

Customizable biopolymers for heavy metal remediation

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Abstract

Nanoscale materials have been gaining increasing interest in the area of environmental remediation because of their unique physical, chemical and biological properties. One emerging area of research has been the development of novel materials with increased affinity, capacity, and selectivity for heavy metals because conventional technologies are often inadequate to reduce concentrations in wastewater to acceptable regulatory standards. Genetic and protein engineering have emerged as the latest tools for the construction of nanoscale materials that can be controlled precisely at the molecular level. With the advent of recombinant DNA techniques, it is now possible to create 'artificial' protein polymers with fundamentally new molecular organization. The most significant feature of these nanoscale biopolymers is that they are specifically pre-programmed within a synthetic gene template and can be controlled precisely in terms of sizes, compositions and functions at the molecular level. In this review, the use of specifically designed protein-based nano-biomaterials with both metal-binding and tunable properties for heavy metal removal is summarized. Several different strategies for the selective removal of heavy metals such as cadmium and mercury are highlighted.

Introduction

Soil and groundwater contamination by heavy metals is prevalent at hazardous waste sites in the United States. Annually, fuel and power industries generate 2.4 million tons of heavy metal waste, while agriculture and waste disposal add another 2 million tons per year. These metals when discharged or transported into the environment may undergo transformations and can have a large environmental, public health, and economic impact (Gadd & White, 1993). More than 60% of sites listed on the National Priorities List (NPL) list heavy metals as contaminants of concern. Many of these heavy metals such as arsenic, lead,

mercury and cadmium are ranked 1st, 2nd, 3rd and 7th, respectively, on the EPA's priority list. Unlike organic compounds such as trichloroethylene (TCE), perchloroethylene (PCE), and polyaromatic hydrocarbons (PAHs), heavy metals cannot be degraded readily to non-toxic end products *in situ*.

Over nearly the past 25 years, a wide variety of different heavy metal remediation technologies have been proposed or tested. 'Polymer Filtration' is an emerging treatment technology that employs water-soluble polymers for metal chelation and ultrafiltration for removing the polymer-metal complexes (Thompson & Jarvinen, 1999). The target metal ions can be recovered from the

concentrated polymer solution for reuse or reclamation and the polymer can be regenerated. One added benefit is the potential of using these polymers for *in situ* extraction of heavy metals from contaminated soil. Although this technology is highly effective, the cost of ultrafiltration and the potential problems associated with membrane clogging make it undesirable for large-scale operation. Preferably, metal removal and polymers separation can proceed without ultrafiltration. Not only can this technology be used to remediate contaminated soils and solid surfaces and treat aqueous wastes, it can also be incorporated into facilities as a pollution prevention and waste minimization technology. The use of stimuli-responsive polymers such as poly(*N*-isopropylacrylamide) (poly-NIPAM) appears to be a promising solution to this problem (Heskins et al., 1968; Galaev et al., 1999). These tunable polymers exhibit reversible phase transitions in response to temperature or pH changes and such properties are ideal for recovery of the metal-polymer complexes. By tuning the process temperature,

reversible network formation between the individual polymers will then enable the recovery of sequestered metals by precipitation (Figure 1). After extraction of metals, the aggregated polymers can be resolubilized and reused for subsequent cycles.

Genetic and protein engineering have emerged as the latest tools in polymer chemistry for the construction of nanoscale materials that can be controlled precisely at the molecular level. With the advent of recombinant DNA techniques, it is now possible to create 'artificial' protein polymers with fundamentally new molecular organization. Unlike the statistical nature of step and chain polymerization reactions, biopolymers are specifically pre-programmed within a synthetic gene template that can be precisely controlled over chain length, composition, sequence, and most importantly properties. In this manner, it is possible to specifically design protein-based nanobiomaterials with both metal-binding and tunable properties that can be used to selectively remove heavy metals from dilute solutions.

