Molecular imprinted nanoPolymer nanomaterials: application in biomolecule recognition

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ABSTRACT

Molecular imprinting has proved to be an effective technique for generating specific recognition sites in synthetic nanopolymers. These sites are tailor-made in situ by copolymerization of functional monomers and cross-linked around the template molecules. The print molecules are subsequently extracted from the polymer, leaving accessible complementary binding sites in the polymer network. Despite significant growth within the field, the majority of template molecules studied thus far are low molecular weight compounds and generally insoluble in aqueous systems. In biological systems, molecular recognition occurs in aqueous media. So, in order to create molecular imprinted polymers capable of mimicking biological processes, it is necessary to synthesize artificial receptors which can selectively recognize the target biological macromolecules such as peptides and proteins in aqueous media. Actually, the synthesis of molecular imprinted polymers specific for biomacromolecules has been a focus for many scientists working in the area of molecular recognition, since the creation of synthetic polymers that can specifically recognize biomacromolecules is a very challenging but potentially extremely rewarding work. The resulting molecular imprinted polymers with specificity for biological macromolecules have considerable potential for applications in the areas of solid phase extraction, catalysis, medicine, clinical analysis, drug delivery, environmental monitoring, and sensors. In this report, the authors discuss the challenges associated with the imprinting of peptides and proteins, and provide an overview of the significant progress achieved within this field. This report offers a comparative analysis of different approaches developed, focusing on their relative advantages and disadvantages, highlighting trends and possible future directions. *Keywords: molecular imprinting, nanopolymers, bionanomolecules, molecular recognition, synthetic receptor*

1 INTRODUCTION

The selective recognition of biologically molecules governs many essential biological interactions. Tailor-made artificial receptors bind with target molecules is known as 'molecular imprinting' or recognized. Molecular imprinting technology has scope of synthetic receptors with binding constants comparable to natural receptors. In molecular imprinting, host-guest complex is formed between a template molecule and one or more functional monomers in an appropriate solvent to make highly cross-linked polymer matrix encapsulating the template molecule. Peptides and proteins are used in creating molecular imprinted polymer targeting these biomacromolecules.

2 MOLECULAR IMPRINTING IN AQUEOUS MEDIA: NOTABLE EXAMPLES

Chitosan in /polymacrylamide in aqueous solution shows $-NH_3^+$ and the hydroxyl groups of chitosan interact and their hydrogen bonding play role in main recognition mechanism. Hemoglobin imprinted polymers onto porous chitosan beads by physically entrapping or chemically grafting of selective polyacrylamide gels. Acrylated β-cyclodextrin was copolymerized with 2-acryoylamido-2,2′ -dimethylpropane sulfonic acid. β-cyclodextrin; tryptophan template imprinting shown in Figure 1.

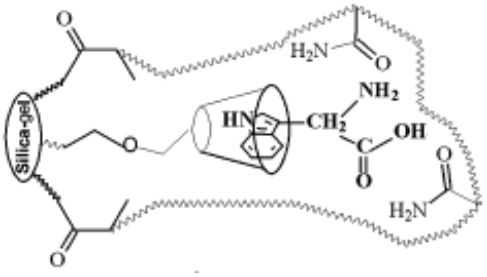

Figure 1. Schematic representation of tryptophan template imprinting.

N-methacryloyl- (L)-histidine-copper(II) form complex with lysozyme template; imprinted cryogel; *N*-Methacryloyl-(L)-histidinemethylester shown in Figure 2. The molecular imprinting is influenced by temperature, pH, and the ionic concentration on the status of biomacromolecules and rebinding of template molecules, covalent immobilization of template proteins shown in Figure 3. The template shape and conformation in a pre-polymerization mixture for protein imprinting provides a suitable alternative for protein imprinting. Fluorescein isothiocyanate derivative-albumin as the protein template molecule in an aqueous phase molecular imprinted polymer was reported the use of a fluorescently labeled template to contribute new understanding of aqueous phase molecular imprinting protocols in situ imaging of real-time protein denaturation events.

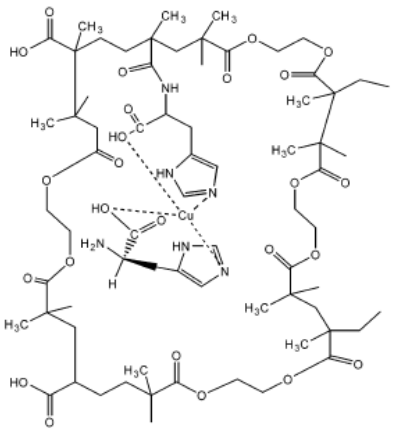

Figure 2. Schematic representation of L-histidine template imprinting.

