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Selection and characterization of a 7-mer peptide binding to divalent cations

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A 7-mer peptide (S-T-L-P-L-P-P) that bound to various divalent cations was selected from a phage display peptide library. Isothermal calorimetric analysis revealed that the peptide bound to Pb^{2+}, Cd^{2+}, Hg^{2+}, and Cu^{2+}. Through the use of CD studies, no secondary structural changes were observed for the peptide upon binding to divalent cations. Ala scanning mutant peptides bound to Hg^{2+} with a reduced affinity. However, no single substitution was shown to affect the overall affinity. We suggest that Pro residues chelate divalent cations, while the structure formed by the peptide is also important for the binding process.
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Keywords: phage display; peptide; divalent cation; ITC; Ala scanning mutant; heavy metal; binding

Introduction

A number of heavy metals such as lead, widely used in industry, are known to pose environmental- and health-related problems. These substances are not easily excreted from the body once they have been absorbed, or otherwise ingested, into it. The atomic radius of lead is similar to that of calcium, thus causing the perturbation of calcium-dependent enzyme systems. Lead is concentrated in, and remains in, the bones for more than 20 years [1,2]. Cadmium is known to cause nephrotoxicity, hepatotoxicity, and oxidative stress [3]. Mercury affects the CNS and easily passes through the blood–brain barrier and remains in the brain for a number of years. It also causes oxidative stress leading to DNA damage and tumorigenesis [4,5].

Phage display is a powerful tool for selecting peptide ligands for use on various target molecules [6]. The targets are not limited to organic molecules such as proteins and peptides, but can also include inorganic molecules such as nanoparticles [7]. Here we extend the application of this technology to the selection of peptides that binds heavy metal ions.

Materials and Methods

Immobilization of Lead Ion on Chelating Gel

Two hundred microliters of Chelation Sepharose Fast Flow gel (GE Healthcare, Stockholm, Sweden) was washed with 1 ml of distilled water three times. One milliliter of 10 mM $PbCl_2$ was added to the gel and incubated at room temperature overnight. The gel was washed with 1 ml of distilled water twice, followed by washing with 1 ml of incubation buffer (0.1 M Tris–HCl, pH 7.5, 0.5% Tween 20) two times to remove any lead ions which were not chelated.

Biopanning

One milliliter of blocking buffer (1% BSA in 0.1 M Tris–Cl, pH 7.5) was added to the Pb^{2+}-charged Chelation Sepharose Fast Flow gel and incubated for 30 min. The gel was then washed with 1 ml of incubation buffer twice. Ten microliters of Ph.D.-7 phage display peptide library (New England Biolabs, Ipswich, MA, USA) was added to the gel and incubated for 1 h. The manufacturer claims that there are 10^{11} PFU in 10 µl phage solution. The gel was washed with an incubation buffer ten times, followed by washing with a Tween 20 buffer (0.1 M Tris–Cl, pH 7.5, 0.5% Tween 20) five times, and a final wash with a glycerol buffer (0.1 M Tris–Cl, pH 7.5, 15% glycerol, 0.5% Tween 20) five times. Bound phages were eluted with 200 mM imidazol solution and amplified in host ER7238 for the next round of biopanning. This panning procedure was repeated three times. The phage DNA was isolated and the displayed amino acid sequence was deduced with nucleotide sequence analysis.

Isothermal Titration Calorimetry

A 7-mer peptide (S-T-L-P-L-P-P) was synthesized with N-terminal acetylation and C-terminal amidation (Peptron, Daejeon, Korea). Solutions of 0.6 mM peptide, 9 mM $Pb(ClO_4)_2$, 9 mM $Cd(ClO_4)_2$, 9 mM $Hg(ClO_4)_2$, and 9 mM $Cu(ClO_4)_2$ were prepared. The peptide solution was added to the sample cell of isothermal titration calorimetry (ITC) (VP-ITC; MicroCal, Piscataway, NJ, USA) and the metal solution was added to the syringe. As a reference, distilled water was added to the sample cell and titration was performed. The thermodynamic parameters were calculated with the MicroCal program.