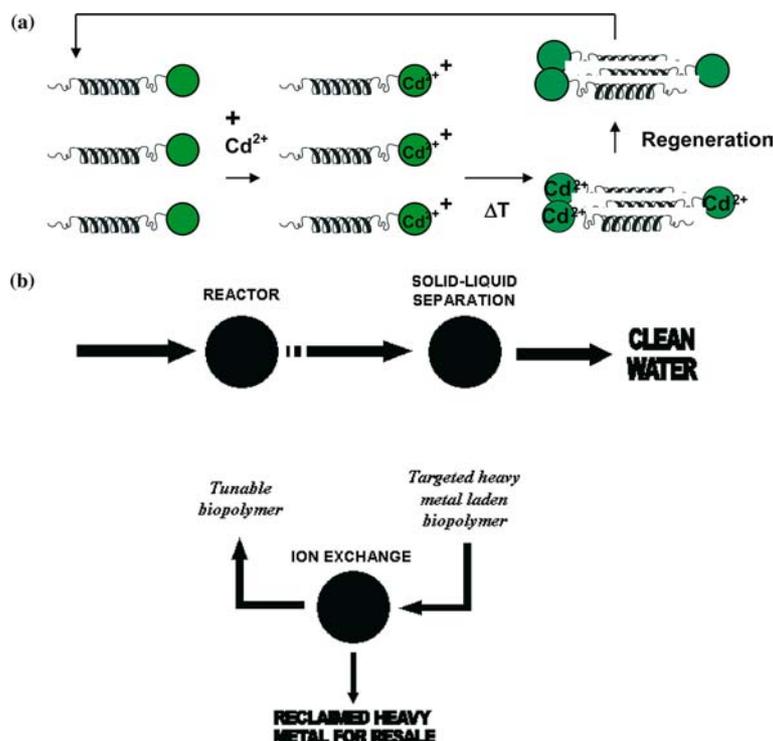


Figure 1. (a) Principle of heavy metal removal by tunable polymers/biopolymers. (b) Schematic of heavy metal removal process for groundwater treatment.

Biopolymers composed of repeating elastin units, for example, behave very similar to poly-NIPAM polymers, and have been shown to undergo phase transitions within a wide range of conditions (Urry et al., 1992). Compare to chemical polymers or chelators, these biopolymers are environmental friendly as no toxic chemicals are required for their synthesis and they can be easily produced in mass quantity and regenerated. Selected metal-binding proteins can be fused to the tunable biopolymers to provide metal-binding functionalities (O'Halloran et al., 1989; Diorio et al., 1995; Sousa et al., 1996; Bae et al., 2000). The use of these metal-binding domains has significant advantages over existing chemical chelators, including higher specificity and affinity. The potential lower limit for heavy metal removal could be on the order of 10^{-10} M, or about eight parts per trillion (Bontidean et al., 1998; Ramanathan et al., 1998), depending on the metal-binding domain employed. In this paper, we will highlight the tunable biopolymer technologies developed in our laboratory and their potential applications and future uses of particular relevance.

Tunable biopolymers based on a polyhistidine metal-binding moiety

The first metal-binding, tunable biopolymer was created based on a polyhistidine tail as the metal-binding moiety (Kostal et al., 2001). Six different biopolymers consisting of an N-terminal 38-, 48-, 58-, 68-, or 78-repeating VPGVG domain, and either one hexahistidine or two hexahistidines at the C-terminal were generated. All biopolymers were easily produced in *E. coli* BLR(DE3) and purified by taking advantage of their inherent temperature responsive properties (Figure 2). The reversible phase transition behaviors of the biopolymers were investigated and a range of transition temperatures (T_t) from $>40^\circ\text{C}$ to $<20^\circ\text{C}$ were obtained by controlling the salt concentration and the chain length of the biopolymers (Kostal et al., 2001). The transition was completely reversible, and total solubilization of the biopolymers was observed when the temperature was decreased below T_t .

