Biochemical and Biophysical Research Communications 374 (2008) 747–751

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

The structural peptidoglycan hydrolase gp181 of bacteriophage φKZ

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ARTICLE INFO

Article history:
Received 7 July 2008
Available online 29 July 2008

Keywords:
Structural lysin
Enzybiotic
Bacteriophage
Pseudomonas aeruginosa
Peptidoglycan

ABSTRACT

Gp181 (2237 amino acids) of *Pseudomonas aeruginosa* bacteriophage φKZ (*Myoviridae*) is a structural virion protein, which bears a peptidoglycan hydrolase domain near its C-terminus. This protein is supposed to degrade the peptidoglycan locally during the infection process. Nine deletional mutants allowed delineation of the peptidoglycan hydrolase domain between amino acids 1880–2042 (gp181M8) and analysis of its biochemical properties. Gp181M8 tolerates a high ionic strength (>320 mM) and is less sensitive to long thermal treatments compared to the similar φKZ endolysin. Gp181M8 lysed all tested outer membrane-permeabilized Gram-negative species. The C-terminal distal end (amino acids 2043–2237) enhances the specific activity of gp181M8 threefold, resulting in a twelve times higher activity than commercial hen egg white lysozyme. These biochemical properties suggest that this novel peptidoglycan hydrolase domain may be suitable for enzybiotic applications.

In the course of the infection bacteriophages transport their genome across the bacterial cell envelope. The major barrier to overcome is the peptidoglycan layer, which provides structural integrity to the cell and prevents free diffusion of globular proteins above ~50 kDa [1]. Phages of both Gram-positive and Gram-negative bacteria often encode virion-associated peptidoglycan hydrolases (structural lysins) to facilitate efficient DNA entry [2]. Since maintenance of cell integrity and viability is essential for further phage replication, the peptidoglycan degradation is restricted to local degradation of the peptidoglycan layer. Diffusion of enzymatic activity is prevented since the peptidoglycan hydrolase is encoded as a domain of a structural protein of the phage virion. Intuitively, one would expect that the functionality of the structural lysin is more stringent in Gram-positive than in Gram-negative infecting phages due to the much thinner peptidoglycan layer in the latter. Indeed, mutations in the structural lysin of PRD1 or T7 (both infecting Gram-negative bacteria) did not stop the infection process but merely delayed it [3,4]. Moreover, in T7 this delay only occurs under conditions in which the peptidoglycan layer is more intensely cross-linked [4]. Contrarily, antibodies directed to the structural lysin of *Lactococcus lactis* bacteriophage Tuc2009 reduced phage infection over 100-fold [5].

φKZ is a giant *Pseudomonas aeruginosa* bacteriophage (280,334 bp) that belongs to the *Myoviridae* family [6]. Recently, the cryo-EM structure of the φKZ virion has been reported [7,8]. The φKZ baseplate has a flat, hexagonal shape with a diameter of ~800 Å and a thickness of ~350 Å. In the middle of the φKZ baseplate a needle-like density is visible which is similar to the cell-puncturing device of bacteriophage T4 [8]. In T4, this needle-like structure is composed of a heteromeric complex of three gp5 and three gp27 molecules. Gp5 has a central lysozyme domain which is similar to T4 lysozyme and digests the peptidoglycan layer locally during phage tail contraction, allowing tube penetration into the periplasmic space [9]. In this report, the identification and the biochemical characterization of the structural lysin gp181 of bacteriophage φKZ is reported. This protein is putatively involved in the infection process as part of the needle-like cell-puncturing device of φKZ.

Materials and methods

Electrospray ionisation MS/MS analysis. Bacteriophage φKZ virions were purified by two consecutive CsCl-gradient centrifugations, disrupted by triple freeze–thaw cycles, reduced by β-mercaptoethanol, heat denatured (95 °C, 5 min) and loaded on a 12% polyacrylamide gel for electrophoretic separation. To visualize the bands, gels were stained with Simply Blue Safestaining solution (Invitrogen, Carlsbad, CA, USA). The expected band was excised from the gel and digested with trypsin. Protein digests were separated by liquid chromatography with a linear 5 to 60 (v/v) % acetonitrile gradient and subsequently identified by ESI-MS/MS (ThermoFinnigan, San Jose, CA, USA) in an *m/z* range from 300 to 1500. All MS/MS data were searched using Mascot (Matrix Sci-

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doi:10.1016/j.bbrc.2008.07.102

ences, London, UK) and Sequest (ThermoFinnigan) against the GenBank non-redundant protein database and a local database of all possible φKZ gene products.

