Metal-Ion-Induced Luminescence Enhancement in Protein Protected Gold Clusters

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Supporting Information

ABSTRACT: We probed the interaction between Au38@BSA and various heavy metal ions using luminescence spectroscopy. Interestingly, Au38@BSA showed luminescence enhancement upon interaction with Cd2+ and Pb2+ at concentrations higher than 1 ppm, due to the formation of cluster aggregates. Such aggregates were detected by dynamic light scattering (DLS) and high resolution electron microscopy (HRTEM) studies. Luminescence enhancement of Au38@BSA in the presence of Cd2+ was due to the interaction of Cd2+ with the cluster core, while Pb2+-induced luminescence enhancement was due to BSA-Pb2+ interaction. Observations were further supported by X-ray photoelectron spectroscopy (XPS) studies. This kind of phenomenon has been observed in protein protected clusters for the first time. We believe that such metal-ion-induced luminescence enhancement can be used to synthesize cluster systems with enhanced optical properties and different ion–cluster interactions can be used to develop metal ion sensors using Au38@BSA.

INTRODUCTION

The study of noble metal nanoclusters consisting of a few to hundred metal atoms has become fascinating due to their unique optical and electronic properties. Especially, Au and Ag metal nanoclusters have been studied extensively due to their attractive optical properties. Such nanoclusters typically use thiols as protecting ligands, and various protocols in their synthesis. Macromolecular templates such as DNA, dendrimers, and most recently proteins, have also been used for the synthesis of such nanoclusters. The most commonly used proteins are bovine serum albumin (BSA), lysozyme (Lyz), lactoferrin (Lf), human serum albumin (HSA), and a few others. Protein protected noble metal clusters (PPCs) have been synthesized under basic pH and are stable over a wide pH range. Typically the core of such nanoclusters is less than 2 nm in diameter. PPCs exhibit attractive optical, electronic, catalytic, and magnetic properties. Luminescence of PPCs is stable under different pH conditions, and their quantum yield is high as compared to their monolayer protected counterparts. Due to the simple synthetic procedure and ease of modification with various functional groups, PPCs have been considered as major candidates for biolabeling, in vivo and in vitro imaging, and various sensing applications. They are biocompatible due to lower metallic content and use of bulky proteins as ligands. High quantum yield and presence of various functionalities of PPCs can be used for highly selective and sensitive detection of analytes in various applications.

Owing to their small size, biocompatibility, luminescence, and low toxicity, PPCs are good candidates for sensing of metal ions and small molecules. Intense photoluminescence (PL) is one of the most interesting properties of PPCs. According to the previous reports, the reason for the high quantum yield of such clusters is FRET between the protein shell and the core of the cluster. Recently, Chevrier et al. have studied the structural and intense luminescence properties of the BSA-stabilized gold cluster in detail. It is also possible to tune the luminescence property of nanoclusters by changing the composition through alloying, doping, etc. Enhancement of luminescence through different routes has been studied by several groups. In our previous report, we have shown that an Au cluster protected by mixed proteins shows 3-fold enhancement in luminescence due to FRET. Luo et al. have reported aggregation-induced luminescence enhancement of Au(I) thiolate where they have shown that the Au(I) thiolate shell surrounding the Au(0) core plays a role in enhancing the luminescence. Metal-induced luminescence enhancement has been reported by Muhammed et al., where they found that the enhancement of luminescence in Au38@BSA was due to Ag nanoparticles where protein acts as a spacer between the gold cluster and the nanoparticles. A similar study has been demonstrated for glutathione-capped Au clusters by Ji et al.,

Received: August 2, 2019
Revised: October 29, 2019
Published: November 4, 2019

DOI: 10.1021/acs.jpcc.9b07370
J. Phys. Chem. C 2019, 123, 28969−28976

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where they have found that the enhancement was due to the formation of aggregates through GSH–Pb²⁺ interaction. Exchanging the core with other metals can significantly change the luminescence property of a cluster as shown by Wang et al., where they observed drastic fluorescence enhancement in an Au cluster when it was doped with Ag atoms.

