Crystal Structure of the Zinc-dependent MarR Family Transcriptional Regulator AdcR in the Zn(II)-bound State

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Supporting Information

Experimental Procedures

Protein Expression

Unlabeled Protein

A pET3a plasmid encoding the *adcR* gene was transformed into *E. coli* BL21(DE3) cells. *E. coli* cultures were grown in LB medium supplemented with 100 µg/ml ampicillin at 37 °C with shaking at 250 rpm until reaching an OD600 = 0.6-0.8. Protein overexpression was induced by isopropyl β-D-1-thiogalactopyranoside, IPTG, (400 µM final concentration) and the cells allowed to grow overnight at 30 °C before harvesting.

Se-Met AdcR

Selenomethionyl AdcR was overexpressed in *E. coli* by inhibition of the methionine biosynthetic pathway followed by supplementation with selenomethionine as described.¹ Overexpression was induced by IPTG (400 µM final concentration) followed by growth overnight at 30 °C.

Methyl-Labeled Protein

E. coli cultures were grown in M9 minimal media prepared in D2O with [²H₇,¹³C₆]-glucose and ¹⁵NH₄Cl as the sole carbon and nitrogen sources, respectively. Once the cells reached an OD600 ≈ 0.6 85 mg of α-[¹³C₅,3-²H₁] ketoisovalerate and 50 mg of α-[¹³C₄,3,3-²H₂] ketoisobutyrate were added to 1 L culture. Protein overexpression was induced one hour later by IPTG (400 µM final concentration). Cells were allowed to grow overnight before harvesting by low speed centrifugation.

Protein Purification

AdcR purification was carried out as previously described² with the exception of the selenomethionyl AdcR which was purified in the presence of 10 mM DTT to avoid selenomethionine oxidation.

NMR Experiments

Methyl group assignments for the apo-, Zn(II)-AdcR and the Zn(II)-AdcR-DNA complex were obtained using a U- ^2H , ^{15}N , ^{13}C] Ile δ - $^{13}\text{CH}_3$] Leu, Val- $^{13}\text{CH}_3$ / $^{13}\text{CH}_3$] AdcR (ILV) sample(s) containing only exchangeable backbone and side chain amide protons, γ -methyl protons of Val residues, and δ -methyl(s) of Ile/Leu residues prepared as described previously³. Methyl groups were assigned using ^1H , ^{13}C HMQC, and HMCM[CG]CBCA experiments.⁴ NMR spectra for the apo-form were collected on a Varian Inova 600 equipped with a room temperature probe at the Texas A&M University Biomolecular NMR Laboratory. All other NMR spectra were acquired on a Varian DDR 800 MHz spectrometer equipped with a cryogenic probe at the Indiana University MetaCyt Biomolecular NMR laboratory. Typical solution conditions were 200–600 μM ^{15}N -labeled or ILV-labeled protein, 10 mM MES- d_{13} (pH 6.0, Sigma) or 10 mM Tris-HCl (pH 8.0, Sigma), 50 mM NaCl, 1mM imidazole, 1 mM TCEP (Gold Biotechnology), 0.02% (w/v) NaN₃ (Fisher) and 10% (v/v) $^2\text{H}_2\text{O}$ (CIL). All spectra were acquired at 35 °C. NMR spectra were processed and analyzed using NMRPipe⁵ and SPARKY⁶. Chemical shift referencing is relative to 2,2-dimethyl-2-silapentene-5-sulfonic acid (DSS; Sigma).⁷

Crystallography

Zn(II)-AdcR samples were prepared by adding a slow addition of excess Zn(II) to apo-AdcR in the presence of 1mM imidazole. Zn(II) AdcR and Zn(II)-SeMet-substituted AdcR were concentrated to 350 μM and crystallized in 0.1 M Tris-HCl (pH 8.5), 18% PEG 10,000, 20% glycerol,

and 0.1 M NaCl (Qiagen) by hanging drop vapor diffusion at 20 °C. Crystals were flash frozen in liquid nitrogen. Intensity data for the native and SeMet-substituted AdcRs were collected at the Advanced Light Source (ALS) Beamline 4.2.2. All data were processed with HKL2000.⁸ Phase calculations were performed by the AutoSol module of PHENIX.⁹ Phases from the SeMet-Zn(II)-AdcR data set were combined with intensities from the native set in AutoBuild to generate an initial model and electron density maps for the native complex. Model building was performed in Coot¹⁰ and refinement in PHENIX.

