Preferential Protein Binding to Peptide-Imprinted Polysiloxane Scaffolds

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Introduction

Molecularly imprinted polymers have the ability to rebind specific molecules. During polymerization, a template molecule is mixed with monomers having complementary functional groups and a crosslinking agent. After the polymer hardens, the template is removed, leaving behind a spatial imprint of its physical shape and surface chemical composition.

Most research on molecular imprinting has logically involved use of whole molecules as templates. Imprinting of large biomolecules, such as proteins, however, is complicated by the size, large number of functional groups, and flexibility of the molecules. Analogous to the way antibodies recognize only a certain portion (the epitope) of an antigen, it may be possible for molecularly imprinted polymers to bind a particular protein based on imprinting of a shorter peptide portion of the molecule.

The objective of this study was to determine if a protein (lysozyme, 14kDa) can preferentially bind to a polysiloxane scaffold imprinted with a 16 residue peptide (lysozyme C, 1.8 kDa).

Materials and Methods

Fabrication of scaffolds by sol-gel processing

Sol-gel techniques were used to prepare porous polysiloxane (silica) scaffolds imprinted with lysozyme C. A solution containing 36.5% tetraethoxysilane, 9.1% 0.1M HCl, 6.5% deionized water, and 11.1% ethanol was shaken for 24 hours. A second solution containing 165µL γ-aminopropyltriethoxysilane and 500µL 0.1M sodiumdocecylsulfate was left at room temperature for 30 minutes without shaking. Fluorescently labeled lysozyme C (0.1 mg/scaffold) and the first solution were added to the second solution and vigorously mixed for 10 seconds. Nonimprinted (blank) scaffolds were formed the same way but without protein. The gels were aged for 24 hours and then dried at 40ºC for 48 hours.

Peptide Loading

To determine peptide loading, imprinted scaffolds were treated with 0.4mg/mL protease in 0.1M carb-bicarb buffer (pH 8.5). The amount of labeled peptide digested was measured using a fluorometric plate reader.

Protein Preferential Binding

To determine if lysozyme preferentially binds to peptide-imprinted scaffolds, a competitor of similar molecular weight (RNase, 13.7 kDa) was also tested. Proteins were fluorescently labeled to enable quantification. In addition to allowing lysozyme and RNase to bind individually to the substrates, a solution containing proteins at a ratio of 1:1 was also used. Following digestion of the template peptide, proteins were left to bind for 24 hours. After digestion with protease, fluorescence in the supernatant was measured.

Results and Discussion

Peptide Capacity

Figure 1 shows results from the loading experiment. Most of the peptide that could be released was removed by 24 hours. About 30% (27µg) of the amount added was released from the scaffold. This amount was the basis for the number of potential binding sites for lysozyme.

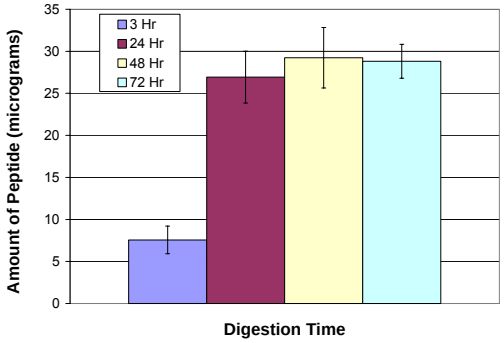

Figure 1. Amount of surface-accessible peptide.

Protein Preferential Binding

Figure 2 shows results from the protein binding experiments. Data are corrected for binding to blank (nonimprinted) scaffolds. Approximately 21µg more lysozyme bound to imprinted scaffolds compared to blank (nonimprinted) scaffolds. In contrast, RNase bound equally to imprinted and nonimprinted scaffolds. When both proteins were present in the solution, nearly 5µg lysozyme bound, but no RNase was measured. The preferential binding of lysozyme to peptide-imprinted materials follows a trend similar to that seen for scaffolds imprinted with the whole lysozyme molecule.

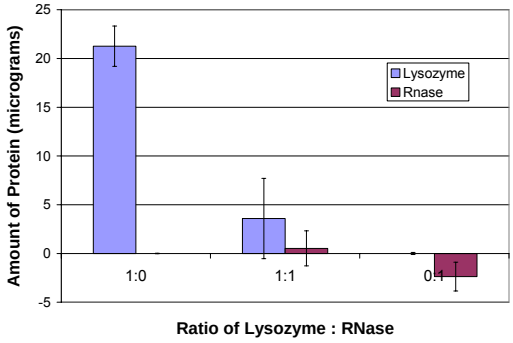

Figure 2. Binding of lysozyme and RNase to peptide-imprinted materials.

Conclusion

Initial results indicate promise for developing scaffolds that preferentially bind particular proteins after imprinting with only a portion of the whole molecule.

Acknowledgment

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