

Crystallization of Designed Three-Stranded Coiled Coil Peptides

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Heavy metals such as arsenic, mercury, cadmium and lead are environmental contaminants known for their toxicity. To understand more fully the interactions between heavy metals and proteins, we have prepared the cysteine substituted derivatives of the peptide TRI [Ac-G(LKALEEK)₄G-NH₂]. This family of peptides has been shown to associate to form a three-stranded coiled coil and binds heavy metals in a three-coordinate, thiolate environment. Such structures are relevant to biological systems such as the bacterial metalloregulatory proteins MerR and ArsR that, respectively, bind Hg(II) and As(III) in such a fashion. To develop our understanding of these designed peptides, it is important to obtain x-ray characterization of the metallated peptides. Initial crystallization screens with the TRI peptides gave beautiful, but poorly diffracting crystals. We next examined the related peptide Coil Ser (CS). This peptide previously was crystallized¹ at pH 5.0 as an anti-parallel three stranded coiled coil. Further solution studies at higher pH showed that it re-ordered into a parallel orientation. As with the TRI peptides, substitution of an interior leucine for a cysteine in CS results in a peptide that can bind heavy metals such as Hg(II) and Cd(II) in a trigonal environment. We have obtained a crystal of As(CS-L9C)₃ at pH 8.0 that diffracted to 1.7 Å. The unit cell is C2 with crystallographic parameters a=77.3 b=29.4 Å c=44.2 Å $\alpha=90^\circ$ $\beta=119.5^\circ$ $\gamma=90^\circ$ Z=4 with one trimer in the asymmetric unit. Analysis of the Matthews coefficient indicates that the crystal contains 41 % solvent. We will report our progress on solving this structure. This work was supported by the NIH grant number R01 ES-012236-02.

1) Lovejoy et al. (1993) *Science* 259 , 1288-1293.

