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Fabrication of Nano-Scaled Structures using Genetically Engineered Tobamoviruses

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1. Introduction

For the fabrication of nanometer-scaled devices, nanowires with diameters less than 10 nm are necessary. In addition to fine control of the size and shape, nanowires need to be selectively placed onto electrodes for a bottom-up fabrication of a device. Biological supramolecules have identical dimensions and their outer-surface can be genetically or chemically modified. The use of a tube-shaped bio-template offers a solution to these requirements [1]. Nanodots made with a bio-templated method have already been successfully utilized to fabricate memory devices [2, 3].

Tobamovirus (TMV) is a plant virus that has been one of the favorite bio-template proteins for making nanowires. The virus is 300 nm in length with outer- and inner-diameter of 18 nm and 4 nm, respectively [4]. Utilizing the central channel of TMV, formation of metallic nanowires such as Cu, Ni, Co, Co/Pt, and Fe/Pt have been reported. However, it has been challenging to obtain 300-nm nanowires with wild-type TMV.

We genetically modified the inner-surface of TMV to make metallic nanowires with new characteristics. We introduced point mutations in the region facing the inner-surface of TMV so that negative (asparatic acid: D) or positive (lysine: K) charges are increased inside. It turned out that increasing positive charge at a specific site resulted in dotted nanowire formation. For selective placement of nanowires on a given surface, functional peptides that have specific binding properties are genetically attached to TMV.

Our ultimate goal is to supply a nano-scaled structure for possible applications in constructing electronic devices by “biomineralization”.

2. Methods

Pt/Co nanowire formation.

Wire formation was performed in a 1.5 ml tube with a final concentration (in 200 μl) of 0.15 mg/ml TMV in the presence of 150 mM NaCl in H2O. To 170 μl of the solution, 3 μl and 1 μl of 100 mM each of $(NH_4)_2Co(SO_4)_2$ and K2PtCl4 were added and ultrasonication was applied for 30 seconds (30 cycles of one second pulse with five second intervals) on ice. Adding of solutions and sonication were repeated followed by addition of 1 μl of freshly prepared 100 mM $NaBH_4$. Adding of reductant and sonication were repeated. These steps (4 sonications) constitute one cycle, and three cycles of the procedure were performed resulting in a final concentration of 9 mM for $(NH_4)_2Co(SO_4)_2$ and 3 mM each for K_2PtCl_4 and $NaBH_4$. Twice volume of $(NH_4)_2Co(SO_4)_2$ or K_2PtCl_4 were added in experiments for two-fold increase in the final concentration of them.

3. Results and Discussion

Genetic Inner-Surface Modification of TMV

We first tried to make recombinant TMV virus-like particles with inner-surface residues modified according to the structural information of TMV. First, serine at position 101 (S101) and threonine at 103 (T103) were changed to lysine to investigate whether increased positive charges inside TMV would affect nanowire formation. In addition to S101 and T103 residues, two glutamic acid residues in the vicinity of the channel at position 97 and 106 (E97 and E106) were changed to lysine residue, respectively. Mutant virus-like particles were obtained in all cases except for T103K mutant. The length of the virus-like particles was apparently not affected, resulting in 300 nm-long particles

Fig. 1. A high-resolution TEM image of dotted nanowires formed inside TMV-E106K mutant stained with Au-glucose. Scale bar is 5 nm

Effects of Inner-Surface Modifications of TMV

Next, Pt/Co nanowire formation was attempted using lysine mutants. Interestingly, TMV-E106K formed dotted Pt/Co nanowires inside the channel (Figure 1). The high-resolution TEM image showed the diameter of each dot to be about 3 nm suggesting that the diameter of the channel had been increased as a result of dotted nanowire formation. Lattice fringes were observed in some dots indicating that those dots are made of a single crystal. Energy dispersive X-ray spectroscopy (EDS) analysis showed that the nanowire formed inside TMV are made mostly of Pt (Figure 2). The percentage of Co content ranged from 8 to 25 %, which appeared to reflect experimental errors of EDS analysis. The result suggests that the dots are likely a mixture of single crystal Pt or $CoPt_3$ dots with some part of the nanowire made of amorphous Pt. The length of the dotted nanowires were similar to those of the nanowires formed inside wild-type TMV. The dotted nanowire was not straight which may have been caused by spontaneous nucleation at many positions which in turn caused the dots to grow and enlarge the inner-channel, resulting in bent virus-like structures. The tube-shaped structure of TMV was maintained, possibly because of the presence of genomic RNA. Changing $(NH_4)_2Co(SO_4)_2$ to K_2PtCl_4 ratio in nanowire forming conditions apparently gave similar results.

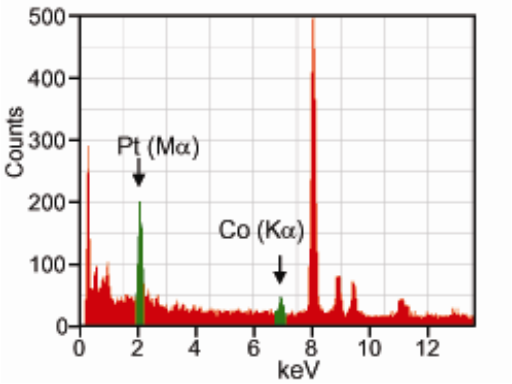

Fig. 2. Energy dispersive X-ray spectroscopy (EDS) spectrum of dotted nanowires formed inside TMV-E106K mutant.

4. Conclusions

By changing the amino acid residues facing the inside channel of TMV, dotted nanowire was made. Outer-surface modification was also tried, and it was found that "read-through" technology is effective for attaching functional peptides to the outside without interfering with nanowire formation inside TMV. Nanowires produced in this study are a promising component for bottom-up fabrication of other nanometer-scaled devices.

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