Self-assembly of Gold Nanoparticles by Hybridization of Two Complementary DNAs

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Abstract

Gold nanoparticles were synthesized by reducing $HAuCl_4$ with trisodium citrate. These gold nanoparticles could react with disulfide group modified DNAs well. The DNAs modified gold nanoparticles assembled into regular array according to the hybridization of two complementary DNAs linked on gold nanoparticles individually. This reaction caused the color of gold nanoparticle solution changed from red to blue. Thus, this phenomenon showed the potential of DNAs modified gold nanoparticles to be applied in biosensing or as DNA probes for diagnosis.

***Keywords*:** Gold nanoparticles, DNA, Hybridization

Introduction

To detect sequence-specific DNA has been attended because of its applications in the diagnosis of pathogenic and genetic diseases [1-3]. Many detection techniques have been developed with radioactive, fluorescent, chemiluminescent and other types of labeled probes [4,5]. In this field, the organization of gold nanoparticles by DNA hybridization reactions reveal a potential for diagnosis.

Gold nanoparticles suspension consist of small granules of this transition metal in a stable and uniform dispersion. The studies of the particle size evolution of the surface plasmon resonance width and maximum position has also been reported [6]. By using this phenomenon, Mirkin and his coworkers have assembled gold nanopaticles by DNA hybridization with a color change from red to blue [7]. In this new nanoparticle-based detection system, gold nanoparticles are capped with thiol-modified DNAs. Using a colorimetric detection method, the responses of targeting special DNAs can be detected [8,9].

In this report, we modified gold nanoparticles with disulfide modified DNAs. Self-assembly of gold nanoparticles and color changed were observed while the hybridization reaction happened.

Experimental

Tetrachloroauric acid ($HAuCl_4$) and trisodium citrate were purchased from Across Chemical Co. The 5'-C_6 disulfide modified DNAs were synthesized by Quality Systems Inc. The sequences of these two DNAs are described as the following:
DNA I: 5'-C_6 disulfide-AAT GAG TGG GCA GGC GGC GA.
DNA II: 5'-C_6 disulfide-TCG CCG CCT GCC CAC TCA TT.
The C_6 disulfide indicates the chemical of 1-O-dimethoxytrityl-hexyl-disulfide 1'-[(2-cyanoethyl)-(N, N-diisopropyl)]- phosphoramidite**.**

Synthesis of Gold Nanoparticles

In a 250 ml round bottom flask equipped with a condenser, a 85 ml of distilled water was mixed with a 3 ml of 1% (w/v) $HAuCl_4$ aqueous solution (This solution was prepared by dissolving 1 g of $HAuCl_4 \cdot 2H_2O$ in 90 ml of distilled water.). The mixture was boiled with vigorous stirring. The rapid addition of 8.8 ml of 38.8 mM trisodium citrate to the above solution resulted in a color change from pale yellow to bright red. The boiling was continued for 10 minutes and the heat was then removed. After the solution reached room temperature, it was stored at 4 ℃ [10,11].

Preparation of DNA-coated gold nanoparticles

A 750 μl aqueous solution of the gold nanoparticles was added to a mixture of a 86 μl aqueous solution of 5'-C_6 disulfide modified DNA (10 μM) and a 144 μl of deionized water. The mixture was allowed to stand for 12 to 24 hours at room temperature. Then a 100 μl of 0.1 M sodium phosphate buffer (with a pH value of 7.0) and a 100 μl of 1.0 M NaCl were premixed and then were added to the previous mixture. After 10 minutes later, a 10 μl aqueous solution of 1 % NaN_3 was added and then was stood for an additional 40 hours. The solution was centrifuged to give the gelatinous precipitate at the bottom of the tube. The clear solution was removed and then the residue was resuspended in a 200 μl of buffer solution

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(containing 10 mM phosphate and 0.1 M NaCl) and recentrifuged. After removal of the aqueous solution, the residue was dissolved in a 1.0 ml of buffer solution and a 10 μl aqueous solution of 1 % NaN_3 for storage.

Hybridization of DNA-coated gold nanoparticles

The hybridization reaction of the DNA I and DNA II coated gold nanoparticle solutions was incubated at 25 ℃ for 24 hours. This solution was dropped onto a carbon coated copper grid for TEM (transmission electron microscopy, JEOL 100CX TEM) investigation. The surface plasmon characteristics of gold nanoparticle solution were measured by UV-VIS spectrophotometer (JASCO V-530).

