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Method for Detection of Thiol-containing Amino Acids Using Gold-Polystyrene Composites

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Abstract

This paper reports the preparation of PS-PEI-Au composite colloids via the utilization of a facile method involving poly(ethylenimine) (PEI). The PEI used in the reaction scheme served the role of a linker between Au and PS and additionally as a reducing agent in the conversion of Au ions to Au NPs. The PS-PEI-Au colloids thus prepared were characterized by scanning electron microscopy, UV-Vis and IR spectroscopy and cyclic voltammetry. The PS-PEI-Au composites were further used for the detection of the thiol-containing amino acids, cysteine and homocysteine, via Attenuated Total Reflection (ATR) spectroscopy. Experimental results revealed interfacial binding of the amino acids to the composites, and correlated with successive additions of the respective amino acids.

Keywords

Attenuated Total Reflection Spectroscopy; Cysteine; Homocysteine; PS-PEI-Au composite colloids

Introduction

In recent times, numerous synthetic mechanisms have been employed to achieve the surface adsorption of metal nanoparticles (NPs) onto colloidal inorganic and organic spheres (1,2). A particularly successful method of depositing the metal NPs on the surface of the colloids involves the use of an intermediate linker Poly(ethylenimine), PEI, which can bind both transition metal ions and negatively charged colloids (3,4). Additionally, the PEI serves as the reducing agent in the conversion of the metal ions to the metal NPs (5). In such a scheme, the core component, usually polystyrene (PS) or silicon dioxide (SiO₂), serves as a supporting structure, and the outer NPs predominantly exhibit the properties that are attributed to the metal NP-core colloid composites. The advantages derived from these hybrid materials can be seen in their remarkable attributes which include enhanced conductivity, temperature stability, optical and catalytic activity (6).

Currently there is tremendous interest in the exploration of metal NPs for the detection of biological molecules. A notable example is DNA detection via complementary interactions of sulfur-containing amino acids with gold nanoparticles (Au NPs) (7). Two of these amino acids, cysteine (Cys) and homocysteine (Hcys), are specifically known to play different roles in a variety of diseases. Cysteine plays an integral role in the body by facilitating crosslinking of proteins via disulfide bonds which support their secondary structures and functions (8). It is also a potential neurotoxin (9–11) and is a biomarker for various medical conditions such as rheumatoid arthritis and AIDS. Homocysteine is generated from the metabolism of methionine, and exists in both its reduced and oxidized forms in blood and tissue. A concentration of

Homocysteine larger than ~15 mM is considered as a risk factor for individuals suffering from heart disease. (13) As a consequence, the analytical detection of these amino acids is of paramount importance.

IR spectroscopy in combination with Attenuated Total Reflection (ATR) spectroscopy is one of the most widely used technique for surface infrared analysis. ATR -IR has been used for studying processes at surfaces or in films, such as adsorption (14–17) and diffusion (18–20). Also, ATR-IR spectroscopy has been applied to the study of the chemisorption of protein on a gold surface (21), cysteine adsorption on copper surfaces (22), and the adsorption of N-Acetyl-L-Cysteine on gold (23). Recent scanning tunneling microscopy (STM) studies of the adsorption of cysteine on gold suggest strong Au-S binding of cysteine to a gold surface via the SH group (24,25).

In this paper, we report a novel method for detecting thiol-containing amino acids by incorporating the enhanced properties of Au NP coated polystyrene core colloid composites with the strong affinity that Au NPs have for sulfur-containing compounds. Polystyrene was utilized for the core component with the outer layer being comprised of Au NPs. We characterized the PS-PEI-Au colloids by using SEM, FT-IR, UV-visible spectroscopy, and an electrochemical method. The prepared PS-PEI-Au colloids were placed on the ATR ZnSe crystal forming a thin layer used for the detection of the thiol-containing amino acids, cysteine and homocysteine.

Experimental Section

Materials

Hydrogen tetrachloroaurate (HAuCl_4), polyethylenimine (PEI) (Branched PEI with a Mw 5,000 g/mol), absolute ethanol, sodium citrate ($\text{C}_6\text{H}_5\text{O}_7\text{Na}_3 \cdot 2\text{H}_2\text{O}$), formaldehyde (HCHO, 37%), ammonium hydroxide solution (NH_4OH , 25%), polystyrene colloids (10%), cysteine (Cys), homocysteine (Hcys), sodium phosphate buffer. All material were obtained from Sigma-Aldrich except polystyrene colloids which were obtained from (Seradyn Particle Technology)

Synthesis

Modification of Core Colloids with PEI—1g of PS colloids was dispersed in 50 mL of phosphate buffer solution. 0.1g of PEI was dissolved in 5 mL of phosphate buffer solution and the resulting PEI solution was added to the PS dispersion. The mixture was then stirred at room temperature for 1 h. After three centrifugation/wash cycles, PS colloids modified with PEI were obtained.

