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Crystallization and preliminary X-ray analysis of gene product 44 from bacteriophage Mu

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Bacteriophage Mu baseplate protein gene product 44 (gp44) is an essential protein required for the assembly of viable phages. To investigate the roles of gp44 in baseplate assembly and infection, gp44 was crystallized at pH 6.0 in the presence of 20% 2-methyl-2,4-pentanediol. The crystals belong to space group $R3$, with unit-cell parameters $a = b = 127.47$, $c = 63.97$ Å. The crystals diffract X-rays to at least 2.1 Å resolution and are stable in the X-ray beam and are therefore appropriate for structure determination. Native data have been collected to 2.1 Å resolution using a DIP6040 image-plate system at beamline BL44XU at the SPring-8 facility in Japan.

1. Introduction

Bacteriophage morphogenesis has been investigated as a model system for macromolecular-assembly mechanisms. As genetic studies are useful for investigation of bacteriophage components and assembly systems, many traditional mutation analyses have been performed on phages and their bacterial hosts. As techniques for the structural determination of supermolecules have advanced, the great amount of background information has generated interest in these viruses from crystallographers. Bacteriophage Mu is a temperate phage that can infect a rather wide range of hosts among the enteric bacteria. This virus consists of an icosahedral head, a knob-like neck, a contractile tail, a baseplate and six tail fibres. Mu phage can be reconstituted and has been investigated like many other long-tailed double-stranded DNA (ds-DNA) phages (Giphart-Gassler *et al.*, 1981). The genetics of phage Mu have been extensively studied since the early 1960s. For example, many amber mutants and several temperature-sensitive mutants have been identified by SDS–PAGE and electron-microscopic analysis (O'Day *et al.*, 1979; Ghelardini *et al.*, 1980; Giphart-Gassler *et al.*, 1981; Morgan *et al.*, 2002). These results have identified genes that are essential for bacteriophage assembly. Recently, the complete genome sequence of phage Mu has been determined (Morgan *et al.*, 2002). However, except for electron microscopy (EM), only a few studies of the three-dimensional structure of bacteriophage Mu and its component subunits have been undertaken. The baseplate is believed to recognize the host-cell surface. The baseplate of bacteriophage Mu is at the distal end of the tail and consists of the gene products of genes Y, N, P, Q, V, W and R (gpY, gpN, gpP gpQ, gpV, gpW and gpR; Grundy & Howe, 1985). Although the structural genes for baseplate construction have been identified, the assembly mechanism of the Mu phage has not yet been elucidated at the molecular level.

Bacteriophage T4 is one of the most well understood phages and, along with Mu, belongs to the *Myoviridae* family. T4 phage has been examined using various techniques. Structural information on T4 has been obtained by complementation studies and cross-linking analysis (Eiserling & Black, 1994). More recent structural investigations using X-ray crystallography of the subunits, cryo-electron microscopy (cryoEM) and reconstruction of the three-dimensional structure have demonstrated the properties of T4 phage structure and the process of T4 infection (Leiman *et al.*, 2003). In particular, the baseplate of bacteriophage T4, which is comprised of at least 15 different subunits, has been investigated in detail to analyze its three-dimensional structure (Kostyuchenko *et al.*, 2003). Surprisingly, the components of

the bacteriophage Mu baseplate have very low homology at the amino-acid level with the T4 baseplate subunits, even though the subunits of both viruses have apparently similar structures based on EM pictures and their function in infection.

The gene product of Mu phage gene 44 (gp44), named gpP in traditional genetic assignment, is an essential protein for baseplate assembly. However, it is not known how gp44 functions in baseplate assembly or the infection process. The structural analysis of supermolecules, such as viruses and phages, has recently become possible for crystallographers. Moreover, these investigations can demonstrate the mechanisms of assembly of the complex structures and the functional systems of a whole virion. We have recently undertaken structural studies of the Mu phage baseplate in order to clarify its structure, compare it with the T4 baseplate and understand its assembly mechanism and role in the process of infection. As a first step, we describe here the crystallization and preliminary X-ray crystallographic analysis of gp44.

