Study and characterization of tobacco mosaic virus head-to-tail assembly assisted by aniline polymerization

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One-dimensional composite nanofibres with narrow dispersity, high aspect ratio and high processibility have been fabricated by head-to-tail self-assembly of rod-like tobacco mosaic virus assisted by aniline polymerization, which can promote many potential applications including electronics, optics, sensing and biomedical engineering.

The fabrication of one-dimensional (1D) nanostructures is of considerable interest for advancing nanoelectronics.[1] Although many synthetic strategies of electronically active 1D materials have been developed, it remains a challenge to prepare long, mono-disperse, nanofibres with good processability. By introducing biological elements into nanomaterial synthesis, it is possible to generate highly ordered and programmable anisotropic structures.[2] For example, genetic engineering of viruses has been used to direct the formation of monodisperse single-crystal nanowires with exact control of composition, size and phase.[3]

Tobacco mosaic virus (TMV) is a classic example of a rod-like plant virus. Native TMV particles consist of 2130 identical protein subunits arranged helically around genomic single-strand RNA. TMV, isolated from infectious tobacco plants in gram quantities, is 300 nm long and 18 nm in diameter, with a 4-nm cylindrical cavity along the central core. The tube-like structure, stability, narrow size distribution, and electrostatic properties of the capsid surfaces make TMV a robust platform for the deposition of inorganic materials on either the interior or the exterior surface of the capsid, which leads to interesting 1D structures.[4] In addition, recent studies showed the surface properties of TMV can also be manipulated chemically or genetically without disrupting the integrity and morphology of TMV capsids.[5] We are particularly interested in the reversible head-to-tail assembly of TMV to produce long nanotubes by surface deposition.[4c] Here, we report our preliminary results on the study of tobacco mosaic virus head-to-tail assembly assisted by aniline polymerization.

A head-to-tail ordered assembly of wild type TMV has been observed, likely a product of complementary hydrophobic interactions between the dipolar ends of the helical structure. In an acidic environment, this 1D assembly is dramatically favored by minimizing the repulsion between the carboxylic residues at the assembly interface.[6] We hypothesize that a molecule with amino group(s) can accumulate on the surface of TMV due to the electrostatic attraction or hydrogen bonding between the negatively charged surface residues of TMV and the amino group of the counterpart molecule at a pH above the isoelectric point of TMV (around 3.2).[7] Therefore, the *in situ* polymerization should be able to produce a thin layer of polymers on the surface of TMV, and fix the head-to-tail assembled tube-like structure. To test this hypothesis, we chose aniline as the monomer, due to the ease of polymerization[8] and the intrinsic anisotropic morphology which polyaniline possesses (Fig. 1).[9] In addition, many successful templated syntheses of polyaniline nanofibres in an aqueous solution under ambient conditions have been reported.[10]

In a typical experiment, distilled aniline (10 μL) was introduced into an aqueous TMV solution (1 mg mL^{-1} at pH 5.5), followed by addition of ammonium persulfate (10 mg). The pH of the reaction solution was around 6.5. The reaction mixture was incubated at room temperature for 24 h to form a yellow suspension. After centrifugation at 8,000 g for 3 min, the pellet was quickly rinsed three times with pure water, and resuspended in deionized water to afford the pure polyaniline/TMV composite.

The TMV head-to-tail assembly was confirmed by transmission electron microscopy (TEM). Upon staining with uranyl acetate, native TMV appeared as rod-like particles with an average diameter of 18 nm (Fig. 2a). Most of the particles were 300 nm long, consistent with the literature. Without negative staining, the long fibre structure can be observed by TEM on carbon-coated copper grids (Fig. 2b). In contrast to native TMV, the length of the fibre can be as long as 10 μm based on the TEM observations (Fig. 2b and 2c). As shown in Fig. 2d, the composite nanofibres are narrow dispersed in width with a diameter of ~21 nm. Due to
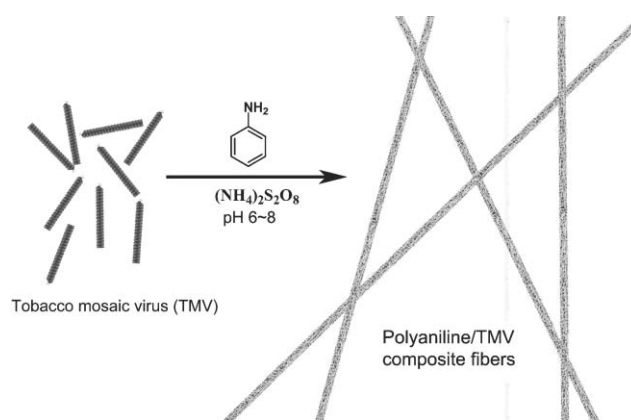

Fig. 1 Schematic illustration of the synthesis of 1D polyaniline/TMV composite fibres.

