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Molecules in focus

The tail lysozyme complex of bacteriophage T4

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Abstract

The tail baseplate of bacteriophage T4 contains a structurally essential, three-domain protein encoded by gene 5 in which the middle domain possesses lysozyme activity. The gene 5 product (gp5) undergoes post-translational cleavage, allowing the resultant N-terminal domain (gp5*) to assemble into the baseplate as a trimer. The lysozyme activity of the undissociated cleaved gp5 is inhibited until infection has been initiated, when the C-terminal portion of the molecule is detached and the rest of the molecule dissociates into monomers. The 3D structure of the undissociated cleaved gp5, complexed with gp27 (another component of the baseplate), shows that it is a cell-puncturing device that functions to penetrate the outer cell membrane and to locally dissolve the periplasmic cell wall.

Keywords: Tail lysozyme complex; Bacteriophage T4; Genes 5 and 27

1. Introduction

Bacteriophage T4, a member of the *myoviridae* family of phages, consists of a head, a contractile tail and six long tailfibers. It has long been thought that the phage particle of bacteriophage T4 might possess lysozyme activity based on the fact that simultaneous adsorption of more than 50 phage particles cause lysis within a few minutes without waiting for phage growth, a phenomenon called “lysis from without”. This lysozyme activity was shown to be encoded by gene 5 [1] whose gene product was located in the phage tail [1,2]. The 42 kDa molecular weight of this tail lysozyme was found to be consistent with the molecular weight of gene product 5 (gp5) [3]. Later, Mosig et al. [4] sequenced gene 5 and found that the predicted amino acid sequence contains 575 amino acid residues which is about 20 kDa larger than the previously reported molecular weight of 42 kDa. The fate of the missing part, which was later shown to be the C-terminal region of the gp5 precursor, was not known until 1997 when Kanamaru et al. [5] demonstrated that the C-terminal domain remains as a structural component of the phage after post-translational cleavage. Kanamaru et al. [6] crystallized and determined the structure of gp5 complexed with the base plate component gp27 and showed it to be a cell-puncturing device.

2. Organization of the gene and the protein structure

The baseplate genes are located in two separate gene clusters, namely the gene cluster that encode

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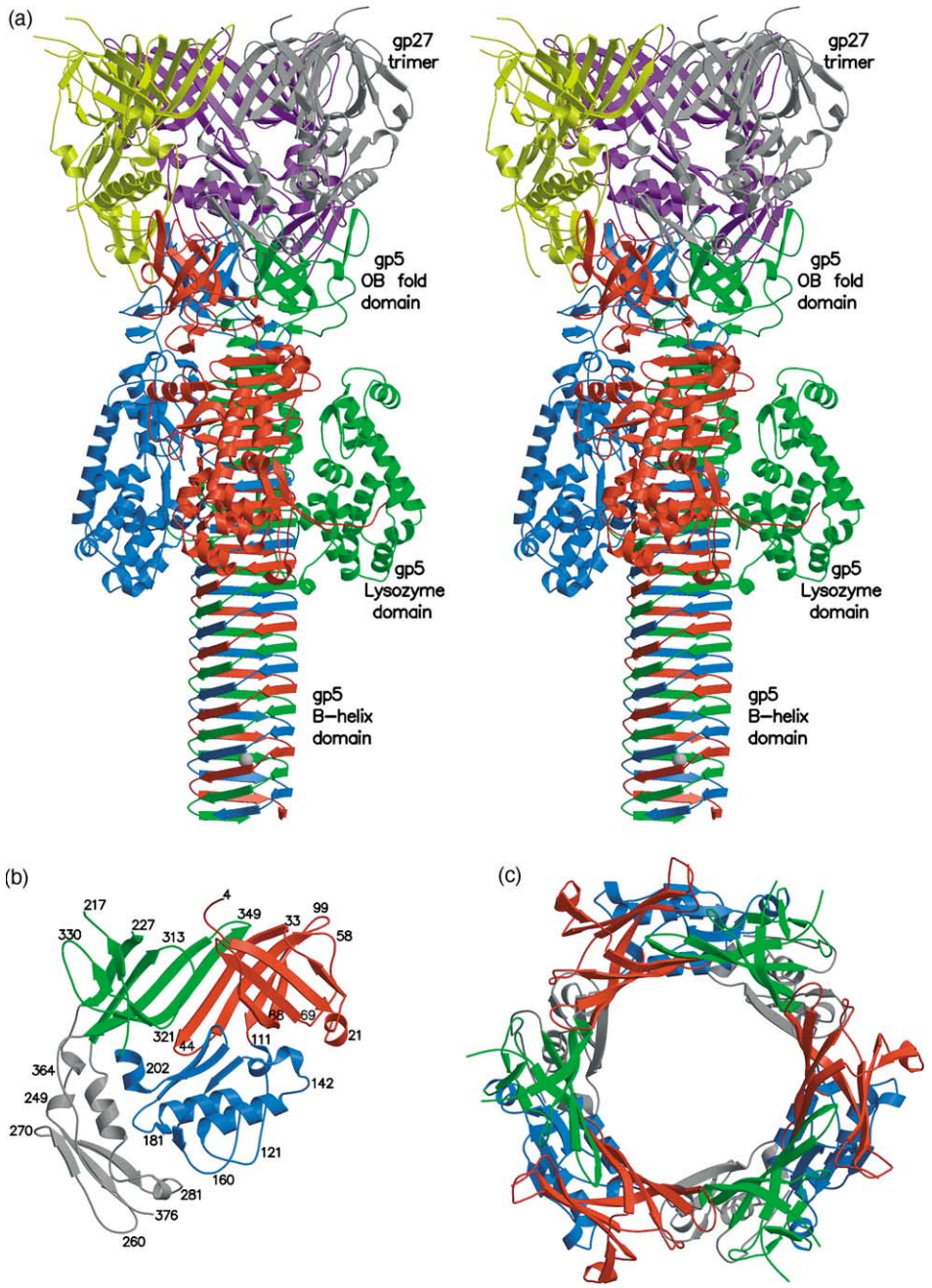

