Metalloregulatory Proteins

International Edition: DOI: 10.1002/anie.201508555
German Edition: DOI: 10.1002/ange.201508555

Specificity of the Metalloregulator CueR for Monovalent Metal Ions: Possible Functional Role of a Coordinated Thiol?

Dániel Szunyogh, Hajnalka Szokolai, Peter W. Thulstrup, Flemming H. Larsen, Béla Gyurcsik, Niels Johan Christensen, Monika Stachura, Lars Hemmingsen, and Attila Jancsó**

Abstract: *Metal-ion-responsive transcriptional regulators within the MerR family effectively discriminate between mono- and divalent metal ions. Herein we address the origin of the specificity of the CueR protein for monovalent metal ions. Several spectroscopic techniques were employed to study Ag^{I}, Zn^{II}, and Hg^{II} binding to model systems encompassing the metal-ion-binding loop of CueR from E. coli and V. cholerae. In the presence of Ag^{I}, a conserved cysteine residue displays a pK_a value for deprotonation of the thiol that is close to the physiological pH value. This property is only observed with the monovalent metal ion. Quantum chemically optimized structures of the CueR metal site with Cys112 protonated demonstrate that the conserved Ser77 backbone carbonyl oxygen atom from the other monomer of the homodimer is "pulled" towards the metal site. A common allosteric mechanism of the metalloregulatory members of the MerR family is proposed. For CueR, the mechanism relies on the protonation of Cys112.*

Metal-ion homeostasis and cellular defense mechanisms against toxic metal ions are central topics in biochemistry.[1] One facet of this theme is the function of metalloregulatory proteins of the MerR family.[1,2] Examples include MerR and ZntR, which act as specific sensors of Hg^{II} and Zn^{II}, respectively, and CueR, which senses monovalent metal ions, such as Cu^{I} and Ag^{I}.[3] The MerR proteins function as DNA-bound homodimers with a dimerization domain, a metal-ion-binding loop, and a DNA-binding domain. A striking feature of the Cu^{I} binding site of CueR is that the metal-ion-coordinating S_{Cys112} atom is positioned 3.6 Å from the backbone carbonyl oxygen of Ser77,[3] that is, at a typical hydrogen-bonding distance for a cysteine SH donor and a backbone amide C=O group.[4] This arrangement has led Brown and co-workers to suggest that Cys112 might be protonated.[5] Metal-ion binding allosterically affects the protein–DNA interaction, changing the DNA structure and thereby regulates transcription.[1,6] In a recent very interesting study, the structure of both the repressor (i.e. metal-free) and activator form of the CueR protein, the Ag^{I}–CueR–DNA complex, was determined, which provided unprecedented insight into the changes in DNA structure accompanying metal-ion binding.[6]

In this study, we employed spectroscopic techniques to explore characteristic differences between the binding of mono- and divalent metal ions to peptides encompassing the metal-ion-binding loops (MBLs) of the CueR proteins from *Vibrio cholerae* (VC) and *Escherichia coli* (EC), as well as quantum-chemical methods to model various possible metal-site structures. The amino acid sequences of the two peptides are shown in Scheme 1. In a series of structures of the CueR protein from *E. coli*, O'Halloran and co-workers have demonstrated that the two cysteine residues (Cys112 and Cys120) coordinate to monovalent metal ions in an almost linear coordination geometry.[3,7]

MBL-VC: Ac-S**C**PGDQGSD**C**PI-NH_2
MBL-EC: Ac-A**C**PGDDSAD**C**PI-NH_2

Scheme 1. Amino acid sequences of the peptides encompassing the metal-ion-binding loop of CueR from *E. coli* (MBL-EC) and *V. cholerae* (MBL-VC), both N- and C-terminally protected. The two cysteine residues that coordinate monovalent metal ions in the CueR protein[3] are indicated in bold face.

The binding of monovalent metal ions was investigated by the use of Ag^{I} as a probe. The binding of divalent metal ions was probed with 1) Zn^{II} as the most relevant biometal against which discrimination must be exercised by CueR, and 2) Hg^{II} as a divalent cation with considerable similarity to Cu^{I} in terms of ligand and coordination-number preferences. Most notably, Hg^{II} commonly binds with high affinity in bisthiolato complexes, similar to the metal site present in CueR.