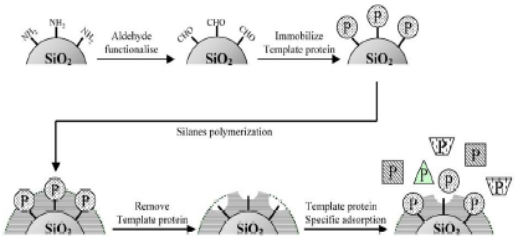

Figure 3. Schematic representation of protein imprinting using immobilized templates.

3 SURFACE IMPRINTING:RECEPTORS

Molecular imprinting has been considered one of the most promising techniques for the preparation of synthetic receptors. In spite of the ease of the conventional imprinting methodology and its associated success with the imprinting of small molecules, the approach has its limititation for the imprinting of protein macromolecules. The major problem during templating of large molecules is the poor efficiency of specific binding due to the multitude of functional sites at these molecules, as well as the limited diffusion of the template molecules into and out of an imprinted polymer network and the incompatibility of the fragile protein template with the imprinting conditions. Confining the recognition sites of molecular imprinted polymers onto the surface of matrix is a technique which has been developed to circumvent the problems associated with the imprinting of biological macromolecules in aqueous solution [1].

Surface imprinting improves the performance of molecular imprinting polymers. Calcium phosphate/alginate hybrid polymer microspheres were prepared with bovine serum albumin embedded and coating on the surface. Organic/inorganic hybrid thin films for protein recognition by the liquid-phase deposition (LPD) are shown in Figure 4.

protein–disaccharide complexes, protein recognition at the quartz crystal microbalance chip surface by surface imprinting is state of art. The outer surface of film is attached to a glass substrate by adhesion facilitating detachment of the mica substrate for selective recognition for a variety of template proteins, including albumin, immunoglobulin G, lysozyme, ribonuclease and streptavidin by homogeneous molecular imprinted polymer films shown in Figure 5.

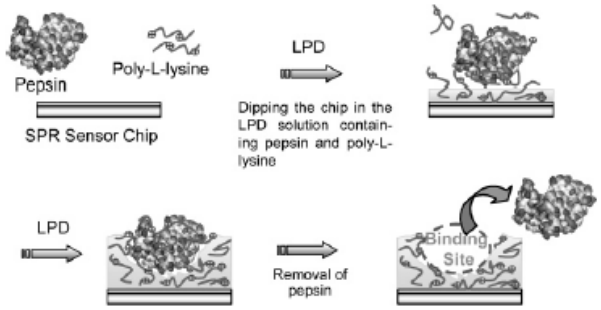

Figure 4. Schematic illustration of protein-templated LPD.

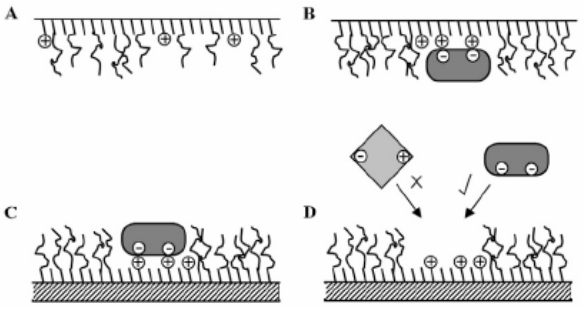

Figure 5. Schematic of protein imprinting in Langmuir monolayers. The native monolayer at the air/water interface (A) interacts with the protein to form imprints (B) that are complementary in shape and charge to the protein. The monolayer is locked into place when transferred to a hydrophobic support (C), followed by removal of the template protein and rebinding studies (D).

4 EPITOPE APPROACH: A SMALL STRUCTURAL ELEMENT FOR THE WHOLE MOLECULE RECOGNITION

An epitope is small active site located within the larger protein structure on an antigen, which combines with the antigen-binding site on an antibody or lymphocyte receptor. A short peptide sequence at the protein surface is used as a template for molecular imprinting polymers preparation. Once the matrix has been polymerized the resultant imprinted material recognizes and bind the whole protein. So, peptide acts as template to recognize its spatial and chemical entity in the polymer such as [Sar1,Ala8]angiotensin II, DNA-imprinted polymers (2-vinyl-4,6-diamino-1,3,5-triazine, hydrogen bond acceptors of A-T base pair), lysozyme, 14kDa peptide.

5 MECHANISM OF THE MOLECULAR IMPRINTING AND RECOGNITION

Molecular imprinting involves polymerizable functional monomers around a template, followed by polymerization and template removal. The influencing factors are physical and chemical nature of the monomers and the interactions between them, the polymerization and its affect on the porous structure, and the rebinding ability of the imprinted cavities.

