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Energetics of Zinc-Mediated Interactions in the Allosteric Pathways of Metal Sensor Proteins

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Abstract

A metal-mediated interprotomer hydrogen bond has been implicated in the allosteric mechanism of DNA operator binding in several metal-sensing proteins. Using computational methods, we investigate the energetics of such zinc-mediated interactions in members of the ArsR/SmtB family of proteins (CzrA, SmtB, CadC and NmtR) and the MarR family zinc-uptake repressor AdcR, each of which feature similar interactions, but in sites that differ widely in their allosteric responsiveness. We provide novel structural insight into previously uncharacterized allosteric forms of these proteins using computational methodologies. We find this metal-mediated interaction to be significantly stronger (~8 kcal/mol) at functional allosteric metal binding sites compared to a non-responsive site (CadC) and the *apo*-proteins. Simulations of the *apo*-proteins further reveal that the high interaction energy works to overcome the considerable disorder at these hydrogen-bonding sites and functions as a “switch” to lock in a weak DNA-binding conformation once metal is bound. These findings suggest a conserved functional role of metal-mediated second-coordination shell hydrogen bonds at allosterically responsive sites in zinc-sensing transcription regulators.

Metal-sensing transcriptional regulators play a central role in the cellular metal-ion homeostasis machinery.¹ While dominant pathways in the allosteric mechanisms of proteins are poorly understood, a metal-mediated hydrogen bond has been implicated in the long-range allosteric mechanism of a number of metal-sensing proteins.^{1,2} The Zn(II)/Co(II) sensor protein *S. aureus* CzrA (Figures 1, SI.1 and SI.2) is a prototypical example of the ubiquitous ArsR family of transcriptional repressors that imparts heavy metal tolerance in many pathogenic organisms.¹ CzrA is a homodimeric protein that binds a zinc ion at each of its two $\alpha 5$ metal binding sites along the dimer interface (Figure SI.2) utilizing two residues from each subunit (Asp84, His86, His97' and His100') to form a tetrahedral coordination environment.³ Under conditions of excess cellular zinc concentrations, CzrA binds zinc which gives rise to transcriptional derepression of *czrB*, encoding a cation diffusion facilitator pump that catalyzes zinc efflux. Zinc is a potent allosteric negative regulator of DNA-binding, inducing CzrA to transition from a high DNA binding-affinity “closed”

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conformation to a more “open” conformation, reducing the DNA binding affinity by ~6 kcal/mol.⁴ Zinc-binding is known to quench the protein’s internal dynamics, forcing CzrA to sample limited conformational ensembles that are electrostatically less favorable for DNA-binding.^{4,5} Insights into the allosteric mechanism in CzrA will aid in targeting metalloregulatory transcriptional repressors in order to impair transition metal resistance and associated anti-biotic resistance in pathogens, defining a novel antibiotic strategy.

A zinc-mediated interprotomer hydrogen bond between the N ϵ face of the conserved metal binding residue His97 and the backbone carbonyl group of His67’ is proposed to nucleate a hydrogen bonding pathway (HBP) that ultimately connects the α 5 metal binding sites (MBSs) to the α R reading heads of the DNA binding regions in Zn(II)-CzrA (Figures 1 and SI.3).³ This hydrogen bond is destabilized in *apo*-CzrA and absent in DNA-bound CzrA^{5,6} and is reminiscent of the Cu(I)-mediated hydrogen bond that contributes to the allosteric mechanism in *Mycobacterium tuberculosis* CsoR.⁷ In Zn(II)-CzrA, the HBP is hypothesized to “lock” the protein in the open conformation, and as such has been implicated in the mechanism of allosteric regulation.^{4,5}