Fig. 1. Structure of the cell-puncturing device, $(gp5^*)_3(gp5C)_3(gp27)_3$ hetero-nonamer (PDB:1K28). (a) Ribbon stereo diagram of the complex. The three gp5 monomers are colored red, green and blue. The three gp27 monomers are colored yellow, grey and purple. (b) The gp27 monomer with its four domains differently colored. (c) Top view of the gp27 cylinder. The overall structure resembles a "torch", where the gp27 trimer and the N-terminal domain of gp5 form the cup, the lysozyme domain forms the supporting base of the cup, and the C-terminal domain is the "handle". It has been postulated that the handle functions as a needle to puncture the cell.

the wedge proteins and those that encode the hub proteins. However, although gp5 is a hub protein, gene 5 is located in the wedge cluster, and although gp25 is a wedge protein, its gene is located in the hub cluster. Gene 5 is transcribed by its upstream late promoter later than 8 to 10 min after infection has been intitated.It has been previously shown from sequence analysis that the precursor tail lysozyme, gp5, consists of 575 amino acids that can be divided into an N-terminal domain (gp5N), a lysozyme domain (gp5Lys) and a C-terminal domain (gp5C). The eight residue sequence VXGXXXX is tandemly repeated 12 times in the C-terminal domain. The lysozyme domain is homologous to "T4 lysozyme" which has 43% identical amino acids and is encoded by gene e of T4. It has been shown that the peptide link between Ser351 and Ala352 in gp5 is cleaved post-translationally, although the C-terminal domain (gp5C) stays associated with the remaining part of the baseplate until infection is initiated.

Sedimentation equilibrium has shown that, as long as the cleaved C-terminus is associated with the remaining part of the baseplate, the (gp5)(gp27) molecule forms a trimeric complex. Gp27 is a monomer in solution, but forms a 1:1 complex with monomeric gp5*, trimetic gp5 or $(gp5^*–gp5C)_3$ (Kanamaru et al., unpublished results). Thus, the baseplate contains a $(gp5^*)_3(gp5C)_3(gp27)_3$ hetero-nonamer.

The 3D structure of the hetero-nonamer has been solved to 2.9 Å resolution (Fig. 1). The overall shape of the hetero-nonamer is like a "torch", where the gp27 trimer and the N-terminal domain of gp5 form the cup, the lysozyme domain forms the supporting base of the cup, and the C-terminal domain is the "handle". The three domains of gp5 are connected by two linker sequences of 44 (linker 1) and 49 (linker 2) residues. The N-terminal domain has a five-stranded β-barrel oligonucleotide/oligosaccharide binding (OB)-fold [7]. The lysozyme domain is similar to T4 lysozyme, gp e, with the RMS deviation of 1.1 Å between equivalent C_α atoms [6]. The C-terminal domain forms a triangular prism with a length of 110 Å and a width of 28 Å. The N-terminal 46 residues in the gp5 C-terminal domain form a 5-stranded β-sheet. The gp5C domain forms a parallel β-helix with a triangular cross-section. The repeating VXGXXXXX sequences form the sides of the triangular prism with the glycine residues forming the corners of the prism. The β-helix is a very stable structure that is resistant to denaturation by SDS at room temperature.

