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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the DNA-binding domain of the essential protein Cdc13 complexed with single-stranded telomeric DNA

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Biological context

Telomeres are the specialized nucleoprotein complexes that cap eukaryotic chromosomes. Telomeres protect chromosomes from degradation and end-to-end fusion and serve as a substrate for the reverse transcriptase telomerase (Blackburn, 2001). In most organisms, telomeres are made up of repetitive, non-coding DNA sequence, ending in a TG-rich single-stranded overhang. The *Saccharomyces cerevisiae* protein Cdc13 is a single-stranded telomere binding protein essential for both chromosomal end protection and telomere replication through recruitment of relevant subcomplexes to the telomere end (reviewed in Lustig, 2001). A 23.5 kDa DNA-binding domain (DBD) has been identified (residues 497–694 of the full-length protein) which is critical for Cdc13 function (Hughes et al., 2000; E.M. Anderson, W.A. Halsey and D.S. Wuttke, in preparation). This domain binds tightly and specifically to single-stranded telomeric DNA. Our goal is to obtain a structural understanding of the requirements for telomere capping and specific interaction with single-stranded telomeric DNA (ssDNA). Towards this goal, we have studied the complex between the Cdc13 DBD and the single-stranded telomeric 11mer dGTGTGGGTGTG using NMR spectroscopy. The complex contains all the necessary elements for examining the structural basis for protein/nucleic acid binding affinity and specificity. Here we present the nearly complete protein resonance assignments for the Cdc13 DBD in the complex.

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Methods and experiments

The DBD (residues 497–694 with an N-terminal methionine) was expressed recombinantly from *E. coli* BL21(DE3) cells using a pET21a vector (E.M. Anderson, W.A. Halsey and D.S. Wuttke, in preparation). Cell pellets were lysed by French press, with the cell supernatant incubated in 0.1% polyethylenimine to precipitate cellular nucleic acids and purified by ion exchange chromatography over SP Sepharose resin (Pharmacia). Uniformly ^{15}N- or ^{15}N,^{13}C-isotopically labeled protein was prepared by growth in minimal media containing $(^{15}NH_4)_2SO_4$ (1.5 g l^{-1}) with or without ^{13}C-glucose (2 g l^{-1}). Yields were approximately 10 mg l^{-1} in minimal media. ssDNA (dGTGTGGGTGTG) was purchased from Operon and purified by reversed-phase HPLC. For NMR spectroscopy, samples of the protein-DNA complex were prepared with 0.7–1.5 mM protein and 0.8–1.7 mM DNA in 50 mM imidazole-d_4 buffer (pH or pD* 7.0) with 150 mM NaCl, 100 mM Na_2SO_4, 0.02% NaN_3 and 2 mM DTT-d_{10} in 10% D_2O/90% H_2O or 100% D_2O. All experiments were acquired on a 500 or 600 MHz Varian Unity^{INOVA} or 800 MHz Bruker DRX spectrometer at 30 or 35 °C. Data were processed using NMRPipe (Delaglio et al., 1995) and analyzed and assigned manually using Ansigv3.3 (Kraulis, 1989).

Backbone $^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$ and $^{13}C'$ resonances and side-chain $^{13}C^{\beta}$ resonances were assigned using 3D HNCACB, CBCA(CO)NH, HNCO, HN(CA)CO, HNCA and HN(CO)CA experiments. $^{1}H^{\alpha}$ assignments were made using the 3D HCACO experiment. Aliphatic side chain assignments were made using 3D HCCH-TOCSY, HCCH-COSY, C(CO)NH and HC(CO)NH experiments, supplemented by a 4D ^{13}C,

