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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of a viral SET domain histone lysine methyltransferase

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Biological context

Evolutionarily conserved SET domains were originally identified in three *Drosophila* proteins: Suppressor of variegation (Su(var)3-9), Enhancer of zeste (E(z)) and Trithorax. Presently, SET domains are known to be present in more than 360 genes in organisms ranging from virus to human as listed in the SMART database. Recent advances in genetic and biochemical studies have revealed that SET domains in many proteins function as histone methyltransferases (HMTases), methylating position-specific lysines within the amino-termini of histones H3 and H4 (Kouzarides, 2002). Site-specific lysine methylation on histone tails in combination with other post-translational modifications, such as acetylation and phosphorylation, are referred to as the 'histone code' which marks for a broad spectrum of chromatin-based biological processes (Turner, 2002). Moreover, it has recently been shown that distinct mono-, di- or trimethylation states of a given lysine in histone tails, modified by SET domain HMTases, are linked to different epigenetic processes *in vivo* (Santos-Rosa et al., 2002). To understand the structural basis of catalysis of SET domain HMTases, we employ heteronuclear multidiemensional NMR techniques to determine the three-dimensional structure of a SET domain protein (referred to as vSET) encoded by *Paramecium bursaria* chlorella virus 1 (PBCV-1). Here, we report the backbone and side-chain ^{1}H, ^{13}C and ^{15}N resonance assignments of the protein.

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Methods and results

The A612L gene (accession number AAC96946) from PBCV-1 encoding the full-length vSET (119 residues) (Li et al., 1997) was subcloned into pET22b(+) expression vector (Novagen) and expressed in *Escherichia coli* BL21(DE3) cells at 37 °C. Uniformly ^{15}N- and ^{13}C/^{15}N-labelled proteins were prepared by growing bacteria in a minimal medium containing $^{15}NH_4Cl$ with or without $^{13}C_6$-glucose. A uniformly ^{13}C/^{15}N-labelled and fractionally deuterated protein sample was prepared by growing the cells in 75% $^{2}H_2O$. The vSET protein was isolated from inclusion bodies and denatured with 6 M Gd-HCl. Protein refolding was accomplished by step-wise dialysis using a 50 mM HEPES buffer of pH 7.5 containing 300 mM NaCl, 2–10% glycerol, 0.1 mM EDTA and 5 mM β-ME. The refolded protein was purified by Source 15S cation exchange chromatography (Amersham) followed by Superose 12/20 gel filtration chromatography (Amersham). NMR samples contained ∼0.5 mM protein in a 50 mM phosphate buffer of pH 6.5 containing 700 mM NaCl, 300 mM urea, 0.1 mM EDTA and 5 mM β-ME in $H_2O/^{2}H_2O$ (9/1) or $^{2}H_2O$. All NMR experiments were conducted at 37 °C on a 500 MHz or 600 MHz Bruker DRX NMR spectrometer equipped with four RF channels and a triple-resonance probe with triple-axis pulsed field gradients. The NMR spectra were processed with NMRPipe (Delaglio et al., 1995) and analyzed by using NMRView (Johnson and Blevins, 1994). The deuterium-decoupled 3D triple-resonance spectra of HNCA, HN(CO)CA, HN(CA)CB and HN(COCA)CB with sensitivity-enhancement (Sattler et al., 1999), recorded with a uniformly ^{13}C/^{15}N-labeled and fractionally (75%) deuterated sample, were used to obtain