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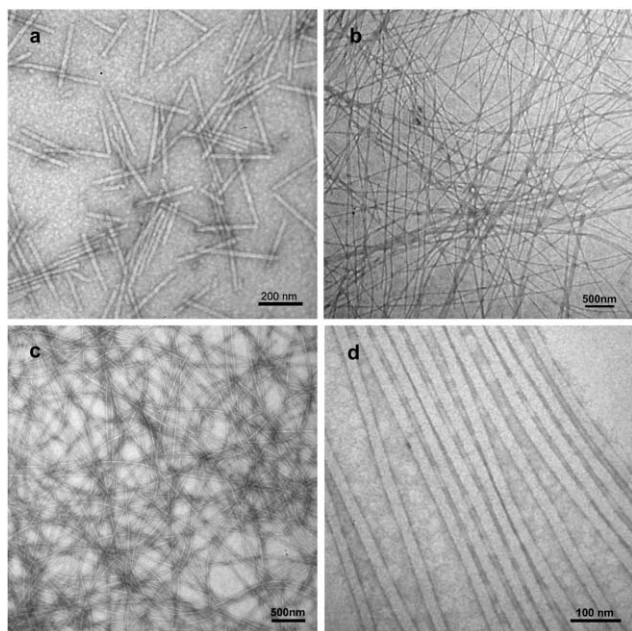
Fig. 2 TEM images of (a) wild type TMV, (b) 1D polyaniline/TMV composite fibres without negative staining, and ((c), (d)) polyaniline/TMV composite fibres after negative staining.

Fig. 3 (a) AFM height image of wild type TMV and (b) corresponding phase image. (c) AFM height image of a single polyaniline/TMV composite fibre and (d) corresponding phase image. (All the images have the same scale bar.)

the nearly neutral pH used for the polymerization, polyaniline formed with this method is in the insulating form, which shows a yellowish color in solution.

Atomic force microscopy (AFM) was also used to characterize native TMV and composite fibres. The samples were drop cast from solution onto silicon wafers, and dried under ambient conditions. The average length of native TMV is around 300 nm, as shown in Fig. 3a and 3b. The small round dots displayed in the images may be the 20S structure of TMV formed by reversible dissociation, or the result of mechanical damage to native TMV.[5a,11] Fig. 3c and 3d show a single 1D assembled TMV/polyaniline composite fibre. It was about 1.6 μm long, with a very smooth surface and no visible gaps. This demonstrated that the head-to-tail protein–protein interaction led to the formation of the fibre-like structures. Such interaction, in principle, is identical to the subunits' interaction at any cross-section of the native TMV structure.[11,12] The surface polymerization of aniline together with the attractive interaction between polyaniline and TMV further stabilized the resulting composite fibres, and compensated for the loss in entropy associated with the assembly process.

The formation of polyaniline was confirmed by UV-vis absorption. Thorough dialysis against deionized water gave a polyaniline/TMV composite sample which had two absorption peaks at 260 nm and 427 nm (Fig. 4a). The peak at 260 nm is attributed to the absorbance of TMV, and the peak at 427 nm is attributed to polyaniline in its branched form.[13]

