

The Structure of the Metal-Binding Motif GMTCAAC Is Similar in an 18-Residue Linear Peptide and the Mercury Binding Protein MerP

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The signature CXXC sequence is found in a wide variety of metal-binding proteins, including rubredoxins, ferredoxins, and metallothioneins.¹ Furthermore, it is part of the highly conserved GMTCAAC metal-binding motif that occurs in many heavy metal-binding and transport proteins found in bacteria and humans.^{2–10} These proteins adopt a compact fold and belong to the “ferredoxin-like” structural family, which also includes acylphosphatases, ferredoxins, and small DNA- and RNA-binding domains.¹¹ Since the primary sequences are so similar, differences in three-dimensional (3D) structures must account for variations in affinities and selectivities among metals. In this contribution, the 3D structures and metal-binding properties of the sequence TLAVPGMTCAACIPITVKK,¹² which corresponds to residues 6–23 of the metal-binding loop of MerP, are compared in an 18-residue linear peptide and in the 72-residue protein in solution.

In the absence of metal ions, the 18-residue peptide does not appear to have a preferred conformation in solution. The peptide folds into a highly stable, unique conformation when it binds a metal; the negative intensity of 200 nm in the CD spectrum is reduced, many ¹H NMR resonances shift and become noticeably broader, and there is a substantial increase in the number and intensity of cross-peaks in the 2D ¹H/¹H NOESY NMR spectrum.¹³

Data derived from NMR experiments on the 18-residue peptide in solution are summarized in Figure 1. The NOE data were converted into distance constraints and the 3D structure determined using the standard protocols of XPLOR.¹⁴ The average structures of residues 6–12 of the peptide and the corresponding

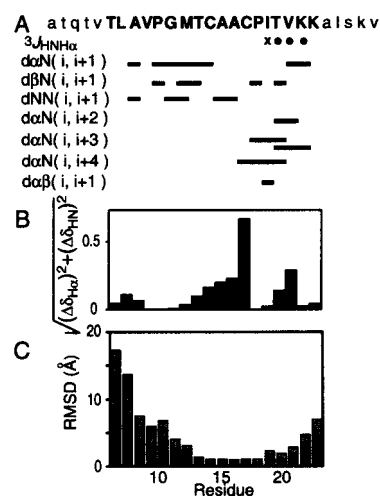


Figure 1. (A) Summary of NMR structural parameters for the metal-bound form of the 18-residue peptide in solution. The filled circles identify residues with $^3J_{\text{HNH}\alpha} < 6.0$ Hz and the crosses identify residues with $^3J_{\text{HNH}\alpha}$ value 6.0–8.0 Hz. (B) ¹H amide and α -hydrogen chemical shift changes upon addition of an equimolar amount of Hg(II) to the peptide solution. The numbering scheme for these same residues in MerP is shown at the bottom. The residues shown in small letters at the beginning and end of the sequence correspond to the surrounding residues in MerP. (C) RMSD from the average of the backbone (black) and side chains (gray) of the 10 lowest-energy structures calculated using XPLOR.

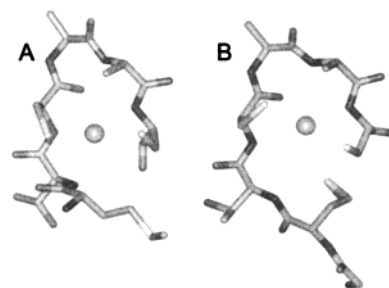


Figure 2. (A) GMTCAAC in the 18-residue peptide (PDB file 1dvw). (B) GMTCAAC in the metal-binding loop of MerP (PDB file 1afj).

