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Letter to the Editor: Backbone assignment of the dimerization and DNA-binding domain of the oncogenic transcription factor v-Myc in complex with its authentic binding partner Max

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Biological context

The protein product (c-Myc) of the protooncogene c-*myc* is a transcriptional regulator playing a crucial role in cellular growth, differentiation, and apoptosis (Blackwood et al., 1992). Myc-dependent interactions are highly regulated to sustain cellular homeostasis and deregulation of *myc* genes leads to, for example, cell transformation. The Myc proteins are sequence-specific DNA-binding proteins (Blackwell et al., 1990; Kerkhoff et al., 1991) of the bHLHZip-type containing helix-loop-helix (HLH) and leucine-zipper (Zip) dimerization domains, and a basic (b) region interacting with DNA. To exert its function, e.g., activation of transcription, direct physical interaction with its authentic binding partner Max is essential (Grandori and Eisenman, 1997). For an understanding of the functional properties of Myc, a detailed knowledge of the three-dimensional structural properties of Myc is indispensable. To date structural information is only available for the unligated (monomeric) form of v-Myc (Fieber et al., 2001) and the ternary complex comprising c-Myc, Max and the cognate E-box recognition sequence (Nair and Burley, 2002). Here, we report the sequence specific backbone assignment for v-Myc in complex with its binding partner Max. This analysis provides for the first time spectral information about a heterodimeric helix-loop-helix oncogenic transcription factor complex displaying a characteristic 4-helix bundle tertiary structure.

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Methods and results

A v-*myc* DNA fragment was obtained by cleaving pET3d-MycMax (Fieber et al., 2001) with *Ncol* and *BamHI.* The fragment was cloned into a pET20b vector (Novagen) predigested with *Ncol* and *BamHI.* The expression plasmid encodes a 103-amino acid protein containing the bHLHZip Domain of v-Myc. The N-terminal leader sequence of the pET20b vector was removed by chemical cleavage with BrCN. Uniformly ^{13}C-, ^{15}N- and ^{2}H labelled protein was obtained by growing E. coli Rosetta(DE3)pLysS bacteria transformed by the pET20b-Myc plasmid in minimal media containing $^{15}NH_4CI$, [^{13}C]-Dglucose and D2O. v-Myc protein was purified and finally renatured with Max by dialysis as previous described (Fieber et al., 2001). For NMR analysis, protein sample was concentrated to 1.1 mM v-Myc/Max.

NMR spectra were obtained at 40 °C on Varian Infinity 800 MHz spectrometer, respectively. The experiments performed included ^{1}H-^{15}N TROSY-HSQC, 3D TROSYHNCO, 3D TROSY-HN(CA)CO, 3D TROSY-HNCA, 3D TROSY-HNCACB, and 3D ^{15}N NOESY-HSQC (Cavanagh et al., 1996). NMR data were processed and analyzed with the NMRPipe (Delaglio et al., 1995) and NMRView (Johnson and Blevin, 1994) software packages, respectively.

Signal assignment was based on the combined analysis of HNCA-connectivities and sequential H^N-H^N NOEs. The performance of experiments with reduced sensitivity (e.g. HNCACO, HNCACB) was limited because of significant transverse ^{15}N and

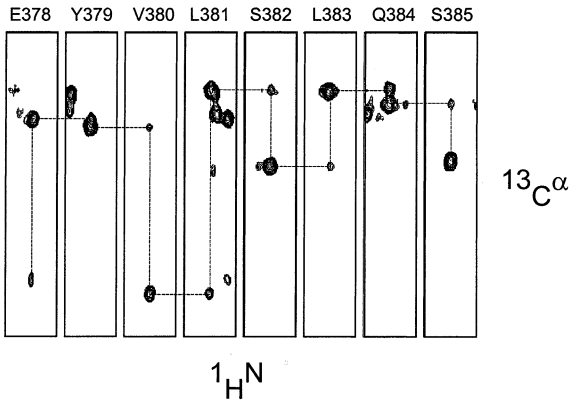

Figure 1. Assigned HNCA strip plot of v-Myc in complex with its protein binding partner Max.

^{13}Cα relaxation. Note that the v-Myc/Max protein complex displays significant relaxation anisotropy (for comparison see the 3D structure of the ternary Myc/Max/DNA complex, Nair and Burley, 2002; pdb-code:1NKP). Given the fact that all the NH vectors are parallel to the long axes of the diffusion tensor, short ^{15}N T_2 values are expected and observed. For example, Cβ and CO connectivities in the HNCACB and HNCACO were only observed for 32 and 79 residues, respectively. In cases were Cβ connectivities could be observed, the spectroscopic information was used to define the amino acid type. Finally, the manual signal assignment of ligated v-Myc was checked by an automated signal assignment tool based on Monte Carlo/Simulated Annealing (MC!SA) (Hoffmann and Konrat, manuscript in preparation). As an input, the method requires predicted NMR parameters such as H^N-H^N NOEs and Cα chemical shifts on the basis of a structural model. For v-Myc ligated with Max, a complete list of short H^N-H^N distances (< 5 Å) representing potential NOEs and Cα chemical shifts were computed from the pdb file of the ternary Myc/Max/DNA complex (Nair and Burley, 2002; pdb-code: 1 NKP). The reliability of the final result was evaluated by the analysis of 20 independent MC/SA cycles. An assignment was accepted provided that the reliability was greater than 75%. In all cases agreement between manual and automated assignment was observed.

Extent of assignments and data deposition

High-quality NMR data for v-Myc was obtained as shown by the ^{1}H-^{15}N TROSYHNCA spectrum collected at 800 MHz in Figure 1. In total, 89 of the 100 non-proline ^{1}H and ^{15}N backbone resonances were assigned. $^1H^N$, ^{15}N, ^{13}CO and ^{13}Cα resonances were assigned for 86 residues. Using the assigned chemical shifts of ^{13}Cα, we have employed the consensus chemical shift index (CSI) (Wishart and Sykes, 1994) to identify the secondary structure of v-Myc. The location of secondary structure elements is in agreement with existing c-Myc structural data (Nair and Burley, 2002) and indicates that even without bound DNA the Myc/Max protein complex displays the characteristic bHLHZip 4-helix bundle structural motif. However, in the basic DNA recognition region conformational flexibility still prevails. The assignments have been deposited in the BioMagResBank (httc://www.bmrb.wisc.edu) under BMRB accession number 6163.

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