Gold Nanocluster Protection of Protein from UVC Radiation: A Model Study on Bovine Serum Albumin

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ABSTRACT: Harmful UVC (200–280 nm) radiation is entirely screened by a combination of dioxygen and ozone in the stratosphere. However, because of environmental pollution, depletion of stratospheric ozone layer is increasing alarmingly, ensuing danger of penetration of the harmful UVC through the earth’s atmosphere to reach the living world. Studies have shown that UVC radiation accelerates aging of albumin solutions along with other qualitative changes. Herein, we have used in situ grown and ex situ added gold nanoclusters (AuNCs) to minimize the damage in the protein structure caused by long-term UVC exposure. The effects were demonstrated in the absence and presence of lipid vesicles to mimic the biological environment. Bovine serum albumin (BSA) has been used as the model protein that contains ~50–60% helicity. It is observed that UVC converts most of α-helix into β-sheet, leading to the aggregation of protein. The ingrown AuNCs could provide about 23–40% protection to the secondary structure, whereas the externally added AuNCs preserved almost 73–82%. To generalize this finding, we have also studied the effect of AuNC protection on the UVC-exposed lysozyme protein. The results show that the proposed method is indeed helpful for life.

1. INTRODUCTION

The minute concentration of ozone in the ozone layer of the stratosphere is extremely important to the living world because it absorbs biologically harmful ultraviolet (UV) radiation coming from the Sun. UV radiation can principally be divided into three categories based on wavelengths: UVA (320–400 nm), UVB (280–320 nm), and UVC (200–280 nm). The most harmful UVC and the moderate UVB are almost entirely blocked by the ozone layer. The less harmful UVA reaches the earth’s crust. However, increase in environmental pollution leads to the depletion of the ozone layer resulting in percolation of UVC. Recently, Michnik et al. have shown that UVC exposure may induce accelerated aging of albumin protein.1 Even, UVC-treated plants show adverse effects on shoot growth, leaf number, protein and carbohydrate contents in leaf as well as chlorophyll a and b, and total carotenoid contents.2 In a study on γD-crystallin protein, present in human eye lens, Wang and Wen reported profuse aggregation of the protein upon prolonged exposure to UVC.3 Reports state that UVC can potentially damage proteins through photolysis-generating reactive species, resulting in protein thiol oxidation.4 Moreover, Seker et al. exposed the alarming effect of UVC induced DNA damage that leads to p53-dependent nuclear trafficking of a promyelocytic leukemia protein, which is a nuclear phosphoprotein that localizes to distinct domains in the nucleus.5

Some remedial measures to the adverse effects of UVC exposure have been proposed. It is reported that treatment with 5-fluorouracil enhances the stability of bovine serum albumin (BSA) protein by 2-fold under UVC irradiation.6 Shih and Cherng showed that treatment by a Chlorella-derived peptide resists UVC-induced cytotoxicity through the inhibition of caspase-3 activity and reduction of phosphorylated Fas-associated protein with death domain (FADD) and cleaved polymerase-1 expression.7 Progressing with the recent trend in nanomaterials research, herein, we present the remedial effect of gold nanoclusters (AuNCs) to the adversity of UVC exposure that induces protein damage. There is hardly any report on this aspect, and we believe that the simplicity in the application of the present method will attract studies in the pharmaceutical field. BSA protein has been used as a model in this study, and AuNCs were either grown in situ or added externally to compare the extent of protein damage by UVC exposure. Experiments on the irradiation effect were also performed in presence of lipid vesicles where the model protein is trapped at the liposomal interface to mimic cell membrane. The results are encouraging as the protein could be protected up to ~70%. The consequences were generalized through studies on the lysozyme (Lys) protein in which similar results were obtained.

