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Letter to the Editor: NMR assignment of the gpU tail protein from lambda bacteriophage

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Biological context

The last step of λ bacteriophage viral assembly involves the attachment of an icosahedral head piece to a non-contractile tail piece which serves as the conduit for transmission of the viral genome to the host bacterium (Katsura and Tsugita, 1977). The protein, gpU, forms the interface between the head and the tail pieces. In this note, we present the near complete backbone and sidechain assignments of this monomeric, 131 residue protein.

In the mature viral particle, gpU forms a hexamer presumably to complement the stoichiometry of the major tail protein, gpV (Katsura, 1987). Consistent with its function as a head/tail piece adaptor, this relatively small protein participates in numerous intra- and inter-molecular interactions. Our overall objective is to use the structure of gpU as a foundation for further high resolution structural studies of viral protein-protein complexes.

Methods and NMR experiments

The bacteriophage λ gene *U*, which encodes gpU, was amplified by PCR methods and placed into the IPTG-inducible expression vector pET-15b (EMD Biosciences; San Diego, CA) creating a hexahistidine tagged protein with an intervening thrombin protease cleavage site. Isotopically labeled preparations of His_6–gpU were purified by nickel affinity and gel filtration chromatography from soluble *E. coli* BL21 (DE3) cell extracts derived from fermentations in M9 minimal media supplemented with (U-99%) ^{15}N-ammonium chloride and (U-99%) ^{13}C-glucose (Spectra Stable Isotopes; Columbia, MD). Optimal sample conditions for NMR spectroscopy were 0.7–0.9 mM protein in 10 mM Tris-d_{11} pH 7.8, 50 mM KCl, and 0.02% NaN_3 and 10% or 99% D_2O as required.

NMR spectra were acquired at 293 K on a Bruker Avance DRX 600 MHz equipped with a room temperature triple resonance probehead and a Varian Unity Inova 800 MHz spectrometer equipped with a cryogenically cooled probehead. Proton chemical shifts were referenced to DSS. Spectra were processed with NMRPipe (Delaglio et al., 1995) and interpreted with NMRView (Johnson and Blevins, 1994). Backbone and aliphatic sidechain assignments were obtained from ^{1}H, ^{15}N-HSQC, ^{1}H,^{13}C-HSQC, HNCO, CBCA(CO)NH, HNCACB, H(C) (CO)NH, (H)C(CO)NH, and HCCH-TOCSY spectra. Aromatic resonances were assigned using a combination of (HB)CB(CGCD)HD, (HB)CB(C GCDCE)HE, and optimized HCCH-TOCSY spectra. Backbone assignments were cor-

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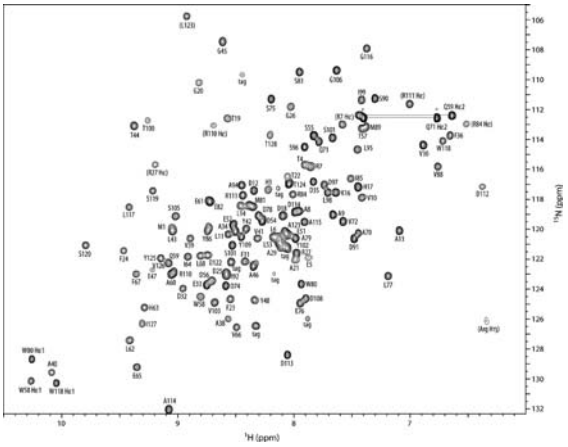

Figure 1. ^{1}H,^{15}N-HSQC of λ bacteriophage gpU. There is an amino terminal 19 aa. affinity tag that was not removed by proteolysis. The majority of the resonances in the affinity tag were not observable with the exception of those marked 'tag'. Bracketed labels indicate that these resonances are aliased in the spectrum.

roborated from main chain amide walks through 3D ^{13}C-NOESY and ^{15}N-NOESY spectra. A representative ^{1}H, ^{15}N-HSQC spectrum is shown in Figure 1.

The secondary structure of gpU was predicted using a combination of chemical shift index analysis (Wishart and Sykes, 1994), TALOS dihedral angle predictions (Cornilescu et al., 1999) and HN-HN NOE observations. Taken together, gpU is comprised of four α-helices (α1: 25–35; α2: 67–72; α3: 94–108; α3: 113–117) and five β-strands (β1: 41–44; β2: 57–63; β3: 80–86; β4: 123–129; β5: 139–144).

Extent of assignments and data deposition

Overall, 96.5 % of the possible ^{1}H and 95.9 % of the possible heavy atom (^{13}C and ^{15}N) assignments were made for gpU, disregarding the amino terminal, 19 amino acid affinity tag and the first and last four amino acids of the native gpU protein. The chemical shift assignments for gpU have been deposited in the BioMagResBank under the accession number 6434.

Spectral quality and additional observations

Upon the addition of Mg^{2+}, gpU forms a hexamer (Katsura and Tsugita, 1977). This stoichiometry is believed to represent the functional form of gpU in mature viral particles as it complements the stoichiometry of the gpV tail protein partner. Addition of 10 mM $MgSO_4$ or $CaCl_2$ to a 0.4 mM ^{15}N-labeled preparation of gpU produced minor chemical changes and line broadening as monitored by ^{1}H, ^{15}N-HSQC spectra. Further addition of Mg^{2+} or Ca^{2+} to 20 mM produced severe line broadening which may indicate the formation of an 88 kDa hexameric species. In contrast, a D74A variant of gpU was not susceptible to line broadening at Mg^{2+} concentrations up to 40 mM. With this simple, ^{1}H,^{15}N-HSQC method, we will be testing additional gpU variants and correlating the results with *in vivo* functional assays.

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