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Immobilized peptides/amino acids on solid supports for metal remediation*

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Abstract: Recently, a significant amount of work has focused on metal binding by natural systems for various applications. This review will focus on the utility of amino acids, short peptides, and proteins that have been immobilized onto solid supports for use in metal binding. These systems include single amino acids, poly-amino acids, and peptides immobilized onto supports such as silica, polymer resins, and membranes. Also included are the studies involving the use of immobilized amino acids in ion-exchange chromatography.

Heavy metals are introduced into the environment through a number of industrial processes [1]. Depending on the chemical form and exposure level, heavy metals can potentially be very harmful to humans and have a negative impact on the environment. Unlike organic pollutants, metal contamination is exacerbated by the fact that metals are a nondegradable, recirculating contaminant and accumulate in the environment [2,3]. As a direct result of this fact, it is necessary to remediate heavily contaminated sites. This can only be accomplished by isolation and recovery of heavy metals since degradation is not an option. As a first attempt at remediation, bulk techniques, such as simple filtration or precipitation, are often utilized [2,4]. Although these techniques are useful in removing a significant fraction of the contaminant, they are unable to reduce the contaminant levels to meet environmental agency regulations for many of the more toxic metals. As a result, a polishing or finishing step must be employed. This finishing step is often in the form of a chemical extraction. The ideal metal extraction and reclamation technique must have the following attributes:

- Selectivity—binding only to the metal of interest, thus allowing for separation from metals that are harmless or beneficial that could overwhelm the available binding sites and significantly reduce the efficiency or capacity of the extracting media
- Strong binding—necessary if effective removal from contaminated areas to an allowable level is to be realized
- Easy release—allowing for efficient preconcentration of the contaminant and rejuvenation or reuse of the media
- Environmental innocuity—preventing further contamination when the media is ultimately discarded
- Stability—ability to be reused with an extended lifetime, ensuring cost-effectiveness

In many instances, the attributes sited for remediation are identical to those desired if preconcentration methodologies are sought as a means of assisting analytical detection methods. With the need to establish concentration levels in the low to sub-ppb levels, validation of the remediation procedure re-

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quires sensitive analytical tools. While techniques exist for all regulated contaminant levels, many labs must resort to less sensitive instrumental capabilities and must employ preconcentration tools to detect regulatory levels.

Currently, the most common chemical modes of metal removal include ion exchangers or removal by chelation with synthetic crown ethers or other macrocylic cage molecules (e.g., [5–9]). The most significant drawback associated with typical ion exchangers is the lack of selectivity in metal binding and/or weak binding characteristics. While crown ethers are both selective and strong binders, due to polydentate chelation within a sized cavity, they often exhibit slow release kinetics [5]. This is a potential problem when metal reclamation is required. In addition, many crown ethers are also very toxic, so using them may simply add to the problem of contamination.

As a result of the inherent problems with most of the current metal remediation strategies, researchers are now turning toward natural systems. For the purposes of limiting the scope of this review, the vast body of research in phytoremediation will not be covered, although it remains a very active and effective approach to remediation for both natural waters and soils. Similarly, the use of immobilized unicellular algae and other microorganisms in metals preconcentration and remediation has a long history with encouraging results but lies outside the scope of this review. However, the likely source of binding in these unicellular organisms are the chemicals that make up the organism. Therefore, one could consider a more focused effort at isolation of these particular biocompounds and direct utilization of only those cellular components that are directly involved in metal binding. While these can range in character from simple cellulose to more elaborate proteins, this review will focus on the potential utility of amino acids, peptides, and proteins that have been immobilized on a substrate for general use in column applications.

A well-known class of metal binding proteins, the metallothioneins, is an example of such biomolecules that are characterized as having a high degree of metal binding specificity and have been isolated in a wide variety of organisms (e.g., [10–13]). Their strong binding characteristics and selectivity seem to fit the criteria of the ideal metal chelator. Upon immobilization, these proteins seemed to lose their metal binding capabilities outside of the pristine cellular environment where they typically function in nature [14]. In this particular instance, closer examination of several metallothioneins showed that their sequences contained a significantly high percentage of cysteine residues and that sulfhydryl groups present on these residues are primarily responsible for metal binding [10,12]. This suggests the possibility of using simpler amino acid chains or synthetic peptides (e.g., poly-amino acids) as metal binding alternatives to natural peptides. Considering only the natural set of amino acids, one can readily recognize a variety of functionalities that could serve as coordination sites for metal chelation. Using amino acids as building blocks with their various side chains and recognizing that peptides are simple polymers of these units using a common amide linkage, a wide variety of interesting chelators could be envisioned. More specifically, these chelators may exhibit the desired characteristics of specificity and have the added side benefit of being nontoxic when discarded.

