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The Spontaneous Formation of Selenium Nanoparticles on Gallic Acid Assemblies and their Antioxidant Properties

Stacey Barnaby FCRH '11
Fordham University, furj03@fordham.edu

Nazmul Sarker FCRH '13
Fordham University, furj03a@fordham.edu

Aaron Dowdell FCRH '12
Fordham University, furj03b@fordham.edu

Ipsita Bannerjee
Fordham University, furj03c@fordham.edu

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Cover Page Footnote

Stacey Barnaby, FCRH 2011, is from Monroe, Connecticut. She is a chemistry major. Stacey is currently working on the development of new biomaterials at the nanoscale for targeted applications in tissue engineering and bioimaging under the direction of Dr. Ipsita Banerjee in the department of chemistry. After graduation, Stacey will be attending graduate school to pursue a Ph.D, where she would like to continue her research in the area of bionanotechnology.

Research

CHEMISTRY

The Spontaneous Formation of Selenium Nanoparticles on Gallic Acid Assemblies and their Antioxidant Properties

Gallic acid (GA) is a naturally occurring plant phenol known for its anti-inflammatory and antioxidant properties. In this work, we probed the molecular self-assembly of GA for the development of GA based nanocomposites for potential device fabrications and enhanced antioxidant applications. We found that the formation of GA nanostructures was pH dependent. Further, we examined the interactions of selenite with GA and subsequently examined the biomimetic formation of selenium (Se) nanoparticles. We found that in the presence of selenite, the yield of nanofibers was significantly higher, and selenium nanoparticle coated nanofibers were formed. The ability of the nanocomposites to scavenge free radicals was also explored. Thus, we have developed a new family of Se nanoparticle coated GA nanofibers, which could not only be applicable as potent antioxidants at the nanoscale but may also have potential applications in optoelectronics and sensors.

Stacey Barnaby, FCRH '11
Nazmul Sarker, FCRH '13
Aaron Dowdell, FCRH '12
Dr. Ipsita Banerjee

Introduction

Nanomaterials have been gaining popularity in recent times due to their wide range of applications in the development of magnetic materials for data storage, optoelectronics, medical diagnostics, sensors, and alternative energy.[1] The potential for nanomaterials to be utilized in a plethora of applications often stems from their ability to self-assemble into supramolecular structures, such as nanotubes, nanofibers, nanocrystals, nanorods, nanocapsules, and nanowires.[2] These nanostructures form the basic building blocks of nanodevices by the bottom-up approach. Succinctly, self-assembly begins at the atomic level, where it relies upon chemical complementarity in order to allow materials on the molecular level to come together and form higher ordered structures. Non-covalent interactions, such as hydrogen bonding and ionic interactions, hydrophobic interactions, Van der Waals forces, and π-π stacking interactions have been known to direct the formation of the aforementioned supramolecular nanostructures.[3] Although these forces are individually weak, they work in tandem to direct the formation of the resulting hierarchical structures, and are seen ubiquitously throughout nature in biomolecules such as proteins, nucleic acids, lipids, and phytohormones.

In this work, we explored the formation of nanostructures of the plant polyphenol Gallic acid (GA) and examined its potential to direct the formation of selenium nanoparticles. The rationale being that, at the nanoscale, the properties of GA may be significantly altered compared to bulk materials due to size and shape control. Gallic acid (3,4,5 –triphydroxyl-benzoic acid) is naturally found in various plants, fruits, and foods such as gallnuts, oak, green tea, grapes, strawberries, pineapples, bananas, lemons, and apple peels.[4] In nature, GA exists in two forms: either in its free state or as an ingredient of tannins,[5] namely gallotannin.[6] Previous studies have indicated a number of biomedical applications for GA due to its antibacterial, antiviral,[7] anti-inflammatory,[8] and antioxidant properties.[9] In addition, GA has been shown to also portray anticancer activity in a multitude of cancer cells such as leukemia, prostate, lung, gastric, colon, breast, cervical, and esophageal.[10]

A variety of metal nanoparticles such as Ag, Au, Pd, and Pt have been synthesized, and GA specifically has been utilized to form Au and Ag nanoparticles.[11-13] However, to our knowledge, no study has been carried out to examine the biomimetic formation of GA – Se nanocomposites. It would be advantageous to develop GA – Se nanocomposities as it might lead to the formation of highly potent antioxidant materials, due to the inherent biological properties of GA and Se indi-

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vidually. The antioxidant abilities of Se are believed to be a result of selenoenzymes such as selenium-dependent glutathione peroxidases, which prevent free radical damage to cells.[14] This property is of prime importance to the biomedical field, particularly in the area of cancer prevention.