In other members of the ArsR family, similar HBPs have been observed in Zn(II)-bound crystal structures of *Synechococcus* SmtB and the Cd/Pb sensor *S. aureus* pI258 CadC.^{3,8,9} NMR data suggest that a HBP may be present in the zinc-bound form of the Ni/Co sensor *M. tuberculosis* NmtR (Figure 2) as well.¹⁰ These proteins have evolved from a common ancestor but have developed unique selectivity and allosteric responses towards different metal ions.¹ CzrA, SmtB, NmtR and CadC share a common DHHX zinc-binding motif (X=H in CzrA and NmtR, X=E in SmtB and CadC) at their α 5 metal binding sites (Figure 2). His67 in CzrA is Arg87 in SmtB, Gly74 in NmtR and Gly84 in CadC.^{3,10,11} Zn(II)-binding to the α 5 sites in SmtB and NmtR triggers an allosteric response that drives the protein to an open conformation that has a low DNA-binding affinity. In contrast, CadC is allosterically non-responsive to Zn(II)-binding at the α 5 sites and adopts a closed DNA binding conformation in its zinc bound state.^{8,11} These factors suggest that HBPs at allosterically functional MBSs may be a diverged evolutionary characteristic of ArsR-family proteins.⁹ In the MarR-family protein *Streptococcus pneumoniae* AdcR (Figures 2), zinc functions as an allosteric *activator* of DNA binding, or opposite to that of ArsR-family repressors; nonetheless, a zinc-mediated hydrogen bond between Glu24 of the high-affinity zinc binding site and Asn38 extends to form a HBP that continues to the DNA binding helices.¹² The presence of such metal ion mediated interactions at functional allosteric sites across protein families for different metal ions suggest a conserved functional role.

In this study, we investigate the impact of zinc binding on the critical His97’-His67 hydrogen bond in Zn(II)-CzrA and examine related allosteric pathways in the other metalloregulatory proteins in an effort to identify common features of their allosteric mechanisms. *Ab initio* calculations were performed using the Gaussian09 program,¹³ while all QM/MM calculations were performed using the Schrodinger suite of programs.¹⁴ Symmetry adapted perturbation theory (SAPT) calculations¹⁵ were performed using the PSI4 program.¹⁶ Molecular dynamics (MD), NMR-refinement and quantum-mechanical/molecular-mechanical (QM/MM) MD calculations of over 2 μ s were performed using the Amber11 suite of programs.¹⁷ Detailed descriptions of all methods and calculations are provided as part of supporting information (SI).

Prior to investigating the role of the metal-ion in these allosteric pathways, we first analyzed the dynamic instability at these sites in the *apo*- proteins. While the structures of *apo*-SmtB and *apo*-NmtR suggest that these interactions are metal-ion dependent, similar structural information is unavailable for apo forms of CadC and AdcR.^{3,10} In an effort to generate an ensemble view of the dynamic instability at these hydrogen-bonding sites and facilitate our

understanding of the conformational dynamics that is overcome by the metal-ion in the course of allosteric regulation, we collected 1.2 μ s of MD data from simulations of the *apo* forms of AdcR, SmtB, NmtR and CadC.^{3,10} In the absence of experimental structures of wild-type *apo* forms of CadC and AdcR, we built models via MD simulations propagated from their zinc-bound structures after removing zinc.¹² In close agreement with our previous studies of CzrA, we find that the HBP is largely broken in the *apo*-forms of these proteins (Figure 3), though metastable conformations do occur with an intact HBP. The calculated free energy profiles^{17,18} shown in Figure SI.4, display an energetic barrier to forming these interactions in the *apo*-form and are indicative of the entropic penalty associated with metal-ion binding in order to form these allosteric networks.¹⁹ In close agreement, a thermodynamic analysis¹⁷ of our previous simulations of the various allosteric forms of CzrA,⁵ reveals that zinc binding indeed reduces the overall entropy of the protein (Table SI. 1), including at the H97-H67' hydrogen binding site. The conformational flexibility at these sites likely allows the transcriptional regulators to visit multiple conformational states that are conducive to DNA or metal-ion binding.

