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

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Supporting Information

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, 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 nonresponsive 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 *Staphylococcus 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 α5 metal binding sites (MBSs) 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 concentration, CzrA binds zinc, which gives rise to transcriptional derepression of *czrB*, encoding a cation diffusion facilitator antiporter 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" conformation to a more "open" conformation, reducing the DNA binding affinity by ~6 kcal/mol.[4] Zinc binding is known to quench the 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 antibiotic resistance in pathogens, defining a novel antibiotic strategy.

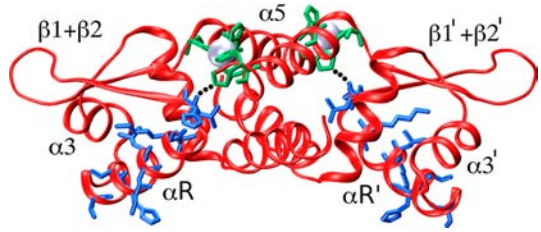

Figure 1. Cartoon representation of Zn(II)-bound CzrA showing major secondary structural features (PDB code: 1R1V). Zinc ions are represented as silver spheres, and its ligating residues are shown in green. The H97−H67′ hydrogen bonds are shown with dotted black lines. Residues forming the HBP are shown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

A zinc-mediated interprotomer hydrogen bond between the Nε 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 α5 MBSs to the αR 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 is 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 it 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

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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]

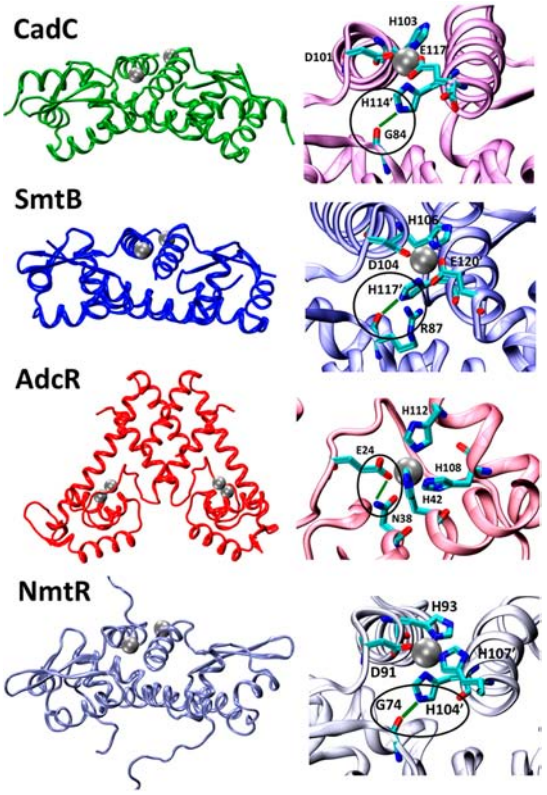

Figure 2. Protein structure and zinc coordination in CadC (PDB code: 1U2W), SmtB (1R22), AdcR (3TGN), and NmtR (computational model) transcriptional regulators. Zinc ions are shown as silver spheres, and the hydrogen bonds are shown with a green line.

These proteins have evolved from a common ancestor but have developed unique selectivity and allosteric responses toward 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 α5 MBSs (Figure 2). His67 in CzrA is Arg87 in SmtB, Gly74 in NmtR, and Gly84 in CadC.[3,10,11] Zn(II) binding to the α5 sites in SmtB and NmtR triggers an allosteric response that drives the protein to an open conformation with a low DNA binding affinity. In contrast, CadC is allosterically nonresponsive to Zn(II) binding at the α5 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 (Figure 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 metal-loregulatory proteins in an effort to identify common features of their allosteric mechanisms. *Ab initio* calculations were performed using the Gaussian09 program,[13] while all quantum mechanical/molecular mechanical (QM/MM) calculations were performed using the Schrodinger suite of programs.[14] Symmetry-adapted perturbation theory (SAPT) calculations[15] were performed using the PSI4 program.[16] Molecular dynamics (MD), NMR refinement, and QM/MM MD calculations of over 2 μs were performed using the Amber11 suite of programs.[17] Detailed descriptions of all methods and calculations are provided in the 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 μs 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

