Chiral meta-molecules consisting of gold nanoparticles and genetically engineered tobacco mosaic virus

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Abstract: We demonstrate a chiral meta-molecule in the ultraviolet (UV) and visible (VIS) regions using a complex of Au nanoparticles (NPs) and rod-shaped tobacco mosaic virus (TMV). Au NPs five nm in diameter are uniformly formed on peptide-modified TMV. The peptide-modified TMV with uniform-sized Au NPs has improved dispersion in solution. A negative circular dichroism (CD) peak is produced around 540 nm, at plasmonic resonance wavelength of Au NPs. Additionally, modification of a CD peak in the UV region is observed. Attaching NPs to a virus causes the enhancement and modification of CD peaks in both the UV and VIS regions. Our results open a new avenue for the preparation of three dimensional chiral metamaterials at optical frequencies.

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1. Introduction

Metamaterials consisting of artificial building blocks much smaller than the wavelength of light are a rich and rapidly growing area in electromagnetism of matter [1,2]. To realize metamaterials operating at optical frequencies, a scaling law suggests that the size of the building blocks, i.e., meta-atoms or meta-molecules which compose metamaterials, must be smaller than several tens of nanometers. Biomolecules, for example, subunits of proteins, are smaller than several tens of nanometers and have identical structures determined by genomic information. Especially, virus proteins are able to self-assemble into three dimensional (3D) structures. Therefore, virus proteins are promising building blocks to construct 3D optical metamaterials.

Proteins are made of amino acids. It is well known that amino acids have chirality, resulting in optical activity. Because of the strong absorption in the ultraviolet (UV) region, proteins show large circular dichroism (CD), which is caused by the absorption difference between left-/right-handed circularly polarized light, in the UV region [3]. This feature is attractive in terms of chiral metamaterials [4], and enables us to realize negative refraction of light even without a negative index of refraction [5]. In the visible (VIS) region, however, proteins are silent in CD spectra.

Enhancement of CD signal in the VIS region is observed by attaching plasmonic nanoparti-



Fig. 1. (a) Schematic representation of TMV. The virus has a right-handed helical structure with 16 and 1/3 coat proteins constituting one helical turn. A coat protein subunit is high-lighted in red. In detail, each TMV is composed of a genomic single-stranded RNA and 2130 identical coat proteins. The RNA molecules make a helical tube about 5 nm diameter, and coat proteins are aligned along the RNA. (b) Atomic coordination of a coat protein subunit of the virus (PDB:2OM3) [13] illustrated using PyMol [14]. A wild-type protein subunit consists of 158 amino acid residues. Amino acid sequence of a titanium-binding peptide (TBP), which was fused to the C-terminus of a coat protein, is shown.

cles (NPs) to the proteins even though the plasmonic NPs do not have optical activity. Several plausible mechanisms of the enhancement in the VIS region have been proposed: chirality imprinting, in which the formation of chiral crystal structures or chiral electronic states of a NP surface induces CD [6–9], and chirality transfer, in which Coulomb interactions between a NP and chiral molecules induce CD [10–12]. Nevertheless, the origin remains obscure. In addition, a numerical calculation [10] implies that intrinsic CD signals due to proteins in the UV region are also modified by attaching plasmonic NPs although this has not yet been observed experimentally. The modification in the UV region cannot be, however, explained either by the chirality transfer or chirality imprinting. Thus, a consistent explanation for the enhancement in both the UV and VIS regions is needed.

In this paper, we report optical properties of a complex made of gold (Au) NPs and tobacco mosaic virus (TMV). TMV is a rod-shaped plant virus consisting of a right-handed helical single-strand ribonucleic acid (RNA) surrounded by 2130 coat proteins [Fig. 1(a)]. We have previously succeeded in the deposition of 5 nm Au NPs on TMV [15]. In this paper, we address the optical properties of the complex by measuring UV-VIS absorption spectra and CD spectra. The complex shows an emergence of a negative CD peak at resonance wavelength of localized surface plasmon in Au NPs. Additionally, a modification of CD peaks in the UV region originated from the proteins in TMV is observed. The physics underlying these CD enhancements is discussed. We propose that propagation and circularly polarized directional differences of refractive indices lead to the modification of optical activity in the UV region with the attachment of Au NPs.

