JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
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Selective Chromium(VI) Ligands Identified Using Combinatorial Peptoid Libraries

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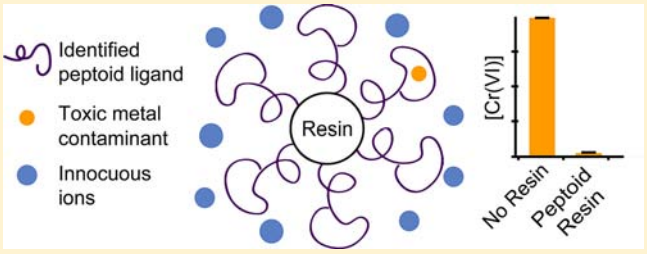
Supporting Information

ABSTRACT: Hexavalent chromium [Cr(VI)] is a worldwide water contaminant that is currently without cost-effective and efficient remediation strategies. This is in part due to a lack of ligands that can bind it amid an excess of innocuous ions in aqueous solution. We present herein the design and application of a peptoid-based library of ligand candidates for toxic metal ions. A selective screening process was used to identify members of the library that can bind to Cr(VI) species at neutral pH and in the presence of a large excess of spectator ions. There were 11 sequences identified, and their affinities were compared using titrations monitored with UV−vis spectroscopy. To identify the interactions involved in coordination and specificity, we evaluated the effects of sequence substitutions and backbone variation in the highest affinity structure. Additional characterization of the complex formed between this sequence and Cr(VI) was performed using NMR spectroscopy. To evaluate the ability of the developed sequences to remediate contaminated solutions, the structures were synthesized on a solid-phase resin and incubated with environmental water samples that contained simulated levels of chromium contamination. The synthetic structures demonstrated the ability to reduce the amount of toxic chromium to levels within the range of the EPA contamination guidelines. In addition to providing some of the first selective ligands for Cr(VI), these studies highlight the promise of peptoid sequences as easily prepared components of environmental remediation materials.



INTRODUCTION

Water contamination from manufacturing and mining activities has been a problem since the industrial revolution, providing a constant need for new technologies that can remove toxic chemicals from drinking water supplies and purify industrial waste streams. While there are practical methods currently in use, there remain many pollutants that are very difficult to remove in a cost-effective fashion. As a particularly notable example, chromium(VI) species produced by leather tanning, chrome plating, and other industrial activities have polluted water supplies in communities worldwide.[1] In some locations, drinking water contamination can reach up to 250 times the limit dictated by the World Health Organization.[2] Though the biological mechanism is unknown, the demonstrated health effects of Cr(VI) exposure include sensitization of the skin and an elevated risk of lung cancer.[3] A variety of methods have been explored for the removal of Cr(VI) and other heavy metal contaminants, including activated carbon adsorption, biosorpents, inorganic particles and membranes, electrochemical treatment, and ion-exchange resins.[4−6] However, many of these methods are expensive due to physical sensitivity of the materials or a lack of selectivity that requires large quantities for effective chromium removal. These limitations have prevented the widespread adoption of a cost-effective strategy for the removal of Cr(VI) and other heavy metals from contaminated areas.

The major challenge in developing materials for remediation is selectivity. Heavy metal contaminants are often found in concentrations that are orders of magnitude lower than innocuous ions in water (e.g. Na^+, Cl^-, Mg^{2+}, SO_4^{2-}, CO_3^{2-}, etc.). Therefore, for the materials to be efficient, they must have a substantially higher affinity for the contaminating ions than for the harmless ones. A few selective metal chelators have been identified for use in biological applications,[7−11] but otherwise ligands have rarely been designed to discriminate between ions. The rational design of a selective ligand for Cr(VI) is particularly difficult due to the limited number of ligands that are currently known.[12] Additionally, because of its potent oxidative reactivity, the pursuit of well-defined Cr(VI) complexes is uncommon. For these reasons, combinatorial chemistry, which has previously been applied to identify new transition-metal complexes and catalysts,[9,10,13,14] provides a particularly attractive approach for the identification of selective binders for Cr(VI) species.

