View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Virology 395 (2009) 312–317

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Characterization of tail sheath protein of giant bacteriophage φKZ *Pseudomonas aeruginosa*

Lidia P. Kurochkina [a,*], Anastasia A. Aksyuk [b], Maria Yu. Sachkova [a], Nina N. Sykilinda [a], Vadim V. Mesyanzhinov [a]

[a] *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, Moscow 117997, Russia*
[b] *Department of Biological Sciences, Purdue University, 915 West State Street, West Lafayette, IN 47907-2054, USA*

A R T I C L E I N F O

Article history:
Received 1 September 2009
Accepted 12 September 2009
Available online 12 October 2009

Keywords:
Bacteriophage φKZ
Bacteriophage T4
Pseudomonas aeruginosa
Tail sheath protein
Immunoelectron microscopy

A B S T R A C T

The tail sheath protein of giant bacteriophage φKZ *Pseudomonas aeruginosa* encoded by gene 29 was identified and its expression system was developed. Localization of the protein on the virion was confirmed by immunoelectron microscopy. Properties of gene product (gp) 29 were studied by electron microscopy, immunoblotting and limited trypsinolysis. Recombinant gp29 assembles into the regular tubular structures (polysheaths) of variable length. Trypsin digestion of gp29 within polysheaths or extended sheath of virion results in specific cleavage of the peptide bond between Arg135 and Asp136. However, this cleavage does not affect polymeric structure of polysheaths, sheaths and viral infectivity. Digestion by trypsin of the C-truncated gp29 mutant, lacking the ability to self-assemble, results in formation of a stable protease-resistant fragment. Although there is no sequence homology of φKZ proteins to proteins of other bacteriophages, some characteristic biochemical properties of gp29 revealed similarities to the tail sheath protein of bacteriophage T4.

© 2009 Elsevier Inc. All rights reserved.

Introduction

Bacteriophage φKZ efficiently infects *Pseudomonas aeruginosa* strains pathogenic to humans. Recently, it has been a subject of an extensive study, including genomic sequencing (280,334 bp), protein identification (Mesyanzhinov et al., 2002), cryo-electron microscopy (cryo-EM) and image reconstruction (Fokine et al., 2005, 2007). Interest in this bacteriophage and its gene products was partially generated by its potential use in phage therapy and other biotechnological applications (Mesyanzhinov et al., 2002; Miroshnikov et al., 2006; Fokine et al., 2007, 2008; Briers et al., 2007; Paradis-Bleau et al., 2007).

According to its morphology, phage φKZ belongs to the *Myoviridae* family (Krylov and Zhazykov, 1978). However, it presents a small group of very large myoviruses that differ from other tailed phages and form a novel genus, named "phiKZ-like viruses" after its type virus *P. aeruginosa* phage φKZ (Krylov et al., 2007). These virions consist of an unusually large head of about 1050–1400 Å in diameter and a 1600–2000 Å long tail. Phage heads contain an internal complex of proteins, an inner body, and a large genome of 210–316 kbp that has no sequence homology to any myovirus genome known (Hertveldt et al., 2005; Krylov et al., 2007; Thomas et al., 2008). However, some of these phages share taxonomically relevant similarity at the proteome level (Lecoutere et al., 2009).

Similarly to all myoviruses bacteriophage φKZ employs a specialized tube-like organelle, called a tail, for recognition and attachment to a host cell, penetration of the cell envelope, and viral DNA translocation into the host cytoplasm (Ackermann, 2003). A tail facilitates high viral infection efficiency and consists of a long co-cylindrical part and a baseplate attached to its distal end. During infection an outer sheath irreversibly contracts, playing a role of a molecular "muscle". As a result of sheath contraction, an inner tail tube penetrates the outer membrane of the cell, creating a channel for DNA ejection from the capsid into the host cell.

Currently, the most genetically, biochemically and structurally studied member of the *Myoviridae* family is bacteriophage T4 that infects *Escherichia coli* (Leiman et al., 2003). The T4 tail sheath is composed of 138 copies of gp18 (71.3 kDa) and the tail tube is thought to have the same number of gp19 (18.5 kDa) molecules (Leiman et al., 2004). The tail structures before and after sheath contraction were previously determined by cryo-EM and image reconstruction methods to 15 and 17 Å resolution, respectively (Kostyuchenko et al., 2005; Leiman et al., 2004). A crystal structure of about three quarters of the tail sheath protein of phage T4, gp18, has been recently obtained (Aksyuk et al., 2009).

The φKZ tail organization is similar to that of phage T4, but its tail is much longer (~2000 Å vs. ~1200 Å). The number of subunits in the φKZ sheath is estimated to be 264 (Fokine et al., 2007), whereas the T4 tail sheath contains 138 subunits. Gene products that encode major φKZ tail proteins have not been previously identified partially due to the lack of sequence homology to proteins of T4 or other *Myoviridae* phages.

* Corresponding author. Fax: +7 495 336 6022.
E-mail address: lpk@ibch.ru (L.P. Kurochkina).