2. Experimental procedures and results

2.1. Expression, purification and crystallization

The Mu phage lysogenic host *Escherichia coli* MH7213 was a kind gift from Professor Martha H. Howe (University of Tennessee). Recombinant gp44 was expressed in *Escherichia coli* BL21(DE3)-pLysS which was transformed with the pET17b vector carrying gene 44 of Mu phage. Purification was performed by three-step chromatography as described elsewhere (Kitazawa *et al.*, 2005). Briefly, gp44 was extracted from host *E. coli* by sonication and purified by DEAE-Sepharose chromatography at pH 8.0, SP-Sepharose chromatography at pH 6.0 and size-exclusion chromatography with Sephacryl S-300. Protein purity and uniformity were confirmed by SDS–PAGE and analytical ultracentrifugation. Purified gp44 formed a 122–124 kDa trimer, a complex of full-length 42 kDa protein and 40 kDa protein which has been processed in the C-terminal region (Kitazawa *et al.*, 2005). Recombinant gp44 was expressed under the same conditions as native protein; because all Mu proteins are expressed by the host cell, in this case *E. coli*, neither native nor recombinant gp44 is glycosylated. After purification, the protein was stored at 243 K. Purified gp44 was concentrated to 20 mg ml^{-1} in 50 m*M* Tris–HCl pH 8.0, 150 m*M* NaCl using Microcon concentrators (Amicon, Beverly, Massachusetts, USA) and was crystallized at 288 K by the hanging-drop vapour-diffusion method. The crystallization droplets were prepared on siliconized cover slips by mixing 1 µl protein solution with 1 µl precipitant solution. A few crystals of gp44 were initially found in the pH range 6–7 with organic solvent, for example 2-methyl-2,4-pentanediol (MPD) or propanol, using commercial crystallization kits from Hampton Research (Laguna Niguel, CA, USA). The most suitable condition for crystallization was determined by comparing the resolution of X-ray diffraction from crystals obtained in the pH range 5.5–6.5 and in 15–25%(*v*/*v*) MPD. After optimizing the conditions, the most suitable precipitant solution for crystallization was found to be a mixture consisting of 20%(*v*/*v*) MPD and 50 m*M* 2-(*N*-morpholino)ethanesulfonic acid (MES). Finally, crystals of gp44 were grown in hanging drops for three weeks at 288 K and grew to up to 0.8 × 0.7 × 0.3 mm in size. Fig. 1 shows a typical crystal.

2.2. Data collection

A crystal of gp44 picked up from a droplet using a mounted nylon loop (Hampton Research, Laguna Niguel, CA, USA) was placed directly into a cold nitrogen-gas stream at 100 K. X-ray diffraction images were collected from the crystal at 100 K under a nitrogen-gas stream using a DIP 6040 image plate with synchrotron radiation of wavelength 0.9 Å at the SPring-8 beamline BL44XU at Hyogo, Japan, which was designed for biological macromolecular-assembly studies. The distance between the crystal and detector was 350 mm. 2° oscillation images were recorded with exposure times of 15 s. A total of 81 images were collected. The diffraction data for the native crystal were obtained in the resolution range 40–2.1 Å and were indexed and scaled using *MOSFLM* (Leslie, 1991) and *SCALA* (Kabsch, 1988). The crystals belong to space group *R*3, with unit-cell parameters $a = b = 127.47$, $c = 63.97$ Å. A total of 23 274 (3416) independent reflections obtained with a redundancy of 5.3 (5.2) at 2.1 Å resolution reached 100.0% (100.0%) completeness with an $R_{\rm merge}$ of 0.047 (0.301) and an $I/\sigma(I)$ of 6.3 (2.5). The values in parentheses are for the highest resolution shell 2.19–2.10 Å. The volume per unit weight ($V_{\rm M}$) was 2.43 Å^3 Da^{-1} for one monomer per asymmetric unit, corresponding to 49.4% solvent content in the unit cell, a value within the range normally observed for protein crystals (Matthews, 1968).

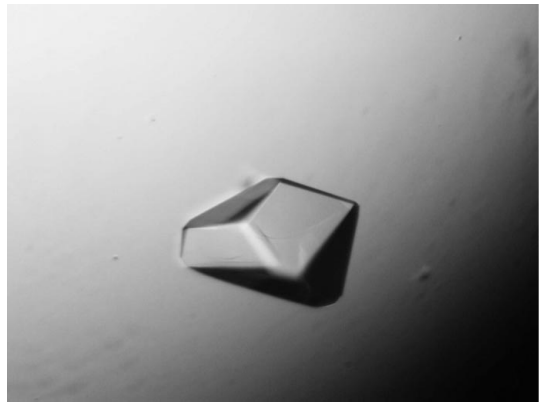

Figure 1
Photomicrograph of a gp44 crystal. The largest crystal dimension was approximately 0.8 mm.

References

Eiserling, F. A. & Black, L. W. (1994). *Molecular Biology of Bacteriophage T4*, edited by J. D. Karam, pp. 209–212. Washington, DC, USA: American Society for Microbiology.

Ghelardini, P., Paolozzi, L. & Liebart, J. C. (1980). *Nucleic Acids Res.* **8**, 3157–3173.

Giphart-Gassler, M., Wijffelman, C. & Reeve, J. (1981). *J. Mol. Biol.* **145**, 139–163.

Grundy, F. J. & Howe, M. M. (1985). *Virology*, **134**, 485–504.

Kabsch, W. (1988). *J. Appl. Cryst.* **21**, 916–924.

Kitazawa *et al.* (2005). Submitted.

Kostyuchenko, V. A., Leiman, P. G., Chipman, P. R., Kanamaru, S., van Raaij, M. J., Arisaka, F., Mesyanzhinov, V. V. & Rossmann, M. G. (2003). *Nature Struct. Biol.* **10**, 688–693.

Leiman, P. G., Kanamaru, S., Mesyanzhinov, V. V., Arisaka, F. & Rossmann, M. G. (2003). *Cell. Mol. Life Sci.* **60**, 2356–2370.

Leslie, A. G. W. (1991). *Crystallographic Computing 5*, edited by D. Moras, A. D. Podjarny & J. C. Thierry, pp. 50–61. Oxford University Press.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Morgan, G. J., Hatfull, G. F., Casjens, S. & Hendrix, R. W. (2002). *J. Mol. Biol.* **317**, 337–359.

O'Day, K., Schultz, D., Ericsen, W., Rawluk, L. & Howe, M. (1979). *Virology*, **93**, 320–328.