Preparation of PS-PEI-Au—0.1g of the modified PS-PEI colloids was dispersed in 100 mL of distilled water, following which 0.15g (3.8 mL of 0.1M) HAuCl_4 was added and the solution heated at 100°C for 1 h. The color of the solution changed from yellow to dark red, indicating formation of Au NPs on the PS surface. After cooling to room temperature, 10 mL of sodium citrate was added followed by dropwise addition of NH_4OH solution to bring the pH of the system to 10. HCHO diluted with ethanol was then added dropwise to reduce residual Au ions. After the reaction, the products were isolated and cleaned by centrifugation/washing/dispersion cycles.

Cysteine/Homocysteine Adsorption on PS-PEI-A—10 μL of the prepared PS-PEI-Au colloids was placed on MIRacle Universal Plate of ZnSe crystal (PIKE Technologies, single reflection horizontal ATR) and allowed to dry forming a thin layer. 10 μL of 1mM cysteine solution was added to the dry PS-PEI-Au layer and the ATR spectrum obtained at a 4 cm^{-1} resolution with a spectral range of 750–4000 cm^{-1} . Following this, additional 10 μL amounts

of the cysteine solution was added at 120 minute intervals to give cumulative 20 and 30 μL quantities, for which the ATR spectrum was obtained in each case. The procedure outlined above for the cysteine solution was then repeated using a 1mM homocysteine solution in its place.

Characterization

UV-Visible spectra were acquired with a *Varian Cary 100 Bio* UV-vis spectrophotometer. The nanoparticles were dissolved in an aqueous solution, and the UV-Vis measurement carried out in a quartz cuvette. FTIR and ATR data were acquired with a *Perkin Elmer Spectrum BX FT-IR System*. The sample for the FTIR measurement was prepared by grounding a dry nanoparticle sample with KBr into fine powders which were then pressed at 20,000 psi into pellets. Scanning electron microscopy (SEM) was performed using an (JSM-5900) at 30 kV. Cyclic voltammograms of the redox of gold nanoparticles were obtained on an electrochemical analyzer (CH Instruments Model 440A) in a standard three-electrode cell comprising a glass carbon electrode as the working electrode, a platinum wire as the counter electrode and Ag/AgCl as the reference electrode. Voltammetry was performed at a sweep rate of 100 mV/s, using KCl as the supporting electrolyte.

Results and Discussion

In the initial step of the synthetic process, the carboxyl modified PS colloids were coated with Poly(ethylenimine) (PEI). In the next step, an aqueous solution of Au precursor ions was mixed with a dispersion of the PEI-modified PS colloids to achieve adsorption. In the final step, the resulting dispersion was heated to 100 $^{\circ}\text{C}$ for 1 hour which ultimately produced the PS-PEI-Au composites. PEI is a cationic polymer with branched structure in which plentiful amine groups can bond with both transition metal ions and negatively charged colloids (3,4). In addition, PEI could act as a reductant in the preparation of metal NPs (5). Recently, by using PEI as both a linker and an “in situ” reductant, a simple method was developed to prepare PS-PEI-Au composites (26). The method is based on the formation of a composite composed of core colloids (PS spheres), reductant (PEI), and metal ions. Heat treatment can transform the composites into PS-PEI-Au colloids. In the synthesis, PEI acted both as a linker between metal ions and core colloids and as the reductant for in situ reduction of metal ions to metal NPs. Formation of the PS-PEIAu colloid composites was seen by the color change from yellow to dark red. This is attributable to electric dipole-dipole interactions and coupling between plasmons of neighbouring particles in the aggregates (27–29). Scanning electron microscopy images were obtained on a JSM-5900 which was operated at 30 kV.