0042-6822/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2009.09.015

However, the recent cryo-EM structure of the φKZ tail in the extended conformation to 28 Å resolution showed that the φKZ tail sheath has similar helical parameters to those of the T4 tail sheath and similar relative changes in the sheath dimensions upon infection were proposed (Fokine et al., 2007). This suggests that two phages have similar mechanisms of the tail sheath contraction despite no apparent sequence homology between the tail proteins, different tail lengths and hosts.

In the present investigation, φKZ tail sheath protein was identified. Using serum against recombinant protein and secondary gold-conjugated antibodies, its localization in the φKZ virion was confirmed by immunoelectron microscopy. Additionally, the recombinant tail sheath protein of φKZ was characterized biochemically, suggesting some differences and similarities to the bacteriophage T4 tail sheath protein, gp18.

Results and discussion

Identification of φKZ tail sheath protein

Structural proteomes of phage φKZ and its tailless mutant have been recently determined by mass spectrometry on total virion particle proteins (Lecoutere et al., 2009). Comparative analysis of these proteomes revealed several proteins most likely located in the tail. It is known that sheath protein is present in multiple copies in the virion, so gene product 29, major component of tail structural proteins, was chosen as a possible candidate. Therefore, a vector for expression of gene 29 in *E. coli* cells was designed in order to obtain serum against recombinant protein and subsequently localize protein on virion particle by immunoelectron microscopy using anti-gp29 serum and secondary gold-conjugated antibodies.

Expression and purification of recombinant protein

A plasmid vector for expression of gene 29 in *E. coli* cells was designed. A soluble protein with a molecular mass of 77.6 kDa was produced in cells at lowered temperature. Using EM, we found that overexpressed recombinant gp29 assembles into regular tubular structures, polysheaths, of variable length similar to the recombinant T4 tail sheath protein, gp18 (Fig. 1). To compare the images of polysheaths, recombinant φKZ gp29 and T4 gp18 were mixed and examined under the electron microscope (Fig. 1C). Polysheaths formed by gp29 have approximately the same diameter as those of gp18. However, gp29 tubular structures are characterized by a better defined internal channel than those of gp18. Probably, it is caused by less compact packing of gp29 subunits in the polysheaths that facilitates the stain penetration into the internal channel.

Polysheaths from the cell lysate were precipitated by addition of ammonium sulfate with subsequent ultracentrifugation followed by purification on a hydroxyapatite column. Electrophoretically pure protein was used for immunization of mice. The titer of antiserum against gp29 determined by ELISA was at least 1:50,000. Serum specificity was analysed by Western blot using the recombinant protein and φKZ structural proteins after SDS-PAGE (Figs. 2B and 3B). It was shown that polyclonal antibodies specifically bind to the recombinant protein as well as phage gp29 and do not recognize other φKZ structural proteins. These antibodies were subsequently used in immunoelectron microscopy.

Localization of gp29 on φKZ virions

In order to directly localize gp29 on the virus particle, immunoelectron microscopy was conducted using the purified φKZ sample which contained intact virions as well as virions with contracted

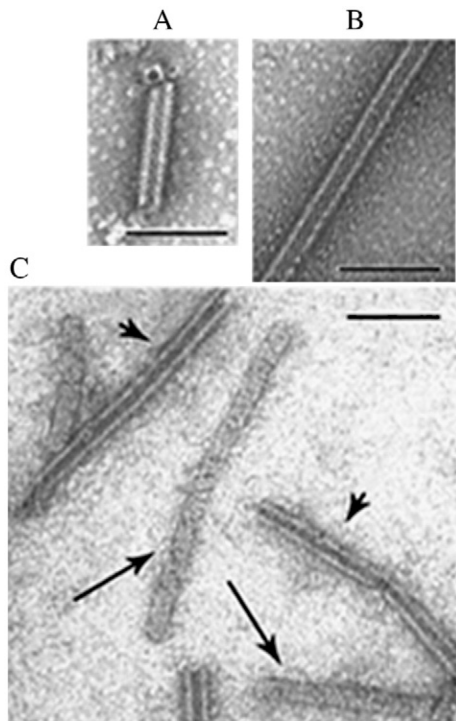

Fig. 1. EM images of φKZ gp29 polysheaths before (A) and after (B) proteolysis. Mixture of T4 gp18 and φKZ gp29 polysheaths (C). Long-tailed arrows indicate gp18 polysheaths. Short-tailed arrows indicate gp29 polysheaths. Specimens were contrasted with 1% uranyl acetate. Scale bar, 100 nm.