3. Biological functions—assembly, disassembly and activation

At least two lysozymes are encoded by the phage T4 genome, namely gp e and gp5. The former is the "T4 lysozyme" and allows the progeny phage to be released from the cell. The latter functions to create a hole in the peptidoglycan layer to permit the tail tube to penetrate the cell wall and reach the inner membrane. The genomic DNA in the head is then transferred through the tube into cytoplasm, a process that might require an electrochemical potential difference across the inner membrane [8].

The C-terminal domain of gp5 plays an essential role in the assembly into a trimeric structure, which in turn is incorporated into the baseplate. The C-terminal domain is dissociated from the rest of the baseplate after infection of the host cell has been initiated (Fig. 2). At the same time, the rest of the molecule dissociates into three gp5*–gp27 hetero-dimers. These dissociate into the constituent monomers at low pH in the periplasm (Kanamaru et al., unpublished result) while the tail lysozyme is activated. It has been suggested that the OB-fold in the gp5 N-terminal domain might bind to the saccharide portion of the peptidoglycan layer preventing gp5* from diffusing throughout the peptidoglycan layer, confining it to the neighborhood of the adsorption point and preventing further harmful destruction of the peptidoglycan.

Unlike monomeric gp5 or C-terminal domain-depleted mature gp5*, the precursor tail lysozyme $(gp5)_3$ or the processed trimeric protein $(gp5^*)_3(gp5C)_3$ do not show lysozyme activity [5]. The structure of the (gp5)(gp27) complex has shown that the enzymatic activity is inhibited by the linker peptide 1 binding into the active site of the neighboring, three-fold related lysozyme domain. The substrate site will become available when the lysozyme dissociates from the gp5 C-terminal β-helix on entry into the periplasm.