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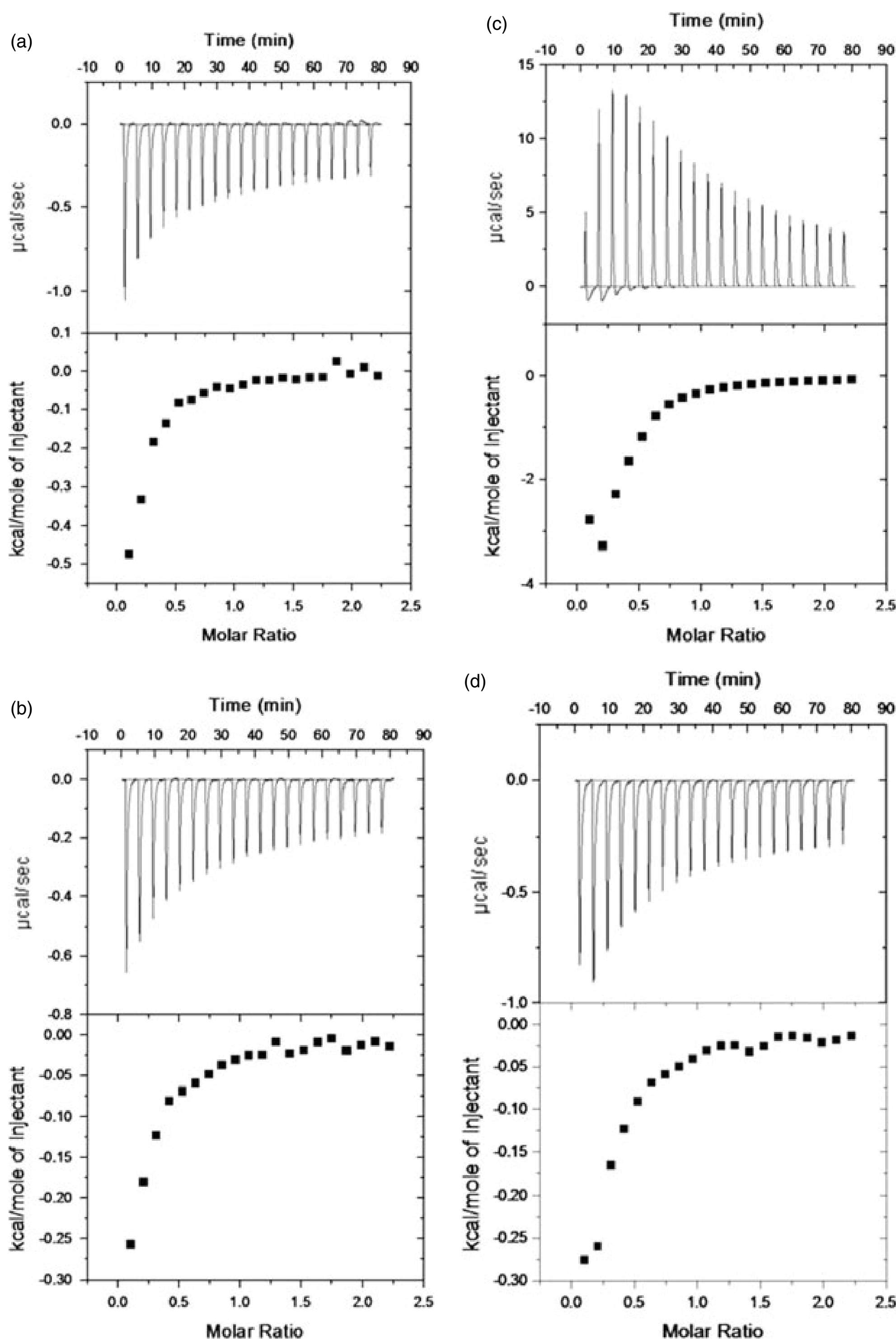

Figure 1. ITC results of binding between the 7-mer peptide (S-T-L-P-L-P-P) and metal ions. (A) 7-mer peptide and lead ion, (B) 7-mer peptide and cadmium ion, (C) 7-mer peptide and mercury ion, and (D) 7-mer peptide and copper ion.

Circular Dichroism

A total of 1.25 mM peptide solution, 1.25 mM $Pb(ClO_4)_2$ solution, 1.25 mM $Cd(ClO_4)_2$ solution, 1.25 mM $Hg(ClO_4)_2$ solution, and 1.25 mM $Cu(ClO_4)_2$ solution were prepared. CD analysis was conducted in ranges between 190 and 260 nm (Jasco, Easton, MD, USA).

