Protein can act as unique template to assemble gold nanoparticles in a linear fashion.

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ABSTRACT

Nanoparticles of metals possess unique chemical, electronic, optical and magnetic properties that differ from their bulk counterpart because of the quantum size effects[1-4]. The spatial orientation and arrangement of nanoparticles is important in the realization of technologically useful nanoparticle-based materials[5-7]. Nevertheless, the assembly of nanoparticles into defined arrays remains a challenge. There are numerous reports of assembly of gold nanoparticles in a defined manner. In most of them, linker molecules were generally added to a preformed gold nanoparticle solution for assembly. Here a facile process for the assembly gold nanoparticles in a linear fashion has been described using protein as templates. The presence of proteins during the reduction of gold salts by sodium borohydride directs the assembly of gold nanoparticles in a linear fashion when cooled to -80^0 C followed by thawing at room temperature. Similar results were obtained by cooling at -20^0C, whereas no assembly was observed when cooled at 0^0C. Assembly was also not observed when proteins were added to gold nanoparticle solution. Therefore, interaction of gold salts with the protein before the reduction is essential for such an assembly process. The assembled products thus obtained were characterized using transmission electron microscopy (TEM), UV-visible spectroscopy (UV-Vis) and thermogravimetric analysis (TGA).

Keywords: Assembly, gold nanoparticles, proteins, templates.

1 INTRODUCTION

An area of considerable current interest has been the development of a practical approach for the assembly of inorganic nanoparticles into well-defined arrays, because it offers immense opportunities leading to applications in microimaging, optoelectronics, therapeutics etc. A key aspect of this approach is the integration of organic self-organization and inorganic assembly such that hybrid materials are constructed by direct or synergistic templating[8]. Most of the methods used so far to assemble gold nanoparticles involve the addition of template molecules to preformed gold nanoparticles[8-16]. Here, we report a simple, one step process where proteins act as a "template", when present in the medium during the reduction process, to assemble gold nanoparticles formed in a rod like fashion. We show here that antibodies (AVF) raised against vascular endothelail growth factor (VEGF), can act as a template when present in solution during the synthesis of gold nanoparticles and direct assembly of the nanoparticles thus formed in a rod like fashion when cooled to -20^0 C followed by thawing at room temperature. A number of other proteins such as antibodies raised against epidermal growth factor receptor (EGFR), Immunoglobulin g (IgG) and BSA also showed the similar templating behavior. Freezing time also plays an important role in directing the assembly process. This paper opens up a new area where shape selective assembly of nanoparticles can be achieved using proteins as a template. These new types of antibody-nanogold bio-conjugates may not only have interesting physico-chemical and opto-electronical properties but also intriguing biological properties due to the presence of the antibody.

2 MATERIALS AND METHODS

In a typical assembly process, aqueous solution of tetrachloroauric acid (HAuCl4, Sigma-Aldrich) was added to an aqueous solution of VEGF-antibody (AVF, Pergerine Pharma) or vice versa under stirring. After 30 min, an aqueous solution of sodium borohydride (Sigma-aldrich) was added to the above solution and the stirring was continued for another 12 h. After 12 h, 10 ml of the conjugates were collected in a 15 ml centrifuge tube, cooled to subzero temperature for a definite time (0-2 h) followed by thawing at room temperature for 24 h. The products thus obtained were characterized using UV-Visible spectroscopy (UV-Vis), transmission electron microscopy (TEM), infrared spectroscopy (IR) and thermogravimetric analysis (TGA).

UV-visible spectra of all the samples were recorded on a Shimadzu UVPC2401 spectrometer.TEM study was carried out by drop coating the conjugates/nanoparticles on a copper TEM grid using a TECNAI G^2 instrument operating at 120 KeV. Fourier Transform Infrared Spectroscopy (FTIR) spectra were obtained on a Nicolet 550 spectrometer. All the samples were analyzed using a zinc selenide ATR crystal. The resolution of the instrument

was specified as 4 cm^{-1} at a wavenumber of 1000 cm^{-1}. Thermogravimetric Analysis (TGA) were done using a TA Instruments Q500 thermal analyst. The TGA data were obtained in flowing nitrogen at a heating rate of 20^0C/min. Amount of drugs attached onto gold nanoparticles will be obtained from weight loss from the TGA curve.