2. Experimental procedures

As shown in Fig. 1(a), the virus is 300 nm in length with outer- and inner-diameters of 18 nm and 4 nm, respectively. We genetically fused a titanium-binding peptide (TBP; Arg-Lys-Leu-Pro-Asp-Ala) [16], which has been shown to promote mineralization of a plasmonic metal [17], to the outer-surface of the virus [Fig. 1(b)]. We call this engineered virus, tbpTMV. The detailed construction procedures of the plasmid encoding tbpTMV, and preparation and purification of viruses have been described elsewhere [15]. Briefly, double-stranded oligonucleotide encoding TBP was inserted to the end of TMV coat protein-coding region so that the peptide is fused to the C-terminus of the translated protein. A plasmid vector pTLW3, which encodes ToMV species of TMV [18], was used as a parent plasmid for tbpTMV expression. The genomic RNA of tbpTMV was transcribed from the template plasmid *in vitro* using T7 RNA polymerase and an mMESSAGE mMACHINE high yield capped RNA transcription kit (Ambion). Leaves from four week-old tobacco plants (*Nicotiana Benthamiana*) were infected with the RNA using carborundom (600 mesh; Nacalai tesque, Kyoto), and infected leaves were harvested after one week. Viruses were purified and finally suspended in a small volume of H₂O.

For Au deposition, potassium chloroaurate (KAuCl₄) was mixed with wild-type TMV (wtTMV) or tbpTMV in the presence of 5% acetic acid, and the solution was reduced by 5 mM sodium borohydride (NaBH₄) for 20 min at ambient conditions [15, 19]. Transmission electron microscopy (TEM) was performed using a 300 kV electron microscope (JEOL JEM-3100FEF). The sample solution was put on a copper (Cu) grid and stained with 3% phosphotungstic acid, which allows clearer visualization of proteins.

UV-VIS absorption spectra of the complex in solution in a quartz cell were measured using a UV-VIS spectrometer (Ultrospec 3300 pro). NP formation was performed in a 1.5 mL tube with a final concentration (in 200 μ L) of 0.15 mg/mL (3.8 nM) TMV in acetic acid. Final concentrations of other reagents were 3.125% for acetic acid, 625 μ M for KAuCl₄ and 6.25 mM for NaBH₄.

CD spectra and absorption spectra between 200 and 700 nm of the dried samples were simultaneously measured using a spectropolarimeter (JASCO J-820). To prepare dried samples, the whole reaction mixture (200 μ L) containing the identical amount of TMVs was dropped onto a 15 mm × 30 mm × 1 mm quartz substrate, and dried at ambient conditions. Data were acquired at 0.5 nm intervals at scanning speed of 50 nm/min with 1 sec response time. A total of 4 scans were performed for each sample and averaged. In the measurements, a CD dip (peak) is defined as rotation of the polarization plane to the left (right) when the light propagation is observed from the detector.

3. Results

3.1. TEM observation

Figure 2(a) shows a TEM image of wtTMV after Au deposition (wtTMV-Au). White rods correspond to the TMV. Black patches attached to the TMV correspond to the Au NPs. TEM at cryogenic temperature confirmed that Au NPs were formed on the surface of TMV in solution, and that they are not artifacts observed when the sample was put on the Cu grid [15]. The size of each NP on representative TEM images was measured. Figure 2(b) shows the size distribution of the Au NPs. The average diameter and standard deviation of the Au NPs diameter was 4.78 ± 2.18 nm. Figure 2(c) shows a TEM image of tbpTMV after Au deposition (tbpTMV-Au). Although the exact mechanism is unclear, Au NPs more uniform in size were deposited using tbpTMV as a scaffold [Fig. 2(d)]. The average diameter of Au NPs on tbpTMV was 4.96 ± 1.06 nm.