Peptoids, or N-substituted glycine oligomers, are uniquely appropriate for this application due to their modular synthesis,

Received: August 24, 2013
Published: November 6, 2013

   dx.doi.org/10.1021/ja408788t | J. Am. Chem. Soc. 2013, 135, 17488−17493

wide variety of potential monomers, resistance to enzymatic degradation, and relatively low synthetic cost.[15,16] Previous work has, in fact, shown that peptoids can be designed to bind to metals.[14,17−19] However, neither their selective binding abilities nor their ability to bind to Cr(VI) species has been explored. In this work, we have developed a library of peptoids that can bind to a wide variety of metal ions. We have also developed a screening method that selectively reports the members of the library that can bind to Cr(VI) ions even in a complex mixture of other ions. This has resulted in a new class of binders for this toxic metal that can be prepared easily on solid supports for use in remediation applications.

RESULTS AND DISCUSSION

Library Design and Synthesis. To identify new peptoid-based ligands for metal ions, the solid-phase library depicted in Figure 1a was first synthesized. Because of its compatibility with both the peptoid synthesis conditions and the acid-induced cleavage of the side chain protecting groups, a photocleavable moiety[20,21] was chosen to link the library members to the 140−170 μm PEG-grafted polystyrene beads. To facilitate library sequencing using MALDI-TOF MS/MS spectrometry, two 6-aminohexanoic acid residues and a 4-bromo-D-phenylalanine residue were also included. These spacing groups added a distinctive isotopic tag (^{79}Br/^{81}Br) to each structure[22,23] while simultaneously increasing the overall mass of the compounds.

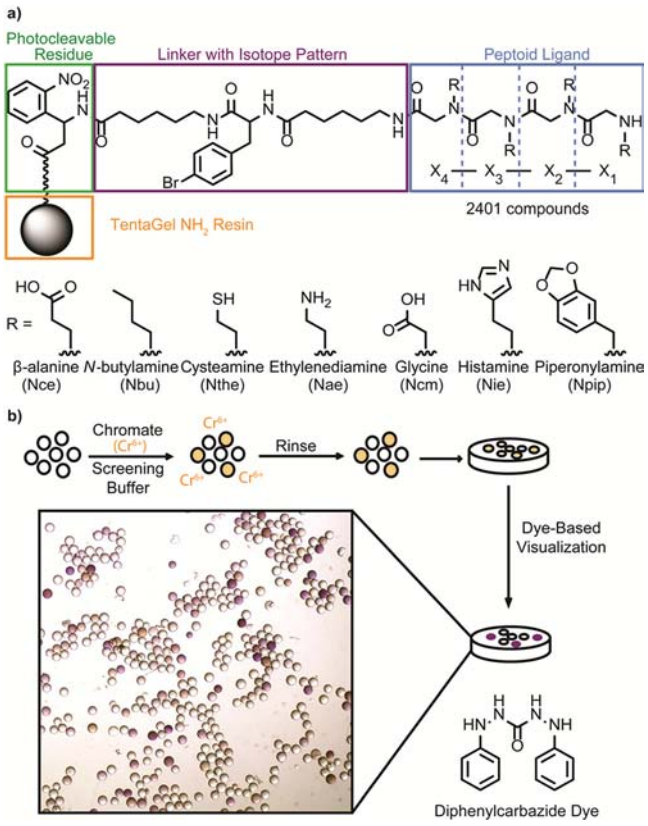


Figure 1. Outline of the peptoid library design and overall screening procedure. (a) The diagram depicts the linking groups and variable positions, as well as the amine monomers that were incorporated into each of the "X_n" sites. (b) The library was screened for metal ion binding as shown. A representative photograph shows a portion of the library after incubation with $Cr(VI)_{aq}$, rinsing, and treatment with a diphenylcarbazide dye.