This review will focus on studies directed at metal binding by immobilized amino acids as well as short chain polypeptides. In some instances, the incorporation of amino acids into short polymeric chains or evaluation of anionic compounds are also included. Figure 1 shows selected amino acids from the standard set of 21 that are relevant to this discussion.

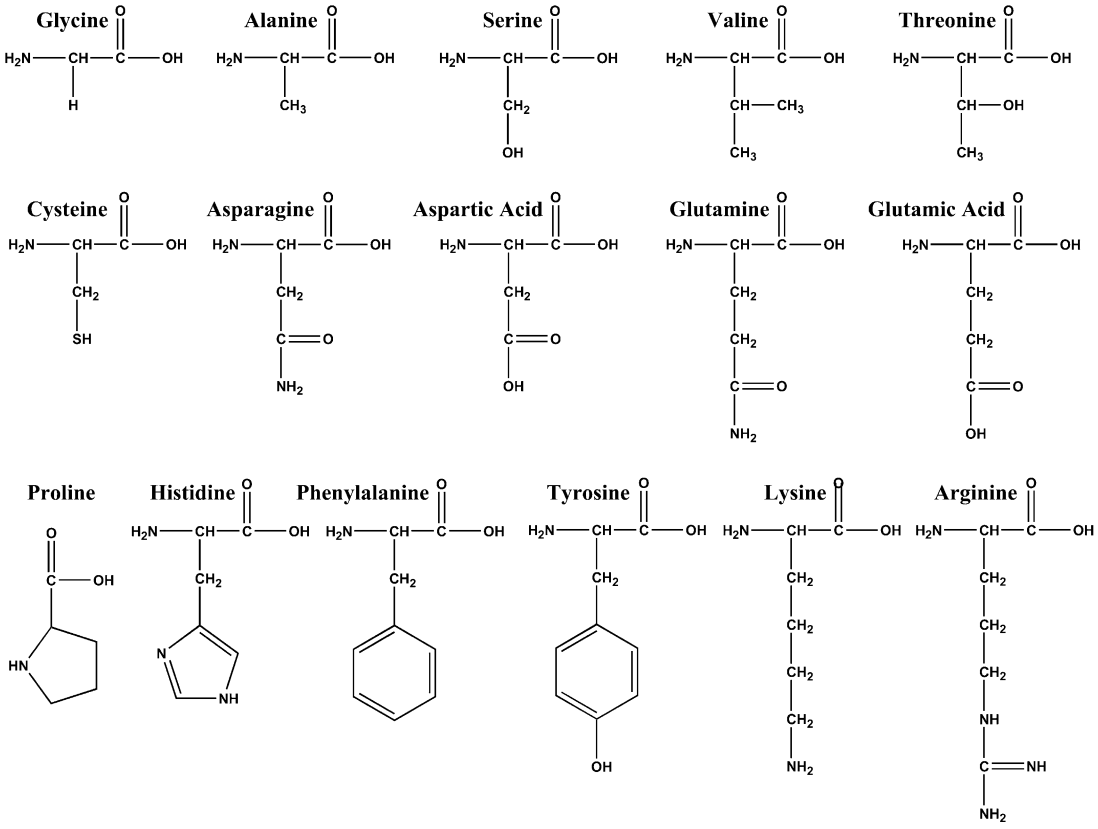

Fig. 1 Selected set of amino acids that have been used by various researchers as immobilized chelators of metals.