It can be concluded from the above discussion that reactivity of clusters with metal ions can change their properties drastically. In particular, heavy metal ions can react with clusters in different ways, either with the core or with the protecting shell. Heavy metal ion contamination is one of the serious threats to human health and environment due to their toxic effects. Some of the heavy metals are biologically essential such as copper (Cu), zinc (Zn), and iron (Fe) but at higher concentrations they can lead to toxicity while other heavy metals, namely, mercury (Hg), cadmium (Cd), and lead (Pb), are not biologically essential, and their presence even at lower concentrations can cause harm to the organism. Although various conventional analytical techniques have been used for analyzing metal ions, PL spectroscopy is one of the simplest tools for such analysis.

Due to strong surface plasmon resonance and its dependence on the surface protection, gold nanoparticles have been employed to detect heavy metal ions. DNAzyme biosensors also showed good selectivity and sensitivity for detecting heavy metal ions. PPCs are highly sensitive to the luminescence property of a cluster as shown by Wang et al., where they have found that the enhancement was due to the formation of aggregates through GSH–Pb²⁺ interaction.

**Experimental Methods**

Reagents and Materials. All the chemicals were commercially available and used without further purification. Bovine serum albumin, sodium hydroxide, tetrachlorauric acid trihydrate, PbCl₂, CdCl₂, CdO, Cd₂⁺, Cu₂⁺, Pb²⁺, and Hg²⁺ were chosen. Metal ion solutions of various concentrations were obtained by serial dilution of the stock solution. For performing metal ion interaction studies with AuQC@BSA, various metal ions such as Cd²⁺, Pb²⁺, Hg²⁺, Fe²⁺, and Cu²⁺ were chosen. Metal ion solutions of various concentrations were obtained by serial dilution of the stock solution. Typically, 5 μL of the cluster solution was diluted 400 times with distilled water. To study the interaction, different concentrations of metal ions were added to the above-mentioned cluster solution. After addition, the solution was allowed to stir for 5 min. Then 100 μL of 1 M NaOH was added to the above mixture and stirred for 12 h until the solution turned golden brown in color. The reaction was carried out at room temperature. The solution of AuQC@BSA was stored at 4 °C for further use.

**Synthesis.** Synthesis of AuQC@BSA. AuQC@BSA was synthesized as reported previously. In a typical synthesis, 1 mL of 6 mM tetrachlorauric acid trihydrate (HAuCl₄·3H₂O) solution was added to 25 mg of BSA powder in 1 mL of distilled water, under vigorous stirring. The mixture was allowed to stir for 5 min. Then 100 μL of 1 M NaOH was added to the above mixture and stirred for 12 h until the solution turned golden brown in color. The reaction was carried out at room temperature. The solution of AuQC@BSA was stored at 4 °C for further use.

**Study of Interaction of AuQC@BSA with Different Metal Ions.** For performing metal ion interaction studies with AuQC@BSA, various metal ions such as Cd²⁺, Pb²⁺, Hg²⁺, Fe²⁺, and Cu²⁺ were chosen. Metal ion solutions of various concentrations were obtained by serial dilution of the stock solution. Typically, 5 μL of the cluster solution was diluted 400 times with distilled water. To study the interaction, different concentrations of metal ions were added to the above-mentioned cluster solution. After addition, the solution was mixed well and incubated for 2 min before recording the luminescence spectrum. It is well-known that Cu²⁺ and Hg²⁺ ions can quench the luminescence of AuQC@BSA but the interaction of AuQC@BSA with other metal ions has not been studied in detail. Therefore, from the perspective of toxic heavy metal ions, interactions of Cd²⁺ and Pb²⁺ were studied in detail.