TABLE S1. Data collection, phasing and refinement statistics for Zn(II)-AdcR

Zn(II)-AdcR	
Data collection	
Space group	P2 ₁ 2 ₁ 2 ₁
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	55.848, 57.621, 85.443
α , β , γ (°)	90.00, 90.00, 90.00
Resolution (Å)	50.00-2.00 (2.03-2.00)*
<i>R</i> _{sym}	0.069 (0.287)
<i>I</i> / σ <i>I</i>	21.49 (3.80)
Completeness (%)	97 (99.7)
Redundancy	5.7 (5.6)
Refinement	
Resolution (Å)	1.998
No. reflections	17718
<i>R</i> _{work} / <i>R</i> _{free}	0.1847 / 0.2497
No. atoms	
Protein	2506
Ligand/ion	4
Water	302
<i>B</i> -factors (Å ²)	
Protein	22.0
Chain A	18.2
Chain B	25.6
Ligand/ion	17.6
Water	31.0
R.m.s deviations	
Bond lengths (Å)	0.004
Bond angles (°)	0.624

*Values in parentheses are for highest-resolution shell

Table S2. Summary of the Metal binding site geometries for sites 1 and 2

Site	Ideal Structure	Bond Distances (Å)	Structure Angles
Site 1		M—Nε2(H112): 2.04	Nε2(H112)-M- Nε2(H108): 100.7°
		M— Nε2(H108): 2.03	Nε2(H112)-M- Nδ1(H42): 108.3°
		M— Nδ1(H42): 2.02	Nε2(H112)-M- Oε1(E24): 118.2°
		M—Oε1(E24): 1.98	Nε2(H108)-M- Nδ1(H42): 105.6°
			Nε2(H108)-M- Oε1(E24): 107.9°
Site 2			Nδ1(H42)-M- Oε1(E24): 114.6°
		M—OH2: 2.09	H2O-M- Oε1(E107): 120.5°
		M— Oε1(E107): 2.00	H2O-M- Oε1(E41): 89.3°
		M—Oε1(E41): 2.01	H2O-M- S(C30): 113.4°
		M—S(C30): 2.23	Oε1(E107): -M-O2: 103.2°
			Oε1(E107): -M- S(C30): 113.8°
			Oε1(E41)-M- S(C30): 113.3°

Table S3. Partial residue-specific (non-stereospecific) methyl ^1H and ^{13}C chemical shift assignments for the apo-, Zn(II)-bound and DNA-bound ternary AdcR complex. Calculation of the chemical shift perturbation (Δppm) shown in Figure 4 was done using the largest perturbation (in italics) when more than one assignment was available for a particular residue. Overall, 45 of 66 total methyl resonances (9 of 10 Ile, 20 of 38 Leu, 18 of 20 Val) were assigned in the apo- form and 56 of 66 total methyl resonances (10 of 10 Ile, 28 of 38 Leu, 19 of 20 Val) were assigned in the Zn(II)- and ternary complex states.

