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Molecularly imprinted polymers for tobacco mosaic virus recognition

Linden D. Bolisay[a], James N. Culver[b], Peter Kofinas[a,*]

[a]Department of Chemical and Biomolecular Engineering, University of Maryland, College Park, MD 20742, USA
[b]Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, MD 20742, USA

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Abstract

Molecular imprinted Polymers (MIP) targeted for Tobacco mosaic virus (TMV) have been synthesized. Batch equilibrium studies using imprinted and non-imprinted polymer hydrogels in TMV and TNV solutions were conducted to determine virus-binding capacities. TMV-imprinted hydrogels showed increased binding to TMV (8.8 mg TMV/g polymer) compared to non-imprinted hydrogels (4.2 mg TMV/g polymer). Furthermore, TMV-imprinted hydrogels exhibited increased binding to TMV compared to TNV, while non-imprinted hydrogels bound similar amounts of TMV or TNV. This research has demonstrated that molecular imprinting of viruses can be used to selectively induce binding of target viruses based on shape differences of their virions.

Keywords: Hydrogel; Molecular imprinting; Virus

1. Introduction

Molecular imprinting is a technique producing synthetic materials containing highly specific receptor sites that have an affinity for a target molecule. Molecular imprinted polymers (MIPs) can mimic the recognition and binding capabilities of natural biomolecules such as antibodies and enzymes. MIPs can be applied in a wide range of technologies, such as catalysis, separation and purification, drug delivery, and detection [1–7]. The removal of viruses could potentially impact a wide range of diseases. Current typical virus removal methods include ultrafine filtration or high-speed centrifugation. Although workable, these solutions are slow and expensive. Molecularly imprinted polymers against viruses may be an attractive and inexpensive alternative to existing techniques. Acting as a viral "sponge", the MIP could be designed to selectively and specifically remove one or more pathogenic viral species from blood or sera.

Experiments with virus MIP have been previously reported [5,6]. These MIP systems contain two-dimensional surface imprinting "stamps" of viruses on a crosslinked polymer surface that is attached to a sensor. When the virus template is imprinted and removed, these sensors are able to re-bind small amounts of the target virus on the polymer surface. Two-dimensional surface imprinting is designed to detect the presence of small amounts of viruses whereas three-dimensional imprinting can be used to bind and extract large amounts of virus from a given solution. Currently no work has been published on the synthesis of MIPs for large virus extraction. The majority of published work in MIP synthesis is conducted in organic solutions. This may be a problem with templates that are naturally found in aqueous solutions because interactions in an organic environment can be different. Conducting the synthesis of viral MIPs in an aqueous environment may lead to better selectivity and binding of aqueous target molecules.

Tobacco mosaic virus (TMV) has been a well-studied and characterized virus, and is used as the target virus in this work [8]. The TMV virion is non-enveloped, rod shaped, and is 300 nm in length and 18 nm in diameter. The virus is composed of one 5130 kb RNA strand surrounded by 2130 protein subunits (153 amino acids per subunit) arranged in a right hand helix. The overall isoelectric point of 3.5 [9]. At neutral pH conditions, the virus particle has an overall negative charge [10]. Tobacco necrosis virus

*Corresponding author. Tel.: +1 301 405 7335; fax: +1 301 405 0523.
E-mail address: kofinas@umd.edu (P. Kofinas).
URL: http://www.glue.umd.edu\~kofinas.

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(TNV) is used as the non-targeted virus in this work. The TNV virus particle is non-enveloped, icosahedral shaped, and is 24 nm in diameter. The virus is composed of a single 3.8 kb RNA strand inside a virus shell consisting of 180 protein subunits (each subunit containing 267–272 amino acid residues). The overall isoelectric point is 4.5, giving the virus particle an overall negative charge at a neutral pH [9].

This paper discusses the non-covalent synthesis and binding capacity of molecularly imprinted polymers, using poly(allylamine hydrochloride) (PAA · HCl) as the polymer matrix and TMV as the template. PAA · HCl was chosen because it is soluble in water and has a high amount of amine groups that can associate with the viral template. In its crosslinked form, the polymer possesses low toxicity [11–13]. Batch experiments using TMV-imprinted hydrogels and non-imprinted polymer (NIP) hydrogels were conducted in aqueous solutions of TMV to determine the binding capacities of each polymer to the target virus. TNV was also used in batch experiments to compare the binding capacity of a non-target virus. These TMV–MIPs show increased binding to the target virus, TMV, compared to the non-target virus, TNV.