Independent of the number of elastin repeats, all biopolymers containing a hexahistidine domain bound Cd^{2+} at a 1:1 ratio, while no binding was

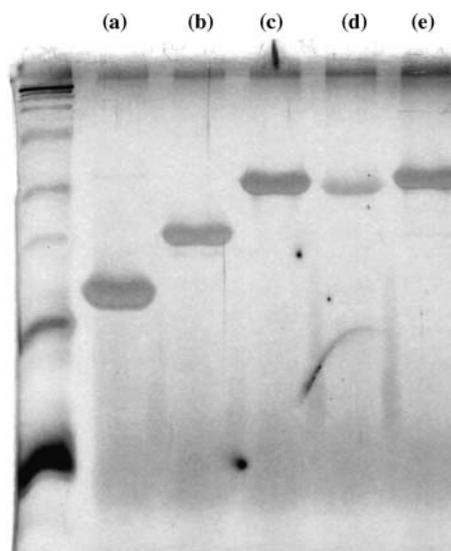


Figure 2. Purification of biopolymers by the reversible temperature transition. (a) ELP38H6, (b) ELP58H6, (c) ELP78H6, (d) ELP78, and (e) ELP78H12.

observed with a similar elastin biopolymer (ELP78) without the hexahistidine tag. The addition of six extra histidines increased the Cd^{2+} binding capacity to a ratio of 1.5:1, suggesting that the metal-binding capability of the biopolymers can be easily modified. Regeneration of the metal binding sites for repeated usage was achieved by incubating the biopolymer–cadmium complexes with a ‘stripping buffer’ composed of a pH 4.0 acetate buffer. The regenerated biopolymer aggregates were resolubilized below 25°C and remained fully functional even after four repeating cycles of cadmium binding. In addition, these tunable biopolymers offer the advantages of being easily regenerated and reused for many repeating cycles. Production and purification of biopolymers are based on the same phase transition principle and could be easily scaled-up to kilogram quantity; therefore, providing a low-cost and environmentally benign technology for heavy metal removal.

Tunable biopolymers based on metalloregulatory proteins

Although a polyhistidine domain was used successfully to create tunable, metal-binding biopolymers, the use of histidine clusters offers no selectivity, low affinity, and a narrow working pH

range. One group of interesting metal-binding proteins that could provide high affinity and specificity is typically associated with metal resistance. Many bacteria develop resistance to heavy metals such as mercury and arsenic by inducing the expression of an array of resistance proteins, many of which have very high affinity and specificity toward these metals. Examples are the MerR protein and the ArsR protein, which are regulatory proteins used for controlling the expression of enzymes responsible for mercury and arsenic detoxification, respectively (O'Halloran et al., 1989; Diorio et al., 1995). The affinity of these proteins for mercury and arsenate, respectively, is typically in the 10^{-10} M range, on par with those of metallothioneins and phytochelatins. However, the clear advantage of these proteins is their specificity. The binding affinity of MerR and ArsR protein is at least 4–5 order of magnitude higher for mercury and arsenate, respectively, than other heavy metals (Bontidean et al., 1998; Ramanathan et al., 1998).

Based on this concept, tunable biopolymers were engineered for the selective removal of mercury. A bacterial metalloregulatory protein, MerR, which binds mercury with an unusually high affinity and selectivity, was fused to ELP to provide the highly selective nature of the biopolymers (Kostal et al., 2003). Selective binding of mercury was demonstrated at an expected ratio of 0.5 mercury/biopolymer with minimal binding of competing heavy metals (cadmium, nickel, and zinc even at 100-fold excess) was observed. The sequestered mercury was extracted easily, allowing reuse of the biopolymers. In repeating operations, mercury in contaminated water was reduced to concentrations below the required drinking water limit. The practical utility of the biopolymers with mercury-contaminated Lake Elsinore water was demonstrated with no decrease in efficiency. As nature offers a wide selection of specific metalloregulatory proteins, this technology offers promising solutions to remediation of other important pollutants such as arsenic or chromium.

