O35 |

SURFACE MODIFICATION OF GALLIUM NANOPARTICLES AND THEIR INTERACTION WITH HUMAN SERUM ALBUMIN

N. V. Falcón-Cruz[1], A. Meléndez[2], I. Ramos[2], and R. Oyola[1]
[1]*Department of Chemistry, University of Puerto Rico-Humacao, Call Box 860, Humacao PR 00792*
[2]*Department of Physics & Electronics, University of Puerto Rico-Humacao, Call Box 860, Humacao PR 00792*
[*]*e-mail: rolando.oyola@upr.edu*

Introduction

Metal nanomaterials, such as gallium nanoparticles, have been found to be considerably valuable in generating new pathways as transducing materials in biosensors and diagnostic approaches.[1] For instance, gallium nitride (GaN) nanoparticles have been proposed as antibacterial, and show biocompatibility and chemical stability.[2] Gallium oxide hydroxide (GaO(OH)) nanoparticles have been used in diagnostic and therapeutic approaches, like cancer treatments.[3] Also, surface conjugation of these materials with the appropriate compounds, like β-cyclodextrin, provides selectivity, facilitates drug delivery, and increases solubility.[3] Recently, we showed that the lag phase of Aβ40 aggregation kinetics is significantly retarded by GaN NPs in a concentration dependent manner, implying the activity of GaN NPs in interfering with the formation of the crucial nucleus during Aβ40 aggregation.[4] Also, the results showed that GaN NPs can reduce the amyloid fibril elongation rate during the aggregation kinetics. It was speculated that the high polarization characteristics of GaN NPs may provoke a strong interaction between the particles and Aβ40 peptide and in this way decrease self-association of the peptide monomers to form amyloids. A better understanding of the interaction of GaNPs with biological components, like proteins, is an important consideration for the improvement of these nanomaterials. Thus, sensing molecular forces that control binding and structural conformations are relevant to develop new nanomaterials.

Human serum albumin (HSA) is the most abundant plasma protein in mammals that serves as ligand carrier and is a model widely used in biophysical and biochemical studies. [5-6] HSA possesses one tryptophan (Trp214) residue in the hydrophobic cavity of Sudlow's site I (subdomain IIA, warfarin binding site), which can be used as a probe for conformational and ligand interactions. HSA has another major binding site, Sudlow's site II (subdomain IIIA, indole/benzodiazepine site). Therefore, it is relevant to probe the interaction of

nanoparticles with HSA to use GaNP as sensors in biological fluids. In the present work, we synthesize and characterize gallium oxide nanoparticles conjugated with β-cyclodextrin (GaOCD) and hydroxyl (GaO(OH)) and probe its interaction with HSA by fluorescence spectroscopy. Our results show that HSA tertiary structure is not affected by GaO(OH) or GaOCD nanoparticles.

Methods

β-Cyclodextrins (βCD), Gallium (III) Nitrate Hydrate [$Ga(NO_3)_3 \bullet xH_2O$], ammonia solution (2.0 M in ethanol), Na_2HPO_4, NaH_2PO_4, and HSA (fatty acids free) were purchased from Sigma-Aldrich (St. Louis, USA). All aqueous solutions contain 20 mM sodium phosphate buffer (PBS) pH = 7.40 and were prepared using nanopure deionized water. The concentration of HSA was determined using molar absorption coefficient of 35,700 $M^{-1}cm^{-1}$ (at 280 nm). Gallium oxide nanoparticles (GaO(OH)) were synthesized as reported by Ganguly et al.[3] Briefly, a 1:4 ammonia water solution was added drop by drop to 10 mL of 2.00 mM gallium (III) nitrate hydrate until pH was maintained at 7.0-7.5 where a white precipitate was observed. Conjugation of gallium oxide nanoparticles with βCD (GaOCD) was achieved adding 2.8 mL of 0.5 mM βCD to 10 mL of gallium (III) nitrate hydrate. After well mixed, ammonia water was added drop by drop until pH was maintained at 7.0-7.5 and white precipitate was observed. For both synthesized NPs, solutions were centrifuged at 1200 rpm for 30 min, a minimum of three times, removing the supernatant and washing with deionized water each time. Precipitate was dried in vacuum oven at 60 °C for two days. NPs were then stored under vacuum for future use. NP aqueous dispersions were prepared, dissolving a weighted amount in deionized water and sonicating for 60 minutes. Scanning electron microscopy (SEM) images were obtained at the PREM Materials Characterization Laboratory at University of Puerto Rico-Humacao. Measurements were done on dried samples of GaO(OH) and GaOCD. The solid was dispersed on sampler tape and images taken at different magnifications. For fluorescence emission spectra, an Agilent Cary Eclipse spectrofluorometer with temperature control (± 1 °C) was used, exciting at 290 nm and scanning between 300 – 500 nm range at scan speed of 120 nm/min. Samples were measured in a 1 cm^2 quartz cell. Measurements for 1.0 μM HSA solutions were made with excitation and emission slits at 10 nm and the photomultiplier high voltage (PMT) set to 600 V. A stirrer was used consistently through the duration of the experiments. The UV–Vis absorption spectra were obtained on an Agilent 8453 spectrophotometer (Agilent, USA) in a 1.0 cm^2 path length cell. The spectra were measured with their appropriate backgrounds. Solutions containing 1.00 μM HSA with varying concentrations of GaOCD up to 76 μg/mL were prepared in a total

