

Metal Ion Coordination Interactions for Biomolecule Recognition: a Review

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ABSTRACT

Molecular imprinting is an effective method to create selective binding sites in polymeric matrices for biomolecule recognition. This review gives recent improvements of the design and preparation of selective binding sites via metal coordination interactions in molecularly imprinted polymers (MIPs) and focuses on particularly metal coordination bonds between biomolecules such as amino acids, peptides, proteins and templated polymers. The discussion will evaluate key parameters for molecular imprinting in the perspective of metal coordination.

Key Words:

Metal ion coordination; Molecular imprinting; Metal chelation; Biomolecule recognition

INTRODUCTION

Molecular imprinting is an effective method to introduce highly selective binding sites into polymeric materials to specifically rebind template molecule in preference to analog molecules. The functional groups complementary to template molecule are led to form an assembly around template. Highly specific polymeric materials are obtained polymerising functional monomers and cross linking agents around this complex. Template molecule is then extracted and thus binding sites complementary to template are established (Figure 1) [1]. The molecular imprinted polymers (MIP)s are resistant to elevated temperature and pressure, inert to chemicals, stable and cheap [2].

The driving forces required for the binding between template and functional monomer are covalent bonds, non covalent bonds and metal coordination. The strength of the interactions between template and monomer is significant for the efficiency of the imprinting process [3, 4]. In covalent imprinting, the template molecule is bound to monomer with functional groups covalently. However, covalent bonding gives strong interactions between template and monomer, it has slow rebinding kinetics and harsh conditions are required for template removal after polymerization step [5].

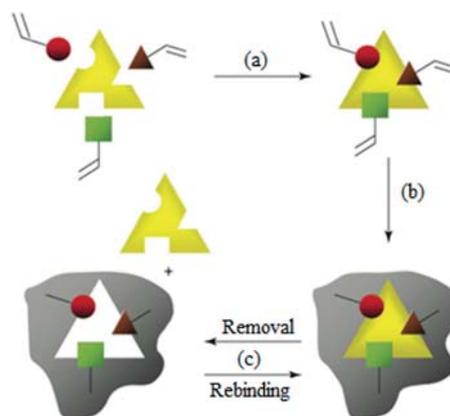


Figure 1. Representation of molecular imprinting process. Reprinted from ref. [1].

Non covalent imprinting relies on secondary interactions between template and monomer such as hydrogen bonding, van der Waals interactions and Coulomb forces. Despite water is common solvent for molecular imprinting since many biomolecules have limited solubility in organic media, the recognition capability is reduced due to weakness of hydrogen bonds. Also imprinting effect may be weakened in aqueous environment since polar solvents compete with hydrogen bonding interactions [6].

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The principles of metal ion coordination in molecular imprinting process

Metal ion coordination with biological molecules is well suited to molecular recognition due to its specificity and stability. In metal coordination during imprinting process, metal chelating monomers are pre-complexed to metal ion, generally a transition metal ion, which, in turn, coordinates the template molecule. Metal ions are employed as mediator that directs functional monomer and template molecule to establish a high fidelity of imprint with high specificity [7-9]. Metal coordination has higher strength with respect to hydrogen bonding which makes it more stable in water for example, binding energy of the Cu^{2+} complex and imidazole residue of histidine is 4.8 kcal/mol and it is less than 1 kcal/mol for typical hydrogen bonding interaction [10]. Additionally metal coordination is a fast binding process and binding strength can be adjusted by choosing appropriate metal ion for a defined template molecule. Furthermore it is possible to replace the metal ion with another one to enhance the selectivity or use the MIP for different aim [11-13]. Therefore within this context metal coordination approach has an important potential for preparation of highly specific MIPs in aqueous medium.

The most important step of the preparation of the MIP is the prearrangement of the functional monomer, the metal ion and the template molecule. This ternary complex is then polymerized with cross linking agents initiated thermally or by UV light. After polymerization the template molecule is removed with appropriate agents. In order to use the material with other metal ions, it can be washed with complexing agents such as ethylenediaminetetraacetic acid (EDTA) to remove all the metal content then the other metal ion can be reloaded [14].

Key parameters for metal coordination

The selection of metal ion is one of the most important parameter to obtain specific recognition. Template molecule dominates the type of the metal ion used for imprinting process. The type of the metal ion defines binding strength of the template, metal ion and monomer complex and the spatial arrangement of this complexation [15]. In order to obtain high specificity, coordination mode of the metal ion and monomer complex should be determined. The molecularly imprinted polymers for the application of analyte extraction from biological fluids was prepared in aqueous medium with tetracyclines—a large family of common antibiotics—as template molecule, Fe^{2+} as mediator and methacrylic acid (MAA) as functional monomer (Figure 2) [12]. Different metal ions such as Mg^{2+} , Fe^{2+} and Cu^{2+} were complexed with tetracycline (TC) in the preparation of MIPs.

