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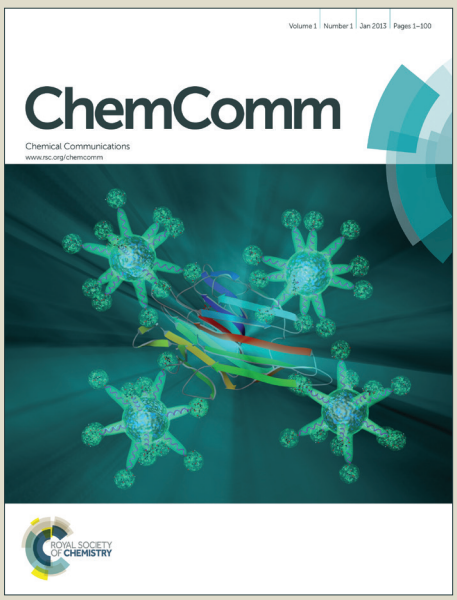

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Serum Albumin Adsorbed on Au Nanoparticles: Structural Changes Over Time Induced by S-Au Interaction

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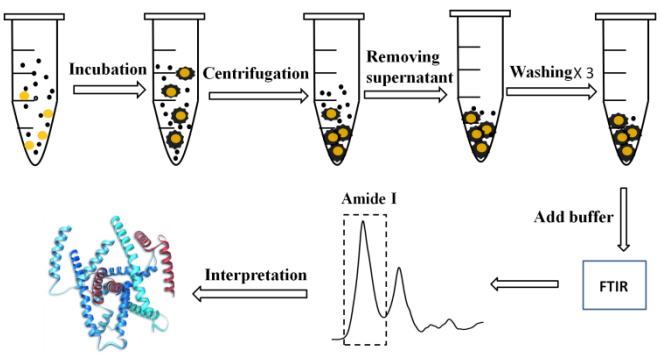
We report the evolution of the protein secondary structure of HSA adsorbed on AuNPs over time. This evolution is in agreement with the S-Au interaction time determined by Raman spectroscopy. The results indicate that the changes in the secondary structure of HSA are induced by the S-Au interaction.

Upon contact with biological matrices, nanoparticle surfaces are modified immediately by the adsorption of biomolecules, particularly proteins, leading to the formation of a protein corona.[1] The protein corona may enhance the uptake of the nanoparticles by cells,[2] and could be exploited for drug delivery.[3] Gold nanoparticles (AuNPs) have been investigated vigorously as a promising drug delivery platform for targeted cancer therapies.[4] For example, AuNPs are quickly coated by serum proteins after injection into the bloodstream,[5] and most cells and tissues never encounter the naked particles.[6] As the most abundant protein in serum, human serum albumin (HSA) plays an important role as a carrier protein for steroids, fatty acids, and thyroid hormones, and is widely used clinically to treat serious burn injuries, fetal erythroblastosis, and other disorders.[7] The structural changes in protein adsorbate over time are still an unexplored but potentially critical issue. Here, we report an interesting observation of changes in structure and stability of HSA absorbed on AuNPs using Fourier transform infrared (FT-IR) and fluorescence spectroscopy. The mechanism underlying time-related HSA structural changes induced by AuNPs binding was also elucidated by Raman spectroscopy. This discovery characterized the mechanism underlying HSA structural changes after adsorption on AuNP and may be of great potential importance in the application of AuNPs in bio-fields.

Au nanoparticles (~40 and ~70 nm in diameter) were fabricated by reducing boiled chloroauric acid solution with sodium citrate.[8] Protein corona (HSA-AuNP) were obtained by incubating HSA and AuNPs. HSA-AuNP separation and investigation were illustrated in Scheme 1. The protein secondary structure is based on analysis of the amide I band of FT-IR spectroscopy.



Scheme 1. Schematic illustration of sample preparation.

The transmission electron microscope (TEM), energy dispersive X-ray (EDX) spectra of AuNPs and HSA-AuNP bioconjugates are compared in Figure S1. After 10 hours incubation, a misty corona was evident around the AuNP particle (Figure S1C, arrows). The EDX spectrum of HSA-AuNP bioconjugates (Figure S1B) shows the appearance of a sulfur element, indicating the absorption of proteins on AuNPs. Dynamic light spectrum (DLS) data show that the average hydrodynamic diameter of HSA-AuNPs bioconjugate is obviously larger than that of unbound AuNPs (Figure S2).

