

Separation of baculoviruses using molecularly imprinted polymer hydrogels

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ABSTRACT

The goal of this research is to develop molecular imprinted polymers (MIP) for biomimetic recognition of viruses. Our experimental results indicate that hydrogels can be produced, which can specifically and selectively bind recombinant baculoviruses. Although it is expected that imprinted cavities will be distorted due to the swelling of the hydrogel in water, our experiments show that even the swollen gels exhibit remarkable affinity toward recombinant baculovirus. The proposed methodologies for the synthesis and characterization of MIPs thus offer exciting avenues for the development of virus recognition techniques. The virus MIPs must function in aqueous environments. Our approach employs a more flexible non-covalent imprinting method, starting from a readily available polyamine polymer, and both MIP synthesis and testing are performed in aqueous solutions. The development of a virus imprinted MIP, which would apply to the identification, classification, and removal of viruses. This is currently a very difficult task, but the need is widespread in diverse sectors, including national security, human and animal health, crop protection, and biologics production. The development of general methods using MIPs capable of specific recognition of biological analytes would have an enormous value in medicine and bioanalytics.

INTRODUCTION

We are aiming to develop molecularly imprinted polymers (MIPs) which can recognize, and potentially differentiate viruses. Biomimetic recognition of recombinant baculovirus (BV-GFP) has been achieved in experiments using polymers while in their water-swollen state, thus addressing a major problem which has long been associated with this area of research in hydrogels. A virus imprinted MIP would apply in the identification, classification, and removal of viruses. This is currently a very difficult task, but the need is widespread in diverse sectors, including national security, human and animal health, crop protection, and biologics production.

The removal of viruses could potentially impact a wide range of diseases. One way to ensure that the production of human biopharmaceuticals is virus-free, is to use virus-specific “sponges”. For example, the removal of hepatitis virus or HIV from the blood supply could be impacted by such MIP technology.

While methods of template fixation vary among research groups, the large majority of molecular imprinting studies to date have concentrated on synthesizing imprinted polymers from a monomer, rather than crosslinking an existing polymer having the appropriate functional groups. Typically, polymerizable, functional monomers, capable of non-covalent interactions

with the template molecule, are mixed in organic solutions with the template and allowed to associate. The virus MIPs must function in aqueous environments. Our approach employs a more flexible non-covalent imprinting method, starting from a readily available polyamine polymer, and both MIP synthesis and testing are performed in aqueous solutions. The development of general aqueous methods using MIPs capable of specific recognition of biological analytes would have an enormous value in medicine and bioanalytics.

Recombinant baculovirus has become a widely used vehicle for transfer of genes into insect cell lines, and is used as a model virus in this work. The most widely used baculovirus is a nuclear polyhedrosis viruses (NPV). NPV are enveloped in a large protein crystal or occlusion body. The occlusion body is dissolved in the host insect, releasing viral DNA which is absorbed in the midgut. Baculovirus used in a protein expression vector system that has advantages in that it is non-infectious to vertebrates, produces proteins under a powerful very late promoter, has a high efficiency of infection, is constructed relatively simply using commercially available kits, and has a unique, well-characterized structure. Variant viruses, including non-occluded but infectious forms are easily constructed offering a variety of virus forms for imprinting studies.

The experimental results presented deal with non-covalent molecular imprinting of poly(allylamine hydrochloride), (PAA•HCl) to produce molecularly imprinted polymer hydrogels (MIP) imprinted against baculovirus. The chosen polymer matrix, poly(allylamine) has good water solubility and presents a high density of amine groups. In its cross-linked form, this polymer possesses low toxicity and is biocompatible [1,2]. It has also been recently employed as a matrix for the molecular imprinting of glucose [3,4].

EXPERIMENTAL DETAILS

Experiments were conducted using a model virus as a template, vPH-GFP [5,6]. This virus utilizes the polyhedrin promoter in a PolIH- background to synthesize green fluorescent protein in infected insect cells [6] and insect larvae [7,8]. The molecular imprinting technique was used to create specific binding sites for baculovirus in crosslinked network polymers. Poly (allylamine hydrochloride) (PAA•HCl) was crosslinked in the presence of the baculovirus template molecule. The template was removed by washing the hydrogel with a base, to create cavities of specific shape size and functionality, which are able to rebind the baculovirus. The randomly crosslinked PAA•HCl networks were prepared by the aqueous reaction of a 25 % w/v solution of linear PAA•HCl chains and epichlorohydrin (EPI), which served as the crosslinker. The PAA•HCl used in the synthesis of the gels had an average molecular weight of 70,000 g/mole. Before imprinting the polymer with baculovirus, a portion of the HCl groups of PAA•HCl were neutralized with NaOH. This was done not only to provide free amine sites for crosslinking, but also to raise the pH of the polymer to the appropriate range for the baculovirus, since the baculovirus collapses at a pH higher than 8.5. Therefore, the pH of the solution before imprinting was adjusted between 6.5 and 8.5. After imprinting the polymer with baculovirus, EPI was added to crosslink the neutralized amine sites.

