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The neck of bacteriophage T4 is a ring-like structure formed by a hetero-oligomer of gp13 and gp14

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Abstract

After packaging of DNA into the head of bacteriophage T4 is completed, a neck is formed at the portal vertex of the head to be ready for the tail attachment. The main components of the neck are gp13 and gp14 (gp: gene product), which consist of 309 and 256 amino acid residues, respectively. In order to elucidate the structure and subunit arrangement in the neck, overexpression systems of gene *13* and gene *14* were constructed and purified to homogeneity. Far-UV circular dichroism (CD) spectra of gp13 and gp14 indicated that gp13 is rich in α-helices whereas gp14 is rich in β-sheets. Sedimentation velocity analysis of gp13 and gp14 revealed that both proteins are present as monomers in solution. The frictional ratios (f/f_0) of the two proteins indicated that gp14 has a more elongated shape than gp13. Although isolated gp13 and gp14 do not interact with each other when mixed under physiological conditions, they form a hetero-oligomer complex with the stoichiometry of 10:5 after treatment with ammonium sulfate. Electron microscopy of this complex has shown that it forms a ring-like structure of 15 nm in diameter.

Keywords: Bacteriophage T4; Neck protein; Virus assembly; Protein–protein interaction; Analytical ultracentrifugation; Transmission electron microscopy

1. Introduction

Bacteriophage T4 is a member of the *Myoviridae* family which infects the host bacterium *Escherichia coli*. The self-assembly pathway of the virion has been extensively investigated as reviewed by a number of authors [1–3]. The phage head, tail and tail fibers assemble independently along well-ordered pathways and after the head and tail join, the tail fibers bind to the tail to form an infectious virion. The head of phage T4 is a prolate icosahedron which is 120 nm long and 86 nm wide in diameter [4]. The prohead formation is initiated by the initiator complex made of twelve subunits of gp20 which form the portal vertex [5]. The genomic DNA of phage T4 is packaged through the portal vertex into the head after the prohead assembly is completed. Before or during the DNA packaging, expansion of the prohead occurs, resulting in a volume increase of about 50% [6]. Upon completion of the DNA packaging, the attachment of gp13 and gp14 to the portal vertex takes place before the tail joining begins [7,8].

In another pathway, tail tube and the sheath polymerization are terminated by gp3 and gp15. Gp3 binds at the proximal end to the tube, forming a hexameric ring structure and stopping tube polymerization [2,9]. The connector protein, gp15, then binds to gp3 and the sheath and forms the connector which protrudes from the top of the sheath by about 6–8 nm [2,7], forming a hexameric ring of 13 nm in diameter [10]. Gp13 and/or gp14 recognize gp15, leading to the joining of the head and the tail.

The structure and the subunit composition of the neck were first studied by Coombs and Eiserling [7]. They observed the "necked" tail by electron microscopy with negative staining. The "necked" tail can be obtained by osmotic shock upon rapid dilution of T4 phage solution from 1.58 M CsCl to a buffer

Abbreviations: bp, base pair; CBB, Coomassie Brilliant Blue; EDTA, ethylenediaminetetraacetic acid; TEM, transmission electron microscope; gp, gene product; IPTG, isopropyl β-D-1-thiogalactopyranoside; PCR, polymerase chain reaction; PMSF, phenylmethylsulphonyl fluoride; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PVDF, polyvinylidine difluoride

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without CsCl, which disrupts the head and yields a tail with a neck [7]. They reported that the neck is 15 nm in length and 16 nm in width and is located between the portal vertex gp20 and the connector protein gp15. Six fibers called whiskers or fibritin, formed by gpwac, are attached to the neck. There are six protein components between the head and tail of which the major structural components are gpwac, gp13 and gp14. Genes *wac*, *13* and *14* are present in this order in the phage genome following the baseplate gene cluster and regulated by a common promoter.

Recently, the structure of the tail including the neck was analyzed by three-dimensional image reconstruction from cryo-electron microscopy to a resolution of 15 Å [11,12], which is not sufficient for the subunit arrangement in the neck region to be discernible. In order to elucidate the structure and interactions of the neck proteins, gene *13* and *14* were cloned and over-expressed. The gene products were purified to homogeneity and the proteins were characterized by analytical ultracentrifugation, circular dichroism and electron microscopy with negative staining. In the present study it is shown that ammonium sulfate induced a specific interaction between gp13 and gp14 to form a hetero-oligomer whose dimension appears consistent with binding to the gp15 connector.

2. Materials and methods

2.1. Bacterial strains and plasmid

E. coli XL1-blue was used for the construction of plasmids. *E. coli* BL21 (DE3) was used for overexpression. Expression vectors pET29a and pET28b (Novagen) were used for cloning of gene *13* and gene *14* respectively.

2.2. Media, chemicals and buffers

LB broth contains 1% (w/v) Bacto Tryptone, 0.5% (w/v) Bacto yeast extract and 0.5% (w/v) NaCl. LB plate contains 1% (w/v) Bacto Trypton, 0.5% (w/v) Bacto yeast extract, 1% (w/v) Bacto agar and 1% (w/v) NaCl. T-buffer had a final concentration of 50 mM Tris pH 8.0. TE-buffer was composed of 10 mM Tris, 1 mM EDTA pH 8.0. TN-buffer consisted of 50 mM Tris pH 8.0 and 0.1 M NaCl. Chemicals and reagents were purchased from Nacalai Tesque, Inc. Japan or Wako Pure Chemical Industries Japan.

