Surface Plasmon Spectral Shifts of Functionalized Gold Nanoparticles for the Use in Biosensors

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ABSTRACT

Recently there has been a great interest in the use of gold nanoparticles to detect the hybridization process of DNA. The method uses the physical phenomenon that describes size-dependent Surface Plasmon Resonance (SPR) of gold nanoparticles and its shift to greater wavelengths upon their aggregation. Here we report on the synthesis of gold nanoparticles coated with functional linker biomolecules and their spectral shift driven by the formation of the avidin-biotin complex towards the use of these materials in the development of DNA biosensors. The gold nanoparticles were covalently functionalized by biotin and BSA-biotin in aqueous solution. UV-vis analysis of the dispersed colloidal nanoparticles resulted in a characteristic absorption spectra. Upon the addition of the avidin, the aggregation of gold nanoparticles was inferred from the alteration of absorption spectra.

Keywords: biomarkers, gold nanoparticles, surface plasmon resonance, biotin, avidin.

1 INTRODUCTION

There is a growing interest in the potential application of nanoparticles to develop novel bio-conjugated nanostructures and biosensing devices [1-7]. The special properties of the nanoparticulates, such as surface plasmon resonance (SPR) play a significant role in elucidating the specific molecular recognition event of biomolecules (protein, DNA) allied with nanomaterials [5-7].

In recent years, gold nanoparticles have been used to detect the hybridization process of nucleic acids, and specific antigen-antibody interactions that can be applied to develop bio-sensing devices [7-8].

The hybridization process of the Au-conjugated DNA has been used to develop a colorimetric detection technique to identify single -base mismatch in a DNA sequence [9]. The thiol-gold linkage was utilized to connect the nucleic acids with gold nanoparticles. The gold nanoparticles allied with DNA have been applied to construct ultra sensitive quartz crystal microbalance and scanometric DNA array detection [9-10].

To construct bio-conjugated nanostructures and devices, the nanoparticles are functionalized with linker molecules, which maintain the connectivity between nanoparticles and biomolecules of interest, (e.g. nucleic acids) [6]. The linker molecules possess specific functional groups, which connect covalently with the DNA strands (or the modified end of the nucleic acid strands) [6-8]. Avidin, streptavidin, and biotin have been employed as linker molecules to establish the covalent connection between biomolecules and nanostructures, since the avidin-biotin complex is highly stable in a wide temperature and pH range [11-12].

Biotin, a water-soluble B-complex vitamin, binds to both streptavidin and avidin, two naturally occurring proteins [13]. Avidin is a basic glycoprotein (MW 68Kd). It has a high isoelectric point of approximately 10, and consequently is positively charged at the neutral pH [13]. Streptavidin is a tetrameric protein (MW 60Kd), and is isolated from the bacterium *Streptomyces avidini*. Like avidin, it contains four affinity-binding sites for biotin. Conversely, streptavidin has an isoelectric point close to neutral pH, and therefore contains a few strongly charged groups near this pH [13,14]. The strong affinity to avidin was documented by the estimation of the dissociation constant, K_d ($K_d = 4 \times 10^{-14}$ M for streptavidin, and 6×10^{-16} M for avidin) [13,14] .

The application of biotin-avidin complex has been extended to construct bio-conjugated nanostructures [6-7]. The attachment of functional linker molecules to the nanoparticles put forth critical effects on the physiochemical properties of the nanoparticles [6-8]. Due to the surface modification of the nanoparticles by the attachment of biomolecules, the optical properties of nanoparticles could undergo a considerable alteration [8]. On the other hand, the attachement of nanoparticles to biomolecules can lead to a modification in the binding property of the linker molecules [2,8].

In this study, we discuss the synthesis and properties of gold nanoparticles functionalized with linker molecules, and their potential applications. Biotin can function as an effective linker molecule, as well as interact with gold nanoparticles by means of non-covalent interactions [11,12], thus, it was used to functionalize gold nanoparticles. To investigate the effect of the linker functional biomolecules on the nanoparticles, we synthesized the biotin functionalized gold nanoparticles by following three different approaches (see experimental procedure).

2 EXPERIEMNTAL PROCEDURE

2.1. **Materials**

Potassium tetrachloroaurate (III) [$KAuCl_4$] (Sigma Aldrich, 99.99% (metals basis), Au 49% min, Packed under argon), Sodium Citrate, Sodium Borohydride [$NaBH_4$] (Aldrich, 99%), Biotin (Sigma-Aldrich, d-Biotin, 99%), BSA-Biotin (Sigma), Avidin (Sigma), PBS buffer (Sigma-Aldrich)

2.2 **Synthesis of Gold Nanoparticles Coated with Biotin**

An aqueous solution of gold nanoparticles was prepared by the reaction of $KAuCl_4$ and sodium citrate (Table1, Experiment A). Thereafter, the gold nanoparticles were modified with the addition of BSA-biotin molecules at room temperature [4, 11]. 5 mL of gold solution was mixed with 0.5 mL of aqueous BSA-biotin solution and incubated for 4 hours at room temperature (Table1, Experiment A). Then the solution was centrifuged at 15^oC and 13500 rpm for 1 hour. The final product of biotinylated gold was dispersed in 10 mM phosphate buffer.