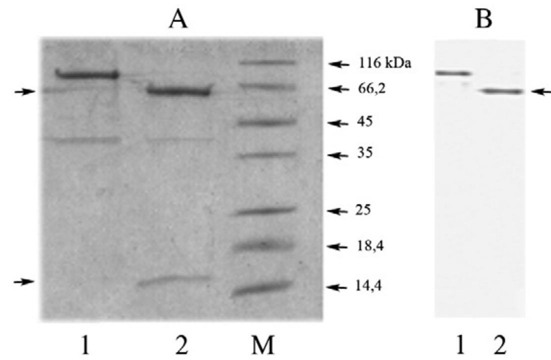

Fig. 2. SDS-PAGE of recombinant gp29 before (1) and after (2) proteolysis. (A) Coomassie staining. (B) Staining with serum against recombinant gp29 after Western blotting of proteins from the gel onto a nitrocellulose membrane. Lane M, protein marker. Arrows indicate the products of trypsin cleavage.

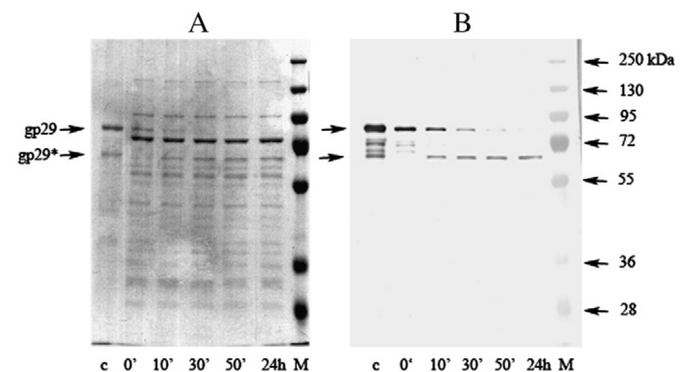

Fig. 3. Proteolysis of gp29 within phage φKZ. Digestion time is indicated under the lanes. Lane c, recombinant gp29, lane M, protein marker. (A) Coomassie-staining. (B) Staining with serum against recombinant gp29 after Western blotting of proteins from the gel onto a nitrocellulose membrane. Arrows indicate full-length gp29 and its 60 kDa fragment after trypsin cleavage (gp29*).

A B

Fig. 4. Localization of gp29 on φKZ virions by immunoelectron microscopy. The purified φKZ virions were incubated with preimmune mouse serum (A) or serum against gp29 (B) followed by incubation with gold-labeled anti-mouse IgG. Specimens were contrasted with 1% uranyl acetate. Scale bar, 100 nm.

sheath. First, the phage sample was incubated with primary antibodies, pre-immune serum (control) or serum against gp29 (sample), and then with secondary antibodies conjugated to gold particles (10 nm). No gold particles were observed in the control (Fig. 4A), whereas most of φKZ virions in the sample were labeled with gold particles (Fig. 4B). The binding was observed with the tail sheaths in both the extended and contracted forms. One, two or three gold particles were found for each labeled virion, indicating that the φKZ tail sheath is composed of gp29 subunits.