We recorded a series of UV absorption spectra at different pH values to monitor metal-ion binding through the observation of ligand-to-metal-charge-transfer (LMCT) bands (Figure 1). The two peptides showed the same qualitative changes in their absorption spectra as a function of the pH. The free pepides displayed a large increase in absorbance

[*] D. Szunyogh
MTA-SZTE Bioinorganic Chemistry Research Group
Dóm tér 7, 6720 Szeged (Hungary)
H. Szokolai, Prof. B. Gyurcsik, Prof. A. Jancsó
Department of Inorganic and Analytical Chemistry
University of Szeged, Dóm tér 7, 6720 Szeged (Hungary)
E-mail: jancso@chem.u-szeged.hu
Prof. P. W. Thulstrup, Dr. N. J. Christensen, Prof. L. Hemmingsen
Department of Chemistry, University of Copenhagen
Universitetsparken 5, 2100 Copenhagen (Denmark)
E-mail: lhe@chem.ku.dk
Prof. F. H. Larsen
Department of Food Science, University of Copenhagen
Rolighedsvej 30, 1958 Frederiksberg C (Denmark)
Dr. M. Stachura
ISOLDE-CERN, 1211 Geneva 23 (Switzerland)
and
TRIUMF, 4004 Wesbrook Mall, Vancouver, BC V6T 2A3 (Canada)
Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under http://dx.doi.org/10.1002/anie.201508555.

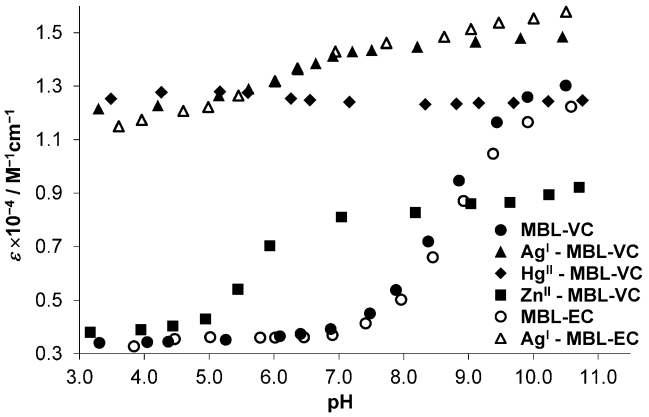

Figure 1. UV absorbance at 230 nm as a function of the pH for various metal-ion–MBL-VC systems and free MBL-VC (filled symbols), and for Ag^{I}–MBL-EC and free MBL-EC (open symbols). The absorbance values have been divided by the total ligand concentration ($c_{MBL\text{-}VC}$ or $c_{MBL\text{-}EC} = 1\text{–}2\times10^{-4}$ M). See Figures S1–S6 for the full UV spectra.

from about pH 7.5 to about pH 9.5, which reflects the deprotonation of the cysteine thiol groups. None of the metal–peptide complexes showed this change in the same pH range, illustrating that the thiol groups are deprotonated at a lower pH value in the presence of metal ions. For the Hg^{II}–MBL-VC complexes, there were only minor changes in the absorption over the entire pH range, indicating that both Cys residues were already coordinated to Hg^{II} at the lowest pH value investigated. The metal-site structure was probed by ^{199m}Hg PAC spectroscopy (PAC = perturbed angular correlation of γ rays; see Figure S7 and Table S1 in the Supporting Information). A characteristic signal of bis(thiolato)mercury-(II) was observed at pH 9.5. At pH 6.0, the signal was very similar, albeit with a marginally lower frequency, which possibly indicates the presence of an additional very weak ligand. In the Zn^{II}–MBL-VC system, the absorbance was very similar to that of the free peptide at low pH values, indicating that the metal ion does not bind at these conditions. An increase in absorbance was observed in the pH range from 5 to 6, thus indicating that the pK_a values of the two Cys residues fall in this range in the presence of Zn^{II}. For the Zn^{II}–MBL-VC complexes, an additional increase in absorbance was observed at high pH values (>10); this increase in absorbance presumably reflects the deprotonation of a coordinating water molecule.[8] In the presence of Ag^{I}, the absorbance at 230 nm of the two peptides was comparable to that of the Hg^{II}–MBL-VC complexes at low pH values and considerably higher than that of the free peptide, thus implying the coordination of both Cys sulfur atoms. However, a surprising change in absorbance, apparently reflecting the deprotonation of an ionizable group, was observed at pH ≈6.5 for both the Ag^{I}–MBL-VC and Ag^{I}–MBL-EC systems. The modest change in absorbance during the titration might imply the deprotonation of a coordinating thiol group, or alternatively the coordination of a thiolate replacing another ligand in the complex with Ag^{I}; the complexes would then have to display similar spectroscopic properties. This pK_a value ≈6.5 is unique for the monovalent metal ion, that is, it was not observed for the two divalent metal ions, and it is intriguing that it occurs relatively close to physiological pH, and it is thus potentially relevant to the function of CueR.