Other advances in the area of selenium based nanotechnology stem from its high photoconductivity; catalytic activity for hydration and oxidation reactions;[15] as well as its high piezoelectric, thermoelectric, and nonlinear optical responses[16]—all of which can be applied to systems such as solar cells and rectifiers. In the past, Se nanoparticles have been synthesized utilizing a variety of methods, including using the redox reaction of selenourea and peroxynitrite.[17] It has also been reported that the decomposition of sodium selenosulfate using acids in solutions of surfactants (sodium dodecylsulfonate, cetylpyridinium chloride) or certain polymers (sodium polyphosphate, gelatin, polyvinyl alcohol, polyethleneglycol) allow for the formation of colloidal Se nanoparticles.[15] This allows for a more uniform size distribution.

While the above methods can be used to synthesize Se nanoparticles in a fairly narrow range of diameters, they require harsh reducing agents or stabilizers.[16] The recent surge in the development of environmentally friendly synthetic methods has led to the development of biomimetic methods, which allow nanoparticles to form spontaneously,[18] and provide control over the formation of size, structure, orientation, and shape of the resulting nanoparticles. This could lead to numerous possibilities for designing nanoparticles with very specific attributes. It has been reported that the protein bovine serum albumin (BSA) is involved in the formation of Se nanoparticles when selenium precursors were combined with BSA and hydrazine in a 6:1 ratio at 85°C in an aqueous solution, forming selenium nanobars; whereas in a 1:1 ratio, nanospheres were formed.[19] Thus, the concentration of the Se precursor, in the presence of the stabilizer, dictated the shape of the nanoparticles formed. Further, it was found that changing the temperature, the shape directing agent, as well as the pH value allowed for control over the formation of the nanoparticles. Herein, we have examined the self-assembly of GA as well as the growth of Se nanoparticles in the presence of GA. We also examined the radical scavenging efficacy of the nanoconjugates.

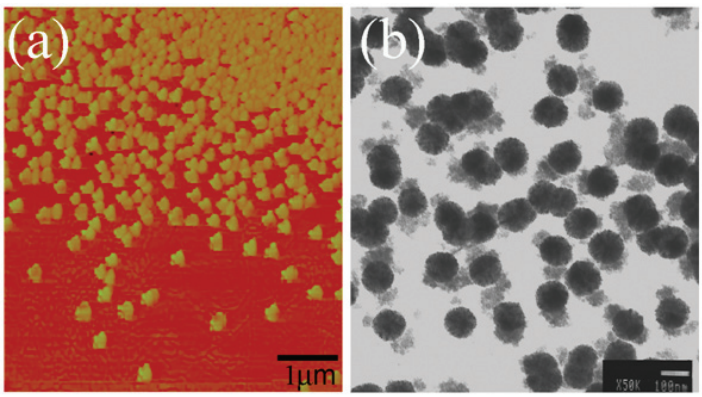

Figure 1- AFM images of self-assembled GA nanostructures grown at a) pH 5; b) pH 7.

Materials and Methods

Materials

2,2-diphenyl-1-picrylhydrazyl (DPPH), dithiothreitol (DTT), and sodium selenite were all purchased from Sigma Aldrich. Buffer solutions of various pH values were purchased from Fisher Scientific. Gallic acid was purchased from Acros Organic.

Methods

SELF-ASSEMBLY OF GALLIC ACID

The growth of GA nanostructures was probed at different concentrations (3 mM- 20 mM). The effect of pH was studied in a range of 2-9. The structures were allowed to self-assemble for a duration of one to four weeks, at which point they were centrifuged and washed thrice using distilled water. Supernatant was removed via micropipette before analysis.