φKZ gp29 primary structure analysis

Based on alignment of amino acid sequences using the ClustalW2 program, the φKZ tail sheath protein, gp29 (695 aa), shares only 11% identity with T4 tail sheath protein, gp18 (659 aa) (Fig. 5). No conserved domains or motifs were observed in the primary structure of two proteins, excepting a short segment (25 aa) in the N-termini with 40% identity of amino acid sequences. Nevertheless, it is possible to assert that tail sheath proteins have no homology at the level of amino acid sequences.

```
18-T4           --------MTLLSPGIELKETTVQSTVVNNSTGTAALAGKFQWGPAFQIKQVTNEVDLVN
29-φKZ          MAYYNAVPRVVFNGIRDRSRRPLIRPDITFAQHCPLLRLFTETGPTETTYVGDSDDGFAS
                         .::.   : .. .:  . :. :   . *    : **:       .: .:..

18-T4           TFGQPTAETADYFMS-----AMNFLQYGNDLRVVRAVDRDTAKNSSPIAGNIDYTISTPG
29-φKZ          IYGQASLDPRSKFFNTQSLLALNLLGRGNGFYVKRLRPEDAANPSRLIVAIEIVEDEIPL
                 :**.: :. . *:.     *:*:*  **.: * *   .*:*: *  *..      . *

18-T4           SNYAVGDKITVKYVSDDIETEGKITEVDADGKIKKINIPTGKNYAKAKEVGEYPTLGSNW
29-φKZ          TIRRLSGFNYPNSVRDIGNAPVPTTDKVDGLKARIILIEDNTSEVGTQRVLPGTLVSDKD
                :   :..    : * *  ::    *:   . * : * *  ... . ::.*   . :..:

18-T4           TAEISSSSSGLAAVITLGKIITDSGILLAEIENAEAAMTAVDFQANLKKYGIPGVVALYP
29-φKZ          GSQSLVYPLFEAPVSFFGKLGDSNGMRVWSTTTADIEEFDEAAMAKFKTRQFRIQLIEKP
                 ::    .   *.*  :**:  ..*: : .  .*:         *::*.  :   :   *

18-T4           GELGDKIEIEIVSKADYAKG-----------------ASALLPIYPGGGTRASTAKAVFG
29-φKZ          EVGTSPVIVKTADQQDYLNITFDKGVYSDMYNADLYVGDVLVDSYSDDGVVSGLSPLYSP
                    . : :: ..: ** :                  ...*:  *...*. :. :

18-T4           YGPQTDSQYAIIVRRNDAIVQSVVLSTKRGEKDIYDSNIYIDDFFAKGGSEYIFATAQNW
29-φKZ          FSQFYVYHENIDLVRQMIYDTEMRVNPAAAAHTTAPGEIDFLTFLAVDGDPYQGIQVLGP
                :.     :  * : *:     .: :..  . :    .:* :  *:* .*. *    . .

18-T4           PEGFSGILTLSGGLSSNAEVTAGDLMEAWDFFADRESVDVQLFIAGSCAGESLETASTVQ
29-φKZ          LDGG-ITLGKDGNIYASGGTDGTTDLEEYAKLVDIENINFGKLNDRYNN--IAEYQFGVL
                 :*    *  .*.: :.. . .   :* :  :.* *.::.  :          *    *

18-T4           KHVVSIGDARQDCLVLCSPPRETVVGIPVTRAVDNLVNWRTAAGSYTDNNFNISSTYAAI
29-φKZ          YDTGLPMESKYRAMRVLSARRDLQYFFTTFVETDSRLPDEATELSRVQQIITRLKAFPES
                 ..    :::  .: : *. *:    :..   .*. :  .::  * .:: :.  .::.

18-T4           DGNHKYQYDKYNDVNRWVPLAADIAGLCARTDNVSQTWMSPAGYNRGQILNVIKLAIETR
29-φKZ          TLYGTGVCRAMIVMQSGKLMDGTYRKYVPQLLDVAMSWARYAGAGTGNLVPGMEMDVSPN
                    .        ::    : .      .:  :*: :*   ** . *:::  ::: :...

18-T4           ------QAQRDRLYQEAINPVTGTGGDGYVLYGDKTATSVPSPFDRIN--VRRLFNMLKT
29-φKZ          NRVTFVKDLNVKFFDDRVRAQAWANGATWSQSYDHRSSYYPCLRSVMLDDTSVLLSPITV
                      :  . ::::: :.. : :.*  :    *: ::  *.  . :   .  *:. :..

18-T4           NIGRSSKYRLFELNNAFTRSSFRTETAQYLQGNK----ALGGIYEYRVVCDTTNNTPSVI
29-φKZ          NICCVLIRLIHKVHAQFSGNATLTPEQLVERCDEYILDLVRDMFGTRVNIIPRTEITPID
                **       :.:::  *: .:  *      : ::     : .::  **   . .: ..:

18-T4           DRNEFVATFYIQPARSINYITLNFVATATGADFDELTGLAG
29-φKZ          ANNGTSWTCNVTVEANNPRTTLNFNLETVRIETPPAQQ---
                 .*    *  :    .    ****   :.  :
```

Fig. 5. Alignment of amino acid sequences tail sheath proteins (T4 gp18 and φKZ gp29) using ClustalW2 program. Identical amino acid residues were indicated with (*), conserved substitutions were indicated with (:), semiconserved substitutions were indicated with (.). Fragments with high homology and gp29 cleavage site were shaded with grey.

Proteolysis of recombinant gp29

As was previously shown, the T4 tail sheath protein is extremely resistant to proteolysis in the assembled form of either extended or contracted sheaths (Arisaka et al., 1990). A test for proteolysis with trypsin was used for structural studies of recombinant gp29. When purified gp29 polysheaths were incubated with trypsin, the protein was cleaved into two fragments, as indicated by disappearance of the full-length protein band (Fig. 2A, lane 1) and appearance of two fragments with molecular weights of about 60 and 15 kDa (Fig. 2A, lane 2) on an SDS-PAGE. As was shown by N-terminal amino acid sequencing, trypsin digestion of gp29 proceeds specifically and results in the cleavage of peptide bond between Arg135 and Asp136. Analysis of the sample by EM showed that protein cleavage does not affect the tubular structure of the polysheaths (Fig. 1B) and their stability. Furthermore, it was found that intact polysheaths are formed by the full-sized protein with the N-terminal sequence of Ala-Tyr-Tyr-Asn-Ala, whereas polysheaths after trypsin treatment are formed by two polypeptides in equimolar ratio, which correspond to Arg135–Asp136 cleavage products with different N-terminal sequences. One of them has the gp29 N-terminal sequence, whereas the other starts with Asp-Ile-Gly-Asn-Ala, suggesting both protein fragments remained a part of the polysheath structure. Thus, the trypsin cleavage site (Arg135–Asp136) is most likely located on a polysheath surface. In bacteriophage T4 the most accessible domain of the sheath protein consists of residues 98–188 (Aksyuk et al., 2009), suggesting a similar domain architecture.

