VIROLOGY 227, 207–210 (1997)
ARTICLE NO. VY968308

SHORT COMMUNICATION

View metadata, citation and similar papers at core.ac.uk
brought
provided by Elsevier

Assembly of Bacteriophage PRD1

ANNA-LIISA HÄNNINEN,* DENNIS H. BAMFORD,*,† and JAANA K. H. BAMFORD†,[1]

*Institute of Biotechnology and †Department of Biosciences, Division of Genetics, Biocenter, P.O. Box 56 (Viikinkaari 5), 00014 University of Helsinki, Finland

Received June 2, 1996; returned to author for revision August 29, 1996; accepted October 21, 1996

Assembly of the broad-host-range bacteriophage PRD1 involves translocation of the virus-specific membrane to the inside of the icosahedral protein shell formed of trimeric coat proteins. The formation of PRD1 particles is, in addition to the virus-encoded assembly factors P10 and P17, dependent on GroEL/GroES chaperonins. The chaperonins assist in the folding of the capsid proteins P3 and P5 and in the assembly of viral membrane proteins. © 1997 Academic Press

Virus capsid assembly, where typically hundreds of subunits assemble accurately and efficiently to form a closed protein shell, provides a good model system to study the control mechanisms of macromolecular assembly in general (*1, 2*). The diverse mechanisms by which different dsDNA viruses reach their final functional structures can include several conformational changes, proteolytic cleavages, and covalent joinings. Most viruses clearly require accessory factors in addition to the capsid subunits to aid their assembly. These factors include scaffolding proteins, dispensable domains of structural proteins, and either virus- or host-encoded chaperone-like proteins, or both.

Bacteriophage PRD1, a Tectivirus, belongs to a family of closely related viruses, which have the special feature of having a membrane as an internal structural component of the virion. These phages infect a variety of gram-negative hosts, including *Escherichia coli* and *Salmonella typhimurium*, carrying a conjugative P-, W-, or N-type plasmid, which encodes the phage receptor (*3, 4*). The PRD1 particle consists of an outer icosahedral protein coat surrounding the viral membrane, which, in turn, encloses the linear 15-kb-long dsDNA genome (*5, 6*). The genome has a terminal protein covalently linked to the 5′ ends and 110-bp-long inverted terminal repeats (*7, 8*), and it replicates via a protein-primed mechanism (*9, 10*).

PRD1 infection leads to the synthesis of some 30 virus-specific gene products, many of which are small membrane-associated proteins. The virion protein coat consists of two proteins: the minor capsid protein P5 and the major capsid protein P3, the latter comprising 70–80% of the total protein mass of the virion (*11*). It has been recently shown by cryoelectron microscopy that there are 240 copies of P3 trimers in the PRD1 coat packed on a $T = 25$ lattice (*12*). The membrane is composed of host-derived phospholipids and phage-encoded proteins (*11*). During the assembly process the virus-specific membrane proteins are inserted into the host plasma membrane, whereas the coat proteins assemble to soluble homotrimers in the cytoplasm. Based on the previous biological–biochemical and recent electron microscopic (*12*) and Raman spectroscopic studies (*13, 14*), a model of PRD1 assembly has been proposed; the viral membrane components at the plasma membrane act as a scaffold for the process where P3 trimers interact laterally to form a closed shell. This process is associated with an auxiliary folding event where about 5% of the amino acid residues in P3 obtain their final fold. The particle formation event is absolutely dependent on the function of viral assembly factors P10 and P17 in addition to the presence of the major capsid protein P3 (*15*). In the final maturation step DNA is packaged into the empty virion, and mature phage particles are released by the host cell lysis. A more detailed description of the PRD1 system is given in a recent review (*16*).

Molecular chaperones are conserved universal cellular proteins involved in a number of essential processes, including protein folding, polypeptide transport, protein disaggregation, and regulation of the heat shock response (*17, 18*). The chaperonin proteins of the hsp60 family are large cylindrical heptameric protein complexes. The bacterial hsp60 chaperonin GroEL with the cochaperonin GroES was originally described as a host function essential for bacteriophage λ head assembly (*19*), but it has later been shown to generally assist protein folding by binding and releasing repetitively the maturing polypeptide in an ATP-dependent reaction (*20, 21*).

[1] To whom correspondence and reprint requests should be addressed. Fax: 358-9-70859098. E-mail: gen_phag@cc.helsinki.fi.