Results and Discussion

The peptide sequence S-T-L-P-L-P-P persistently appeared after three rounds of biopanning (Table 1). We chose this peptide for further analysis. Chemically synthesized peptide was used for ITC analysis [8]. As the peptide was selected for binding to the lead ion, we first performed ITC against this ion and confirmed the binding (Figure 1(A)). However, there was a possibility of peptide

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Table 1. Selected peptide sequences after three rounds of biopanning against lead

Peptide sequence	Number of hits
S-T-L-P-L-P-P	9 out of 62
S-P-M-T-L-Y-G	6 out of 62
T-M-Q-M-T-R-Y	6 out of 62
L-V-T-T-G-P-L	4 out of 62
Q-T-H-S-W-W-P	2 out of 62

binding to other divalent cations. Accordingly, we performed ITC against cadmium, mercury, and copper. They were all shown to bind to the 7-mer peptide (Figure 1(B–D)). The thermodynamic parameters are shown in Table 2. The binding affinity was highest for mercury followed by lead, copper, and cadmium, respectively. The calculated ΔH shows that all the binding between the peptide and metal ions are exothermic reactions.

Next, we performed CD analysis to determine if the binding of metals caused any secondary structural changes in the peptide. As seen in Figure 2, little change was observed for each metal ion binding.

Of the seven amino acids, it was possible that one was the critical residue for the binding of the metal ions. Thus, seven different Ala scanning mutant peptides [9] were synthesized and ITC analysis was performed (Figure S1, Supporting Information). As seen in Table 3, substituting Leu at the fifth position with Ala resulted in a 48% decrease in binding affinity, followed by a 42% decrease by substituting the first position of Ser with Ala. Substituting the third position of Leu with Ala resulted in the least change in binding affinity (15%). No one substitution showed any fold-decrease in binding affinity, suggesting that there are more than one metal binding sites and that the overall structure formed by the peptide assisted the binding.

Generally, short peptides have flexible structures. The 7-mer peptide in this experiment has three Pro residues which can make the peptide rather rigid. The two amino acids at the N-terminus (S-T) are polar, but all other amino acids are hydrophobic. This hydrophobicity may be responsible for the low solubility of the peptide. More than two molecules of peptides can together form a certain structure, which has polar amino acids on the external surface and hydrophobic ones on the inside. Metal ions may be chelated inside this structure. The main chelator in this case may be Pro. It has been reported that Pro produced from heavy metal stress metabolism acted as metal chelators in plants [10]. Another report has stated that plants with overexpressed Pro exhibited stronger heavy metal tolerance [11]. It has also been noted that heavy metal protection via Pro-dependent enzymes resulted from the formation of metal–Pro complexes [12]. Mass spectroscopic analysis showed the production of cadmium–Pro complexes. Metal–Pro chelation was also reported in the case of ACE [13].

There are reports that certain cyclooctapeptides interacted with various heavy metal ions [14,15]. The 7-mer peptide in this study is much easier to synthesize chemically and is more easily expressed on cell surfaces than cyclooctapeptides. Accordingly, the peptide could be useful for detecting heavy metal ions in the environment. It can also be used as a sensor in an electromagnetic circuit. Similarly, it may be utilized in the form of whole cell biosensor [16]. In addition to metal detection, its use could be extended to biosorption of heavy metals from the environment. For example, it can be displayed on the surfaces of yeast [17] or bacteria [18,19] and used to remove heavy metals.

