Synthetic Peptide Receptors: Molecularly Imprinted Polymers for the Recognition of Peptides Using Peptide−Metal Interactions

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Molecular imprinting is a process for synthesizing organic polymers that contain recognition sites for small molecules.[1] The imprinting process consists of a template molecule that organizes functional and cross-linking polymerizable monomers during the polymerization process. The template is extracted from the insoluble network material leaving behind domains that are complementary in size, shape, and functional group orientation to the template molecule. We now report protocols for molecular imprinting that create macromolecular receptors for small peptides. Importantly, *both polymerization and recognition are carried out in an aqueous environment*. The peptide recognition sites have been achieved by incorporating two types of interactions. One consists of strong, specific binding between an *N*-terminal His residue and Ni(II) bound to the polymer.[2,3] This binding, which relies on a metal−ligand interaction, is not compromised by water or other protic solvents. The second bonding motif comprises multiple weaker interactions between the network polymer chains and the imprinting peptide molecule that are established during the polymerization.

Peptide−macromolecule interactions are ubiquitous in nature. Examples include sensory neuropeptides, such as enkphalins that play a role in signaling, peptide hormones (e.g. corticotropin, vasopressin), and peptide antibiotics (e.g., gramicidins).[4] A critical element of the above processes is the recognition of a specific peptide by a macromolecular receptor. The preparation of artificial binding sites for such peptides may provide insight into recognition processes. In addition these artificial receptors may facilitate the screening of peptide mixtures or assist in the evaluation of peptidomimetics that can be used to either enhance or inhibit receptor responses.

The preparation of molecularly imprinted polymers (MIPs) selective for amino acids and small peptides (up to a maximum of three residues) has been limited to traditional imprinting formulations such as polyacrylates with methacrylic acid as the functional monomer in organic solvents.[5] With few exceptions.[6,7] these systems have utilized protected amino acids and peptides. These formulations employ free radical polymerization and rely on the use of hydrogen-bonding interactions between the template and functional monomers as the selectivity-providing interaction.[8] Although protic solvents such as alcohols and water are compatible with free radical polymerization, these have been largely excluded from use in imprinting due their ability to compete with hydrogen-bonding interactions. However, as targets with more biological relevance such as peptides and oligonucleotides are identified, these traditional organic formulations are no longer effective or suitable. Here, practical matters such as lack of solubility of peptides in organic media and more subtle effects such as peptide conformation, make water the solvent of choice.

Metal ions have been used as templates in MIP systems. The resulting polymers exhibit selectivity for the template cations.[9] Polymer-bound metals have also served as the recognition element for imprinted polymers. MIPs based on coordination complexes have been utilized most notably as sensors and catalysts.[10] The investigation of coordination complexes in conjunction with imprinted polymers as artificial receptors has been limited, with a few notable exceptions.[7,11]

Our strategy for creating peptide receptors using molecular imprinting takes advantage of the affinity of *N*-terminal histidine residues for Ni(II). Hochule et al. introduced an adsorbent, based on a Ni(II)−nitrilotriacetic acid complex, for protein purification.[12] This ligand occupies four positions in the octahedral coordination sphere of Ni^{2+} leaving the remaining two for selective interactions. Histidine has been shown to bind to these complexes at the terminal amine and pyridine nitrogen of the imidazole ring with high affinities. We have developed a polymerizable acrylamide functionalized NTA ligand. By incorporating a Ni(II)−NTA complex into the polymer we have provided a "handle" to bind peptides containing *N*-terminal His residues in water. We find that the multiple interactions between the peptide and the polymer matrix that are developed during the polymerization are sufficient to provide sequence selectivity between the imprinted peptide and other amino acid sequences containing *N*-terminal His residues.

