Biomimetic, Molecularly Imprinted Hydrogels for Recognition and Capture of High Molecular Weight Proteins

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ABSTRACT

A novel molecularly imprinted (MIP) hydrogel consisting of methacrylic acid (MAA), 2-dimethylaminoethyl methacrylamide (DMAEM), and acrylamide (Aam) were fabricated using free radical polymerization in the presence of the template molecule lysozyme. Kinetic binding studies revealed a rapid recognition of template molecule where 95% of lysozyme was bound after 20 min. The imprinting efficiency of these matrices also exhibited 21% more binding of lysozyme after 20 min compared to non-imprinted control polymer. To study the specificity of the MIP hydrogel formulation to lysozyme, MIP polymers were placed into solution with similarly sized molecules pepsin and soybean trypsin inhibitor. These binding studies indicated less than 2% binding of these molecules to MIP polymers and validated our MIP process. These novel polymers have the potential as tissue engineering scaffolds and drug delivery devices.

Introduction

Although molecularly imprinted materials have shown promise for the recognition of small molecular weight molecules, the development of these materials for the recognition of macromolecules such as high molecular weight proteins or even whole cells has been sparse. Problems existing in the imprinting of such molecules include low solubility in organic solvents, sensitivity to polymerization conditions, and low percentage of template extraction due to diffusional limitations. Nonetheless, a biomaterial that can recognize these macromolecules would be advantageous in areas ranging from separations research to biological assays and even as a tissue engineering scaffold to recognize free blood proteins.

In recent years, molecularly imprinted polymer systems have come to the forefront of biomedical applications. First used in chromatography columns for the separation of specific small molecular weight compounds (analytes), imprinted polymers now have applications ranging from molecular sensors to water purification. MIP involves forming a pre-polymerization complex between the template molecule and functional monomers with specific chemical structures designed to interact with the template either by covalent chemistry, non-covalent chemistry (self-assembly), or both. Once the pre-polymerization complex is formed, the polymerization reaction occurs by free radical initiation in the presence of a crosslinking monomer and an appropriate solvent. When the template is removed, the product is a porous matrix with specific recognition elements for the template molecule. Thus, MIP creates stereo-specific three-dimensional binding cavities based on the template molecule of interest. The resulting polymer networks can then specifically recognize the template molecule in solution even when in a mixture of other similar molecules.

Materials and Methods

Synthesis of MIP Lysozyme Polymers

All materials were purchased from Sigma-Aldrich (St. Louis) unless otherwise indicated. MAA was vacuum distilled for eight hours to remove hydroquinone inhibitor. All other

chemicals were used as received. Hydrogel matrices were prepared via free radical polymerization in the presence of lysozyme. Functional monomers AAm, MAA, and DMAEM were investigated to determine their efficiency on imprinting the template. In a typical synthesis, .575 mol of each monomer was dissolved in Tris buffer (0.020M, pH 7.4) to obtain a 30 wt% monomer solution. To insure proper folding of the protein and simulate physiological pH, the pH of the resulting solution was adjusted to ~7.4 using concentrated NaOH. Lysozyme powder was then added (10 wt% monomers) and agitated for 5 min to allow dissolution of the protein. N,N'-methylenebisacrylamide (MBA) as crosslinker was added at 10 wt% of monomer and the resulting solution was gently agitated for 20 min to allow for the formation of the polymer-template complex. The photoinitiator 2,2-dimethoxy-2-phenylacetophone (DMPA) was dissolved at 1 wt% of monomers, and the resulting solution was pipetted into a mold consisting of two glass slides separated by a .7mm Teflon spacer. Exposure of molds to UV light (Dymax UV Light Source, New Jersey) at 23 mW/cm^2 for 30 min initiated a free radical polymerization of the polymer. Control films were made in the same method excluding protein addition.

Batch Recognition Studies

To determine if lysozyme was successfully imprinted into our matrices, 30 mg of polymer was placed in 50 mL conical tubes and 10 mL of Tris buffer (0.020 M, pH 7.4) was added and then placed on a rotating mixer at 25 rpm. Polymers were allowed to swell and equilibrate for 20 min. Then, 10 mL of a 0.3 mg/mL solution of lysozyme in Tris buffer was added to the particles to give a final lysozyme concentration of .15 mg/mL and a final particle concentration of 1.5 mg/mL. At 20 min. supernatant was removed and analyzed for lysozyme concentration using UV-Vis spectroscopy at 285 nm.