The peptoid segment was synthesized using the submonomer method[24] with chloroacetic acid.[25] Amine monomers were incorporated at each of four variable positions to generate structures with the potential to form metal ion binding pockets. Seven different amines were selected for incorporation: five with heteroatoms capable of metal ion coordination (Nce, Nthe, Nae, Ncm, and Nie in Figure 1a) and two chosen to add nonbonding interactions to the library (Nbu and Npip). Before inclusion, the incorporation ability of each amine monomer was validated through the synthesis of repetitive "sandwich" sequences with benzylamine, as previously described.[26] Using split-and-pool synthesis techniques,[27,28] a library of 2401 unique structures was produced. Following synthesis, the side chain protecting groups were cleaved using trifluoroacetic acid.

Screening for Selective Metal Affinity. The key objective of this work was the identification of ligands that could bind chromium(VI) ions in complex ionic solutions with high affinity. This was achieved using the screening procedure outlined in Figure 1b. Portions of the library were first incubated with solutions containing 2 mM CrO_3 and a high salt screening buffer (1 M NaCl, 1 M $MgSO_4$, 20 mM $NaHCO_3$, pH 7). Upon dissolving CrO_3 in water, a mixture of chromate and dichromate ions is formed,[29] which will be referred to as $Cr(VI)_{aq}$ in this work. After 1 h, the beads were isolated via filtration and rinsed briefly with water. A number of the library members were observed to have taken on a pale yellow color at this point. To facilitate the unambiguous identification of the chromium complexes, a solution of diphenylcarbazide[30] was next added. This resulted in the formation of a bright pink precipitate, which remained trapped in the resin matrix.[31] As this detection method relies on the oxidation of the carbazide group to generate the color,[32] it also confirmed that the bound metal ions were in the (VI) oxidation state. The darkest pink beads were individually selected from the library for metal removal, photocleavage, and sequencing by mass spectrometry, as described in the Supporting Information.

There were 11 sequences identified in the screen (Figure 2a; see also Supporting Information, Figure S1, for a sequencing example and Figure S2 for all structures), with sequence 5 being identified twice. There was a substantial degree of similarity among the ligands, with the Nthe monomer consistently being present in position X_2 or X_3. This is consistent with previous studies of the interactions of Cr(VI) ions with thiol containing metabolites.[12] However, the absence of structures in which this monomer was in position X_1 or X_4 suggested that other interactions were also participating. Additionally, each sequence contained at least one nonchelating residue, and almost always in position X_1.

To confirm that the coordination of the isolated structures was metal-specific, the library was also screened for nickel(II) binding under similar conditions. In this case, dimethylglyoxime was used to visualize the complexes.[33] As would be expected from previous studies involving peptide-based ligands,[31] the identified peptoids contained a large number of imidazole groups (sequences appear in Supporting Information, Figure S3). They bore no resemblance to the $Cr(VI)_{aq}$-binding ligands, confirming the ability of the library design and screening approach to provide successful coordination motifs for widely varying species.

Binding Affinity Characterization. To validate the ability of the identified peptoids to interact with Cr(VI) species and to compare their relative affinities, all of the 11 identified sequences were resynthesized for use in titration experiments.