A gel electrophoresis followed by western blot analysis using TMV antibody demonstrated



Fig. 2. TEM images and schematic representations of Au NPs aligned along with (a) wtTMV and (c) tbpTMV. The samples were stained with 3% phosphotungstic acid, which allows clearer visualization of proteins. Viruses are observed as white rods and Au NPs as black patches. The size distribution of Au NPs of wtTMV-Au (b) and tbpTMV-Au (d) are also shown.

that less than 1 in 20 coat proteins were modified with the peptide [15,20]. Because 16 and 1/3 coat proteins constitute one helical turn, less than one coat protein per turn should have been modified in the tbpTMV. The number of the TBPs displayed on the surface of TMV is smaller than that of Au NPs formed. It is thus unlikely that individual TBP promotes uniform Au NP formation. We believe that mineralization was affected by the overall electrical potential on the virus surface.

3.2. UV-VIS absorption measurements in solution

Figure 3 shows absorption spectra for wtTMV-Au and tbpTMV-Au. An absorption peak around 540 nm was observed, which can be attributed to localized surface plasmon of Au NPs. Although the same amount of reagents was used, tbpTMV-Au showed the largest absorbance at 540 nm. This indicates that tbpTMV is a better scaffold for constructing TMV-Au complexes. In addition, different from the complex prepared with wtTMV, tbpTMV-Au can be dispersed in solution for a longer period (Inset of Fig. 3). Absorbance at 540 nm of the wtTMV-Au decreased after 10 to 15 min, probably because the complex flocculates and forms sediment quickly. On the other hand, absorbance of the tbpTMV-Au remained the same after 60 min; tbpTMV-Au with uniform-sized Au NPs has improved dispersion in solution. When carefully observing where Au NPs are attached in TEM images [Fig. 2(c)], it appears that they are accumulated on only one of the TMVs in a cluster of TMV particles. In this respect, we assume that TBP peptides displayed on tbpTMV have some effect on avoiding the formation of a larger cluster,



Fig. 3. UV-VIS spectra of tbpTMV-Au (red) and wtTMV-Au (blue). Black line corresponds to a spectrum obtained using reaction mixture without virus. Inset shows time dependence of absorbance at 540 nm.

leading to better dispersion observed in the UV-VIS experiment shown in Fig. 3.

3.3. CD measurements

Figure 4(a) shows CD spectra of the TMV-Au complexes. Enlarged CD spectra between 200 and 300 nm are shown in the inset. Strong CD bands are shown in the UV region. It is known that right-handed α -helices, which consist of amino acids, in TMV coat proteins show a positive CD peak at 190 nm and a negative peak at 209 nm deriving from the excitation of bonding π orbital to anti-bonding π^* orbital (π - π^* transition) with optical dipoles perpendicular and parallel to the axis, respectively [3]. The α -helices also show a negative CD peak at 222 nm owing to the excitation of the non-bonding n orbital to π^* orbital (n- π^* transition) with parallel dipole. These dipoles interact with light, bringing about optical activity. Absorption in this region (240 nm and below) is due principally to the peptide bond connecting amino acids. In addition to these peaks, a positive CD peak between 275 and 282 nm is known to be attributed to aromatic tyrosine residues of the coat protein [21]. These peaks are observed for tbpTMV without Au NPs as shown by black crosses in Fig. 4(a). The band shape of wtTMV without Au NPs is indistinguishable from that of tbpTMV. The band shape changes when TMV-Au complexes are formed. Particularly for the tbpTMV-Au (red open squares in the inset), the negative CD peak at 222 nm is considerably enhanced compared to the negative peak at 209 nm.

In the region between 300 and 700 nm of Fig. 4(a), tbpTMV without Au NPs shows no obvious CD peak. Contrastingly, for the tbpTMV-Au, a negative CD peak in plasmon wavelength around 540 nm was observed even though optical activity in this region is negligible for TMV or Au NPs themselves. A similar negative CD peak was observed for the wtTMV-Au (blue filled circles). It is noteworthy that the absorption peak derived from localized surface plasmon



Fig. 4. (a) CD spectra of the tbpTMV-Au (red open squares) and wtTMV-Au (blue filled circles). Black crosses correspond to tbpTMV only. Inset shows enlarged spectra between 200 and 300 nm. (b) Simultaneously measured UV-VIS spectra of the complexes.

of Au NPs was also observed around 540 nm as shown by the solid red line and dashed blue line in Fig. 4(b). This indicates the interaction of plasmonic resonance and optical activity. It should be noted that the CD band between 200 and 300 nm also changed shape and a negative peak at 222 nm was enhanced after the formation of Au NPs, particularly when using tbpTMV. By attaching Au NPs to TMV, optical activity was enhanced at two different wavelengths.