To further explore the pK_a value of the Ag^{I}–MBL-VC system, we conducted circular dichroism (CD) and potentiometric experiments (Figures 2 and 3). The CD spectra display an isodichroic point at approximately 218 nm, thus demonstrating that Ag^{I} is bound to the peptide, as the peptide is the

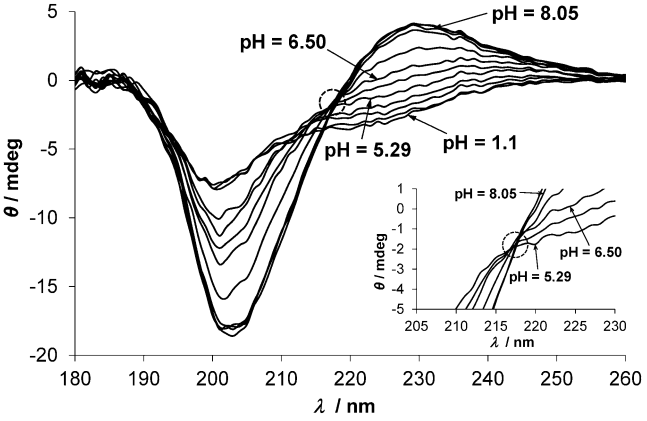

Figure 2. CD spectra for the Ag^{I}–MBL-VC system at different pH values. The isodichroic point (marked by a dashed circle) in the pH range from 5 to 8 at approximately 218 nm is highlighted in the insert. $c_{MBL\text{-}VC} = 1\times10^{-4}$ M.

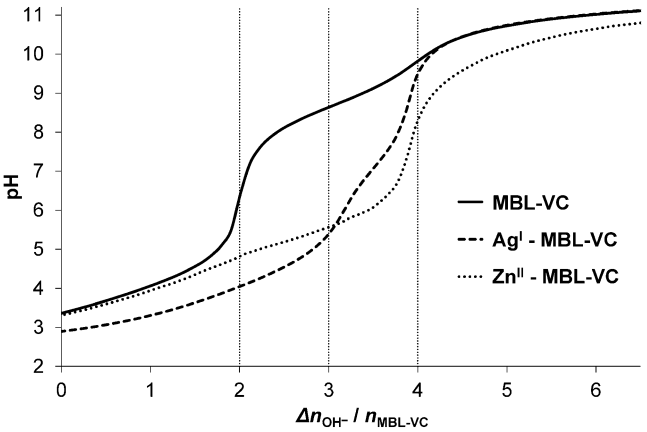

Figure 3. Titration curves for MBL-VC in the absence (solid line) and presence of metal ions (Ag^{I}: dashed line; Zn^{II}: dotted line). Volumes are normalized as equivalents of consumed base per ligand. The vertical lines identify the points of the curves at which 2, 3, and all the 4 hydrogen atoms of the ligand are titrated. $c_{MBL\text{-}VC} = 1\times10^{-3}$ M.

only chiral molecule present, and that the titration in the range of pH ≈5–8 mainly involves two species, that is, the protonated and deprotonated forms. The potentiometric data (Figure 3) further support the presence of a unique pK_a value of about 6.5 for the Ag^{I}–MBL-VC system. Furthermore, the data clearly demonstrate that the first deprotonation process for Ag^{I}–MBL-VC takes place at a pH value much lower than that for the free peptide (p$K_{a,Cys}$ = 8.25 and 9.06; see Table S2) and the Zn^{II}–MBL-VC complex, thus implying that the pK_a value for the first thiol is low for the Ag^{I}–MBL-VC

complex. The titrations of the Ag^{I}–MBL-VC complex and the free peptide at high pH values were very similar, thus indicating that no more than the expected four groups undergo titration (2 Asp, 2 Cys). In contrast, the Zn^{II}–MBL-VC system titrated in a highly similar manner to the free peptide at low pH values, thus indicating that Zn^{II} binds only above about pH 4 (see also Figure S8), after which the deprotonation of the two thiol groups occurs at pH ≈ 5–6. It also showed an additional group titrating at high pH values, assigned as a proton release of a metal-bound water ligand ($pK_a = 9.53$; see Table S2), in agreement with the UV data (see above). (A more detailed description of the speciation in the Zn^{II}–MBL-VC system can be found in the Supporting Information.)