GROWTH OF SE NANOPARTICLES IN THE PRESENCE OF GALLIC ACID

GA solution was prepared at different concentrations (3 mM- 20 mM) in pH 5, 7, and in distilled water. Sodium selenite was added to the GA solution in a 1:1 ratio. The reducing agent dithiothreitol was added dropwise (≈10 µL) until a brick red color was observed. The formation of nanoparticles was monitored over a period of twenty-four hours using fluorescence spectroscopy, followed by centrifugation, washing solution twice, and removal of supernatant via micropipette before analysis.

DPPH RADICAL SCAVENGING ASSAYS

Studies were carried out with selenium nanoparticle bound GA nanostructures as well as on GA nanofibers alone. For the assays, the concentrations of GA nanofibers as well as the GA fibers coated with Se nanoparticles was varied from a range of 1.0 mM - 3.0 mM. The concentration of DPPH was kept constant at 0.75 M and the solutions were brought to a constant volume

by addition of buffer solution (pH 7.4). Immediately upon addition of the DPPH stock solution, absorbance spectroscopy readings were carried out for a period of one hour.

Characterization

FTIR Spectroscopy

Analyses of the GA monomer and self-assembled GA were performed using Matteson Infinity IR equipped with DIGILAB, ExcaliBur HE Series FTS 3100 software. The washed samples were dried, mixed with spectroscopic grade KBr, and pressed into pellets. The measurements for the samples were carried out at 400-4000 cm-1.

UV Vis (Absorbance) Spectroscopy

UV Vis spectroscopy was carried out using a Thermo Scientific NanoDrop 2000 spectrometer. Readings were taken at a wavelength range of 190 nm to 700 nm using a 1-2 µL solution. A buffer solution was utilized as the solvent. All samples were repeated in triplicates.

Fluorescence Spectroscopy

Analyses of the presence of Se nanoparticles in various GA solutions were carried out using a Jobin Yvon Fluoromax 3 fluorimeter. Samples were excited at 597 nm.

Atomic Force Microscopy

The samples were centrifuged and washed twice using distilled water. The supernatant was removed using a micropipette and then the sample was placed on mica sheets and dried for analysis using a Quesant Universal SPM microscope, which was used in contact mode in air using a silicon nitride cantilever.

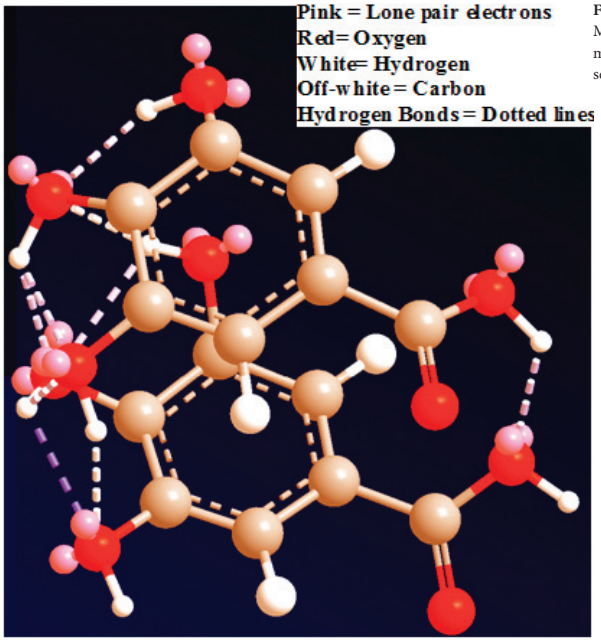

Figure 2- Molecular model of GA self-assembly.

Transmission Electron Microscopy

The morphologies of the samples were analyzed by TEM (JEOL 1200 EX), operated at 100 KV. Samples were washed twice in distilled water and air-dried on carbon-coated copper grids for analysis.

