Silica Coating on a Bionanorod

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Abstract

Wild-type tobacco mosaic virus (TMV), the bionanorod, is reproducibly coated with silica via the reaction of tetraethylorthosilicate (TEOS) in an aqueous methanolic solution. Previous investigations have shown that smaller silica nanostructures are formed when methanol rather than ethanol is used as a solvent in this reaction. The uncoated TMV particles are stable in aqueous methanolic solutions with a methanol concentrations less than 60 wt%. Several techniques are used to analyze the silica coated particles, including electron microscopy, EELS, x-ray scattering, and light scattering. A thin and consistent silica shell is found to exist around the intact virion particle which allows the TMV nanorod to be stable in pure methanol. The goal of this research is to characterize the silica-coated TMV structure for use in future applications.

Introduction

Preparation of nanoscaled materials with complex structures, such as nanowires and nanotubes, are of great importance in regards to miniaturization. Currently there are several methods of creating nano-wires [1], including the use of biological structures, such as virions [2-4], as templates for the synthesis of specific nanostructures. *Tobacco mosaic virus* (TMV) produces a tube-like virus particle, 300nm long by 18nm wide, which is stable over a wide range of pH and temperatures, thus making it an ideal nanostructure template. The coating of various metals and metal sulfide nanoparticles on the surface of wild-type and genetically modified TMV have been previously reported [5-10]. Additionally, silica oxide gels (from tetraethylorthosilicate (TEOS) with a strong acid in ethanol) formed on wild-type TMV has been presented by researchers, along with the creation of mesoporus silica structures formed using a wild-type TMV template [5,11].

The tubular structure of the TMV particle consists of ~2130 identical protein subunits of molecular weight 17.5 kDa wrapped in a helix around a single strand of RNA [12]. These particles are stable in solutions of approximately pH 2 to 10 and at temperatures up to 60° C [13]. The surface of the assembled virus particle contains numerous glutamic acid, aspartic acid, lysine and arginine residues, giving an overall negative surface charge above the isoelectric point, pH 3-3.5, or a positive charge when below. The length of the TMV particle is controlled by the length of the encapsulated viral RNA.

Previous examples of silica coatings created by Shenton *et al.* were conducted in strong acidic conditions (pH 2.5) where the acid catalyzed hydrolysis of silica in an ethanol solvent (between the isoelectric points of TMV (pI 3.5) [14] and silica (pI 2) [15]) produced a charge-charge interaction between the negatively charged silica and the positively charged TMV surface [5]. However, a search for milder reaction conditions in addition to a more detailed

characterization of the silica-coated TMV is needed for the future application of TMV-based silicate oxide nanotubes. Milder reaction conditions are not only safer but provide greater control over the gelation reaction by slowing the process [16]. The research presented here therefore achieves silica layer formation in aqueous methanolic solutions in a pH range of pH 5-7. Furthermore, the hydrolysis and condensation reactions of TEOS in aqueous methanolic solutions produce denser nucleating particles about half the diameter of those formed under similar reaction conditions in ethanol [17]. These particles may help produce smoother coatings with increased density as a result of the increased mobility and smaller negatively-charged surface area of the smaller particles.

Characterization of the silica-TMV nanotubes is performed using electron energy loss spectroscopy (EELS), small angle X-ray scattering (SAXS), and dynamic light scattering (DLS). EELS provides high spatial resolution elemental analysis of the silica coating, while SAXS and DLS provide in situ information on the structures of the system. The detailed structural information resulting from this research will be useful in utilizing silica-TMV nanotubes in other applications, such as multi-layered coatings.

Results and Discussion

A TEM image of unstained silica-coated wild-type TMV particles re-suspended in 100% methanol is shown in Figure 1. The methanol re-suspension is important to note as normally alcohols denature proteins by disrupting the intramolecular hydrogen bonding, where new hydrogen bonds are formed instead between the alcohol molecule and the protein side chains [18]. However, in this case, the TMV structure is preserved. The lengths of the nanotubes are consistent with those of wild-type TMV (300±29nm) template [19]. Due to the low electron density of silica, the thin silica shell on the TMV is difficult to observe clearly. TEM observation at higher magnification resulted in blurring of the image and failure to show a discrete silica shell.

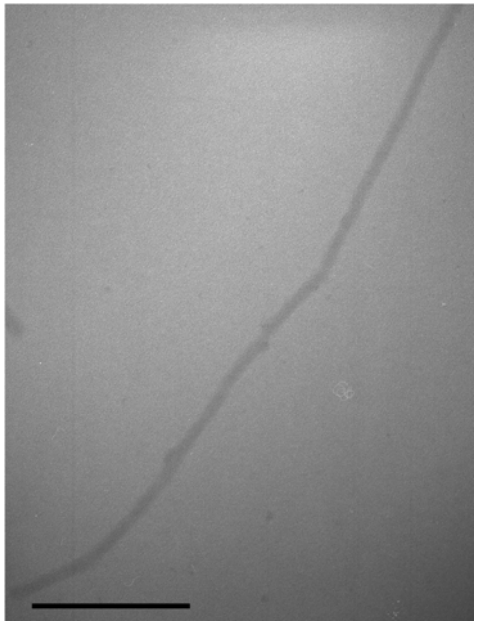

Figure 1: TEM image, unstained, of silica-coated wild-type TMV suspended in 100% MeOH. The scale bar is 200 nm.

DLS studies help show that the dispersion and stability of the virus particles remain unaffected by the coating process. The hydrodynamic radius, $R_{h,}$ of the silica-coated wild-type TMV before centrifugation and re-suspension in MeOH is found to be 50±20nm. Using Broersma's equation for the theoretical diffusion coefficient for a cylinder [20] and the given values for the size distribution for the uncoated virus, the expected value for the R_h of wild-type TMV was calculated, falling between 50 - 60nm. The experimental DLS values for R_h are in agreement with the theoretical values.

Electron Energy Loss Spectroscopy (EELS) was used to confirm the presence and location of silica and TMV in the sample. EELS was chosen for its high spatial resolution (as low as 0.3-0.5 nm in some cases), unlike x-ray emission spectroscopy which suffers from beam broadening due to backscattering, secondary fluorescence, and the generation of fast secondary electrons in the specimen [21]. Previous EELS studies conducted on TMV have shown the presence of carbon and phosphorous (from the RNA) to be indicators of TMV [22]. Both phosphorous and silica were found on the structures, confirming the presence of silica-coated TMV.

SAXS studies clearly show the difference between the silica-coated TMV and uncoated TMV particles in the presence of preformed silica particles. The preformed silica particles are made using the same reaction condition as that used for the silica-coated TMV.

Conclusions

Silica-coated TMV has been successfully produced by the reaction of TEOS in methanol and water at pH values above those that have been previously reported. Stabilization of the TMV in alcohol has been achieved through the silica coating. TEM studies combined with EELS analysis confirm the presence of silica on the surface of the TMV while SAXS studies show the differences between uncoated and coated TMV particles.

Acknowledgements

This research was support by the U.S. Department of Energy (DEFG02-02-ER45975 and DEFG02-02-ER45976). EELS analysis was carried out in the Center for Microanalysis of Materials, University of Illinois, which is partially supported by the U.S. Department of Energy under grant DEFG02-91-ER45439. Special thanks go to Dr. Ray D. Twesten for his assistance in collecting the EELS data. SAXS studies were supported by the NSF funded (MRI program award 0321118-CTS) facility for In-situ X-ray Scattering from Nanomaterials and Catalysts.

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