Figure 1 illustrates our strategy for preparing highly cross-linked polyacrylamides containing binding sites which incorporate a Ni(II)−NTA complex. The polymerizable methacrylamide−NTA−Ni^{2+} mixed complex was prepared by combining aqueous solutions of NTA monomer with $NiSO_4$. The pre-polymerization complex was then formed by addition of the *N*-terminal histidine peptide His-Ala. Copolymerization of this complex (5 mol %) with *N,N′*-ethylenebisacrylamide cross-linking monomer[13] (82 mol %) and acrylamide (13 mol %) provided a pale blue monolith. The polymer, which was formed in quantitative yield, was ground and washed with water (pH = 3−4) to remove the template. This was followed by a wash with methanol and drying. The polymer particles were dry-sieved, and those particles larger than 425 μm were used for binding.

The polymer-bound NTA−Ni(II) complex in the absence of peptide is pale green. Upon binding an *N*-terminal histidine peptide the color changes to pale blue. This change lends itself to a colorimetric assay of binding of peptides to these materials.

Binding studies were performed to evaluate uptake of the template and non-template peptides. Aqueous solutions of peptide were added to vials containing 20 mg of polymer. After equilibration, the concentration of unbound peptide was measured by

(1) (a) Wulff, G. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1812−1832. (b) Shea, K. J. *Trends Polym. Sci.* **1994**, *2*, 166−173. (c) Ramstrom, O.; Ansell, R. J. *Chirality* **1998**, *10*, 195−209. (d) Mosbach, K.; Ramstrom, O. *Bio/Technology* **1996**, *14*, 163−170.

(2) Anderegg, G. *Pure Appl. Chem.* **1982**, *54*, 2693−2758.

(3) Hopgood, D.; Angelici, R. J. *J. Am. Chem. Soc.* **1968**, *90*, 2508−2513.

(4) (a) Hokfelt, T.; Johansson, O.; Ljungdahl, A.; Lundberg, J. M.; Schultzberg, M. *Nature* **1980**, *284*, 515−521. (b) Hancock, R. E. W. *Lancet* **1997**, *349*, 418−422.

(5) (a) Ramstrom, O.; Nicholls, I. A.; Mosbach, K. *Tetrahedron Asymmetry* **1991**, *5*, 649−656. (b) Kempe, M. *Anal. Chem.* **1996**, *68*, 1948−1953. (c) Yu, C.; Mosbach, K. *J. Org. Chem.* **1997**, *62*, 4057−4064.

(6) Klein, J. U.; Whitcombe, M. J.; Mulholland, F.; Vulfson, E. N. *Angew. Chem., Int. Ed.* **1999**, *38*, 2057−2060.

(7) Sundaresan, V.; Ru, M.; Arnold, M. *J. Chromatogr.* **1997**, *775*, 51−63.

(8) (a) Spivak, D.; Gilmore, M. A.; Shea, K. J. *J. Am. Chem. Soc.* **1997**, *119*, 4388−4393. (b) Kempe, M.; Mosbach, K. *J. Chromatogr.* **1995**, *694*, 3−13.

(9) (a) Dai, S.; Burleigh, M. C.; Ju, Y. H.; Gao, H. J.; Lin, J. S.; Pennycook, S.; Barnes, C. E.; Xue, Z. L. *J. Am. Chem. Soc.* **2000**, *122,* 992−993. (b) Yu, K.; Tsukagoshi, K.; Maeda, M.; Takagi, M. *Anal. Sci.* **1992**, *8*, 701−703. (c) Kuchen, W.; Schram, J. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1695−1701.

(10) (a) Chen, G. H.; Guan, Z. B.; Chen, C. T.; Fu, L. T.; Sundaresan, V.; Arnold, F. H. *Nat. Biotechnol.* **1997**, *15*, 354−357. (b) Jenkins, A. L.; Uy, O. M.; Murray, G. M. *Anal. Chem.* **1999**, *71*, 373−378. (c) Polborn, K.; Severin, K. *Chem. Commun.* **1999**, 2481−2482.

