



Letter to the Editor: Assignments of ^1H and ^{15}N resonances of the bacteriophage λ capsid stabilizing protein gpD

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Biological context

The *Escherichia coli* bacteriophage λ virion is comprised of an icosahedral head and a long flexible non-contractile tail, and has been extensively studied as a model system for virus assembly (Hendrix et al., 1983; Dokland and Murialdo, 1993). Like all icosahedral dsDNA bacteriophages, the morphogenesis of bacteriophage λ proceeds through the assembly of an empty prohead, which is subsequently filled with DNA. The shell of the procapsid is composed mainly from gpE. DNA packaging is accompanied by expansion of the shell and binding of the 109 amino-acid protein gpD. The binding sites for gpD are created or exposed only after the prohead expands (Imber et al., 1980). gpD is not required for prohead assembly itself but is essential if a full-length genome is to be stably accommodated in the λ capsid. Thus, it is believed that gpD is necessary for stabilizing the capsid. In addition, N- and C-terminal fusion peptides and proteins of gpD have been used in λ phage display (Sternberg et al., 1995; Mikawa et al., 1996). The homo-trimeric structure of gpD in crystals has been determined and this structure is also present on the phage capsid, as observed by cryo-electron microscopy of empty capsids at 15 Å resolution (Yang et al., 2000). In the crystal structure, however, the functionally required N-terminal region is not defined. Interestingly, gpD exists stably as a monomer even at millimolar concentrations in solution in contrast with the crystal structure. The present NMR assignments will allow the detailed characterization of the

N-terminal region and provide a basis for further studies of the interaction between gpD and the phage λ capsid, and hence, for the understanding of the virion assembly process.

Methods and experiments

The full length gpD protein of bacteriophage λ was expressed in *E. coli* and purified as published previously (Yang et al., 2000). Isotopically labeled gpD protein was prepared from cells grown in M9-based minimal medium supplemented with $^{15}\text{NH}_4\text{Cl}$ as the sole nitrogen source. The final NMR samples contained approximately 1.3 mM unlabelled or uniformly ^{15}N -labelled gpD dissolved in 90% $\text{H}_2\text{O}/10\%$ D_2O , or 100% D_2O , containing 20 mM sodium phosphate, pH 6.0. All the NMR measurements were performed at 25 °C on either Bruker DRX 600 or Bruker AV800 spectrometers. Sequence-specific assignments of the polypeptide backbone resonances were obtained using standard techniques with 2D homonuclear NOESY, TOCSY, and DQF-COSY (Wüthrich, 1986), as well as 3D ^{15}N -resolved [$^1\text{H},^1\text{H}$]-NOESY and [$^1\text{H},^1\text{H}$]-TOCSY spectra. 3D HNHB was used for the assignment of $\text{H}\beta$ protons (Archer et al., 1991). All pulse sequences in H_2O incorporated the WATERGATE sequence using 3-9-19 composite pulses for the water suppression (Piotto et al., 1992).

Sequence-specific assignments of aromatic side chains were obtained using NOEs between the aromatic protons and the βCH_2 group or the α -proton (Wüthrich, 1986), using [$^1\text{H},^1\text{H}$]-NOESY in D_2O . Proline residues and methionine ϵCH_3 groups were assigned based on the analysis of NOEs. The NMR spectra were processed using the program PROSA

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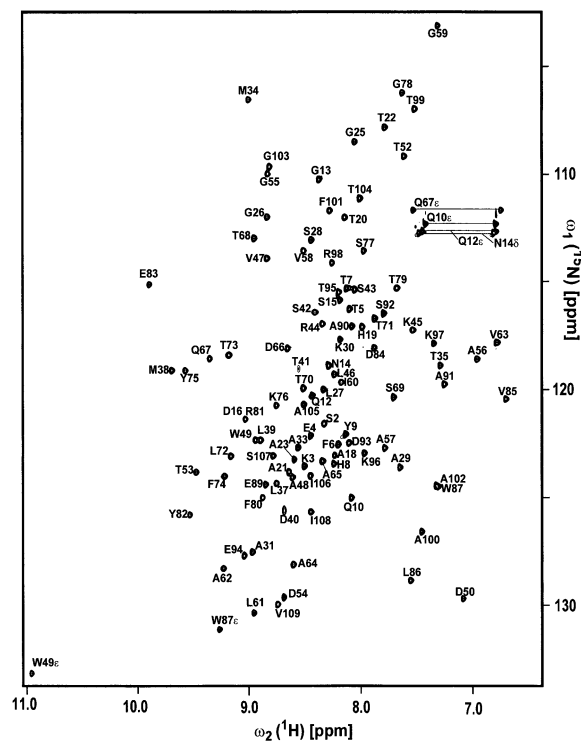


Figure 1. $[^1\text{H}, ^{15}\text{N}]$ -COSY spectrum of uniformly ^{15}N -labeled bacteriophage λ gpD protein in 90% $\text{H}_2\text{O}/10\%\text{D}_2\text{O}$ at 800 MHz, pH 6.0, and 25 °C. Backbone amide, Trp N ϵ 1, Gln and Asn amide side chain resonance assignments are indicated by the one-letter code for amino acids and the sequence number.

(Güntert et al., 1992), and the spectral analysis was supported with the software package XEASY (Bartels et al., 1995).

Extent of assignments and data deposition

High-quality NMR data for the gpD protein were obtained as shown by the $[^1\text{H}, ^{15}\text{N}]$ -HSQC spectrum collected at 800 MHz in Figure 1. More than 97% of the backbone amide and non-labile ^1H chemical

shifts could be assigned. Backbone ^1H and ^{15}N assignments are complete except for the N-terminal two residues and for Gly51. The amino acid side-chain assignments of non-labile hydrogen atoms are complete except for Thr 1, Ser 2, Lys 3, H δ and H ζ of Phe 6, H δ and H ϵ of His8, and H β and H γ of Pro 11. The ^1H and ^{15}N chemical shifts for the bacteriophage λ gpD monomer have been deposited in the BioMagResBank database (<http://www.bmrb.wisc.edu>) under BMRB accession number 5807.

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