We next performed *ab initio* calculations to evaluate these interactions in the zinc-bound forms of CzrA, AdcR, NmtR SmtB and CadC. The strength of the H97-H67' hydrogen bond in Zn(II)-CzrA was calculated for a number of crystallographically and computationally derived protein conformations (Table SI.2). Using the counterpoise method, we calculated the basis set superposition error-corrected interaction energies between His67 and the metal-bound residues, Zn-D84H86H97'H100' (Figures 4 and SI.3) for the zinc-bound crystal structure geometry of CzrA.¹³ These calculations were performed on representative model systems at the second order Møller–Plesset perturbation level of theory while employing the augmented Dunning correlation-consistent polarized aug-cc-pVDZ and aug-cc-pVTZ basis sets.^{20,21} These data were then used to extrapolate to the complete basis set limit.²² In order to incorporate sampling and solvation effects, we calculated the interaction energies for additional conformations of Zn(II)-CzrA obtained from MD and QM/MM²³ (M06-2X/LACVP*/OPLS 2005)^{14,24,25} calculations (Table SI.1). Furthermore, we coupled MD simulations of Zn(II)-CzrA in explicit solvent using NMR-derived restraints with QM/MM MD (SCC-DFTB3/FF99SB)^{26,27} calculations to obtain an accurate NMR-based solution description of the metal bound form of the protein. Interaction energies were similarly calculated for zinc-bound crystal structure geometries of AdcR, SmtB and CadC (Figure SI. 7). In the absence of a reported structure of Zn(II)-NmtR, 500 ns of MD and 2 ns of QM/MM MD simulations were employed to derive its structure for these calculations.^{10,23,28} The interaction energies were calculated for additional conformations of CzrA and CadC obtained from QM/MM MD simulations. Interaction energy calculations were additionally performed for representative models using the universal implicit solvation model based on density (SMD).²⁹ SAPT calculations were performed to analyze the contributing factors to these interaction energies.

We determined the His97-His67' inter-fragment interaction energy to be on the order of -20 kcal/mol for Zn(II)-CzrA (Figures 4 and SI. 7, and Tables SI.3 to SI.8). Charge distribution calculations performed on the entire zinc-bound protein structure (M06L-LC/3-21G*/LANL2DZ)^{24,30,31} show that zinc binding locally polarizes residues around the metal binding sites, strengthening neighboring interactions (Figure SI.6). To address the impact of zinc binding on this specific interaction and estimate the associated stabilization afforded by metal-ion binding, we calculated the interaction energy for conformations of *apo*-CzrA with an intact H97-H67 hydrogen bond. It is noted that such conformations of *apo*-CzrA are transiently possible though NMR experiments and recent MD simulations find this interaction to be destabilized or absent.^{3–6} The metal binding residues in the *apo*-state were modeled in protonation states that agreed with the experimentally determined +1 charge increment upon binding a zinc ion.¹⁹ Our calculations found that zinc binding stabilized the

interaction by more than 10 kcal/mol compared to conformations of *apo*-CzrA with a structurally intact HBP (Figures 5 and SI.7). Such a large interaction energy is able to sufficiently overcome the dynamic instability observed in the *apo*-forms of these proteins and, coupled with entropic effects, provides a rationale for the absence of this hydrogen bond in the other allosteric forms of CzrA.

Our calculations further show that the zinc-mediated interaction energies are stronger at the functional allosteric metal binding sites of CzrA, AdcR, NmtR and SmtB compared to the non-functional site in CadC, which itself is comparable to those calculated for our *apo*-CzrA model. (Figures SI.7, and SI.8, and Tables SI.3 to SI.8). Zinc-binding fails to strengthen the interaction compared to *apo*-CadC (Figure SI.8) and is not sufficiently strong to overcome the protein's intrinsic conformational dynamics and thereby lock it into a weak DNA-binding open conformation observed in the Zn(II)-bound forms of other ArsR-family sensors. Indeed in some CadCs, the allosterically-inhibited $\alpha 5$ Zn(II) sites are not retained, and structural analysis of one of these by NMR is also consistent with a closed conformation.³ While SmtB and CadC share identical Zn(II)-binding motifs, the Arg87-Glu120 interaction contributes ~25 kcal/mol to the uniquely high interaction energies observed in Zn(II)-SmtB (see Figure 2). This strong Arg-Glu salt-bridge interaction is present in *apo*-SmtB as well, and possibly plays a role in forming the HBP on binding zinc. In sharp contrast, similar calculations show that the imidazole side chain of His67 contributes only ~1 kcal/mol towards this interaction energy and thus can not be responsible for the modest interaction energy observed in Zn(II)-CadC. CzrA and NmtR share identical zinc binding motifs, but significantly differ in their zinc binding affinities and allosteric responses.^{19,32} While Zn(II) is a strong negative allosteric regulator of CzrA, it has a weaker allosteric effect on NmtR as a result of not coordinating the N-terminal residues.¹⁰ No significant difference is observed between their model system interaction energies, emphasizing the additional role that conformational dynamics and protein structure play in the allosteric mechanism. Remarkably, the interaction energies for Zn(II)-AdcR are of a magnitude that is similar to other functional allosteric MBSs, despite the fact that Zn(II) is an allosteric *activator* of AdcR.¹² Furthermore, an SAPT energy-decomposition analysis found these interactions to be electrostatics driven (Figure SI.9 and Table SI.5). These calculations taken collectively, suggest that such zinc-mediated interactions may be a common feature of the allosteric mechanisms of metal-sensing repressors irrespective of the sign on the allosteric coupling free energy ($+\Delta G_c$ for ArsR/SmtB proteins; $-\Delta G_c$ for AdcR).²