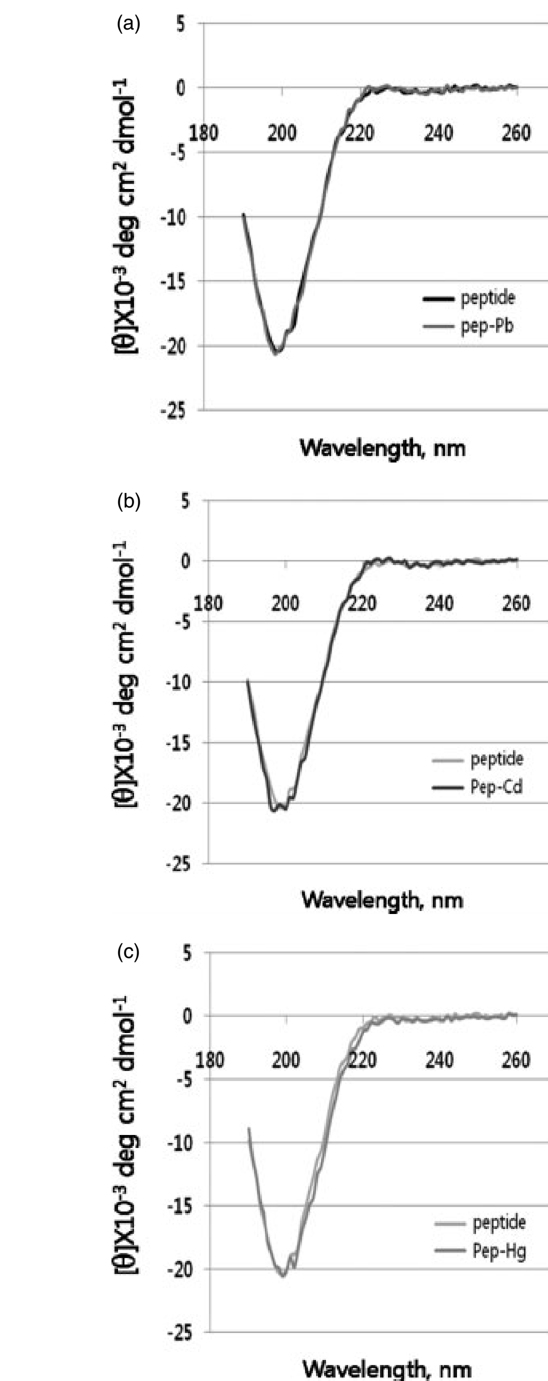

Figure 2. CD spectra of binding between the 7-mer peptide (S-T-L-P-L-P-P) and metal ions. (A) 7-mer peptide and lead ion, (B) 7-mer peptide and cadmium ion, and (C) 7-mer peptide and mercury ion.

Conclusion

The 7-mer peptide composed of S-T-L-P-L-P-P was selected from phage display peptide library and shown to interact with various divalent cations. Its interaction was confirmed by isothermal calorimetry. The interaction induced little secondary structural

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Table 2. The thermodynamic parameters of binding between 7-mer peptide and various metal ions

Metal ion	Ka (M^{-1})	ΔH (Kcal mol^{-1})	ΔS (cal(mol K)$^{-1}$)	ΔG (Kcal mol^{-1})
Pb^{2+}	$9.791 \times 10^3 \pm 2.065 \times 10^3$	$-1.255 \times 10^3 \pm 3.366 \times 10^2$	14.05	-5.442×10^3
Cd^{2+}	$4.075 \times 10^3 \pm 7.233 \times 10^2$	$-8.547 \times 10^3 \pm 3.482 \times 10^3$	−12.13	-4.933×10^3
Hg^{2+}	$1.574 \times 10^4 \pm 1.891 \times 10^3$	$-5.474 \times 10^3 \pm 3.022 \times 10^2$	0.8175	-5.718×10^3
Cu^{2+}	$7.124 \times 10^3 \pm 1.765 \times 10^3$	$-6.065 \times 10^2 \pm 1.432 \times 10^2$	15.59	-5.252×10^3

Table 3. Binding affinity changes between Ala scanning mutant peptides and mercury ion

Peptide	Ka (M^{-1})
STLPLPP	$1.574 \times 10^4 \pm 1.891 \times 10^3$
ATLPLPP	$9.069 \times 10^3 \pm 2.770 \times 10^3$
S**A**LPLPP	$1.114 \times 10^4 \pm 4.603 \times 10^3$
ST**A**PLPP	$1.338 \times 10^4 \pm 3.601 \times 10^3$
STL**A**LPP	$1.061 \times 10^4 \pm 4.169 \times 10^3$
STLP**A**PP	$8.146 \times 10^3 \pm 2.989 \times 10^3$
STLPL**A**P	$1.004 \times 10^4 \pm 2.727 \times 10^3$
STLPLP**A**	$1.191 \times 10^4 \pm 5.579 \times 10^3$

change as determined by CD analysis. The presence of many Pro residues seems to confer the peptide a rigid structure. As no single peptide substitution with Ala showed substantial decrease in binding affinity, the whole structure formed by the peptide seems the most important determinant for this interaction. As Pro are known chelators of cations, the heavy metal ions may be chelated by Pro residues inside a multimeric structure of the peptides created by their amphiphilic nature.

Acknowledgement

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Supporting information

Supporting information may be found in the online version of this article.

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