited (San Bruno, California, USA). HSA was purchased from Sigma. *N*-Acetyl-L-cysteine, L-mecysteine, and D-penicillamine were from Aladdin Reagent (Shanghai, China). Silver nitrate was from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). All solutions were freshly prepared before use.

Instrumentation and characterization: The FT–Raman spectra were measured with an RFS 100 FT–Raman spectrometer (Brüker, Germany) equipped with a 1064 nm Nd:YAG laser system with a maximum power out of 2 W, a quartz beamsplitter, and a cryogenically cooled Ge detector. The FTIR spectra (of 400–4000 cm^{-1}) were measured with a Nicolet IR AVATAR-360 (Nicolet, USA) spectrometer with pure KBr as the background. The XRD spectra were collected on a Rigaku D/max-2500 X-ray diffractometer (Rigaku, Japan) with $Cu_{K\alpha}$ radiation. The TEM images of the nanoparticles were recorded on a JEOL 100 CXII (JEOL, Japan) microscope with an accelerating voltage of 100 kV. The AFM measurements were performed with an AFM (Veeco Company, nano IIIa) in tapping mode. All CD (Jasco J-715) spectra were measured in water in a 10 mm quartz cell at ambient temperature. The solution was shaken before every CD measurement.

Biological studies: ***Samples***: The human urine samples were analyzed in this work were obtained with informed and signed consent from volunteers.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (no. 20935001) and the National Basic Research Program of China (2006CB705703). We greatly appreciate help from Prof. Yu Liu (College of Chemistry, Nankai University, China) with the AFM measurements.

Keywords: circular dichroism · cysteine · nanoparticles · self-assembly · silver

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Received: September 8, 2009
Published online: November 20, 2009

