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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments and secondary structure of the c-Myc binding domain (MBD) and the SH3 domain of the tumor suppressor Bin1

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Biological context

The Myc family of nuclear proto-oncogenes is involved in many types of human malignancies. Most investigations have focused on the product of the c-Myc gene, which is overexpressed in many cancers and leukemias. c-Myc contains two domains: the C-terminal domain (CTD) mediates interaction with Max and physiological recognition of DNA target sequences, whereas the N-terminal domain (NTD) contains the transcription activation domain (TAD) and regions required for transcriptional repression, cell-cycle regulation, transformation, and apoptosis. Most of the biological properties of c-Myc involve interactions with different proteins through its NTD or CTD (reviewed in Sakamuro and Prendergast, 1999).

Bin1 is a 482 amino acid protein consisting of three domains: an N-terminal region termed the BAR domain (Bin1/Amphiphysin/Rvs167, amino acids 1–260) because of its similarity to two polypeptides, amphiphysin and Rvs167, a central region that has been implicated as the c-Myc binding domain (MBD, amino acids 260–413), and a C-terminal SH3 domain (amino acids 413–482). Bin1 is a nucleocytosolic adapter protein (Sakamuro et al., 1996) and its function is complex and varied by tissue-specific splicing. It was identified originally through its ability to interact with and inhibit malignant transformation by c-Myc. Later on, it was established that there are two ubiquitous splice isoforms of Bin1 and several other splice forms that are restricted in expression to muscle or brain (Butler et al., 1997). Bin1 polypeptides, and in particular its brain isoforms, are related in their terminal domains to amphiphysin, a neuronal protein involved in endocytosis. Outside the brain, Bin1 has functions that are not related to endocytosis processes. This is due to the fact that the brain isoforms of Bin1 are cytosolic, whereas the ubiquitous Bin1 isoforms localize to the nucleus as well as to the cytosol (Kadlec and Pendergast, 1997). Isoforms that localize to the nucleus and bind c-Myc exhibit tumor suppressor properties which are inactivated or missing in malignant melanoma, breast cancer and prostate cancer (Ge et al., 1999). In contrast, brain isoforms of Bin1 lack tumor suppressor activity. In fact, it has been shown that Bin1 is functionally inactivated by the missplicing of one of its brain-specific exons (Ge et al., 1999).

Here, we report the near complete ^{1}H, ^{13}C and ^{15}N assignment and secondary structure of the MBD and SH3 domain of one of the ubiquitous Bin1 isoforms. These data provide a basis for determining the solution structure of the Bin1/c-Myc complex, and for gaining a deeper insight into the regulatory mechanisms of tumor suppression and how they are modified in cancer.

Methods and experiments

A recombinant protein consisting of the MBD and the SH3 domain of Bin1 (170 amino acids long) was expressed in *E. coli* strain BL21-CodonPlus (Stratagene) containing the pET-15b expression vector (Novagen). The protein was purified to homogeneity using metal affinity chromatography (CLONTECH). U-^{15}N and

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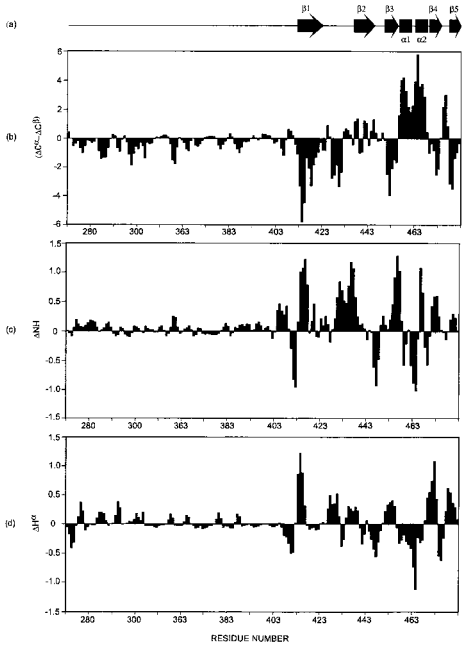

Figure 1. Summary of NMR data for secondary structure determination. (a) Cartoon of secondary structure elements. (b) The values of $(\Delta C^{\alpha} - \Delta C^{\beta})$ are plotted versus the amino acid sequence, where ΔC^{α} and ΔC^{β} were obtained by subtracting the corresponding random coil chemical shifts of $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ from the shifts measured in the spectra. The value $(\Delta C^{\alpha} - \Delta C^{\beta})$ for a particular residue i represents an average over three consecutive residues: $(\Delta C^{\alpha} - \Delta C^{\beta})_i = (\Delta C^{\alpha}_{i-1} + 2{*}\Delta C^{\alpha}_{i} + \Delta C^{\alpha}_{i+1}\text{-}\Delta C^{\beta}_{i-1} - 2{*}\Delta C^{\beta}_{i} - \Delta C^{\beta}_{i+1})/4$. (c) and (d) indicate deviations from random coil for NH and H^{α} resonances, respectively, and are calculated following the same procedure as indicated in (b).

U-^{13}C,^{15}N samples were produced in standard M9 media supplemented with ^{15}N ammonium chloride (1 g/L) and ^{13}C glucose (2 g/L). Cells were grown at 37 °C to an OD_{600} of 0.6 and induced with 1 mM IPTG for 5 h at 25 °C. ^{15}N-labeled or $^{13}C/^{15}N$-labeled protein solution was prepared in 25 mM sodium phosphate (pH = 6.5), 150 mM NaCl, 1 mM DTT, 95% H_20/5% D_2O. The concentration of the purified protein ranged between 1.0–1.5 mM.

All NMR spectra were recorded at 25 °C on a Varian INOVA 600 MHz spectrometer equipped with pulsed field gradient triple-resonance probes. Linear prediction was used in the ^{13}C and ^{15}N dimensions to improve the digital resolution. Spectra were processed using the NMRPipe software package (Delaglio et al., 1995) and analyzed with XEASY (Bartels et al., 1995). The assignments of the ^{1}H, ^{15}N, ^{13}CO, and ^{13}C resonances were based on the following experiments: CBCA(CO)NH, HNCACB, CC(CO)NH-TOCSY, HNCO, HNHA, 3D ^{15}N-edited TOCSY-HSQC, and HCCH-TOCSY (Bax et al., 1994; Kay, 1997). The backbone resonance assignment was achieved mainly by the combined analysis of the HNCACB and CBCA(CO)NH data. The side-chain resonances were identified mainly by the analysis of HCCH-TOCSY. Aromatic ring resonances were assigned based on the analysis of heteronuclear NOESY optimized for the detection of aromatic $^{13}C/^{1}H$ resonances.

Extent of assignments and data deposition

In the ^{1}H-^{15}N HSQC, 99% backbone amide resonances were assigned. Of the other backbone resonances, 99% have been assigned for C^{α}, 99% for H^{α} and 97% for C′. Moreover, 97% aliphatic side-chain resonances have been assigned for the SH3 domain of Bin1. Secondary structure assignment has been made based on secondary chemical shifts for C^{α}, C^{β}, NH and H^{α} resonances (Figure 1). The ^{1}H, ^{13}C and ^{15}N chemical shifts of Bin1 (residues 270–482) have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 4871.

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