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단 신

Lectin 주형을 이용한 탄수화물 기능화된 금 나노입자의 자기조립에 관한 연구

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Self-Assembly of Carbohydrate-Functionalized Gold Nanoparticle Composites via lectin templates

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INTRODUCTION

The unique structure produced by biological systems provides inspiration for the fabrication of functional materials. Proteins themselves are nanoscopic entities as well as can be used as useful nanomaterial components possessing novel properties. In addition to their exquisite features, they are obtained easily from the natural sources. There are a number of examples which employ viruses and protein assemblies for the design of new functional materials. Freeman and co-workers at Tel Aviv University have shown the formation of the predesigned diamondlike protein crystals using the self assembly of a tetrahedral letin.[1] This strategy can be applied for the controlled fabrication of new three-dimensional protein lattices and scaffolds. Rotello and co-workers at the University of Massachusetts demonstrated the controlled assembly of protein-nanoparticle composites via protein surface recognition.[2] These protein-mediated assemblies are also important tools for the detection of biomolecules as well as for the manufacturing of functional nanomaterials with their unique properties.[3]

Herein, we report the use of the lectin protein for the spontaneous self-assembly of carbohydrate- functionalized gold nanoparticles. We envisage the precise control over the spatial arrangements of carbohydrate-functionalized gold nanoparticles using the specific carbohydrate-protein interaction. This interaction plays a crucial role in a wide variety of intercellular processes in our nature. Gold nanoparticles were suggested as excellent fluorescence quenchers.[4] In this report, we have observed the recognition process via quantitative fluorescence quenching experiments by monitoring of the fluorescence of Tritc-Concanavalin A (Con A) as the addition of the nanoparticles. Self-assembly process was observed using qualitative TEM, dynamic light scattering technique as well. We observed the aggregation process can be reversed by the addition of free mannose sugar which was not observed by the addition of galactose.

RESULTS AND DISCUSSION

The approach we use in this study is to design and synthesize the materials for the ordered nanoparticle array via protein linkers. We have chosen the lectin, concanavalin A (Con A), as the template and mannose-functionalized gold nanoparticles as the building blocks for the supramolecular entities. Previously, we have used the lectin-mannose interaction for the creation of an NSL (nanosphere lithography)-fabricated Ag nanoparticle sensor.[5] The synthesis of gold nanoparticles was performed by the colloid-synthetic methodologies- Brust[6] and Murray displacement reactions.[7]

The assembly of gold nanoparticles has been monitored using dynamic light scattering (DLS), which yields an average hydrodynamic radius (R_h) of nanoparticle conjugates in solution. In case of aggregation corresponding to Fig. 1, addition of Con A results in a rapid increase in the average hydrodynamic radius from 5.3 nm (±0.2 nm) to 43.5 nm (±10 nm). The dramatic change of R_h values

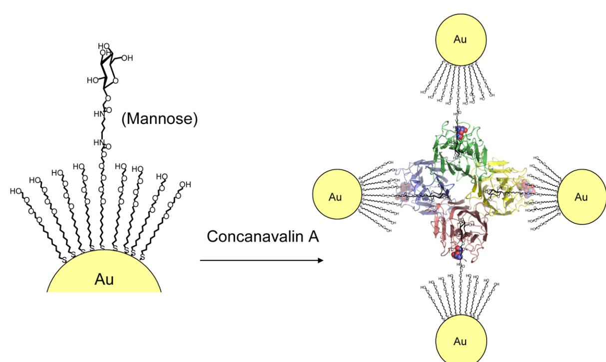

***Fig.* 1.** Schematic representation of protein-carbohydrate composites. Mannose-functionalized gold nanoparticles specific bind to the four recognition sites of the lectin (Con A) template.

upon the addition of protein demonstrated gold nanoparticles self assembled via recognition process with the lectin template. It only provide a qualitative measure of the average size distribution of the particles in solution, because DLS is performed on the basis of a nearly monodispersed homogeneous solution.[8]

Electron microscopy provides an accurate determination of the morphology and the size distributions of the aggregates. Transmission Electron Microscopy (TEM) micrographs of mannose-functionalized gold nanoparticle and Con A are shown in Fig. 2. Con A is stained for visualization on TEM grids. TEM micrographs showed the average size of mannose-functionalized Au nanoparticles of relatively uniform size distribution (2-3 nm) which are well-separated. It has been reported Con A is a 104kDa mannose specific plant lectin comprised of a tetramer with dimension of 63.2, 86.9, and 89.3Å and has four binding sites.[9] The binding constant of Con A to a mannose- functionalized surface was found to be 5.6×10^6 M^{-1}.[10] Considering those data, the change of R_h values in

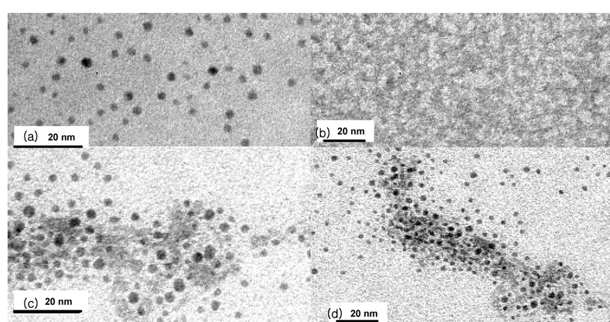

***Fig.* 2.** TEM micrographs showing mannose-functionalized Au/lectin conjugates (a) before (c), (d) after addition of lectin. The TEM micrographs of the stained Con A is shown in (b).