3 RESULTS AND DISCUSSION

Figure 1 describes the UV-visible spectra of gold nanoparticles prepared in the presence and/ or absence of the AVF. An increase in absorbance and slight red shift in the λ_{max} value of Au0-AVF (λ_{max} = 519 nm) and Au-AVF (λ_{max} = 521 nm) samples were observed, compared to control gold nanoparticles (GNP, λ_{max} = 512 nm). AVF was added to gold nanoparticles solution in Au0-AVF, whereas AVF was present during the reduction of gold salts to gold nanoparticles in Au-AVF sample. An increase in absorbance and red shift in the λ_{max} value with broadening of the spectrum confirm the attachment of antibody with gold nanoparticles. According to the Mie theory, the observed shift in the Y_{max} value with an increase in plasmon resonance coincides with a rising dielectric constant of the medium surrounding the gold nanoparticles [16].

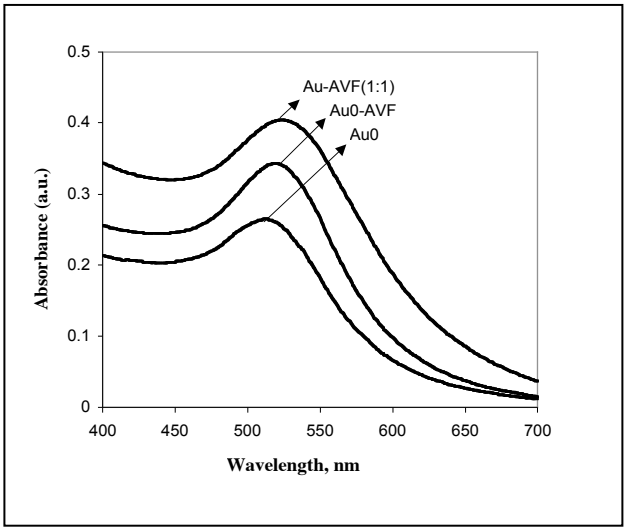


Figure 1. UV-Visible spectra of gold nanoparticles and its nano-conjugates.

Figure 2 describes the TEM picture of gold-AVF conjugates with and without cooling. Assembly of nanoparticles in a specific orientation was not observed without cooling, (data not shown). But, when cooled to subzero temperature (-80^0C) followed by thawing at room temperature, a unique assembly of gold nanoparticles in a rod like fashion was observed (Fig. 2a). Higher magnification image of one edge of the rod shows assembly of discrete nanoparticles in a rod like fashion (Fig. 2b). However, when AVF was added to an a preformed nanoparticles solution, no assembly of gold nanoparticles were observed even after cooling (2c).

