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Letter to the Editor: Assignment of the ^{1}H, ^{13}C, and ^{15}N resonances of the DNA binding domain of gpNu1, a genome packaging protein from bacteriophage λ

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Biological context

Terminase enzymes are vital DNA packaging 'machines' common to the double-stranded DNA bacteriophage, and eukaryotic viruses such as the adenoviruses and the herpes virus groups (Casjens, 1985). In bacteriophage λ, this terminase is composed of two virally encoded proteins, gpA (641 amino acids) and gpNu1 (181 amino acids) which form a $gpA_1 \bullet gpNu1_2$ holoenzyme complex (see Catalano (2000) for a review of λ terminase). The holoenzyme inserts individual copies of the viral genome into preformed, empty capsids by the combined efforts of its site-specific endonuclease activity, its duplex strand separation activity, and a DNA translocase activity. ATP plays important roles in all of the catalytic activities of the enzyme: Nuclease activity is modulated by ATP binding while strand separation and translocation are dependent on ATP hydrolysis. Mutagenesis and kinetic studies have identified ATPase catalytic sites within each subunit of the holoenzyme (Hwang et al., 1995).

GpNu1 promotes site-specific binding to the DNA packaging initiation site, which is presumably mediated by a predicted helix-turn-helix (HTH) DNA binding motif (Lys5-Glu24) (Kypr and Mrazek, 1986). A putative ATP-binding P-loop sequence lies immediately downstream of this HTH motif, suggesting an interaction between the two sites. Indeed, DNA stimulates the ATPase activity of gpNu1 within the context of the holoenzyme complex, while ATP stimulates the nuclease activity and modulates the nuclease fidelity of the enzyme (Catalano, 2000). Interestingly, however, mutation of the 'critical' lysine within the P-loop of this subunit (Lys35) does not significantly impair the ATPase activity of the subunit (Hwang et al., 1995), and appears to affect the communication between the DNA and ATP binding sites of the protein instead.

Structural studies on the terminase gpNu1 subunit have been hampered by aggregation of the full-length protein (Meyer et al., 1998). We therefore engineered a gpNu1 construct in which the region that is believed to mediate self-association has been deleted (manuscript in preparation; see (Yang et al., 1999). This construct (gpNu1ΔE68) contains the first 68 amino acids of gpNu1, in which both the HTH and P-loop motifs are located. GpNu1ΔE68 possesses site-specific DNA binding activity, is fully soluble at elevated concentrations, and forms a stable 15.6 kDa symmetric dimer. Structural analysis of gpNu1ΔE68 in the absence and presence of ATP and DNA will shed light onto the mechanisms by which the nucleotide and polynucleotide binding sites interact during DNA packaging. Here, we report the assignment of the ^{1}H, ^{13}C, and ^{15}N resonances of the DNA binding domain of gpNu1.

Methods and results

Sample preparation. The gpNu1ΔE68 construct was expressed and purified to homogeneity essentially as described previously for gpNu1ΔK100, which contains the first 100 amino acids of gpNu1 (Yang et al., 1999). ^{15}N- and $^{13}C/^{15}N$-labelled samples were pro-

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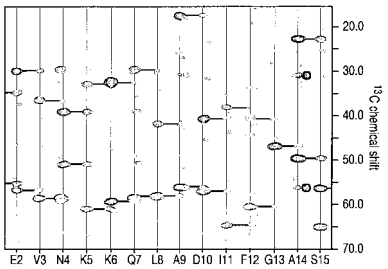

Figure 1. Strips from the HNCACB spectrum, showing sequential connectivities from amino acids E2 to S15. Lines are drawn horizontally to connect the C^{α} and C^{β} resonances.

duced by growing the bacteria in M9 minimal media, containing $^{13}C_6$-D-glucose and/or $^{15}NH_4Cl$ as sole sources of carbon and nitrogen, respectively. NMR samples typically contained 1.0 mM gpNu1-ΔE68 dissolved in 20 mM perdeuterated Tris, pH = 7.2, containing 1 mM NaN_3 and either 10 (v/v)% or 99.99% ^2H_2O.

NMR spectroscopy. Triple resonance experiments including HNHA, HNCO, CBCA(CO)NH, HNCACB, H(CO)-TOCSY, and C(CO)-TOCSY, spectra were recorded at 298 K using a Varian Inova 500 MHz spectrometer. A 2D (Hβ)Cβ(CγCδ)Hδ experiment, all 3D and 4D ^{13}C- and ^{15}N-edited NOESY and the 3D HCCH-TOCSY experiments were performed on a Varian Inova 600 spectrometer (for a review on the NMR experiments, see Muhandiram and Kay, 1994; Kay, 1997). Spectra were processed using NMRPipe (Delaglio et al., 1995), and analyzed using Pipp (Garret et al., 1991). In short, the acquisition domain was multiplied with a Gaussian function and a 90° shifted sine bell function, while indirect time domains were multiplied by a 90° shifted squared sine bell function. In addition, zero-filling and, where appropriate, linear prediction preceded Fourier transformation. Polynomial baseline corrections were employed in data processing schemes. ^{1}H chemical shifts were referenced to internal DSS at 0 ppm, while the ^{13}C and ^{15}N chemical shift reference points were calculated based on the $^{13}C/^1H$ and $^{15}N/^1H$ frequency ratios relative to the ^{1}H chemical shift of internal DSS.

Extent of assignments and data deposition

The assignment of the ^{1}H, ^{15}N, and ^{13}C backbone resonances is complete, except for the ^{13}CO resonance of Met27, which precedes a Pro residue, and the resonance position could therefore not be determined from the HNCO spectrum. Of the non-labile side chain protons, only the Lys5 and Lys6 $^1H^{\epsilon/\epsilon'}$ are left unassigned, while of the side chain carbons the $^{13}C^{\gamma}$ atoms of Ile11, Leu40, and Ile47 remain to be assigned. The majority of the sequence specific assignments were obtained by analysis of the HNCACB, CBCA(CO)NH, C(CO)TOCSY, H(CO)TOCSY and HCCH-TOCSY spectra, while a few of the side chain ^{1}H resonances could only be assigned using the ^{15}N- and ^{13}C-edited NOE spectra. The connectivity pattern of residues 2–15 of gpNu1ΔE68 in a HNCACB spectrum is shown in Figure 1 as an example of the assignment procedure. Backbone and side chain carbonyl ^{13}C chemical shifts were readily assigned in a 3D HNCO spectrum, while the positions of the aromatic resonances followed from the combination of a homonuclear 2D TOCSY spectrum, a ^{1}H-^{13}C HSQC spectrum optimized for aromatic residues and a 2D (Hβ)Cβ(CγCδ)Hδ spectrum. The full analysis of the 3D solution structure of the DNA binding domain of gpNu1ΔE68 is currently in progress. The chemical shift assignments have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number 4752.

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