dx.doi.org/10.1021/ja408788t | J. Am. Chem. Soc. 2013, 135, 17488−17493

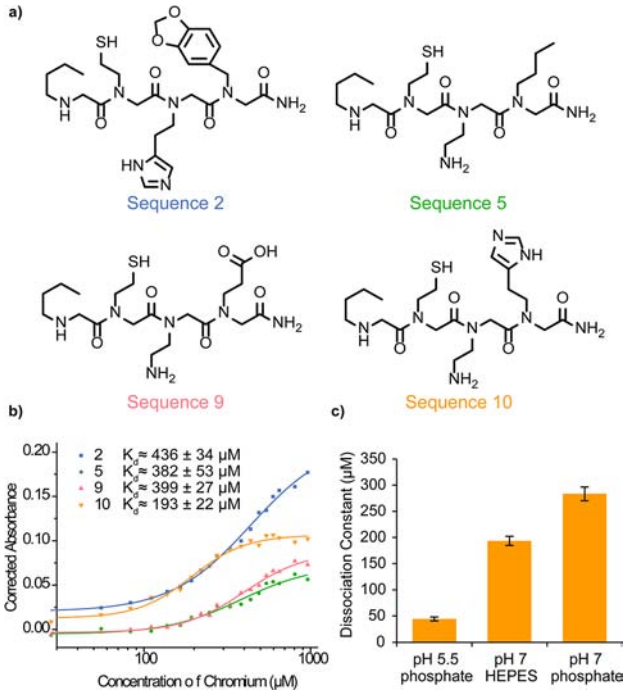


Figure 2. Summary of identified sequences and their Cr(VI) binding affinities. With UV−vis spectroscopy, binding constants were determined for each sequence at 300 μM in 10 mM HEPES buffer (pH 7). (a) The four highest affinity sequences identified from the screen are shown. (b) Binding curves are plotted for each of the four sequences in HEPES buffer as $Cr(VI)_{aq}$ was added. The data were fit using a logistic function and the K_d values were approximated by the inflection points of the curves. (c) The affinity of sequence 10 for $Cr(VI)_{aq}$ was also measured in phosphate buffer at pH 5.5 and 7 (shown) and pH 8 (not shown). The error bars represent the standard error in the logistic fit.

The ligand candidates were prepared on Rink Amide resin and purified by reverse-phase HPLC (Supporting Information, Figure S4). Each of the sequences was characterized through the addition of $Cr(VI)_{aq}$ while monitoring complex formation using UV−vis spectroscopy. At each concentration, the spectrum of the peptoid−$Cr(VI)_{aq}$ mixture was compared to a control sample that lacked the ligand. An increased absorbance due to complex formation was found at 457 nm (Supporting Information, Figure S5a−c), similar to wavelengths previously established for a S → Cr charge-transfer.[12] A corresponding decrease in the absorbance of free $Cr(VI)_{aq}$ was also observed. The dissociation constants were approximated as the midpoints of sigmoidal fits to the data assuming one-to-one association of the peptoid and $Cr(VI)_{aq}$. It is recognized the accuracy of these values may be confounded by competing processes, such as the oxidation of the thiols to disulfides or sulfonates, but the NMR spectra discussed below suggest that complex formation is the most significant reason for the observed spectral shifts. The measured values should be taken as approximations; nonetheless, they provide a ready means by which to compare the performance of the identified binding sequences.

The structures, binding curves, and estimated dissociation constants for the highest affinity sequences are shown in Figure 2, and the full set of binding data appears in Supporting Information Figure S5d. Each of the best sequences possessed a side chain group in position X_3 that is positively charged at pH 7, an Nthe in position X_2, and an Nbu in position X_1. The significance of the positively charged residues can be rationalized by interactions with the negatively charged chromate and dichromate anions in neutral solution. To support this assertion, we evaluated the binding affinity of sequence 10, the sequence with the highest affinity, at various pH values. As expected, a lower pH of 5.5 resulted in stronger observed binding between the ligand and the metal, whereas the affinity at pH 8 was too low to measure accurately with this technique (Supporting Information, Figure S6).

Previous examples of $Cr(VI)_{aq}$ complexes have involved thiol ligands.[34] To verify that additional moieties of sequence 10 were also required for binding, a solution of glutathione was evaluated using the same titration method. A dissociation constant above 1 mM was observed at pH 7, and a dissociation constant of 708 ± 170 μM was measured at pH 5.5 (Supporting Information, Figure S6e). The affinity has been previously reported at low pH, measuring a dissociation constant of 673 ± 23 μM at pH 1.[35] This dissocation constant is 10-fold higher than that of sequence 10 under the same conditions, confirming the importance of nonthiol residues in the structures.