Small angle X-ray scattering (SAXS) was used to measure the increase in the diameter of TMV quantitatively upon surface polymerization. Interestingly, TMV and polyaniline/TMV composite fibres show similar scattering data in water (data not shown), probably because the average electron density of the polymer layer is similar to that of water. We therefore embedded the fibres into a silica gel matrix. The scattering is now mainly due to the silica
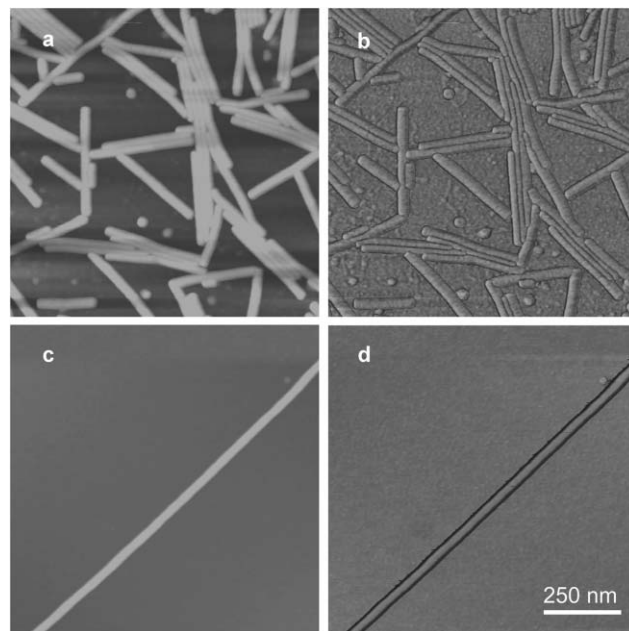
volume that is excluded by the fibres (Fig. 4b) and is sensitive to the overall diameter of the fibres. The form factor of rod-like particles depends on both the length and diameter of the particle. Since the fibre length is much greater than its diameter, the position of the first minimum in the scattering curve corresponds to the first zero of Bessel function $J_1(qR)$. The diameter of native TMV was found to be 16.7 nm, and that of the composite fibre was 17.7 nm. Although these values are slightly lower than those obtained by TEM, they are consistent with a surface coating of TMV with polyaniline.

A very interesting feature of the synthesis is that the polymerization of aniline takes place only on the surface of TMV. We used scanning force microscopy (SFM) to analyze the reaction mixture before purification (Fig. 5). The reaction solution was diluted 10 times with pure water, then spin-coated onto a silicon wafer. Both height and phase images showed that the length of composite fibre can be longer than 20 μm. However, it is
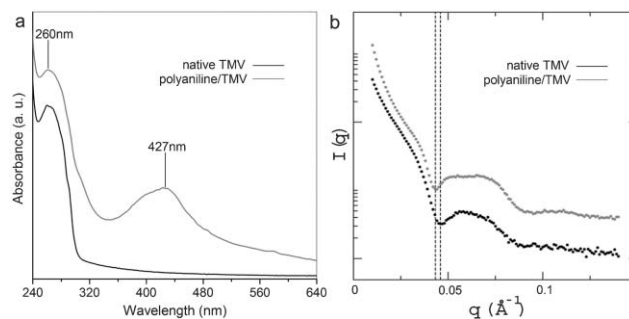
Fig. 4 (a) UV-vis spectra of native TMV (black) and polyaniline/TMV composite fibre (grey). (b) SAXS data of the polyaniline/TMV composite fibre (grey) and native TMV (black) dispersed in a silica gel matrix.
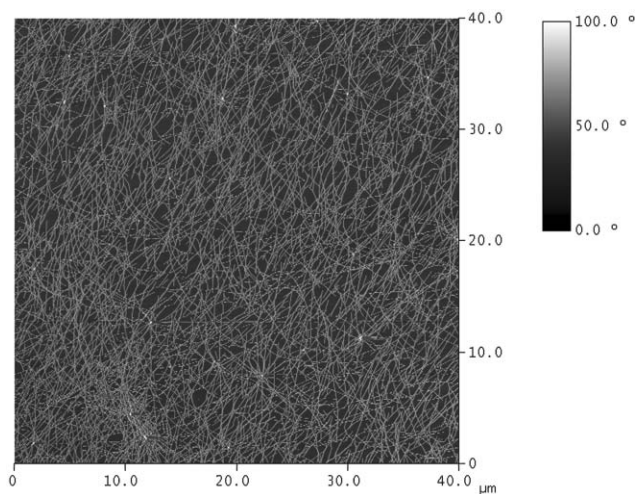
Fig. 5 SFM phase image of polyaniline/TMV composite fibres.

difficult to determine the exact diameter for the soft protein samples.[14] No bulk polyaniline or pure polyaniline nanofibres were formed in the reaction. Similar results were observed with TEM (data not shown). Without the presence of TMV as a template, highly aggregated polyaniline with a small amount of nanofibrillar polymers was produced, in accordance with previous reports.[8a,8d] The electrostatic interactions between aniline (and polyaniline) and TMV led to the polymerization of aniline on the virus surface exclusively.

In summary, 1D composite nanofibres have been generated by head-to-tail self-assembly of rod-like TMV particles assisted by aniline polymerization on the TMV surface. These results open a unique way to produce composite fibrillar materials with narrow size dispersity, high aspect ratio and high processibility, which can promote many potential applications in electronics, optics, sensing and biomedical engineering.

Notes and references

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