We further examined the role of the HBP in the switch-dynamics of CzrA by propagating a molecular dynamics trajectory of *apo*-CzrA from its closed DNA-bound conformation. In this simulation, the protein structure successfully “opened” to achieve a low-DNA binding conformation without forming the critical H67-H97' hydrogen bond (Figure SI.10). *Apo*-CzrA was able to transition back to a moderately open conformation over the course of 150 ns of MD exhibiting the conformational flexibility of this allosteric form. In striking contrast, Zn(II)-CzrA, which maintains the HBP throughout, adopts an open conformation over 125 ns of MD in our previous simulations.⁵ This strongly suggests that this interprotomer HBP primarily functions as a “lock” that holds the CzrA dimer in the low DNA binding affinity open conformation.

In this study, we provide support for the general idea that the formation of a second coordination shell interaction is an energetically important feature of allosteric activation or inhibition of DNA binding by metal sensor proteins. Our calculations reveal that allosterically functional metal binding sites have stronger zinc-mediated interaction energies (~20 kcal/mol or greater) compared to the non-functional allosteric sites in CadC (~10 kcal/mol). An interaction energy on the order of the non-functional site in CadC (~10 kcal/mol)

characterizes the HBP-bearing conformation of *apo*-CzrA, indicating that this interaction would be unable to lock CzrA into an open conformation. Our MD simulations show that the HBP is not essential for CzrA to achieve a low-affinity open conformation, but upon metal binding functions primarily to rigidify or “lock” the protein into the low affinity open state.^{2–4} This study provides a novel energetic and dynamic perspective into the evolutionary adaptation of zinc-mediated hydrogen bonds at functional allosteric sites in bacterial metalloregulatory proteins. Understanding such allosteric pathways in proteins in detail is of tremendous intrinsic interest and may allow development of a new anti-microbial strategy based on perturbation of transition metal ion homeostasis that could be used to combat bacterial pathogens.³³

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

MD	molecular dynamics
MBS	metal binding site
QM/MM	quantum-mechanical/molecular-mechanical
SAPT	symmetry adapted perturbation theory
HBP	hydrogen-bonding pathway

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Figure 1.

Cartoon representation of Zn(II)-bound CsrA showing major secondary structural features (PDB code: 1R1V). Zinc ions are represented as silver spheres and its ligating residues are shown in green. The H97-H67' hydrogen bonds are shown with dotted black lines. Residues forming the HBP are shown in blue.

Figure 2. Protein Structure and zinc coordination in CadC (1U2W), SmtB (1R22) AdcR (3TGN) and NmtR (computational model) transcriptional regulators. Zinc ions are shown as silver spheres and the hydrogen bonds are shown with a green line.

Figure 3. Normalized population distribution of zinc-mediated hydrogen bond distances from MD simulations of the *apo* allosteric forms of CadC (H114-G84'), SmtB (H117-R87'), NmtR (H104-G74') and AdcR (E24-E38). A purple arrow indicates the hydrogen bond distance in crystallographically determined zinc-ion bound conformations of these proteins.

Figure 4.

Metal coordination environment at the $\alpha 5$ metal binding site of CzrA represented by a model system (PDB code:1R1V). Zn^{2+} is represented as a sphere. It coordinates with D84, H86, H97' and H100' residues. The H97-H67' hydrogen bond is indicated by a dashed green line. A red line separates the system into two-fragments used in the interaction energy calculation.

Figure 5. Inter-fragment interaction energies for *apo* and Zn(II)-bound conformations of CzirA calculated for protein structures obtained from crystallographic, QM/MM, QM/MM MD and MD methods. The data is shown in Tables SI.3 to SI.8.