Varying Peptoid Structure To Elucidate Binding Interactions. As a first step toward probing the interactions of sequence 10 with $Cr(VI)_{aq}$, we synthesized a series of sequence analogues with different monomer orders and substitutions (Figure 3, full structures in Supporting Information, Figure S2). Since the sequences shown in Figure 2 had a consistent order for the X_2-X_3 groups, we first looked at the impact of switching the Nae and the Nthe residues. The binding affinity appeared to be negligibly affected by this change; however, when the entire sequence was reversed the measured dissociation constant increased by nearly 2-fold. Since the peptoid backbone is achiral, this is likely due to the N-terminal residue switch from Nbu to Nie. To confirm the involvement of this component, the affinity of sequence 10 was measured after acylation of the secondary amine in position X_1. The dissociation constant was even higher than that of the reversed sequence. To investigate the contribution of the X_4 and X_3 residues, they were sequentially replaced with nonbinding Nbu. One of these substitutions (Nbu in place of the initial Nie) was identified from the screen (sequence 5), but the other substitutions were not identified from the screen and were therefore additionally synthesized. The sequences with Nbu substituted for Nae and Nie retained their affinity, with measured dissociation constants similar to that of the reversed sequence; however, the sequence in which the Nthe had been replaced had a significantly decreased affinity that could not be determined with the titration. This and the prevalence of the Nthe in the identified sequences suggested that the thiol is more directly involved in the metal binding than the other residues.

Relative to peptides, which contain chiral α-amino acids, peptoids can sample a substantially greater conformational space. This could be viewed as both a positive and a negative feature: the additional conformations could allow multidentate binding interactions that peptides cannot accommodate, but a significant entropic penalty could accompany complex formation. In an effort to introduce some degree of conformational bias into the structures, a "turn" sequence, similar to the D-Pro-Gly sequence used in peptides,[36−38] was introduced into the identified binding motif of sequence 10, Figure 3b. A pair of extended glycine analogues with internal ethylene glycol groups (1PEG and 2PEG) were also

dx.doi.org/10.1021/ja408788t | J. Am. Chem. Soc. 2013, 135, 17488−17493

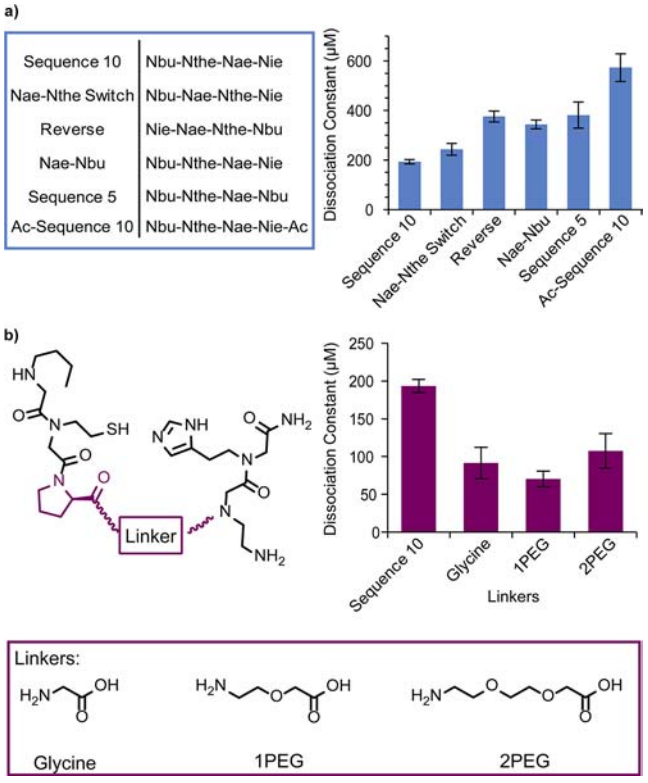


Figure 3. Enhancing binding affinity through variations of sequence 10. Full structures of all sequences are shown in the Supporting Information. (a) A series of sequence 10 variants was synthesized to examine the importance of sequence order and to verify the binding contributions of the individual residues. The binding affinity of each ligand was determined at 300 μM in 10 mM HEPES buffer (pH 7) through titration with $Cr(VI)_{aq}$. (b) To explore the influence of enhanced flexibility, several sequence 10 derivatives were prepared with a D-proline turn and a glycine, 1PEG, or 2PEG linker. The affinity of the synthesized sequences is compared to that of the original sequence 10. The binding affinity of each new ligand (measured at 150 μM) was determined by titration with $Cr(VI)_{aq}$ in 10 mM HEPES buffer (pH 7).

