An elastin-like polypeptide (ELP) composed of a polyhistidine tail (ELPH12) was exploited as a tunable, metal-binding biopolymer with high affinity toward cadmium. By taking advantage of the property of ELPH12 to undergo a reversible thermal precipitation, easy recovery of the sequestered cadmium from contaminated water was demonstrated as the result of a simple temperature change. In this study, batch soil washing experiments were performed to evaluate the feasibility of using ELPH12 as an environmentally benign strategy for removing cadmium from contaminated soil. The stability constant (log K) for the cadmium–ELPH12 complex was determined to be 6.8, a value similar to that reported for the biosurfactant rhamnolipid. Two washings with 1.25 mg/mL of ELPH12 were able to remove more than 55% of the bound cadmium as compared to only 8% with 1.25 mg/mL of ELPH12. As a result, a significantly lower concentration (in the mM range) typically required to achieve similar extraction efficiencies. However, cadmium recovery by simple precipitation was incomplete due to the displacement of bound cadmium by zinc ions present in soil. Owing to its benign nature, ease of production, and selective tailoring of the metal binding domain toward any target metals of interest, ELP biopolymers may find utility as an effective extractant for heavy metal removal from contaminated soil or ore processing.

Introduction

Soil contamination with heavy metals is a common and serious environmental problem at the majority of National Priority List (Superfund) sites (1). Commonly found heavy metals include lead, mercury, arsenic, chromium, cadmium, nickel, zinc, and copper (2). Unlike most other organic pollutants, heavy metals cannot be chemically or biologically degraded. Remediation of recalcitrant or sorbed metals in soil in an efficient and economic way is of increasing interest and importance worldwide. Many techniques are available for decontaminating soils containing heavy metals and most of them fall under two major categories: immobilization (3, 4) and extraction (5). Immobilization involves fixation of heavy metals by solidification or stabilization, thereby, preventing their migration into the groundwater. These techniques render the treated soil unfit for future applications and are typically used as a last resort. Extraction procedures employ a combination of physical/chemical/biological methods for the actual removal of heavy metals from soil. Soil washing is a promising technique that involves the transfer of heavy metals into a wash solution either by desorption or solubilization (6). Different extractants such as acids, bases, chelating agents, oxidizing agents, and surfactants have been used for this purpose (7). The use of biological extractants is of particular interest because of their environmentally friendly and biodegradable nature (8). The feasibility of using biosurfactants, such as surfactin (9) from Bacillus subtilis ATCC 21332 and rhamnolipid (10) from Pseudomonas aeruginosa ATCC 9027, to enhance the removal of heavy metals from soil has been demonstrated. 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 cadmium.

One emerging alternative to biosurfactants is the use of metal-binding biopolymers based on elastin-like polypeptides (ELP) (11). ELPs are biopolymers consisting of a repeating pentapeptide VPGVG that undergo a reversible phase transition from water-soluble forms into aggregates upon increasing the temperature (12). The temperature transition (Tc) can be precisely controlled by varying the chain length and peptide sequence (13). Unlike the statistical nature of step and chain polymerization reactions, ELP biopolymers are specifically preprogrammed within a synthetic gene template that can be easily customized with the desired properties (14). More importantly, ELP has been successfully fused to metal-binding peptides or proteins while retaining the temperature responsive property as well as the functionality of the fusion partner (11, 14). They can be produced in large quantities by E. coli and easily purified to homogeneity by taking advantage of the temperature responsive characteristics of ELP. Previously, ELP-based biopolymers, containing a polyhistidineterminel (ELPH12) as the metal chelating domain, were generated (11) demonstrating the possibility of easy recovery and regeneration of metal–biopolymer complex for many repeating cycles of cadmium removal. In this paper, we demonstrate the feasibility of using the ELPH12 biopolymer to enhance the removal of heavy metals from contaminated soil. The results presented here should pave the way for the use of ELP-based biopolymers for the environmental friendly cleanup of contaminated soil.

Experimental Methods

Soil Characterization. The soil used in this study was coarse, slightly acidic, subsurface soil obtained from the City of San Bernardino Rapid Infiltration and Extraction (RUX) wastewater treatment facility. Grain size distribution (Table 1) was performed according to the sieving and hydrometer ASTM D422 method (15). Soil was found to be mostly sand, 91%, with low amounts of silts and clay. Other soil characteristics were as follows: pH 6.5, 0.27% total organic carbon (TOC), and a cation exchange capacity of 10.1 mequiv 100 g⁻¹.