APPLICATION OF IMMOBILIZED AMINO ACIDS AND PEPTIDES

Single amino acids

As mentioned previously, cysteine (Cys) is a major component of a group of metal binding proteins, called metallothioneins [10]. As a result, researchers have investigated Cys immobilized onto a solid support for use as a metal chelator. Elmahadi and Greenway utilized Cys immobilized onto silanized controlled pore glass (CPG) through a gluteraldehyde linker for preconcentration of Cd^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, and Zn^{2+} [15]. Capacities for these metals were determined through breakthrough curve analysis and calculated at 12.48, 5.50, 7.86, 6.06, 11.66, and 7.88 mmol of metal/g of dry resin, respectively.

Denizli and coworkers [16,17] and Disbudak et al. [18] also utilized immobilized Cys for metal preconcentration and remediation. In each of these studies, 2-methacryloylamindocysteine (MAC) was allowed to react with 2-hydroxyethylmethacrylate (HEMA) in an aqueous medium. The product was spherical beads, with an average size of 150–200 μm, of poly(2-dyroxyehylmethacrylate–methacryloyl-amidocysteine) [p(HEMA-MAC)]. The beads were characterized according to their swelling ratio, FTIR analysis, and elemental analysis. The spectroscopic studies were conducted in the absence of metal to characterize the beads and confirm the incorporation of MAC, not to study the metal binding characteristics. In separate studies, binding characteristics were determined for As^{3+}, Cd^{2+}, Cr^{3+}, Cu^{2+}, Hg^{2+}, and Pb^{2+}; and it was shown that while the pHEMA beads exhibited negligible Cd^{2+} binding, p(HEMA-MAC) beads exhibited significant Cd^{2+} capacity. The microbeads can be regenerated with an acidic solution. Several studies have been conducted using glycine (Gly) residues supported on various

cross-linked resins. George and coworkers [19] and Vinodkumar and Matthew [20] studied the metal binding capabilities and the effects of the degree of cross-linking on metal uptake on polyacrylamide cross-linked with *N*,*N*′-methylene-bis-acrylamide (NNMBA) with supported Gly. Gly was incorporated into the resin by transamidation using a solution containing an excess of the sodium salt of glycine. The metals studied include Co^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, and Zn^{2+}. Metal binding increased with an increase in cross-linking, until 8 % cross-linking and then decreased. Interestingly, the metal desorbed resins showed specificity toward the previously desorbed metal over other metals. This was attributed to "pockets" left by the desorbed metal or the "memory" of the ligand for the metal.

George et al. also studied the metal binding ability of divinylbenzene (DVB)-cross-linked polyacrylamide supported Gly toward Co^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} [21]. Once again, Gly residues were introduced through transamidation with Gly. Interestingly, as the degree of cross-linking increases from 2–20 %, the metal complexation decreases due to a decrease in the available carboxylate ligands for metal binding with an increase in DVB content. The resin does show enhanced specificity toward the desorbed metal over other metals in subsequent runs, and the time for rebinding of the desorbed metal is significantly less for rebinding than it is for initial binding as seen with the NNMBA cross-linked resin.

Finally, George et al. directly compared the metal-ion complexation characteristics between Gly functionalities supported on DVB-cross-linked polyacrylamide and NNMBA-cross-linked polyacrylamide toward Co^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+} [22]. DVB was chosen because it is more rigid and hydrophobic than NNMBA. The NNMBA-cross-linked polyacrylamide was shown to be more effective at metal complexation than the DVB-cross-linked resin, while DVB showed increased selectivity over NNMBA. Again, metal rebinding is much faster and more specific than initial binding on both resins. Each of these resins can be regenerated by acid washing and reuse.

In a procedure similar to that described previously by Denizli [16,17] and Disbudak [18], Say et al. prepared poly(hydroxyethyl methacrylate-*co*-methacrylamidohistidine) p(HEMA-*co*-MAH) beads for metal complexation [23]. Again, these beads were fully characterized without metals bound by swelling studies, FTIR, and elemental analysis of the bead. In additional experiments, the metal binding affinity was demonstrated to be $Cu^{2+} > Cr^{3+} > Hg^{2+} > Pb^{2+} > Cd^{2+}$, and the beads could be easily regenerated with 0.1 M HNO_3.