FT-IR is a useful technique for characterizing the structures of proteins in various environments. The second-derivative spectrum of the amide I band (1700-1600 cm^{-1}) was used to analyze the secondary structural components of proteins. In order to obtain high-quality FT-IR spectra, a high concentration of protein (>10 mg/ml) was used. After 20 min incubation, the HSA-AuNP sample was

washed and injected into a CaF_2 cell and the FTIR spectra collected at 0.5 h intervals. FT-IR analysis of native HSA showed that HSA consisted of ~75% α-helix (Figure 1A), which is consistent with the X-ray crystallography data (PDB ID: 1E78).

Structural changes in HSA absorbed on AuNPs are shown in Figure 1. The second-derivative spectra of the amide I region showed significant changes in the secondary structural composition as a function of incubation time, the intensities of bands assigned to α-helix (1658, 1665 cm^{-1}) decreased, whereas the β-structures (β-sheet at 1630 and 1640cm^{-1}, β-turn at 1683 and 1689 cm^{-1}) and random coil (1649cm^{-1})[9] increased. Figure 2 shows the relative amounts of different structural component of HSA in HSA-AuNP obtained by quantitative analysis of second-derivative FT-IR spectra. These results indicate that the secondary structure of HSA underwent changes in HSA-AuNP bioconjugates over time. With extended incubation, the structure of HSA in HSA-AuNP reached a relatively stable value (about 8-9 hours). We also investigated the interaction of HSA with 70 nm AuNPs and obtained similar results (Figure S3, Figure S4).

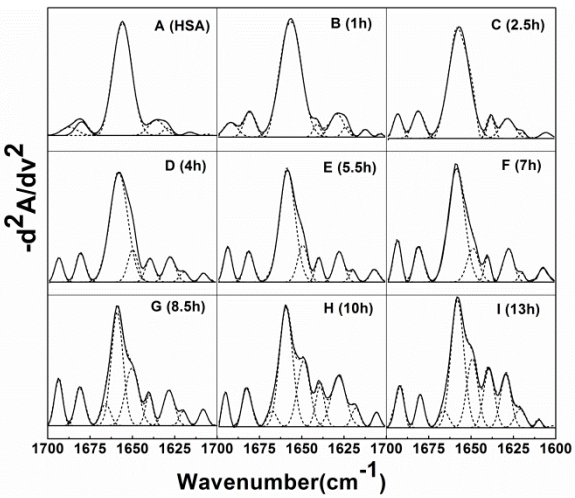


Figure 1. Curve-fitted inverted second-derivative amide I spectra of HSA absorbed on 40 nm AuNPs at different incubation times.

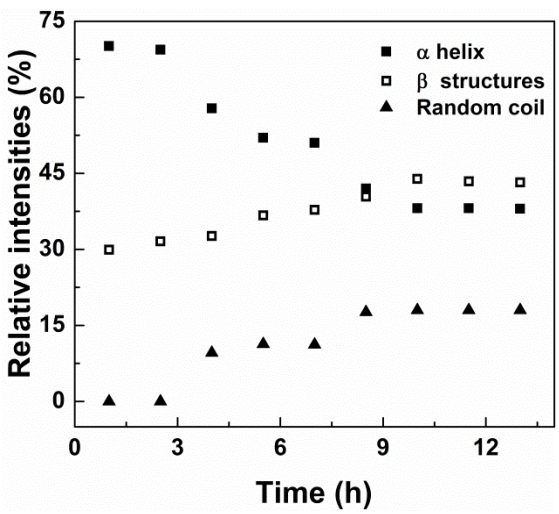


Figure 2. Effects of AuNPs on the relative amount of α-helix structure (■), β-structures (β-sheet and β-turn, □), and random coil (▲).

The stabilization of HSA absorbed on AuNPs can be determined by monitoring the degree of unfolding induced by guanidinium hydrochloride (GnHCl). Figure 3 shows the change in protein fluorescence intensity at 340 nm as a function of GnHCl concentration for 40 nm HSA-AuNPs at different incubation times. HSA was significantly stabilized by AuNP binding. A similar result was obtained for 70 nm HSA-AuNP (Figure S5). These results indicate that the overall structure of HSA was stabilized, although the random structure component was increased by AuNPs binding.

