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Crystal structure of the C-terminal domain of Mu phage central spike and functions of bound calcium ion

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ABSTRACT

Bacteriophage Mu, which has a contractile tail, is one of the most famous genus of *Myoviridae*. It has a wide host range and is thought to contribute to horizontal gene transfer. The *Myoviridae* infection process is initiated by adhesion to the host surface. The phage then penetrates the host cell membrane using its tail to inject its genetic material into the host. In this penetration process, *Myoviridae* phages are proposed to puncture the membrane of the host cell using a central spike located beneath its baseplate. The central spike of the Mu phage is thought to be composed of gene 45 product (gp45), which has a significant sequence homology with the central spike of P2 phage (gpV). We determined the crystal structure of shortened Mu gp45Δ1-91 (Arg92–Gln197) at 1.5 Å resolution and showed that Mu gp45 is a needlelike structure that punctures the membrane. The apex of Mu gp45 and that of P2 gpV contained iron, chloride, and calcium ions. Although the C-terminal domain of Mu gp45 was sufficient for binding to the *E. coli* membrane, a mutant D188A, in which the Asp amino acid residue that coordinates the calcium ion was replaced by Ala, did not exhibit a propensity to bind to the membrane. Therefore, we concluded that calcium ion played an important role in interaction with the host cell membrane.

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1. Introduction

The temperate bacteriophage Mu has a wide host range that includes certain strains of *E. coli* K12, *Citrobacter freundii*, and some strains of *Shigella dysenteriae* [1,2]. Mu phage is the most efficient transposable genetic element known. It is also demonstrated that Mu phage plays an important role in horizontal gene transfer between species, because of its broad host range. This is supported by observations that Mu prophages are present in several bacterial species [3,4]. Most temperate bacteriophages integrate into one or a small number of specific sites in the host chromosome, whereas Mu phage integrates at many random sites. Therefore, Mu phage-induced mutations are caused by the random insertion of the phage DNA within the host genome, resulting in its inactivation of targeted genes [1]. Those mutations are extremely stable, with few revertants [5]. Although most integrations of Mu do not cause loss of host DNA, Mu may cause the deletion and double-insertion mutations [6]. Mu recombination systems therefore have been investigated by many researchers, especially structural biologists [7].

Mu phage shares the morphology of *Myoviridae*, which is composed of an icosahedral head filled with double-stranded DNA, a contractile tail, a baseplate located at the distal end of the tail, and tail fibers [8,9]. The tail fibers are responsible for host recognition, and thus the host selectivity is determined by tail fiber specificity [10]. The baseplate of *Myoviridae* carries small protruding rod structures, tail spikes and/or tail pins, that are involved in the infection process. The P2 phage gpV was found to be a tail spike beneath the baseplate by immune-electron microscopy [11]. Recently, we determined the crystal structure of a P2 gpV C-terminal domain [12]. The structure was a triangular pyramid and looked like a spearhead. Moreover, using cryo-electron microscopy, Browning et al. showed that P2 gpV was located in a central part of the baseplate; they named it a "central spike". They also reported the atomic structures of full-length central spikes for P2 and ϕ92 phages, and proposed that these central spikes were instrumental in the puncturing of host cell membrane and in the injection of phage DNA into the cell [13]. In fact, the other central spike, T4 phage gp5 that folds into a rigid piercing needle, is required to penetrate the lipid bilayer [14]. Since the C-terminal region of the Mu phage gp45 (Ser64–Gln197)

Abbreviations: DSC, differential scanning calorimetry; EDTA, ethylenediaminetetraacetic acid; gp45Δ1-63, gp45Δ1-91, gp45Δ1-99, the recombinant C-terminal fragment of Mu phage gp45 corresponding to Ser64–Gln197, Arg92–Gln197, and Glu100–Gln197, respectively, fused to a histidine tag; PCR, polymerase chain reaction; PEG, polyethylene glycol; MAD, multi wavelength anomalous dispersion; MES, 2-morpholinoethanesulfonic acid; QCM, a quartz crystal microbalance; rmsd, root mean square deviations; SAM, self-assembling monolayer; Tris, tris(hydroxymethyl)aminomethane

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has significant amino acid sequence similarity (45.5%) with that of the P2 phage gpV (Ser87–Leu211) (Fig. 1), gp45 is believed to be a Mu phage central spike subunit. We have also reported that these C-terminal domains of P2 gpV and Mu gp45 were sufficient for binding to the *E. coli* membrane by using quartz crystal microbalance (QCM) [15,16].

This paper presents the crystal structure of truncated Mu gp45 (Arg92–Gln197) at 1.5 Å resolution; it is a needlelike shaped homotrimer consisting of an intertwined β-sheet and β-helix. The apex of Mu gp45 includes iron, chloride, and calcium ions as well as P2 gpV. Although the C-terminal domain of the Mu gp45 D188A mutant that was expected to be free from calcium ion had the same thermo-stability as the wild type in differential scanning calorimetry (DSC) measurement, the mutant did not have any binding properties to the *E. coli* membrane. It is therefore proposed that the calcium ion of Mu gp45 was related to the interaction with the membrane of the host cell.