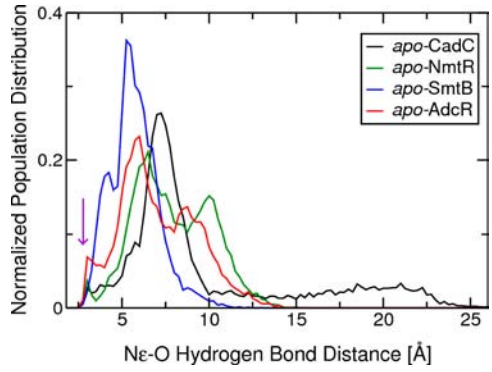

Figure 3. Normalized population distribution of zinc-mediated hydrogen bond distances from MD simulations of the *apo* allosteric forms of CadC (H114−G84′), SmtB (H117−R87′), NmtR (H104−G74′), and AdcR (E24−E38). A purple arrow indicates the hydrogen bond distance in crystallographically determined zinc-ion-bound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

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 analysis[17] 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 crytallographically and computationally derived protein conformations

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(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/MM[23] (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] In order to differentiate between the allosteric mechanism in CzrA and CadC, interaction energies were calculated for additional conformations of these proteins 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 factors contributing 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 5 and SI.7, and Tables SI.3−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 MBSs, 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 His97-His67′ 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 MBSs of CzrA, AdcR, NmtR, and SmtB compared to the nonfunctional 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−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 CadC's the allosterically inhibited α5 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 toward this interaction energy and thus cannot be responsible for the modest interaction energy observed in Zn(II)-CadC. CzrA and NmtR share identical zinc binding motifs, but they differ significantly 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

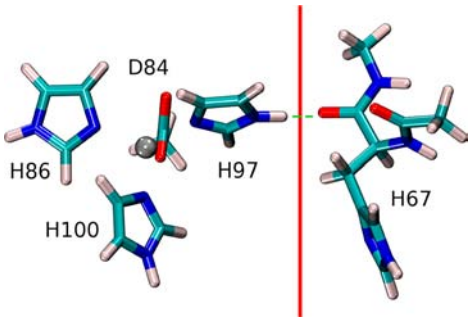

Figure 4. Metal coordination environment at the α5 MBS of CzrA represented by a model system (PDB code: 1R1V). Zn^{2+} is represented as a sphere. It coordinates with D84, H86, H97′, and H100′ residues. The H97−H67′ hydrogen bond is indicated by a dashed green line. A red line separates the system into two fragments used in the interaction energy calculation.

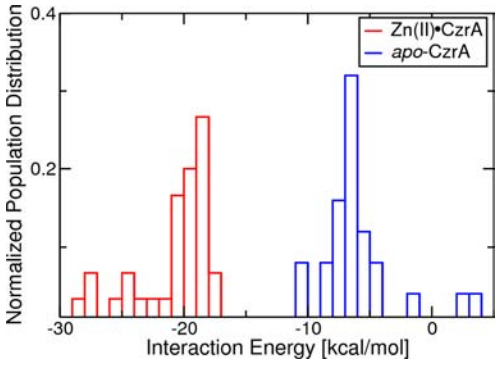

Figure 5. Inter-fragment interaction energies for *apo* and Zn(II)-bound conformations of CzrA calculated for protein structures obtained from crystallographic, QM/MM, QM/MM MD, and MD methods. The data are shown in Tables SI.3−SI.8.

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decomposition analysis found these interactions to be predominantly driven by electrostatics (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 conformation that binds to DNA weakly without forming the critical His97-His67′ 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] These results strongly suggest that the interprotomer HBP primarily functions as a "lock" that holds the CzrA dimer in the open conformation that has a lower DNA binding affinity.

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 MBSs have stronger zinc-mediated interaction energies (~20 kcal/mol or greater) compared to the nonfunctional allosteric sites in CadC (~10 kcal/mol). An interaction energy on the order of the nonfunctional 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. The detailed understanding of allosteric pathways in proteins is of tremendous intrinsic interest and may facilitate the development of a new antimicrobial strategy based on perturbation of transition metal ion homeostasis that could be used to combat bacterial pathogens.[33]

■ ASSOCIATED CONTENT

Supporting Information

Detailed descriptions of calculations, figures and tables, and complete refs 13, 16, and 17. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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