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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments and secondary structure of ADR6 DNA-binding domain

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Biological context

SWI-SNF complex is an ATP-dependent chromatin-remodeling complex that has an important role in the transcription control in the yeast *Saccharomyces cerevisiae* (Peterson et al., 1995). This complex facilitates activator function by antagonizing chromatin-mediated transcriptional repression. Components of this complex include SNF2/SWI2, SNF5, SNF6, SWI1/ADR6, SWI3 and at least five additional polypeptides. In which, ADR6 was studied to be required for sporulation and expression of alcohol dehydrogenase II isozyme from *Saccharomyces cerevisiae*.

ADR6 gene of *S. cerevisiae* has an open reading frame which could encode a polypeptide of 1314 amino acids. A DNA-binding domain is located at residue 405 to residue 506 of ADR6 protein. This domain belongs to a recently discovered ARID (AT-rich interaction domain) family, first recognized in the murine Bright and the *Drosophila* Dead ringer (DRI) gene products (for review see Kortschak et al., 2000). The highly conserved ARID domain that corresponds to a stretch of approximately 90 amino acids is found in a wide range of important regulatory proteins. ARID-encoding genes are involved in a variety of biological processes including embryonic development, cell lineage gene regulation and cell cycle control. A number of ARID proteins including P270, a human counterpart of ADR6 (Dallas et al., 2000), exhibit non-sequence-specific DNA binding, but Bright and Dri, two members of the extend ARID subfamily, and Mrf-2 (Whitson et al., 1999) exhibit sequence-specific DNA binding to AT-rich sites. Until now, only two

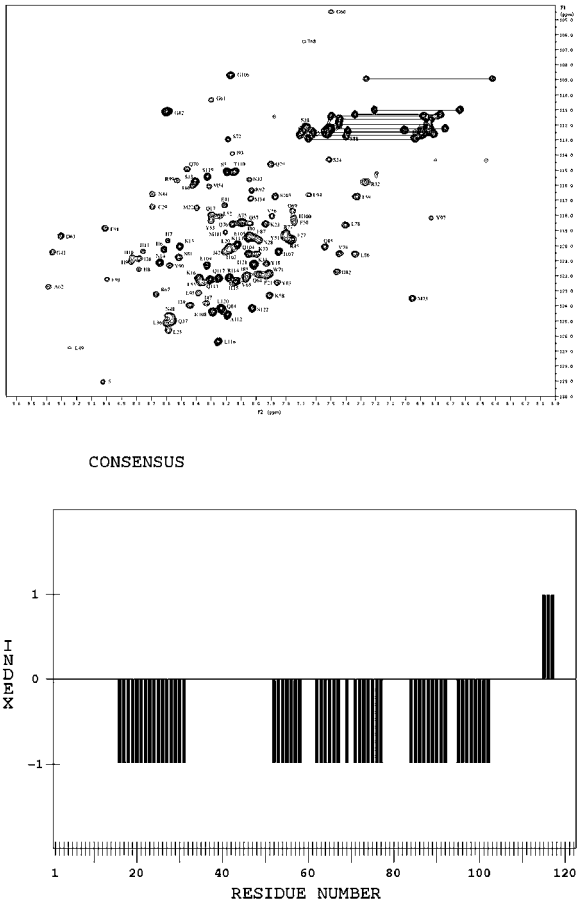

Figure 1. (A) A 500 MHz ^{1}H, ^{15}N-HSQC spectrum of recombinant DBD-ADR6 obtained at 300 K. The NMR sample contained about 1 mM DBD-ADR6 in 50 mM phosphate buffer, pH 4.9. The resonance assignments are indicated with the one-letter amino acid code and residue number. Side-chain amide protons of Asn and Gln are indicated by horizontal lines. (B) CSI consensus plot for recombinant DBD-ADR6, generated using $^{1}H^{\alpha}$, $^{13}C^{\alpha}$and $^{13}C^{\beta}$ and ^{13}CO chemical shifts.

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solution structures of the Mrf-2 and DRI ARIDs have been determined by NMR. They reveal significant differences to known DNA-binding domain structures (Iwahara et al., 1999; Yuan et al., 1998). Comparison of MRF-2 and DRI ARIDs structures shows significant discrepancies between these two structures. Study of the solution structure of ADR6 ARID domain will aid to the resolution of this quandary and help to exploit the structures of ARID family and the relationship between structures and functions of this family more deeply.

We therefore focused our attention on this ARID domain of ADR6 (DBD-ADR6) protein with the aim of obtaining its 3D structure in solution and of analysing the relationship of its structure and function. The DBD-ADR6 gene has been cloned and successfully expressed in *E. coli*. Here we report backbone and side-chain resonance assignment, as well as the secondary structure predicted by the NOE interactions and chemical shift analysis.

Methods and experiments

Uniformly labeled recombinant DBD-ADR6 protein was overproduced in *E. coli* using minimal medium containing 0.5 g/l 99% ^{15}N-ammonium sulfate and 2.5 g/l 99% ^{13}C-glucose as the sole nitrogen and carbon source, respectively. The protein was purified as described earlier (Tu et al., 2000).

The NMR samples were prepared with 50mM phosphate buffer at pH 4.9 in 90% H_2O/10% D_2O and contained 0.45 ml of about 1 mM protein. All NMR experiments were recorded at 300 K on a Bruker DMX 500 spectrometer. The following experiments were carried out: ^{15}N labeled sample: 2D TOCSY, 2D ^{1}H, ^{15}N-HSQC, 3D ^{15}N-edited TOCSY-HSQC (67 ms mixing time), 3D ^{15}N-edited NOESY-HSQC (130 ms mixing time); ^{15}N, ^{13}C labeled sample: 2D ^{1}H, ^{13}C-HSQC, 3D HNCO, 3D HNCA, 3D CBCA(CO)NH, 3D CBCANH, 3D H(C)(CO)NH-TOCSY, 3D (H)C(CO)NH-TOCSY, 3D HBHA(CBCACO)NH, 3D HCCH-TOCSY, 3D HCCH-COSY and 3D ^{13}C-edited NOESY-HSQC (130 ms mixing time). NMR data processing was achieved using NMRPipe and NMRDraw software (Delaglio et al., 1995), and analyzed with PIPP (Garrett et al., 1991). The chemical shift indices (CSI) were obtained using the CSI software (Wishart and Sykes, 1994).

Extent of assignment and data deposition

The 2D ^{1}H, ^{15}N-HSQC spectrum (Figure 1A) of DBD-ADR6 illustrates the good dispersion of the proton and nitrogen resonances in the amide groups.

Complete backbone assignments of residues from G12-S122 were made for $^{1}H^{N}$, ^{15}N, $^{1}H^{\alpha}$, $^{1}H^{\beta}$, $^{13}C^{\alpha}$and $^{13}C^{\beta}$ (the first 11 amino acids belong to the His-tag). Most ^{13}CO assignments were determined (about 95%), excluding those of residues preceding prolines, and G43, K113 and I121. Assignments of side-chain resonances (G12-S122) were mostly completed, excluding the ^{1}H and ^{13}C resonances in the aromatic rings and the partial ^{1}H and ^{15}N resonances of Gln and Asn side-chain NH_2 groups. The side-chain ^{1}H and ^{13}C resonances of L52, L86 and L95 need to be further determined.

The secondary structure prediction based on CSI (Figure 1B) and short-range NOEs analysis show the existence of six α-helices characteristic of minimal ARID proteins. The chemical shift values of the proton, nitrogen and carbon resonances have been deposited in the BioMagResBank (accession number: 5061).

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