Having established a unique pK_a value of approximately 6.5 for the Ag^{I} peptide systems, we conducted ^{1}H NMR experiments to identify the titrating group (Figure 4; see the Supporting Information for the assignment of the resonances, as deduced from 2D ^{1}H–^{1}H COSY, TOCSY, and ROESY experiments). In Figure 4 we focus on the $C_\beta H_2$ hydrogen atoms of the two Asp and two Cys residues of the MBL-VC peptide in the absence and presence of the metal ions. The free peptide showed the expected deprotonation of the carboxyl and thiol groups with pK_a values of about 4.0 and 8.5 (see also Table S2). In the presence of Ag^{I} and Hg^{II}, the pK_a values of the two carboxylic acids were only marginally perturbed, thus indicating that they do not participate in metal-ion binding, whereas coordination of at least one of the Asp carboxylate groups is likely in the Zn^{II}-containing system.

In the presence of Hg^{II}, the $C_\beta H_2$ proton resonances of the two Cys residues were shifted significantly downfield at all pH values, thus reflecting the coordination of both thiolate groups from pH 2 upwards. Similarly, in the presence of Zn^{II}, above pH 5–6, the $C_\beta H_2$ proton signals of the two Cys are affected, thus demonstrating that these residues coordinate to the metal ion. For the Ag^{I}–MBL-VC system, a somewhat different change as a function of the pH value was observed, with broad downfield resonances already at pH 2, thus revealing the coordination of at least one thiolate. More interesting is the group of resonances near 2.9 ppm at pH 5