**Results and Discussion**

Spectroscopic and Microscopic Characterizations of AuQC@BSA. Synthesis and characterization of gold cluster within BSA template was reported by Xie et al. Briefly, the addition of HAuCl₄·3H₂O to BSA forms Au⁺–BSA complex. BSA contains 21 tyrosine residues which can reduce Au⁺ to Au⁻. Further reduction of Au⁻ to Au⁺ occurs by adding NaOH into the mixture. At alkaline pH, BSA acts both as a reducing and as a capping agent for Au cluster synthesis. Due to the bulkiness of BSA, it provides steric protection to the cluster. AuQC@BSA has been characterized using different spectroscopic and microscopic techniques (Figure 1), and the core of the cluster has been assigned using MALDI MS study (Figure S1).
A comparison between UV–vis absorption spectra for pure BSA and AuQC@BSA is depicted in parts A and B of Figure 1, respectively. BSA shows an absorption feature at 280 nm due to the presence of aromatic amino acids such as tyrosine and tryptophan residues. In the case of AuQC@BSA, a decrease in the absorption intensity at 280 nm was observed along with a shoulder at 375 nm as compared to that of pure BSA. The absence of a well-defined absorption feature in the case of clusters has been attributed to the encapsulation of the cluster by bulky BSA.13 The PL excitation and emission spectra of the cluster are presented in parts C and D of Figure 1, respectively. Two excitation maxima around 365 and 500 nm were observed for the cluster as previously reported.12 When the cluster was excited at 365 nm, two emission maxima, one around 450 nm which is due to weak luminescence from the protein and the other at 645 nm because of emission from the cluster, were found. The cluster showed bright red luminescence and photographs of the cluster under visible light and UV light are shown in Figure 1, parts E and E’, respectively. To study the size of clusters, HRTEM analysis was performed (Figure 1F). The core of the cluster was found to be below 2 nm, and no particles of bigger size were found. MALDI MS study was performed to assign the core of the cluster. The calculated mass difference between the parent protein and the cluster formed provides the number gold atoms present in the core of the cluster. AuQC@BSA has a peak at m/z ~74 210 and the mass difference between AuQC@BSA and BSA (m/z 66 700) was ~7.5 kDa, suggesting the formation of Au38@BSA. So, henceforth AuQC@BSA will be referred as Au38@BSA.

With this background, our studies on the interaction of different metal ions with the cluster are discussed in the next section.

**Metal-Induced Enhancement of Photoluminescence in Au38@BSA.** Clusters can interact with various metal ions through chemical functionalities of the protein or through the metal core. Interaction between different metal ions and clusters can bring changes in the PL as well as other properties of the cluster. Here, we have studied the effect of various metal ions such as Cd2+ and Pb2+ on the PL of Au38@BSA. The emission spectra for Au38@BSA and BSA in the presence of Cd2+ and Pb2+ ions at different concentrations are shown in Figure 2.

As shown in Figure 2A, different concentrations of Cd2+ were added to Au38@BSA to monitor its effect on the luminescence profile. A decrease in the PL intensity was observed upon addition of 100 ppb Cd2+. However, at 500 ppb, instead of further decrease, it leads to an increase in the PL intensity. Moreover, the addition of 1 ppm of Cd2+ resulted in higher emission intensity as compared to the parent cluster. With further increase in the concentration of Cd2+ from 1 to 10 ppm, luminescence intensity increased systematically. A gradual blue shift in the emission peaks from 650 to 630 nm was also noticed in the process, and when the concentration of Cd2+ reached at 10 ppm, it resulted in a ~2.7 fold enhancement of emission intensity. To find out the role of BSA on Cd2+ and Au38@BSA interaction, similar concentrations of Cd2+ were added to BSA, and emission spectra were collected (Figure 2B). BSA showed an emission maximum ~335 nm when excited at 280 nm. At lower concentrations of Cd2+, no change in the protein emission was noticed. Upon addition of increasing concentrations of Cd2+, changes in the luminescence of BSA were less marked and were opposite to that of Au38@BSA. Thus, it is suggested that PL enhancement in Au38@BSA due to the interaction of Cd2+ with the cluster core and not with the protein shell. Further studies with DLS and HRTEM were performed to understand the effect of Cd2+ on Au38@BSA. These results are discussed in the next section of the paper.