4. Discussion

Kuwata-Gonokami and co-workers [22] and Plum and co-workers [23] reported that metallic gammadia fabricated on a substrate using electron-beam lithography on a substrate show optical activity. 3D gammadia themselves have space-inversion symmetry and show no optical activity. The gammadia, however, exhibit optical activity when they are placed onto an achiral substrate. These are called planar-chirality structures. Several chiral metamaterials fabricated using top-down and bottom-up methods have also been reported [24–27]. The physics in these chiral metamaterials are very similar to the planar-chirality. However, our experimental results have a different physical origin from these planar-chirality. Our sample is a complex of an achiral material and a chiral material, which originally shows optical activity. A naive assumption may be that the achiral material has nothing to do with the optical activity. However, this is not true.

In the present experiments, the CD peaks should originate from the chiral structures in the complex of TMV and Au NPs. In the complex, TMV is made of right-handed helical RNA and right-handed helically aligned coat proteins consisting of right-handed amino acids (Fig. 1).

We thus consider TMV as a chiral medium, in which the indices of refraction for right-handed and left-handed polarizations propagating toward one direction are different. Additionally, the refractive indices depend on propagating directions. Such a directional difference of the indices of refraction plays an essential role in our results. Because Au NPs are achiral themselves, we suppose here that a chiral medium is connected to a normal achiral medium.

In the visible region, the plasmon resonance due to the coupled mode between light and electrons in Au NPs leads to the considerable enhancement of optical activity. This is consistent with a scheme of chirality transfer [10]. A strong electric field owing to localized surface plasmons probably enhances the CD signals of chiral molecules at plasmon wavelength. This is likely to be a CD counterpart of surface-enhanced Raman scattering.

On the other hand, previously, the enhancement of the CD bands in the UV region has not been observed experimentally nor discussed theoretically, although numerical calculation indicates the possibility. The schemes of chirality imprinting or chirality transfer cannot explain this observation because the Au NPs are very similar to dielectric in the UV region [28]. Although possibilities for a profound modification of the virus and a Fano-type resonance by attaching the NPs cannot be excluded, we have another explanation.

In optical activity, refractive indices depend on the propagation direction of light as well as the direction of circular polarization. An attachment of Au NPs to a chiral medium affects both propagation and circular-polarized directions of refractive indices. This situation is different from other phenomenon such as a magneto-optical (MO) effect. In MO effect, refractive indices depend only on the direction of circular polarization. Because refractive indices are independent of the propagation direction of light in MO effect, attaching Au NPs does not affect the light unless light interacts with electrons like plasmon.

In this study, we consider that the intrinsic resonance of the α -helix couples with the normal achiral medium and should enhance the optical activity of the complex; the optical activity of the complex in the UV region becomes larger than that of the chiral medium alone. As a result, attaching a NP to a protein leads to the enhancement and modification of CD peaks in both the UV and VIS regions. This scheme provides a consistent explanation to the present experimental results.

5. Conclusions

We succeeded in fabricating an optically active chiral meta-molecule in both the UV and VIS regions utilizing an engineered TMV and Au NPs. The engineered TMV, tbpTMV, is a better scaffold to deposit uniform-sized Au NPs; tbpTMV-Au has improved dispersion in solution. A plasmon-enhanced CD effect is observed in the VIS region. In addition, CD signals in the UV region are also modified. Proteins can be considered as chiral meta-atoms, and achiral plasmonic NPs enhanced the CD response of the proteins: the complex of virus and Au NPs is thus a chiral meta-molecule in both the UV and VIS regions. The meta-molecule – structurally defined complex of Au NPs and an engineered TMV – constructed here is thus a promising building block for 3D chiral metamaterials at optical frequencies.

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