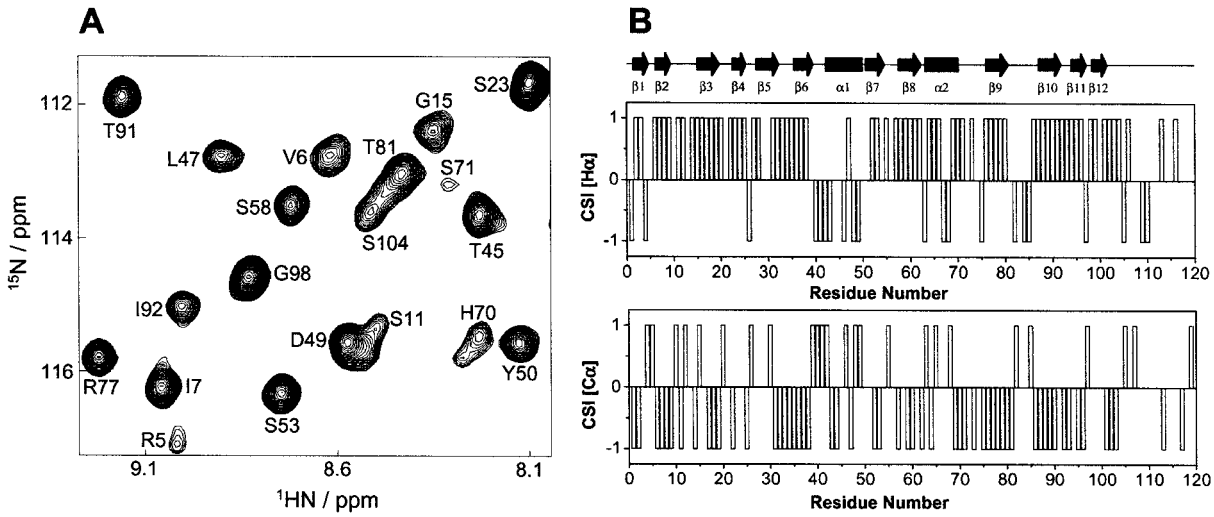

Figure 1. NMR spectral analysis of vSET. (A) A central region of 2D ^{1}H-^{15}N HSQC spectrum of the protein collected at pH 6.5 and 37 °C. The assignments are annotated by the resonance peaks. (B) The chemical shift index of backbone C_α and H_α atoms of the protein residues. The secondary structural elements of the protein determined by a collection of the NMR data, including chemical shifts of H_α and C_α atoms, backbone amide hydrogen exchange rate and sequential NOE patterns.

backbone resonance assignments. The backbone assignments were confirmed through sequential NH-NH and NH-Hα NOEs identified in the ^{15}N-edited 3D NOESY-HSQC spectrum collected with a mixing time of 100 ms. The side chain ^{13}C atoms were assigned using a 3D (H)C(CO)NH-TOCSY (Sattler et al., 1999) spectrum recorded on the ^{2}H(75%)/^{13}C/^{15}N-labeled sample. Side chain ^{1}H resonances were assigned using a 3D HCCH-TOCSY spectrum (mixing time = 18 ms) using a fully protonated ^{13}C/^{15}N-labeled sample in $^{2}H_2O$, and confirmed with a 3D ^{15}N-edited TOCSY-HSQC experiment (mixing time = 60 ms), in which the intra-residue correlations of nearly all non-proline residues were observed. The side chain ^{1}H and ^{13}C resonances for aromatic residues were assigned using a combination of 2D ^{1}H NOESY and TOCSY in addition to ^{13}C HSQC and 3D HCCH-TOCSY recorded in the aromatic carbon region. $^{3}J_{\mathrm{NH-H}\alpha}$ coupling constants were measured in a 3D HNHA-*J* spectrum (Sattler et al., 1999).

Extent of assignment and data deposition

The high quality of the 3D triple-resonance spectra allowed us to obtain nearly complete backbone assignments of $^{1}H^{N}$ and ^{15}N for the entire protein except residues M1, F2, G83, Y105 and R115. The $^{13}C_\alpha$ and $^{13}C_\beta$ atoms for the entire protein were assigned. Figure 1A displays part of the 2D ^{1}H-^{15}N HSQC spectrum for vSET. The side chain ^{1}H and ^{13}C resonance assignments were obtained for over 90% of the residues. A total of 39 slowly exchanging amide protons have been identified with a series of ^{15}N-HSQC spectra recorded on a uniformly ^{15}N-labeled sample after the H_2O buffer was changed to $^{2}H_2O$ buffer. A total of 62 $^{3}J_{\mathrm{NH-H}\alpha}$ coupling constants were obtained with a 3D HNHA spectrum. Deviations of the $^{13}C_\alpha$ and $^{1}H_\alpha$ chemical shifts from random coil values, characteristic sequential and medium range NOEs and $^{3}J_{\mathrm{NH-H}\alpha}$ coupling constants indicate that vSET consists mainly of β-strands. A table of the ^{1}H, ^{13}C and ^{15}N chemical shift assignments of vSET has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 5567.

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