Similar measurements were conducted to know the effect of Pb2+ on the PL properties of Au38@BSA. Upon addition of 100 ppb of Pb2+, a decrease in the PL intensity of Au38@BSA was observed (Figure 2C) which was similar to that of Cd2+. Further addition of Pb2+ (500 ppb to 10 ppm) resulted in a gradual increase in the luminescence intensity. Addition of 10 ppm Pb2+ caused a ~1.6 fold enhancement of the cluster. To understand the role of protein in this interaction, similar concentrations of Pb2+ were added to BSA. A systematic decrease in the protein emission was observed with increase in concentration of Pb2+ from 100 ppb to 10 ppm without any change in the position of emission. Pb2+ has a tendency to bind to proteins which could induce the aggregation of clusters through protein–protein interaction.35 Such aggregation can result in aggregation-induced enhancement in luminescence. Although enhancement was observed in the presence of both the metal ions, Cd2+ showed a higher enhancement than Pb2+ at similar concentration, although reasons for enhancement could be different in both the cases. In the case of Cd2+, major interaction was with the core and in the case of Pb2+, major interaction was with the protein shell of the cluster. A shift in the cluster emission toward the blue region upon interaction with Cd2+ also suggests that the core itself is changing after interaction with Cd2+. The calculated quantum yields for Au38@BSA and Au38@BSA in the presence of Pb2+ and Cd2+ were found to be 8.0%, 13.0% and 15.0%, respectively.

Control PL study was performed to check the sensitiveness of the incubation time (Figure S2A). Same parameters were maintained during each measurement. No enhancement in the emission intensity was observed in the parent cluster over time. During the course of time, no precipitates were seen either in the solution of parent cluster or in the presence of Pb2+ and Cd2+. Time-dependent changes in I650 of Au38@BSA upon
addition of 1 ppm concentration of Pb^{2+} and Cd^{2+} were measured to check the stability of the cluster. When Pb^{2+} (Figure S2B) and Cd^{2+} (Figure S2C) were added to the cluster solution, a large increase in I_{550} counts was observed, which was stable over long time. This suggested that the enhancement in emission intensity was due to the presence of Pb^{2+} and Cd^{2+}.

UV–vis absorption spectra of Au_{38}@BSA in the absence and presence of metal ions have been measured (Figure S3). As mentioned earlier, prominent absorption features were absent in protein protected gold clusters due to encapsulation by the bulky protein. The absorption peak at 280 nm is the characteristic feature of aromatic amino acids of the protein. No prominent change in the absorption features was seen upon adding Cd^{2+} to the cluster solution while a significant change was found upon Pb^{2+} addition. This change indicated that there is an interaction of Pb^{2+} with the protein shell of the cluster which also supports the PL studies.

**Metal-Ion-Induced Aggregation Studies of Au_{38}@BSA by DLS and HRTEM.** To investigate the effect of metal ions on the size of Au_{38}@BSA, DLS measurements were performed (Figure 3, parts A and B). From the DLS study, the size of BSA was found to be ~7.6 nm and parent Au_{38}@BSA has a size of ~9.7 nm, which are closely matching with the values reported (Figure S4).50 This suggested that the cluster core to be of 2.1 nm and this value is slightly more than the size observed in HRTEM analysis (Figure 1F). The larger size observed in DLS measurement than by HRTEM is due to the presence of a solvation shell around the cluster in water. The volume fraction-dependent DLS measurement was carried out for the parent cluster. But no change in the size was observed with increase in concentration of the cluster (Figure S5). Figure 3A shows the changes in the size distributions of Au_{38}@BSA when different concentrations of Cd^{2+} were added to the former. Size of the cluster (9.7 nm) increased gradually upon interaction with increasing concentrations of Cd^{2+}, and finally, it reached ~40 nm at 10 ppm of Cd^{2+}. Similar results were also obtained in the case of Pb^{2+} (Figure 3B) but at 10 ppm, it led to bigger aggregates of the clusters. Changes in the size of the parent cluster in the presence of Cd^{2+} and Pb^{2+} implied that the interaction of both the metal ions with the cluster induced their aggregation.