In another method, the gold nanoparticles were prepared by reduction of $KAuCl_4$ with $NaBH_4$ in the presence of biotin (Table1, Experiment B). 1mL of $KAuCl_4$ solution was added to 89 mL of deionized water. Next, 1 mL biotin solution was added with constant stirring. Thereafter, 10 mL $NaBH_4$ solution was added drop wise and the solution was allowed to stir for 3 hours, maintaining the temperature within 0-5^oC to obtain final product.

Correspondingly, the biotin functionalized gold nanoparticles were prepared by the reaction of $KAuCl_4$ and $NaBH_4$ in aqueous solution containing BSA-biotin (Table1, Experiment C). 2 mL of BSA-biotin solution was added to 88 mL of $KAuCl_4$ solution with constant stirring at room temperature. Then 10 mL of $NaBH_4$ solution was added drop wise and the mixture was allowed to stir for 4 hours at -5^oC to get the final product.

EXP	$KAuCl_4$ (M)	Sodium Citrate (M)	$NaBH_4$ (M)	Biotin (M)	BSA Biotin (mg/ mL)
A	$2.4*10^{-4}$	$8.5*10^{-4}$	-----	----	1.44
B	$3.0*10^{-4}$	---	$9.8*10^{-5}$	$3.6*10^{-6}$	---
C	$1.1*10^{-4}$	---	$3.1*10^{-4}$	---	1.7

Table 1: Concentration of the chemicals for the preparation of gold nanoparticles

2.3. **Aggregation experiment of the BSA-biotinylated gold nano particles with the addition of avidin**

A 100 mM avidin stock solution was prepared in PBS buffer. 2 mL of avidin solution was added to an equal amount of BSA–biotinylated gold solution (prepared by citrate method) and the solution was incubated for 1 hour at room temperature.

2.4. **Characterization of gold solutions**

Dynamic light scattering (DLS) method was utilized to measure the effective diameter and polydispersity of the biotin functionalized gold nanoparticles. 90Plus/BI-MS-Particle Size Analyzer (Brookhaven Instrument Corporation) was used to determine the size of gold nanoparticles in an aqueous solution at room temperature (25^oC). The DLS method employs spatial and temporal variation of the scattered light to measure the size of the particles suspended in a liquid medium. The effective diameter of the nanoparticles represents an average size of the particles in the samples, and the polydispersity is a measure of the non-uniformities that exist in the particle size distributions.

The UV-vis absorption spectrum is a sensitive way to analyze the nanoparticles environment [1]. Ocean Optics HR2000, High Resolution Spectrophotometer was used to obtain the absorption spectra of gold nanoparticles solution. The change of UV-vis spectra of bioconjugated nanoparticles provides qualitative information of the stability of the nanomaterials system. The characteristic absorption spectrum portrays the size-dependent optical behavior of the nanoparticles [1].

3 RESULTS AND DISCUSSIONS

Table (2) shows the sizes of gold nanoparticles allied with different biotin molecules. The attachment of linker biotin molecules causes a change in the hydrodynamic diameter of the nanoparticles as indicated by the progression of the diameter size of the nanoparticles with the addition of biotin from 30 nm to approximately 3 times its size.

Sample	Effective Diameter	Standard Error	Poly-dispersity
Au NP in aqueous solution	30nm	0.1	0.113
Biotinylated Au NP	59 nm	1.9	0.260
BSAbiotinylated Au NP	97nm	0.4	0.186

Table2: Measurement of gold nanoparticles (Au NP) by DLS

The UV-vis absorption spectra of the aqueous solution of gold prepared by the citrate method exhibited characteristic absorption at the wavelength approximately 530 nm (Figure1). When the gold nanoparticles were combined with biotin, the absorption spectrum of the gold did not alter significantly from its original spectral nature. This result affirms that the surface condition of the gold nanoparticles did not change appreciably after the association with the biotin molecules.