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2. RESULTS AND DISCUSSION

BSA-coated near-infrared (NIR)-emitting AuNCs (NC1) and L-glutathione (GSH)-coated AuNCs (NC2) were synthesized for the in situ and the ex situ studies, respectively. NC1 and NC2-treated BSA (NC2@BSA) were separately embedded in 1,2-dimyristoyl-sn-glycero-3-phosphoglycerol (DMPG) vesicles for the liposomal studies. Dynamic light scattering (DLS) experiments (Figure 1) show the hydrodynamic diameters for BSA = 6.6 nm, NC1 = 8.8 nm, NC2 = 2.7 nm, and NC2@BSA = 9.3 nm. The data show that the AuNC produced in BSA (NC1) is 2.2 nm in diameter. The sizes correlate well with the obtained emission spectra.8,9

The AuNCs were characterized by their absorption and fluorescence spectra (Figure 2). The 280 nm band in each of the absorption spectra is due to BSA. NC1 shows two peaks at 435 and 650 nm in its emission spectrum (Figure 2a). NC2 shows its emission maximum at 650 nm (Figure 2b) that shifts to 626 nm in NC2@BSA because of its encapsulation in the less polar protein environment (Figure 2c). The long-wavelength bands are due to the AuNCs. The blue emission is due to the oxidative states of BSA in the presence of HAuCl4.10

Prolonged exposure of BSA to UVC (up to 3 h) shows the appearance and enhancement of a distinct absorption band at 318 nm in aqueous medium in addition to the band at 278 nm (Figure 3a). Increase in 280 nm absorption band indicates structural changes induced in BSA and that in 318 nm band shows aggregation of the protein.1,11,12 We have also intercalated the protein in DMPG vesicle to biomimic the system and performed the same set of experiments as

Figure 1. DLS histograms of (from left to right) (upper panel) BSA (6.6 nm) and NC1 (8.8 nm) and (lower panel) NC2 (2.7 nm) and NC2@BSA (9.3 nm).

Figure 2. Absorption (black) and emission (red, ex = 280 nm and blue, ex = 370 nm) spectra of (a) NC1, (b) NC2, and (c) NC2@BSA in the aqueous medium at room temperature and neutral pH.
mentioned above, where changes were found to be similar (Figure 3b). Emission from the embedded tryptophan in BSA concomitantly decreases along with a consistent bathochromic shift upon exposure to UVC (Figure 3c,d). As the fluorescence yield of tryptophan is much lower in bulk water compared with that while embedded in BSA, progressive quenching of emission from BSA with UVC exposure indicates a change in conformation of the protein resulting in exposition of tryptophan toward the bulk environment.13,14 The bathochromic shift shows that polarity around the fluorophore increases as time progresses.

Far-UV circular dichroism (CD) spectroscopy provides quantitative information on the secondary structure. The CD spectra of BSA and BSA-incorporated DMPG vesicles indicate a change in the secondary structure of BSA (Figure 4). Initially, the CD spectrum of BSA gave a large value of molar ellipticity at 222 and 208 nm, indicating ∼52% α-helix content in the BSA structure and presence of ∼13% β-sheet.15,16 Upon exposure to UVC for about 3 h, we found a decrease in the α-helix content in BSA both under free and intercalated conditions to ∼1.72% along with an augmentation of β-sheet to ∼29%. Such circumstances are known in other modes of denaturation of BSA protein as well. Holm et al. showed that BSA aggregation takes place at an elevated temperature where the thermal aggregates have amyloid properties.17 Fibrillation, which occurs over minutes to hours, does not have a lag phase and is independent of seeding.

While admiring the previous reports on the effect of UVC on proteins4–5 and the remedies to lower the adverse effect of the radiation,6,7 we propose a simpler AuNC-based model that can be applied to reduce the unwanted curse of UVC exposure due to environmental pollution considerably. Hence, we have synthesized the in situ and ex situ variants of BSA-protected AuNCs and mimicked the biological cells by intercalating them at the DMPG interface. AuNC generation in situ (NC1) changes the secondary structural motif of BSA, where we recorded the percentages of α-helix and β-sheet to be 41.43 and 3.2, respectively. This is in accordance with the previous reports.18,19 Absorption of the ex situ generated GSH-protected AuNCs (NC2) inside of BSA, which can be achieved by incubating the aqueous solution of NC2 with BSA for 6 h, marginally changes the α-helix and β-sheet percentages to ∼50 and ∼11, respectively, compared to the parent BSA. Comparing the status of NC1 and NC2@BSA, it becomes apparent that the latter could be a better candidate because the change in the secondary structure of BSA is much less.