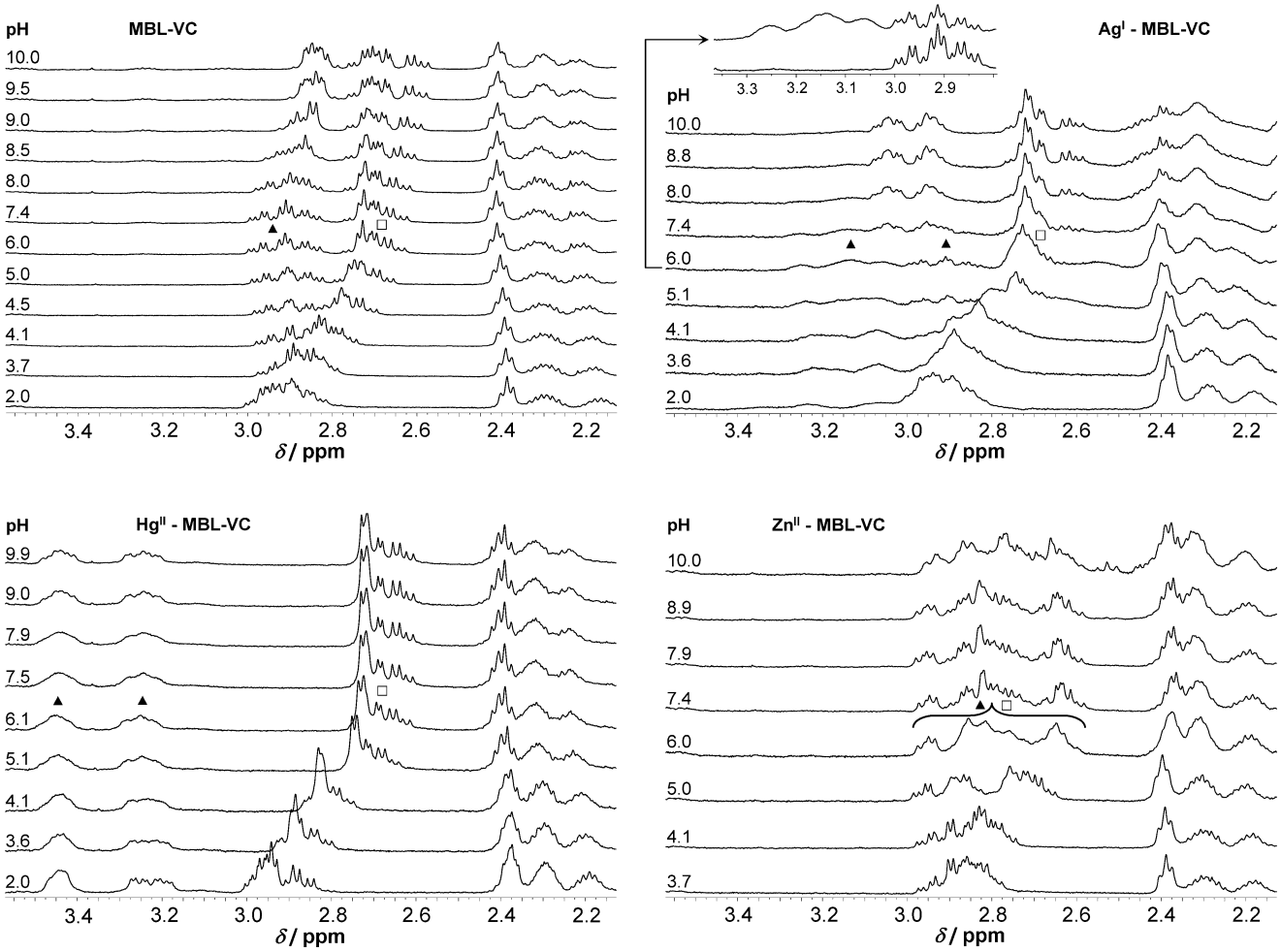

Figure 4. pH-dependent series of ^{1}H NMR spectra recorded for free MBL-VC and metal-ion–MBL-VC systems, in the range of the Cys and Asp $C_\beta H_2$ resonances ($\delta \approx 2.5$–3.5 ppm). ▲ and □ denote the resonances of Cys $C_\beta H_2$ and Asp $C_\beta H_2$, respectively. Comparison of spectra in the absence and presence of Ag^{I} ions at pH 6.0 in the insert (upper right series, recorded at higher concentration) demonstrates that free-ligand-like signals are clearly present even if one equivalent of Ag^{I} is added. (Signals appearing in the spectra between $\delta \approx 2.15$–2.45 ppm originate from Gln 6 $C_\beta H_2$, Pro 3 and Pro 11 $C_\beta H_2$, and Gln 6 $C_\gamma H_2$.) $c_{MBL\text{-}VC} = 1.3 \times 10^{-3}$ M, $H_2O/D_2O = 90{:}10$ v/v.

and 6. These resonances very closely resemble those observed for the free peptide, which strongly suggests that one of the thiols is protonated at this pH value. At pH 6.0, the integral of the broad bands associated with Ag^I-bound thiolate (3.01–3.29 ppm) corresponds to slightly more than two hydrogen atoms, whereas the resonances resembling thiol in the free peptide (2.80–3.01 ppm) correspond to slightly less than two hydrogen atoms (as determined by using the Asp $C_\beta H_2$ proton signals at 2.65–2.79 ppm as a reference, corresponding to four hydrogen atoms), which is consistent with a pK_a value of about 6.5. This analysis demonstrates that the pK_a value of approximately 6.5 for the Ag^I–MBL-VC peptide originates from deprotonation of the second Cys thiol. The same conclusions may be drawn for the MBL-EC peptide, but the spectra display more line broadening (see Figures S9 and S10). Whether the protonated thiol coordinates cannot be concluded, as the NMR data suggest that this is not the case, whereas the UV absorption data might indicate the contrary. A search in the Cambridge Structural Database[9] demonstrates that Cu^I and Ag^I coordination by protonated thiols is rare, but does exist.[10–12] Furthermore, the aqua ion of Ag^I is a rather weak acid, with a pK_a value of about 12 (extrapolated to zero ionic strength) and of about 10 at an ionic strength above 0,[13] that is, it decreases the pK_a value of water by about 4–6 pH units. Therefore, it seems plausible that Ag^I, in a formally neutral Ag^I–SSH unit, will be a very weak acid and will lower the pK_a value of the second thiol group by only a few pH units. Thus, the spectroscopic, structural, and thermodynamic data indicate that the presence of a protonated Cys residue at the metal site of CueR cannot be excluded.

