



Synthesis and characterization of mixed ligand chiral nanoclusters

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Received,
Accepted

DOI: 10.1039/x0xx00000x

www.rsc.org/

Chiral mixed ligand silver nanoclusters were synthesized in the presence of a chiral and an achiral ligand. The ratio of the ligands was changed to track the formation of these clusters. While the chiral ligand lead to nanoparticles, Presence of the achiral ligand induced the formation of nanoclusters with chiral properties.

Clusters of metal atoms have attracted attention since they provide a size scale that can cover the properties in between atomic and nanoscale behavior. In this size scale (2 nm and below),¹ unique properties such as intense absorption peaks covering visible and near IR spectra,^{2–4} tunable fluorescence,^{5–8} and discreet electronic transitions^{9–11} can be observed. Due to these properties and their small size possible applications in catalysis,^{12–15} sensing,^{16,17} and light harvesting^{2,18} are sought.¹⁹ Cluster/biomolecule complexes to detect phenol compounds or drugs^{20,21} and cluster doped nanoparticles/nanowires to increase catalytic activity of nanostructures^{22,23} were also studied.^{8,24,25}

Introduction of chiral properties to these clusters could extend their applications to chiroptical devices,^{26–28} enantioselective catalysis,^{29–31} as well as to new sensing methods.^{20,32,33} To impart chirality, usually a chiral ligand, such as D-L-penicillamine,³⁴ D-L glutathione^{33,35} or N-acetyl-L-cysteine,³⁶ is used to stabilize the cluster of atoms. While several biomolecules provide chiroptical activity in near UV, these metal/chiral ligand complexes are active in the UV-

Visible (UV-Vis) range due to discrete energy bands of the metal part.^{37–39} Hybrid complexes of metals and chiral ligands can facilitate the design of biosensors since these complexes have amplified CD signals compared to biomolecules.³⁵ Kitaev and Cathcart also reported the generation of mixed ligand chiral silver clusters and showed that the presence of a non-chiral ligand enhanced the chiroptical behavior of these clusters.⁴⁰

Here, we show the synthesis and characterization of chiral silver nanoclusters that are stabilized with a mixed ligand system: L-cysteine (L-cys) and mercaptohexanoic acid (MHA). In the reaction conditions that are tested, the use of MHA was critical for the formation of chiral nanoclusters compared to homoligand L-cys-stabilized silver nanoparticles. A nanocluster of ~10 kDa was consistently encountered even at different starting ligand ratios suggesting the stability of the complex of metals atoms with the ligands.

Mixed ligand silver nanoclusters (Scheme 1) were synthesized by reducing silver nitrate in methanol and water mixture at room temperature (RT). The double distilled water was used throughout the experiments. First, we mixed equal volumes (12.5 ml) of methanol and water; purged this mixture with nitrogen, and adjusted its pH to 11 by sodium hydroxide solution. We dissolved 4.45 mg (0.036 mmol) of L-cys in this solution and then added 5.08 μl (0.036 mmol) of MHA. In silver to thiol ligand ratio of 1, we used 12.5 mg (0.073 mmol) of silver nitrate and reduced silver by addition of 13.91 mg (0.36 mmol) sodium borohydride in water. The reaction was kept under vigorous stirring for 6 hours.

The color of the solution became yellow immediately and gradually turned into dark brown, then into red/pink. To end the

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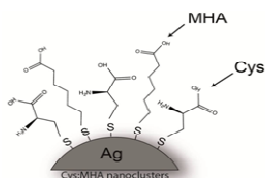
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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See

DOI: 10.1039/x0xx00000x

reaction, the product was precipitated by the addition of tetrahydrofuran (THF) and collected through centrifugation. For optical characterization, the product was resuspended in water. We followed the same procedure to synthesize nanoclusters with different ligand ratios of L-cys:MHA (1:2, 1:3, 2:1, and 3:1) always at a silver to total thiol ratio of 1:1. We also changed the silver to thiol ratio to track the formation of nanoclusters at the same L-cys: MHA ratio of 1:1.



Scheme 1 Schematics of Ag nanoclusters with cysteine and mercaptohexanoic acid.

The raw product was separated via PAGE (Cleaver, OmniPAGE mini vertical electrophoresis system) with a separating gel of 30 wt% and a stacking gel of 8 wt% acrylamide monomers (acrylamide/bisacrylamide=93/7), respectively. The size of the gel was 10 cm × 10 cm × 2 mm. A buffer solution of Tris-HCl with pH 8.8 for the separating gel and pH 6.8 for the stacking gel was used. The running electrode buffer was an aqueous mixture of glycine (192 mM) and Tris (25 mM). We took 1 ml of the reaction mixture, precipitated the product in THF, redissolved it in 50 μl water, and added 16% (v/v) of glycerol to this solution. We loaded 5 μl of this solution into the well of the stacking gel and eluted the sample for 3 hours at 4 °C with constant 200 V (Cleaver, CS-300V) to separate the product. To extract silver nanoclusters, the band at each fraction was cut and left in water for a day. Filters with 0.2 μm pore size were utilized to remove remaining lumps of the gel.

