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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the N-terminal 16 kDa domain of *Escherichia coli* Ada protein

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Biological context

The N-terminal 16 kDa domain of *Escherichia coli* Ada protein (residue 1-146, N-Ada16k) specifically demethylates the Sp diastereomer of DNA methyl phosphotriester lesions by a direct and irreversible methyl transfer to its cystein residue, C38. Upon the C38 methylation of N-Ada16k, its sequence-specific DNA binding affinity for the promoter regions of its own gene and other methylation resistance genes is enhanced dramatically by 10^3-fold. Then the C38 methylated N-Ada16k (meC38 N-Ada16k) acts as a transcriptional regulator for the methylation damage. It is of interest to understand how the C38 methylation can induce the sequence-specific DNA binding activity. N-Ada16k contains a single, tightly bound zinc ion that is necessary for proper folding *in vitro* and *in vivo* (Myers et al., 1992). The ligand residues for the zinc ion are the four cystein residues: C38, C42, C69, and C72 (Myers et al., 1993). ^{113}Cd NMR studies revealed that the thiols of four cystein residues are coordinated to the zinc ion both before and after the C38 methylation (Ohkubo et al., 1994). Thus the Ada protein is the first example of which a zinc ion coordinated cystein residue acts as a nucleophilic catalytic ligand. Recently, a mutation study showed that the replacement of C42, C69 and C72 dramatically decreases the methyltransferase activity (Sun et al., 2001). This result indicates that the nucleophilicity of C38 is enhanced by other ligand residues through the coordinate bond.

For the N-terminal domain of Ada protein, the solution structure of the 10kDa fragment (residue 1-92, N-Ada10k), which possesses the methyltransferase activity, has been reported by NMR (Myers et al., 1993). It has a four-stranded β-sheet sandwiched between two α-helices. The Zn binding site is located at the edge of the β-sheet. The refined solution structure of N-Ada10k showed a remarkable cavity around the zinc center to trap the methylated phosphate on DNA (Lin et al., 2001). However, since N-Ada10k lacks the sequence-specific DNA binding activity, the mechanism of the transcriptional regulation by N-Ada16k remains unclear. Here we report the ^{1}H, ^{13}C, and ^{15}N NMR assignments of N-Ada16k and meC38 N-Ada16k from *E. coli* and compare the chemical shift differences between them as the first step in determining the solution structure by NMR.

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Methods and results

The DNA fragment encoding N-Ada16k sequence of *Escherichia coli* K12 strain was cloned into *Nde*I/*Bam*HI sites of the pET22b plasmid vector. The recombinant plasmid was introduced into *E. coli* strain BL21(DE3). The bacteria were grown at 37 °C and protein expression was induced by addition of 1 mM isopropyl-β-D-thiogalactopyranoside. After induction for 2 h, the bacteria were harvested by centrifugation. The cell pellets were sonicated and centrifuged. The supernatant was purified using the phosphocellulose P-11 column according to the reported procedure (Sakashita et al., 1995). MeC38 N-Ada16k was prepared by incubation of N-Ada16k with methylnitrosourea-treated DNA for 30 min. Purity of the protein was confirmed by SDS-PAGE. ^{15}N labeled proteins or ^{15}N/^{13}C doubly labeled proteins were prepared using M9 minimal media with ^{15}N ammonium chloride and/or ^{13}C glucose as the sole nitrogen and carbon sources. The proteins were dis-