DNA manipulations and plasmid construction. Purified φKZ genomic DNA [6] was used as template to amplify the different coding sequences of the deletion mutants of gp181 with *Pfu* polymerase (Fermentas, Ontario, Canada). *Gp181M1*, *M2*, *M4*, and *M6* were cloned into pET26 (cleaved with NdeI/XhoI) (Novagen, San Diego, CA, USA), *gp181M3*, *M7*, *M8*, and *M9* into pQE30 (cleaved with BamHI/HindIII) (Qiagen, Hilden, Germany), and *gp181M5* into pET23 (cleaved with NdeI/HindIII) (Novagen). Insertion created an in-frame fusion with an N-terminal (pQE30) or C-terminal His_6-tag (pET26, pET23).

Protein expression and purification. Protein expression was carried out in BL21(DE3) (Invitrogen) (pET constructs) and AD494 (DE3) (Invitrogen) or XL1 Blue MRF' (Stratagene, La Jolla, CA, USA) (pQE30 constructs) after induction of exponentially growing cells (OD_{600nm} = 0.6) in 1 L Luria–Bertani medium at 37 °C (4 h) with 1 mM isopropyl-β-D-galactopyranoside (IPTG). Cells were harvested (3300*g*, 15 min, 4 °C) and pellets were resuspended in 20 mL lysis buffer (10 mM imidazole, 20 mM NaH_2PO_4, 0.5 M NaCl, pH 7.4). This suspension was frozen/thawed four times prior to sonication (3 × 15 s, amplitude 40% on a Vibra Cell™, Sonics, Dandurry, CT, USA) and filtered successively through 0.45 and 0.22 μm Durapore membrane filters (Millipore, Billerica, MA, USA). Purification of the recombinant protein was performed on an Äkta FPLC (GE Healthcare, Uppsala, Sweden), using a HisTrapTM HP 1 mL column (GE Healthcare). The lysate was loaded after column equilibration with washing buffer (50 mM imidazole, 20 mM NaH_2PO_4, 0.5 M NaCl, pH 7.4; 10 column volumes). The column was washed with 15 column volumes of washing buffer. Protein was eluted in elution buffer (500 mM imidazole, 20 mM NaH_2PO_4, 0.5 M NaCl, pH 7.4) and dialyzed using Slide-A-Lyzer MINI Dialysis units (Pierce Biotechnology, Rockford, IL, USA).

Peptidoglycan hydrolytic assay. Peptidoglycan hydrolytic activity was assayed quantitatively as described previously [13]. Briefly, the outer membrane of exponentially growing *P. aeruginosa* PAO1 cells (or other species) was permeabilized by incubation (45 min) in chloroform-saturated 0.05 M Tris–HCl buffer pH 7.7 at room temperature. Subsequently, the cells were washed to remove chloroform and concentrated to an OD_{600nm} = 1.5. Addition of 30 μl protein to outer membrane permeabilized cells (270 μl) resulted in a decrease of optical density which was measured spectrophotometrically using a Bioscreen C Microbiology Reader (Labsystems, Oy, Finland). Negative controls with only buffer were subtracted from the sample measurement. A standardized calculation method of the enzymatic activity to warrant a maximal reliability has recently been described [11 – http://www.biw.kuleuven.be/logt/Activity-Calculator.htm]. For the biochemical characterization, 50 ng gp181M8 was used per tested condition (total volume = 300 μl) and all conditions were performed in triplicate. To assess the influence of pH and ionic strength on activity, the permeabilized cells were washed and resuspended in Na_3–citrate–HCl (pH 5–5.6) or KH_2PO_4/K_2HPO_4 (pH 6.2–8.0) with different ionic strengths. To investigate the heat stability, aliquots of protein (50 ng/30 μl) were heated for 10 or 60 min at fixed temperatures (between 25 and 90 °C) and subsequently cooled on ice for 10 min before measurement of the residual activity at 25 °C. To screen the substrate spectrum, different Gram-negative species were treated similarly as *P. aeruginosa* to permeabilize the outer membrane and used as substrate in the enzymatic assay (=270 μl). An amount of 14 ng (gp144) or 62 ng (gp181M8) (in a volume of 30 μl) was added to the different substrates and their respective specific activities were expressed and compared in U/mol [11].

