Journal of Biomolecular NMR, **14:** 95–96, 1999.
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Letter to the Editor: Resonance assignments of the Tn*916* integrase DNA-binding domain and the integrase:DNA complex

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Received 8 January 1999; Accepted 17 February 1999

Key words: integrase, NMR assignments, protein-DNA complex, Tn*916*

Biological context

Conjugative transposons are promiscuous genetic elements that spread antibiotic resistance in pathogenic bacteria (reviewed in Scott and Churchward, 1995). Excision and integration of the conjugative transposon Tn*916* is performed by integrase, a type I topoisomerase related to the tyrosine family of site-specific recombinases (Argos et al., 1986). Like the functionally homologous integrase from the lambda bacteriophage, Tn*916* integrase is a heterobivalent DNA binding protein that consists of a carboxyl-terminal catalytic domain that recognizes and cleaves inverted repeats at the transposon/chromosome junction, and an amino-terminal domain that site-specifically binds to directly repeated sequences within the transposon arm. We have recently shown that the unbound amino-terminal DNA binding domain of the Tn*916* integrase protein (INT-DBD) adopts an unusual LβββLα fold (L = loop) (Connolly et al., 1998). Here we report the ^{1}H, ^{13}C, and ^{15}N chemical shifts of the INT-DBD in both its free and DNA-bound forms, and the ^{1}H assignments of its DNA binding site within the complex.

Methods and results

The preparation of ^{15}N- and ^{15}N,^{13}C-labeled INT-DBD has been described previously (Connolly et al., 1998). NMR samples of free INT-DBD (residues 1–81) contained ∼1.3 mM protein, 50 mM phosphate (pH 6.0), 100 mM NaCl, and 5 mM DTT. NMR samples of the 1:1 INT-DBD:DNA complex were ∼1 mM INT-DBD in 25 mM phosphate (pH 6.0), 5 mM NaCl, and 25 μM EDTA. The DNA had the nucleotide sequence dGAGTAGTAAATTC/dGAATTTACTACTC, and the INT-DBD protein in the complex consisted of residues 1–74, with a cysteine-to-alanine substitution at position 57.

NMR experiments were performed at 300 K (free INT-DBD) or 305 K (INT-DBD:DNA) on Bruker DRX 500 and 600 MHz spectrometers. Detailed descriptions of the experiments used for the assignment of the protein and DNA resonances, along with their original references, have been reviewed elsewhere (Bax and Grzesiek, 1993; Clore and Gronenborn, 1993; Cavanagh et al., 1996). Protein resonances (^{1}H, ^{13}C, ^{15}N) were assigned using 3D HNCO, HN(CA)CO, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, C(CO)NH, HCCH total correlation spectroscopy (TOCSY), HCCH-COSY, ^{15}N-edited TOCSY, ^{15}N- and ^{13}C-edited NOESY, and 4D HCCH-NOESY experiments (mixing times 70 to 150 ms). 3D ^{15}N- and ^{13}C-edited ROESY experiments were used to stereospecifically assign a total of 6 and 16 β-methylene groups within the INT-DBD:DNA complex and the free INT-DBD, respectively. Spin echo difference CT-HSQC experiments were used to stereospecifically assign a total of 6 isoleucine and valine side-chain methyl groups in the free protein. DNA assignments were obtained from 2D [F1 and F2 ^{13}C-filtered]-NOESY and TOCSY spectra. ^{1}H chemical shifts were referenced to external DSS in D_2O (0.00 ppm), while ^{15}N and ^{13}C chemical shifts were referenced indirectly to the absolute frequency ratios ^{15}N/^{1}H = 0.101329118 and ^{13}C/^{1}H = 0.251449530

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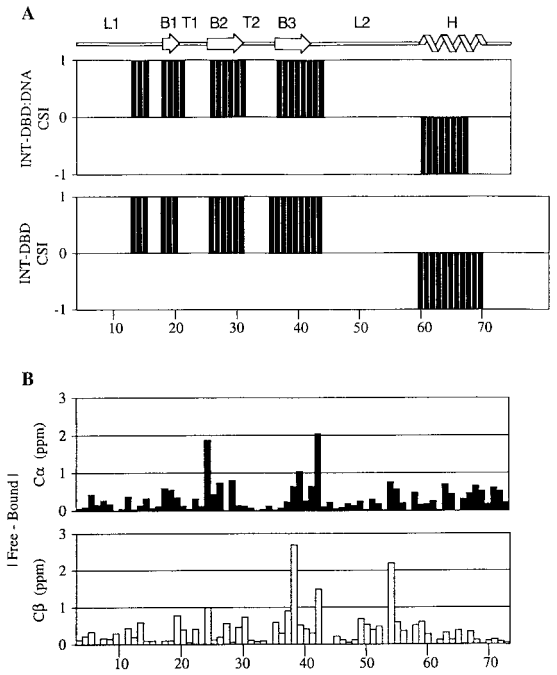

Figure 1. (A) Plot of the chemical shift index (CSI) (Wishart and Sykes, 1994) as a function of residue number for the INT-DBD:DNA complex and the free INT-DBD. An index of −1 indicates helical structure, 0 indicates coil, and 1 indicates β-sheet/strand structure. (B) Absolute chemical shift differences between the free and DNA-bound forms of the INT-DBD as a function of residue number.

(Live et al., 1984). Spectra were processed using NMRPipe (Delaglio et al., 1995) and analyzed using the programs PIPP, CAPP, and STAPP (Garrett et al., 1991).

Extent of assignments and data deposition

Nearly complete assignments of the ^{1}H, ^{13}C, and ^{15}N resonances for both the free INT-DBD (9.5 kDa) and the INT-DBD:DNA complex (16.7 kDa) were obtained. The chemical shifts of ∼95% and ∼97% of the protonated carbon atoms were assigned for the free INT-DBD and the INT-DBD:DNA complex, respectively. Assignments for ∼85% and ∼89% of the non-labile ^{1}H resonances were made for the free INT-DBD and the INT-DBD:DNA complex, respectively. All non-labile DNA hydrogens were assigned in the INT-DBD:DNA complex, with the exception of the ribose 5′ and 5″ resonances and the adenine H2 resonances.

Analysis of the chemical shift indices of the free and DNA-bound forms of the integrase DNA binding domain reveals that the secondary structure within this protein is not significantly altered by DNA binding (Figure 1A). However, there is some variability in the lengths of these structural elements; strands B2, B3 and the α-helix differ by 1–2 residues. A comparison of the $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ chemical shift differences between free and DNA-bound INT-DBD reveals significant perturbations as a result of DNA binding (Figure 1B). Of particular note are the $^{13}C_{\beta}$ shifts of F38, W42, and K54, which exhibit the largest chemical shift differences and reside at the protein-DNA interface (Wojciak et al., *Nat. Struct. Biol.*, in press).

The ^{1}H, ^{13}C, and ^{15}N chemical shifts for the free INT-DBD and the INT-DBD:DNA complex have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession numbers #4160 and #4165, respectively.

Acknowledgements

We thank Dr. Thorsten Dieckmann and Marek Grzeskowiak for technical support. This work was supported by grants from the U.S. Department of Energy (DE-FC-03-87ER60615) and National Institutes of Health (R01GM57487).

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