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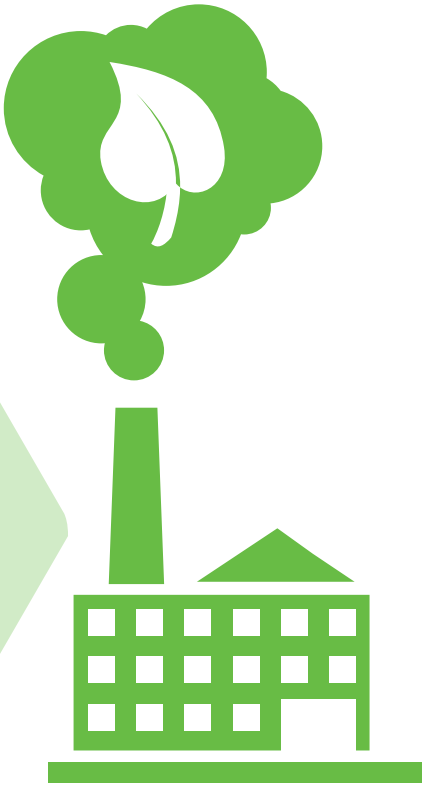

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OLIGOPEPTIDE SEQUENCES OF THE METAL BINDING DOMAIN OF CueR METALLOREGULATORY PROTEINS AS CANDIDATES FOR TOXIC METAL ION CAPTURE

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ABSTRACT

Various toxic metal ion resistance systems operate from bacterial level up to higher plants and animals. In bacteria, metalloregulatory proteins are key factors in the control of metal ion level. Inspired by the metal binding domain of these highly sensitive metal ion sensor proteins we have designed artificial oligopeptides, containing two cysteine residues, and investigated their interaction with cadmium(II) and mercury(II) ions. The studied ligands bound both metal ions with a rather high stability. The composition and solution structure of the various metal ion complexes have been determined. The genetic code of one of the oligopeptide sequences has been introduced into E. coli BL21 cells and (over)produced in the form of a fusion protein. Preliminary investigation of the viability and potential metal ion accumulation of the modified bacteria, compared to control cells, in the presence of cadmium(II) and mercury(II) has also been performed.

INTRODUCTION

The environmental hazard of toxic metal ions and the problem of removing them from contaminated area have been a hot subject of environmental research in the recent years. Besides the traditional physicochemical technologies aiming at the discharge of toxic metal ions, bioremediation of contaminated areas is a very promising, alternative approach, which is based on the use of microorganisms (bacteria, algae, yeasts or fungi) able to extract the metal ions from the target media by biosorption or other ways [1].
Different organisms have evolved various defense mechanisms towards toxic metal ion shocks, e.g. exclusion, compartmentalization, complex formation or the enhanced production of metal-binding proteins or oligopeptides (like metallothioneins (MT) or phytochelatins (PC)) in the case of toxic stress. The complex defense mechanisms that operate at prokaryotic level often use metal-responsive MerR family member proteins [2]. These are transcriptional regulators that respond to the change of intracellular metal ion concentration or availability by balancing the expression of cellular metal uptake and efflux/detoxification systems [3]. Different metalloregulatory proteins were associated with the sensing of various metal ions including Cu^{+}, Ag^{+}, Au^{+}, Hg^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+} [2–4]. A characteristic feature of metalloregulatory MerR family member proteins is the presence of a short multiple Cys-containing metal binding loop at their C-terminal region. The sensing of mono- and divalent metal ions occurs via coordination of a set of donor ligands (Cys/His) from this loop and in some cases from other regions, too [3,4].

Metalloregulatory MerR proteins may have a great potential in the detection, capture and removal of toxic metal ions from contaminated media, owing to their outstanding metal ion sensitivity and selectivity. We have commenced a research on exploiting these remarkable features by focusing on the properties of the metal binding domain of these proteins. We have designed a number of relatively short (10-15 amino acid residues) oligopeptides, inspired by the metal binding domains of selected proteins of the metalloregulatory MerR family (i.e. *V. cholerae* CueR or *E.coli* CueR). The presented work describes our efforts on the characterization of some of these metal-binding loop motifs and their interaction with different metal ions. Besides, we also present our first results with genetically modified bacteria adapted for toxic metal ion accumulation.