It is found that Fe^{2+} could obtain high recognition capability due to specific coordination interactions between TC and MAA.

As mentioned the self assembly of template-monomer is the pre-organization and first step for the fabrication of MIPs. Utilizing metal ion as mediator assembles a bridge between template and monomer through coordination bond. (S)-Naproxen was complexed with 4-vinylpyridine through coordination with Co^{2+} and thus MIP with high selectivity was prepared [16]. Figure 3 shows the schematic presentation of this complex and also the binding of template and monomer via hydrogen bonding. It was shown that without metal ion which means by using only hydrogen bonding interactions, selectivity for template was reduced significantly. The stoichiometry of the complex used was determined with UV spectrum by titrating monomer and template. The optimization of monomer amount is required for the design of MIP since high amount of monomer results low adsorption capacity due to inefficient template removal and low amount of monomer causes incomplete organization of template in templated

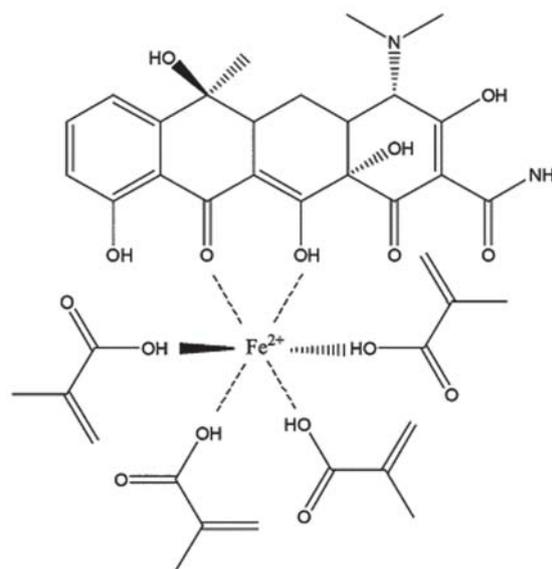


Figure 2. The schematic representation of complexation of TC/ Fe^{2+} /MAA. Reproduced from ref. [12].

polymer matrix. In the same manner, the amount of metal ion plays important role to enhance selectivity. In order to form highly specific recognition sites complementary to template, the stoichiometric amount of metal ion to bridge the specific interaction between template and monomer with high stability is necessary due to structural and spatial complementary to template [17].

The other parameter is the influence of the anion used since it may participate in the recognition process.

Molecularly imprinted solid phase microextraction fiber was developed to recognize thiabendazole (TBZ) - a kind of fungicide- via the metal coordination interaction and it was found that enrichment properties in aqueous solutions were improved with respect to hydrogen bonding interactions. Four different copper salts as acetate, sulfate, nitrate and chloride were studied with respect to adsorption capacity and copper(II) acetate was found to show the highest adsorption capacity. This

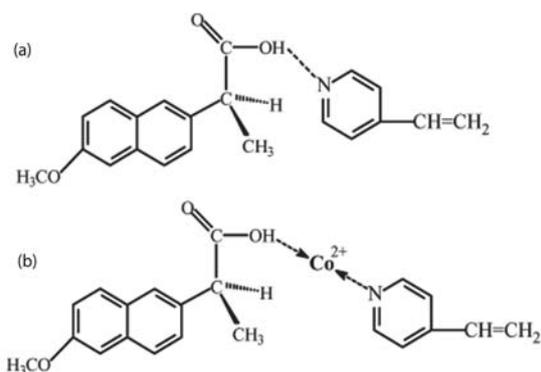


Figure 3. The binding interactions of MIP via a) H-bonding and b) metal coordination. Reprinted from ref. [16].

demonstrates that anion has an effect on the recognition process since it changes the size and shape of the metal-template-monomer complex [18].

Metal coordination for bis(imidazole) recognition

Metal ion coordination with its specificity and stability is well suited procedure for molecular recognition of biological molecules which is exemplified by the chromatographic method namely IMAC (Immobilized Metal Affinity Chromatography) [19-21]. It has been developed by Porath and protein purification was achieved via binding of electron donor groups on protein surface and metal ion immobilized on the support surface [22]. The amino and carboxyl groups of amino acid participate in the fabrication of metal-amino acid complex. Especially, histidine containing peptides form more stable complex with metal ions due to metal coordination between metal ion and imidazole side chain of amino acid. The common chelating ligands are iminodiacetic acid (IDA), nitrilotriacetic acid (NTA) and tris carboxymethyl ethylene diamine (TED) are shown below (Figure 4).