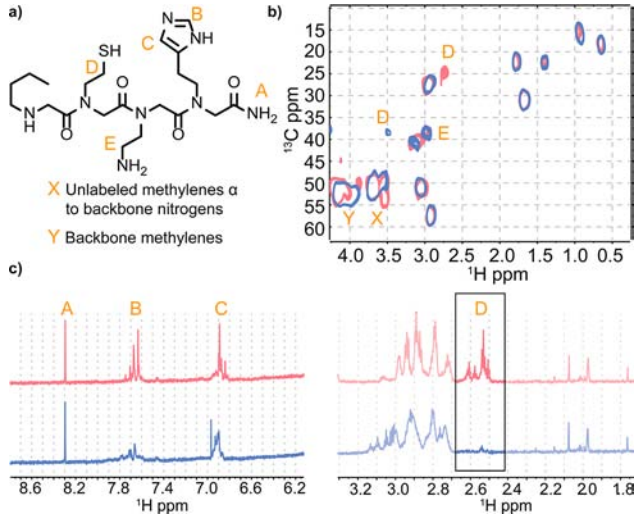


Figure 4. NMR characterization of sequence 10 in the presence of $Cr(VI)_{aq}$. All spectra were obtained in 10 mM phosphate buffer (pH 7) with 10% D_2O. (a) Key resonances appearing in the NMR spectra of sequence 10 are annotated. (b) HSQC NMR (500 MHz, 60 °C) spectra are shown of sequence 10 alone (pink) and in the presence of excess $Cr(VI)_{aq}$ (blue). (c) ^{1}H NMR (900 MHz, 25 °C) spectra are shown for sequence 10 alone (pink) and with excess $Cr(VI)_{aq}$ (blue).

incorporated to explore the effects of conformational flexibility further. The binding of each of the three structures was evaluated with UV−vis, and indicated that all of them possessed a 2-fold improvement in affinity over the parent peptoid (sequence 10). The similarity of the measured dissociation constants suggested that the D-proline was the main contributor to the increased affinity, but more characterization would be necessary to determine the specific role of the moiety. For example, the simple extension of the backbone chain could also allow a better binding pocket to be formed and thus produce increased affinity.

Complex Characterization by NMR. Several Cr(VI) complexes have been structurally characterized,[12] and the interactions of peptoids with other metal ions (such as Zn^{2+}) have been studied.[17,19] However, these ligands bear little resemblance to peptoid sequence 10, and therefore this complex was characterized using NMR spectroscopy (Figure 4, full spectra in Supporting Information, Figure S7). Overall, the large number of conformers and overlapping signals in the ^{1}H NMR spectrum of sequence 10 severely limited interpretation of the backbone resonances. However, the spectrum revealed multiple singlets that clearly corresponded to the two aromatic protons of the imidazole group (resonances B and C in Figure 4c), as well as a distinct signal for the protons α to the thiol group (resonance D at 2.6 ppm). The latter assignment was confirmed by the shift of the resonance upon disulfide formation (Supporting Information, Figure S8). After the addition of $Cr(VI)_{aq}$ the imadazole peaks broadened, suggesting weak interactions with the chromate. In addition, the peak at 2.6 ppm shifted substantially, as would be expected if the thiol were directly involved in metal ion binding. Most notably, the peaks in the overall spectrum remained sharp, indicating that a diamagnetic Cr(VI) species was being bound and that minimal amounts of paramagnetic chromium ions (which would result if the ligand had been oxidized) were present.