Results and Discussion

Growth of GA nanostructures

The GA moiety contains a phenolic ring system as well as three hydroxyl groups and a single carboxyl group, making it a pH sensitive molecule potentially capable of aromatic stacking interactions under appropriate growth conditions due to its ring system. Self-assembly processes involving a range of aromatic ring systems such as azopyridine side chain polymers,[20] tetra-aryl derivative of bimesityl,[21] and different linear triblock

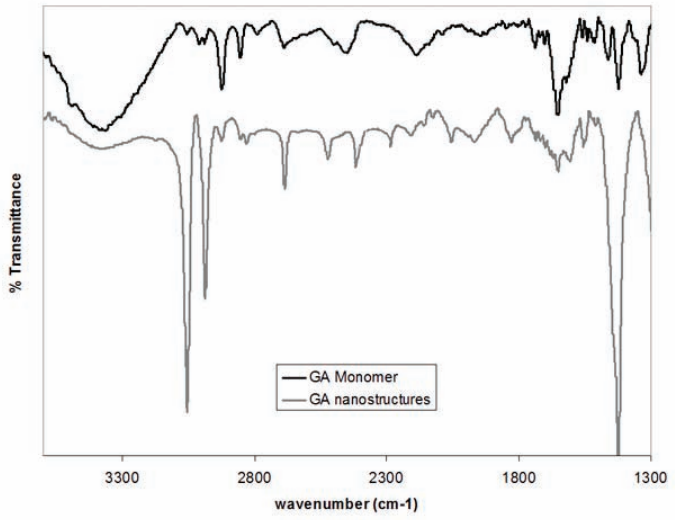

Figure 3- Comparison of FTIR spectra of GA monomer wtih self-assembled nanostructures.

copolymers of poly(tert-butoxystyrene)-b-polystyrene-b-poly(4-vinylpyridine) have been investigated.[22] For example, in the case of phenolic -OH groups in bimesityl derivatives, various packing motifs were formed via self-assembly, wherein the helical motif was the predominant form observed.[21,23] In contrast, the carboxyl groups mainly form a dimeric catemeric motif.[24] In the case of triblock copolymers, the addition of pentadecylphenol and/or nanodecylphenol allowed for cylindrical assembly.[22] Thus, aromatic residues play an important role in the self-assembly process due to stacking interactions.[25] Phenolic ring systems have also been reported to play vital roles in the self-assembly of long and hollow peptide nanotubes of Trp-Phe,[26] due

to π-stacking interactions.[27] Therefore, GA is primed to be an ideal molecule for the formation of unique nanostructures via self-assembly. In fact, Faggi and coworkers found that the synthesis of fluorous derivatives of GA self-assemble into nanosized fibers or balls.[28] It was seen that the semi-perfluorinated chains played a vital role in the aggregation.

The growth of GA nanostructures was probed by AFM and TEM. When grown under mildly acidic to neutral conditions (pH 5-7), we observed a high propensity of nanospheres (Figures 1a and 1b). However, when grown at pH 7, nanospheres larger in diameter (>500 nm) were obtained. It was observed that GA showed color changes, which were dependent on the pH in which it was grown. Specifically, at pH 5 a yellow-orange color was observed while at pH 7, it turned dark brown. This demonstrates the potential use of GA as a pH sensor.[29] The pKa values of GA are 4.10 and 8.38, indicating that it undergoes step-wise deprotonation as the pH increases.[30] At elevated pH values, there is a higher propensity of negative charges, which is inhibitory to the self-assembly due to repulsion. This is consistent with our results, as the most abundant supramolecular structures were observed at lower pH values. Mechanistically, this confirms that hydrogen bonding plays a pivotal role in the self-assembly process, wherein at lower pH, hydrogen bonding is higher. The proposed self-assembly of GA at low pH is shown in Figure 2.