We recorded the absorption spectra of the clusters with a Shimadzu UV-3150 UV-Vis spectrometer. We used a Jasco J-810 CD spectrometer to track chiroptical properties of the clusters. Rectangular quartz cuvettes with a 1 mm path length were utilized throughout the measurements.

For the characterization of size, PAGE was utilized; nanoclusters that are synthesized with L-cys:MHA ratio of 1:1 was run against clusters of known size, Ag₃₂ and Ag₄₄, which are synthesized according to literature with glutathione and 5-mercapto-2-nitro benzoic acid (MNBA), respectively.^{41,42} Mass spectroscopy was carried out in an Autoflex speed MALDI-TOF with 1 kHz smartbeam-II laser in linear positive mode.

We systematically changed i) ligand and ii) silver to thiol ratio to determine the matrix of conditions that produce clusters. In UV-Vis spectrum, the peaks approximately around 385 and 550 nm indicate the formation of clusters.⁴¹ The peak below 400 nm is the characteristic of electronic interaction of metal core and ligands⁴³ and peak at 550 nm is attributed to large HOMO-LUMO gap due to discrete energy levels.⁴¹ At constant silver to thiol ratio (1:1), ligand ratios of 2:1, 1:1, 1:2, 1:3 provided almost overlapping UV-Vis spectra (Fig. 1); this overlap implied the formation of very similar structures even if different amounts of ligands were present in the reaction mixture. However, the use of solely L-cys lead to the formation of silver nanoparticles that revealed a characteristic peak at 440 nm due to surface plasmon resonance of silver.

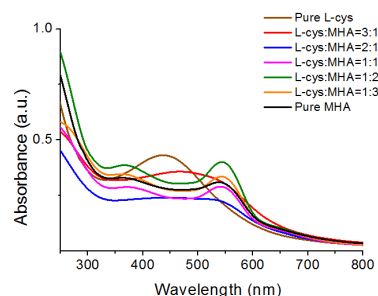


Fig. 1 UV-Vis spectra of the structures with different ligand ratios.

The reaction products were separated via PAGE to determine the nanocluster that is present in the synthesis with 2:1, 1:1, 1:2, 1:3 ligand ratios (Fig. 2a). UV-Vis spectroscopy revealed that Band 4 appeared in the aforementioned ratios is shown to contain nanoclusters (Fig. 2b).

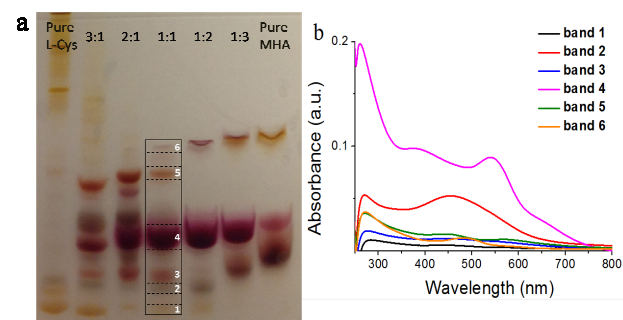


Fig. 2 (a) PAGE of the as-synthesized structures with different ligand ratios, (b) UV-Vis spectra of PAGE fractions of the sample with L-cys: MHA ratio of 1:1.

We monitored the chirality of this dominant band via CD spectroscopy in each synthesis. This band in each sample produced similar UV-Vis and CD spectrum underscoring the synthesis of the same cluster at mixtures with different ligand ratios.

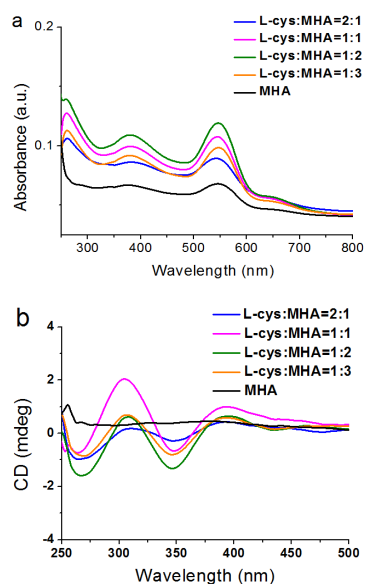


Fig. 3 (a) UV-Vis spectra of the Band 4 from the PAGE of the samples with different L-cys: MHA ratios, (b) CD spectra of the same samples.

