Adsorption-induced Self-fusion of Cationic Gold Nanoparticles on Tobacco Mosaic Virus (TMV)

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The surface of tobacco mosaic virus (TMV) is densely coated by quaternary ammonium-modified gold nanoparticles. By dropping gold nanoparticle dispersions onto TMV pre-cast substrates ("after-staining method"), nanoparticles are spontaneously adsorbed on TMVs. The adsorbed nanoparticles were fused to form semi-continuous structures. This observation indicates the possibility of preparing 1-D arrays from wide variety of nano-sized materials, by using TMV tubules as templates.

Recently, much attention has focused on colloidal metal nanoparticles because of their highly potential applications in areas of electronics, photonics, and catalysis.[1] In particular, thiol-stabilized gold nanoparticles provide important nanomaterials due to their stability, facile preparation, and their chemical versatility.[2]

On the other hand, bio-nanomolecular chemistry has been attracting much interest as a powerful tool to introduce functionality and nanostructural complexity into materials chemistry. Especially, the design of nanoparticle–biomolecules conjugates has been intensively studied.[3] For example, well-defined two dimensional arrays of metal nanoparticles was prepared by using ferritins.[4] Oligonucleotides or antibodies-modified nanoparticles successfully formed nanoparticle arrays.[5] 1D arrays of metal nanoparticles was obtained by reduction of pre-adsorbed metal ions in the presence of DNAs or TMVs.[6]

We have recently reported that quaternary ammonium-modified metal nanoparticles having long[7] and short[8] spacer alkyl chains are adsorbed densely onto rigid anionic polymers such as DNA, in spite of charge repulsions operating between the particles.[7a,8] Similar self-assembly of cationic nanoparticles have been reported subsequently.[9,10] As biomolecules usually possess negative surface charges, they are good candidates for the preparation of densely packed nanoparticle layers with ordered structures.

One of the other potential applications of gold nanoparticles is staining of TEM samples. Gold nanoparticles have been often used as a stain reagent for biological transmission electron microscopy because of their stability and low electron transmission efficiency. Small gold nanoparticles are suited for high resolution observations, but it requires a great deal of skill. In order to overcome this problem, silver enhancement is used. But self-fusion of nanoparticles themselves at room temperature will enhance the contrast automatically and we do not need such a process.

Thiocholine bromide ($HS\text{-}(CH_2)_2\text{-}N(CH_3)_3{}^+Br^-$), a small cationic thiol compound, was synthesized by hydrolyzing acetylthiocholine bromide (Sigma) with concd HBr and was purified by recrystallization. Its purity was checked by ^1H NMR, IR, and elemental analysis. TCB-stabilized gold nanoparticles are highly stable in aqueous solutions with average diameter of ca. 2.7 nm (cuboctahedral, Figure 1).[8] From this size information and elemental analysis of obtained nanoparticles, each particle contains 610 gold atoms and is covered by 100 TCB molecules in average. Therefore, the particle surface is surrounded by 100 positive charges and is highly positively charged. The occupied area of one TCB molecule is calculated as 22.1 $Å^2$, which is a slightly larger than alkylthiol-SAM on Au(111) (21.4 $Å^2$).[11] It is probably ascribed to statistic hindrance of the large trimethylammonium groups and electrostatic repulsions among them. These nanoparticles are prepared in methanol and are readily dispersed in water. We have shown that these particles could be used for DNA metallization.[8]

Tobacco mosaic virus (TMV), which was selected as the target molecule in this study, is a rod-shaped virus, with a diameter of 15 nm and a length of 300 nm. In the center, one single strand RNA molecule is located and unique amide acid subunits surround the RNA. Surface coating of TMV by TCB-stabilized gold nanoparticles was carried out on a carbon-coated TEM copper grid. After putting a drop of 0.05 mg cm^{-3} TMV solution, a drop of aqueous dispersion of TCB-stabilized Au ($[Au] = 6 \times 10^{-3}$ mol dm^{-3}) was successively placed.

Figure 2 displays TEM images of the nanoparticle-bound TMV molecules. Rod-type structures typical of TMV's are clearly observed in the image. Only contour of TMV molecules was stained with nanoparticles, whose structure is probably generated during the evaporation of aqueous nanoparticles. It seems that cationic gold nanoparticles are immobilized on TMV molecules by electrostatic interactions. At pH = 7, TMV surface is anionic and adsorb cations.[6] Comparing the size of gold nanoparticles (2.7 nmϕ) and TMV (15 × 300 nm), TMV is much

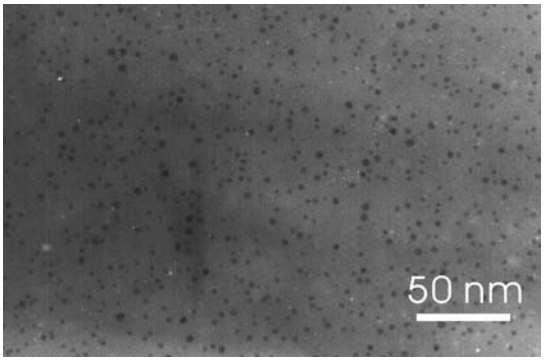

Figure 1. TEM image of TCB-stabilized gold nanoparticles prepared by $NaBH_4$-reduction of $HAuCl_4$ in the presence of TCB. Au:TCB = 1:3 (mol/mol), prepared in methanol. Samples were obtained by casting a water drop of the molecule on carbon-coated Cu TEM grid.