In an attempt to prepare a novel molecular imprinted adsorbent to remove heavy metals, Say et al. synthesized Cu^{2+}-imprinted poly(ethylene glycol dimethacrylate-methacryloylamidohistidine/Cu^{2+} [poly(EGDMA-MAH/Cu^{2+})] microbeads by dispersion polymerization of EGDMA and MAH/Cu^{2+} [24]. After removal of the Cu^{2+}, these beads exhibited a maximum Cu^{2+} capacity of 48 mg of Cu^{2+}/g of support and excellent selectivity of Cu^{2+} over Zn^{2+}, Ni^{2+}, and Co^{2+}. Metal binding exhibited a strong dependence on pH, with increased binding at increased pH. Cu^{2+} was easily desorbed with EDTA and the beads were reusable without a significant loss in capacity.

Phenylalanine (Phe) has also been immobilized onto spherical macroreticular styrene-divinylbenzene beads to create a chelating resin [25]. This resin was capable of separating Cu^{2+} from Co^{2+} and Ni^{2+}. Co^{2+} and Ni^{2+} are not retained on the column at pH 3 while copper is and can be eluted with 1 M HCl. The beads are also capable of removing Cu^{2+} from seawater.

Immobilized poly-amino acids on silica supports

In addition to single amino acids, poly-amino acids and peptides have been immobilized onto solid supports for use in metal chelating systems. Jurbergs and Holcombe attached poly-L-Cysteine (PLCys) ($n \sim 50$ residues) to CPG via a procedure described by Masoom and Townshend [26] and characterized it according to its Cd^{2+} binding capabilities [27] Using breakthrough analysis, it was determined that PLCys was an effective chelator for Cd^{2+}. Through competitive binding studies using ethylenediaminetetraacetic acid (EDTA) and ethylenediamine dihydrochloride (en) as competing ligands, conditional stability constants were calculated at 10^{13} for the very strong binding sites, 10^{9}–10^{11} for the

strong binding sites, and 10^6 for the intermediate sites. Although there is very strong binding, the metal can be quantitatively recovered using 0.1 M HNO_3, making the column fully regenerable and reusable. A study of Cd^{2+} capacity at various pHs revealed that the affinity of PLCys for Cd^{2+} had a significant dependence on pH. There was very little binding in acidic pHs, and binding increased as pH increased. They postulated that at elevated pHs, the PLCys is more hydrophilic due to the sulfhydryl groups being deprotonated. As a result, the peptide chain would be unfolded due to an increased hydration from ion-dipole interactions, and the side chains may be more accessible in an unfolded peptide, thus leading to an increase in metal capacity. They were also able to determine that the metal binding of PLCys may be mass transport-limited since they observed the Cd^{2+} capacity increase as the solution flow rate was decreased. Various concentrations of hard acid metals in the influent stream (e.g., alkali and alkaline earth metals, Co^{2+} and Ni^{2+}) had very little effect on PLCys–Cd^{2+} binding.

Later, a comparison of the metal binding capabilities of PLCys (n ~ 50 residues) and 8-hydroxyquinoline (8HQ), both immobilized onto CPG, was conducted [28]. Once again, using breakthrough analysis in metal capacity determination, PLCys showed more selectivity against harder acid metals than 8HQ. While 8HQ strongly complexes a broad range of metals (Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, and Pb^{2+}), PLCys isolated soft acid metals such as Cd^{2+} and Pb^{2+} and had very little affinity for Co^{2+} or Ni^{2+}. Thus, they reasoned that PLCys should be efficient in isolating many of the heavy metals from complex matrices containing hard acid metals. The conditional stability constants, again determined through competitive binding studies with EDTA and en, agreed with the previously reported values reported by Jurbergs and Holcombe [27].

In the process of studying various supports, Miller and Holcombe evaluated Cys immobilized on porous carbon, a more inexpensive support [29]. Both PLCys (n ~ 50 residues) and the Cys monomer were tethered to Carbopack™-X, a commercially available porous carbon, by derivitizing the carbon with carboxylate functionalities by acid activation and linking the PLCys or Cys through the use of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC). Breakthrough analysis and competitive binding studies demonstrated that porous carbon is an effective support for immobilized ligands. In fact, the capacities for all metals tested were consistently higher on the porous carbon than on CPG. It is suggested that this may be due to the immobilization efficiency. The immobilization procedure is much simpler for porous carbon than for CPG, possibility resulting in greater coverage of the polymer onto the support. Conditional stability constants were in good agreement with previous work done on CPG.