To assign the relevant peaks further, ^{1}H−^{13}C HSQC and ^{1}H TOCSY spectra were obtained in DMSO at 60 °C to coalesce the rotamer signals (Supporting Information, Figure S9). Following this, a second round of high-temperature HSQC analysis was performed in phosphate buffer (10 mM, pH 7) for samples both with and without $Cr(VI)_{aq}$. Analysis of the spectra showed that the methylene neighboring the thiol (Figure 4, signal D) had shifted from 2.6 to 3.5 ppm. The new peak position is similar to previously reported characterizations of thiol−Cr(VI) complexes.[39,40] Additionally, the formation of two equivalent peaks corresponding to the methylene α to the primary amine appeared upon addition of $Cr(VI)_{aq}$ (Figure 4, signal E). This could be due to differentiation of the two hydrogens upon the formation of a new chiral center when the terminal amine binds to the chromate, or due to stabilization of two different rotamers. Furthermore, shifts in the remaining methylenes that were α to the side chain nitrogens (Figure 4, signal X) and the backbone methylenes (Figure 4, signal Y) were also apparent, possibly due to a change in the shift of the methylene group beta to the thiol.

Chromium Depletion Assays. As the peptoid ligands were prepared on polymer supports, a convenient platform was already available for the selective solid phase extraction of $Cr(VI)_{aq}$ species from contaminated water sources. This ability

dx.doi.org/10.1021/ja408788t | J. Am. Chem. Soc. 2013, 135, 17488−17493

was tested using water samples taken from Strawberry Creek (Berkeley, CA) and Ocean Beach (San Francisco, CA). Contamination was simulated by adding $Cr(VI)_{aq}$ to the samples at concentrations of 20 and 200 μM (1 and 10 ppm). A third pair of chromium-containing samples was prepared using 10 mM phosphate buffer (pH 7) with the same $Cr(VI)_{aq}$ concentrations to evaluate metal ion removal under more controlled conditions. Sequence 10, the highest affinity ligand of the identified sequences, and 1PEG-S10 (sequence 10 with the D-Pro and 1PEG turn) were synthesized on water-compatible TentaGel beads. The sequence identities were again confirmed by photocleavage of a small portion of each resin, followed by MALDI-TOF MS (Supporting Information, Figure S7). In addition to the sequence 10 and 1PEG-S10 ligands, a commercially available anion-exchange resin, DOWEX Monosphere 550A, was evaluated for comparison. Each resin sample was incubated with each of the different contaminated solutions. The ligands were added in approximately 10-fold molar excess of the $Cr(VI)_{aq}$ in solution. After a 2 h incubation period, the concentration of chromium in the supernatant was measured using ICP-OES (Figure 5).

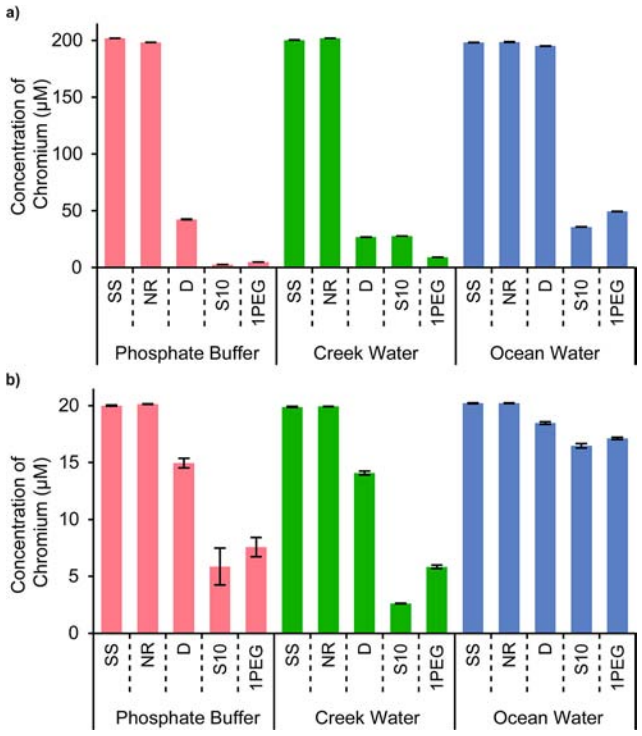


Figure 5. Use of peptoid ligands for the depletion of Cr(VI) species from environmental samples. After exposure to various resin samples, the supernatants were analyzed using ICP-OES with five replicates. The error bars represent the standard error of each sample set (n = 5). SS = starting solution, NR = no resin added, D = Dowex Monosphere 550A, S10 = sequence 10, and 1PEG = 1PEG-S10. Data are shown for initial $Cr(VI)_{aq}$ concentrations of (a) 200 and (b) 20 μM.