MATERIALS AND METHODS

We have synthesized a 12-mer oligopeptide fragment of the metal binding loop of *V. cholerae* CueR and two slightly modified variants of the native sequence (**PP**: Ac-SC**P**GDQGSDC**P**I-NH_2, **PS**: Ac-SC**P**GDQGSDC**S**I-NH_2, **HS**: Ac-SC**H**GDQGSDC**S**I-NH_2), see Figure 1. The cadmium(II) and mercury(II) binding features of **PS** and **HS** have been investigated by pH-potentiometric titrations and by UV, SRCD (synchrotron radiation circular dichroism), ^{1}H NMR and PAC (perturbed angular correlation of γ-rays) spectroscopies. Genetically modified bacteria have been constructed by the tools of molecular biology.

ACPGDDSADCPI — ***E. coli* CueR**

SCPGDQGSDCPI — ***V. cholerae* CueR**

SCPGDQGSDCSI

SCHGDQGSDCSI

SCHGDQGSDCPI

} **mutant sequences**

Figure 1. The metal binding loop of two metalloregulatory MerR proteins and variants of the native sequences with mutations at the Pro residues

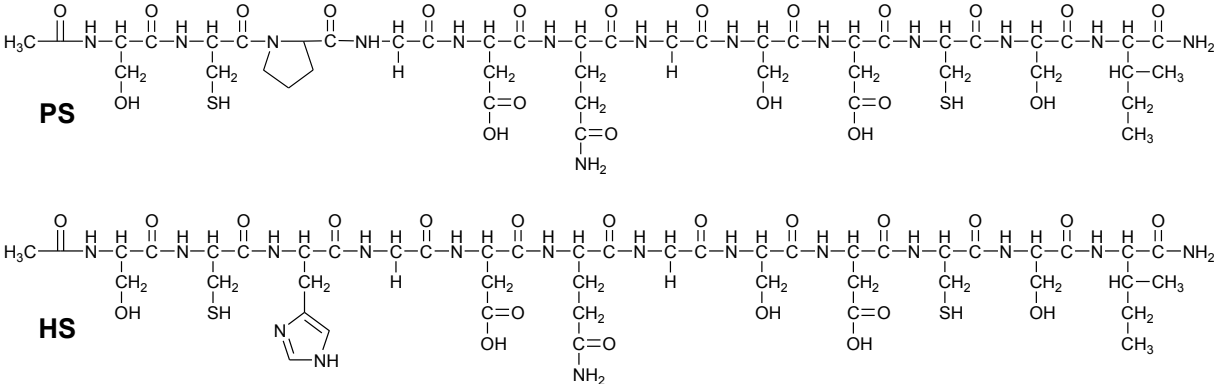

Figure 2. Schematic structure of the studied oligopeptides, Ac-Ser-Cys-Pro-Gly-Asp-Gln-Gly-Ser-Asp-Cys-Ser-Ile-NH_2 (**PS**) and Ac-Ser-Cys-His-Gly-Asp-Gln-Gly-Ser-Asp-Cys-Ser-Ile-NH_2 (**HS**)

RESULTS

We have realized that the two 12-mer peptides (see Figure 2) possess a significant disorder in their structure based on the recorded SRCD spectra of the ligands, showing a coil-like signature. However, this disorder radically changes in the presence of metal ions.
Detailed pH-potentiometric studies with cadmum(II) ions showed the formation of various, fairly stable species with both peptides, depending on the applied metal ion – peptide ratio and pH (Figure 3.). Combining these data with UV-titrations and ^{1}H NMR experiments, we could clearly prove the coordination of the two thiolate groups of the peptides to the metal ion both in their monocomplexes, or in cadmium(II)-bridged structures. Nevertheless, the histidine residue of **HS** also plays a role in cadmium(II) binding (Figure 4.) [5]. It is, however, somewhat surprising that the lack of the histidne residue does not cause any significant decrease in the stability of the **PS** complexes (see Figure 3). Beside other possible causes, it might originate from the coordination of the aspartate donors that may, indeed, take over the role of histidine in the cadmium(II) – **PS** complexes.