2. Experimental

2.1. Materials

Poly(allylamine hydrochloride) (M_w 15,000), NaOH (99.998%), Epichlorohydrin (99%), and ascorbic acid were purchased from Sigma-Aldrich (Milwaukee, WI). Sodium chloride (A.C.S. grade), sodium phosphate (dibasic, anhydrous, enzyme grade), ethylenediaminetetraacetic acid (disodium salt, electrophoresis grade), polyethylene glycol ($M_w = 8000$, molecular biology grade), potassium chloride (A.C.S. grade), and sucrose (ultracentrifugation grade) were purchased from Fisher Chemicals (Suwanee, GA). Chloroform (A.C.S. grade) was purchased from J.T. Baker (Phillipsburg, NJ). Ethyl alcohol (200 proof) was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY). All chemicals were used as received. Deionized water was obtained using the Millipore Super-Q water system.

2.2. Virus purification

Wildtype TMV from infected *Nicotaina tabacum* were isolated and purified as described by Gooding and Hiebert [14]. Virions were further purified by centrifugation at 22,000 rpm in a Beckman SW28 rotor for 2 h in a 10–40% sucrose density gradient at 4 °C. The white band corresponding to the virus layer was extracted, and pelleted by centrifugation at 30,000 rpm in a Beckman TI80 rotor for 2 h in a solution of water at 4 °C. The pellet was resuspended in water and analyzed for virus concentration using a Perkin Elmer Lambda 25 UV/Vis spectrophotometer. Virus concentrations for TMV were determined by measuring absorbance at 260 nm, corrected for light scattering at 325 nm, using an extinction coefficient of 3.01 $mg^{-1} cm^2$. Wildtype TNV was isolated and purified using a similar procedure. For this virus, the centrifugation of the sucrose density gradient was conducted at 25,000 rpm for 2 h. Virus concentrations for TNV were determined by absorbance at 260 nm, using an extinction coefficient of 5 $mg^{-1} cm^2$.

2.3. Hydrogel synthesis

The virus-imprinted polymer hydrogel was synthesized using the following procedure: 0.5 mL of 50% (w/v) poly(allylamine hydrochloride), PAA · HCl, in water, was placed into a 1.5 mL microcentrifuge tube, and 0.3 mL of 1 M sodium hydroxide was added and mixed. Next, 1 mg of virus was added and then water was used to bring the total volume to 0.8966 mL, and mixed for 1 h. Then, 0.1034 mL of epichlorohydrin was added and mixed for 4 h. After mixing, the hydrogel was allowed to cure for 5 days.

2.4. Template extraction

The hydrogel was cut and placed in a 50 mL plastic tube filled with 70% ethanol. The tube was then placed on a rotator and rotated for 24 h using a Barnstead International Labquake Shaker. Next, the ethanol solution was discarded and the tube was filled with 0.001 M sodium chloride and heated in boiling water for 1 h. The salt solution was then discarded and replaced with 1 M sodium chloride and the tube was heated again in boiling water. The cut hydrogel was transferred to a 600 mL beaker, filled with 500 mL of water, and washed for 3 days, changing the water every 8 h. Finally, the cut hydrogel was transferred to a petri dish and dried in a 55 °C oven.

2.5. Virus-binding test

Dried polymer hydrogel and virus solution were placed in a microcentrifuge tube in a ratio of 1 mg polymer weight to 1 mg/mL virus concentration. The virus solution and polymer were mixed in the microcentifuge tube for 4 h. The solution was then filtered using a 0.45 μm syringe filter and analyzed for viral concentration using a UV/Vis spectrophotometer.

3. Results and discussion

The synthesis of MIPs involves three steps, as seen shown Fig. 1. The first step involves the association of functional groups between the template molecule and monomer units on the polymer chains. This association can be by covalent or non-covalent. In our case, polymer chains are used to non-covalently interact with the template. Next, polymer chains are frozen in place by a crosslinking reaction, resulting in a polymeric network molded around the template. The last step involves the removal of the template from the polymeric network, resulting in cavities that have the shape, size, and functional group orientation that is complementary to the target molecule.

