The mechanism of dsRNA bacteriophage genome delivery into the host cell

A. Gaidelytė,
R. Daugelavičius,
G. Žiedaitė,
R. Petraitytė,
E. Bakienė

Department of Biochemistry and Biophysics, Vilnius University, M. K. Čiurlionio 21, LT-2009 Vilnius, Lithuania

It is shown that three steps of phage-induced changes in the cell envelope permeability can be registered at the initial stages of infection of bacteria *Pseudomonas syringae* by the phage ϕ6 and the related phage ϕ13. Permeabilization of the host outer membrane was followed by depolarization and subsequent repolarization of the plasma membrane. The duration of these stages correlated well with the time required for the interaction between the virion components and successive layers of the host cell envelope during phage penetration into the host cell.

Key words: *Cystoviridae*, genome delivery, cell envelope permeability

INTRODUCTION

Initiation of a viral infection requires delivery of viral genome into the host cell. Most bacterial viruses have an adapted entry mechanism where the viral nucleic acid is delivered through the host cell envelope leaving the capsid outside [1]. However, having a dsRNA genome imposes specific demands for the virus. As the cells do not possess the enzymes that are capable of transcribing and replicating dsRNA templates, viruses with dsRNA genomes have to bring their own virion-associated RNA-dependent RNA polymerase into the cell along with their genome [2]. So, the entry mechanism of dsRNA bacteriophages should differ considerably from those of other bacterial viruses. Members of the family *Cystoviridae* are the only bacterial dsRNA viruses known thus far [3]. These unique bacteriophages are enveloped with a lipid-containing membrane which surrounds the nucleocapsid. The genome composed of three dsRNA segments (L, M and S) resides inside the virion-associated polymerase complex (nucleocapsid core) [4, 5]. To carry the viral core into the cytoplasm of their host *Pseudomonas syringae* they have to overcome a complicated Gram-negative cell envelope with two membranes and a peptidoglycan layer between them. It has been shown earlier, that upon infection of the type organism, phage ϕ6, virion penetrates into the periplasmic space of the cell by a protein-targeted fusion of the phage envelope with the bacterial outer membrane (OM) [6]. The viral muramidase digests the peptidoglycan layer, and the nucleocapsid transfers through the plasma membrane (PM) via membrane invagination and intracellular vesicle [7, 8]. It is a unique example of endocytosis-like process in bacteria. Successive interaction of phage components with the layers of cell envelope should result in changes of the envelope permeability. For a long time ϕ6 has been alone in the family *Cystoviridae*. However, eight different bacteriophages related to ϕ6 have been isolated recently [4]. The structure and life cycle of these phages is the subject of considerable investigation. The aim of this work was to elucidate the mode of action of bacteriophage ϕ6 and related phage ϕ13 on the integrity and functions of cellular membranes during phage genome delivery into the host cell.

MATERIALS AND METHODS

Reagents. Tetraphenylphosphonium chloride was purchased from Aldrich. Potassium phenyldicarbaundecaborane was synthesized by Dr. A. Beganskienė (Faculty of Chemistry, Vilnius University), polymyxin B sulfate was purchased from Sigma, EDTA was purchased from Serva, all other reagents were of analytical grade.

Bacteria. *Pseudomonas syringae* pv. *phaseolicola* strains HB10Y and LM2509, and bacteriophages ϕ6 and ϕ8 were kindly provided by Prof. D. H. Bamoford (University of Helsinki, Finland).

General methods. The cells were grown in Luria–Bertani broth at 23 °C with aeration to mid-log phase, pelleted by centrifugation (5000 × g) for 10 min at 4 °C and resuspended in 0.1 M sodium phosphate buffer (pH 7.0) at the cell density approximately $1 \times \times 10^{11}$ cfu/ml. The cell suspension was kept on ice un-

til used (maximally 3 hours). 25 μl of this preparation was added to an appropriate medium in a thermostated reaction vessel (5 ml volume) and the cell suspension was aerated by magnetic stirring. The concentration of lipophilic ions TPP^+ or PCB^- in the medium was monitored using selective electrodes, as described earlier [9].