Artificially contaminated soil was prepared by soaking the soil in 1 mM cadmium nitrate for 3 months while maintaining the pH at 7.0. The soil was then rinsed with distilled water, recovered by centrifugation for 20 min at 17 700g, and dried in a vacuum oven at 120 °C overnight (Isothermal model 281A, Fisher Scientific). Dried cadmium-
TABLE 1. Particle Size Distribution of the Sandy Soil

<table>
<thead>
<tr>
<th>class name</th>
<th>particle size (mm)</th>
<th>fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>very coarse</td>
<td>1–2</td>
<td>41</td>
</tr>
<tr>
<td>coarse</td>
<td>0.5–1</td>
<td>36</td>
</tr>
<tr>
<td>medium</td>
<td>0.25–0.5</td>
<td>14</td>
</tr>
<tr>
<td>fine</td>
<td>0.25–0.1</td>
<td>7</td>
</tr>
<tr>
<td>very fine</td>
<td>0.1–0.05</td>
<td>1</td>
</tr>
<tr>
<td>silt and clay</td>
<td>&lt;0.05</td>
<td>1</td>
</tr>
</tbody>
</table>

contaminated soil was stored in closed plastic containers prior to experiments. The final cadmium content in the soil samples was determined to be 118 mg/kg soil using the USEPA Method 3050.

Production and Purification of ELPH12 Biopolymer. All cultivations were carried out in Luria Bertani (LB) media supplemented with 100 μg/mL of ampicillin. E. coli BLR (DE3) cells containing pET-Ela78h12 (11) coding for ELPH12 were grown in 3 L of medium in a BIOFLO 3000 fermentor (New Brunswick Scientific, Edison, NJ) until the late exponential phase. Cells were harvested, washed in 20 mL of 0.9% NaCl, and resuspended in 20 mL of 10 mM Tris pH 8.0 (Tb8) buffer. Cells were lysed with a French press, and cell debris was removed by centrifugation for 15 min at 30 000g. Purification of ELPH12 by repeated temperature transition was achieved by adding NaCl to the cell free extracts to a final concentration of 1 M. The sample was then heated to 30 °C and centrifuged at 30 000g at 30 °C. The pellet containing the ELPH12 protein was dissolved in ice-cold Tris buffer, pH 7 (Tb7). This temperature transition cycle was repeated twice more times, and the pellet containing the biopolymer was finally dissolved in ice cold water. The purity of the protein was determined by SDS PAGE electrophoresis (16), followed by silver staining (Bio-Rad, Hercules, CA). The molecular weight of elastin was confirmed by MALDI-TOF mass spectrometry. The molar ratios presented were calculated based on the theoretical molecular weight of 34369 Da for ELPH12 and 32368 Da for ELP (without the histidine tag).

Temperature Transition Profile of ELPH12. The concentration of ELPH12 in solution was determined by measuring the absorbance at 215 nm using a Beckman DU-60 spectrophotometer. An extinction coefficient of 64.1 g/mL/cm (Shimadzu AA6701) with 1.25 mg/mL of ELPH12. Batch washings with 0.035 mM EDTA and 0.035 mM HCl were carried out at similar conditions above. Washed samples were dried in a vacuum oven at 120 °C overnight and analyzed for total cadmium content. The percentage of metal removal was determined based on the initial metal content of 118 mg/kg of cadmium. All experiments were performed in triplicates, and the average results are presented.

Results and Discussion

Characteristics of ELPH12. A biopolymer (ELPH12) composed of 78 ELP repeats and a tandem hexahistidine cluster (His-tag) was used in this study to demonstrate the feasibility in soil washing applications. Since the transition behavior of ELP biopolymers is known to be dependent on ionic strength (17), the reversible phase transition temperature (Tt) of ELPH12 was investigated to determine the conditions required for easy aggregation and recovery. The turbidity profile was measured, and the onset of transition was determined at a temperature where the turbidity reached 50% of the maximum (Figure 1). In accordance to previous reports (11, 18), the transition temperature (Tt) decreased from 33.5 °C in the absence of salt to 19 °C in the presence of 1 M NaCl. Using this information, over 98% recovery of ELPH12 was obtained at all concentrations tested by precipitating at 37 °C in 1 M NaCl. Aggregation was reversible, and the precipitates resolubilized when the temperature was decreased below Tt.

FIGURE 1. The transition temperature profiles of ELPH12 in the absence or presence of 1 mM NaCl. In both cases, the turbidity of solutions containing 1.25 mg/mL of ELPH12 dissolved in water was measured at 215 nm. Relative turbidity is defined as (A300(A300(min)) × 100.