Figs. 2 shows the binding capacity of TMV-imprinted crosslinked polymers. Two crosslinked polymers, one imprinted with TMV and a control sample that was non-imprinted were examined for their TMV and TNV binding capabilities. The expectation is that the TMV-imprinted gel, having cylindrical cavities, would preferentially bind the virus of the same shape (TMV) compared to another tobacco virus of different shape (TNV). Experiments were performed for 3 different sets of imprinted/non-imprinted gels and each gel was tested in triplicate. The TMV-imprinted polymer displayed a 2.1-fold increase in affinity to the TMV virus compared to the non-imprinted control polymer. The TMV imprinted polymers, on the other hand, did not exhibit any significant change in TNV affinity compared to the non-imprinted control. These experiments thus demonstrated that a virus-imprinted

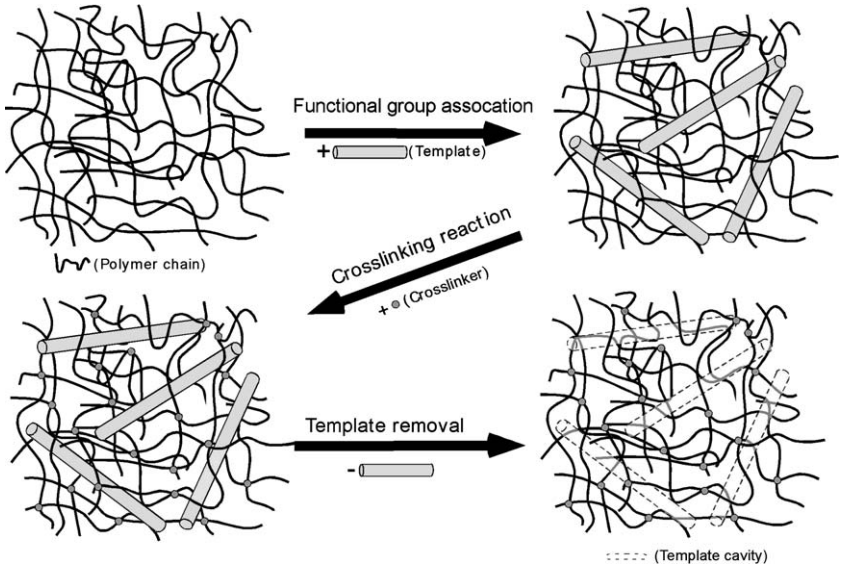

Fig. 1. Schematic of a virus imprinting processes.

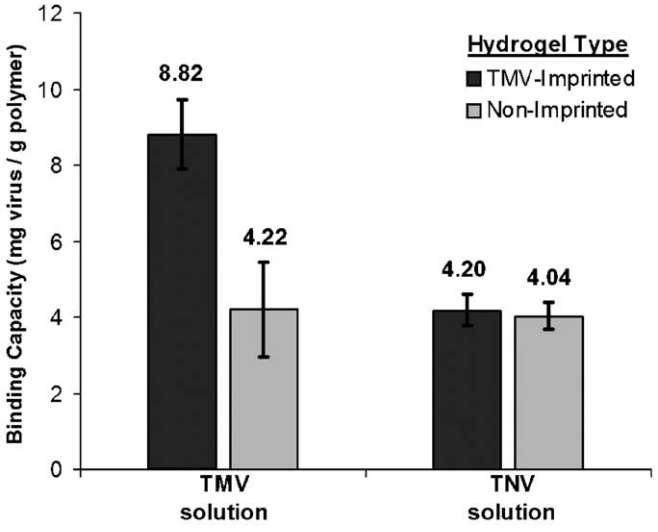

Fig. 2. Virus-binding capacity of TMV-imprinted polymers compared to non-imprinted control polymers. The TMV-imprinted polymer preferentially binds the cylindrical TMV virus over the icosahedral TNV virus.

polymer can be synthesized which is selective to a specific virus based on shape (geometrical) factors (cylindrical TMV virus versus spherical TNV virus). The experimental results overall indicate the ability of the crosslinked polymer gel matrix to conformally map viral surface features, retain these features when the gel is swollen, and specifically and selectively capture a virus based on these features. The effect of cavity shape will be investigated in future experiments with other crosslinkers of varying size and chemical structure.

4. Conclusions

Crosslinked polymers imprinted against TMV via non-covalent interactions were synthesized. The TMV-imprinted polymer exhibited an increase affinity to the target virus compared to the control polymer. Furthermore, the TMV-imprinted polymer demonstrated a preferential affinity, based on shape, to the target virus compared to the non-target virus. In contrast, there was no significant increase in binding of the control polymer to either target or non-target virus. The mechanism of virus imprinting involves the creation of cavities of similar shape to the original virus particle, providing a spatially defined site for binding of the templated virus.

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