RESULTS AND DISCUSSION

In the present study, with the intent to provide a deeper insight into the mechanism of dsRNA bacteriophage genome delivery into the host cell, we investigated the interaction of viral and cellular membranes during the penetration of phages ϕ6 and ϕ13 into cells of bacteria *P. syringae*. Changes in cell envelope permeability to lipophilic ions and the PM voltage (Δψ) of bacteria upon interaction with the phages were studied.

Assays on the distribution of lipophilic ions are widely used for the quantification of Δψ of biological membranes of different origin. Thus, tetraphenylphosphonium (TPP^+) and phenyldicarbaundecaborane (PCB^-) are used for estimation of Δψ in biological microobjects having a negative (*e.g.*, bacterial cells) and positive (*e.g.*, inverted bacterial membrane vesicles) membrane voltages, respectively [10, 11]. Furthermore, analysis of TPP^+ and PCB^- uptake by bacterial cells is a simple but informative way to estimate the cell envelope permeability [9]. The cell envelope of Gram-negative bacteria such a *P. syringae* is hardly permeable to lipophilic ions. TPP^+ easily penetrates phospholipid bilayers. However, the outer layer of OM of Gram-negative bacteria consists of lipopolysaccharide (LPS), which forms an effective permeability barrier against various hydrophobic and lipophilic compounds, including TPP^+ and PCB^- [12, 13]. Besides, it has been clearly demonstrated that an alive bacterial cell imposes additional constraints on the permeability of the cell envelope to lipophilic anions, such as PCB^-, depending on the metabolic activity of the cell [14]. It has been shown that certain bacteriophages are able to break the bacterial envelope permeability barrier to lipophilic compounds [9, 15].

In the following set of experiments the fluxes of lipophilic cation TPP^+ and anion PCB^- across the envelopes of bacteria HB10Y (the host of phage ϕ6) or LM2509 (the host of phage ϕ13) cells upon interaction with appropriate phages were studied. Intact cells bind only small amounts of TPP^+ and PCB^- (Fig. 1, A and B, curves 1, 3 and 4). Enhanced TPP^+ influx into the cells in the presence of phages ϕ6 and ϕ13 reflects an increase in cell OM permeability to lipophilic ions caused by the phages (Fig. 1, A and B, curves 2) (compare with the effect of EDTA, the common OM permeabilizing agent, presented in Fig. 1, A and B, curves 1). It has been shown previously that during the penetration of phage ϕ6 into the host cell a fusion between the phage membrane and the host OM occurs [6, 16]. The fusion was shown to be nonleaky and resulting in the establishment of a continuum between the phage and host membranes [6]. It seems likely that the phospholipid bilayer patches formed in the OM by viral membranes act as channels through which lipophilic ions can diffuse. As a consequence, TPP^+ acquires the possibility to distribute between the cell cytoplasm and external medium according to Δψ. In the light of our results presented in Fig. 1, B, as well as preliminary electron microscopy analysis (data not shown), there is a reason to believe that quite an analogous mechanism is implicated in the phage ϕ13 entry. Control experiments revealed that less than 70% of the cells were infected in the given conditions (data not shown), and this fact explains the smaller effect of the phages on TPP^+ uptake by the cells compared with the effect caused by EDTA (Fig. 1, A and B, curves 2 and 1). The following efflux of TPP^+ reflects the depolarization of the PM (Fig. 1, A and B, curves 2). Both enhancement of the OM permeability and depolarization of the PM resulted in