(11) (a) Dhal, P. K.; Arnold, F. H. *Macromolecules* **1992**, *25*, 7051−7059. (b) Matsui, J.; Higashi, M.; Takeuchi, T. *J. Am. Chem. Soc.* **2000**, *122*, 5218−5219.

(12) Hochuli, E.; Dobeli, H.; Schacher, A. *J. Chromatogr.* **1987**, *411*, 177−184.

(13) Shea, K. J.; Stoddard, G. J.; Shavelle, D. M.; Wakui, F.; Choate, R. M. *Macromolecules* **1990**, *23*, 4497−4507.

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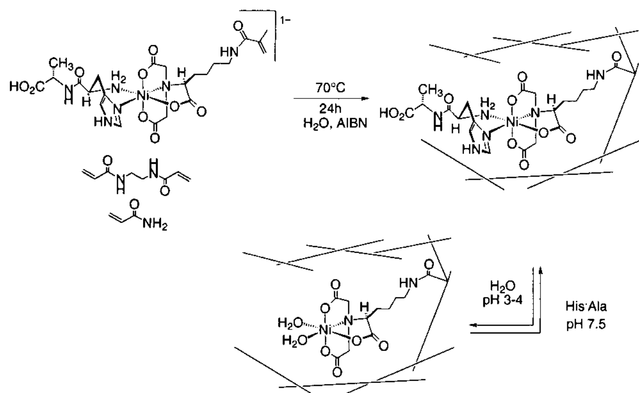

Figure 1. Schematic representation of the peptide imprinting process. Copolymerization of the (His-Ala)−Ni−NTA complex with mono- and bisacrylamides followed by extraction of the peptide at pH 3−4 provides a polymer containing Ni−NTA complexes capable of rebinding the template peptide.

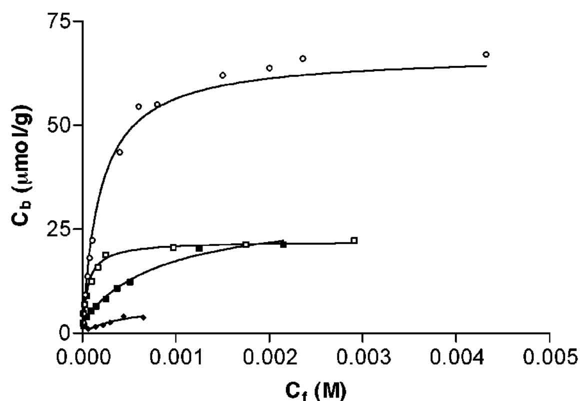

Figure 2. Binding isotherms for the rebinding peptides in aqueous solution to a polymer prepared using His-Ala as the template peptide. His-Ala (○), His-Phe (□), His-Ala-Phe (■), Ala-Phe (◆). A large increase in capacity is found for the template peptide (His-Ala) over other *N*-terminal histidine di- and tripeptides. Binding of a non-histidine-containing peptide (Ala-Phe) is minimal.

HPLC. Binding isotherms were obtained for the template peptide (His-Ala) as well as three other peptide sequences (Figure 2).

It is clear from the binding data that the (His-Ala)-imprinted polymer has a significantly higher capacity for the template peptide over the other sequences examined and that non-histidine-containing peptides have almost no affinity for the polymer. In addition, the ordering of the remaining peptides seems to conform to the hypothesis that, during polymerization, a region is being formed within the cross-linked polymer that is complementary to the template species in both size and shape. With a (His-Ala)-imprinted polymer the binding capacity is cut by over a third for His-Phe binding. This would be expected if the additional steric bulk of the phenyl group limits access of the peptide to the binding site prepared by His-Ala. The tripeptide His-Ala-Phe appears to achieve the same binding capacity as His-Phe, although uptake at lower substrate concentrations is less, indicating that there are fewer strong binding sites available for the tripeptide. This position is further supported by examining the Scatchard plots for these peptides. The plot for His-Phe (Figure 3b) appears to represent binding sites which are relatively homogeneous with respect to binding affinity.