The absorption spectra of NC1 and NC2 show an augmentation of a shoulder peak at ∼320 nm as before, but the extent of increase is remarkably low (Figure 5). The relative absorbance plots of the AuNC-based species show that the secondary structure destruction of BSA is reduced by 40 times (Figure 6a). Comparing the activities of NC1 and NC2 and their liposome-intercalated counterparts, it is obvious that the action of NC2@BSA in a DMPG vesicle is most superior in the group (Figure 6b). This is encouraging in the sense that NC2 is added externally to BSA and the absorbed composite is intercalated in a DMPG vesicle to provide a suitable mimic to the mechanism in the biological cellular environment. Hence, it is apparent that the application of ex situ NC2 would minimize...
the adverse effect of UVC radiation on a biological cellular protein.

Excitation of native BSA at 280 nm yields emission maximum at 340 nm (Figure 3c). The DMPG-vesicle-intercalated BSA emits at 330 nm (Figure 3d). This provides evidence about the existence of BSA in much lesser polarity inside of the liposomes. We observed that NC1 shows multiple emission bands at 345, 400, and 665 nm (Figure 7a). The 345 nm emission is that of BSA in which a 5 nm bathochromic shift indicates a higher micropolarity due to openness in the protein structure. It is known that while synthesizing AuNC inside of BSA, the protein not only acts as a reducing agent but also develops oxidative states of BSA. The 400 nm band is thus attributed to the oxidative states of BSA, and the 665 nm band denotes emission from the AuNCs. The oxides of BSA are generated by HAuCl₄ during the synthesis process while developing the AuNCs in situ. Production of the GSH-protected AuNCs (NC2) uses HAuCl₄ ex situ and thus does not produce the oxidative states in BSA as mentioned above. This is clearly reflected in the emission spectrum of NC2@BSA, where we find two emission bands at 340 and 665 nm for BSA and the AuNCs, respectively (Figure 7b). Fluorescence spectral changes in the liposome-intercalated AuNC variants are also similar. The relative decrease in BSA emission is found to be the highest for the unprotected BSA and the lowest for NC2 in BSA intercalated in a DMPG liposome (Figure 8). Simultaneously, the 665 nm band of AuNCs also suffers a decrease in fluorescence because of the change in microenvironment.

We did far-UV CD spectroscopy with the AuNC samples to compare and provide an idea about the quantitative structural changes in BSA upon exposure to UVC for a prolonged period of time (Figure 9). The change in ellipticity at 222 and 208 nm indicates α-helix percentage that decreases from 41.43 to 11.38 and 16.84 for bare NC1 and in liposomes, respectively (Figure 9a). Encouragingly, we observed that the decrease in α-helix percentage is from 49.88 to 38.40 and 38.85 for bare NC2@BSA and in liposomes after UVC exposure, respectively (Figure 9b). The β-sheet populations in the above cases are 25.97, 22.46, 14.93, and 14.64. These figures are far better in comparison with those obtained from the action of UVC on BSA (vide supra). NC2@BSA intercalated in DMPG vesicles provides maximum resistance to the adverse effects of UVC.

The results were generalized by studying the effect of prolonged exposure of UVC on Lys. We synthesized AuNCs in Lys and GSH-protected AuNCs and modified with Lys. Similar characterization of the Lys–AuNC composites was performed by DLS. The experiment shows that the hydrodynamic diameter of Lys is 4.8 nm, the size of AuNC@Lys is 7.3 nm, and the size of Lys + AuNC@GSH is 7.5 nm. Hence, the
size of AuNCs produced in Lys is 1.5 nm. The AuNCs in Lys were characterized by their absorption and fluorescence spectra (Figure 10). The 280 nm band in each of the absorption spectra is due to Lys. AuNC@Lys shows two peaks at 445 and 650 nm in its emission spectrum (Figure 10a), and the GSH-protected AuNC in Lys shows its emission band at 616 nm (Figure 10b). The long-wavelength bands are due to the AuNCs. The blue emission is due to the oxidative states of Lys in the presence of HAuCl₄.