The synthesized PS-PEI-Au composites were suspended in water, dispersed them on glass, and coated with approximately 10 nm of AuPd. Figure 1 shows an SEM image of the as-synthesized PS-PEI-Au composites bound together in clusters, where the small Au NPs can be seen immobilized on the surface of the PS-PEI spheres. The lighter edges of the spheres are due to electrical charging that is an issue in SEM imaging. The lighter edges is an edge effect, that is, secondary electrons can be collected from the top and bottom surfaces of the sphere edges, leading to an enhanced secondary electron signal. In addition, the UV-Vis spectra in Figure 2 show a characteristic absorption peak at $\sim 550\text{ nm}$ for the composite, further indicating the formation of Au NPs on the PS surface. The origin of the SP band for gold nanoparticles is the coherent excitation of free conduction electrons due to polarization of the electrons induced by the electrical field of incident light which is largely isotropic for well-isolated spherical nanoparticles (30). A change in absorbance or wavelength provides a measure of particle size, shape and aggregation or elongation properties. Encapsulation of the nanoparticles by adsorption or binding of organic species on the particle surface and subsequent cross-linking at binding sites of the encapsulating shells are important factors that influence the optical property.

Figure 3 shows the IR spectra of PS, PS-PEI and PS-PEI-Au composites. The peak at 2918 cm^{-1} is attributable to the stretching vibration of C-H from PS, PS-PEI and PS-PEI-Au composites but the intensity of the peak of PS-PEI-Au composites was sharply decreased. The stretching vibration of C=O of carboxyl about 1710 cm^{-1} is clearly seen on the PS curve, however it could not be observed that it drastically decreases in PSPEI curve and disappears entirely in the PS-PEI-Au curve. This can be explained by the coordination of the carboxyl group of PS with PEI and Au NPs in the synthetic colloidal modifications. The two peaks at 1493 and 1452 cm^{-1} are as a result of the antisymmetric and symmetric stretching of O-C-O respectively and are seen intensely in the PS curve, but decrease in the PS-PEI curve, and decrease even more in the PS-PEI-Au curve. This is again due to the consumption of the functional group in the synthetic coordinations to form PS-PEI and PS-PEI-Au respectively. Also observable is a peak at 1614 cm^{-1} which is present on the PS curve and persists through the subsequent synthesis of PS-PEI and ultimately PS-PEI-Au. This peak can be assigned to the benzene ring which comprises the PS core structure. For PS-PEI, a new peak emerged at 3429 cm^{-1} which was not appreciably present in PS. This can be ascribed to the N-H stretching vibration from the PEI. It can be seen that the intensity change of this N-H vibration is not significant after the formation of metal NPs on the surface. (i.e. PS-PEI-Au). These results support the recently reported formation procedures of Au NPs on PEI (31–32). In the proposed mechanism, the protonated secondary amine of branched PEI was formed by mixing PEI and HAuCl_4 solutions. It was suggested to predominantly induce the reduction AuCl_4^- precursor to zero charge gold atoms which formed Au NPs by colliding itself.

As a further means of characterization, a thin film of the PS-PEI-Au colloids was characterized by cyclic voltammetry (CV) in order to probe the oxidation and reduction behavior of Au NPs. A typical voltammetric response of the film at a sweep rate of 100 mV/s , using KCl as the supporting electrolyte is presented in Figure 4. The CV shows the peaks corresponding to gold oxide formation at approximately 1.1 V and its subsequent reduction at a potential of 0.4 V . The redox potentials were shifted to more negative potential direction compared to redox potential of gold nanoparticles, oxidation peak potential at 1.2 V , and reduction potential at 1.0 V in acid solution (33).