Qualitative control of enzyme activity in the course of protein isolation and purification was performed essentially as described in [12]. A drop of the protein solution was applied to a small circle of filter paper and placed onto a chloroform vapour-treated lawn of *P. aeruginosa*. An appearance of a cleared zone around the paper in 5–10 min was an indication of peptidoglycan hydrolytic activity. A circle moisted with corresponding buffer was used as a control.

Results

Gp181 is a structural component of the φKZ particle

Purified phage φKZ particles were disrupted and proteins were separated by standard denaturing gel electrophoresis. The ~245 kDa protein band was excised, the proteins were eluted and subjected to trypsin digestion. Electrospray ionisation-MS/MS analysis led to the experimental identification of 72 significant peptides of gp181 (2237 amino acids), originating from throughout the entire predicted gp181 sequence. A protein sequence coverage up to 24% was reached, positively identifying gp181 as a structural component of the φKZ phage particle.

Cloning, expression and purification of gp181 derivatives

Gp181 shows protein similarity (66%) in its C-terminal part (residues 1866–2051) to the catalytic domain of the endolysin gp144 of phage φKZ which has been described recently [12–15]. The φKZ endolysin is involved in cell lysis at the end of the replication cycle of φKZ to release the progeny virions. Gp144 contains an additional N-terminal specific peptidoglycan binding domain which is absent in gp181. The catalytic glutamate residue [14,15] of gp144 is conserved in gp181 (Glu1906). However, the N-terminal part of gp181 (residues 1–1865) does not show homology to known sequences in public databases, except for gp183 of phage EL, a relative of φKZ which also infects *P. aeruginosa* [16].

To delineate functional domains in gp181, nine different deletion mutants of the gp181 C-terminus were constructed (Table 1) according to an algorithm based on the increased degree of information coordination between residues (IDIC-sites) [17] (Fig. S1). All constructs were expressed in *Escherichia coli* and the solubility of the produced recombinant protein was assessed. Recombinant gp181M3 production was toxic for *E. coli*, which might indicate the presence of cell wall degrading activity. Expression of the shortest deletion mutants resulted in soluble and purifiable protein (gp181M5 to gp181M9). The low solubility of gp181M7 was greatly enhanced by extension of its N-terminus with 17 residues (gp181M6). The soluble recombinant proteins were purified by the C-terminal His_6-tag using Ni^{2+} affinity chromatography. The purity was at least 95% as assessed visually on SDS–PAGE. Circular dichroism spectroscopy analysis revealed a predominantly α-helical secondary structure, corroborating a similar folding of the different mutants (data not shown).

Peptidoglycan hydrolytic activity of gp181 truncated proteins

To confirm qualitatively the peptidoglycan hydrolytic capacity, the different purified proteins (M5 to M9) were dropped on a *P. aeruginosa* cell lawn. This field was pretreated with chloroform vapour to permeabilize the outer membrane, exposing the peptidoglycan layer. Hen egg white lysozyme was included as a positive control. The resulting clear lysis zones support the functional annotation of gp181 as structural lysin of φKZ. All tested truncated gp181 variants were active, except gp181M9 which did not lead to a lysis zone. These findings show that the minimal catalytic domain is located between residue 1880 and 2042 (gp181M8). It is

Table 1
Overview of deletional mutants of gp181

Name	Start	End	Vector	Insertion site	Expression	Soluble	Active
gp181M1	1594	2237	pET26	NdeI–XhoI	Yes	No	n.a.
gp181M2	1613	2237	pET26	NdeI–XhoI	Yes	No	n.a.
gp181M3	1661	2237	pQE30	BamHI–HindIII	Toxic	n.a.	Indicative
gp181M4	1738	2237	pET26	NdeI–XhoI	Yes	No	n.a.
gp181M5	1744	2237	pET23	NdeI–HindIII	Yes	Yes	+
gp181M6	1863	2237	pET26	NdeI–XhoI	Yes	Yes	++
gp181M7	1880	2237	pQE30	BamHI–HindIII	Yes	Poorly	++
gp181M8	1880	2042	pQE30	BamHI–HindIII	Yes	Yes	++
gp181M9	2043	2237	pQE30	BamHI–HindIII	Yes	Yes	–

n.a., not applicable.