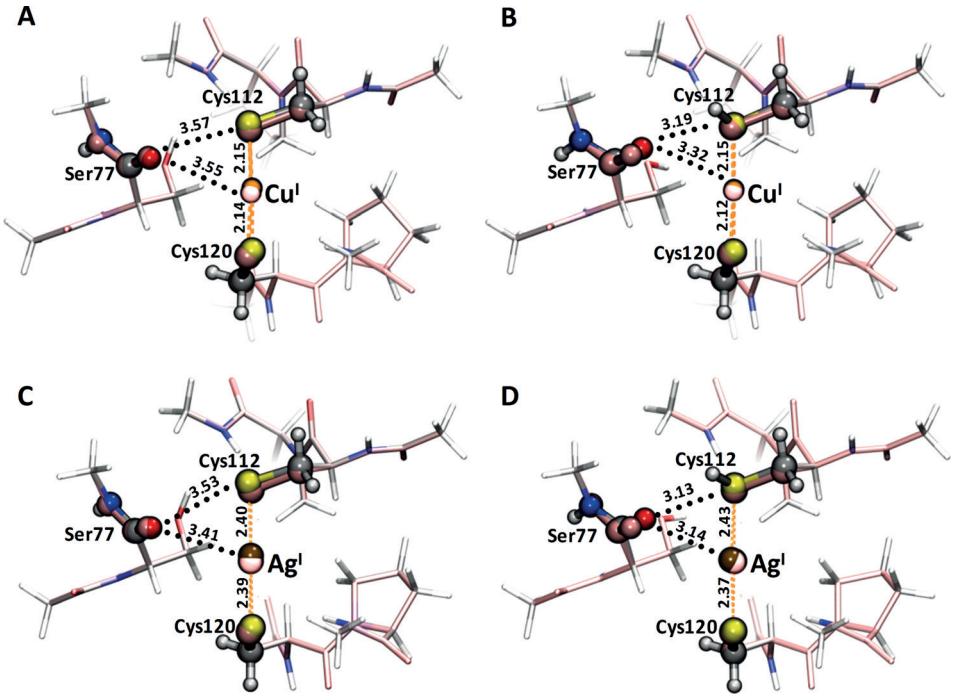

Figure 5. Overlay of the experimentally determined structures (pale red, PDB ID 1Q05 in A, B and 1Q06 in C, D) and geometry-optimized structures of the CueR metal site. Atoms allowed to relax in the geometry optimization are shown as colored spheres (H white, C grey, N blue, O red, S yellow, Cu orange, Ag brown). A,B) Cu^I models with deprotonated Cys112 (A) and protonated Cys112 (B). C,D) Ag^I models with deprotonated Cys112 (C) and protonated Cys112 (D). The geometry optimizations were carried out in ORCA[14] at the BP86-D3/def2-TZVP level of theory.[15–19] Selected interatomic distances for the optimized structures are indicated. Note that the Ser77 backbone oxygen atom is "pulled" towards both Cu^I (Ag^I) and the Cys112 sulfur atom upon protonation, even in this constrained optimization. The figures were made with VMD.[20]

To explore the effect of protonating Cys112 and if a thiol may coordinate Cu^I and Ag^I at the CueR metal site, we carried out quantum-chemical geometry optimizations of various model systems (Figure 5; see also Figures S11–S14). The metal site was optimized with and without protonated Cys112, both within a fixed protein-structure model, in which only the Cys side chains, Cu^I or Ag^I, Ser77 backbone atoms, and hydrogen atoms were able to relax, and for small model systems allowed to relax completely, that is, with no constraints from the protein. The large model system with both cysteine residues deprotonated is highly similar to the experimentally determined structure. Thus, the latter structure probably represents the deprotonated state for both coordinating cysteine residues, despite the peculiar proximity of the Ser77 backbone oxygen atom. Importantly, the effect of protonating Cys112 is to "pull" the Ser77 backbone oxygen atom about 0.2–0.4 Å closer to the metal site in a combined interaction involving both the hydrogen bond to Cys112 and a shorter Cu^I–O and Ag^I–O distance. Surprisingly, no other major structural change accompanies the protonation, that is, the Cu^I–S and Ag^I–S bond lengths only change marginally (< 0.03 Å), as is also the case for the small model systems (see Figures S11–S14). In this way, the metal-site reorganization energy upon protonation is minimized, while giving rise to considerable movement of Ser77, thus allowing for an efficient allosteric mechanism.

Finally, we investigated whether the metal site with Cys112 protonated was strained. The overall structures of the small model systems optimized without any constraints (see Figures S11–S14) are similar to those of the large model systems, and the thiol remains coordinated, although the hydrogen-bond geometry is more favorable. The strain energy is 55 kJ mol^{-1} for the large Cu^I model system and 57 kJ mol^{-1} for the large Ag^I model system. These values are comparable with estimates of strain energies at metal sites in other proteins,[21,22] although the strain energy is defined slightly differently in this study (see the Supporting Information). This result indicates that the CueR metal site may exist with

a coordinating protonated Cys112 residue. Similarly, in a thorough theoretical study of the binding of various metal ions to the CueR metal site,[23] it was demonstrated that the metal site discriminates against formal positive charge, but that discrimination between $Cu^{I}SS$ and $Cu^{I}SSH$ is less obvious. That is, one of the thiols might be protonated in the metal-bound form.