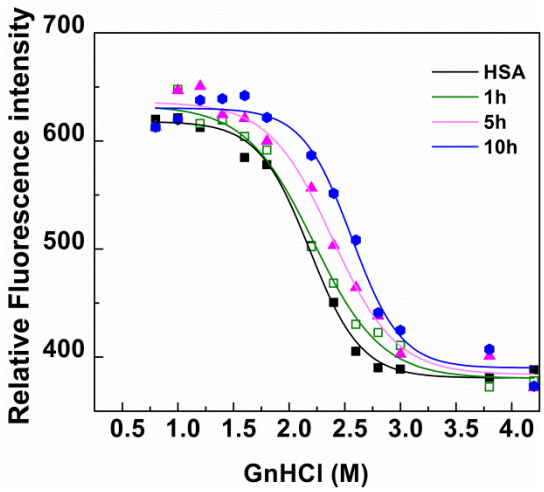


Figure 3. Fluorescence emission intensity of HSA adsorbed to 40 nm AuNPs as a function of the concentration of guanidinium hydrochloride (GnHCl) at incubation times of 1 h (□), 5 h (▲), and 10 h (●).

HSA contains a single cysteine and 17 pairs of disulfide bonds.[10] The thiol group in HSA can be easily attached to the surface of gold nanoparticles to form a stable S-Au bond.[11] This covalent interaction between S in HSA and Au in AuNPs can be determined by Raman spectroscopy. A Raman band occurs at ~292 cm^{-1} for the S-Au interaction.[12] In order to explore the possible relationship between the formation of the S-Au bond and the evolution of the structure of HSA, the surface enhanced Raman spectrum (SERS) of the HSA-AuNP bioconjugates was determined as a function of incubation time (Figure 4). AuNPs or HSA had no Raman band at 292 cm^{-1}, whereas the S-Au stretch mode at 292 cm^{-1} appeared with a greater incubation time. A more intense Raman signal was obtained for the S-Au bond as a function of incubation time, and the S-Au bond up-shifted until approximately 8-9 h. These changes interestingly agree with the time needed to achieve a relatively stable structure, which was shown by FT-IR.

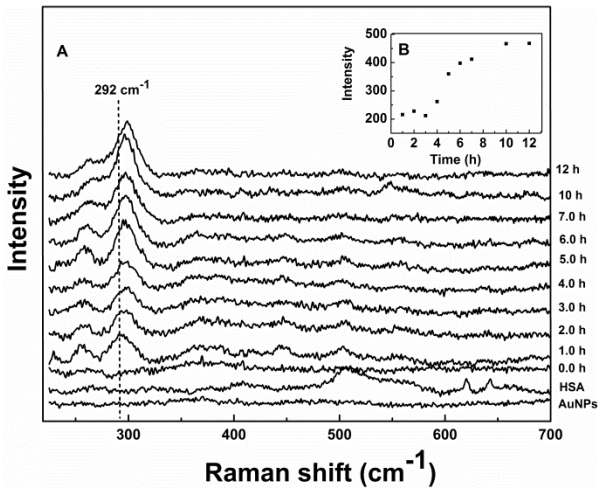
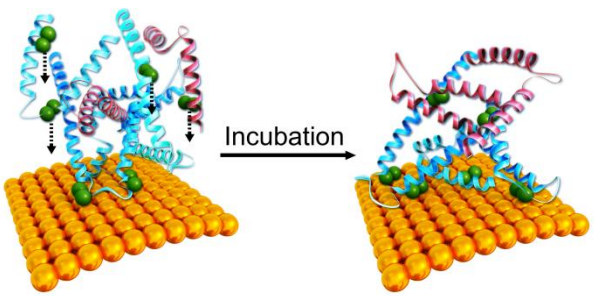


Figure 4. Raman spectra of raw HSA, AuNPs, and SERS spectra of HSA-AuNPs (A) and the intensity of HSA-AuNPs (B) as a function of incubation time.

The absorption of HSA on AuNP is due to an electrostatic mechanism,[13] hydrophobicity[14] and S-Au interaction.[15] The electrostatic interaction may occur immediately, but the interaction with S-Au takes 8-9 hours and changes the overall structure of HSA. The mechanism for structural changes of HSA over time induced by S-Au interaction can be illustrated as Scheme 2. The disulfide bonds of HSA are located in different places, some are most likely on the surface and easily contact Au, whereas some are located in flexible buried regions and take time to come into contact with Au, and others are located in the core region of the HSA, which might take even longer to come into contact with Au. Meanwhile, the formation of the S-Au bond may lead to the evolution of the secondary structure of HSA, and these reactions take approximately 8-9 h.



Scheme 2. Schematic illustration of interaction between Au (yellow) in AuNPs and S (green) in HSA. HSA is rendered as a cartoon representation.

In summary, the structure and stability of HSA adsorbed to AuNPs changed as a function of incubation time. Raman spectroscopy analysis showed that this evolution was induced by the interaction between S in HSA and Au in the nanoparticle. These results provide fundamental insights into the effects of nanoparticles on the adsorbed protein structure and a somewhat cautionary note about selecting nanoparticle-based applications in bioapplications.

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Notes and references

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