hypothesized that gp181M9 may constitute the needle-like structure which protrudes from the φKZ baseplate as is also visible in the cryo-EM structure of the φKZ baseplate [8]. Interestingly, secondary structure predictions suggest the presence of long α-helices in gp181M9, similarly as observed in the membrane-puncturing needle of bacteriophage P22 [18].

Biochemical characterization of gp181M8

The peptidoglycan hydrolytic activity of gp181M8 was characterized and compared to the highly similar φKZ endolysin (gp144) which has been characterized recently [14]. Both buffer pH and ionic strength influence the enzymatic activity of phage peptidoglycan hydrolases significantly and were investigated simultaneously to include interdependent effects, varying the ionic strength between 20 and 320 mM and pH between 5.0 and 8.0 (Table 2). The activity of both gp181M8 and gp144 peaks at pH 6.2. However, in general gp181M8 prefers more acidic conditions compared to gp144 (e.g., 51% residual activity at pH 5.0 at 140 mM compared to 24% in case of gp144; 42% residual activity at pH 8.0 at 140 mM compared to 78% in case of gp144) [14]. Although the optimal ionic strength for both proteins is quite similar (140 mM for gp181M8 and 120 mM for gp144), gp181M8 can tolerate a much wider range of ionic strengths (up to at least 320 mM).

The enzymatic activity of gp181M8 was accurately quantified [2] under the optimal conditions as determined from Table 2 and compared to the activity of gp181M6 (which combines the catalytic site and the putative C-terminal needle). An activity saturation curve was determined with incremental amounts of both proteins and the enzymatic activity was derived from the linear region of this curve (Fig. 1). Gp181M8 displays 1.25×10^{12} units/mol enzymatic activity on outer-membrane permeabilized *P. aeruginosa* cells, compared to 3.77×10^{12} U/mol for gp181M6. Thus, the presence of the distal C-terminal end enhances the enzymatic activity about threefold. The activity of gp181M6 is about 12 and 37 times higher than commercially available hen egg white lysozyme and the previously described structural lysin domain of *P. aeruginosa* bacteriophage φKMV [10,11], respectively, but about 2 times less than gp144. As mentioned above, the latter acquired an additional N-terminal substrate binding module, resulting in a greater substrate proximity which explains the higher enzymatic activity.

Table 2
Influence of varying pH values and ionic strengths on the enzymatic activity of gp181M8

Ionic strength (mM)	pH					
	5.0	5.6	6.2	6.8	7.4	8.0
20	27	26	39	46	30	36
80	27	83	68	66	42	15
140	51	31	100	65	36	42
200	20	12	81	84	16	16
260	17	6	22	40	24	18
320	12	3	14	16	14	14

The relative enzymatic activity of gp181M8 at different conditions (pH, ionic strength) compared to the optimal conditions (=100%) is represented.

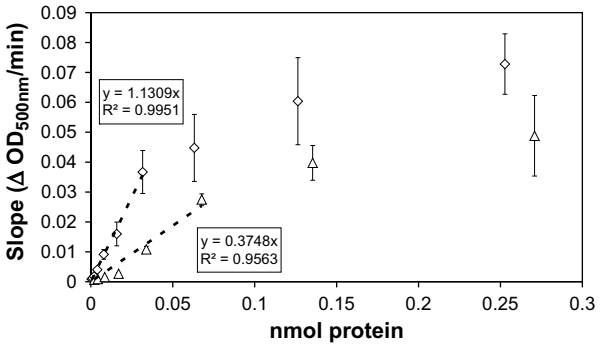

Fig. 1. Saturation curves of gp181M6 and gp181M8 under optimal buffer conditions. The activity in ΔOD_{500nm}/min (*Y*-axis) for incremental amounts of gp181M6 (diamond) and gp181M8 (triangle) (*X*-axis) is depicted. A linear regression of the demarcated linear region of the saturation curves gives an activity of 3.77×10^{12} and 1.25×10^{12} U/mol for gp181M6 and gp181M8, respectively.