Soil–Biopolymer Adsorption Characteristics. The adsorption characteristic of ELPH12 was studied with uncontaminated soil. Experiments were performed with 4 g of soil using different soil-to-solution (wt/vol) ratios of 1:5, 1:10, and 1:20 and ELPH12 concentrations ranging from 0.65 to 5 mg/mL. Samples were mixed in a mechanical tumbler at 30 rpm for 24 h, and the soil pellet was recovered by passing the samples through a 0.22-μm filter. The ELPH12 concentrations remaining in the supernatants were determined. A control experiment with no soil was also performed. The minerals and other constituents released from the soil had no interference at the wavelength (215 nm) used to measure the ELPH12 content.

Biopolymer–Cadmium Extraction Studies. All batch soil washing experiments were carried out with 4 g of soil at a constant 1:10 (wt/vol) soil-to-solution ratio. Samples were collected after 24 h of tumbling in a mechanical tumbler (30 rpm) and centrifuged for 20 min at 17700g. Single batch washings were conducted with 1.25, 2.5, and 5 mg/mL of ELPH12. Two and three sequential batch washings were done with 1.25 mg/mL of ELPH12. Batch washings with 0.035 mM EDTA and 0.035 mM HCl were carried out at similar conditions above. Washed samples were dried in a vacuum oven at 120 °C overnight and analyzed for total cadmium content. The percentage of metal removal was determined based on the initial metal content of 118 mg/kg of cadmium. All experiments were performed in triplicates, and the average results are presented.

VOL. 38, NO. 11, 2004 / ENVIRONMENTAL SCIENCE & TECHNOLOGY • 3149
and the amount of bound cadmium was measured. The experiment with error bars showing the standard deviations. The results represented the average of three sets of experiment with error bars showing the standard deviations.

**FIGURE 2. Cadmium binding stoichiometry of ELPH12 in 50 mM Tris buffer, pH 7.** One nmol of ELPH12 was mixed with various amounts of cadmium. Results represented the average of three sets of experiment with error bars showing the standard deviations.

**Biopolymer–Metal Complexation.** The maximum binding capacity of ELPH12 was investigated by incubating the biopolymers over a range of cadmium concentrations. After 1 h incubation, the biopolymer–cadmium complex was recovered by precipitation, and the amount of Cd(II) associated with the aggregates was measured. Binding increased rapidly at lower concentrations and reached a maximum value of 3.2 cadmium per biopolymer (Figure 2). This result is consistent with the reported coordination number of 4 for histidine–cadmium complexes (19), which translates into 3 molecules of Cd(II) per 12 histidines.

The affinity of ELPH12 toward cadmium was investigated by determining the stability constant ($K_f$) of the biopolymer–metal complex in aqueous solution. After incubating different amounts of ELPH12 with 50 nmol of cadmium, the cadmium–biopolymer complex was recovered by precipitation, and the amount of bound cadmium was measured. The stability constant ($K_f$) was determined from the slope of a straight line relationship obtained by plotting the moles of cadmium bound per mol of ELPH12 vs the free metal concentration (Figure 3). A log $K_f$ value of 6.8 was obtained, which is comparable to that obtained with a rhamnolipid biosurfactant previously used successfully for cadmium removal from soil (8, 10). When comparing to the values for cyanide (5.3), citrate (5.0), and EDTA (18.2), all of which are considered to be strong chelators for Cd(II), our result indicates very strong complexation between ELPH12 and cadmium (20).

**Soil–Biopolymer Sorption Characteristics.** Extensive sorption of biosurfactants onto soil particles affects the effectiveness of the soil washing procedure. Higher adsorption implies lesser availability of the biopolymer for complexation to metals. The sorption behavior of the ELPH12 biopolymer to soil was investigated using a 1:10 soil-to-solution ratio and ELPH12 concentrations of 0.63, 1.25, 2.5, and 5 mg/mL, respectively. The sorption isotherm was nicely described using a Freundlich isotherm (Figure 4) as defined

$$\log \frac{x}{m} = \log K_f + \frac{1}{n} \log C_{eq}$$

where $x$ is the amount of ELPH12 adsorbed (in mg), $m$ is the mass of soil (in g), $K_f$ is the Freundlich adsorption coefficient, $n$ is a measure of nonlinearity, and $C_{eq}$ is the concentration of ELPH12 in solution after adsorption is complete (equilibrium).

A $K_f$ value of 1.3 was obtained, and the isotherm was nonlinear with $1/n = 0.85$. A maximum of 19% of the added ELPH12 was adsorbed to the soil particles. This is significantly lower than the sorption values of 50–75% observed with surfactin (9) and rhamnolipid (10). Since there is very little adsorption of the biopolymer to soil, we expect that the use of ELPH12 would afford a similar extraction efficiency at much lower biopolymer concentrations than those required for the biosurfactants.