DLS is more than the expected values. TEM examination of mannose-functionalized Au/lectin conjugates revealed large disordered 3D networks of Au nanoparticles. This is consistent with the previous results of DLS experiments. It verifies the lectin template functions as protein linker of Au nanoparticles and extends the network three dimensionally rather than forming isolating aggregates.

Even TEM and DLS are suitable methods to provide the rapid characterization of the system, we need to perform the quantitative investigation to understand the manner by which nanoscale particles assemble. Fluorescence experiments were used to quantify the extent of the binding of mannose-functionalized Au particles to lectin template upon recognition. The lectin template is fluorescence-tagged and fluorescence was measured by the addition of mannose-functionalized Au nanoparticles. It has been reported the fluorescence of the fluorescence-tagged oligonucleotide is quenched by the gold nanoparticles with DNA probes upon binding.[11] It can be expected the fluorescence of the fluorescence-tagged Con A will be decreased by specific binding of carbohydrate-modified Au nanoparticles in a similar fashion. We have observed the decrease of the fluorescence of Tritc-ConA by the addition of the nanoparticles (Fig. 3). The dissociation constant, $K_d = 0.18 \pm 0.015$ (mg/ml), of mannose-functionalized Au nanoparticles with the lectin template was calculated by Hill equation. For the typical nanoparticles, there are 12 recognition units and 90 background thiols.[7] The binding constant of Tritc-Con A to mannose-functionalized gold nanoparticles would be 2.2×10^5 M^{-1}, assuming the molecular weight of mannose-functionalized gold nanoparticles is 40000 g/mol.[12] This value is slightly lower than the binding constant of Con A to a mannose- functionalized surface (5.6×10^6 M^{-1}) but much

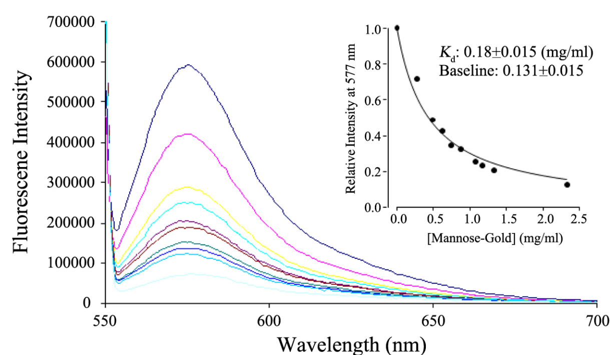

***Fig.* 3.** Change in the fluorescence spectra of Tritc-Con A on addition of mannose-functionalized Au nanoparticles. Spectra were obtained in pH 7.4 PBS buffer at 25 °C of 10 nM Tritc-Con A (1.4 dyes/protein).

higher than the binding constant of Con A to mannose-functionalized dendrimers (7.5×10^3 M^{-1}).[13]

The possibility exists that the fluorescence quenching is achieved by non-specific Au nanoparticle adhesion to Tritc-ConA. To verify this was not the case, we have performed the experiments for the modulation of the assembly by the addition of "free" mannose and "free" galactose after the formation of protein-carbohydrate conjugates. We expected the increase of fluorescence of Tritc-ConA by the competitive binding with "free" mannose, since Tritc-ConA template would bind to "free" mannose as well as mannose-functionalized gold nanoparticles. Control experiment was conducted with "free" galactose which is known as a sugar which does not specifically bind to Con A recognition site. In contrast to the case of "free" mannose, when "free" galactose were added into the assembly there was a little or no change of the fluorescence of Tritc-Con A/mannose-functionalized Au conjugates. It demonstrates that the specific recognition of Con A to mannose would induce the change of the extent of fluorescence of Tritc-Con A. In this system, nanoscale particles assemble and dissemble by the addition of the competitive binding molecules. Even though the complete recovery of fluorescence is not accomplished, it will be the useful tools to control supramolecular processes by the modulation of binding molecules.