Proteolysis of gp29 within φKZ virion

We have studied gp29 resistance to proteolysis within the extended sheath in the intact phage particles. Products of gp29 degradation were visualized by staining with Coomassie Brilliant Blue and serum against recombinant gp29 in immunoblotting after SDS-PAGE of φKZ structural proteins. The results show that trypsin digestion of the purified phage results in gradual disappearance of the gp29 full-sized band and gives rise to the band corresponding to the 60 kDa fragment similar to N-truncated protein detected after trypsinolysis of recombinant gp29 (Fig. 3A, lanes 3–6). Immunostaining confirmed that this band corresponds to the tail sheath protein fragment (Fig. 3B, lanes 3–6). The low-molecular band corresponding to another tryptic 15-kDa fragment is well detected by Coomassie-staining only in trypsinized sample of pure polysheaths (Fig. 2A, lane 2), but not detected in the phage treated with trypsin (data not shown). It was not visualized by immunostaining in either phage (data not shown) or recombinant gp29 (Fig. 2B) both subjected to proteolysis. No apparent difference of the sheath and/or the phage morphology was detected by EM in the negatively stained sample of trypsinized φKZ (data not shown). Additionally, the titer of infectious virions after φKZ trypsinolysis resulting in gp29 cleavage within the sheath remained the same as in the wild type virion (about 7×10^{10} plaque forming units per ml). The results suggest that in the intact virion gp29 region sensitive to proteolysis is also located on sheath surface, whereas the intra- and intermolecular interactions providing functional properties of protein do not change after its cleavage. It should be noticed that gp29 cleavage can occur during storage of phage suspension due to endogenous proteolysis and result in disappearance of the full-length protein band in the gel after SDS-PAGE.

Proteolysis of gp29 in monomeric form

Earlier we have found that C-terminus is responsible for T4 gp18 self-assembly into polysheaths (Poglazov et al., 1999). To elucidate the role of the C-terminus in protein polymerization, the plasmid for expression of gp29 deletion mutant was designed. The C-terminally truncated fragment shortened by 181 residues (CΔ181) was produced by *E. coli* cells in soluble form. However, the removal of a quarter of the polypeptide chain from the C-terminus affects the polymerization properties of the protein, preventing self-assembly into polysheath-related structures. Furthermore, CΔ181 fragment is sensitive to proteolysis. Limited trypsinolysis of CΔ181 results in the trypsin-resistant fragment with molecular weight of about 35 kDa. Results of CΔ181 trypsinolysis are shown in Fig. 6. It was found that the trypsin-resistant fragment is stable in solution and does not degrade further during long incubation with trypsin, suggesting a compact 3D structure. Thus, φKZ gp29 is readily cleaved in monomeric form resulting in the formation of protease-resistant fragment having approximately the same molecular weight as a similar fragment of T4 gp18 (Efimov et al., 2002).

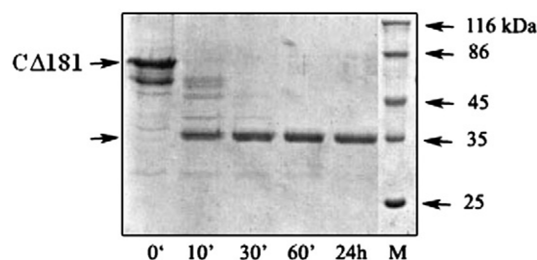

Fig. 6. Limited trypsinolysis of C-truncated gp29 fragment shortened by 181 amino acid residues (CΔ181). Trypsinolysis time was indicated under the lanes. Lane M, protein marker. Arrows indicate CΔ181 and trypsin-resistant fragment.

In the absence of apparent sequence homology, the tail sheath proteins performing similar functions in the phages φKZ and T4 have approximately the same size and biochemical properties. For comparison major characteristics of T4 gp18 and φKZ gp29 are summarized in the Table 1. The C-terminal part of their molecules is responsible for protein polymerization. Full-length proteins assembled into polysheaths maintain their polymeric structure during proteolysis. Their C-truncated mutants in monomeric form are sensitive to proteolysis resulting in stable protease-resistant fragments of approximately identical molecular weights. These data point to similar structural organization of the tail sheath proteins. Furthermore, similar structural elements are necessary for sheath proteins from different phages for the protein assembly (polymerization) around the tail tube as well as for sheath contraction during infection. Considering similarity in tail sheath organization of phages T4 and φKZ, we can suggest that genes coding sheath proteins have been formed as a result of divergence homologous genes and their gene products have similar 3D structures.

Genes passed through divergent evolution and lacked similarity of amino acid sequences, but maintained similar 3D structures, are widespread among tailed phages (Jiang et al., 2003; Benson et al., 2004; Fokine et al., 2005). Structural similarities between phage and viral capsid proteins point to a common evolutionary origin for the heads of tailed dsDNA phage in bacteria and archea (Fokine et al., 2005; Morais et al., 2005), and eukaryotic viruses of the Herpes family

Table 1
Major characteristics of the φKZ and T4 tail sheath proteins.