In nearly every solution, sequence 10 and 1PEG-S10 were substantially more effective than the DOWEX resin for $Cr(VI)_{aq}$ removal. This effect was particularly evident as the ionic strength of the solution increased relative to the metal ion concentration, which was consistent with the selection criteria used in the binding screen. In many cases, the peptoid ligands reduced the metal ion concentrations to values close to the EPA limit for drinking water, 2 μM.[41] Even in the ocean water sample with 200 μM $Cr(VI)_{aq}$, where competing ions such as sodium and chloride are in ~10 000-fold excess, more than 80% of the chromium was removed. The remediation ability of sequence 10 was inhibited only when the chromium concentration in the ocean water sample was reduced to 20 μM. Interestingly, the binding ability was still retained at 20 μM in the freshwater and phosphate buffer samples. In the Strawberry Creek water sample, the 1PEG-S10 ligand was more effective than sequence 10 at 200 μM $Cr(VI)_{aq}$, which corresponds with its lower dissociation constant. However, as the ratio of chromium to other ions decreased in the 20 μM samples, sequence 10 became more effective than 1PEG-S10. A possible explanation is the loss of some selectivity when the turn was incorporated. This observation reinforces the necessity of screening all of the ligands with an excess of competing ions to achieve maximum selectivity.

Final experiments sought to confirm our previous analysis of the binding moieties. The first task was to demonstrate that the thiol group, and not a sulfonate group produced through oxidation, was interacting with the Cr(VI) species. When sequence 10 was preoxidized using sodium periodate (confirmed to produce the sulfonate group, as shown in Supporting Information Figure S10), the resulting beads were ineffective in removing the metal ions.

There are many other commercially available products that contain thiols and thioureas; however, our results clearly indicate that the additional components supplied by the peptoid significantly contribute to the binding. As further confirmation, we incubated the resin with solutions of chromium and glutathione, both at a concentration equal to that of the $Cr(VI)_{aq}$ and additionally at a 1:1 molar ratio with the peptoid (Figure S10). Our sequence retained its ability to remove more than 80% of the chromium in both solutions, reaffirming the increased effectiveness of the identified structure.

■ CONCLUSIONS

Through this work, we have developed a new platform for the identification of selective ligands using a combinatorial peptoid library. The chromium(VI) ligands that were identified provide some of the first examples of selective binders for this pollutant, and they demonstrate the promise of peptoid sequences as easily prepared components of environmental remediation materials. Further improvements in binding affinity may be achievable through further exploration of the backbone structures, including the introduction of conformationally restricted spacing groups. This strategy is currently being explored in ongoing studies. Moreover, the chromogenic screening method is likely to be successful for the identification of selective binders for a wide variety of different metal ions. We are currently applying this method to the discovery of new chelators for lanthanides, actinides, and other species that would benefit from the availability of new ligand structures.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full experimental procedures and additional characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Funding for this work was generously provided by the NSF (CHE 1059083). Work at the Molecular Foundry was supported by the Office of Science, Office of Basic Energy Sciences, of the U.S. Department of Energy under Contract DE-AC02-05CH11231. Additionally, Dr. Ronald Zuckermann and Michael Connolly at the LNBL Molecular Foundry are gratefully acknowledged for instrumentation support and helpful advice. A.S.K. was supported by the Berkeley Chemical Biology Graduate Program (NIH Training Grant 1 T32 GMO66698) and a Philomathia Fellowship in Environmental Sciences. Summer stipend support for E.Y.Z. was provided by the UC Berkeley Chemistry Department. We also acknowledge support for the Central California 900 MHz NMR facility through grant GM68933 from the National Institutes of Health.

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