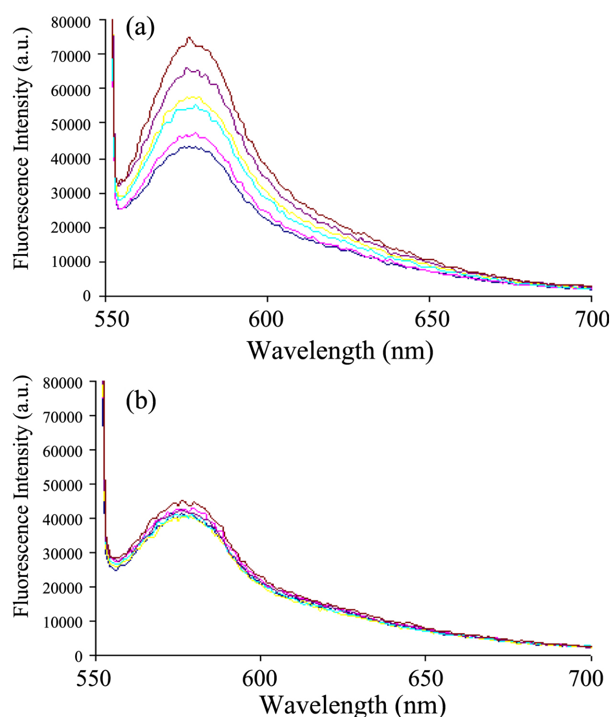

***Fig.* 4.** Change in the fluorescence spectra of Tritc-Con A/mannose-functionalized Au nanoparticle conjugates upon the addition of (a) "free" mannose and (b) "free" galactose.

In conclusion, we have succeeded in the organization of the self-assembly of mannose-functionalized gold nanoparticles using lectin (Con A) template. The recognition process was monitored by DLS, TEM and fluorescence quenching experiments. Especially, the quantitative detection of the assembly process via fluorescence quenching of Tritc-ConA has lead to the possibility of the moderate modulation of the recognition process. The key feature of this system was the reversibility of the recognition process by the external factors, such as the addition of the competitive binding molecules.

The notion of the fabrication of nanoparticle assemblies from biomolecular template, is an important step towards generating a variety of tailored geometric structures by using various biomolecules with their own unique properties without the complicated and time-consuming synthetic procedures. We have used biomolecular template as the basis for bottom-up fabrication of supramolecular assembly of nanoparticles. In this study, we have developed the bio-derived routes to organize metallic nanoparticles using the specific recognition of biomolecules, rather than nonspecific interactions. Our system is interesting for its potential uses for the biomolecular sensing devices. This will provide new possibilities for the improvement of existing technologies for fabricating biosensors and nanodevices as well as fundamental understanding of this process. Further optimization is necessary to improve the shape, size and interparticle distance of the assemblies.

EXPERIMENTAL SECTION

All chemicals were reagent grade, and were used without further purification. The lectin concanavalin A (Con A), isolated from Canavalia ensiformis (jack bean) and Tritc-ConA were purchased from Sigma-Aldrich. Proton NMR spectra (300 MHz) were recorded using TMS as the internal standard. Column chromatography was performed over silica gel (Merck, 230-400 mesh).

Carbohydrate synthesis

Thiol-terminated ethylene glycol-derivative mannose was prepared analogously to those described in the literature.[14]

Synthesis of tri(ethylene glycol) thiols

Tri(ethylene glycol) thiols were prepared according to literature methods using tri(ethylene glycol) and 11-bromo-1-undecene.[15]

Synthesis of ethylene glycol-terminated gold nanoparticles

Ethylene glycol-terminated gold nanoparticles were prepared by chemical reduction of hydrogen tetrachloroaurate (III) hydrate ($HAuCl^-_4 \cdot xH_2O$). Briefly, $HAuCl^-_4 \cdot 3H_2O$ was dissolved in deionized water. Toluene containing trioctylammonium bromide was then added and mixture stirred vigorously for 15 minutes. Tri(ethylene glycol) thiol was added, and the organic layer became turbid. Sodium borohydride ($NaBH_4$) solution were next added with rapid stirring. The reaction was then allowed to stir for three hours at room temperature, and then the organic phase was collected. The resulting nanoparticles were recrystallized using toluene and ethanol, then characterized by transmission electron microscopy and ^{1}H NMR. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.67 (bt, 10H), 3.49 (m, 4H), 1.59 (bs, 6H), 1.25 (vbs,14H).

Synthesis of mannose-functionalized gold nanoparticles

Ethylene glycol-terminated gold nanoparticles and thiol-terminated ethylene glycol mannose were dissolved in the mixtures of 10 mL methanol and 20 mL CH_2Cl_2. Argon is bubbled through the solution for 40 minutes, and the dark brown solution is then stirred for 48 hours under argon. Solvent removal yielded a dark brown solid which was washed extensively with ethanol. The product was recrystallized as many times as was necessary to give the nanoparticle free of unbound thiols. ^{1}H NMR (300 MHz, MeOH) δ3.76 (bs), 3.41 (s), 1.76 (bs), 1.42 (m), 1.01 (m).

Transmission electron microscopy (TEM) measurements

Transmission electron microscopy (JEOL 2000EX operating at 100 kV) was used to characterize the morphology and the structure of the gold nanoparticles. Samples were prepared by evaporating a 5 uL drop of the nanoparticle solution on a carbon-coated 200 mesh copper grid. Excess liquid was removed by the contacting the edge of the grid with absorbent paper.

Dynamic light scattering (DLS) measurements

Dynamic light scattering experiments were performed using a Malvern PCS-4700 instrument. The laser source was the 488.0 nm line of a Coherent Innova-70A Ar ion laser at a power of 20 mW. The temperature was maintained at 23 ºC.

Fluorescence measurements

Fluorescence spectra were obtained by using a scanning spectrometer (Spex, Edison, NJ) at 543-nm excitation for fluorescein and at 575-nm excitation.

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