Phage	Tail sheath protein	
	φKZ	T4
Gene number	29	18
Amino acid residue number	695	659
Molecular weight, kDa	77,641	71,331
Number of protomers in the tail	264	138
Protease susceptibility in polysheaths/monomeric form	+/+	-/+
Molecular weight of trypsin-resistant fragment, kDa	35	30

(Duda et al., 2006). An evolutionary connection between two distinct types of tails – non-contracted and contracted – based on structures of two proteins forming tail tubes, and their similarity to Hcp1, a component of the bacterial type VI secretion system, has been recently established (Pell et al., 2009). Similar comparative studies for tail sheath proteins for phages with contracted tails have not yet been possible, because currently only one structure of phage T4 tail sheath protein has been determined (Aksyuk et al., 2009). Further crystallographic studies of gp29 from φKZ are essential to compare the structures of sheath proteins from two *Myoviridae* phages.

Materials and methods

Cloning of gene 29

Gene 29 was amplified from the φKZ genomic DNA by PCR using oligonucleotide primers containing the point base substitutions to generate suitable cloning sites. We used 5′-AATCAAAACCATGGCA-TATTACAACGC-3′ (Nco I restriction site is underlined) as forward primer, and 5′-TCTGAAAGGGATCCACTCATTGCTGAGCA-3′ (Bam HI restriction site is underlined) as reverse primer, respectively. The reverse pimer 5′-TTAGGATCCTCATGACATAGCTACGTCTAAAAG-3′ (Bam HI restriction site is underlined) was used for amplification of gene 29 fragment truncated from 3′-end. The amplicons were digested with Nco I and Bam HI and cloned in Nco I/Bam HI digested pET-23d(+) vector (Novagene, USA).

Protein expression, purification and antibody preparation

The protein expression was carried out in *E. coli* strain BL21(DE3) as described previously (Studier et al., 1990) at 25 °C.

To purify soluble gp29, the cell pellet from 200 ml of culture was resuspended in 3 ml of TE buffer (0.02 M Tris–HCl (pH 7.8), 0.001 M EDTA), sonicated for 2–3 min using Virsonic 100 disintegrator (Virtis, USA) and centrifuged at 12,000 rpm (Eppendorf, Germany) for 10 min to remove the debris. Nucleic acids were precipitated by addition of the streptomycin sulfate solution to a final concentration of 3% (w/v). The recombinant protein was precipitated from the supernatant by addition of saturated ammonium sulfate to a final concentration of 25–30%. The protein precipitate was pelleted by centrifugation as above, and dissolved in 2 ml of TE buffer. Polysheaths were pelleted on TL-100 ultracentrifuge (Beckman, USA) at 75,000 rpm for 3.5 h, resuspended in TE buffer and analysed by SDS-PAGE (Laemmli, 1970). The protein was additionally purified on a hydroxyapatite column (Bio-Rad, USA) equilibrated with 0.01 M sodium phosphate (pH 7.8). The protein was eluted by a step gradient from 0.01 to 0.3 M sodium phosphate (pH 7.8). Gp29 was eluted at 0.025–0.05 M of sodium phosphate and analyzed by SDS-PAGE.

To produce antibodies, female mice BALB/C (14–17 g) were immunized intraperitoneally with a suspension containing 50–100 μg of pure gp29 in 0.15 M NaCl and 0.2 ml complete Freund's adjuvant. Two booster injections were given with the same protein preparation in incomplete Freund's adjuvant with 2- to 3-week intervals. Serum was recovered 7 days after the third immunizations.

Western blot

After separation by SDS-PAGE proteins were transferred by electroelution from the gel onto a nitrocellulose (Bio-Rad) or Immobilon-P membrane (Millipore) in electroblotting buffer (0.1 M Tris, 0.1 M boric acid, 0.01 M EDTA) at 200 mA for 60 min. Antigen was detected with antiserum followed by rabbit anti-mouse HRP conjugated antibody (Sigma, USA). Antibodies were diluted in 3% milk/PBS/0.05% Tween-20 and incubations were for 1 h at room temperature with gentle rocking. Blots were developed by 0.02% 3′,3′-diaminobenzamide (Sigma).

Phage propagation and purification

Psedomonas aeruginosa PAO1 cells were grown in 50 ml of 2xTY medium at 37 °C to a density of 2×10^8 cells/ml and infected by bacteriophage φKZ suspension with multiplicity of 1 and incubated until cellular lysis. The cells were additionally lysed by adding of chloroform to a final concentration of 0.5% (v/v) for 4 h at 4 °C. Phage suspension was incubated with DNase I (final concentration 20 μg/ml) for 1 h at room temperature. Cell debris was removed by centrifugation at 3500 rpm for 20 min (Megafuge 2.0 R, Heraeus Instruments, Germany). The phage was concentrated by centrifugation at 15,500 rpm for 1 h at 4 °C (Sorvall RS 5 Plus, USA). The phage pellet was resuspended in 2 ml of medium and purified by a CsCl step gradient centrifugation (Dickson, 1974). The band with the highest opalescence was collected and dialysed overnight against 0.01 M Tris–HCl (pH 7.5), 0.01 M $MgSO_4$, 0.15 M NaCl at 4 °C. The titer of wild type phage was about 10^{11} plaque forming units/ml.

