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Block copolymer nanotemplating of tobacco mosaic and tobacco necrosis viruses ☆

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Abstract

This paper examines the interaction between a block copolymer and a virus. A poly(styrene-*b*-4-vinylpyridine) block copolymer was loaded with nickel, and cast from a selective solvent mixture to form a cylindrical microstructure (PS/P4VP–Ni). The nickel ions were confined within the P4VP block of the copolymer. The binding of tobacco mosaic virus (TMV) and tobacco necrosis virus on microphase-separated PS/P4VP–Ni was examined. A staining technique was developed to simultaneously visualize virus and block copolymer structure by transmission electron microscopy. Electron microscopy revealed virus particles associated with block copolymer microphase-separated domains, even after extensive washes with Tween. In contrast, virus associated with PS/P4VP block copolymers lacking Ni were readily removed by Tween. The cylinder long axis of the microstructure was oriented using a hot press and a cooled channel die for quenching, resulting in PS/P4VP cylinders that had a strong anisotropic directional preference. When exposed to flowing solutions of TMV, the PS/P4VP–Ni surface exhibited an ability to retain TMV in a partially aligned state, when the direction of flow coincided with the long axis of the PS/P4VP–Ni cylinders. These results suggest that Coulombic interactions provide a robust means for the binding of virus particles to block copolymer surfaces.

Keywords: Block copolymer; Nanopatterning; Virus

1. Introduction

This paper investigates the interaction between a block copolymer surface and viruses. A polystyrene-*b*-poly(4-vinylpyridine) copolymer surface was investigated for its ability to bind and pattern two different plant viruses, tobacco mosaic virus (TMV) and tobacco necrosis virus (TNV). This study is unique in its use of a self-assembled block copolymer template for the creation of regularly shaped metal-loaded domains for the purpose of interacting with biological species such as viruses.

Research has shown that nanotextured surfaces can influence the behavior of viruses and cells [1,2]. Because much of the chemistry involved in signaling and recognition in biology involves surface interactions, having a tunable surface that can be altered to study different organisms or to elicit different reactions could be a valuable research tool [3–5,7,8,6]. A nanopatterned block copolymer surface seems a natural fit for a microfluidic system, as its ease in processing should allow it to be integrated, especially in the capacity of binding studies, species detection or structural characterization. A number of methods on the use of polymers to assist in the patterning of viruses have been reported. Niu et al. [9] have fabricated one-dimensional nanofibers with high aspect ratio, narrow dispersity and high processibility. The self-assembly of TMV is assisted by aniline polymerization. The electrostatic attraction of the amine group on the monomer to the negative charge on the virus causes the monomer to accumulate on the TMV surface, inducing head-to-tail assembly. Clark et al. [2] have utilized the electrostatic interactions of self-assembled monolayers to order viruses on the surface of thin films.

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Much current research on this topic of biological-abiotic surface interfaces involves patterning that exists on the microscale. Lithographic techniques can produce a wide variety of structures, such as arrays of lines and dots [10], that have been proven to affect the direction of the growth of cells, the adhesion of cells to a substrate, and the shape and health of cells attached to the substrate [11]. Lithographic techniques have also been used to pattern viruses and direct their assembly onto surfaces [12,13]. Rather than using lithography to pattern an inorganic surface, block copolymers were employed in this study to create a surface decorated with nanometer-sized periodic structures. Block copolymers microphase separate into periodic domains of nanometer dimensions. Control over domain morphology can be exerted through simple choice of block length and composition, allowing a wide range of available morphologies and functional groups. In a block copolymer, single blocks can be chosen so that they have the chemical ability to take up metal ions. Metal loading occurs through metal ion sequestration in the receptive block. Previous work [6] has shown that nickel-templated block copolymers preferentially interact with recombinant proteins. Such block copolymers have been used for the directed self-assembly of metal nanoparticles [14]. Divalent metal cations have been found to promote TMV precipitation from aqueous solutions [15]. The block copolymer of this study, polystyrene-*b*-poly(4-vinylpyridine), was exposed to nickel salts while in solution, resulting in high specificity in targeting the nickel ion to the receptive block of each block copolymer. Transmission electron micrographs showed that nickel metal nanoparticle formation is not observed. Rather, the receptive poly(4-vinylpyridine) block complexes the nickel ion and confines it with apparently minimal leakage between blocks. Tween detergent was used to examine the strength of the binding of the virus to the copolymer surface by disrupting weak functional group interactions between the virus and the copolymer.