Tunable biopolymers for soil remediation

Over nearly the past 25 years, a wide variety of different heavy metal remediation technologies have been proposed or tested for soil remediation. These technologies fall into one of two different

categories or combinations of both: (1) contaminant removal via extraction (Semer & Reddy, 1996) or (2) stabilization/immobilization (Singh & Oste, 2001) of the contaminants to prevent migration of the heavy metals from the contaminated areas. Generic technologies in this first category include soil washing (*ex situ*) and soil flushing (*in situ*). Stabilization and/or immobilization technologies include solidification (metal contaminants are physically bound or trapped within a stabilized mass) and stabilization (chemical reactions are induced between a precipitating or strongly sorptive agent and contaminants to reduce mobility).

Despite considerable research and testing efforts, significant uncertainties remain regarding which approach is more likely to achieve remedial success for heavy metal contaminated sites (Vinogradov, 1959). These uncertainties stem from the complexities associated with metal speciation and complexation in the subsurface environment. For this reason also, most remediation technologies for heavy metals, whether for extraction or for stabilization/solidification, employ a 'shotgun' approach. Remediation technologies for heavy metals are generally non-specific. In addition to the target contaminants of concern, sizeable quantities of non-toxic inorganic species such as calcium and magnesium are also affected. One of the more common strategies is to lower the subsurface pH environment (to as low as 1) by either the addition of acids (Neale et al., 1997) or electrokinetically (Sah & Chen, 1998) generated protons to extract the target contaminant metals. While effective, this radical alteration has a major impact on the soil matrix as a whole. For these reasons, the use of biological extractants is of particular interest because of their environmentally friendly and biodegradable nature. Biosurfactants, such as surfactin (Mulligan et al., 1999) and rhamnolipid (Herman et al., 1995) have been used to enhance the removal of heavy metals from soil. The major drawbacks of these biosurfactants are their extensive adsorption onto the soil and the high concentrations (in the mM range) typically required to successfully extract soil-bound metals.

By taking advantage of the property of the ELP biopolymers to undergo a reversible thermal precipitation, easy recovery of the sequestered cadmium from contaminated soil was recently demonstrated (Prabhukumar et al., 2004) in *ex situ*

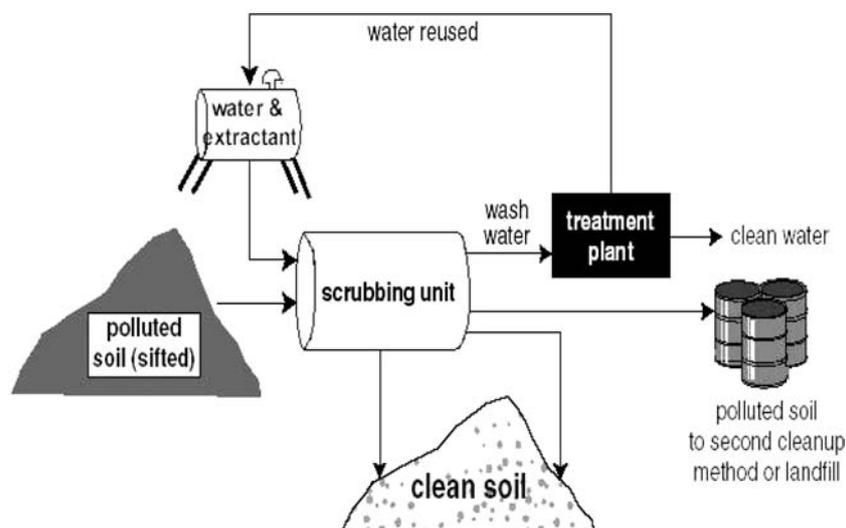


Figure 3. Schematic of the soil washing process.

soil washing experiments, which could be used for remediation and possibly metal extraction as an alternative to cyanide heap leaching. After mixing the soil with the biopolymer for 24 h, the extractant solution is separated from the washed soil. The separated extractant solution is then treated to recover contaminants and, if cost effective, to reuse the extractant. A schematic of the process is shown in Figure 3.