Results and discussion

At present, there are many methods to synthesize gold nanoparticles. Most of these methods described the reducing processes using tetrachloroauric acid ($HAuCl_4$) as precursor[10] and the synthesis processes are simple. In our work, we chose nontoxic reagent, sodium citrate, as reductant. Figure 1A shows the UV-VIS spectrum of synthesized gold nanoparticles. The sharp absorption peak at 526 nm with narrow bandwidth means the narrow size distribution of gold nanopatticles in solution. The solution of gold nanoparticles appeared dark red because of the surface plasmon resonance phenomenon of gold nanoparticles. The increase of nanoparticle size occurred a red shift in plasmon band. Thus, when gold nanoparticles were coagulated, the plasmon peak shifted toward lower energy and the bandwidth increased.[6] The red shift was attributed to the decrease of spacing between gold nanoparticles. The increase of bandwidth was due to the coagulation of gold nanoparticles. Insomuch, a blue solution was obtained when red gold nanoparticles were coagulated.

Scheme 1 shows the strategy to assemble gold nanoparticles by complementary DNAs. The disulfide functional group reacted with gold [12] and introduced DNAs onto the surface of gold nanoparticle. When gold nanoparticles reacted with disulfide DNA, only a little shift of the absorption peak was observed (Figure 1B). As compared with the spectrum before reaction, the same bandwidth was appeared in both of the spectra. Thus, the gold nanoparticles were still well suspended in solution after being coated with DNAs.

When the gold nanoparticles were coated with 5-C6 disulfide modified DNA I and DNA ?? individually, they were still well suspended in aqueous solutions. These two DNAs could hybridize while the two kinds of the modified gold nanoparticles were mixed. Figure 2 shows the TEM micrograph of this result. There were separations between gold nanoparticles caused by the length of hybridized double strand DNAs.

A red shift happened while the coagulation of gold nanoparticles appeared. Consequently, the absorption peak in UV-VIS spectra shifted to longer wavelength with increasing the hybridizing time. Figure 3 shows the peak wavelength shifts of gold nanoparticles versus hybridization time. While the coagulation happening, the color of gold nanopraticle solution changed from dark red to blue. In this paragraph, the peak shifted from 525 nm to 560 nm. Figure 4A shows the

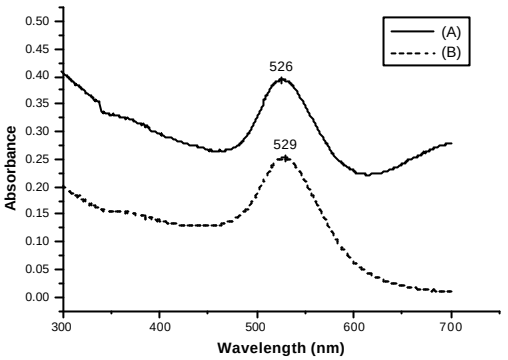
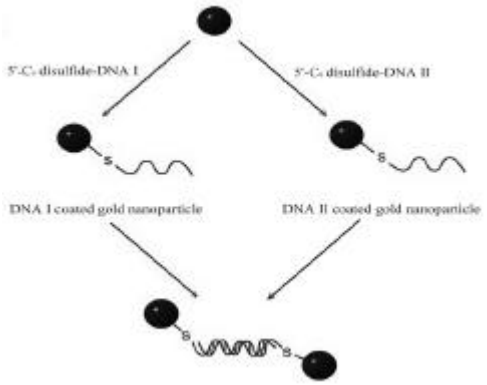


Figure 1. UV-VIS spectra of (A) synthesized gold nanoparticles and (B) DNA modified gold nanoparticles.



Scheme 1. Schematic depiction of the strategy to assemble gold nanoparticles by complementary DNAs.

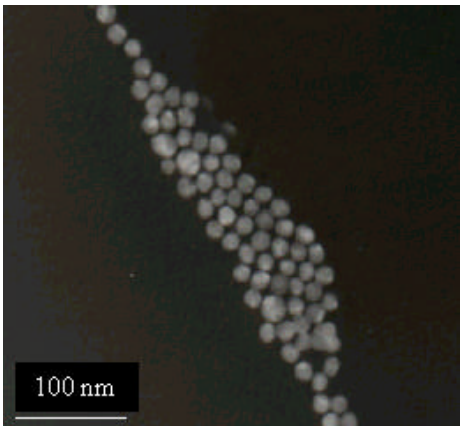


Figure 2. TEM micrograph of self-assembled gold nanoparticles by the hybridization of two kinds of complementary DNAs. The particle size is 14.7±1.6 nm and the scale bar is 100 nm.

spectra of the gold nanoparticle solution at different hybridization times. Evidently, the absorption peak shifted to lower energy while increasing hybridization time. The peak almost smeared after reacting for 19 hours. During the reacting time, the absorbance at 526 nm became lower and lower. Oppositely, the absorbance at 700 nm was raised as shown in Figure 4B. Consequently, the color of gold nanoparticle solutions changed from red to blue (Figure 3).