FTIR spectroscopy was also utilized to further confirm the formation of self-assembled nanostructures. As seen in Figure 3, in the case of the GA monomer, the hydroxyl group peak is observed at 3385 cm-1. However, in the case of the nanostructures, the peak is shifted to 3396 cm-1, most likely due to the fact that the hydroxyl groups are hydrogen bonded intramolecularly, as well as intermolecularly with other -OH groups and with carboxyl groups of GA moieties in the vicinity. In addition, strong peaks are observed at 3054 cm-1 and 2990 cm-1 due to the presence of hydrogen bonded carboxyl groups and aromatic C-H stretch of phenolic groups. The C=C ring stretch is shifted from 1655 cm-1 to 1652 cm-1 in the case of the nanostructures. An intense C-O stretching peak is also observed at 1425 cm-1 in the case of the nanostructures, which are shifted compared to the relatively less intense peak at 1423 cm-1 in the case of the monomer. These results further confirm the formation of nanostructures.

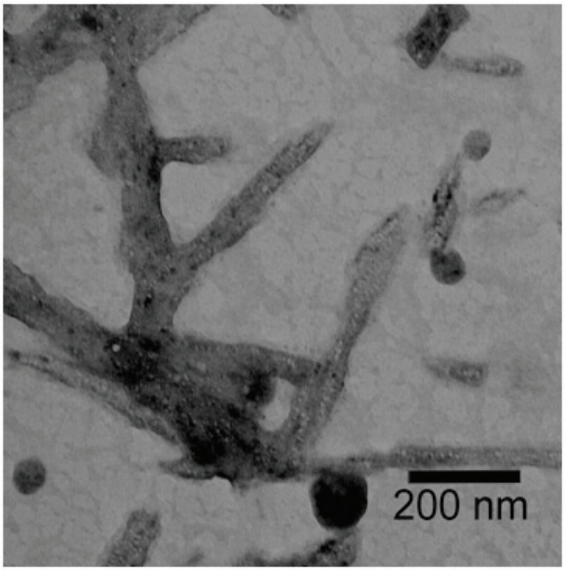

Figure 5- TEM image of Se nanoparticle coated GA nanofibers.

Formation of Se Nanoparticles in the Presence of Gallic Acid

In the presence of Se ions, we observed a remarkable transformation in the growth of the nanostructures, as nanofibers of uniform diameter 50-75 nm were formed upon incubation with Se within one week at pH 7. Depending on the growth period, the nanofibers were an average of 5-10 μm. This is most likely because selenium ions form complexes with GA. Previous studies have shown that a 1:1 reaction of iron (III) and GA forms a binary complex, where the Fe is complexed with GA via the hydroxyl groups.[31] It is likely that similar interactions between GA and Se2+ occur, where the Se ion complexes with GA. The proposed structure for formation of the complex is shown in Figure 4.

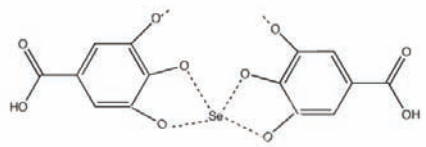

Figure 4- Complexation of GA with selenium.

The formation of GA-Se complexes was also confirmed visually, wherein a blue color was observed upon formation of the complexes. Furthermore, FTIR spectroscopy of the complexes showed that the peak at 570 nm was red shifted by 20 nm, most likely due to coordination with GA.[32] Since Se successfully bound to GA, resulting in the formation of nanofibers, we explored the coating of nanoparticles on the GA nanofiber templates. Thus, DTT was added to the GA nanofibers complexed with Se in order to induce the formation of Se nanoparticles. We observed a color change to brick red, thus confirming Se nanoparticle formation. TEM analyses revealed that the quantity and organization of nanofibers is much greater in the presence of Se nanoparticles, (Figure 5) indicating that interactions with Se accelerate the growth of the nanofibers, due to binding interactions between Se

Figure 6- Emission spectrum of Se nanoparticle coated GA nanofibers.