Kinetic stability

To assess the sensitivity to thermal inactivation, aliquots of gp181M8 were heated for either 10 or 60 min at elevated temperatures between 25 and 90 °C. Subsequently, the samples were cooled to allow refolding and the residual activity was measured

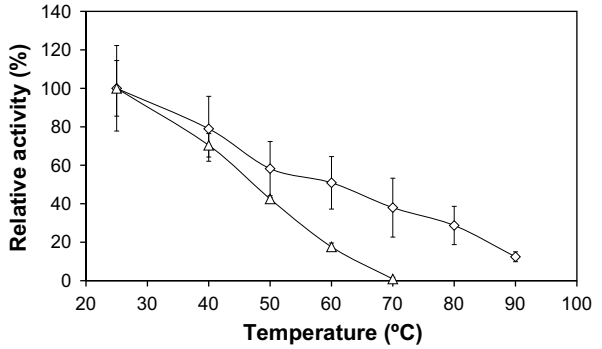

Fig. 2. Kinetic stability of gp181M8. Samples of gp181M8 (50 ng/30 μl) were heated at different temperatures between 25 and 90 °C for 10 (diamond) or 60 (triangle) minutes. Subsequently, the samples were cooled on ice and the residual activity was measured at 25 °C. All conditions were measured in threefold and averages/standard deviations are depicted.

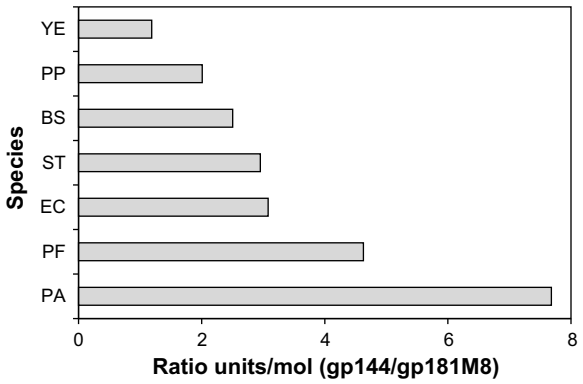

Fig. 3. Substrate spectrum gp181M8 compared to gp144. The ratio of the specific activities (in U/mol) of the endolysin (gp144) and the structural lysin domain (gp181M8) of phage φKZ on different species (YE, *Yersinia enterocolitica*; PP, *Pseudomonas putida*; BS, *Burkholderia solanacearum*; ST, *Salmonella typhimurium*; EC, *Escherichia coli*; PF, *Pseudomonas fluorescens*; PA, *Pseudomonas aeruginosa*) is depicted.

at 25 °C (Fig. 2). Thermal inactivation of gp181MA occurs gradually between 25 and 90 °C. A residual activity of 12% is measured after 10 min at 90 °C and 1 h incubation at 70 °C is necessary for complete inactivation of the enzyme. Interestingly, the similar gp144 is completely inactivated after 10 min at 60 °C [14]. These data show a prolonged stability for the structural catalytic domain, which possibly mirrors the need to withstand severe mechanical forces during the infection process. A high thermoresistance has previously been reported for the structural lysin of *P. aeruginosa* bacteriophage φKMV, which still keeps 21% residual activity after 2 h at 100 °C [10,19].

Determination of the substrate spectrum

Seven different Gram-negative species (*P. aeruginosa*, *Pseudomonas fluorescens, Pseudomonas putida, E. coli, Salmonella typhimurium, Burkholderia solanacearum, Yersinia enterocolitica*) were tested in triplicate for sensitivity to gp181M8. All species were susceptible to lysis, showing that the substrate spectrum of the structural lysin does not impose restrictions to the bacterial host spectrum of phage φKZ which is more narrow and limited to several *P. aeruginosa* strains. The endolysin gp144 affects all tested Gram-negative species as well [14]. The ratios of the enzymatic activity of gp144 and gp181M8 on the different species show that gp144 is more active on all tested substrates. However, an increasing specificity of gp144 towards the host *P. aeruginosa* (ratio 7.7) is observed, whereas the activity of gp144 and gp181M8 on *Y. enterocolitica* is almost equal (ratio 1.1) (Fig. 3). This suggests that either the acquisition of the peptidoglycan binding module by gp144 and/or subtle differences in the catalytic domain must influence the specificity towards the substrate.