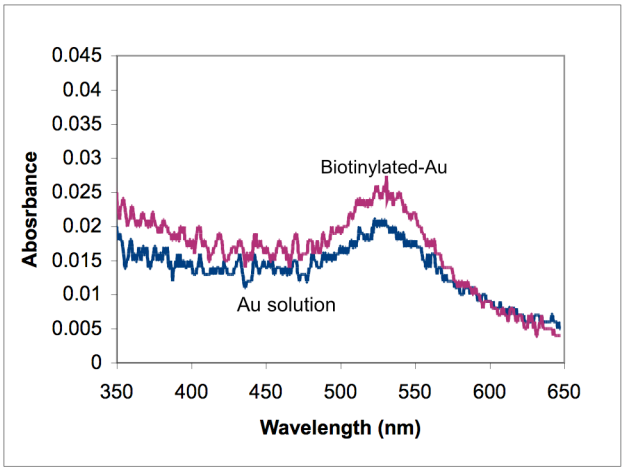

Figure.1: Comparison between Au solution and Biotinylated –Au solution

The biotinylated gold nanoparticles prepared by the sodium borohyhride method exhibit the absorption at the wavelength 539 nm (Figure 2). Similarly, BSA-biotinylated gold nanoparticles show the absorption at 526 nm (Figure2). From the UV-vis absorption study, it can be inferred that the methods of synthesis, as well as the nature of functional linker biomolecules considerably control the optical property of the nanoparticles as shown by the wavelength change of approximately 13 nm (Figure 2).

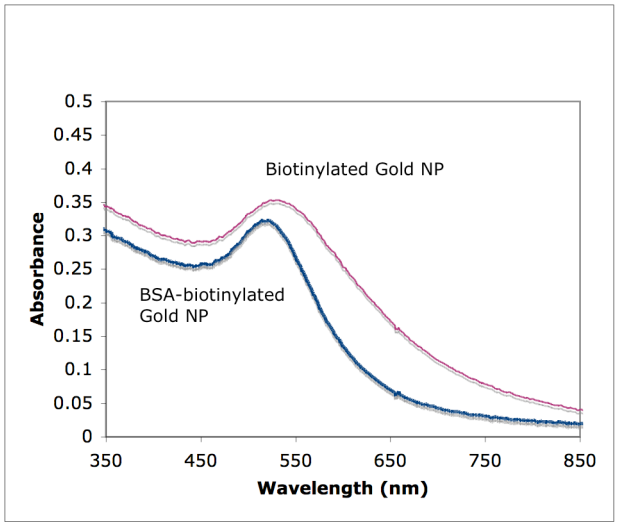

Figure 2: UV-vis absorption spectra of biotinylated gold and BSA-biotinylated gold nanoparticles (Gold NP)

Since avidin forms a strong complex with biotin, it initiates the aggregation of the biotinylated nanoparticles. The noncovalent interaction between the biotin and avidin causes an alteration in the absorption spectra of the gold nanoparticles by changing the effective size of the bio-conjugated nanostructures (Figure 3).

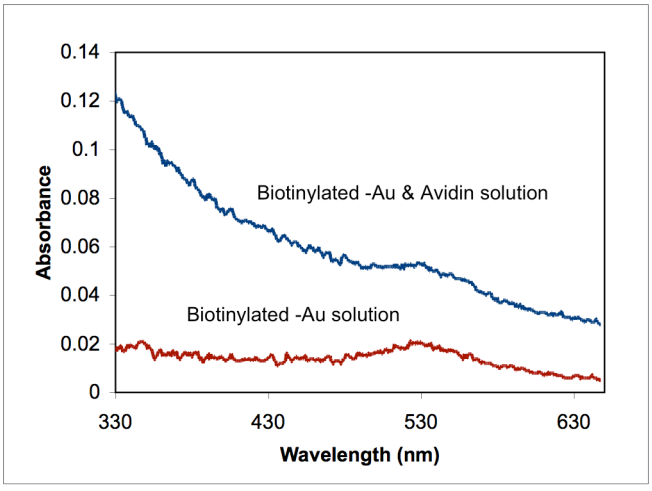

Figure 3: The change in the UV-vis absorption spectra of biotinylated gold after addition of avidin

In figure 3, a drastic shift of the absorption spectra of gold is not observed; it exhibits inadequate binding of biotin to avidin and consequently incomplete aggregation of the gold nanoparticles. Although the formation of the

avidin-biotin complex is highly probable under a wide range of experimental conditions, the attached nanoparticles can cause a change in the binding activity of biotin.

The linker biomolecules (avidin, biotin) connect the target molecules (e.g., nucleic acids, proteins) to the nanostructures and the physical features of the nanomaterial system depend on the association method. Although, the colorimetric DNA detection technique generally depends on the specific hybridization of DNA to trigger the change in optical properties of nanomaterials [8,15], the method of attachment of nucleic acids to nanostructures can likely influence the process as discussed in this study. As a result, the coupling of nanoparticles and biomolecules plays an important role in bio-template reactions, nanoparticle assembly and biosensing devices.

4 CONCLUSIONS

The linker molecules can effectively modulate the sensitivity and selectivity of biosensing devices. Since the attachment of nanomaterials can modify the selective molecular recognition property, and physiochemical behavior of linker molecules, proper conditions must be maintained to preserve the active functionality of the linker biomolecules.

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