Res. #	Assignment	Apo-AdcR		Zn(II)-AdcR			Zn(II)-AdcR-DNA		
		$\delta^{13}\text{C}$ (ppm)	$\delta^1\text{H}$ (ppm)	$\delta^{13}\text{C}$ (ppm)	$\delta^1\text{H}$ (ppm)	$\Delta\delta_{\text{apo-Zn}}$ (ppm)	$\delta^{13}\text{C}$ (ppm)	$\delta^1\text{H}$ (ppm)	$\Delta\delta_{\text{Zn-DNA}}$ (ppm)
L4	CD1-MD1	nd	nd	22.946	0.774	nd	22.871	0.711	0.081
L4	CD2-MD2	22.891	0.788	23.307	0.814	1.093	23.389	0.801	0.082
I8	CD1-MD1	14.739	0.916	14.701	0.892	0.040	14.567	0.802	0.141
L12	CD1-MD1	23.289	0.878	23.438	0.870	1.531	23.399	0.850	0.040
L12	CD2-MD2	nd	nd	nd	nd	nd	nd	nd	nd
V15	CG1-MG1	22.252	1.088	23.646	1.100	1.432	23.636	1.035	0.034
V15	CG2-MG2	20.864	1.040	20.863	1.073	0.017	20.899	1.000	0.051
I16	CD1-MD1	13.160	0.773	13.181	0.553	0.112	13.217	0.502	0.044
L17	CD1-MD1	nd	nd	23.372	0.943	nd	nd	nd	nd
L17	CD2-MD2	nd	nd	25.150	0.978	nd	25.247	0.889	0.107
I25	CD1-MD1	12.761	0.755	11.992	0.745	0.769	12.452	0.740	0.460
L26	CD1-MD1	nd	nd	20.967	-0.417	nd	20.971	-0.486	0.035
L26	CD2-MD2	26.458	0.645	27.024	0.549	0.568	27.413	0.508	0.390
I27	CD1-MD1	nd	nd	10.879	0.801	nd	10.707	0.741	0.175
V34	CG1-MG1	20.635	0.865	20.549	0.889	0.087	20.987	0.777	0.442
V34	CG2-MG2	20.631	0.810	21.102	0.859	0.472	21.245	0.806	0.145
L36	CD1-MD1	nd	nd	27.072	0.820	nd	26.986	0.765	0.090
L36	CD2-MD2	nd	nd	nd	nd	nd	nd	nd	nd
I43	CD1-MD1	15.512	0.642	14.093	0.614	1.419	13.774	0.641	0.319
L44	CD1-MD1	nd	nd	25.620	0.398	nd	25.467	0.321	0.158
L44	CD2-MD2	19.743	-0.176	19.614	-0.226	0.131	19.498	-0.320	0.125
L46	CD1-MD1	nd	nd	22.205	0.783	nd	22.293	0.713	0.095
L46	CD2-MD2	nd	nd	nd	nd	nd	nd	nd	nd
L47	CD1-MD1	23.717	1.215	23.413	1.210	0.304	23.697	1.229	0.284
L47	CD2-MD2	nd	nd	nd	nd	nd	nd	nd	nd
L52	CD1-MD1	23.228	0.843	23.307	0.814	0.080	23.389	0.801	0.082
L52	CD2-MD2	26.490	0.742	26.410	0.737	0.080	26.404	0.712	0.014
L57	CD1-MD1	25.741	0.806	25.597	0.800	0.828	25.391	0.740	0.208
L57	CD2-MD2	nd	nd	nd	nd	nd	nd	nd	nd
L61	CD1-MD1	23.962	0.697	23.963	0.704	0.601	23.945	0.630	0.041
L61	CD2-MD2	25.603	0.631	25.590	0.675	0.026	25.613	0.579	0.053
V63	CG1-MG1	19.235	0.678	19.141	0.656	0.095	18.605	0.529	0.540
V63	CG2-MG2	21.134	0.688	20.851	0.630	0.654	20.267	0.440	0.592
V68	CG1-MG1	22.163	0.890	21.270	0.558	0.908	21.328	0.500	0.065
V68	CG2-MG2	24.000	0.792	23.915	0.814	0.538	23.469	0.780	0.325
I72	CD1-MD1	12.898	0.188	11.614	0.286	1.285	10.694	-0.158	0.946
L75	CD1-MD1	23.363	0.754	23.222	0.736	0.713	23.264	0.670	0.053
L75	CD2-MD2	nd	nd	nd	nd	nd	nd	nd	nd
V76	CG1-MG1	20.864	1.040	20.865	1.027	0.011	20.746	0.965	0.123

V76	CG2-MG2	22.145	0.891	22.152	0.929	<i>0.902</i>	22.152	0.929	0.000
L81	CD1-MD1	24.812	0.795	24.965	0.759	0.154	24.928	0.748	0.037
L81	CD2-MD2	nd	nd	nd	nd	nd	nd	nd	nd
V92	CG1-MG1	20.631	0.810	20.590	0.806	0.041	20.385	0.808	<i>0.205</i>
V92	CG2-MG2	21.478	0.666	21.327	0.663	<i>0.151</i>	21.504	0.700	0.178
I93	CD1-MD1	12.243	0.730	12.391	0.717	0.148	12.007	0.672	0.385
L97	CD1-MD1	24.740	0.866	24.777	0.836	0.010	24.530	0.795	0.248
L97	CD2-MD2	nd	nd	25.411	0.826	nd	25.411	0.826	0.000
L100	CD1-MD1	24.036	0.944	23.959	0.963	0.078	24.014	0.896	0.064
L100	CD2-MD2	nd	nd	24.103	0.907	nd	24.014	0.896	<i>0.089</i>
I104	CD1-MD1	13.361	0.894	13.940	0.959	0.580	13.839	0.885	0.108
L116	CD1-MD1	23.228	0.843	24.149	0.851	0.921	23.950	0.836	<i>0.199</i>
L116	CD2-MD2	26.431	0.773	26.329	0.773	<i>2.619</i>	26.324	0.750	0.013
L117	CD1-MD1	25.349	0.740	25.149	0.730	0.200	25.071	0.658	0.086
L117	CD2-MD2	nd	nd	nd	nd	nd	nd	nd	nd
V122	CG1-MG1	22.802	1.149	22.799	1.131	1.291	22.835	1.065	0.049
V122	CG2-MG2	21.509	1.021	21.460	1.011	<i>1.344</i>	21.389	0.932	<i>0.081</i>
V133	CG1-MG1	21.511	0.786	21.576	0.780	<i>0.422</i>	22.007	0.726	<i>0.432</i>
V133	CG2-MG2	22.832	1.236	22.938	1.236	0.150	22.943	1.160	0.038
I134	CD1-MD1	12.172	-0.416	12.130	-0.437	0.043	12.048	-0.518	0.091
L138	CD1-MD1	nd	nd	nd	nd	nd	nd	nd	nd
L138	CD2-MD2	nd	nd	nd	nd	nd	nd	nd	nd
L141	CD1-MD1	24.740	0.866	24.579	0.883	0.161	24.551	0.839	0.036
L141	CD2-MD2	28.285	0.705	27.187	0.702	<i>1.098</i>	27.045	0.738	<i>0.143</i>
V142	CG1-MG1	22.253	0.465	22.490	0.354	0.438	22.738	0.256	<i>0.253</i>
V142	CG2-MG2	23.082	0.974	22.803	0.931	<i>1.931</i>	22.803	0.931	0.000
I145	CD1-MD1	13.387	0.805	13.750	0.728	0.365	13.261	0.758	0.489