Discussion

During the replication cycle of a bacteriophage, the cell wall has to be passed twice. To facilitate both passages, most double-stranded DNA phages encode two distinct peptidoglycan hydrolases. Analysis of the φKZ genome revealed two peptidoglycan hydrolases, gp144 (260 amino acids) and gp181 (2273 amino acids). Interestingly, they share a high similarity (66%) within their catalytic domain. All available data previously confirmed gp144 as the lysis-related peptidoglycan hydrolase (endolysin) of φKZ [12–15].

Here, we show that gp181 (2237 amino acids) can be functionally annotated as the infection-related structural lysin of phage φKZ, based on the mass spectrometric identification of gp181 in φKZ phage particles and the biochemically characterized peptidoglycan hydrolase domain which is embedded near the C-terminus of gp181. It is hypothesized that gp181 constitutes the distal end of the tail tube of φKZ as visualized by cryo-EM studies [8], facilitating tail tube penetration through the cell wall by local peptidoglycan degradation and allowing subsequent injection of phage genomic DNA into the host cytoplasm.

Phage-encoded lysis-related endolysins receive increasing attention as 'enzybiotics' for prevention and treatment of bacterial infections. Exogenous addition of these enzymes to Gram-positive pathogens results in an efficient reduction of cell viability through cell lysis [20,21]. Biochemical properties of the structural lysin domain gp181M8 were extensively characterized and compared to endolysin gp144 to evaluate its potential as enzybiotic candidate. Although the enzymatic activity of the latter is significantly higher, the infection-related catalytic domain is more resistant to increased ionic strength and heat. Increased heat stability seems to be a recurring property of structural lysins [10,19]. From these results, we conclude that it is conceivable to tap the pool of structural lysins for enzybiotics besides endolysins as well. A recombinant fusion between the peptidoglycan binding domain of gp144 and the catalytic domain of gp181 will possibly combine the best of what phages offer to attack the peptidoglycan layer to develop highly stable and active enzymes.

Acknowledgments

This work was financially supported by the Flemish FWO (research grant G.0308.05 and 1.5.184.05 N), the research council of the K.U.Leuven (OT/04/30 and OT/05/47) and the Russian foundation for basic research (05-04-50829-MF-a and 07-04-12224-OFI). Yves Briers holds a predoctoral fellowship of the '*Instituut voor de aanmoediging van Innovatie door Wetenschap en Technologie in Vlaanderen*' (I.W.T., Belgium). Rob Lavigne is postdoctoral fellow of the '*Fonds voor Wetenschappelijk Onderzoek-Vlaanderen*' (FWO-Vlaanderen, Belgium).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.07.102.

References

[1] P. Demchick, A.L. Koch, The permeability of the wall fabric of *Echerichia coli* and *Bacillus subtilis*, J. Bacteriol. 178 (1996) 768–773.
[2] M. Moak, I.J. Molineux, Peptidoglycan hydrolytic activities associated with bacteriophage virions, Mol. Microbiol. 51 (2004) 1169–1183.
[3] P.S. Rydman, D.H. Bamford, Bacteriophage PRD1 DNA entry uses a viral membrane-associated transglycosylase activity, Mol. Microbiol. 37 (2000) 356–363.
[4] M. Moak, I.J. Molineux, Role of the gp16 lytic transglycosylase motif in bacteriophage T7 virions at the initiation of infection, Mol. Microbiol. 37 (2000) 345–355.
[5] J.G. Kenny, S. McGrath, G.F. Fitzgerald, D. van Sinderen, Bacteriophage Tuc2009 encodes a tail-associated cell wall-degrading activity, J. Bacteriol. 186 (2004) 3480–3491.
[6] V.V. Mesyanzhinov, J. Robben, B. Grymonprez, V.A. Kostyuchenko, V.A. Bourkaltseva, N.N. Sykilinda, V.N. Krylov, G. Volckaert, The genome of bacteriophage phiKZ of *Pseudomonas aeruginosa*, J. Mol. Biol. 317 (2002) 1–19.
[7] A. Fokine, V.A. Kostyuchenko, A.V. Efimov, L.P. Kurochkina, N.N. Sykilinda, J. Robben, G. Volckaert, A. Hoenger, P.R. Chipman, A.J. Battisti, M.G. Rossmann, V.V. Mesyanzhinov, A three-dimensional cryo-electron microscopy structure of the bacteriophage phiKZ head, J. Mol. Biol. 9 (2005) 117–124.
[8] A. Fokine, A.J. Battisti, V. D Bowman, A.V. Efimov, L.P. Kurochkina, P.R. Chipman, V.V. Mesyanzhinov, M.G. Rossmann, Cryo-EM study of the *Pseudomonas* bacteriophage φKZ, Structure 15 (2007) 1099–1104.
[9] S. Kanamaru, P.G. Leiman, V.A. Kostyuchenko, P.R. Chipman, V.V. Mesyanzhinov, F. Arisaka, M.G. Rossmann, Structure of the cell-puncturing device of bacteriophage T4, Nature 451 (2002) 553–557.
[10] R. Lavigne, Y. Briers, K. Hertveldt, J. Robben, G. Volckert, Identification and characterization of a highly thermostable bacteriophage lysozyme, Cell. Mol. Life Sci. 61 (2004) 2753–2759.