Gutierrez et al. used the same approach as Jurbergs and Holcombe [27] by attaching poly-L-aspartic acid (PLAsp) (n ~ 50 residues) to CPG to test its metal binding capabilities [30]. The binding affinity of PLAsp is $Cu^{2+} > La^{3+} \approx Ce^{3+} \approx Eu^{3+} > Pb^{2+} > Cd^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Ca^{2+} > Na^{2+}$, which is somewhat complimentary to PLCys and consistent with carboxylate functionality complexing.

In an attempt to find a cheaper alternative to PLAsp, Miller et al. compared immobilized PLAsp to immobilized poly-acrylic acid (PAA), a synthetic polymer [31]. The results for PLAsp-CPG are similar to those reported above. Additionally, metal binding was measured as a function of pH and capacity again decreased with a decrease in pH due to protonation of the carboxylates and possible conformational changes at low pH (ca. <pH = 4) for PLAsp-CPG. Stability studies show that PLAsp-CPG exhibited minimal loss of capacity upon exposure to 0.05 M ammonium acetate buffer, 5 % H_2O_2, and elevated temperature (60 °C).

Miller and Holcombe also studied gold as a support for PLAsp [32]. Gold was chosen because it acts as an inert surface, acting only as an anchor for the polymer and remaining unreactive toward the metals in solution. The gold used initially was in the form of gold transmission electron microscopy (TEM) grids that were stacked in a microcolumn with thin PTFE spacers between each grid to promote mixing and flow. The immobilization of the polymer was conducted online with an FI system, using a modification of a procedure described by Leggett [33]. The metal binding trend remained the same as for PLAsp on CPG but the capacities were considerable higher for the PLAsp on gold for all of the metals studied; Al^{3+}, Ce^{3+}, Cu^{2+}, Eu^{3+}, Fe^{3+}, and La^{3+}. This is possibly due to a more efficient immobi-

lization procedure. The same authors also attempted to employ gold-coated CPG substrates for immobilization prepared by electroless coating techniques, but the results were not encouraging due to patchy gold coverage and possible pore blockage by the deposited gold [34].

Membrane-immobilized poly-amino acids

In addition to silica, gold, and carbon, poly-amino acids have also been immobilized onto membranes. Membrane technology is very well developed in the area of separations and remediation (e.g., [35–37]). Often used in the passive separation of contaminants from solution, popular membrane technologies include reverse osmosis, nanofiltration, and ultrafiltration. In heavy metal sorption, the membrane is often functionalized with a metal chelating group such as iminodiacetate, amidoxime, phosphoric acid, or sulfonic/carboxylic groups to facilitate metal removal [38–45]. Thus, the membrane serves as a support for the metal binding material and with the attachment of these groups the membrane can be tuned to exclude specific solutes.

Recently, researchers have investigated the attachment of amino acids and poly-amino acids to membrane surfaces for metal extraction purposes. For example, Bhattacharyya and coworkers attached poly-L-glutamic (PLGlu) ($n \sim 93$ residues) acid to several different microfiltration membranes (both silica- and cellulose-based) to study the heavy metal sorption characteristics [46]. The polymer was attached to the membranes through an aldehyde functional group on the surface of the membrane. It was shown that the PLGlu-functionalized membrane is capable of binding heavy metals with the affinity following the order of $Pb^{2+} \geq Cu^{2+} > Ni^{2+} \approx Cd^{2+}$. The membrane also exhibited preferential binding of Pb^{2+} and Ni^{2+} over Ca^{2+}. The binding characteristics were dependent upon the type of membrane used, the degree of PLGlu functionalization, pH, and metals present.

Poly-D-aspartic acid (PDAsp) and poly-L-aspartic acid (PLAsp) have also been successfully immobilized onto both cellulose- and silica-based microfiltration membranes [47]. These functionalized membranes show capacities for Cu^{2+}, Cd^{2+}, and Pb^{2+} that are consistently higher than conventional ion-exchange and chelation resins, in the range of mmol of metal/g of sorbant. Not unexpectedly, little difference was seen in the performance in PDAsp and PLAsp. Ritchie and coworkers outlined the three primary mechanisms for metal sorption to include ion exchange, chelation, and electrostatic interactions [47]. Due to the polymeric nature of these ligands attached to a membrane, electrostatic interactions take the form of counterion condensation. Condensation zone binding is an important factor in the increase in binding capacity of functionalized membranes over conventional ion exchange systems due to the high charge density within the membrane pores.