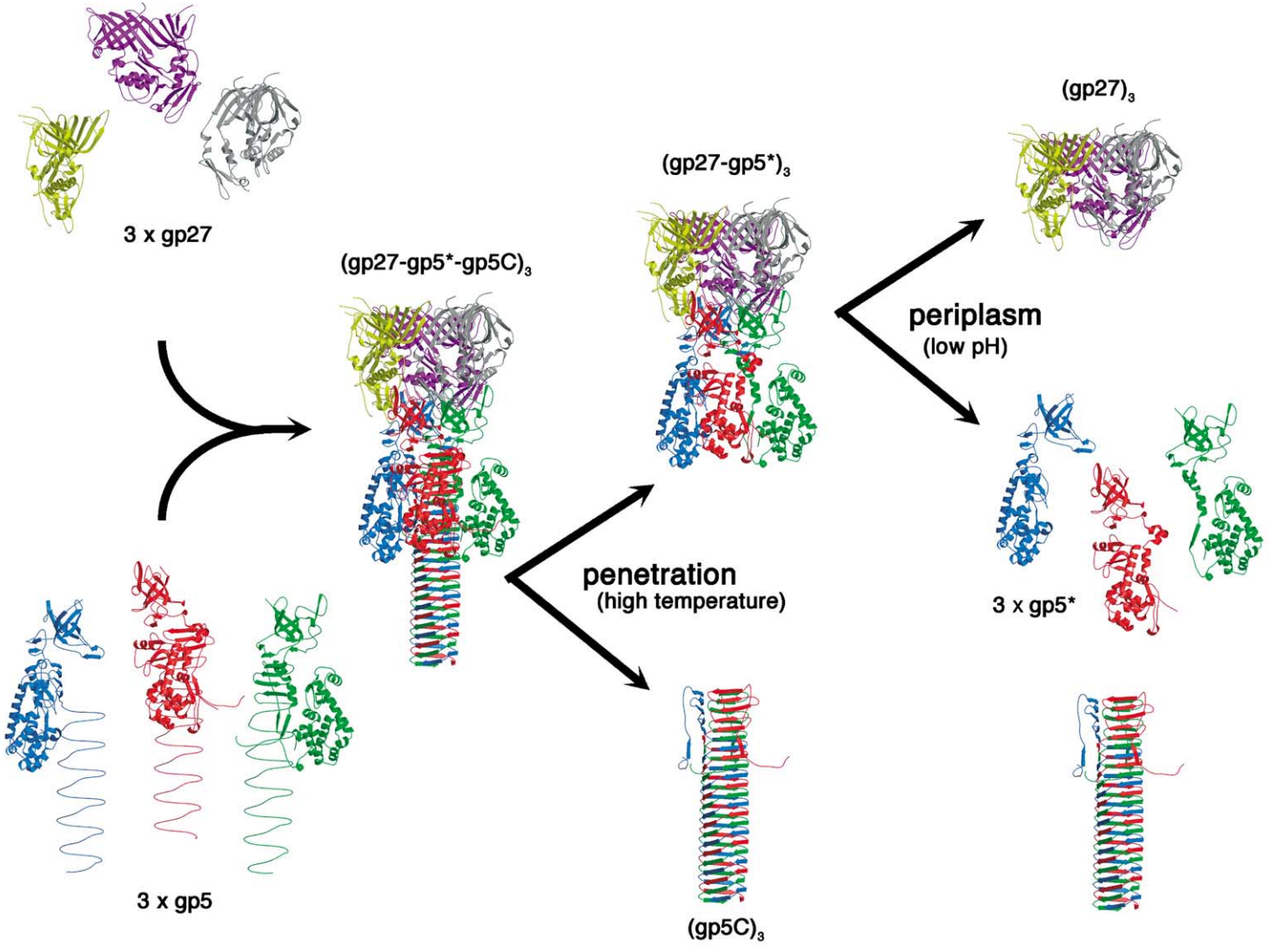

Fig. 2. Processing and assembly of gp5. After gp5 has been synthesized in the cell, it trimerizes to form the triple-stranded β-helix of the C-terminal domain and binds gp27. Gp5 is then cleaved between Ser351 and Ala352 by an unknown protease, although the C-terminal domain remains attached to the rest of the molecule and is incorporated into the baseplate together with gp27. In the infection process, presumably during penetration, the C-terminal domain, gp5C, is detached. The activated gp5* will be dissociated into monomers at low pH in the periplasm. The C-terminal domain can be detached in vitro by incubation at 37 °C for several hours.

4. Structure–function relationship based on mutations and variants

The discovery of the tail-associated lysozyme was based on the isolation of a mutant by Kao and McClain [1]. The mutation was later shown to be a replacement of glycine 322 by an aspartic acid [9]. Residue 322 in the lysozyme domain is in contact with the triple-stranded β-helix of the gp5 C-terminal domain. Presumably, the resultant steric hindrance as well as the charge repulsion makes the detachment of the C-terminal domain and lysozyme activation easier. Another temperature sensitive mutant, 5tsDH6318, that has a mutation in gene 5 has been isolated by Hall et al. [10]. This is a pseudo-revertant of gene 63 amber mutant. Gp63 is essential and facilitates the tailfiber attachment to the baseplate. The phage particle of this pseudo-revertant can efficiently attach the tailfibers without the aid of gp63. At non-permissive temperatures, this mutation inhibits tailfiber attachment. The mutation has been identified as Ala65Thr, whereas, Ala65 was replaced by Thr in the N-terminal domain of gp5. Although the phenotype cannot be readily explained by the structure, Ala65 is localized at the subunit interface between the N-terminal domains and the structural change by the mutation may be transmitted to gp9 which is the socket of the tailfibers so that the tailfiber attachment is facilitated [11,12].

The genomes of a number of T4-related phages are currently being sequenced. Among these is the RB49 phage which was found to have approximately 50% of the amino acids in gp5 identical to those of T4 (Namiki et al., unpublished result). This phage has an extra repeat of the well preserved C-terminal VXGXXXX sequence and, thus, has one-third turn longer β-helix. Some other T4-related phage appears to have a shorter β-helix.

Processing of gp5 is important for the later activation of the lysozyme domain. At this time, the enzyme responsible for the gp5 cleavage has not yet been identified. It is either an auto-catalytic or a host-enzyme cleavage. However, there is no structural evidence in favor of auto-catalysis. Site-directed mutagenesis of residues in the neighborhood of the cleavage site might elucidate the substrate specificity of the enzyme.

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