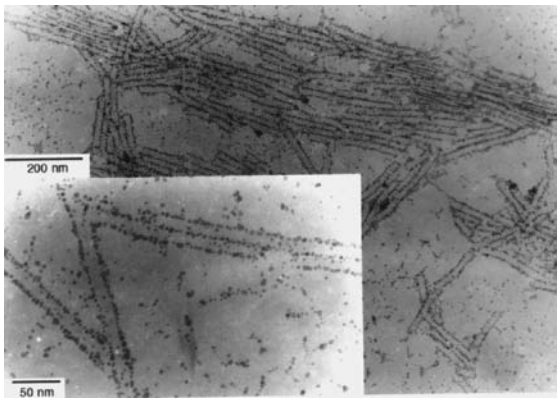

Figure 2. TEM image of TMV molecules adsorbed with TCB-stabilized gold nanoparticles. The gold nanoparticles were fused automatically at room temperature simply by adsorption onto TMV molecules.

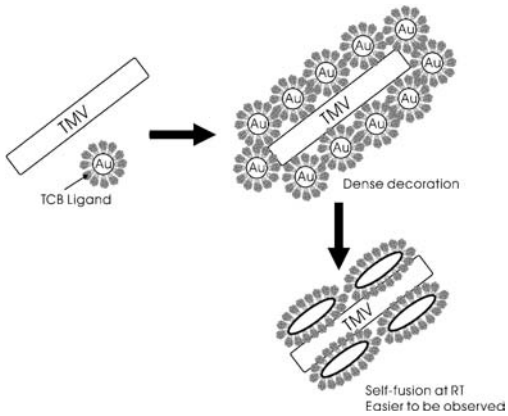

Figure 3. A schematic figure of adsorption-induced self-fusion of cationic gold nanoparticles at room temperature on TMV.

larger than the nanoparticles. The nanoparticles are densely immobilized on TMVs and it is clear that they overcomed the electrostatic repulsion. The excess charges of the surface of the gold nanoparticles would be compensated by counter ions when the nanoparticles were immobilized onto TMV molecules. Though in many cases, charged nanoparticles cannot be densely adsorbed on the substrate surfaces as a result of electrostatic repulsions. According to a theory, the attached nanoparticles were covered by their counter ions and repulsive force between the particles becomes lower.[12] Then, high interaction between DNA and TCB-stabilized gold nanoparticles can give a densely packed adsorption.

It should be noted that the particles display self-fusion into merged arrays rapidly even at room temperature. In Figure 2, not only spherical nanoparticles but also larger semi-continuous structures are observed. TCB-stabilized gold nanoparticles in dispersions form no precipitates or aggregates for years (Figure 1). It indicates that self-fusion of nanoparticles never occurs in dispersions where nanoparticles are dispersed independently. However, once densely immobilized onto a substrate, the nanoparticles are instantly fused to generate larger rod-like structures. This is probably due to the decreased stability of smaller particles[13] and the small stabilizer (TCB) molecule (length $= 0.8$ nm).[8]

Commercially available gold nanoparticles with the size of 0.8–2.4 nm are often employed to stain TEM samples or to label biomolecules. However, such nanoparticles are isolated in TEM images and are too small to be facilely observed. Thus, these particles are often enlarged by addition of Ag^+ ions and deposition of Ag on these particles to generate several tens or hundreds nm sized nanoparticles (silver enhancement).

On the contrary, our TCB-stabilized gold nanoparticles could be densely accumulated on the target surface and they are readily self-fused even at room temperature for a very short time (<10 min) (Figure 3). Therefore, no Ag enhancement is required and by using small molecular recognition molecules on the particles, this self-fusion process will make the observation of biomolecules much easier.

In conclusion, self-fusion of gold nanoparticles can be applied to the TEM staining for biomolecules. Small gold nanoparticles with a very small ligand length (ca. C3) are amenable to fuse spontaneously when they are immobilized on surface at the same temperature. This staining method will substitute uranium compounds and gives a superior staining technique. Furthermore, TMV is an excellent candidate of biomolecular templates to align 1-D nanowires from smaller nanoparticles, which is difficult for flexible DNA templates.

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