In similar studies, Hestekin et al. show the differences that result from PLGlu and PLAsp bound to either pure cellulose or cellulose acetate-based membranes [48]. Counterion condensation was more closely evaluated, and a continuous flow system was employed with the membrane system. Results indicate that pure cellulose membranes with a higher surface area provide more aldehyde linkage groups for greater polymer attachment and that counterion condensation is an important mechanism for metal ion sorption in membrane systems.

Ritchie et al. have also immobilized PLCys onto both silica- and cellulose-based membranes and evaluated it according to its metal binding capabilities [49]. They showed that PLCys was an effective chelator for heavy metals such as Hg^{2+}, Pb^{2+}, and Cd^{2+}. Other parameters examined in this study included the efficiency of PLCys deprotection, the efficiency of PLCys functionalization, effects of flow rate and metal concentration, and the effects of the presence of mercury counterions on PLCys metal binding. Also investigated was the metal selectivity of membrane immobilized PLGlu in the presence of a multimetal solution containing both Cd^{2+} and Pb^{2+}.

Denizli et al. synthesized a poly(2-hydroxyethylmethacrylate-*co*-methacrylamidophenylalanine) membrane for copper adsorption [50,51]. The membranes were prepared through UV-initiated photopolymerization of 2-methacrylamidophenylalanine (MAPA) and 2-hydroxyethylmethacrylate (HEMA) with azobisisobutyronitrile present as an initiator. Characterization of this membrane revealed the order

of metal affinity to be $Hg^{2+} > Ni^{2+} > Cu^{2+}$, with metal adsorption increasing with increased pH, leveling off at pH 5.0. The capacities of these membranes were reported as mmol of metal/m^2 of membrane. The membranes can be regenerated with 0.1 M HNO_3 and reused without significant loss of capacity.

Researchers have also attached Phe to a polyethylene membrane, in the form of a hollow fiber, through radiation-induced graft polymerization [52]. The attachment of the polymer was conducted using two different reaction schemes in an effort to determine which method would produce the highest density of functional groups. The first method involved grafting glycidyl methacrylate (GMA) to the fiber and then coupling the Phe to the GMA. The second method involved attaching the Phe to the GMA first and then grafting the Phe-GMA to the fiber. Although the fiber was not fully characterized for metal binding capacity, the Cu^{2+} binding along the cross-section was monitored and a uniform distribution of Cu^{2+} through the fiber was found. This demonstrated a homogeneous distribution of Phe through the fiber. It was also concluded that the second reaction scheme (grafting the Phe-GMA complex to the fiber) occurred at a rate 180-fold less than grafting the GMA alone. The preliminary results from this study indicate that with further investigation, this technology may be applicable to heavy metal remediation.

Immobilized peptides

Peptides and short chains of amino acids have also been immobilized for metal extraction. Terashima et al. immobilized a fusion protein synthesized from maltose binding protein (pmal) and human metallothionein (MT) onto Chitopearl resin [53]. This resin was evaluated for its Cd^{2+} and Ga^{2+} binding capabilities. Interestingly, the optimal pH for Cd^{2+} binding was determined to be 5.2 while for Ga^{2+} it was 6.5. Based on the hard and soft acid-base theory and the analysis of the adsorption isotherms of these metals, the results indicate that the cysteine residues of the MT moiety of the immobilized protein are responsible for Cd^{2+} binding. Other negatively charged residues such as Asp, Glu, lysine (Lys), serine (Ser), threonine (Thr), glutamine (Gln), and asparagines (Asn) bind Ga^{2+}. As a result of this strong metal binding dependence on pH, this system can distinguish between these two metals.