Another important aspect is how the local structure around the metal site in the protein may affect and tune the pK_a value of Cys112. The metal site is shielded from the solvent,[3] and should be stabilized in the uncharged $Cu^{I}SSH$ form. Similarly, the Cys120 sulfur atom accepts hydrogen bonds from two amide nitrogen atoms and is positioned directly over the N terminus of a small helix; thus, it may be stabilized as a thiolate by hydrogen bonding and the helix dipole. Although the argument is the same as that put forward by O'Halloran and co-workers, we argue in this case that the effect of the helix dipole is not to stabilize the negative charge of the $Cu^{I}SS$ species, but to selectively lower the pK_a value of Cys120 and thus ensure that Cys112 is the least acidic of the two cysteine residues. In the crystal structure,[3] S_{Cys112} takes part in only one other hydrogen bond: It accepts a hydrogen bond from the Gly114 amide nitrogen atom. In analogy to thiol coordination, thioether coordination to Cu^{I} is well established in cellular compartments, in which the conditions are less reducing than in the cytosol,[24] thus implying that the affinity of such sites—although presumably lower than that of bisthiolato–Cu^{I} coordination—is high enough to have physiological significance. Finally, EXAFS data recorded at pH 8.0 demonstrate that Cu^{I} is bound in a bisthiolato complex.[7] Under such conditions, both cysteine residues are expected to be deprotonated, in agreement with the experimental results in this study. For the MerR family members responding to divalent metal ions, the conserved Ser residue is substituted by a Cys residue,[3,25] which has been demonstrated to coordinate to Zn^{II} in ZntR.[3] Very recently, it was demonstrated that the mutation of Ser77 to Ala77 or Cys77 did not significantly affect the response of CueR to Cu^{I}, but that the introduction of a Cys residue at position 77 gave rise to sensitivity to divalent metal ions as well.[25] This result agrees well with a functional unit for the transmission of the effect of monovalent-metal-ion binding through the backbone carbonyl group of the amino acid at position 77. Thus, the model proposed herein (see above) coherently accounts for the sensitivity profile observed by Ibáñez et al.[25] On the basis of a previously observed change from a bisthiolato to a tristhiolato coordination mode in the Hg^{II} complexes of de novo designed TRIL9C peptides,[26] it is conceivable that a pH-dependent structural switch is also operational in the Hg^{II}-dependent MerR protein, in which the corresponding Cys79/Cys82 residue completes the trigonal-planar coordination geometry.[27–29] Thus, it seems plausible that a common allosteric mechanism may be functional in the metalloregulatory MerR family members, in which metal-ion specificity and the transmission of altered structure and dynamics upon metal-ion binding is propagated by "pulling" a specific residue from the other monomer of the homodimer: Either by direct metal-ion coordination of a conserved Cys residue for divalent metal ions, or through a hydrogen bond to a conserved Ser residue for monovalent metal ions. O'Halloran and co-workers recently demonstrated that amino acids 75–77 of one monomer undergo considerable movement upon Ag^{I} binding to the other monomer of the CueR–DNA complex.[6] Similarly, the structure of a MtaN, a MerR protein family member, displays the largest difference in backbone Φ/Ψ angles upon binding to DNA for residues 71–75, which constitute a hinge region. A structural alignment of CueR (PDB ID 1q05) and MtaN in complex with DNA (PDB ID 1r8d; see Figure S15)[30] demonstrates that the same hinge region is present for CueR,[30] and that it encompasses the conserved Ser residue, which we propose to be "pulled" upon metal-ion binding, thus further supporting the hypothesis put forth herein.

In summary, in a spectroscopic study of Ag^{I}, Zn^{II}, and Hg^{II} binding to model systems of the CueR metal-ion binding site, we observed a unique thiol pK_a value close to physiological pH for the Ag^{I} complexes. By using quantum-chemical methods, we explored the structural consequences of protonating Cys112. We propose a functional model that may be general to MerR family members, whereby the allosteric mechanism involves a conserved residue (Ser77 in the case of CueR) in the other monomer of the homodimer.

Acknowledgements

This research was supported by the Danish Council for Independent Research (Nature and Universe) of the Ministry for Higher Education and Science through the NICE grant, the Hungarian National Research Development and Innovation Office (Grant No: ANN 116390), and ISOLDE/CERN through beam-time grant IS488. We thank Prof. Morten J. Bjerrum and Prof. Erik Larsen (University of Copenhagen) for fruitful discussions.

