Selective Recognition of Angiotensin II

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Summary

The overexpression of several peptides and proteins in the body often lead to catastrophic physiological conditions. Ultimately it would be beneficial to be able to reduce the circulation of these peptides. One possible method of achieving this is by creating systems that use synthetic biomaterials to mimic natural biological recognition processes. By using synthetic biomaterials, one can design the next generation of materials for therapeutic and diagnostic devices by overcoming barriers posed by natural macromolecules and ligands, which are expensive and relatively unstable. These recognitive polymer systems can be fabricated with molecular architectures possessing specific chemical moieties that provide a framework for selective recognition of a target analyte in aqueous environments. A particular peptide that would benefit from a system such as this is angiotensin II. This is an octapeptide hormone which has been implicated in arterial fibrosis when present in increased levels. This paper reports on a novel recognitive system that uses synthetic biomaterials in order to recognize and capture the undesirable anaylte. Our method uses configurational biomimesis which produces polymeric surfaces or polymeric recognitive networks that have three dimensional, stereospecific binding micro- and nanocavities based on the given template molecule angiotensin II. To achieve destruction of the overexpressed peptide for further therapeutic effects, we have incorporated biodegradable components into the polymer backbone which creates an acidic microenvironment upon hydrolytic cleavage at ester bonds. This microenvironment will therefore create suitable conditions to destroy the peptide.

Introduction

In creating a synthetic system that mimics naturally occurring recognition processes it is important to understand how the network structure behaves in physiological conditions. The performance of the configurationally imprinted biomimetic network networks ((CIBP) depends largely on the composition and spatial orientation of the bulk structure. Therefore it is beneficial to investigate and characterize the structure, and functionality of the networks. Examining details such as the macro-porous, micro-porous and non-porous regions of the network, the polymer's free functionalities and its physical response in solution will greatly aid in understanding the performance and will ultimately help in optimizing the system. Investigating the functional group conversion, the crystalline structure and the overall heterogeneity of the network will give insight to the polymers' biocompatibility and long term stability.

Several methods can be used to evaluate the polymer's structure, function and physical response in solution. Namely the polymers should be analyzed by SEM, FTIR and DSC. Finally, the suitability of hydrogels as biomedical materials and their performance in a particular application depend to a large extent on its bulk structure. Therefore the porosity of the polymer networks should be evaluated. This can be done by calculating the number-average molecular weight between crosslinks $\overline{M}_c$, network mesh

size ξ crosslinking density ρ and equilibrium weight swelling ratio q by standard techniques developed in our laboratory. In the case of the CIBPs it is important to show that the functional monomer functionalities are present in the final polymer network. This information can be used to understand and optimize binding sites and overall recognition.

In imprinted networks, FTIR has been used to determine the nature of the template-monomer complex for small molecular weight drugs and for determining the change in composition of polymers that are templated FTIR spectroscopy also has the potential to show the difference between imprinted polymer networks without the presence of template and the networks that contain bound molecules. FTIR uses the intrinsic properties of covalent bonds and their thermal energy vibrations to provide useful information about the structure and function of polymers. The molecular vibrations contain characteristic quantized vibrational energy levels that can absorb infrared radiation with energies that correspond to vibrational energy level transitions. The bonds of functional groups such as carboxyl and amine groups absorb infrared radiation at characteristic energies and can be easily identified. The most significant portion of the FITR is the frequency range from 1200 cm^{-1} and 600 cm^{-1}. This is known as the fingerprint region of the IR spectrum because small changes in the structure of the molecule can significantly impact the location and shape of the absorption peaks in this range. By identifying characteristic peaks in this range, the spectrograms can provide powerful insight into the overall chemical compounds present in a particular sample.

Results

Polymers were prepared for SEM analysis by cutting swollen, wet polymers into disks with a 12mm diameter. One corner of the polymer was then sectioned at a 45° angle. This was done in order to image the morphology of the inside of the polymers and not the surface layer which is smoothed during polymerization between two glass plates. The disks were then dried in a vacuum oven at a temperature of 35°C for two days. The disks were then placed on an SEM stage and sputter coated with a AU/Pd target for 30sec. The coating was approximately 22nm thick. The samples were then analyzed by SEM (Hitachi S-4500 field emission scanning electron microscope) at 10kV (see Figures 1 and 2)

The polymers were prepared for spectroscopic analysis by crushing the dry polymer films to particle sizes ranging from 75-150 μm. 1mg of crushed polymer was then placed with 150mg of dry KBr (Sigma-Aldrich, St. Louis Missouri). This mixture was then compressed into a pellet. A KBr pellet that did not contain polymer was also made as a control. The pellets were then run through a FTIR spectrophotometer (Infinity Gold Series Spectrometer, Mattson, Wisconsin). The final spectra were obtained using 128 scans with a resolution of 1 cm^{-1}. FTIR spectroscopy was run on both the imprinted polymers and the nonimprinted controls (see Fig 4).