**Batch Soil Washing Studies.** Artificially contaminated soil was prepared by soaking a sandy soil in 1 mM cadmium nitrate for 3 months. The final cadmium content was determined to be 118 mg/kg soil. To evaluate the feasibility of extracting soil-bound cadmium with the ELPH12 biopolymers, contaminated soil was incubated overnight with various concentrations of ELPH12 dissolved in water. The pH of the wash solution remained near neutral even without the addition of buffer. As shown in Figure 5, cadmium removal increased with increasing ELPH12 concentrations; 38%, 44%, and 55% of the soil-bound cadmium was removed by 1.25, 2.5, and 5 mg/mL of ELPH12 solutions, respectively. For comparison, soil washing with distilled water or with 1.25
mg/mL (0.036 mM) of ELP biopolymers containing no His-
tag removed only 8% of the bound cadmium, indicating that
the histidine cluster on ELPH12 is mainly responsible for the
improved metal chelation. More importantly, the fraction of
ELPH12 adsorbed onto soil was less than 10% in all soil
washing experiments, a value slightly lower than soil not
contaminated with cadmium. This reduction in sorption is
important as a significantly lower concentration of ELPH12
(0.036 mM as compared to 5–10 mM of biosurfactants) was
required to achieve similar extraction efficiencies when
comparing to biosurfactants (10).

To compare the efficiency of the ELPH12 biopolymers
with a commonly used chelating agent—ethylenediamine-
tetraacetic acid (EDTA), batch washing experiments were
also performed using 0.036 mM EDTA. The amount of
cadmium removed was only 50% of the value obtained using
a similar concentration of ELPH12, suggesting that the
ELPH12 biopolymer may be more efficient as a chelator for
soil washing than EDTA on a molar basis.

To explore the possibility of sequential extraction using
the minimum amount of biopolymers, three batches of soil
washing with 1.25 mg/mL of ELPH12 were performed. The
extent of cadmium removed increased to 55% in the second
wash to a level comparable with the removal by 5 mg/mL
of ELPH12 in a single batch washing (Figure 5). Sequential
washes with water, in contrast, did not improve the removal
efficiency. Any additional wash, however, did not yield any
higher level of cadmium removal. This may be due to the
presence of cadmium inside the pores of soil particles
minimizing complexation with the biopolymers.

When the amount of cadmium extracted by ELPH12 was
calculated for the batch washing experiments, a ratio of 1.3,
0.7, and 0.5 Cd/ELPH12 was obtained for the 1.25, 2.5, and
5 mg/mL of ELPH12 solutions, respectively. These values
are significantly lower than the maximum binding capacity
of 3.2, indicating potentially empty binding capacities on
the ELPH12 biopolymers. However, when the actual cadmium
associated with ELPH12 was analyzed, only 25% of the
extracted cadmium was associated with the biopolymers
while the remaining was found in solution. Apparently, the
soil-bound cadmium was first extracted by the histidine
cluster and subsequently desorbed probably due to the
presence of other competing agents in the wash solution.

To determine whether stronger complexing agents
presented in the wash solution were responsible for displacing
cadmium from the biopolymers, ELPH12 was first removed
from the washing solution by precipitation. Additional
ELPH12 (5 mg/mL) was added, and essentially all cadmium
left in the wash solution was removed by coprecipitation
with ELPH12. This result suggests that most of the cadmium
was indeed free in solution and not bound to other
complexing agents. To address whether other cations
extracted from soil were completing with cadmium for the
histidine cluster, the mineral content of the wash solution
was analyzed by Inductively Coupled Plasma (ICP), batch washing experiments were
taken in the same ratio in the absence of Zn2+ did not have
any significant effect. Analysis of zinc revealed that 2.5 mol
of Zn2+ were bound to each mol of ELPH12 in all Zn2+-
containing samples. This indicates that zinc, even if present
in low quantities, would affect cadmium binding due to its
higher affinity toward the histidine cluster. This observation
is not surprising as histidine clusters are known to bind
erspecifically to many different cations (19). However, the
good news is that cations that are common in soils such as
Fe, Mg, Ca, and Al did not interfere with cadmium binding.
Because of the affinity of the histidine cluster toward other
heavy metals such as lead and mercury, this His-tag based
ELP biopolymer may be useful for the removal of soil
contaminated with mixed-metal wastes.

Although the results demonstrated here using a tandem
hexahistidine cluster as the metal-binding domain was
promising, the percentage removal was relatively modest.
Moreover, the idea of inexpensively recovering the bound
cadmium by simple precipitation was not complete due to
stronger displacement by the zinc ions present in the soil
and the need of NaCl for precipitation. 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 (14, 21). Metal-binding peptides such
as metallothionein (22) and synthetic phytochelatins (23)
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. These feasibilities are
currently under investigation.

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Literature Cited


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