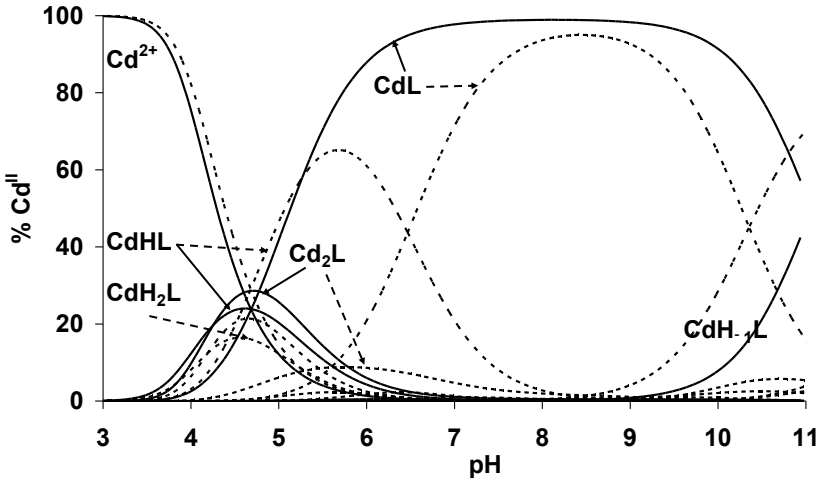

Figure 3. Comparison of species distribution diagrams in the Cd(II) – **PS** (continuous lines) and Cd(II) – **HS** (dotted lines) 1:1 systems. L denotes the ligand, c(**PS**,**HS**) = 0.001 moldm^{-3}

In the case of the other studied metal on, mercury(II), there is a remarkable difference as compared to the cadmium(II) containing systems: complex formation occurs well below pH 2. The coordination environment of the metal ion has been studied by SRCD, ^{1}H NMR and ^{199m}Hg PAC spectroscopies. We could conclude, that bis-ligand species were not formed with either of the ligands and in contrast to cadmium(II) the histidine moiety of **HS** most probably does not participate in the coordination of mercury(II).
The studied oligopeptides, owing to their high affinity towards cadmium(II) and mercury(II), were judged to be good candidates for the construction of genetically modified bacteria able for the intracellular overproduction of metal binding fusion proteins. In our first attempt, one of the 12-mer metal ion capturing fragments (**PS**) was attached to the C-terminus of a carrier (maltose binding) protein.The genetic code of the oligopeptide sequence has been transformed into the cells in a pMAL-B plasmid. Our preliminary studies showed that the prolification of bacteria overexpressing the fusion protein was inhibited as compared to control cells that were either overexpressing the carrier protein without the attached metal binding fragment or expressing only the original proteins of the cell. This phenomenon was independent of the cadmium(II) or mercury(II) content of the medium. This may suggest that the metal binding

fragment withdrew some of the metal ions being essential for the prolification. However, when overexpression of the fusion protein was initiated in cultivated medium, the modified bacteria survived and were able to absorb toxic metal ions.

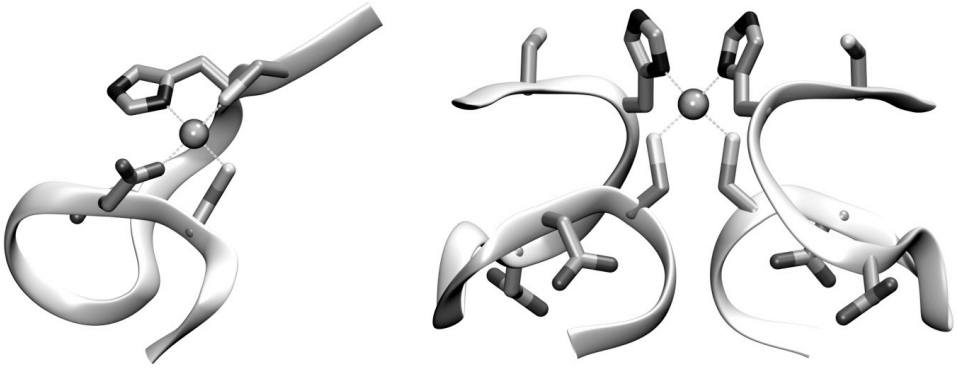

Figure 4. Possible structures of the CdL and $Cd(HL)_2$ complexes of **HS** calculated by molecular mechanics geometry optimization [5]

CONCLUSIONS

Two oligopeptides, inspired by the metal binding domain of CueR metalloregulatory proteins, showed excellent affinity towards mercury(II) and cadmium(II) ions. Genetically modified bacteria overproducing the metal binding oligopeptides in a form of fusion proteins were successfully constructed and showed a potential the accumulation of toxic metal ions.

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