Prolonged exposure of Lys and Lys trapped in DMPG to UVC shows structural changes similar to those observed in the case of BSA as evidenced from the spectroscopic data (Figure 11). Far-UV CD spectra of Lys and Lys-incorporated DMPG vesicles indicate a change in the secondary structure of Lys (Figure 12). The α- and β-helix contents in Lys are calculated to be 32.16 and 15.09%, respectively. Upon exposure to UVC for about 3 h, the α-helix content in free Lys reduced to 23.50% and the β-sheet content increased to 21.70%.

Similar to BSA, we observe that the change in the absorbance at 322 nm for Lys, which develops due to protein aggregation, drops remarkably on application of AuNCs (Figure 13). This effect seems to be identical on the ex situ addition of AuNCs to the DMPG-trapped Lys. However, the relative changes in the emission indicate that the ex situ application of GSH-protected AuNCs to Lys in DMPG vesicles is more effective than the in situ production of the NC. Far-UV CD spectroscopy shows a similar behavior for Lys as seen in the case of BSA (Table 1) (see Figure 14).

3. CONCLUSIONS

In summary, we have proposed a simple and ready remedy to the adverse effects of UVC exposure on a model protein, BSA.
Percolation of UVC radiation through the stratosphere is probable because of the depletion of the protective ozone layer because of the environmental pollution. In the present report, we have shown that AuNCs can serve as measures to considerably reduce protein aggregation due to UVC radiation. The effect of UVC on bare BSA results in considerable changes in the secondary structure, leading to aggregation. The spectroscopic results show that the in situ grown AuNCs in BSA reduce changes in the protein structure, which, in turn, is enhanced when the AuNCs are produced ex situ and applied to protein. Moreover, intercalation of the AuNC-absorbed BSA in a liposomal bilayer serves best in this context. These results were confirmed by the application of AuNCs to Lys protein as well under identical exposure to UVC radiation.

4. EXPERIMENTAL SECTION

4.1. Materials. Gold(III) chloride hydrate, BSA (lyophilized powder, ≥98%), Lys from chicken egg white (lyophilized powder, ≥90%), DMPG, and GSH reduced (≥98%) were purchased from Sigma-Aldrich. Sodium hydroxide pellets were obtained from Merck. High-performance liquid chromatography (HPLC) water was used in all experiments.

4.2. Synthesis of AuNC@BSA (NC1). The BSA-coated NIR-emitting AuNCs were prepared as described elsewhere.\textsuperscript{25} Briefly, an aqueous solution of HAuCl\textsubscript{4} (5 mL, 10 mM, 37 °C) was mixed with a BSA solution (5 mL, 50 mg mL\textsuperscript{−1}, 37 °C) under vigorous stirring. After 5 min, 0.5 mL of NaOH solution (1.2 M) was added, and the reaction was allowed to continue at 37 °C for 12 h. An intense red-colored solution was obtained and stored at 4 °C.

4.3. Synthesis of GSH-Protected AuNC (NC2) and BSA Modification. The GSH-coated AuNC was synthesized by following a previous protocol.\textsuperscript{26,27} Briefly, freshly prepared

Figure 10. Absorption (black) and emission (red, ex = 280 nm and blue, ex = 370 nm) spectra of (a) AuNC@Lys and (b) GSH-protected AuNC@Lys in the aqueous medium.

Figure 11. Absorption spectral changes in (a) Lys and (b) Lys trapped in DMPG vesicles upon exposure to UVC radiation for around 3 h in the aqueous medium. The emission spectral changes are shown in (c) and (d) for Lys and Lys trapped in DMPG, respectively, upon excitation at 280 nm.

Figure 12. CD spectra of BSA and BSA-intercalated DMPG vesicles under different conditions. The UVC exposure time is about 3 h.
AuNC (NC2) could be stored at 4 °C. The aqueous solution of GSH-protected AuNC could be stored at 4 °C for 6 months with negligible changes in the photophysical properties. NC2 was embedded in DMPG vesicles as reported earlier. DMPG was dissolved in a chloroform/methanol mixture in a 2:1 ratio, which was then evaporated using a rotary evaporator. A thin lipid film was produced, which was kept overnight in vacuum for further drying. Tris buffer (pH 7.4) along with the aliquots of NC1 at a temperature above the phase-transition temperature (24 °C) of DMPG was added to the dry lipid film to achieve a final lipid concentration of 4 mM. The suspensions were then homogenized by vortexing followed by heating/cooling in five cycles. In a single cycle, the samples were kept at 30 °C for 10 min and at 4 °C for further 10 min. For spectroscopic measurements, the samples were diluted 10 times in deionized water to stop light scattering. A similar procedure was followed for NC2@BSA. 