The complementarity between metal ion and template molecule forms specific assembly prior to polymerization. After polymerization when template is removed from polymer matrix, a very specific binding cavity is formed as complementary to template due to this specific arrangement of functional groups around template molecule. In order to prepare selective abiotic

receptors for 1,4-Bis(imidazol-1-ylmethyl)benzene (2 in Figure 5) which is analog of surface histidine bearing proteins, metal chelating monomer copper(II) (N-(4-vinylbenzyl)-imino) diacetic acid was pre-organized with template molecule and then polymerized in the presence of cross linking agent [5]. The affinity for molecule 2 was determined higher than the analog molecules 4,4'-Bis(imidazol-1-ylmethyl)biphenyl (4) and 1-Imidazol-1-ylmethyl-4-(pyrrol-1-ylmethyl)benzene (6) which contain single imidazole. This bigger affinity may explain two

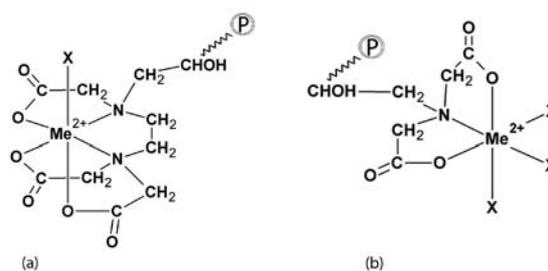


Figure 4. Metal coordination between metal ion (Me) and a) TED and b) IDA. Reproduced from ref. [22].

point binding mechanism of the template to MIP and it shows that the templating is very critical for distribution of metal ions through the polymer matrix.

Metal coordination for amino acid, peptide and protein recognition

Amino acid imprinted polymers were developed via metal coordination for chiral separation of various amino acids from aqueous solutions [23]. MIPs were prepared with the complex of Cu^{2+} -N-(4-vinyl benzyl) iminodiacetic acid as functional monomer and template amino acid in

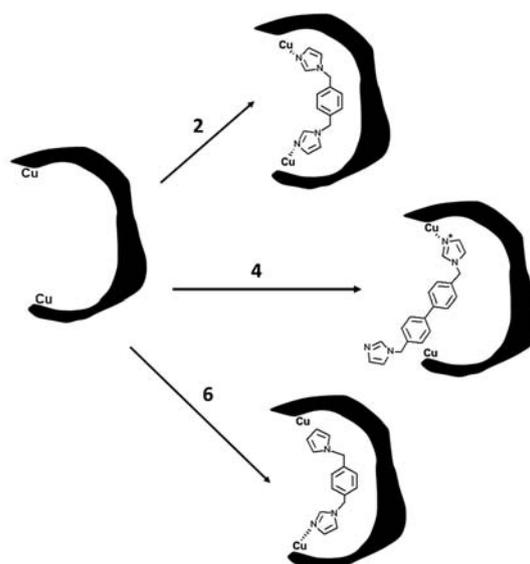


Figure 5. Schematic representation of binding of various bis(imidazole) molecules. Reproduced from ref. [5].

the presence of cross linkers. All the MIPs showed high enantioselectivity for the template amino acid and also it was reported that enantioselectivity depends on both size and shape of the side chain of the amino acid.

Hochuli et al. has been firstly developed a new quadridentate chelating ligand nitrilotriacetic acid (NTA) for protein purification via metal chelate chromatography [24]. It was reported that NTA forms more stable coordination interaction with both Cu^{2+} and Ni^{2+} than the interaction with IDA and each of these metals. NTA when complexed with Ni^{2+} occupies four positions in metal octahedral coordination sphere and leaving two for dipeptide His-Ala interaction [25]. It was achieved by selective polymeric receptors to separate between peptide sequence via strong coordination NTA, Ni and His-Ala.

Due to the complex and flexible structure of proteins, molecular imprinting process of these large molecules is still challenging task and reviewed by many scientists [26-29]. Proteins are not compatible with MIP process since MIP is synthesized in organic media and proteins are generally soluble in aqueous solutions [30]. Proteins are not resistant to environmental changes such as pH, temperature etc. [31]. Kempe et al. was first developed molecularly imprinting procedure for protein imprinting using the advantages of metal ion coordination interactions [32]. MIPs were prepared to recognize ribonuclease A (RNase A) through metal coordination utilizing N-(4-vinyl)-benzyl iminodiacetic acid (VBIDA) as metal chelating monomer and copper ion as mediator in the presence of the protein onto methacrylate-derivatized silica particles (Figure 6). There has been growing attention in metal mediated protein imprinted polymers [33-36]. All these studies show promising properties of metal coordination for the preparation of highly selective MIPs via stable and specific arrangement of metal ions with protein surface for protein separation and recognition.

