Nanoscale Biopolymers with Customizable Properties for Heavy Metal Remediation

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Heavy Metal Contamination

- 2.4 million tons of metal wastes per year from industrial sources
- 2 million tons per year from agriculture and domestic waste
- \( \text{Pb}^{2+}, \text{Hg}^{2+} \) and \( \text{Cd}^{2+} \) are ranked 2nd, 3rd and 7th, respectively, on the EPA’s priority list
Metal Chelating Polymers

- Requires toxic solvents for synthesis
- Ultrafiltration is required
- Membrane clogging

Solution: Develop metal-binding materials that can be recovered by environmental stimuli
Metal Chelating Biopolymers

- Based on biological building blocks
- Nanoscale biopolymers that are specially pre-programmed within a DNA template
- Economically produced by bacteria
- Environmentally friendly
- Tunable properties based on changes in environmental conditions – pH or temperature
Elastin Biopolymer

- Structurally similar to the repeating elastomeric peptide sequence of the mammalian protein, elastin
- VPGVG are the most frequently repeating units
- Undergo a reversible phase transition from water soluble forms into aggregates as the temperature increases

High temperature: twisted filament of β-spirals
Low temperature: β-turn
Genetic and Protein Engineering Methodology

- Plasmid
- Enzyme
- Synthetic gene
- Recombinant plasmid
- ELP biopolymers
Customizable Metal Binding Biopolymers

Metal Binding Domain

Elastin Domain

Fine tune the transition temperature by controlling amino acid sequence and number of repeating unit \((VPGXG)_n\)

Fine tune affinity with different binding sequences
Heavy Metal Removal by Tunable Biopolymer

Heavy Metal Removal by Tunable Biopolymer

Regeneration

soluble protein

insoluble protein

Cd$^{2+}$

Cd$^{2+}$

Cd$^{2+}$

Cd$^{2+}$
Elastin Biopolymers with Metal-Binding Affinity

Biopolymer
A. ELP38H6
B. ELP58H6
C. ELP78H6
D. ELP78
E. ELP78H12

Transition temperatures can be fine tuned from 20 to 40°C by controlling the chain length and salt concentration.
# Cadmium Removal

<table>
<thead>
<tr>
<th>Biopolymer</th>
<th>Cd$^{2+}$ binding (mol protein : mol Cd$^{2+}$±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ela38H6</td>
<td>1 : 1.04 ± 0.04</td>
</tr>
<tr>
<td>Ela58H6</td>
<td>1 : 0.97 ± 0.01</td>
</tr>
<tr>
<td>Ela78H6</td>
<td>1 : 0.95 ± 0.01</td>
</tr>
<tr>
<td>Ela78</td>
<td>1 : 0.01 ± 0.00</td>
</tr>
<tr>
<td>Ela78H12</td>
<td>1 : 1.50 ± 0.02</td>
</tr>
</tbody>
</table>

SD - standard deviation

50 nmol of biopolymer were incubated with 100 nmol Cd$^{2+}$
Repeated Cycles of Metal Binding

5 µg added each cycle

Cadmium (µg)

S  P  S  P  S  P  S  P  AS
Cycle 1  Cycle 2  Cycle 3  Cycle 4
Practical Applications: Soil Flushing/Washing

- Environmental friendly extractant
- Simple separation and recycling

In-situ Soil Flushing

Ex-situ Soil Washing
Soil-Biopolymer Adsorption Characteristics

- Soil-Biopolymer Sorption Characteristics
- Freundlich isotherm

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

Maximum 19% adsorption

Biosurfactant – typically in the 70% range

Biopolymer-Cadmium Extraction Studies

- Single batch washing with distilled water
- Two sequential batch washings with distilled water
- Single batch washing with 1.25 mg/ml (0.036 mM) ELP without the histidine tag
- Single batch washing with 0.036 mM EDTA
- Single batch washing with 1.25 mg/ml ELPH12
- Single batch washing with 2.5 mg/ml ELPH12
- Single batch washings with 5 mg/ml ELPH12

Less than 10% adsorption
First generation biopolymers

• $\text{His}_6$ or $\text{His}_{12}$ tag can serve as a simple metal binding domain

• Both $T_t$ and metal binding capacity can be easily regulated

• Biopolymers can be recycled

• *Lack specificity and affinity*
Customizable Metal Binding Biopolymers

Elastin Domain

Metal Binding Domain

Fine tune ΔT by controlling amino acid sequence and number of repeating unit (VPGXG)_n

Fine tune affinity with different binding sequences
Bacterial \textit{mer} Systems

\textit{mer} genes on \textit{E. coli} plasmids or transposons

MerR \quad MerT \quad MerP

\text{IM} \quad \text{OM}

Hg^{2+} \quad \text{organomercury}

Hg^{0}
MerR can serve as a specific mercury binding domain

MerR-Hg complex
Production and purification of ELP153-MerR

A, cell free extract
B, purified protein (80.1 kDa)
C, size marker
Binding of Mercury to ELP153-MerR

Similar binding from pH 4 to 9
Selective Binding of Mercury by Ela153-MerR Biopolymer

Acidic waste water (pH 4)

Other Metal-binding Domains

Metallothioneins (MTs)
- MDRNCSCAACDSCTCAGSCKCKECK
- CTSCKKSCCCCPVGCAKCAQGCICK
- GASDKCSCCA

Synthetic Phytochelatinss (ECs)
- $\gamma$ECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECEC
- ECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECECG

Higher affinity toward Hg and Cd
Cadmium Binding

![Graph showing cadmium binding with ELPH12 and ELPEC20, indicating preference for Hg over Cd.](image)

Prefer Hg over Cd
Differential Precipitation and Separation – Strategy

- ELP78
- Histag
- Cd$^{2+}$
- ELP105K21
- Hg$^{2+}$
- EC20
- Cd$^{2+}$
- ELP105K21
- Hg$^{2+}$
- EC20

$\Delta T_1$ or $\Delta T_2$ or NaCl
Customizable Metal Binding Biopolymers

Metal Binding Domain

Elastin Domain

Fine tune ΔT by controlling amino acid sequence and number of repeating unit (VPGXG)_n

Fine tune affinity with different binding sequences
Differential precipitation and recovery by tuning the elastin composition: Lysine as the guest residues
Differential Precipitation and Separation

1) Marker.
2) Biopolymer mixture
3) 32 °C heat spin pellet
4) 48 °C heat pellet
5) 48 °C supernatant

ELPH12
ELPKEC20

Supernatant heat to 48°C
Centrifuge at 48°C
Pellet dissolved in cold buffer

Mixture Heat to 32°C
Centrifuge at 32°C
Pellet dissolved in cold buffer
Conclusions

- Nanoscale ELP biopolymer can be designed with customized properties:
  - Specificity
  - Tunable transition

- Provide a novel, environmental friendly, and green engineering method for removal of heavy metal from water and soil.
Acknowledgement

Exploratory Research: Nanotechnology