To further confirm the aggregation of the cluster, HRTEM analysis was performed at a higher concentration of metal ions (Figure 3, parts C, C′, D, and D′). TEM images of the clusters with Cd^{2+} are shown in Figure 3, parts C and C′. It clearly shows aggregation of the clusters. Similar aggregates were also found in the case of Pb^{2+} (Figure 3, parts D and D′). Compact aggregation of clusters was seen in this case (Figure 3D). These aggregates are mostly spherical in shape. Magnified image of one such spherical aggregate is shown in Figure 3D′. In both the cases, sizes of the clusters are much larger than the parent one (Figure 1F). This result confirms the metal-ion-induced aggregation of the cluster. The presence of Cd^{2+} and Pb^{2+} has been confirmed by EDS analyses (Figures S6 and S7).

**XPS Studies of Au_{38}@BSA in the Presence of Cd^{2+} and Pb^{2+}** X-ray photoelectron spectroscopy (XPS) is an important tool to reveal the oxidation states of elements in the sample. XPS analysis of parent Au_{38}@BSA has shown that Au 4f_{7/2} appears at 84.1 eV, confirming the presence of a stable metallic core (Au^{0} state) in the cluster (Figure S8A).17 The binding energy of S 2p_{3/2} at 162.1 eV suggested Au–S bonding which stabilizes the core through cysteine residues of the protein (Figure S8B). The PL data shown in Figure 2 proposed that the major interaction of Cd^{2+} is with the core of the cluster whereas Pb^{2+} interacts with the BSA shell of the cluster. To further investigate metal-ion-induced changes in the oxidation states of cluster core and interacting elements, we carried out XPS analysis of Au_{38}@BSA upon interaction with Cd^{2+} and Pb^{2+} (Figure 4).

Due to the interaction of Cd^{2+}, Au 4f_{7/2} at 84.1 eV got shifted to 85.3 eV suggesting the oxidation of the core to Au^{1+}. However, two peaks at 406.4 and 406.6 eV binding energy were seen in the Cd 3d region and assigned as Cd^{2+} and Cd^{0}, respectively. After interaction with Pb^{2+}, only a change of 0.4 eV in the binding energy of Au 4f_{7/2} was observed (from 84.1 to 84.5 eV), indicating the core to be closer to its metallic state, and the binding energy at 138.9 eV was the characteristic feature of Pb^{2+}. The XPS data have shown that Cd^{2+} induced a
change in the oxidation state of the cluster core, i.e. from Au\(^0\) to Au\(^+\) whereas Pb\(^2+\) did not bring a significant change in the core of the cluster. This also supported the results obtained from PL studies discussed earlier.

Figure 3. DLS spectra of Au\(_{38}@\)BSA at various concentrations of (A) Cd\(^2+\) and (B) Pb\(^2+\). Data for parent Au\(_{38}@\)BSA are also shown. TEM images showing the aggregation of clusters upon adding (C) Cd\(^2+\) and (D) Pb\(^2+\). The corresponding higher magnification TEM images are shown in parts C\(^′\) and D\(^′\), respectively.

Figure 4. (A and B) Au 4f region of Au\(_{38}@\)BSA upon interaction with Cd\(^{2+}\) and Pb\(^{2+}\), respectively. (C and D) Corresponding Cd 3d and Pb 4f regions, respectively.