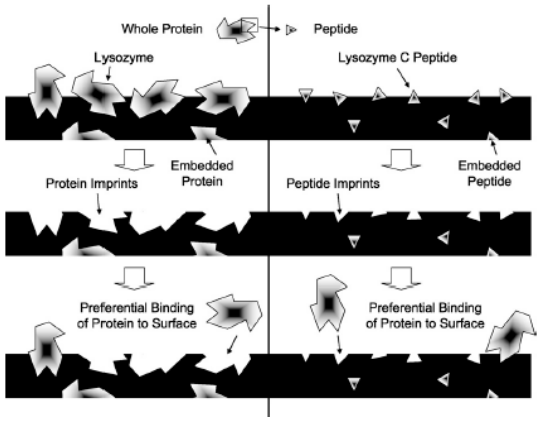

Figure 6. Presentation of whole protein imprinting (traditional approach) and peptide imprinting (epitope approach). On the left, the entire protein molecule creates large imprints with high surface availability. On the right, a small polypeptide sequence from the protein creates small imprints that bind the template molecule at only a specific location.

The binding process may be represented as the following reaction:

$$P + \sum_{i=1}^{n} T_i \rightarrow P - T_k \; + \sum_{i \neq k} T_i$$

Polymer P specifically recognizes the target T_k from a mixture of molecules T_i (i=1, n) and forms a polymer/target complex.

Interactions between the template molecule and the functional monomer, molecular imprinting technology can be covalent and non-covalent approach. In order to combine the superiority of both covalently and non-covalently imprinting approach, hybrid techniques are emerging named as semi-covalent and sacrificial-spacer methods. These approaches exploit covalent template-monomer complexes in the imprinting step, but entirely non-covalent interactions for rebinding. For example, Whitcombe et al. prepared a cholesterol imprinted polymers using a carbonate derivative as sacrificial linker motif [2].

Molecular imprinted polymer receptor, the microenvironment like buffer surrounding the binding site can have a important role in determining how effective the molecular imprinted polymer will be in recognizing the target molecule. Ionic strength of the binding solution, not the buffer composition itself, plays a important role in determining the effectiveness of the molecular imprinted polymers. Inhibition of binding capacity is most likely can result from the change of macromolecule conformation or the hindrance of template-receptor interaction caused by the high salt concentration. Variation of buffer composition and ionic strength both show a large effect on the binding systems, even when ionic interaction is not the dominant recognition factor. Complex protein conformations relying on the environment, further complicate quantitative comparison between molecular imprinted polymers. The high template/functional monomer concentration during preparation affects a larger number of available binding sites. Optimum distance between functional groups on the synthetic receptor favors a maximal binding capacity. Study of lysozyme imprinted silica beads indicated that the amount of lysozyme adsorbed onto the beads depends on the composition of functional monomers used during synthesis of the particles. The zeta-potential of lysozyme molecules and the imprinted silica beads (observed. Zeta potential matching) may be an important factor in the design of synthetic receptors.

Type and concentration of the cross-linking agent is also a critical factor in creating synthetic receptors with high affinity for their target molecules. When synthesizing molecularly imprinted polymers, ethyleneglycol dimethacrylate has been the most commonly used cross linking agent. The effect of other cross linking agents on the recognition by molecular imprinted polymers is still foggy. It is notable that ethyleneglycol dimethacrylate is structurally related to polyethylene glycol, which has long been used to provide resistance to non-specific protein binding. Cross-linking agents in molecular imprinted polymers, the isothermal titration of monomers to protein templates, including lysozyme, ribonuclease A and myoglobin screened the functional monomer for molecular imprinted polymers. Four different cross-linking monomers, ethyleneglycol dimethacrylate, tetraethyleneglycol dimethacrylate, polyethylene glycol 400 dimethacrylate, and polyethyleneglycol 600 dimethacrylate synthesized protein imprinted polymers. The size of the protein template correlates with the optimal number of ethyleneglycol-repeat units in the cross-linking monomers. Comparing the poly(ethyleneglycol dimethacrylate) or poly(tetraethyleneglycol dimethacrylate) protein-imprinted polymers, it is found that the non-specific hydrophobic binding appears to be the cause of the high binding to short polyethylene glycol-repeating units, especially since non-imprinted films bind lysozyme equally well, if not better than, "imprinted" films. The effective protein binding and imprinting can be obtained with a cross-linking agent containing more ethylene glycol repeat units—even though poly(ethylene glycol) is often used to prevent protein binding. It may be that the greater flexibility of the longer cross linking agent is important in allowing the polymeric matrix to conform to the protein molecule. Microcalorimetry of the interaction between monomers and protein stamps can be analyzed to determine styrene monomer as a good candidate as functional monomer with tetraethyleneglycol

dimethacrylate to form poly(styrene-co-tetraethyleneglycol dimethacrylate) or lysozyme-imprinted polymers or poly(styrene*co*- polyethylene glycol 400 dimethacrylate) RNase A-imprinted polymers, or myoglobin, methyl methacrylate.