Specific Recognition Studies

To establish the specificity of our imprinted network, similarly sized molecular weight proteins pepsin or soybean trypsin inhibitor were substituted for lysozyme and recognition was determined according to the batch recognition study protocol described previously.

Results and Discussion

Recognition studies of certain hydrogel formulations revealed a higher level of template binding compared to non-imprinted control microparticles (Figure 1). In addition, when MIP particles were placed into solution with similarly sized molecules, pepsin and soybean trypsin inhibitor, the binding of these two proteins was less than 1% for the MIP polymers while the imprinted polymer bound over 90% of the lysozyme over 20 min. These results give convincing proof of the specificity of our technique to the lysozyme template. However, control polymers yielded less than 2% binding to either pepsin or trypsin inhibitor but showed a high binding affinity toward lysozyme. This result could be contributed to the differences in the total net charge that each protein carries. Lysozyme carries a net positive charge of +7 at neutral pH. This positive charge can lead to a greater affinity for complexation with the negatively charged MAA functional monomer and it is this complexation that could be driving not only the specific binding of lysozyme to MIP polymers, but also the binding of lysozyme to non-imprinted controls. In the same way, both pepsin and soybean trypsin inhibitor carry a net negative charge at neutral pH (-4 and -6 respectively) which could lead to electrostatic repulsion between the negatively charged carboxylic acid group of the methacrylic acid and lead to the low binding. If this explanation is correct, the results indicate that the imprinting technique is not based upon size but is based upon a specific polymer-template complex that

involves a balance of positive and negative charges and hydrogen bonding. However, these results are promising toward our end goal of an imprinted system that can quickly recognize and bind free-blood proteins either in biomedical applications such as assays. We believe the non-specific binding of the lysozyme to the control polymer is due to the presence of functional groups that are randomly polymerized throughout the polymer and can attract and bind free template over time. Future studies will determine if lowering the concentration of functional monomers or increasing the percent crosslinker added will lead to better recognition of lysozyme compared to controls.

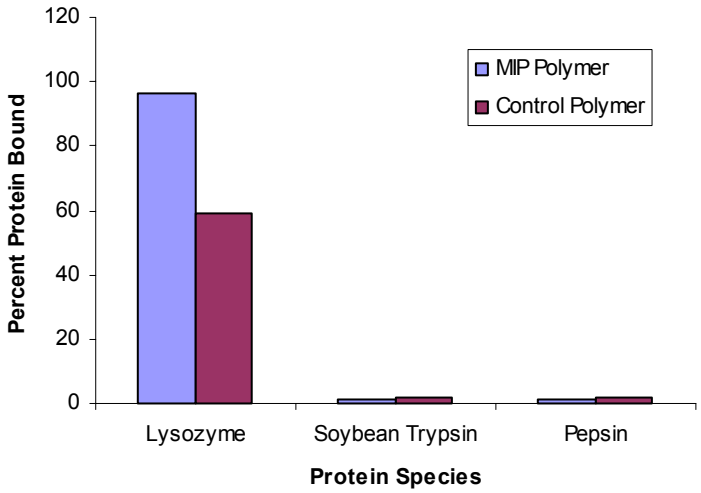

Figure 1: *Recognition studies of MIP polymers:* MIP polymers exhibited higher recognition over control polymers and exhibited little recognition to similar sized molecules.

Conclusions

Due to the specific recognition of lysozyme to our MIP polymers, we feel that these results are promising toward our end goal of an imprinted system that can quickly recognize and bind free-blood proteins in biomedical applications such as assays. We believe the non-specific binding of the lysozyme to the control polymer is due to the presence of functional groups that are randomly polymerized throughout the polymer and can attract and bind free template over time. Future studies will determine if lowering the concentration of functional monomers or increasing the percent crosslinker added will lead to better recognition of lysozyme compared to controls.

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