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Backbone and stereospecific methyl side chain resonance assignments of the homodimeric zinc sensor AdcR (32 kDa) in the apo- and Zn(II)-bound states

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Abstract

Streptococcus pneumoniae adhesin competence repressor (AdcR) is a Zn(II)-dependent 32 kDa homodimer that controls the transcription of a zinc-specific ABC uptake system (AdcABC), three pneumococcal histidine triad proteins (PhtA, PhtD and PhtE), and an AdcA homolog AdcAII. AdcR is the first metal-dependent member of the MarR family of prokaryotic transcriptional repressors. Two-dimensional NMR studies reveal large changes in the spectrum upon Zn(II) binding. Near complete backbone and stereospecific methyl group resonance assignments for apo- and Zn(II)-AdcR are presented here.

Keywords

Metalloregulatory protein; Winged helix; Zinc sensor; Allosteric regulation; *S. pneumoniae*

Biological context

Prokaryotes have evolved a myriad of metal sensor proteins that collectively control the intracellular concentrations of transition metal ions. These metalloregulatory proteins function as transcriptional regulators of genes that encode metal-specific, membrane bound uptake and efflux systems as well as cytosolic metal chelators. *Streptococcus pneumoniae* AdcR is a Zn(II)-specific metalloregulatory transcriptional repressor and the first metal-dependent member of the MarR family of prokaryotic transcriptional regulators to be characterized (Reyes-Caballero et al. 2010). The coordination of Zn(II) by AdcR results in the repression of the *adcRCBA* operon, which encodes a presumptive Zn(II)-specific importer, AdcABC, that has been shown to be necessary for virulence (Hava and Camilli 2002). The MarR family of transcriptional regulators is a widespread family in bacteria and is comprised of winged helix-turn-helix homodimeric proteins that are known to regulate multidrug resistance and oxidative stress.

Two dimensional NMR experiments have shown that binding of Zn(II) results in large global changes in the spectrum. The regulatory Zn(II) site in AdcR is a four-coordinate site that involves the direct coordination by the imidazole side chain of H42 in the $\alpha 2$ helix as well as H108 and H112 in the $\alpha 5$ helix (Guerra et al. 2011). In fact, mutation of any of these residues results in little or no changes in the spectrum consistent with the idea that Zn(II)

binding to these mutants fails to induce the appropriate conformational change to achieve a high-affinity DNA binding state. Also, in vitro binding assays have shown that mutation of any of these residues results in a diminished binding affinity for Zn(II) while in vivo experiments show that mutation of either H108 or H112 results in a growth phenotype indistinguishable from a $\Delta adcR$ strain, due to an elevated cell associated Zn(II) concentration. This reveals that these residues are critical for the in vivo function of AdcR (Reyes-Caballero et al. 2010). Although we previously reported the crystal structure of Zn(II)-bound AdcR to 2.0 Å resolution (Guerra et al. 2011), the structures of the other allosteric states of AdcR (apo- and DNA-bound), as well as insights into changes in conformational dynamics that characterize interconversion between states, are as yet undefined. As a first step in this process, here we report sequential resonance assignments for the backbone ($^1H^N$, ^{15}N , $^{13}C\alpha$, $^{13}C'$), side chain $^{13}C\beta$, and methyl side chain ^{13}C resonances for both apo- and Zn(II)-bound AdcR in order to gain new insights into allosteric switching by AdcR.

Methods and experiments

Protein expression and purification

Recombinant AdcR (146 residues) was expressed in *E. coli* BL21(DE3) cells as described in our previous work (Reyes-Caballero et al. 2010; Guerra et al. 2011). The samples used for backbone assignments of both the apo and Zn(II)-loaded forms of AdcR were isotopically enriched during protein expression using $^{13}C_6$ -glucose and $^{15}NH_4Cl$ as the sole carbon and nitrogen sources, respectively, at a level of 70 % random fractional deuteration. After overnight induction at 30 °C the cells were harvested by low speed centrifugation. For His-labeled AdcRs, a pET3a plasmid encoding the *adcR* gene was transformed into a histidine auxotrophic *E. coli* BL21(DE3) strain (generous gift from Dr. Waugh, NCI, Bethesda, MD). *E. coli* cultures were grown in M9 minimal media supplemented with 0.1 g of each unlabeled amino acid and 0.02 g of unlabeled histidine. Cultures were allowed to grow until an $OD_{600} \approx 0.6$ at which time 0.1 g of ^{13}C , ^{15}N -histidine was added. Cultures were incubated at 37 °C with shaking for 10 min before protein overexpression was induced by IPTG (400 μM final concentration). Cells were allowed to grow overnight at 30 °C before harvesting by low speed centrifugation. Purification was carried out as described above. For Ile, Val, and Leu methyl-labeled samples, *E. coli* cultures were grown in M9 minimal media prepared in D_2O with $[^2H_7, ^{13}C_6]$ -glucose and $^{15}NH_4Cl$ as the sole carbon and nitrogen sources, respectively. Once the cells reached an $OD_{600} \approx 0.6$ 85 mg of α - $[^{13}C_5, 3-^2H_1]$ ketoisovalerate and 50 mg of α - $[^{13}C_4, 3, 3-^2H_2]$ ketoisobutyrate were added to 1 L culture. Protein overexpression was induced 1 h later by IPTG (400 μM final concentration). Cells were allowed to grow overnight at 30 °C before harvesting by low speed centrifugation. In all cases purification was performed as previously described (Reyes-Caballero et al. 2010).