2. Experimental procedures

2.1. Purification of shortened gp45 wild type and mutants

The truncated g45 encoding the wild type gp45Δ1-63 (Ser64–Gln197) and derived missense mutants was amplified using polymerase chain reaction and cloned into expression vector pET16 (Novagen), gp45Δ1-91 (Arg92–Gln197) was cloned into pET21b (Novagen), and gp45Δ1-99 (Glu100–Gln197) was cloned into pET28b (Novagen). The expression constructs were transformed into *E. coli* BL21(DE3)pLysS. Cells were grown at 310 K in Luria broth medium containing 50 μg/ml kanamycin or 100 μg/ml ampicillin. Overexpression was induced by adding isopropyl β-D-thiogalactopyranoside to a final concentration of 0.4 mM at an optical density at 600 nm of 0.5 to 0.6. Cells were incubated at 303 K for 3.5 h under vigorous shaking, harvested by centrifugation, and suspended in buffer (20 mM phosphate buffer pH 7.5, 300 mM NaCl, 20 mM imidazole). The suspension was sonicated in the presence of 1 mM phenylmethanesulfonyl fluoride. After centrifugation, the supernatant was loaded onto a nickel affinity column (Qiagen), which was equilibrated with the same buffer. The protein was eluted with a buffer (20 mM phosphate buffer pH 7.5, 300 mM NaCl, 500 mM imidazole) and was purified by gel filtration on Superdex 200 (GE Healthcare). For gp45Δ1-91, protein fractions were dialyzed against 20 mM TrisHCl pH 8.0, 50 mM NaCl, and were further subjected to Mono-Q (GE Healthcare) for purification. The specimen was collected in flow through fractions. Gp45Δ1-91 and gp45Δ1-99 were concentrated to 5 mg/ml and 10 mg/ml, respectively, and used for crystallization. Protein concentrations were determined using UV absorption measurements at 280 nm.

2.2. Crystallization and crystallographic data collection

The hanging-drop vapor-diffusion method was used for crystallization. Each drop consisted of 1 μl of protein and 1 μl of reservoir solution and suspended over 0.5 ml of reservoir solution at 293 K. Gp45Δ1-99 formed hexagonal plane crystals of about 0.2 mm × 0.2 mm × 0.1 mm within 4 days using a reservoir solution of 21% PEG 8000, 0.1 M acetate pH 4.5, and 0.2 M NaCl. The crystals were transferred into 100 μl buffer solution (21% PEG 8000, 0.1 M acetate pH 4.5, 0.2 M NaCl). The PEG 8000 concentration was gradually increased to 35% in 5% increments. The crystals were frozen in liquid nitrogen. The heavy atom derivative crystals for phase determination were prepared by soaking to the saturated K_2PtCl_6 solution containing 35% PEG 8000 for 2 h. Gp45Δ1-91 formed tetragonal prism crystals of about 0.2 mm × 0.1 mm × 0.1 mm within 7 days using a reservoir solution of 16% PEG 20000, 0.1 M MES pH 6.5, and 0.15 M NaCl. Diffraction data sets were collected at 100 K on a Rayonix MX-225HE CCD detector supported by NSSRC at the BL44XU beamline at SPring-8 (Harima, Japan). To determine the phase, multiwavelength anomalous dispersion (MAD) data collection

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P2 gpV    MNTLAN------IQELARALRNMIRTGIIVETDLNAGRCRVQTGGMCTDWLQWLTHRAGR  54
Mu gp45   MERVNDSALNRLLTPLMRRVRLMLARAVVNVINDGRKVQNLQVG-LLDDEESDEVERLQN  59
          *: : :      :  * * :* *:  .::   : .    .:*.* :  *  .  ..*  .

Mu gp45#                                              GDAGIYHHEGHRIRLTK 118
P2 gpV    SRTWWAPSVGEQVLILAVGGELDTAFVLPGIYSGDNPSPSVSADALHIRFPDGAVIEYEP 114
Mu gp45   YGHFSVPLPGAEALIACVGAQRDQGIAVVVEDRRYRPTN------------------LEP 101
             : .*  * :.** .**.: * .:.:       .*:                    **

Mu gp45# D--GRCIIT-C---------------KTVEVYADESMTVDTPRTTFTGDVEIQKGLGVKG 160
P2 gpV    -ETSALTVSGIKTASVTASGSVTATVPVVMVKASTRVTLDTPEVVCTNRLITG-TLEVQK 172
Mu gp45   -GDAGIYHHEGHRIRLTKDGRCIITCKTVEVYADESMTVDTPRTTFT-----G-DVEIQK 154
             :.:     :   :* .*    *  .* * *.  :*:***... *     *  :*:**

Mu gp45# KSQFCS----NITAP--DAIINGKSTDKHIHRGDSGGTTGPMQ                  197
P2 gpV    GGTMRG----NIEHTGGELSSNGKVLHTHKHPGDSGGTTGSPL                  211
Mu gp45   GLGVKGKSQFDSNITAPDAIINGKSTDKHIHRGDSGGTTGPMQ                  197
          *  ::*    : : *. :   ***  ..* * ********.
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Fig. 1. Sequence alignment of Mu gp45 and P2 gpV. Primary sequence of Mu gp45 is four residues shorter than that of P2 gpV, and the C-terminal domain (Ser64–Gln197) of Mu gp45 has 45.5% sequence identity with that of P2 gpV (Ser87–Leu211). Primary sequence alignment of "P2 gpV" vs "Mu gp45" was resulted from ClustallW [25]. An asterisk indicates positions which have an exactly conserved residue. A colon indicates conservation between groups of strongly similar properties, and a period indicates weakly similar properties. The N-terminal residue of crystallized gp45Δ1-91 is shown in bold type. The structure-based alignment of the C-terminal domains of "P2 gpV" vs "Mu gp45#" does not match with the primary sequence alignment. The intertwined β-sheet region is underlined, the triple β-helix region is double underlined, and the ion binding region is dashed underlined.

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