The following procedure was used to demonstrate successful biomimetic recognition of baculovirus imprinted polymer gels: Ordinary disposable syringes (3 mL) were packed with approximately 1 mg glass wool (to retain contents) and 0.2 g of the appropriate hydrogel

(baculovirus-imprinted or non-imprinted). As a control, one syringe was packed only with glass wool. After packing, syringes were washed three times with a 70 % ethanol solution. 6 mL sterile media (SF900-II™ serum free media (SFM) Life Technologies, San Francisco) was used to wash each column following sterilization. Recombinant baculovirus was obtained as described in [5]. 1.5 mL of baculovirus stock solution (6.89×10^8 pfu / mL) was passed through each column 10 times and the effluent collected. Baculovirus stock dilutions of 10^6 , 10^5 , 10^4 , and 10^3 pfu / mL were prepared from 6.89×10^8 pfu / mL column-washed stock solution in SFM. At the end of the procedure, four dilutions of baculovirus-imprinted, non-imprinted, and non-hydrogel column-washed solutions were prepared.

Cell infection and infection scoring *Spodoptera frugiperda* 9 (*Sf9*) cells were cultured to a final density of 5×10^6 cells / mL in SFM. Cells were inoculated with baculovirus dilutions in 96-well plates (10 μ L virus dilution into 100 μ L cells). Each dilution of virus was inoculated into 36 wells. Infection frequency (green fluorescent protein expression) was scored using a stereomicroscope with a fluorescent filter (510 nm) (Leica, Germany) and the protocol of Cha et al [7].

RESULTS

Figure 2 was generated using a standard baculovirus titer procedure [7]. One treatment (virus-imprinted polymer) and two control solutions (non-imprinted polymer and no polymer at all) were examined for their ability to infect *Sf9* cells. That is, 2 mL of 6.89×10^6 pfu/mL stock was passed repeatedly through 0.2 grams of each polymer or through no polymer at all. The percentage infection frequency reflects how many wells out of twelve were infected for each dilution. Experiments were performed in triplicate (graph includes average and standard deviation). None of the samples washed through virus-imprinted polymers were able to infect *Sf9* cells, suggesting complete retention of baculovirus in the imprinted polymer. Note, however, this is preliminary data, suggesting potential for this work, but does not preclude the many other interpretations of this result (eg., deactivation of virus in imprinted polymer - although identical procedures were used) in the absence of additional controls and independent measurement of virus quantity. A key issue in MIPs is the conformational relaxation of the binding pocket that is left exposed when the template is dissociated from the matrix polymer. Although it is expected that the imprinted cavities will be distorted due to the swelling of the hydrogel in water, the experimental results show that even the swollen gels show remarkable biomimetic recognition for BV-GFP.

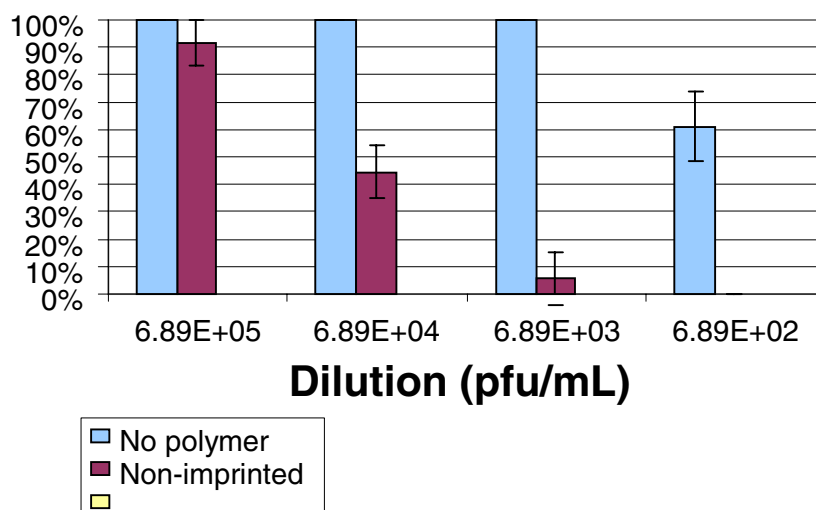


Figure 2. Infectivity data for GFP baculovirus

It is important to highlight that the preliminary results which show baculovirus recognition and binding were imprinted using virus suspended in serum-free cell culture media (where from it was originally synthesized from Sf-9 insect cells). Additional biomimetic recognition experiments will be performed in other medias to examine the limits of MIP/virus recognition (eg., w/ w/o 5% serum, added glucose, added surfactants such as Triton or lipids). Radiolabeled or fluorescently labeled virus will be also used, as needed, to provide an unambiguous characterization of the virus-MIP interaction.

CONCLUSIONS

The technique presented here not only employs the more flexible non-covalent approach to imprinting, but also begins with a polymer having an appropriate functionality, instead of functional monomer, and is performed in aqueous solution under air. Our experiments have shown that the proposed MIP gels have overcome the main problem of recognition in a water-swollen state, i.e. the influence of flexibility of the polymer sequence between two crosslinking points. It is clear that the molecular imprinting procedure has produced cavities in a water-swollen state with an affinity for the recombinant baculovirus imprint. Note, however, this is preliminary data, suggesting potential for this work, but does not preclude the many other interpretations of the imprinting result (eg., deactivation of virus in imprinted polymer - although identical procedures were used) in the absence of additional controls and independent measurement of virus quantity.

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