residues (11–17) of MerP are shown in Figure 2. The backbone structure of the peptide is relatively well defined for those residues shown in Figure 2A, with an RMSD to the mean of 0.41 Å for their α -carbons. The N-terminal five residues of the peptide are unstructured, although the corresponding residues (6–10) are highly structured in MerP where they are sandwiched between the third and fourth β -strands.¹⁵ The 3D structures of the GMTCAAC residues bound to Hg(II) are remarkably similar in the 18-residue peptide and the 72-residue protein. The RMSD between these residues in the peptide and in the protein is 0.49 Å. In both structures the two cysteine side-chains are very well defined, and point toward the interior of the loop and coordinate the metal ion. In addition, the methyl group of A16 points toward

(14) Structure calculations were performed using the computer program XPLOR version 3.1 and a hybrid distance geometry/simulated annealing protocol. Two independent calculations were performed in the presence and in the absence of metal. A total of 77 NOEs were assigned and classified as strong, medium, and weak corresponding to distance restraints of 1.9–2.7, 1.9–3.3, and 1.9–5.0 Å, respectively. Three dihedral angles constraints were used in the calculations. Both calculations converged in the same folding. No NOE violations greater than 0.35 Å or dihedral angle violations greater than 5° were found. The 10 lowest-energy structures were used for further analysis. See: Brunger, A. T. In *X-PLOR*, version 3.1; *A System for X-ray Crystallography and NMR*; Yale University Press: New Haven, CT, 1992; Nilges, M.; Clore, M. Gronenborn A. *FEBS Lett.* **1988**, *239*, 129–136.

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(12) The 18-residue peptide was synthesized by solid-phase method using Fmoc-chemistry and purified by reverse phase HPLC using a linear gradient H₂O/acetonitrile buffer system on a C-18 column. See: King, D. S.; Fields, C. G.; Fields, G. B. *Int. J. Pept. Protein Res.* **1990**, *36*, 255–266.

(13) The NMR sample was prepared by dissolving peptide in 90% H₂O and 10% D₂O, 20 mM phosphate buffer (pH ~6.5), 1 mM DTT for a final concentration of 1.5 mM. All of the experiments were performed on a Bruker DMX 500 spectrometer.

Table 1. Dissociation Constants of the 18-Residue Peptide and MerP

metal	peptide K_d (μ M)	MerP K_d (μ M)
Hg(II)	90	2.8
Cu(II)	120	5.2
Ni(II)	180	20.0
Zn(II)	350	4.0
Cd(II)	430	18.0
Ag(I)	430	13.0
Ca(II)	> 10 ⁵	> 10 ⁵

the metal, and the methyl group of A15 is located on the outside. As in MerP, the side-chain of M12 is not involved in metal binding. This is also the case for mbd4¹⁶ and Atx1,¹⁷ whose metal-binding loops have sequences virtually identical to that of MerP. In the structures in Figure 2 A and B, T13 points away from the metal, ruling out possible interactions with its side chain oxygen. In contrast, the T14 and T13 side chains of Atx1 and mbd4 are significantly closer to the metal-binding site. The metal-binding loop of the peptide is somewhat less extended than that of MerP. The dissociation constants (K_d) of the 18-residue peptide and MerP for seven different metals are listed in Table 1. Metal-binding to the peptide was characterized by measuring the reduction of negative ellipticity at 200 nm in the far-UV CD spectra¹⁸ upon addition of the various metals. The corresponding K_d 's for MerP were determined by measuring the reduction in the CD absorbance at 222 nm, which can be correlated with a slight unwinding of the first helix upon metal binding.¹⁵ The values of K_d for Hg(II) binding are in the range previously reported for the reduced form of MerP.¹⁹ The binding affinities of the 18-residue peptide are reduced by less than 2 orders of magnitude compared to MerP, and the order of affinities is similar for the two polypeptides.