volume of 1.5 mL and left stirring for 10 min. Fluorescence spectra were taken at temperature intervals of 5 °C in the range of 20 – 40 °C. Quenching constants were determined using the Stern-Volmer equation: $F_0/F = K_{SV}[Q] + 1$ where F_0 and F are the fluorescence emission intensity of HSA at 340 nm in the absence and presence of the quencher (GaOCD), [Q] is the quencher concentration, and K_{SV} is the Stern-Volmer quenching constant. Corresponding backgrounds were subtracted for each NP concentration. Thermal denaturation of HSA was determined using a 1.00 μM HSA with 0.0, 20, 40, and 76 μg/mL GaNP in a total volume of 1.5 mL. The HSA emission spectra were taken at 5 °C intervals in the range of 20 – 100 °C, allowing 8 minutes for equilibration before measurement. The results were the average of three independent measurements. Thermodynamic parameters were obtained based on two-state system.

Results

Morphological Characterization

Dried NPs samples have been characterized using SEM images as well as Energy Dispersive X-ray Spectroscopy (EDS) to determine the morphology and atomic composition of synthesized NPs. **Figure 1a-d** shows SEM image of GaO(OH) and GaOCD. Width and length measurements shows that GaO(OH) NPs are larger than GaOCD (~0.7 μm vs. 0.4 μm). Chemical composition of both synthesized NPs was determined by EDS analysis. The mass percent distribution of carbon, oxygen, and gallium in GaO(OH) is 0.00, 14.83, and 85.17, respectively. Capping of GaO(OH) with βCD was validated by the increase in mass % of carbon in the EDS signal: C = 3.44, O = 20.30, and Ga = 76.26 %, respectively.

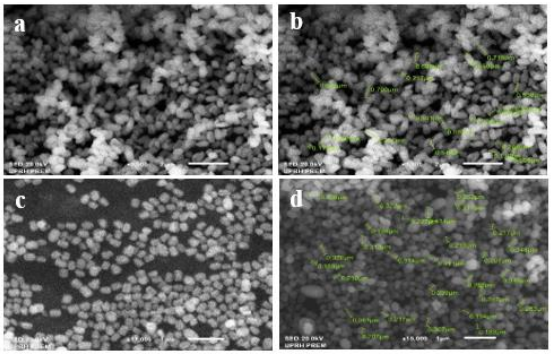

Figure 1. SEM imaging of nanoparticle samples. (a) Low magnification (x9,500) image of GaO(OH) showing rod-like morphology. (b) Length and width measurements indicating GaO(OH) NPs are no larger than 0.7 μm. (c) High magnification (x17,000) image of GaOCD as nanodisk-like structures with (d) (x15,000) length and width measurements, indicating most are

smaller than 0.4 μm.

Fluorescence Quenching of HSA in the Presence of GaOCD

Quenching of HSA was probed using Trp214 emission in the presence of a variety of GaOCD concentrations for determination of binding constants between 20 - 40°C. **Figure 2** shows that increasing concentration of GaOCD quenches Trp emission, an indication of protein interaction with NP. The Stern Volmer plots' slopes decrease with temperature from 6.1 x 10^3 to 4.4 x 10^3 ($\mu g/mL^{-1}$), indicative of static quenching mechanisms. [4,6] Fluorescence quenching experiments resulted in low K_{SV} values (10^3 $(\mu g/ml)^{-1}$) relative to gold nanoparticles (>10^8 M^{-1}). van't Hoff analysis of the quenching data for determination of thermodynamic parameters of binding suggest the presence of electrostatic forces governing HSA and GaOCD interactions.

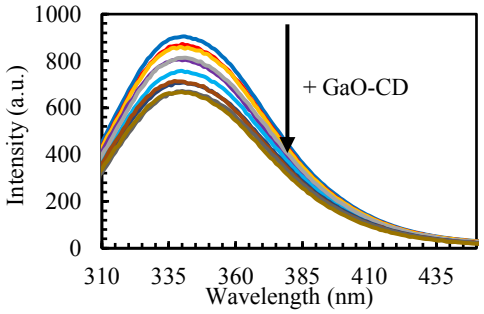

Figure 2. Fluorescence emission spectra of HSA with different concentrations of GaOCD, taken at 20 °C. Increasing concentration of NP causes decrease in emission.