The purpose of experiments presented here was to provide a better understanding of the nature of the virus–copolymer interaction. It was necessary to resolve the tendency of rod-like virus particles to lie randomly on the copolymer surface rather than self-orienting to maximize contact with the attracting block of the copolymer surface. To have any chance of large-scale ordering of the virus, the surface of the block copolymer itself must have a degree of long-range microstructural order. This was accomplished through the use of applied stress above the glass transition temperature of the polymer. Tween detergent was again used to disrupt weak bonding between the copolymer and the virus.

2. Materials and methods

2.1. Poly(styrene-b-4-vinylpyridine)

The PS/P4VP block copolymer purchased from Polymer Source had a molecular weight ratio of 20.0 K/19.0 K for the polystyrene and poly(4-vinylpyridine) blocks, respectively. The polystyrene block had a degree of polymerization of 192, and the poly(4-vinylpyridine) had a degree of polymerization of 180. The total block copolymer had a polydispersity of 1.09.

2.2. Tested tobacco virus species

Two different virus species, TMV and TNV, were used in order to examine the interaction between viruses and the block copolymer surface. The TMV virion is a rigid rod 300 nm long with a diameter of 18 nm. The isoelectric point of TMV is 3.5; therefore at pH 7, TMV has an overall negative surface charge, with a linear charge density of 0.5–2 electrons/Å [16]. TMV forms head-to-tail oligomeric string aggregates that are much longer than the length of a single TMV virion. Unlike the TMV rod, TNV has a regular icosahedral shape with an average diameter of 26 nm [17]. TNV has an isoelectric point of 4.5, thus, like TMV, TNV has a negative charge at pH 7.

2.3. Ultramicrotoming and electron microscopy

A Leica EM UC6 ultramicrotome was used to section samples for electron microscopy. PS/P4VP–Ni and PS/P4VP specimens were fixed in Spurr's fast-curing resin. Embedded specimens were microtomed at room temperature. Specimens were cut to 100 nm thickness so that they would be visible in the transmission electron microscope and able to endure the planned virus exposure and Tween detergent wash procedures. Microtomed films were mounted on 600 mesh copper grids, model number 600TT, purchased from Ted Pella. Electron microscopy was performed on a Hitachi H600AB electron microscope with 100 kV accelerating voltage.

2.4. Nickel metal complexation and film preparation

PS/P4VP was dissolved in chloroform, a good solvent for both polystyrene and poly(4-vinylpyridine) blocks. A polymer/chloroform concentration of 1 mg/ml was used. In order to dissolve $Ni(NO_3)_2$ in the PS/P4VP/chloroform system, the metal salt was added to the solution at a concentration of 100 μg/ml, and the mixture was titrated with tetrahydrofuran until a clear solution was formed. At the point of solution formation, the amount of tetrahydrofuran added was 8.5% of the total solution volume. The amount of nickel ion provided for complexation was in excess to the number of available surface pyridines. However, it is believed that the ratio of nickel ions complexed in the vinly pyridine block should be close to 1:6, as nickel normally has hexavalent complexation behavior with nitrogen. While we did not explicitly examine the amount of nickel uptake, the transmission electron microscopy (TEM) images suggest that there is not an excess of nickel remaining in the film. Bulk films of the copolymer and nickel were cast in flat-bottomed basins formed from Bytac film in a chloroform-saturated desiccator to slow the evaporation process. Film formation via evaporation occurred

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