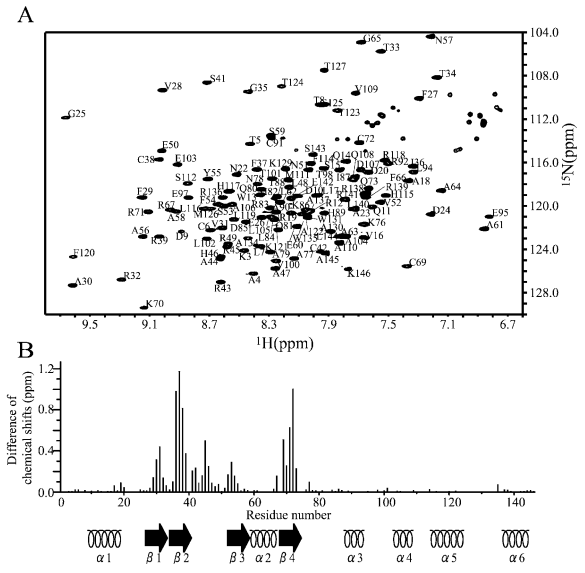

Figure 1. (A) ^{1}H-^{15}N HSQC spectrum of 1 mM meC38 N-Ada16k from *Escherichia coli*. Backbone amide cross peaks are indicated with their one letter code and number. (B) Top: Comparison between N-Ada16k and meC38 N-Ada16k chemical shifts (HN, N). The combined chemical shift differences, $\Delta\delta_{tot}$, on all residues were calculated according to the equation following. $\Delta\delta_{tot} = ((\Delta\delta_{HN} \times W_{HN})^2 + (\Delta\delta_N \times W_N)^2)^{1/2}$. The weighting factors used were $W_{HN} = 1$, $W_N = 0.154$ (Ayed et al., 2001). Bottom: Summary of the secondary structure elements (α-helix, spiral; β-strands, arrow).

solved in 50 mM sodium phosphate buffer of 85% H_2O/15% D_2O at pH 6.5 with 300 mM NaCl and 5 mM 2-mercaptoethanol to the protein concentrations of 1 mM.

All 2D and 3D NMR spectra were acquired at 30 °C using a Varian INOVA 600 MHz spectrometer equipped with a tripleresonance z-gradient probehead. The NMR experiments performed included 2D ^{1}H-^{15}N HSQC, 3D HNCO, HNCA, HN(CO)CA, HNCACB and CBCA(CO)NH. All the NMR data were processed with NMRPipe (Delaglio et al., 1995) and analyzed with the PIPP program (Garrett et al., 1991). The sequential resonance assignments were established by the combined analysis of the 2D and 3D NMR spectra. Figure 1A shows the ^{1}H-^{15}N HSQC spectrum of meC38 N-Ada16k with assignments for the cross peaks indicated by residue number. To estimate the secondary structure, the consensus CSI data for both N-Ada16k and meC38 N-Ada16k were calculated from C_α, C_β and CO chemical shifts. Figure 1B shows the chemical shift differences, $\Delta\delta_{tot}$, between N-Ada16k and meC38 N-Ada16k. The four-stranded β-sheet region in the vicinity of C38 (residue 27-32, 36-39, 52-55 and 67-69) and the loop region (residue 40-51) exhibit more striking chemical shift changes ($\Delta\delta_{tot} > 0.1$ p.p.m.). On the other hand, the methylation of C38 does not affect signals derived from the four helices of C-terminal regions (residue 83-94, 103-109, 115-124 and 132-143 $\Delta\delta_{tot} < 0.1$ p.p.m.).

Extent of assignments and data deposition

For N-Ada16k, a total of 99% of C_α, 96% of C_β and 89% of CO resonances were unambiguously assigned. 94% of backbone amide resonances, except for 7 prolines plus M1, were assigned. The eight backbone amide resonances of K2, E75, H82, R83, Q96, W131, Q132 and Q133 were unassigned, presumably due to conformational exchange or rapid exchange with solvent. Over 88% of the side-chain resonances were assigned. For meC38 N-Ada16k, a total of 99% of C_α, 99% of C_β and 91% of CO resonances were unambiguously assigned. 95% of backbone amide resonances, except for 7 prolines plus M1, were assigned. The unassigned backbone amide resonances were K2, E75, H82, R83, Q96, Q132 and Q133. Over 90% of the side-chain resonances were assigned. The chemical shifts of ^{1}H,^{15}N, and ^{13}C resonances for *E. coli* N-Ada16k and meC38 N-Ada16k at 30 °C and pH 6.5 have been deposited in BioMagResBank (http://www.bmrb.wisc.edu) under accession code 6053 and 6054, respectively.

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