Journal of Biomolecular NMR, **12:** 459–460, 1998.
KLUWER/ESCOM

Complete heteronuclear NMR resonance assignments and secondary structure of core binding factor β (1-141)

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Received 12 May 1998; Accepted 1 June 1998

Key words: core binding factor, secondary structure, sequence-specific assignment

Biological context

Core-binding factor (CBF) is a heteromeric transcriptional enhancer that is essential for a number of developmental processes, particularly hematopoiesis (Speck and Stacy, 1995). CBFβ modulates the affinity of the CBFα subunit for DNA without establishing additional contacts on the DNA or changing the magnitude of DNA bending. The CBFβ subunit is essential for the *in vivo* function of at least one of the CBFα subunits, that encoded by the *CBFA2* (also known as the *Acute Myeloid Leukemia* 1, or *AML1*) gene. Knockouts of the genes encoding either subunit of CBF in mice result in embryonic lethality and a profound block in hematopoietic development.

The primary structures of CBFβ and its *Drosophila* homologues Bro and Bgb are not similar to those of any other proteins, and the mechanism by which CBFβ stabilizes the CBFα-DNA complex is unusual in that contacts to DNA are not substantially altered. The CBFβ subunit is an essential component of the CBF complex and is mutated in a substantial percentage of human leukemias making it an interesting and important target for structural studies. A truncated CBFβ protein containing amino acids 1–141 [CBFβ(141)] which includes the region of homology to Bro and Bgb, binds to the CBFα subunit *in vitro* with the same affinity as a full length isoform of CBFβ, CBFβ(187) (Huang et al., 1998). Herein we report the complete heteronuclear NMR resonance assignments and secondary structure of the CBFβ heterodimerization domain, CBFβ(141).

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Methods and results

CBFβ(141) was expressed and purified according to the procedure described by Huang et al. (1998). Celtone media (Martek, Inc.) was employed for isotopic enrichment of the protein with ^{13}C and ^{15}N. CBFβ(141) samples were exchanged into 25 mM potassium phosphate, pH 6.5, 0.1 mM EDTA, 1 mM DTT, and 0.1% NaN_3 by size-exclusion chromatography on a 2.5 × 36 cm column of Sephacryl S-100. A sample of 1.5 mM $^{13}C/^{15}N$-labeled CBFβ(141) in 25 mM potassium phosphate, pH 6.5, 0.1 mM EDTA, 5 mM DTT, 0.1% NaN_3 and 5% D_2O was employed for all measurements. All NMR measurements were made at 20 °C on a Varian UnityPlus 500 MHz NMR spectrometer equipped with an actively shielded gradient triple resonance probe (Nalorac Corp.).

Cα, Cβ, N, and NH assignments were obtained primarily from two 3D experiments, HNCACB and HN(CO)CACB (Muhandiram and Kay, 1994). H_α assignments and additional confirmation of the sequential assignments was obtained from an HN(CA)HA experiment modified from the original sequence to include the use of pulsed field gradients and sensitivity-enhancement. CO assignments were obtained from gradient sensitivity-enhanced HNCO spectra. Aliphatic side-chain assignments were obtained from 3D HCCH-TOCSY (Kay et al., 1993), 3D C(CO)NNH and 3D H(CCO)NNH (Grzesiek et al., 1993) spectra. Aromatic proton and carbon spin systems were assigned using 3D HCCH-TOCSY (Kay et al., 1993), 2D ^{1}H-TOCSY-relayed-ct-[^{13}C,^1H]-HMQC (Zerbe et al., 1996), and 2D $(H_\beta)C_\beta(C_\gamma C_\delta)H_\delta$ and 2D $(H_\beta)C_\beta(C_\gamma C_\delta C_\epsilon)H_\epsilon$ (Yamazaki et al., 1993) spectra.

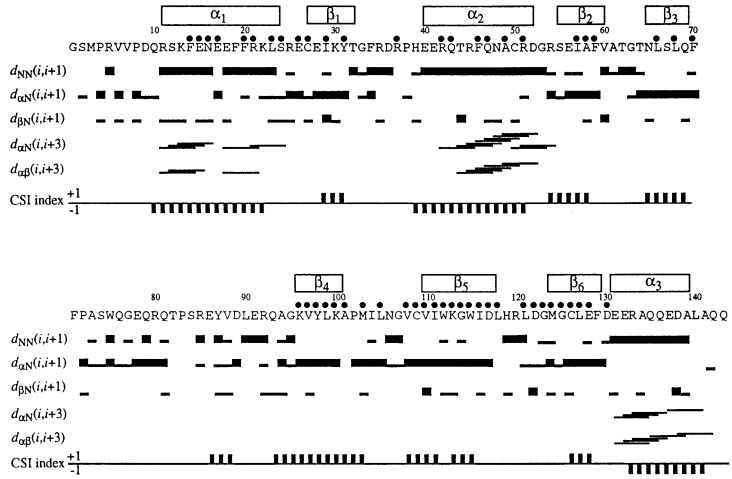

Figure 1. Primary sequence of CBFβ(141) with a summary of observed short and medium range NOEs, NH exchange data, and CSI data (Wishart and Sykes, 1994). No $d_{\alpha N}(i, i+2)$ or $d_{\alpha N}(i, i+4)$ NOEs were observed. A total of 8 $d_{NN}(i, i+2)$ NOEs were observed which are not shown in the figure. Circles above the sequence indicate residues with amide protons still visible in the two-dimensional [^{15}N, ^{1}H] HSQC spectrum after 1 h of solvent exchange in D_2O buffer at pH 6.5, 10 °C. Regular secondary structures are shown above the sequence. The consensus CSI data obtained from $^{1}H_{\alpha}$, $^{13}C_{\alpha}$, $^{13}C_{\beta}$, and C′ chemical shift data are represented by square bars. Zero represents the chemical shift of random coil, and −1 and +1 square bars represent the consensus positive and negative deviations of the chemical shifts from random coil values observed in α-helices and β-strands, respectively.

Regular secondary structure elements in proteins give rise to characteristic NOEs that can be used to identify the boundaries of these elements. Figure 1 shows the characteristic NOEs identified in 3D ^{15}N- and ^{13}C-edited NOESY spectra recorded with a mixing time of 120 ms on CBFβ(141). Also shown in Figure 1 are the NH exchange data and chemical shift index (CSI) (Wishart and Sykes, 1994) data for CBFβ(141). CBFβ(141) contains a total of three α-helices and six β-strands.

Extent of assignments and data deposition

We have obtained assignments for all the aliphatic nuclei of CBFβ(141) with the exception of the γCH_2 and δCH_2 resonances of Arg 42. A complete assignment of the aromatic nuclei of CBFβ(141) has been obtained with the exception of the C_ζ and H_ζ resonances of Phe 34, Phe 71 and Phe 129. All of the side-chain NH_2 resonances have been assigned, and 12 of the 15 ϵ-amino moieties of the Arg residues have been assigned.

The ^{1}H, ^{13}C and ^{15}N chemical shifts for CBFβ(141) at pH 6.5 and T = 293K have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4092.

Acknowledgements

J.H.B. is supported by grants from United States Public Health Service K02 AI01481 and R29 AI39536 from the Institute for Allergy and Infectious Disease. N.A.S. is a Leukemia Society of America Scholar, supported by grants from United States Public Health Service R01 CA/GM 58343 and R01 CA75611.

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