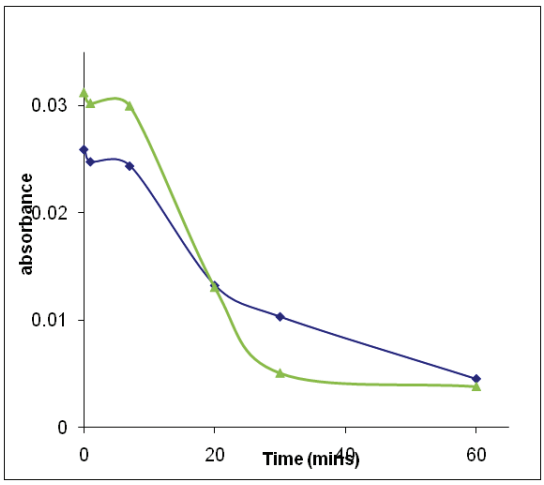

Figure 7- Comparison of (DPPH) radical scavenging activity of Se nanoparticle coated GA nanofibers with GA. The wavelength at 517 nm was monitored over time.

and GA.

Excitation and emission spectroscopic analysis were also conducted to confirm the formation of Se nanoparticles. In the excitation spectrum recorded, the lambda max was obtained at 597 nm. The emission spectrum (Figure 6) shows a peak at 682 nm, confirming the formation of Se nanoparticles.[33]

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The development of antioxidant materials to quench reactive oxygen species (ROS) is essential because of the damage caused by ROS in the body, which plays a role in the development of cancer, cardiovascular disease,[34-36] malarial anemia,[37] and age-associated health problems.[38] The DPPH radical scavenging assay has been effectively utilized to assess the ability of a polyphenol to transfer an H atom to a free radical and is thus a means of measuring a system's capacity to serve as an antioxidant in the body.[33] Past studies have utilized the DPPH assay to measure the antioxidant capacity of polyphenols such as flavonols, tannins, caffeic acid, caffeoyl asters,[34] conjugated linoleic acids,[39] bromophenols from the marine red alga *Polysiphonia urceolata*,[41] red wine pigments,[41] as well as isoflavones and their metabolites.[42] Kawabata and co-workers have assessed the formation of oxidative dimers produced from GA using the DPPH assay.[29] The DPPH assay works by measuring the reduction of the DPPH radical. Therefore, this modified spectrophotometric approach measures a decrease in absorbance of the peak as the electron becomes scavenged and thus measures its action as a free radical scavenging antioxidant. We compared Gallic acid control to Se nanoparticle coated nanofibers. As shown in Figure 7, over time we observed that the absorbance decreased and in 60 minutes, a reduction was observed for the Se nanoparticle coated GA nanofibers. Furthermore, the absorbance spectrum of the DPPH radical scavenging activity of GA over time is shown in Figure 8. These results indicate that Se nanoparticle coated GA nanofibers could potentially work as potent antioxidants.

Conclusions

We have developed a new family of GA based nanostructures. We found that the growth was pH dependent where, in general, self-assembly occurred in one to four weeks. Further, selenium nanoparticles were formed biomimetically over time in the presence of GA, leading to the formation of GA-Se nanocomposites. Complexation with Se resulted in a significant alteration in the shape of GA nanoparticles from nanospheres to nanofibers. The results were confirmed through various spectroscopic and microscopic methods. Finally, we examined the antioxidant abilities of the nanocomposites using the DPPH radical scavenging assay, where the nanostructures were found to be highly potent.

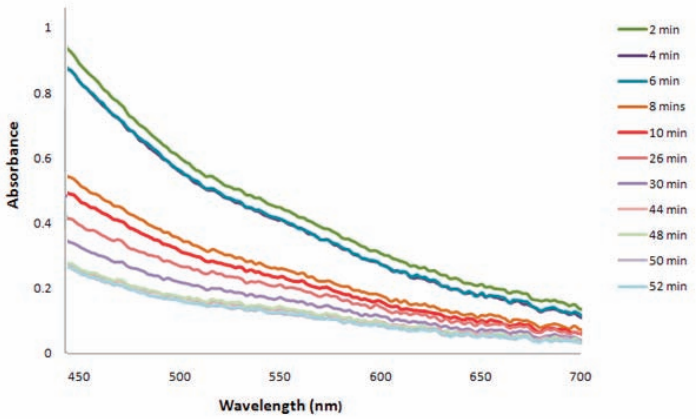

Figure 8- Absorbance spectra showing the1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of GA.

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