Immunoelectron microscopy

The sample of phage particles was incubated with antiserum against the recombinant protein (or pre-immune antiserum as a control) in 1% BSA/PBS for 1 h at room temperature. Unbound antibodies were removed by centrifugation of phage particles on TL-100 ultracentrifuge at 30,000 rpm for 30 min. The pellet was subjected to three cycles of resuspending/centrifuging (once in PBS/0.02% Tween-20 and twice in PBS) before application of the mixture to farmvar film on 200-mesh grid. Then grid was incubated on a drop of goat anti-mouse gold (10 nm) conjugated antibodies (Sigma) in 0.1% BSA/PBS for 15 min and rinsed with water. The specimen was then contrasted with 1% uranyl acetate and observed in a Jeol 100 CXII electron microscope (Japan).

Limited trypsinolysis of gp29

Trypsin (TPCK-treated) was added into the protein solution or phage suspension in 0.025 M Tris–HCl (pH 7.8) in the weight ratio of 1:100 and incubated at 25 °C. The reaction was stopped by adding of phenylmethylsulfonyl fluoride to a final concentration of 0.001 M.

Edman degradation

Individual protein bands after separation by SDS-PAGE followed by transferring onto immobilon membrane were subjected to the N-sequencing. Recombinant gp29 (untreated and treated) after ultracentrifugation in solution were also subjected to the N-sequencing. Not less than five amino acid residues were determined in each case and they were completely identical with gp29 primary structure. The N-terminal amino acid sequence was determined with a Procise cLC 491 Protein Sequencing System (PE Applied Biosystems, USA). Phenylthiohydantoin derivatives of the amino acids were identified by a 120A PTH Analyzer (PE Applied Biosystems).

Acknowledgments

This research was supported by grant from the Russian Fund for Basic Research (08-04-01260) to L.P.K. We are grateful to Dr. V.A. Kadykov for help with the electron microscope.

References

Ackermann, H.W., 2003. Bacteriophage observations and evolution. Res. Microbiol. 154, 245–251.

Aksyuk, A.A., Leiman, P.G., Kurochkina, L.P., Shneider, M.M., Kostyuchenko, V.A., Mesyanzhinov, V.V., Rossmann, M.G., 2009. The tail sheath structure of bacteriophage T4: a molecular machine for infecting bacteria. EMBO J. 28, 821–829.

Arisaka, F., Takeda, S., Funane, K., Nashijima, N., Ishii, S., 1990. Structural studies of the contractile tail sheath protein of bacteriophage T4. Structural analyses of the tail sheath protein, gp18, by limited proteolysis, immunoblotting, and immunoelectron microscopy. Biochemistry 29, 5057–5062.

Benson, S.D., Bamford, J.K.H., Bamford, D.H., Burnett, R.M., 2004. Does common architecture hypothesis reveal a viral lineage spanning all three domains of life? Mol. Cell 16, 673–685.

Briers, Y., Volckaert, G., Cornelissen, A., Lagaert, S., Michiels, C.W., Hertveldt, K., Lavigne, R., 2007. Muralytic activity and modular structure of the endolysins of *Pseudomonas aeruginosa* bacteriophages φKZ and EL. Mol. Microbiol. 65, 1334–1344.

Dickson, R.C., 1974. Protein composition of the tail and contracted sheath of bacteriophage T4. Virology 59, 123–138.

Duda, R.L., Hendrix, R.W., Huang, W.M., Conway, J.F., 2006. Shared architecture of bacteriophage SPO1 and herpesvirus capsids. Curr. Biol. 16, R11–R13.

Efimov, A.V., Kurochkina, L.P., Mesyanzhinov, V.V., 2002. Engineering of bacteriophage T4 tail sheath protein. Biochemistry (Moscow) 67, 1366–1370.

Fokine, A., Kostyuchenko, V.A., Efimov, A.V., Kurochkina, L.P., Sykilinda, N.N., Robben, J., Volckaert, G., Hoenger, A., Chipman, P.R., Battisti, A.J., Rossmann, M.G., Mesyanzhinov, V.V., 2005. A three-dimensional cryo-electron microscopy structure of the bacteriophage phiKZ head. J. Mol. Biol. 352, 117–124.

Fokine, A., Battisti, A.J., Bowman, V.D., Efimov, A.V., Kurochkina, L.P., Chipman, P.R., Mesyanzhinov, V.V., Rossmann, M.G., 2007. Cryo-EM study of the *Pseudomonas* bacteriophage φKZ. Structure 15, 1099–1104.

Fokine, A., Miroshnikov, K.A., Shneider, M.M., Mesyanzhinov, V.V., Rossmann, M.G., 2008. Structure of the bacteriophage phiKZ lytic transglycosylase gp144. J. Biol. Chem. 283, 7242–7250.