[11] Y. Briers, R. Lavigne, G. Volckaert, K. Hertveldt, A standardized approach for accurate quantification of murein hydrolase activity in high-throughput assays, J. Biochem. Biophys. Methods 70 (2007) 531–533.
[12] K.A. Miroshnikov, N.M. Faizullina, N.N. Sykilinda, V.V. Mesyanzhinov, Properties of the endolytic transglycosylase encoded by gene 144 of *Pseudomonas aeruginosa* bacteriophage phiKZ, Biochemistry (Mosc.) 71 (2006) 300–305.
[13] C. Paradis-Bleau, I. Cloutier, L. Lemieux, F. Sanschagrin, J. Laroch, M. Auger, A. Garnier, R.C. Levesque, Peptidoglycan lytic activity of the *Pseudomonas aeruginosa* phage phiKZ gp144 lytic transglycosylase, FEMS Microbiol. Lett. 266 (2007) 201–209.
[14] Y. Briers, G. Volckaert, A. Cornelissen, S. Lagaert, C.W. Michiels, K. Hertveldt, R. Lavigne, Muralytic activity and modular structure of the endolysins of *Pseudomonas aeruginosa* bacteriophages φKZ and EL, Mol. Microbiol. 65 (2007) 1334–1344.
[15] A. Fokine, K.A. Miroshnikov, M.M. Shneider, V.V. Mesyanzhinov, M.G. Rossmann, Structure of the bacteriophage phiKZ lytic transglycosylase, gp144, J. Biol. Chem. 283 (2008) 7242–7250.
[16] K. Hertveldt, R. Lavigne, E. Pleteneva, N. Sernova, L. Kurochkina, R. Korchevskii, J. Robben, V.V. Mesyanzhinov, V.N. Krylov, G. Volckaert, Genome comparison of *Pseudomonas aeruginosa* large phages, J. Mol. Biol. 354 (2005) 536–545.
[17] A.N. Nekrasov, Analysis of the information structure of protein sequences: a new method for analyzing the domain organization of proteins, J. Biomol. Struct. Dyn. 21 (2004) 615–624.
[18] L. Tang, W.R. Marion, G. Cingolani, P.E. Pervelige, J.E. Johnson, Three-dimensional structure of the bacteriophage P22 tail machine, EMBO J. 24 (2005) 2087–2095.
[19] Y. Briers, R. Lavigne, P. Plessers, K. Hertveldt, I. Hanssens, Y. Engelborghs, G. Volckaert, Stability analysis of the bacteriphage phiKMV lysin gp36C and its putative role during infection, Cell. Mol. Life Sci. 63 (2006) 1899–1905.
[20] J. Borysowski, B. Weber-Dabrowska, A. Górski, Bacteriophage endolysins as a novel class of antibacterial agents, Exp. Biol. Med. (Maywood) 231 (2006) 366–377.
[21] M.J. Loessner, Bacteriophage endolysins-current state of research and applications, Curr. Opin. Microbiol. 8 (2005) 480–487.