Figure S1. Superimposition of the two AdcR protomers within the homodimer. Chain A is shaded green and chain B is shaded cyan. The two protomers superimpose with a r.m.s.d. of 1.2 Å for the corresponding 129 C α atoms, excluding the β -wing which is not observed in chain A.

Figure S2. Close up view of sites 1 and 2. Metal binding residues are colored yellow (site 1) and blue (site 2) and highlighted in stick representation. Second coordination sphere residues N38 (zinc site 1) and S32 (zinc site 2) are shown in line representation. Hydrogen bonds are shown as black dashed lines.

Figure S3. Backbone amide chemical shift perturbation upon Zn(II) binding to apo-E41A AdcR (A) and apo-E107A AdcR (B) s obtained from analysis of ^1H - ^{15}N TROSY spectra each AdcR with and without 1.0 monomer mol•equiv Zn(II). Both plots are indicative of large global changes in the spectrum consistent with what is observed in WT-AdcR.²

Figure S4. Surface representation of zinc site 1. Metal-binding side chains are highlighted in stick representation with the carbon atoms colored yellow. (A) Orientation is the same as the cyan chain B in Fig. 1. (B) 180° rotation along the z -axis. Both panels illustrate the extent to which site 1 is solvent exposed. The non-liganding Oε2 from E24, Nε2 from H108 and Nδ1 from H112 form hydrogen bonds with water molecules on the surface (see panel B).

Figure S5. Effect of metal binding on apo AdcR. (A) Co(II) (top) or Zn(II) (bottom) titration into apo-AdcR (pH 6.0, 500 μ M AdcR, $K_{Co} = 2.4 \times 10^5 \text{ M}^{-1}$)². Representative crosspeaks (L52, L81, T127, V133) are colored *blue* for apo-AdcR, *orange* for 0.5 mol equiv metal added to apo-AdcR, and *green* for fully metallated AdcR. (B) Backbone amide chemical shift perturbation upon Co(II) (left) and Zn(II) (right) binding to apo-AdcR. Co(II) perturbations are colored from cyan to red and Zn(II) perturbations are colored from blue to red. Grey residues represent no data, which for Co(II) includes the paramagnetic broadening by Co(II) at the metal binding site. (C) Backbone amide chemical shift perturbation represented as a bar graph. The grey horizontal bar in the Co(II) bar graph (left) represents the area broadened beyond detection by the paramagnetic effect of Co(II). Perturbations were calculated using $\Delta\delta = [(\Delta\text{ppm } ^1\text{H})^2 + (\Delta\text{ppm } ^{15}\text{N} / 7)^2]^{1/2}$

Figure S6. Primary structure alignment of *S. pneumoniae* AdcR with putative AdcRs from *S. gordonii*, *S. pyogenes* and *S. mutans*; *L. lactis* ZitR;¹¹ as well as two structurally characterized MarR family members, *E. coli* MarR and *S. enterica* SlyA.^{12,13} The alignment was made using ClustalW and colored by percent identity using Jalview^{14,15}. Secondary structure elements of Zn(II)-AdcR are indicated above the alignment as defined by the crystal structure and the metal binding residues are enclosed in red dashed boxes.

Figure S7. Superimposition of the winged helical domains of chain B of Zn(II)-AdcR and one protomer of SlyA in the DNA-bound form. AdcR chain B is colored cyan and the SlyA protomer is colored orange. The DNA has been omitted for clarity.

Supplementary References

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