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^{1}H, ^{15}N, and ^{13}C NMR resonance assignments for the DNA-binding domain of the BPV-1 E2 protein

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Biological context

Papillomaviruses infect epithelial cells and cause tumor formation. The E2 protein, 410 amino acids long in bovine papillomavirus strain 1 (BPV-1), regulates viral replication and transcription (Androphy, 1994). The N-terminal half of the molecule constitutes the transactivation domain and the C-terminal one-third is the DNA-binding domain (DBD). The crystallographic structure of the minimal DBD (326–410) bound to DNA reveals a homodimeric protein that comprises an eight-stranded β-barrel flanked on each side by two α-helices; one recognition helix from each monomer interacts with the major groove of DNA (Hegde et al., 1992). The solution structure of the corresponding 83-residue region of the human papillomavirus strain 31 has been determined 31% sequence identity to be similar (Liang et al., 1996). We are investigating the solution structure of BPV-1 E2 DBD (310–410). This 10-residue DBD is more stable to urea denaturation (denaturation midpoint = 3.5 M) relative to the 85-residue E2 DBD (denaturation midpoint = 2.0 M) (Pepinsky et al., 1996) and binds DNA with higher affinity than the 85-mer. Our goal is to elucidate the structural changes induced in the BPV-1 E2 DBD by DNA binding and to examine the structural determinants of improved protein stability. Here, we describe the strategies that were used to obtain the resonance assignments.

Methods and results

Proteins used in NMR experiments were unlabeled, labeled uniformly with ^{15}N or ^{13}C, or labeled in specific residues and were purified as described previously (Lee et al., 1995). Cloning procedures resulted in the addition of Met-Ala to the N-terminus. Amino acid sequencing revealed that the Met residue was removed (75%) or that both residues were removed (25%). Resonance assignments are given for residues 310 to 410 of BPV-1 E2, and are renumbered as −1 to 100.

Rich medium (Luria-Bertani; LB) was used in preparing unlabeled protein. ^{15}N- or ^{13}C-labeled protein samples were prepared from cells grown on filter-sterilized modified from CM medium that was published media (Neidhardt et al., 1974; Jansson et al., 1996) but modified for complete isotopic labeling. CM medium contained KH_2PO_4 (9 g/l), K_2HPO_4 (6 g/l), NaCl (2.9 g/l), $MgCl_2$ (5.4 g/l), trace element solution (10 ml/l), thiamine (0.001 g/l), glucose (4 g/l unlabeled or 2 g/l $^{13}C_6$-labeled), NH_4Cl (0.5 g/l, unlabeled or ^{15}N-labeled), and ^{13}C- or ^{15}N-labeled algal lysate (1.5 g/l) (Celtone powder, Martek Biosciences Corp., MD). The trace element mixture contained concentrated HCl (8 ml/l), $FeCl_2 \bullet 4H_2O$ (5 g/l), $NaMoO_4 \bullet 2H_2O$ (0.61 g/l), $ZnCl_2$ (0.34 mg/l), $CaCl_2 \bullet 2H_2O$ (0.18 g/l), H_3BO_3 (64 mg/l), $MnCl_2 \bullet 4H_2O$ (40 mg/l), $CoCl_2 \bullet 6H_2O$ (18 mg/l), and $CuCl_2 \bullet 2H_2O$ (4 mg/l).

NMR spectra were recorded using a Bruker AMX-500 NMR spectrometer. Spectral widths were 7500, 2000, and 10 000 Hz and centers were 4.66, 116.5, and 47.5 ppm in the ^{1}H, ^{15}N and ^{13}C dimensions, respec-

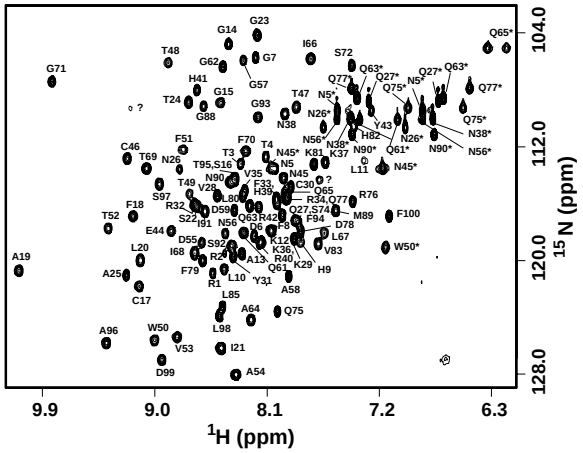

Figure 1. Two-dimensional ^{1}H-^{15}N HSQC spectrum of 1.7 mM BPV-1 E2 DBD at 35 °C and pH 5.79, collected at a proton resonance frequency of 500 MHz. Residues 311 to 410 are renumbered as 1 to 100. Side chain peaks of asparagine and glutamine are marked with asterisks. A resonance from the side chain of an Arg residue is observed near 6.7/127 ppm (folded-in). Peaks labeled with a ? were not assigned.

tively. Sample conditions were: 1.7–4.5 mM protein, 20 mM sodium phosphate, 0.1 M NaCl, 50 μM EDTA, 5 mM DTT (d_{10}) and 0.01% NaN_3 at pH 5.75 ± 0.1 and at 35 °C. Protein samples layered with argon prior to capping and sealing the NMR tube were stable for at least one week at 35 °C and four weeks at 4 °C.

Although the E2 DBD has a mass of 23 kDa, the homonuclear 2D spectra were sufficiently resolved to allow identification of side chain resonances (Wüthrich, 1986) for about 70% of the residues. Rapid identification of Ala, Val, Gly, Leu, Phe, and Lys resonances (40 out of 101 residues) was possible using ^{15}N-filtered TOCSY and NOESY and ^{1}H-^{15}N HSQC spectra acquired with selectively labeled protein. Sequential backbone assignments were possible using ^{1}H-^{15}N HSQC and 3D ^{1}H-^{15}N-^{1}H NOESY-HSQC data. The unusually shifted Hϵ1 resonance of the lone Trp50 (7.12 ppm) was identified from the ^{1}H-^{15}N HSQC spectrum collected using a sample labeled using 250 mg/l of $^{15}NH_4Cl$ in synthetic rich medium (Lee et al., 1995) lacking Gln and Trp, which showed specific labeling of the side chains of Asn, Gln, and Trp. The ^{1}H-^{13}C HSQC and HCCH-TOCSY data were used to determine the carbon chemical shifts, and to identify or confirm the chemical shifts of side chain protons attached to carbons.

Extent of assignments and data deposition

Figure 1 illustrates the ^{1}H-^{15}N HSQC of BPV-1 E2 DBD. Backbone assignments are complete for all residues except the imino nitrogens of prolines. Side chain proton assignments are complete for all residues except for Arg42. The carbon chemical shifts of Leu10 β; Lys36 β, γ; Phe33 ε, ζ; Ile66 β, γ; Phe70 δ, Phe79 side chain, Pro73 β, γ, Pro87 β, γ; and Ile91 γ are assigned tentatively because of spectral overlap. The F70 side chain rotates slowly on the NMR time scale, sufficient to give separate resonances, but broadening due to exchange was observed The chemical shift assignments have been deposited in the BioMagResBank database in Madison, WI, U.S.A. (accession code 4087).

We observe NOE cross peaks that show some residues of the hinge region, namely, Thr4, Asp6, His9, and Leu11 contact residues in the core DBD. From the solution structure, we will determine how these contacts stabilize the longer form relative to the core E2 DBD.

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