DOI: 10.1021/acs.jpcc.9b07370
J. Phys. Chem. C 2019, 123, 28969−28976
Selective Luminescence Enhancement of Au38@BSA Due to Metal Ions. The interactions of Au38@BSA with Cd2+ and Pb2+ were discussed in the earlier sections and enhancement of luminescence was observed at higher concentrations of Cd2+ and Pb2+ while luminescence was quenched at lower concentration. The luminescence response of Au38@BSA was studied with other divalent metal ions such as Fe2+, Hg2+, and Cu2+, and the changes were compared with Cd2+ and Pb2+ ions. Figure 5 shows the change in the luminescence of the cluster after treatment with metal ion concentrations starting from 1 ppb to 1 ppm. Area under the emission spectrum of Au38@BSA (550−710 nm wavelength range) is plotted against the molar ratio of metal ion to Au38@BSA, for different analyte ions.

After addition of each concentration of metal ion to cluster, the cluster solution was incubated for 2 min before collecting the emission spectra. Only in the presence of Cd2+ and Pb2+ luminescence enhancement was seen but not in the case of Fe2+. Whereas at lower concentrations of Cd2+, Pb2+, and Fe2+, a decrease in the emission intensity was noticed. Enhancement in emission started from 0.1 ppm of Cd2+ and Pb2+ onward. In the earliest sections, we have already discussed the effect of increase in the concentration of Cd2+ and Pb2+ (Figure 2, parts A and C). Unlike Hg2+, Cu2+ also is known to quench the luminescence of Au38@BSA.13,24 In both cases, at lower concentrations (up to 100 ppb), a decrease in the luminescence intensity was observed, but complete quenching occurred only at 1 ppm of ions. The interaction of Cu2+ with BSA shell of the cluster has been proposed as a possible reason for this luminescence quenching, whereas a similar quenching effect in the case of Hg2+ is due to the interaction between the metal ion and the core of the cluster.13,24

CONCLUSIONS

The interaction of Au38@BSA with Cd2+ and Pb2+ has been investigated in great detail. Au38@BSA was found to have aggregation-induced emission enhancement in the presence of Cd2+ and Pb2+, for concentrations higher than 1 ppm. This phenomenon has been studied for the first time in the case of PPCs. The enhancement of luminescence is due to aggregation of clusters, and such aggregates were detected by DLS and HRTEM analyses. PL studies have shown that, in the case of Cd2+, enhancement in the luminescence is due to interaction between Cd2+ and cluster core whereas PL enhancement in Au38@BSA upon Pb2+ is due to Pb2−protein shell interaction. These observations were further supported by XPS data, where it was shown that interaction with Cd2+ resulted in the oxidation of the cluster core from Au0 to Au+ along with changes in binding energy of Cd2+, but interaction of Pb2+ did not affect the core of the cluster. Interactions of other metal ions such as Fe2+, Hg2+, and Cu2+ with Au38@BSA were also studied and it showed that such interaction is selective to Cd2+ and Pb2+. Difference in the nature of interactions between heavy metal ions and Au38@BSA may be used to develop a sensor with a logical readout for identifying different metal ions. Also, such metal-ion-induced aggregation of clusters leading to emission enhancement will open up the possibility of developing clusters with enhanced optical properties and associated applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcc.9b07370.

Comparative MALDI MS of BSA and ∼Au38@BSA, control PL study and time dependent change in I650 of Au38@BSA, UV−vis absorption spectra of Au38@BSA in the presence of Cd2+ and Pb2+, DLS data, HRTEM EDS spectra, and XPS data of Au38@BSA (PDF)

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Notes

The authors declare no competing financial interest.
ACKNOWLEDGMENTS

We thank the Department of Science and Technology, Government of India for their continuous support on our research program on nanomaterials. J.S.M. thanks Council of Scientific and Industrial Research (CSIR) for her research fellowship. C.S. thanks IIT Madras for his research fellowship.

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