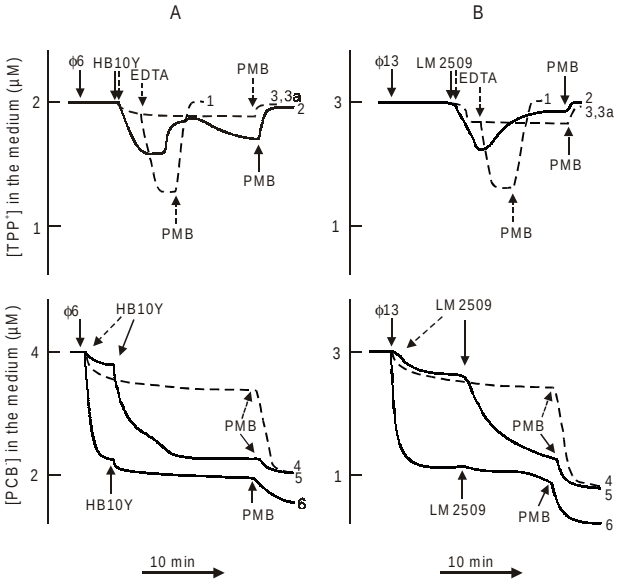

Figure. Effects of phages ϕ6 (A) and ϕ13 (B) on TPP^+ and PCB^- uptake by *P. syringae* cells. The measurements were performed at 28 °C in 0.1 M sodium phosphate, pH 8.0 (B), or in the same buffer containing 10% of LB broth (A). The cells were added to obtain the final concentration of 5×10^8 cfu/ml. Phage additions were: intact (A, curves 2 and 5) or temperature-inactivated (75 °C, 10 min) (A, curves 3a and 6) phage ϕ6 – to $1.25 \times \times 10^{10}$ particles/ml; intact (B, curves 2 and 5) or temperature-inactivated (75 °C, 10 min) (B, curves 3a and 6) phage ϕ13 – to 1×10^9 particles/ml. Curves 1, 3 and 4 – controls without phages (curves 3 and 3a coincide). EDTA was added to the final concentration of 0.2 mM (A) or 0.4 mM (B), polymyxin B (PMB) – to the final concentration of 120 μg/ml

additional PCB^- binding to *P. syringae* cell (Fig. 1, A and B, curves 5). The subsequent stage of TPP^+ uptake by HB10Y cells indicates repolarization of the PM at the later stages of ϕ6 infection (Fig. 1, A, curve 2). Bacteriophage ϕ6-induced changes in *P. syringae* envelope permeability were observed only in a rich medium proper for maintaining and retraction of the pilus, the cell–surface receptor for ϕ6 infection. ϕ6 neither infects the cells suspended in buffered saline nor causes any changes in their envelope permeability (data not shown). Unlike ϕ6, phage ϕ13 attaches directly to the OM, presumably to the LPS [4]. The results of experiments presented in Fig. 1 B, curves 2 and 5 suggest that at least the initial stages of ϕ13 infection are able to proceed in buffered saline and result in permeabilizaton of the OM and depolarization of the PM of the host cell. However, partial repolarization of the PM of LM2509 cells during ϕ13 infection could be observed only in the rich medium (data not shown). The subsequent addition of polycationic antibiotic polymyxin B (PMB) depolarizes the PM and enables to estimate the remaining $\Delta\psi$. The fluxes of TPP^+ were not observed, if heat-inactivated phage particles (75 °C, 10 min) were used in the experiment (Fig. 1, A and B, curves 3a). However, inactivated phages bound a significant amount of PCB^- (Fig. 1, A and B, curves 6; see also [17]).

The time course of phage-induced events in the bacterial cell envelope permeability correlates well with the intervals required for the interaction between phage components and successive layers of the host cell envelope [7, 8]. The enhancement of *P. syringae* OM permeability to the lipophilic ions coincides with the stage of fusion of the cell OM with the phage lipid envelope. It is plausible that phages depolarize the host cell PM during penetration into the cytoplasm. Later on, in the favourable conditions the membrane voltage gradually restores.