Removing target molecules from molecular imprinted polymers is a key factor in the rebinding capability of the receptor. The removal of template proteins is complicated process due to the high molecular weight of proteins and highly cross-linked matrix. Example: polyacrylamide incorporated with methacrylic acid and 2-(dimethylamino)ethyl methacrylate in imprinting of lysozyme. Some lead template removal methods, include the use of sodium dodecylsulphate:acetic acid and trypsin digest, for removing template bovine haemoglobin from a polyacrylamide imprinted hydrogel. Other method is 15%:15% of sodium dodecylsulphate:acetic acid for initial template removal, subsequent re-binding studies to reduce imprinting efficiency. Trypsin solutions also do template removal. Horseradish peroxidase imprinted polymers treatment with alkaline, neutral, or acidic solutions. Recently, the utilization of molecular modeling procedures and current state-of-the-art suggest selecting combinations of template, functional monomers, cross-linkers, and solvents providing the most stable complex in the pre-polymerization solution, selected for molecular dynamics simulations investigating the interaction and conformation of the pre-polymerization complex such as copolymerization of functional ligands (that interact with the imprint molecule) and backbone monomers. Post-crosslinking in the presence of the target molecule allows the imprinted copolymer gel to approach its free energy minimum and affinity to target molecule increases with increasing concentrations of post-crosslinking agent. Using mean-field theory, porosity of polymer networks in various imprinting approaches, suggests the cross-linking induced microphase separation accompanied by polycondensation.

Recently, new facts came out as following:

1.Imprinting efficiency (a measure of the degree of complexation) depends on the effect of size and functionality of the imprinting agent on the imprinting efficiency using a lattice model, concentrating on the effect of solvent-template-monomer interactions on the equilibrium population of monomer-template complexes.

2.The extent of template complexation at equilibrium is governed by the change in Gibbs free energy of formation for each mode of template-monomer interactions, generally resulting in a population of sites with various affinities for the imprinted molecule.

3.Neglecting template-template association, it was found that stronger template-monomer interactions resulted in higher functional complexes and thus higher imprinting efficiencies. However, the efficiency strongly depended on the preparation conditions.

4. Molecular dynamics simulations through a topological analysis of the imprinted network predict configuration before and after removal of the templates, the imprinting quality of cross-linked polymer networks.

5.Low qualities of the imprinted polymers can be attributed to the aggregation of the templates in the pre-polymerization solution, aggregation of the imprinting-induced sites with small pores inherent to the polymer, and deformation of the binding sites due to relaxation of the gel after removal of the templates.

6.The formation of distinct individual cavities that retained the size and shape of the template is enhanced by high degrees of cross-linking and low template concentrations.

7.All-atom kinetic gelation simulation technique offers a recognitive polymeric network formation which incorporates both intramolecular as well as intermolecular interactions and the subsequent effects they have on the end network structure.

6 CONCLUSION

Molecular imprinting is a valuable technique for the preparation of synthetic materials for the selective recognition of biologically relevant molecules such as amino acids, peptides, and proteins. The key factors for the advancement in molecular imprinted polymer research for biomacromolecular targets lie in the preparation of molecular imprinted polymers providing high affinity to the macromolecular template molecule without disrupting the bioreactivit or biofunctionality of the target analyte. However, despite the recent advancements made in the synthesis and characterization of molecular imprinted polymers, there is still an enormous amount of work that needs to be conducted in the field. The exact recognition mechanism, and the way in which the interactions cooperate to recognize target molecules in many molecular imprinted polymer systems is not clearly understood. In addition, many fundamental properties of the molecular imprinted polymers prepared, such as the dependence of the affinity on cross-linking agent concentration, functional monomer concentration, buffer composition, ionic strength, pH, and temperature, have not yet to be systematically studied. It is evident that the development of the molecular modeling and analytical techniques will enable the better understanding the effective molecular imprinted polymer-based receptors.
The art of mimicking biological processes, the study of molecular imprinted polymers capable of the selective recognition of peptides, proteins, and other biological macromolecules will continuously receive considerable attention in the next decades.

7 REFERENCES

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