Thermal Denaturation of HAS

Figure 3 shows representative melting curves for the normalized integrated intensity change for HSA as a function of temperature (20 – 100 °C) in the presence of 0 and 76 μg/mL GaOCD. For determination of T_m values and thermodynamic parameters for thermal denaturation, data was fitted to two-state system.

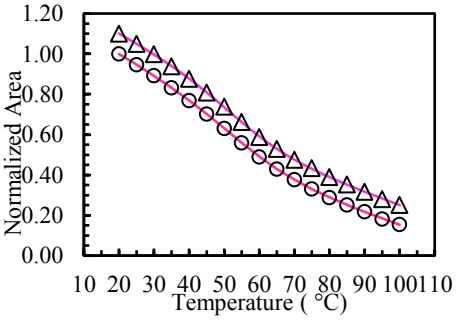

Figure 3. Representative melting curve of HSA (Δ) and in the presence of 76 μg/mL GaOCD (○). Solid lines represent the best fit to two-state equilibrium system. The HSA curve is offset by 0.10 in the Y-axis for presentation purposes.

Melting curves and their corresponding T_m values obtained in the presence of different concentrations of GaOCD are similar. T_m obtained for HSA is in agreement with literature, where two melting point temperatures of 56 (T_{m1}) and

62 (T_{m2}) °C have been reported. [7-9]. These two T_m values have been attributed to sequential denaturation of domain II and domain I, respectively. The presence of GaOCD (up to 76 μg/mL) results in similar T_m, within experimental error, relative to HSA, suggesting that GaOCD does not affect the tertiary structure of the protein in great extent.

Conclusions

This project had the aim of synthesizing and characterizing GaOCD and GaO(OH) and study its interaction with HSA through fluorescence spectroscopy. The preliminary results indicate that the interaction between the GaOCD nanoparticles (up to 76 μg/mL) and HSA is weaker than for gold nanoparticles. Additionally, tertiary structure of HSA is not affected by presence of GaOCD. More studies are required to better understand the interaction of gallium nanoparticles with biomolecules. Proteins and peptides with different physicochemical properties should provide more information about the relative forces governing the NPs and biomolecules interactions.

References

[1] Li, X.; Liu, X. Group III nitride nanomaterials for biosensing. *Nanoscale* **2017**, 9, 7320-7341.
[2] Bain, L. E.; Hoffmann, M. P.; Bryan, I.; Collazo, R.; Ivanisevic, A. Adsorption and adhesion of common serum proteins to nanotextured gallium nitride. *J. Name*., **2013**, 00, 1-3.
[3] Ganguly, B. N.; Verma, V.; Chatterjee, D.; Satpati, B.; Debnath, S.; Saha, P. Study of Gallium Oxide Nanoparticles Conjugated with β-Cyclodextrin: An Application to Combat Cancer. *ACS Appl. Mater. Interfaces,* **2016**, 8, 17127– 17137.
[4] Torres, K.M., Delgado, A.S., Serrano, E.R., Falcón-Cruz, N.V., Meléndez, A., Ramos, I., Du, D., and Oyola, R. Gallium nanoparticles as novel inhibitors of Aβ40 aggregation. Mater. Adv., **2021**,2, 5471-5478.
[5] Flora, K.; Brennan, J. D.; Baker, G. A.; Doody, M. A.; Bright, F. V. Unfolding of Acrylodan-Labeled Human Serum Albumin Probed by Steady-State and Time-Resolved Fluorescence Methods. Biophys. J. **1998**, 75, 1084−1096.
[6] Li, Q.; Yang, W. Y.; Qu, L. L.; Qi, H. Y.; Huang, Y.; Zhang, Z. Interaction of Warfarin with Human Serum Albumin and Effect of Ferulic Acid on the Binding. Journal of Spectroscopy, **2014**, Article ID 834501, 7 pages**.**
[7] Picó, G. A. Thermodynamic Features of the Thermal Unfolding of Human Serum Albumin. Int. J. Biol. Macromol. **1997**, 20, 63−73.
[8] Farruggia, B.; Picó, G. A. Thermodynamic features of the chemical and thermal denaturations of human serum albumin. Int. J. Bio. Macromol. **1999**, 26, 317-323.
[9] Das, N. K.; Ghosh, N.; Kale, A. P.; Mondal, R.; Anand, U.; Ghosh, S.; Tiwari, V. K.; Kapur, M.; Mukherjee, S. Temperature Induced Morphological Transitions from Native to Unfolded Aggregated States of Human Serum Albumin. J. Phys. Chem. B, **2014**, 118, 7267−7276.

Acknowledgments

We gratefully acknowledge financial support from the NSF-DMR-2122102.