Keywords: metal-sensor proteins · metal-site structure · molecular modeling · peptides · thiol coordination

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 15756–15761
Angew. Chem. **2015**, *127*, 15982–15987

[1] H. Reyes-Caballero, G. C. Campanello, D. P. Giedroc, *Biophys. Chem.* **2011**, *156*, 103–114.
[2] N. L. Brown, J. V. Stoyanov, S. P. Kidd, J. L. Hobman, *FEMS Microbiol. Rev.* **2003**, *27*, 145–163.
[3] A. Changela, K. Chen, Y. Xue, J. Holschen, C. E. Outten, T. V. O'Halloran, A. Mondragon, *Science* **2003**, *301*, 1383–1387.
[4] P. Zhou, F. Tian, F. Lv, Z. Shanghou, *Proteins Struct. Funct. Bioinformat.* **2009**, *76*, 151–163.
[5] J. L. Hobman, J. Wilkie, N. L. Brown, *Biometals* **2005**, *18*, 429–436.
[6] S. J. Philips, M. Canalizo-Hernandez, I. Yildirim, G. C. Schatz, A. Mondragón, T. V. O'Halloran, *Science* **2015**, *349*, 877–881.
[7] K. Chen, S. Yuldasheva, J. E. Penner-Hahn, T. V. O'Halloran, *J. Am. Chem. Soc.* **2003**, *125*, 12088–12089.
[8] D. Szunyogh, B. Gyurcsik, F. H. Larsen, M. Stachura, P. W. Thulstrup, L. Hemmingsen, A. Jancsó, *Dalton Trans.* **2015**, *44*, 12576–12588.
[9] F. H. Allen, *Acta Crystallogr. Sect. B* **2002**, *58*, 380–388.

[10] A. Bharti, P. Bharati, M. K. Bharty, R. K. Dani, S. Singh, N. K. Singh, *Polyhedron* **2013**, *54*, 131 – 139.
[11] M. Heller, W. S. Sheldrick, *Z. Anorg. Allg. Chem.* **2004**, *630*, 1869 – 1874.
[12] S. Hu, F.-Y. Yu, P. Zhang, D.-R. Lin, *Dalton Trans.* **2013**, *42*, 7731 – 7740.
[13] J. Burgess, *Metal Ions in Solution*, Ellis Horwood, Chicester, UK, **1978**.
[14] F. Neese, *WIREs Comput. Mol. Sci.* **2012**, *2*, 73 – 78.
[15] A. D. Becke, *Phys. Rev. A* **1988**, *38*, 3098 – 3100.
[16] J. P. Perdew, *Phys. Rev. B* **1986**, *33*, 8822 – 8824.
[17] S. Grimme, J. Antony, S. Ehrlich, H. Krieg, *J. Chem. Phys.* **2010**, *132*, 154104.
[18] F. Weigend, M. Häser, H. Patzelt, R. Ahlrichs, *Chem. Phys. Lett.* **1998**, *294*, 143 – 152.
[19] F. Weigend, F. Furche, R. Ahlrichs, *J. Chem. Phys.* **2003**, *119*, 12753.
[20] W. Humphrey, A. Dalke, K. Schulten, *J. Mol. Graph.* **1996**, *14*, 33 – 38.
[21] U. Ryde, L. Hemmingsen, *J. Biol. Inorg. Chem.* **1997**, *2*, 567 – 579.
[22] U. Ryde, M. H. M. Olsson, *Int. J. Quantum Chem.* **2001**, *81*, 335 – 347.
[23] L. Rao, Q. Cui, X. Xu, *J. Am. Chem. Soc.* **2010**, *132*, 18092 – 18102.
[24] A. V. Davis, T. V. O'Halloran, *Nat. Chem. Biol.* **2008**, *4*, 148 – 151.
[25] M. M. Ibáñez, S. K. Checa, F. C. Soncini, *J. Bacteriol.* **2015**, *197*, 1606 – 1613.
[26] O. Iranzo, P. W. Thulstrup, S. B. Ryu, L. Hemmingsen, V. L. Pecoraro, *Chem. Eur. J.* **2007**, *13*, 9178 – 9190.
[27] J. D. Helmann, B. T. Ballard, C. T. Walsh, *Science* **1990**, *247*, 946 – 948.
[28] L. M. Utschig, J. W. Bryson, T. V. O'Halloran, *Science* **1995**, *268*, 380 – 385.
[29] C.-C. Chang, L.-Y. Lin, X.-W. Zou, C.-C. Huang, N.-L. Chan, *Nucleic Acids Res.* **2015**, *43*, 7612 – 7623.
[30] K. J. Newberry, R. G. Brennan, *J. Biol. Chem.* **2004**, *279*, 20356 – 20362.

Received: September 12, 2015
Published online: November 13, 2015