4.5. Synthesis of AuNC@Lys. The Lys-coated AuNC was synthesized by following the same protocol as used for the preparation of BSA-coated AuNCs with a slight modification. Briefly, an aqueous solution of HAuCl4·3H2O (2 mL, 10 mM, 37 °C) was added to Lys solution (50 mg mL−1) under vigorous stirring. About 0.2 mL of NaOH (1 M) was added to adjust the acidity of the solution at pH 12, and the reaction was continued at 37 °C for 8 h. The color of the solution turned from light yellow to deep brown, indicating the formation of Lys-coated AuNCs. It was stored at 4 °C. Synthesis of GSH-coated AuNC, Lys modification, and intercalation in DMPG vesicles were performed similar to that for the BSA protein.

4.6. Instrumentation. The absorption spectra were collected using a U-2900 spectrometer from Hitachi, and the emission spectral studies were performed in a QM-40 spectrofluorometer from PTI. The particle size of NCs was measured by DLS using a Malvern Zetasizer Nano equipped with a 4 mW HeNe laser operated at λ = 633 nm. All measurements were recorded at room temperature with a scattering angle of 173°. Size distribution calculated using Nano software was derived from non-negative least-square analysis. The samples were almost 10 times more diluted than those used in the spectroscopic measurements. Hence, the concentration used for the DLS experiment was 10 mM. The samples were kept in a UV chamber under 254 nm radiation with an 8 W lamp placed 5 cm away from the samples for 3 h of UVC exposure.

4.7. Calculation of UVC Dose. Energy, $E = h\nu/\lambda$, where $h$ is Planck’s constant $= 6.626 \times 10^{-34}$ m² kg/s, $\nu$ is the velocity of light $= 3 \times 10^8$ m/s, and $\lambda$ is the wavelength of radiation $= 254$ nm. The duration of irradiation is 3 h or 10 800 s. Hence, $E = 7.8260 \times 10^{-19}$ m² kg/s² or $E = 7.8260 \times 10^{-19}$. The duration of irradiation is 3 h or 10 800 s. Hence, $E = 7.8260 \times 10^{-19} \times 10^8 = 8.4521 \times 10^{-15}$ J is falling on a meter square. The source is 5 cm away. Hence, consideration of squared law as the source is 5 cm or 0.05 m away, which means the radiation only covers (0.05)² = 0.0025

| systems | α-helix (%) | β-sheet (%) |
|---------|-------------|-------------|
| Lys     | 32.16       | 15.09       |
| Lys@DMPG | 30.83       | 15.75       |
| Lys under UVC | 23.50     | 21.70       |
| Lys@DMPG under UVC | 17.80     | 23.30       |
| AuNC@Lys | 7.44        | 24.73       |
| AuNC@Lys + DMPG | 6.86        | 25.12       |
| AuNC@Lys under UVC | 4.66        | 26.34       |
| AuNC@Lys + DMPG under UVC | 5.30       | 26.05       |
| Lys + AuNC@GSH | 35.16     | 12.50       |
| Lys + AuNC@GSH + DMPG | 32.88     | 13.74       |
| Lys + AuNC@GSH under UVC | 29.30     | 15.40       |
| Lys + AuNC@GSH + DMPG under UVC | 30.51     | 14.08       |

Figure 13. Absorption spectral changes in AuNC incorporated in situ (a) Lys and (b) Lys trapped in DMPG vesicles upon exposure to UVC radiation for around 3 h in the aqueous medium.

Figure 14. Relative changes in fluorescence emission ($F_0/F$) with time for various systems. All data are within 5% error limit.
This means that the energy has to be divided by 1/0.0025 = 400 so as to make it in 1 m². Hence, \( E = 8.5421 \times 10^{-15}/400 = 2.1130 \times 10^{-17} \) J is the UVC dose at 254 nm.

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