Batch soil washing experiments were performed to evaluate the feasibility of using ELP biopolymers as an environmentally benign strategy for removing cadmium from contaminated soil. The stability constant ($\log K_L$) for the cadmium–biopolymer complex was determined to be 6.8, a value similar to that reported for the biosurfactant rhamnolipid. Sequential washings with biopolymers were able to remove more than 55% of the bound cadmium. Unlike other biosurfactants, which adsorb extensively to soil, less than 10% of the ELP biopolymer was adsorbed under all washing conditions. As a result, a significantly lower amount of biopolymer was required to achieve a similar extraction efficiency.

Future applications

Although the results demonstrated so far using a tandem hexahistidine cluster as the metal-binding domain was promising, the level of cadmium

removal was relatively modest. Moreover, the idea of inexpensively recovering the bound cadmium by simple precipitation was not complete due to stronger displacement by other metals ions present. It should be noted that the flexibility of tailoring the desired metal-binding domain in the ELP biopolymer is a unique property that could be easily exploited for improved affinity and specificity for the target metals (Figure 4) (Kostal et al., 2001, 2003). Metal-binding peptides such as metallothionein (Sousa et al., 1998) and synthetic phytochelatins (Bae et al., 2000) have been shown to have much improved affinity for cadmium than polyhistidine. Incorporation of these cysteine-rich peptides into the ELP biopolymer could significantly enhance its ability to extract heavy metals from soil. For real-world applications, biopolymers could be designed to enable precipitation in the absence of NaCl. This is what makes the ELP technology so flexible because of the ability to control the transition properties precisely (Figure 4). Some of these strategies are currently under investigation.

Because of the versatility of the biopolymers that we can create, we also envision that tunable biopolymers will be ideal for *in situ* applications such as soil flushing or as affinity barriers. In the case of soil flushing, flush water containing tunable biopolymers may be added prior to injection into the contaminated soil or pile. As the flush passes through the soil, metal contaminants will be

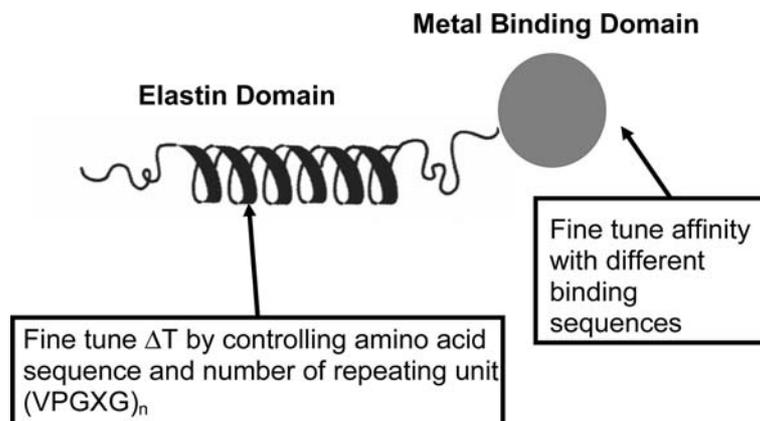


Figure 4. Customization of metal-binding and phase transition properties of the biopolymers.

extracted in its soluble form. After percolation the recovered water containing the metal laden biopolymer solution is subjected to a temperature shift and induces precipitation. The precipitated biopolymers would then be separated from the water by centrifugation or gravity sedimentation for subsequent regeneration. Regenerated biopolymers would then be added to the treated flush water for reinjection into the contaminated soil or pile.

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