Figure 5 shows the ATR spectra for consecutive addition of $10\text{ }\mu\text{L}$ aliquots of 1 mM cysteine solution to a thin layer of the PS-PEI-Au colloids. The NH_3^+ symmetric deformation mode for cysteine adsorbed on the PS-PEI-Au composite was assigned to the peak at 1510 cm^{-1} . After the addition of an initial $10\text{ }\mu\text{L}$ of cysteine there was no visible peak at this wavenumber, however after a cumulative amount of $20\text{ }\mu\text{L}$ a peak evolved which increased in intensity after the addition of a subsequent $10\text{ }\mu\text{L}$. The C=O stretching vibration of the carboxyl group is observed at about 1710 cm^{-1} , and it can thus be surmised that cysteine is coordinated in a nonzwitterionic form to the gold. Additionally, carboxylate groups may be presented, however their visibility in the spectrum may be overshadowed by the NH_3^+ degenerate deformation mode around 1640 cm^{-1} . The presence of the carboxylate groups is supported by the splitting of the COO^- symmetric stretching mode typically at 1400 cm^{-1} to give peak assignments of 1388 cm^{-1} and 1416 cm^{-1} respectively. This data correlates with previous chemisorption data of cysteine on a gold surface as described by Ihs and Liedberg (34). No characteristic S-H stretching band for cysteine was observed around the normal value of 2250 cm^{-1} in the spectra. The most logical explanation of this phenomenon is that the cysteine molecules are coordinated to the gold on the nanocomposites through the sulfur and the proton leaves the sulfur during formation of the gold-cysteine complex. The characteristic peaks aforementioned were absent at the initial $10\text{ }\mu\text{L}$ cysteine amount but increased in intensity with further $20\text{ }\mu\text{L}$ and $30\text{ }\mu\text{L}$ amounts respectively. These results were attributed to the binding of cysteine to the Au NPs on the PS-PEI-Au composites effecting chemical modifications since it can be clearly seen that the spectrum of a $20\text{ }\mu\text{L}$ cysteine droplet yielded no peaks without PS-PEI-Au colloid film. Based on the response of the PS-PEI-Au nanocomposites to the experimental parameters of

volume and concentration, the minimum sensitivity limit of this platform for the detection of cysteine is approximately 2.0 PPB.

Figure 6 shows the ATR spectra for consecutive addition of 10 μL aliquots of 1 mM homocysteine solution to a thin layer of the PS-PEI-Au colloids. The NH_3^+ symmetric deformation mode for homocysteine adsorbed on the PS-PEI-Au composite was assigned to the peak at 1540 cm^{-1} , however the intensity was weak and could represent a site of interfacial binding to the gold. This peak was observed after the addition of an initial 10 μL of homocysteine, and became more clearly defined with cumulative additions of 10 μL aliquots of the homocysteine solution. The COO^- symmetric stretching mode for homocysteine was assigned to the peaks at 1394 cm^{-1} and 1418 cm^{-1} respectively and increased in intensity with further 20 μL and 30 μL cumulative amounts. Additionally, the COO^- asymmetric stretching mode was clearly visible at 1592 cm^{-1} . Similar to the results derived from cysteine, the increase in peak strength was due to the adsorption of homocysteine to the Au NPs inherent in the PSPEI-Au composites. The peaks at 1292 and 1336 cm^{-1} respectively were assigned to the C-O and C-N stretches respectively from the chemical linkages in the homocysteine molecule. These peak intensities increased with successive additions of the homocysteine due to adsorption. As observed with the cysteine and gold, no S-H stretching band (2250 cm^{-1}) was observed in the spectra, which suggests that the SH group is a coordination site for homocysteine where the proton leaves the sulfur in the formation of the gold-homocysteine complex at the surface of the nanocomposite. Analogous to cysteine, the detection limit of homocysteine was found to be 3.0 PPB, whereby the nanocomposites can be reasonably used to detect the presence of homocysteine based on specific binding.

Conclusions

This paper reports the investigative findings of a novel and facile method to detect sulfur-containing amino acids, namely cysteine and homocysteine, by using Au-NP composite colloids. Sulfur-containing amino acids, particularly with thiol groups, have a strong affinity for gold and this is the underlying basis for the reactivity and subsequent detection platform. Results from the investigation revealed that there was cumulative binding of cysteine and homocysteine to the PS-PEI-Au colloids with successive additions on a microliter range. Though the work presented is primarily an elucidatory study, it holds tremendous potential for analytical biodetection, considering the strong physiological link between cysteine and homocysteine and various disease conditions. The Au-NP composites and corresponding detection platform has numerous advantages, including ease of production, simple operation and variability of metal coverage which contribute to increased tunability in a broader spectrum range.

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Figure 1.
SEM images of PS-PEI-Au colloids

Figure 2.
UV-Visible Spectrum of PS-PEI-Au Colloids

Figure 3.
IR Spectra of (a) PS, (b) PS-PEI and (c)PS-PEI-Au

Figure 4.
Cyclic Voltammogram Showing Oxidation/Reduction of PS-PEI-Au Colloids

Figure 5.
ATR Spectrum of 1 mM cysteine droplet on PS-PEI-Au colloid film

Figure 6.
ATR Spectrum of PS-PEI-Au colloids with successive addition of homocysteine