Hertveldt, K., Lavigne, R., Pleteneva, E., Sernova, N., Kurochkina, L., Korchevskii, R., Robben, J., Mesyanzhinov, V., Krylov, V.N., Volckaert, G., 2005. Genome comparison of *Pseudomonas aeruginosa* large phages. J. Mol. Biol. 354, 536–545.

Jiang, W., Li, Z., Zhang, Z., Baker, M.L., Previlige, P.E., Chiu, W., 2003. Coat protein fold and maturation transition of bacteriophage P22 seen at subnanomolecular resolutions. Nature Struct. Biology 10, 131–135.

Kostyuchenko, V.A., Chipman, P.R., Leiman, P.G., Arisaka, F., Mesyanzhinov, V.V., Rossmann, M.G., 2005. The tail structure of bacteriophage T4 and its mechanism of contraction. Nat. Struct. Mol. Biol. 12, 810–813 M.G.

Krylov, V.N., Zhazykov, I.Z., 1978. Pseudomonas bakteriophage φKZ-possible model for studying the genetic control of morphogenesis. Genetika 14, 678–685 (in Russian).

Krylov, V.N., Dela Cruz, D.M., Hertveldt, K., Ackermann, H.W., 2007. "phiKZ-like viruses", a proposed new genus of myovirus bacteriophages. Arch. Virol. 152, 1955–1959.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature 227, 680–685.

Lecoutere, E., Ceyssens, P.J., Miroshnikov, K.A., Mesyanzhinov, V.V., Krylov, V.N., Noben, J.P., Robben, J., Hertveldt, K., Volckaert, G., Lavigne, R., 2009. Identification and comparative analysis of the structural proteomes of phiKZ and EL, two giant *Pseudomonas aeruginosa* bacteriophages. Proteomics 9, 3215–3219.

Leiman, P.G., Kanamaru, S., Mesyanzhinov, V.V., Arisaka, F., Rossmann, M.G., 2003. Structure and morphogenesis of bacteriophage T4. Cell. Mol. Life Sci. 60, 2356–2370.

Leiman, P.G., Chipman, P.R., Kostyuchenko, V.A., Mesyanzhinov, V.V., Rossmann, M.G., 2004. Three-dimensional rearrangement of proteins in the tail of bacteriophage T4 on infection of its host. Cell 118, 419–429.

Mesyanzhinov, V.V., Robben, J., Grymonprez, B., Kostyuchenko, V.A., Bourkaltseva, M.V., Sykilinda, N.N., Krylov, V.V., Volckaert, G., 2002. The genome of bacteriophage φKZ of *Pseudomonas aeruginosa*. J. Mol. Biol. 317, 1–19.

Miroshnikov, K.A., Faizullina, N.M., Sykilinda, N.N., Mesyanzhinov, V.V., 2006. Properties of the endolytic transglycosylase encoded by gene 144 of *Pseudomonas aeruginosa* bacteriophage φKZ. Biochemistry (Moscow) 71, 300–305.

Morais, M.C., Choi, K.H., Koti, J.S., Chipman, P.R., Anderson, D.L., Rossmann, M.G., 2005. Conservation of the capsid structure in tailed dsDNA bacteriophages: the pseudoatomic structure of phi29. Mol. Cell 18, 149–159.

Paradis-Bleau, C., Cloutier, I., Lemieux, L., Sanschagrin, F., Laroche, J., Auger, M., Garnier, A., Levesque, R.C., 2007. Peptidoglycan lytic activity of the *Pseudomonas aeruginosa* phage φKZ gp144 lytic transglycosylase. FEMS Microbiol. Lett. 266, 201–209.

Pell, L.G., Kanelis, V., Donaldson, L.W., Howell, P.L., Davidson, A.R., 2009. The phage λ major tail protein structure reveals a common evolution for long-tailed phages and the type VI bacterial secretion system. Proc. Natl. Acad. Sci. U. S. A. 106, 4160–4165.

Poglazov, B.F., Efimov, A.V., Marco, S., Carrascosa, J., Kuznetsova, T.A., Aijrich, L.G., Kurochkina, L.P., Mesyanzhinov, V.V., 1999. Polymerization of bacteriophage T4 tail sheath protein mutants truncated at the C-termini. J. Struct. Biol. 127, 224–230.

Studier, F.W., Rosenberg, A.N., Dunn, A.H., Dubendorff, J.W., 1990. Use of T7 RNA polymerase to direct expression of cloned genes. Methods Enzymol. 185, 60–89.

Thomas, J.A., Rolando, M.R., Carroll, C.A., Shen, P.S., Belnap, D.M., Weintraub, S.T., Serwer, P., Hardies, S.C., 2008. Characterization of *Pseudomonas chlororaphis* myovirus 201varphi2-1 via genomic sequencing, mass spectrometry, and electron microscopy. Virology 376, 330–338.