NMR spectroscopy

NMR samples of apo- and Zn(II)-AdcR were ≈ 0.3 mM dimer in 10 mM MES-*d*13 (Sigma), pH 6.0, with 50 mM NaCl, 1 mM TCEP (Gold Biotechnology), 0.02 % NaN_3 (Fisher) and 10 % (v/v) 2H_2O (CIL). Apo-AdcR samples were prepared in the presence of 1 mM EDTA and Zn(II)-AdcR samples were prepared in the presence of 2 mol equiv of Zn(II) to ensure saturation of both zinc sites. All NMR spectra were acquired at 35 °C on Varian VNMRs 600 and 800 MHz spectrometers equipped with cryogenic probes at the Indiana University METACyt Biomolecular NMR laboratory. Spectra were processed using NMRPipe (Delaglio et al. 1995) and analyzed using Sparky in combination with the automated assignment program PINE (Bahrami et al. 2009). The sequential backbone resonance assignments were obtained from 1H - ^{15}N TROSY, and triple resonance HNCA, HN(CO)CA, HN(CA)CB, HN(COCA)CB and HNCO spectra (Kay et al. 1992; Yamazaki et al. 1994).

Sequential backbone resonances of all 9 histidines were confirmed by transforming wild type protein expression vectors into a histidine auxotroph strain of *E. coli* BL21(DE3) (Waugh 1996) and utilizing the same strategy described above. Methyl groups of Ile, Val, and Leu residues in both apo- and Zn(II)-AdcR were assigned using ^1H - ^{13}C HMQC and HMCN [CG]CBCA (Tugarinov and Kay 2003). Stereospecific assignments for Leu and Val methyl groups were obtained using a ^1H - ^{13}C HSQC spectrum acquired on a 10 % ^{13}C labeled sample (Hilty et al. 2003). ^1H chemical shifts were referenced to 3-(trimethylsilyl)propane-1,1,2,2,3,3- d_6 -sulfonic acid, sodium salt (DSS) included as an internal standard in each sample. ^{13}C and ^{15}N chemical shifts were indirectly referenced to DSS using their absolute frequency ratios.

Assignments and data deposition

Apo-AdcR (146 residues) purifies as a dimer on a G75 gel filtration column. The ^1H - ^{15}N TROSY spectrum of apo-AdcR (Fig. 1a) shows 133 cross peaks consistent with a two-fold symmetric homodimeric protein under these conditions. Backbone ^1H , ^{13}C , and ^{15}N of apo-AdcR have been completed to 92 %. In total 131 out of 143 expected non-proline residues have been assigned with backbone amide resonances corresponding to Arg2, Gln3, Asn21, Cys30, Thr37, Asn38, Thr39, Leu75, Glu113, Tyr119, Arg136, Lys146 not assignable in this state. Additionally, 9 of 10 Ile δ -methyl, 19 of 38 Leu δ -methyl, and 17 of 18Val γ -methyl groups have been assigned in this state (Fig. 2a). All 36 assigned Leu and Val methyl groups have been stereospecifically assigned. The ^1H - ^{15}N TROSY spectrum of Zn(II)-AdcR (Fig. 1b) is also consistent with a two-fold symmetric homodimeric protein under the experimental conditions. Backbone ^1H , ^{13}C , and ^{15}N of Zn(II)-bound AdcR have been completed to 97 %. In total 139 out of 143 expected non-proline residues have been assigned with backbone amide resonance corresponding to Asp7, Asn13, Ala66, and Leu117 not assignable in this state. Additionally, 10 of 10 Ile δ -methyl, 28 of 38 Leu δ -methyl, and 18 of 18Val γ -methyl groups have been assigned in this state (Fig. 2b). Of the 66 assigned Leu and Valmethyl groups 64 have been stereospecifically assigned with the exception of Leu36 and Leu57, for which just one methyl group was observed (see Fig. 2). A CSI analysis of C α , C β and C' of both apo- and Zn(II)-bound AdcRs (Fig 3a, b respectively) reveals small but significant changes in the secondary structure of AdcR upon Zn(II) binding. The relatively large shifts observed for residues 52, 53, and 54 (Fig. 1) may be reporting on stabilization of a β 0 strand in the winged helix DNA binding motif and/or local tertiary structure upon metal binding (see Fig. 3). Chemical shift assignments have been deposited in the BioMagResBank with accession numbers 18413 (apo-AdcR) and 18414 (Zn(II)-AdcR).

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Fig. 1. ^1H , ^{15}N TROSY spectra of AdcR in the apo- (**a**) and Zn(II)-bound (**b**) states. Crosspeaks from the middle of the spectrum are shown in the insets (*top left* of each spectrum)

Fig. 2.
 ^1H , ^{13}C HMQC spectra of $^{15}\text{N}/^{13}\text{C}/^2\text{H}$ (Val-Leu-Ile)-methyl-protonated AdcR in the apo-
 (a) and Zn(II)-bound (b) allosteric states. Stereospecifically assigned methyl cross peaks are
 labeled with single letter code. The two methyl groups that could not be stereospecifically
 assigned are labeled with an *asterisk* (see text for details)

Fig. 3. Chemical shift indexing (CSI) of the $C\alpha$, $C\beta$ and C' nuclei for both apo-AdcR (**a**) and Zn(II)-AdcR (**b**). The results illustrate small but significant changes in the secondary structure of AdcR upon metal binding