We also changed silver to thiol ratio of these clusters at equimolar contributions from L-cys and MHA. We observed the formation of clusters at ratios of 1:1, 1:1.25, and 1:1.5 but at the ratio of 1:2 only a small amount of clusters was formed. (Fig. 3a) All of these samples produced chiral signals in CD spectroscopy demonstrating the formation of nanoclusters with similar chemistry (Fig. 3b). In addition, when we carried out the experiments with a molecule of reverse chirality, D-cysteine (D-cys), we again observed the formation of clusters but of reverse chirality (Fig. 1S). This result indicates that chirality of these clusters is due to chiral ligand-based formations.

We compared migration of as-synthesized silver nanoclusters with known glutathione-Ag32 and MNBA-Ag44 clusters in the same gel.^{41,42} Irrespective of the cluster loading concentrations, we found that the distance travelled by either band of L-cys:MHA (1:1) cluster in the gel is between Ag32 and Ag44 bands. The respective masses of glutathione-Ag32 and MNBA-Ag44 clusters are known to be 9.27 kDa and 10.68 kDa suggesting the mass of the L-cys:MHA clusters should be in this range.

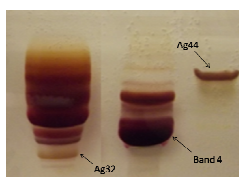


Fig. 4 PAGE of as-synthesized L-cys:MHA (1:1) silver nanoclusters with glutathione-Ag32⁴¹ and MNBA-Ag44 clusters⁴² in the same gel.

To verify the mass range obtained from PAGE, we also characterized L-cys:MHA clusters using mass spectrometry. Nanoclusters in Band 4 are extracted into water. We were unable to get a conclusive mass spectrum with ESI and MALDI (aqueous saturated solution of 2, 5 dihydroxybenzoic acid and α -cyano 4-hydroxycinnamic acid as a matrix). However, using laser desorption ionization (LDI), we found a single broad cluster peak at $\sim 12.5 \pm 3$ kDa (Fig. 5), given the uncertainties in the measurement this result is not inconsistent with the PAGE one.

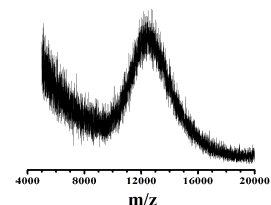


Fig. 5 LDI mass spectrum of band 4 of L-cys:MHA (1:1) nanoclusters.

During synthesis, the formation of red/pink nanoclusters starts after an hour and we stop the reaction after 6 hours. If the clusters are left at room temperature (RT) in the reaction mixture, they aggregate irreversibly. To examine the effect of temperature and solvent on the stability of the clusters, we kept the clusters in i) their initial reaction environment (water/methanol solution at a pH of 11), ii) water/methanol solution, and iii) water. We also stored these solutions at -18 °C, 4 °C, and RT.

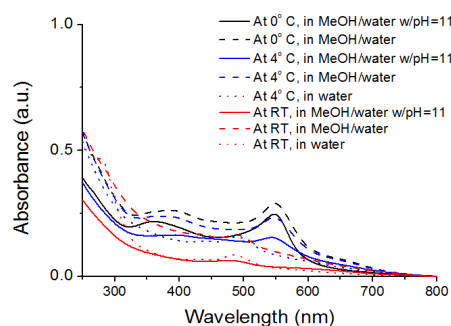


Fig. 6 UV-Vis spectra of samples with L-cys: MHA ratio of 1:1 that are kept at -18 °C, 4 °C, and RT in water, water/methanol solution, and initial reaction conditions for 3 weeks.

We observed a color change in the samples that are kept at RT just after 4 days while the others preserved their color. To monitor the long-term stability, we performed UV-Vis spectroscopy after 3 weeks. The absorption peaks of clusters at 550 nm were preserved in the samples that were kept at -18 °C and 4 °C in methanol/water solution at high pH and in the initial reaction environment.

In conclusion we synthesized chiral nanoclusters with a binary ligand system. We observed that formation of nanoclusters is linked to the presence of the achiral ligand, MHA, whereas the chirality results from the presence of L-Cys. Additionally, regardless of ligand ratio, in most of the binary ligand systems we observed the formation of a chiral nanocluster with similar chemistry.

O.A. acknowledges FP7 Marie Curie Reintegration Grant and UNESCO/L'Oreal Women in Science Fellowship. Z.P.G. acknowledges The Scientific and Technological Research Council of Turkey (TUBITAK)-BIDEB-2210 Scholarship. F.S. acknowledges support of the Swiss National Science Foundation. The authors thank Hasan Kurt and Prof. Cleve Ow Yang for valuable discussions and insights.

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