A thermoresponsive macroporous hydrogel for lysozyme recognition was developed via molecular imprinting method based on metal coordination interaction between protein and metal ion [37]. (N-(4-vinyl)-benzyl iminodiacetic acid) (VBIDA) as metal chelate monomer was pre-organized with Cu^{2+} as mediator and lysozyme as template and then polymerized with NIPAAm (N-isopropylacrylamide) for thermoresponsiveness, AAm (acrylamide) for mechanical strength and N,N-methylenebisacrylamide for cross linking. The Lysozyme-MIP prepared via metal coordination between VBIDA, Cu^{2+} and lysozyme demonstrated higher protein recognition than Lysozyme-MIP prepared via electrostatic interactions obtained with VBIDA and protein. Also it was confirmed with selectivity tests that metal coordination

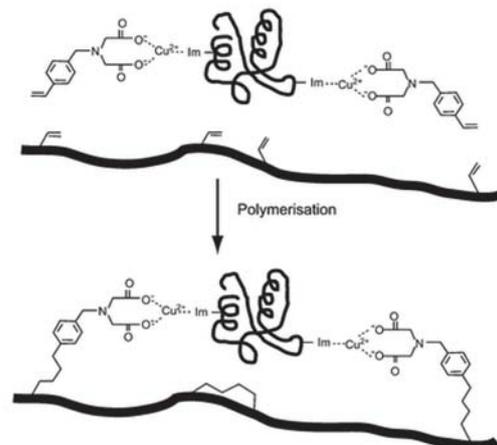


Figure 6. The schematic presentation of protein imprinting process with metal coordination. Reproduced from ref. [32].

was important to positioning the binding groups around the template molecule. Porcine serum albumin (PSA) imprinted silica particles were prepared using IDA as metal chelating agent and copper as metal ion in the presence of (3-Aminopropyl)triethoxysilane (APTES) [38]. It was shown that PSA-MIP demonstrates highly specific recognition for template molecule in comparison with NIP and MIP-no metal ion coordination.

Proteins may undergo conformational changes in the presence of monomers and crosslinkers due to its vulnerable nature. It was reported that for lysozyme and bovine hemoglobin there was a significant change on conformational states of both proteins when in solution with various common monomers and crosslinkers [39]. This conformational change would impair the recognition sites targeting a certain protein molecule. In that study, epitope approach was offered to achieve efficient protein imprinting process. L-histidine imprinted polymers were developed via metal coordination between Cu^{2+} as mediator, L-histidine as template and N-methacryloyl-(L)-histidine (MAH) as metal-chelating monomer for selective separation of cytochrome c [40]. The chromatographic separation of L-histidine, cytochrome c and ribonuclease A was achieved.

Surface imprinted silica particles were prepared for hemoglobin recognition using a novel approach [41]. The template protein was immobilized onto surface via metal coordination prior to silanes polymerization. Hemoglobin was removed using different strategies and it was determined that the highest recognition capacity was obtained when 22% of hemoglobin was removed. These experimental results show that soft imprinted cavities were achieved to create which induce recognition of protein molecules by sub-stoichiometrically removal of template protein.

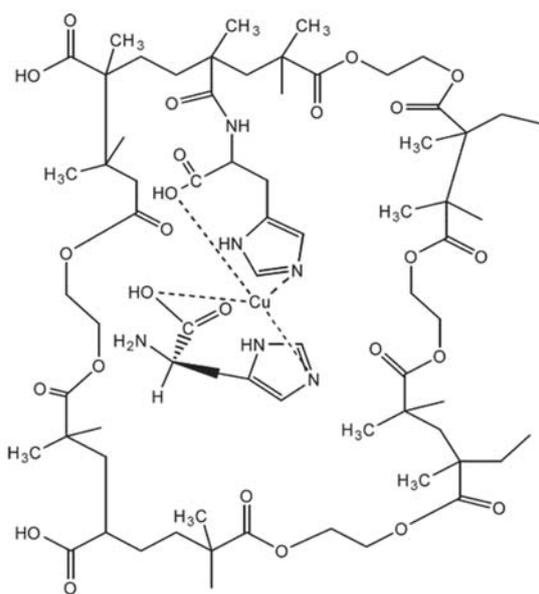


Figure 7. The representation of MAH-Cu²⁺-L-histidine formation. Reprinted from ref. [40].

CONCLUSIONS

Molecular imprinting is considered as an important method to obtain highly specific recognitive polymeric matrices for protein recognition [42-47]. Within this review article many MIPs showing high selectivity prepared utilizing metal coordination interactions templating various molecules such as bis(imidazoles), amino acids, peptides and proteins have been mentioned. Molecular imprinting method utilizing metal coordination interactions is attractive to obtain highly specific and stable recognition sites for separation of biomolecules even like proteins.

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