The DSC was used to run kinetic studies and determine the Tg's of the recognitive polymers. All of the kinetic studies were run using the TA 90 differential scanning calorimeter as a miniature reactor (TA instruments, New Castle, Delaware). First, approximately 4.5 to 6.0 mg of the monomer sample was weighed using a digital scale and pipetted into a small aluminum pan using a volumetric pipette. The volume of sample added was approximately 3 μL. The sample pan was then sealed with a piece of polyethylene and placed into the calorimeter. Before the monomer was initiated, the

atmosphere was made inert by purging the system with nitrogen gas at a pressure of 20 psia for twenty minutes. Then, UV light with an intensity of 1.0 mW/cm^2 was allowed to shine on the system for 699 seconds. The heat produced by the reaction with reference to the empty pan was collected over the period of time. Three data points were collected for each second of passing time. (see Fig 3)

For the T_g experiments approximately 5 mg of polymer sample was placed into an aluminum pan. The pan was then sealed and crimped with an aluminum lid. The pan was placed into the calorimeter and a reference pan was placed beside it. The atmosphere was then made inert by purging with nitrogen gas. The DSC scans were started at 30°C and increased to 120°C at a rate of 10°C/min.

All DSC scans were run using four different formulations of polymers which were previously determined to show the best recognition. The four different formulations were divided into two sets of polymers, poly(ethylene glycol)-g-acrylamide (PA) and poly(ethylene glycol)-g-acrylamide-g-methacrylic acid (PAM). Within each set of two polymers, an imprinted polymer which contained Angiotensin II as a template and a control polymer which did not contain the template molecule were made. The polymers all contained a crosslinker percentage of 40% and a template to functional monomer ratio of 1:8.

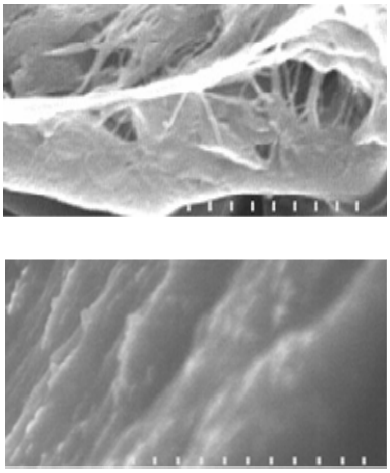

Figure 1: SEM analysis of 1:8 imprinted polymer with 40% cross-linker (top) and control non-imprinted polymer (bottom). Scale bar = 3.00•m

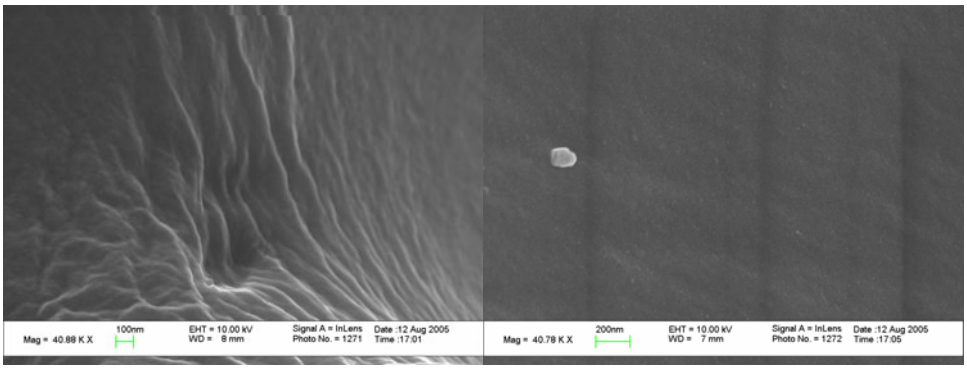

Figure 2: SEM analysis of 1:8 imprinted polymer with 40% cross-linker (top) and control non-imprinted polymer (right).

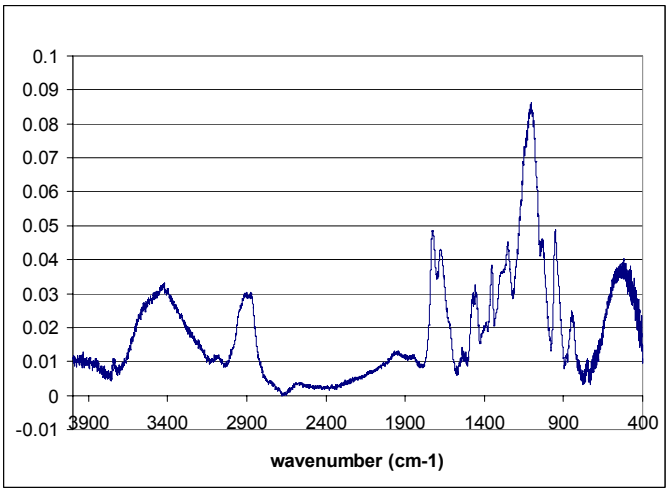

Figure 3: FTIR spectra of CIBP with a 1:8 ratio and a 20%crosslinker percentage

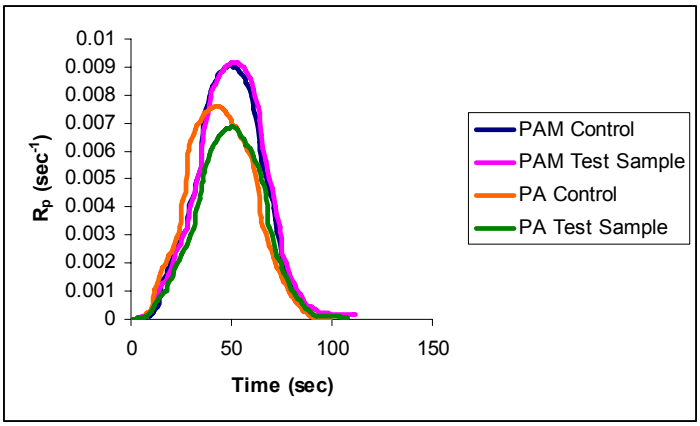

Figure 4: Rates of Polymerization of the Four Samples versus Time