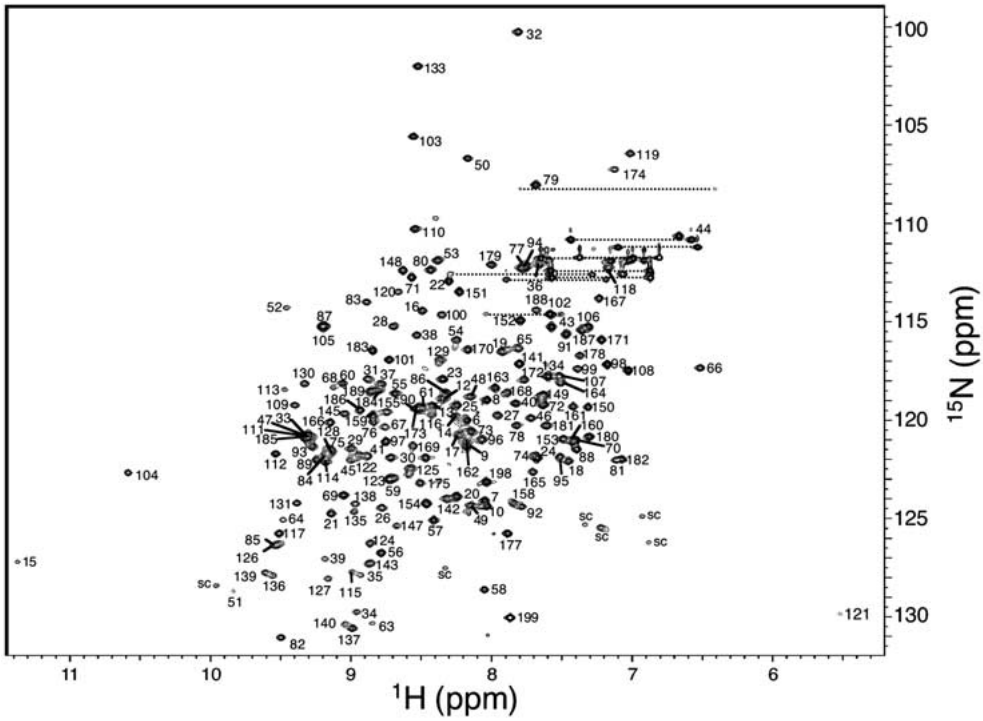

Figure 1. Sensitivity-enhanced 2D ^{1}H-^{15}N HSQC spectrum of the Cdc13 DBD in complex with dGTGTGGGTGTG acquired at 600 MHz and 30 °C. Cross peaks are marked by residue number in the DBD construct. Side chain NH_2 resonances of Asn and Gln residues are connected by horizontal bars, and the side chain cross peaks of Arg residues are indicated by sc.

^{15}N-edited NOESY experiment (mixing time 100 ms). The pulse sequences for all 3D experiments were implemented as provided from Varian ProteinPack with minor modifications. The high number of phenylalanine (17) and tyrosine (11) residues in the domain confounded aromatic resonance assignment. Partial aromatic side chain assignments were thus obtained from aromatic ^{1}H-^{13}C HSQC, 2D (HB)CB(CGCD)HD, 2D (HB)CB(CGCDCE)HE and NOESY experiments (Yamazaki et al., 1993).

Extent of assignments and data deposition

Backbone resonances ($^{1}H^{N}$, ^{15}N, $^{1}H^{\alpha}$, $^{13}C^{\alpha}$ and $^{13}C'$) have been assigned for all residues of the protein from residues 5 to 189 except Asn132*, Pro156, Ser 157* and Ser176* (*indicates that only $^{1}H^{N}$ and ^{15}N were not assigned). The $^{1}H^{N}$ and ^{15}N assignments for the Cdc13 DBD in the protein/ssDNA complex can be seen in Figure 1. Ninety-three percent of all aliphatic side chain ^{1}H and ^{13}C resonances from residues 5 to 189 have been assigned. Fifty-nine percent of aromatic ^{1}H and ^{13}C resonances have been assigned (73% of tyrosine resonances). The ^{1}H, ^{13}C and ^{15}N chemical shifts have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5232.

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