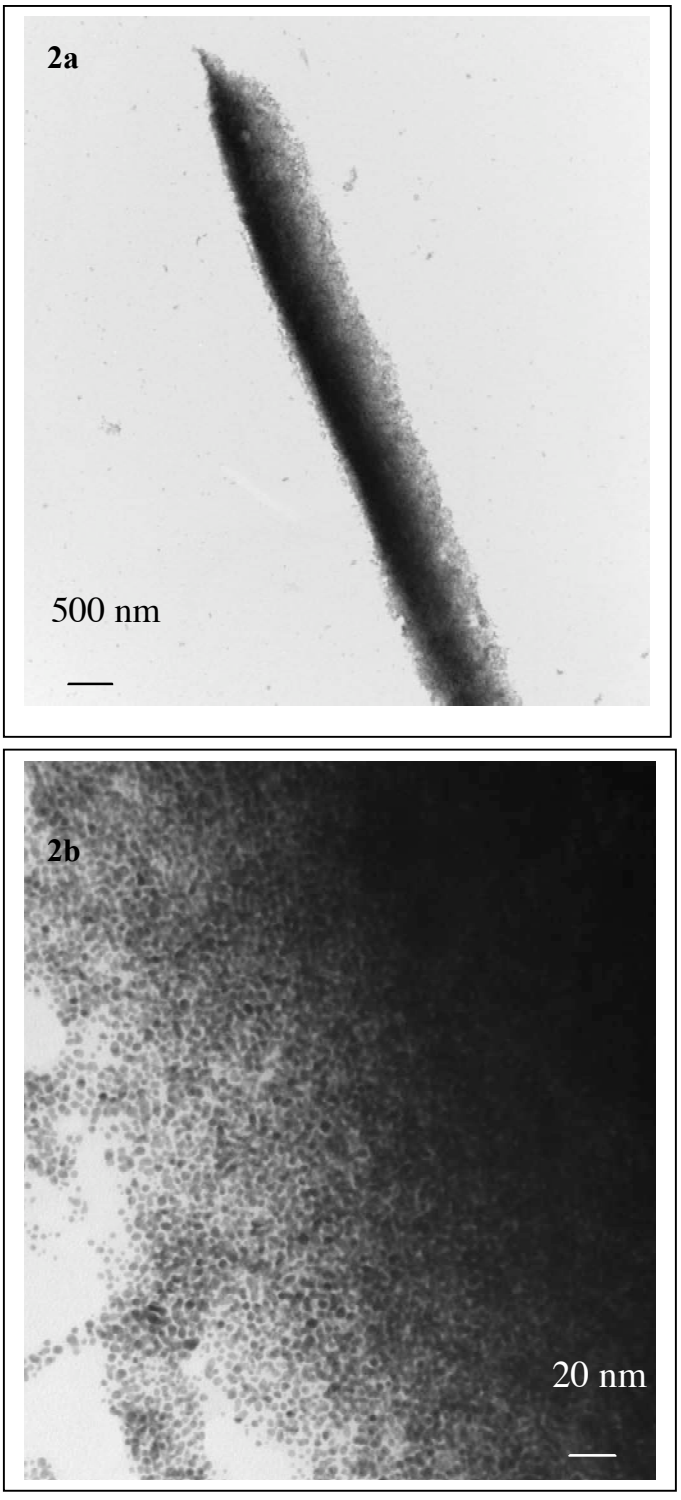
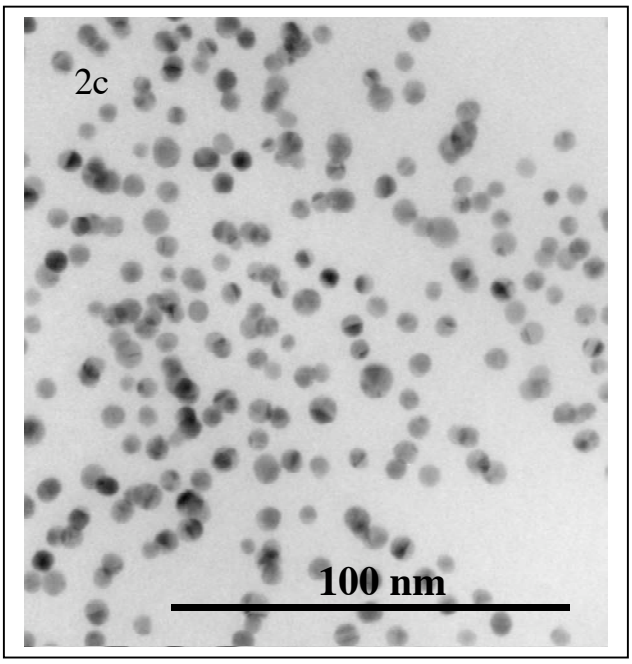
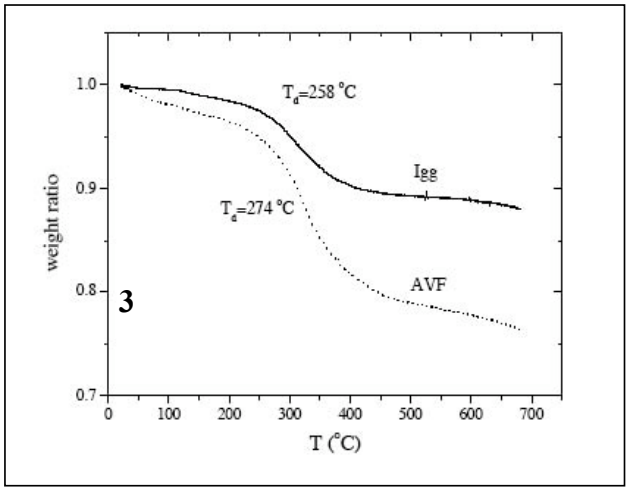


Figure 2. TEM picture of gold nanoparticles a) Au-AVF after freezing to subzero temperature followed by thawing at RT, b) higher magnification image of 2a; c) AVF added to gold nanoparticles.



Association of AVF with the gold nanoparticles was further confirmed by IR spectroscopy. The presence of Amide I vibration (mostly peptide bond C=O stretch) which falls between 1600 and 1700 cm^{-1} is considered to be the characteristic feature of proteins[19]. Prominent Amide I band at 1633 cm^{-1} and Amide II band at 1540 cm^{-1} were present in both AVF and Au-AVF samples. It is well known that 80% of the potential energy of Amide I mode comes from the C=O stretch[19]. Amide II band at 1540 cm^{-1} is attributed to the coupling of N-H bending and C-H stretching vibrations[20, 21]. The presence of amide I and II bands in Au-AVF conjugates confirms the association of proteins with gold nanoparticles.

Figure 3 describes the thermogravimetric analysis of Au-AVF and Au-IgG samples. The weight loss in the temperature range 200-400^{0}C further confirms the attachment of the proteins on the gold nanoparticles. The



weight loss observed in the case of Au-AVF sample (~ 15%) is almost double than that observed in case of Au-IgG sample (~7.5 %). This may be due to the stronger bonding between AVF than IgG with gold nanoparticles.