In general, the affinity for divalent ions varies as a function of ion size, ion radius and charge effect, liganding atoms, spin-pairing stabilization, and preferential coordination geometry.^{20,21} The results in Table 1 suggest that geometric requirements play an important role in metal binding to CXXC-containing motifs. In both oligopeptides and proteins, the sequence CXXC has been shown to bind Hg(II) with linear bicoordinate geometry.^{22–24} Similarly, the MTCXXC sequences have been shown to bind Hg(II) with linear bicoordinate geometry in MerP,¹⁵ mbd1 (unpublished results), and Atx1.²⁵ Therefore, it is not surprising that the 18-residue peptide containing the same sequence and 3D structure as MerP binds Hg(II) with the highest affinity. The coordination

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(18) CD spectra were recorded with a Jasco J-600 spectropolarimeter using a Quartz cuvette with a 0.1 cm path length at 300 K. The peptide concentration was 0.027 mM in 20 mM sodium acetate buffer (pH ~6.5) and 0.1 mM DTT. The protein concentration was 0.008 mM in 20 mM acetate buffer (pH ~6.5) and 0.1 mM DTT. Each titration was repeated three times to calculate a standard deviation for the measured K_d , which was $\pm 3 \mu$ M for the peptide and $\pm 0.2 \mu$ M for MerP. The indirect measurements of K_d were performed as previously described; Ghadiri, M. R.; Choi, C. *J. Am. Chem. Soc.* **1990**, *112*, 1630–1632.

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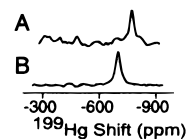
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**Figure 3.** ¹⁹⁹Hg NMR spectra. (A) Hg(II) bound to the 18-residue peptide in solution. (B) Hg(II) bound to MerP.¹⁵

geometry of Hg(II) in the 18-residue peptide was confirmed to be linear bicoordinate by the ¹⁹⁹Hg NMR spectrum.²⁶ Neither the peptide nor the protein bind the metal through dimerization since ¹⁹⁹Hg in a tetracoordinate complex would have a very different chemical shift (~ -200 ppm).²⁶

Copper tends to bind to proteins in tetrahedral and square planar configurations; in the presence of reducing agents, bidentate biomolecules have been shown to bind Cu(I)²⁷, although that cannot be established from the data presented here. The high affinity of the GMTCAAC motif for Ni(II) is somewhat surprising since it prefers octahedral geometry.²⁸ Silver binds the GMTCCXC motif in a bicoordinate manner similar to that of mercury.¹⁶ The lower affinity of the metal-binding loop for cadmium and zinc is expected since Cd(II) has a marked preference for histidine residues and tetrahedral or octahedral geometry,¹⁶ and Zn(II) has been found almost exclusively in tetrahedral arrangements.²⁸ When the peptide binds Cd(II), its 3D structure is substantially different from that shown in Figure 2 (unpublished results). Dimerization of the peptide can be excluded on the basis of the ¹¹³Cd NMR spectrum. Remarkably, a similar order of affinities for the various metals has been found for MerR, which binds mercury trigonally with three thiols.² Even though its binding site and apparent affinity for mercury are different ($\sim 10^{-8}$ M),^{29,30} the concentrations of cadmium, zinc, and silver required to turn on transcription are 2–3 orders of magnitude greater than for mercury.²⁹

Tertiary structure affects the metal-binding affinity of the highly conserved GMTCCXC metal-binding motif. In particular, the higher affinity of MerP for metals compared to the 18-residue peptide may be attributable to the effects of the conserved hydrophobic residues F38 and Y66, which may be involved in stabilizing and subtly altering the shape of the metal-binding loop.¹⁵ The finding that F38 undergoes a substantial conformational change upon binding Hg(II) also suggests that it may alter metal-binding affinity and specificity.¹⁵ Significantly, neither the 18-residue peptide nor MerP bind calcium. Although this is an expected result because calcium requires a completely different binding site.³¹ It demonstrates that high levels of discrimination among metal ions can be achieved with a linear peptide.

The differences in metal-binding affinities between the peptide and the protein show that, although there is a context dependence for the coordination of metal ions, peptides with high affinities and specificities can be designed on the basis of sequences and 3D structures of metal-binding proteins.

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