Another class of metal binding proteins, synthetic phytochelatins, has shown improved Cd^{2+} binding over metallothioneins [54]. Xu et al. used a novel approach by attaching a cellulose-binding domain (CBD) to a synthetic phytochelatin (EC20). The CBD attached itself to a cellulose support, thus immobilizing the phytochelatin [55]. Upon addition of Cd^{2+}, the CBD-EC20 membrane bound the metal at a ratio of ~10 Cd^{2+}/immobilized CBD-EC20, while the membrane with only the CBD attached did not bind any Cd^{2+}. Upon addition of EDTA, Cd^{2+} was removed and the membrane capacity was restored.

Immobilized amino acid for use in ion-exchange chromatography

In contrast to remediation applications where it is desirable to have extremely strong binding, other applications have interest in moderate binding so that the substrate can be used in chromatographic separations via partitioning. Single amino acids immobilized onto silica surfaces have been used extensively for ligand-exchange [56], metal chelation [57], and affinity chromatography [58]. The use of these materials for ion-exchange chromatography has not been as widely explored. Amino acids by nature are zwitterions meaning they possess both positively and negatively charged sites. Zwitterion-exchangers are of particular interest as new stationary phases for high-performance liquid chromatography (HPLC) as they may separate both anionic and cationic species in a single solution. These materials also show increases in mass transport and ion selectivity. Attachment of amino acids to silica is a simple way to achieve a variety of zwitterionic stationary materials.

In the last decade, research in this area has been advanced by Nesterenko [59], who initially explored L-hydroxyproline (Hypro) bonded silica as an anion-exchange material. The amino acid was at-

tached to silica particles through the secondary amine via 3-glycidoxypropyltriethoxysilane. Separation of nine anions (SCN^-, ClO_4^-, I^-, NO_3^-, Br^-, Cl^-, IO_3^-, $H_2PO_4^-$, and NO_2^-) was observed at pH 3.13 with citric acid as the eluent. Significant changes in retention times were observed for different eluents and small adjustments in pH.

Nesterenko expanded his investigations using L-arginine (Arg), L-valine (Val), L-tryrosine (Tyr), L-proline (Pro), and Hypro [60–62]. Amino acids were again attached to the silica through the *N*-terminus, and the ligand's acid-base properties were used to tune ion interactions. Cation and anion-exchange properties of each amino acid were determined in addition to varying effects of carboxylic acid eluent concentration and pH. Immobilized Pro and Hypro successfully separated 6–8 various anions in a single solution under acidic conditions. Immobilized Val and Tyr were characterized as pure cation exchangers as the secondary amine interactions with surface silanol groups make anion–ligand interactions negligible. Surprisingly, Arg was also characterized as a cation exchanger with poor separation of anions. The amine functionalities of Arg are also hindered as a result of interactions with surface silanol groups. Interactions between surface silanol groups and charged sites of the amino acid in these systems tend to dictate ion selectivity. It was postulated that the basicity of the amino groups enhances charge localization due to a change in the multilayer structure at the silica surface and ultimately establishes the exchange properties of the attached amino acid. Asp and Glu, amino acids possessing additional carboxylate functionalities, were also studied [63]. Various solutes such as alkali and alkaline earth metal cations were used in the study along with six benzene derivatives for sorbent evaluation. Asp and Glu were shown to be efficient cation exchangers.

Investigations of bound amino acid-metal cation interactions (i.e., complex forming or ion-exchange) were done using Glu [64]. Glu was chosen because of the relatively stable complexes it forms with metal cations and was evaluated using alkali, alkaline earth, and transition metals. Conditions such as pH, ionic strength, organic solvent, and temperature were varied. An increase in the nonpolar and also the proton-accepting character of organic solvents showed marked increases in capacities and changes in the metal binding character. Also, at both high ionic strength and pH, the chelate effect was shown to prevail over ion-exchange mechanisms.

In recent studies, Kiseleva et al. examine the zwitterionic-exchange properties of commercially available silica-bound poly-aspartic acid (PAsp) [65]. PAsp is attached to surface amine groups through the carboxylate functionalities and thus aligns parallel to the silica surface. The stationary phase contains neutral amide groups along with residual aminopropyl and unreacted carboxylate groups. The poly-amino acid was able to simultaneously separate anions and alkali and alkaline earth metal cations showing the utility of using zwitterionic-exchange column for both cation and anion separations. The optimal pH range for PAsp bound silica is 3.0–3.5 due to the zwitterionic character of the various surface groups.