To prove that the gold nanoparticles were coagulated by hybridization of the two complementary DNAs, the mixture was heated to 80 ℃ to destroy the hydrogen bonds between

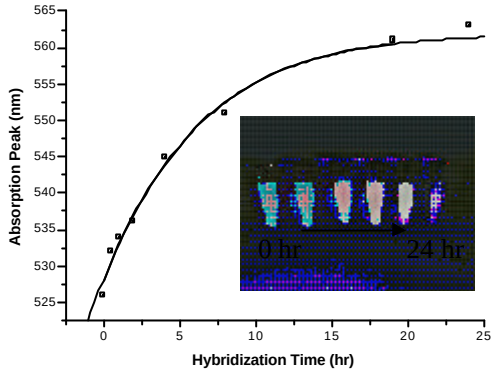
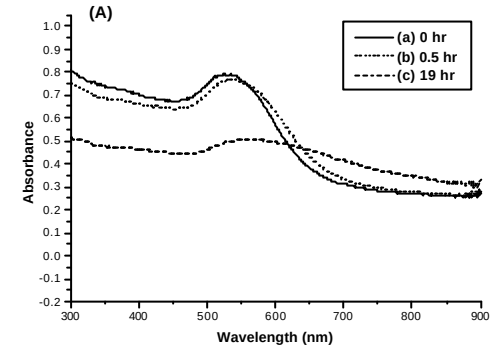
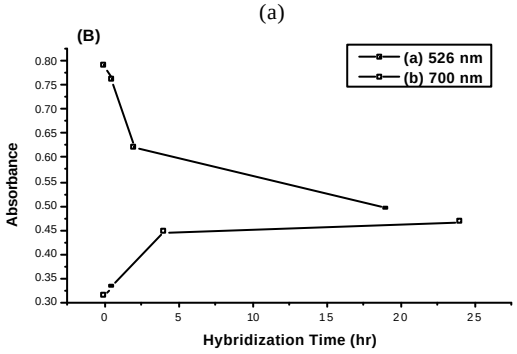


Figure 3. The absorption peak wavelength shifts of gold nanoparticles versus hybridization time. The image shows the colors change from red to blue.



(a)



(b)

Figure 4 (a) UV-VIS spectra and (b) absorbance at 526 nm and 700 nm of the DNAs modified gold nanoparticles at different hybridization times.

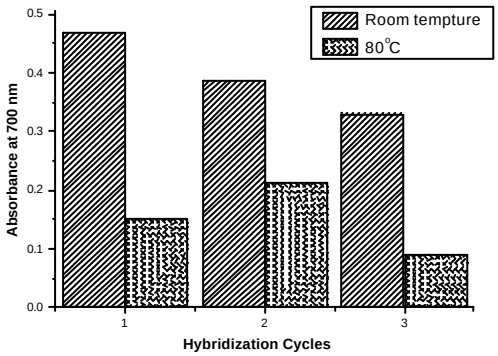


Figure 5. Absorbance at 700 nm of the DNAs modified gold nanoparticles with the treatments of several cycles between 80 ℃ and room temperature .

the double strand DNAs. Therefore, the absorbance at 700 nm became smaller after being heated and the color of gold nanoprticle solution turned to be red. That meant the dehybridization reaction happened and gold nanoparticles were resuspended in water. After being heated, the solution was stood at room temperature for 24 hours to rehybridize the complementary DNAs. Thus, the color of solution became blue as being observed preciously. This phenomenon could be repeated with several cycles between 80 ℃ and room temperature as shown in Figure 5. Evidently, the gold nanoparticles were coagulated with the hybridizing reaction of two complementary olgonucleotides.

Conclusion

We have prepared gold nanoparticles for using in biosensing. The gold nanoparticles could react with disulfide modified DNAs well. Two complementary DNAs could hybridize while they linked on gold nanoparticles. This reaction caused the color of gold nanoparticle solution changed from red to blue. Thus, this phenomenon showed the potential of DNAs modified gold nanoparticles to be applied in biosensing or as DNA probes for diagnosis.

Acknowledgement

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