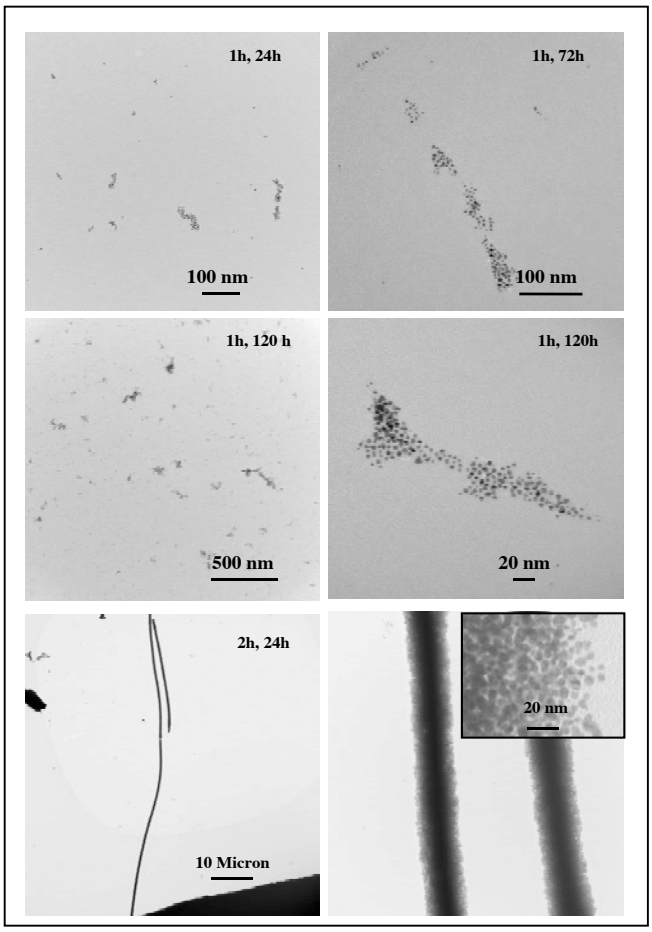


Fig 4. Formation o assembled structure of gold nanoparticles using proteins as templates, a) cooled to –200C for 1h followed by thawing at RT for 24h, b) cooled to –200C for 1h followed by thawing at RT for 72h, c) cooled to –200C for 1h followed by thawing at RT for 120 h, d) higher magnification image of c, e) cooled to –200C for 2h followed by thawing at RT for 24h, f) Higher magnification image of e and inset picture is the higher magnification image of f.

To find out the role of freezing and thawing time on the assembly process, the nanoconjugates Au-AVF was kept at –20^{0}C for 1h, 1.25h, 1.5h and 2h. The effect of thawing time (24 h, 72h, 120 h) was studied on the Au-AVF samples that are kept in the subzero temperature for 1h. The TEM micrographs show that freezing for 1h followed by thawing at room temperature up to 120 h did not produce any assembled product. However, small aggregates of gold nanoparticles were seen. The number and size of aggregates increased with increase in thawing temperatures (Fig. 4a-4d). Similar aggregates were obtained for 1.25 h and 1.5 h freezing time. However, when the freezing time was increased to 2h followed by thawing at room temperature

for 24h, it led to the assembly of gold nanoparticles in a rod like fashion (Fig. 4e, 4f). These observations clearly indicate that the freezing time plays a critical role in the protein mediated assembly process when protein is used as a template.

It can be concluded from the above figures that association of $AuCl_4^-$ with the antibody/protein before the reduction was essential for such a unique organization. When C225, IgG, BSA were employed as templates separately, similar organization was observed. When AVF (or any protein like molecules) was added to an aqueous solution of $HAuCl_4$, initial assembly of $AuCl_4^-$ on AVF occurred due to electrostatic attraction. Such an initial assembly of $AuCl_4^-$ on AVF provided the nanoparticles thus formed after reduction, a unique platform to orient themselves to a particular arrangement. The association between the protein and gold nanoparticles were further confirmed by IR spectroscopy and TGA analysis. It is also important to mention here that the assembled structure was obtained only after freezing to subzero temperature followed by thawing and only when antibodies/proteins was present in solution during the reduction process. The freezing time also plays a critical role in the assembly process. This phenomenon clearly indicated that the electrostatic barrier to particle approach was high enough at room temperature to inhibit the self-assembly process[12]. Freezing to subzero temperature might facilitate the assembly by reducing the Brownian motion of the particles, decreasing the electrostatic barrier and increasing the hydrophobic interaction due to the presence of proteins. The nanoconjugates obtained by this method may find wide application in materials science and biology due to the presence of antibody.

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