Additionally, Liu and Sun have shown that Cys immobilized onto a polyacrylonitrile-divinylbenzene resin has a significant affinity for Ag^+, Hg^{2+}, Au^{3+}, Pt^{4+} with capacities in the range of 0.39–1.22 mmol of metal/g of resin [66]. It was also shown that the immobilized Cys resin is capable of separating these metals chromatographically. In a mixed solution, Pt^{4+}, Hg^{2+}, and Ag^+ were eluted sequentially and Au^{3+} was retained by the column and eluted off with 0.1 % thiourea in 0.1 M hydrochloric acid.

These researchers also conducted a comparison of the ability of three chelating ion-exchange resins to separate Mo^{6+} and W^{6+} [67]. The three functionalities immobilized onto the polyacrylonitrile-divinylbenzene resins were thioglycollic acid linked by 1,6-hexanediol, thioglycollic acid linked by ethylene glycol, and Cys linked by 1,6-hexanediol. After the initial run of Mo^{6+} and W^{6+}, which was unsuccessful in the separation, the Cys resin was not investigated further.

Immobilized amino acid/peptide for Cd^{2+} removal from human plasma

Removal of heavy metals from water is certainly a significant environmental problem. If water supplies were contaminant free, heavy metal poisoning would not be a concern. Unfortunately, this is not the case, and there is currently no specific affinity adsorbent treatment for Cd^{2+} poisoning [68]. Bektas and coworkers immobilized cysteine onto poly(2-hydroxyethylmethacrylate) (PHEMA) microspheres. The PHEMA microspheres were synthesized from a suspension of HEMA and EGDMA [69]. They demonstrated that these microspheres were capable of binding 0.065 mmol Cd^{2+}/g of support from human plasma. Additionally, they can be reused without significant loss of capacity.

In another attempt to develop a method for removal of Cd^{2+} from human plasma, Denizli et al. immobilized cysteinylhexapeptide (CysHP) to poly(2-hydroxyethylmethacrylate) beads [68]. The sequence of the hexapeptide was Lys-Cys-Thr-Cys-Cys-Ala (alanine), and it was immobilized to the beads through a monochlorotriazinyl dye ligand, Cibacron Blue F3GA. The maximum Cd^{2+} bound from human plasma onto these beads in a packed-bed column-based system was determined to be 11.8 mg of Cd^{2+}/g of support.

RELATED STUDIES

Several researchers have focused on more in depth studies pertaining to the metal binding capabilities of these systems. These include the effects of oxidation on Cys chelation and preconcentration [70], the effects of temperature on Lys and Glu retention of cations [71], and the effects of heats of adsorption on PLAsp cation binding [72]. Additionally, a study was conducted in which atomic force microscopy was used to examine conformational changes in immobilized PLCys in various environments [73]. By measuring the height of immobilized PLCys from the surface of a glass slide, it was confirmed that in neutral solutions the polymer chain was generally oriented perpendicular to the surface. With the addition of a metal, the height decreased ca. 15 nm. The addition of acid decreased the height another 10–15 nm, thus supporting the idea that a significant tertiary structure change had occurred. At low pHs, the PLCys likely exists as a tight random coil on the surface. Raising the pH returned the structure to its original form.

CONCLUSION

Research involving the use of immobilized amino acids, peptides and proteins for metal remediation and similar purposes has shown great promise. Amino acids are ideal building blocks for metal chelation systems. They provide a wide range of binding functionalities and are attached to one another through simple amide linkages. These novel binders are easily attached to silica, carbon, gold, and polymeric particles; silica and cellulose based membranes; and incorporated into polymerized resins. The amino acid of interest can be immobilized through either the amine or carboxylate terminus or modified to provide other possible linkage chemistries. Studies show that immobilized amino acids, peptides, and proteins are all capable of metal capacities in the μmole–mmole/g of resin range. Much research involving the use of amino acids as zwitterion-exchange materials has also proved fruitful. In addition, metal selectivity and specificity can be achieved by altering the amino acid functionalities and/or immobilization procedures used. There are still many directions that continuing research in this area could head, including the use of peptide libraries and increased metal-template studies.

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