On the metal site stabilizing role of the C-terminal CCHHRAG fragment of the metalloregulatory protein CueR


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The bacterial copper efflux regulator CueR senses and responds to Cu\(^{1+}\), Ag\(^{1+}\) or Au\(^{1+}\) by binding these ions in a linear coordination geometry via two cysteinate residues located at the ends of a metal binding loop (MBL) near the C-terminus of the protein.\(^1\) The MBL is disordered in the metal ion free repressor state of the protein\(^2\) and flexible enough to accommodate non-cognate, divalent metal ions, e.g. Cd\(^{2+}\) or Hg\(^{2+}\).\(^3\) The CueR protein of *Escherichia coli* possesses a C-terminal CCHHRAG fragment presenting additional, potential metal ion binding ligands. It was demonstrated that an additional cysteinate, besides those of the MBL, may actually participate in the coordination of Hg\(^{2+}\) resulting in a trigonal metal coordination environment and ultimately in a structural disorganization of the metal ion binding site\(^3\) that can possibly inhibit the transcriptional activity of the protein.

Here we present our latest efforts in exploring what role the CCHHRAG fragment might play in the interaction of CueR with cognate metal ions by comparing the Ag\(^{1+}\)-binding of the wild type *E. coli* CueR (WT-CueR) and of its C-terminally truncated variant (\(\Delta C7\)-CueR) lacking the last 7 amino acid residues. While none of our experimental results indicated the direct involvement of ligands of the CCHHRAG fragment in Ag\(^{1+}\)-coordination, CD-spectroscopic and ESI-MS data indicated that Ag\(^{1+}\) is bound by a significantly lower affinity to the \(\Delta C7\)-CueR protein. In line with these, \(^{111}\)Ag PAC spectroscopic results reflected that the linear AgS\(_2\) structure, characterizing the metallated form of WT-CueR, is less populated for the truncated variant. Accordingly, the CCHHRAG fragment, inspite being relatively remote, was shown to stabilize the metal site structure by intramolecular interactions.