This is not the case for His-Ala-Phe which shows a distinctly bimodal Scatchard indicating two populations of binding sites, each with different affinities for the peptide (Figure 3c). The data indicates that, while the overall number of binding sites is similar for His-Phe and His-Ala-Phe, the number of high affinity sites for the tripeptide is less than that available for the dipeptide. The Scatchard plot for the template peptide His-Ala (Figure 3a) resembles the plot for His-Phe in that it is monomodal and results

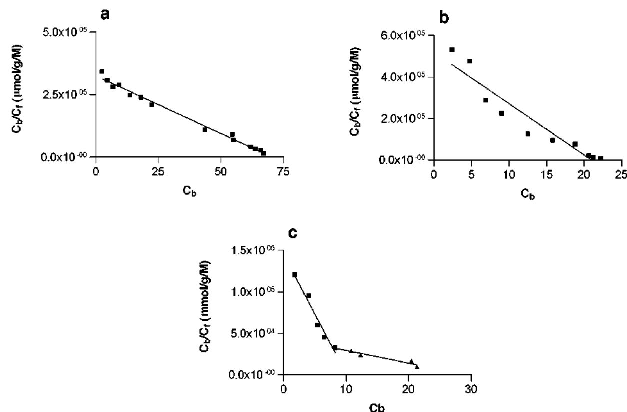

Figure 3. Scatchard plots of data obtained by rebinding peptides to a polymer prepared using His-Ala as the template peptide: (a) template dipeptide His-Ala, (b) dipeptide His-Phe, (c) tripeptide His-Ala-Phe.

in a similar affinity constant for the available sites. However, the total number of sites is much higher for the template peptide.

Control polymers were prepared to provide information about nonspecific (non-metal) interactions between the peptides and the polymer matrix and to determine the importance of template structure on selectivity. Polymers containing the NTA ligand but no nickel were prepared by two methods. One was formed by replacing the pre-polymerization complex in the formulation with the NTA ligand alone; the other was prepared by removing the nickel from the (His-Ala)-imprinted polymer by washing it with a solution of EDTA at pH 8. Binding isotherms of His-Ala to these polymers indicated a B_{max} of 10−20 μmol/g. This capacity is close to the values obtained for some of the nonimprinted peptides on the His-Ala imprinted polymer. This low level binding may be due to electrostatic interactions between the triacid NTA and basic sites on the peptide. An additional control polymer was prepared using the same formulation as the (His-Ala)-imprinted polymer, but instead histamine was used as the template. The capacity of this polymer for His-Ala and His-Phe was nearly identical (36−37 μmol/g). In addition, these values were similar to the capacity of the (His-Ala)-imprinted polymer for His-Phe (26 μmol/g).

The role of the metal and its coordination sphere in peptide rebinding was evaluated by the "bait-and-switch" method. The nickel in the polymer was replaced by copper, and the binding capacities were measured. Washing the nickel-containing polymer (1.0 wt % Ni by elemental analysis) with EDTA (pH 8) resulted in a white polymer which contained 0.17 wt % residual nickel. Washing this polymer with 20 mM $CuSO_4$ solution resulted in a blue polymer which contained 0.87 wt % copper. Uptake of three *N*-terminal histidine-containing peptides including the template sequence HisAla was measured. It was found that, while uptake of the template peptide was essentially the same for the nickel- and copper-containing polymers, uptake of the two non-template sequences was substantially higher for the copper-containing polymer. This result indicates that the metal bound to its coordinating ligands must influence the steric environment around the complex.

We have developed protocols for creating macromolecular receptors for peptides using molecular imprinting. The use of water in the polymer synthesis and recognition steps has obvious advantages over organic systems. These materials may be helpful in analyzing the modes of peptide recognition processes. They may also find use as artificial receptors for screening of peptides and peptidomimetics.

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Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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