We demonstrate here that monitoring of the fluxes of lipophilic ions across *P. syringae* envelope provides an information about separate stages of penetration of phages into the host cell. The similarities between the phage-induced permeabilization of the OM and changes in $\Delta\psi$ at the initial stages of infection suggest similar ϕ6 and ϕ13 genome delivery mechanisms.

ACKNOWLEDGEMENTS

This work was supported by Lithuanian State Science and Studies Fundation grants No. T-477 and T-575 and by EC through its programme Centres of Excellence, project CEBIOLA ICA1-CT-2000–70027.

Received
11 October 2002

References

1. Dreiseikelmann BJ. Microbiol Rev 1994; 58: 293–316.
2. Cann AJ. Principles of Molecular Virology. Academic Press, London, 2001.
3. Van Regenmortel HV, Bishop DHL, Van Regenmortel MH et al. (eds). Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses. Academic Press, 2000.
4. Mindich L, Qiao X, Qiao J et. al. J Bacteriol 1999; 181: 4505–8.
5. Semancik JS, Vidaver AK, Van Etten JL. J Mol Biol 1973; 78: 617–25.
6. Bamford DH, Romantschuk M, Somerharju PJ. EMBO J 1987; 6: 1467–73.
7. Poranen MM, Daugelavičius R, Ojala PM et al. J Cell Biol 1999; 147: 671–81.
8. Romantschuk M, Olkkonen VM, Bamford DH. EMBO J 1988; 7: 1821–9.
9. Daugelavičius R, Bamford JKH, Bamford DH. J Bacteriol 1997; 179: 5203–210.
10. Rottenberg H. Methods Enzymol 1989; 172: 63–84.
11. Skulachev VP. Methods Enzymol 1979; 55: 751–76.
12. Snyder S, Kim D, McIntosh T. J Biochem 1999; 38: 10758–67.
13. Vaara M. Microbiol Rev 1992; 56: 395–411.
14. Daugelavičius R, Bakienė E, Beržinskienė J, Bamford DH. Bioelectrochem Bioenerg 1997; 42: 263–74.
15. Bakienė E, Daugelavičius R, Beržinskienė J et. al. Biologija 1995; Nr. 1–2: 9–13.
16. Bamford DH, Palva ET, Lounatmaa KJ. Gen Virol 1976; 32: 249–59.
17. Daugelavičius R, Bakienė E, Beržinskienė J, Bamford DH. Biotechnol Bioengineer 2001; 71: 208–16.

A. Gaidelytė, R. Daugelavičius, G. Žiedaitė, R. Petraitytė, E. Bakienė

DVIGRANDĘ RNR TURINČIŲ BAKTERIOFAGŲ GENOMO PERNAŠOS Į LĄSTELĘ-ŠEIMININKĘ MECHANIZMAS

S a n t r a u k a

Siekiant išsiaiškinti dvigrandę RNR turinčių bakteriofagų genomo pernašos į ląstelę-šeimininkę dėsningumus, **t**irti bakterijų *Pseudomonas syringae* išorinės (IM) ir plazminės (PM) membranos barjerinių savybių pokyčiai sąveikos su bakteriofagais ϕ6 ir ϕ13 metu. Registruotas lipofilinio katijono tetrafenilfosfonio (TPP^+) pasiskirstymas tarp terpės ir *P. syringae* ląstelių bei lipofilinio anijono fenildikarbaundekaborano (PCB^-) kaupimas ląstelių ir virusų membranose. Paveikus ląsteles bakteriofagais ϕ6 ar ϕ13, išsiskyrė trys ląstelės-šeimininkės apvalkalėlio laidumo pokyčių stadijos. Infekcijos pradžioje fagai didina IM laidumą lipofiliniams jonams, antrojoje stadijoje depoliarizuoja ląstelių PM, vėliau atkuriama PM membranos įtampa. Šių stadijų trukmė atitinka fago komponentų sąveikos su atskirais ląstelės apvalkalėlio sluoksniais trukmę fago RNR ir polimerazės kompleksui skverbiantis į ląstelės citoplazmą.