2.3. Plasmid construction and overexpression of proteins

Gene *13* was amplified by PCR from T4D phage genome with two primers, 5′-AACATG**GGTACC**TAAGGCCCCAA-3′ (*Kpn*I site in bold) and 5′-CCCCCC**CCATGG**TCTGAGAATAACCTGTGC-3′ (*Nco*I site in bold). The 927-bp PCR product was cloned into a pET29a vector at the same restriction sites and *E. coli* XL1 blue cells were transformed with the plasmid. This expression vector was named pLZ101. Gene *14* was amplified from T4D DNA with two primers, 5′-**CCATGG**CTACTTATGATAAA-3′ (*Nco*I site in bold) and 5′-**CTCGAG**TTAATCCATGAAGATC-3′ (*Xho*I site in bold). The 768-bp PCR product was purified using QIAquick (Qiagen) and was cloned into pET28b at the same restriction site and *E. coli* XL1 blue cells were transformed with the plasmid. The resulting plasmid was named pTA114. All amplified and cloned gene segments were verified by DNA sequencing.

E. coli BL21 (DE3) cells containing pLZ101(for gp13) or pTA114(for gp14) were grown by shaking at 30 °C in 1 l of LB broth in the presence of kanamycin at a final concentration of 60 μg/ml. Protein expression was induced by addition of IPTG to a final concentration of 1 mM when the optical density at 600 nm reached 0.5. The culture was continuously grown for an additional 4 h at 30 °C for gp13 and 37 °C for gp14.

2.4. Purification of gp13 and gp14

The purification procedure for gp 13 and gp14 was basically the same. Three grams (wet weight) of harvested cells were resuspended in 30 ml TE-buffer and sonicated on ice for 10 min and the procedure repeated four times (out power, 3; duty cycle 50%, Branson sonifier 250). Before sonication, PMSF was added to the crude extract of gp13 at a final concentration of 1 mM. For gp14, a "Complete" protease inhibitor cocktail tablet (Roche Applied Science, USA) was added. The extract was centrifuged at 20,000×*g* for 20 min to remove cell debris. Ammonium sulfate was added to the supernatant and the mixture stirred for 30 min before centrifugation at 20,000×*g* for 20 min. Gp13 and gp14 were precipitated by ammonium sulfate saturation between 15 and 20%, and between 10 and 15%, respectively. The pellet was resuspended in TE-buffer and insoluble materials were removed by centrifugation (20,000×*g* for 20 min). The sample was loaded onto a HiTrap Q HP (5 ml, Amersham Biosciences Corp., USA) anion exchange column which had been equilibrated with T-buffer. The bound proteins were eluted with a 0- to 1-M NaCl linear gradient in T-buffer. Gp13 and gp14 were eluted around 0.35 M and 0.4 M NaCl, respectively. The fractions containing gp13 or gp14 were pooled and mixed with 1 M ammonium sulfate to make a final concentration of 0.5 M. Samples were loaded onto a HiTrap Butyl FF (5 ml, Amersham Biosciences Corp., USA) hydrophobic interaction column equilibrated with T-buffer containing 0.5 M ammonium sulfate. The adsorbed proteins were eluted with an ammonium sulfate linear gradient (0.5–0 M) in T-buffer. The eluted protein was collected and dialyzed against T-buffer. The desalted sample was applied to a HiTrap Q HP (5 ml, Amersham Biosciences Corp., USA) anion exchange column which had been equilibrated with T-buffer. Gp13 or gp14 was eluted with a 0- to 1-M NaCl linear gradient in T-buffer. Fractions were collected and subsequently run on a HiLoad 16/60 Superdex 200 pg (120 ml, Amersham Biosciences Corp., USA) gel filtration column in TN-buffer. Elution volumes of gp13 and gp14 were 86 ml and 80 ml, respectively. Purified gp13 and gp14 were both run on 13% SDS-PAGE to confirm that the purity was about 95% (Fig. 1). All the purification steps were performed at 4 °C. The yield of gp13 and gp14 was around 7 mg and 4 mg per liter of bacterial cell culture, respectively. Protein concentration was determined either by measuring absorbance at 280 nm with the extinction coefficient of 1.42 for gp13 and 1.11 for gp14 or by BCA Protein Assay Reagent Kit (PIERCE Biotechnology Inc., USA) with BSA as a standard.

2.5. Purification of gp13–gp14 complex

To purify the gp13 and gp14 complex, the purified proteins gp13 and gp14 were mixed at the molar ratio 2:1. Five mol/L ammonium sulfate stock solution was added to 5 ml of protein mixture (0.78 mg/ml) to a final concentration of 0.8 M to induce association and incubated for approximately 5 h at 20 °C. The samples were then dialyzed against TN-buffer. Before application to HiLoad 16/60 Superdex 200 pg (120 ml, Amersham Biosciences Corp., USA) gel filtration column, samples were centrifuged at 20,000×*g* for 20 min. No visible

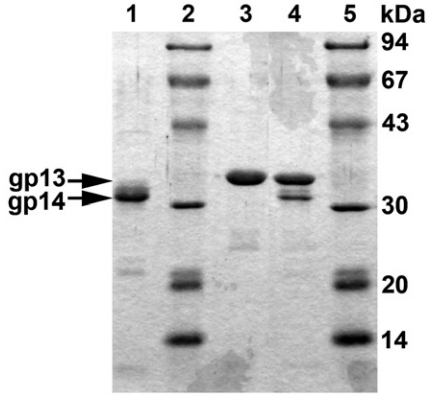

Fig. 1. 13% SDS-PAGE of purified gp13, gp14 and $(gp13)_{10}(gp14)_5$ complex. Lane 1: gp14, lane 3: gp13, lane 4: $(gp13)_{10}(gp14)_5$ complex. Lanes 2 and 5: molecular mass standard as indicated.