Journal of Biomolecular NMR, **14:** 291–292, 1999.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{15}N and ^{13}C resonance assignments for the bromodomain of the histone acetyltransferase P/CAF

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Received 19 April 1999; Accepted 5 May 1999

Key words: histone acetyltransferase bromodomain, ^{1}H, ^{15}N and ^{13}C NMR resonance assignments

Biological context

Bromodomains are modules of about 110 amino acid residues that are found in a large number of chromatin-associated proteins, many of which are involved in transcriptional activation (Haynes et al., 1992; Brownell and Davis, 1996a). Particularly, the bromodomains are present in nearly all known histone acetyltransferase (HAT)-associated transcriptional coactivators, which play a central role in the regulation of nucleosome remodeling and gene activation via histone acetylation (Brownell et al., 1996b; Jeanmougin et al., 1997). It has been suggested on the basis of its modular nature that bromodomain may be involved in protein–protein interactions (Jeanmougin et al., 1997). However, no conformational studies and binding partners of the bromodomain family are available. We have recently initiated structural studies of a bromodomain from the human HAT coactivator P/CAF (p300/CBP-associated factor), using heteronuclear multidimensional NMR spectroscopy techniques. Here, we report the nearly complete sequence-specific backbone and side-chain ^{1}H, ^{15}N, and ^{13}C resonance assignments of the P/CAF bromodomain.

Methods and results

The bromodomain of the histone acetyltransferase P/CAF (residues 719–832) was subcloned into the pET14b vector and expressed in *E. coli* BL21 (DE3) cells. Uniformly ^{15}N-labeled or ^{13}C/^{15}N-labeled protein samples were prepared by growing the bacteria in minimal media containing $^{15}NH_4Cl$, with or without [U-^{13}C]-glucose (Cambridge Isotope Labs). Uniformly ^{13}C/^{15}N-labeled, fractionally deuterated proteins were prepared in a similar fashion by using 75% $^{2}H_2O$. Additionally, a fractionally (15%) ^{13}C-labeled protein was prepared. The proteins were purified by affinity chromatography on a nickel-IDA column (Invitrogen) followed by the removal of poly-histidine tag by thrombin cleavage. The final purification of the proteins was achieved by size-exclusion chromatography. NMR samples of the bromodomain protein (∼1 mM) were prepared in 100 mM sodium phosphate buffer of pH 6.5, containing 0.5 mM EDTA, 5 mM perdeuterated DTT, in $H_2O/^{2}H_2O$ (90%/10%) or $^{2}H_2O$. All NMR experiments were carried out at 30 °C on Bruker DRX500 and DRX600 spectrometers equipped with four rf channels, and a triple-resonance probe with three-axis pulsed field gradients. The NMR data were processed and analyzed using the NMRPipe (Delaglio et al., 1995) and NMRView (Johnson et al., 1994) programs. Deuterium-decoupled triple-resonance experiments HNCACB and HN(CO)CACB (Yamazaki et al., 1994), which were recorded with a uniformly ^{13}C/^{15}N-labeled and fractionally (75%) deuterated sample, were used to obtain the backbone resonance assignments. The backbone assignment was confirmed through sequential NH-NH and NH-Hα NOEs identified in the ^{15}N-edited NOESY-HSQC spectrum (100 ms mixing time). The side chain ^{13}C nuclei were assigned using a 3D (H)C(CO)NH-TOCSY (Logan et al., 1993) experiment recorded on the ^{2}H(75%)/^{13}C/^{15}N-labeled sample. Side chain ^{1}H

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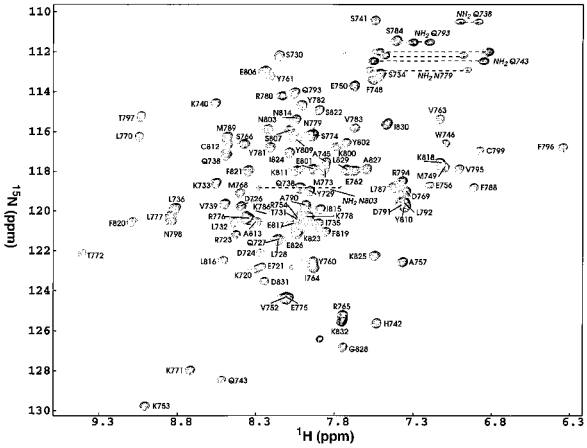

Figure 1. ^{1}H-^{15}N-HSQC spectrum for the bromodomain of the histone acetyltransferase P/CAF (residues 719–832) collected at pH 6.5 and 30 °C. The assignments are annotated by the resonance peaks. Side chain NH_2 peaks of Asn and Gln residues are marked with a dashed line.

resonances were assigned from a 3D HCCH-TOCSY spectrum using a fully protonated ^{13}C/^{15}N-labeled sample in $^{2}H_2O$, and were confirmed with a 3D ^{15}N-dispersed TOCSY-HSQC. The side chain ^{1}H and ^{13}C resonances for the aromatic residues (8 Tyr, 6 Phe and 1 Trp) were assigned using a combination of experiments, including 2D ^{1}H-NOESY and TOCSY, ^{13}C-HSQC, CT-^{13}C-HSQC, and 3D HCCH-TOCSY recorded in the aromatic carbon region with the doubly labeled sample. Stereospecific assignments of the methyl groups of the Leu and Val residues (9 Leu and 5 Val) were obtained from a ^{13}C-HSQC spectrum of a fractionally (15%) ^{13}C-labeled protein (Neri et al., 1989).

Extent of assignments and data deposition

The high quality of the spectra from the 3D HNCACB and HN(CO)CACB experiments allowed us to obtain nearly complete backbone assignments of $^{1}H^{N}$, ^{15}N, $^{13}C\alpha$, and $^{13}C\beta$ atoms (98%). Figure 1 shows the ^{1}H-^{15}N-HSQC spectrum for the P/CAF bromodomain at pH 6.5 and 30 °C. The side chain ^{1}H and ^{13}C resonance assignments were obtained for about 95% of the residues. A total of 14 slowly exchanging amide protons have been identified with a series of ^{15}N-HSQC spectra recorded on an uniformly ^{15}N-labeled sample after the H_2O buffer was changed to $^{2}H_2O$ buffer. A total of 54 $^{3}J_{NH-H\alpha}$ coupling constants were measured with a 3D HNHA spectrum (Vuister et al., 1993). Deviations of the $^{13}C\alpha$ and $^{1}H\alpha$ chemical shifts from random coil values, characteristic sequential and medium range NOEs, and $^{3}J_{NH-H\alpha}$ coupling constants indicate that the bromodomain consists mainly of four α-helices. These secondary structure results are consistent with the structure prediction for the bromodomain family (Jeanmougin et al., 1997). A table of the ^{1}H, ^{15}N, and ^{13}C chemical shift assignments of the P/CAF bromodomain has been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number 4312.

Acknowledgements

We thank M. Sattler and M. Rosen for technical advice, and O. Plotnikova for assistance in the protein preparation. This work was supported by discretionary funds from the Mount Sinai School of Medicine (to M.M.Z.) and NIH grants (to A.A.).

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