0042-6822/97 $25.00
Copyright © 1997 by Academic Press
All rights of reproduction in any form reserved.

TABLE 1

PRD1 Titers (PFU/ml) Obtained on *E. coli* Strains (Sherwood Casjens, University of Salt Lake City) Carrying Mutations in GroEL or GroES[a] in the Presence or Absence of Overexpressed *wt* GroEL or GroES

	37°	40°	40° + GroES[b]	40° + GroEL[c]
DW720 *wt* (pLM2)	5×10^{11}	4.3×10^{11}	2.8×10^{11}	3.6×10^{11}
DW719 *groES619* (pLM2)	3.7×10^{11}*	$<10^{4}$*	4.7×10^{11}	$<10^{4}$
DW715 *groEL764* (pLM2)	2.5×10^{11}	1.9×10^{11}	ND	6.8×10^{9}
DW716 *groEL44* (pLM2)	3.4×10^{11}	3.2×10^{5}*	ND	4.9×10^{11}
DW717 *groEL59* (pLM2)	3.8×10^{10}*	$<10^{4}$*	$<10^{4}$	2.7×10^{11}
DW721 *groEL673* (pLM2)	1.9×10^{11}	$<10^{4}$*	ND	7.8×10^{10}

Note. ND, not determined; *reduced plaque size.
[a] The used mutant set represents backcrossed mutations that do not share the same mutational change.
[b] wt groES allele was introduced to the cells by transforming with plasmid pJBES4.
[c] wt *groEL* allele was introduced to the cells by transforming with plasmid pJBEL6.

Besides phage λ also at least phages T4 and T5 have been shown to depend on GroE in their morphogenesis. However, there is no universal GroE function in bacteriophage assembly. In this investigation we studied the dependence of PRD1 assembly on GroEL/GroES by examining PRD1 infections in different *groE* mutant hosts.

We first tested the effects of *groEL* and *groES* mutations on the plaque formation of PRD1. The PRD1 receptor encoding plasmid pLM2 (*22*) was conjugated to five different mutant strains and to their isogenic parental strain with wild-type GroEL/GroES. PRD1 was grown on these strains at +37° and at +40° (Table 1). At 37° the only effect detected was a diminished plaque size on strains DW717 *groEL59* (pLM2) and DW719 *groES619* (pLM2) and somewhat lowered titer on DW717 (pLM2). At 40° all the mutant strains except DW715 *groEL764* (pLM2) showed considerably reduced titers (up to seven logs) and reduced plaque sizes. To verify the cause of the observed titer reduction, wild-type *groEL* and *groES* genes were cloned to the low copy plasmid pSU18 (*23*) by PCR from *E. coli* chromosome or plasmid pOF39 (*24*), respectively. The resulting plasmids, pJBEL6 and pJBES4, were transformed to the corresponding mutant strains and the ability of these strains to support PRD1 plaque formation was tested at +40° (Table 1). In all of the cases the *wt* gene rescued the mutant effect. However, in the presence of the *wt groEL* gene in DW715 *groEL764* (pLM2) the PRD1 titer was somewhat lower than in the absence of the *wt* gene.

Thin section electron microscopy of the infected (at +40°) mutant strains indicated that the number of intracellular particles, when compared to the infected *wt* strain, was reduced about hundred-fold, much less than expected on the basis of the reduced titers. The morphology of the particles formed in mutant strains did not differ from that of the wild-type PRD1 (not shown). We examined in SDS–PAGE (*25*) the protein composition of PRD1 particles formed in *E. coli* strains DW720 (pLM2), DW717 *groEL59* (pLM2), and DW719 *groES619* (pLM2) (Fig. 1). Cells were infected with wild-type PRD1 using multiplicity of infection (m.o.i.) of about 40 and the cultures were grown at +40°. To remove nonadsorbed phage particles, the cells were washed 15 min after infection (3000 rpm, 5 min, Sorvall GSA rotor, +30°, 50 m*M* Tris–HCl, pH 7.4, 150 m*M* NaCl). After washing, the incubation was continued in the original culture volume and medium at +40° until lysis occurred. Virus particles were purified by rate zonal sucrose gradient centrifugation followed by an ion exchange method as described by Walin *et al.* (*26*). Although this extensive purification method was used, the virus material obtained was not as pure as the particles obtained using *S. typhimurium*

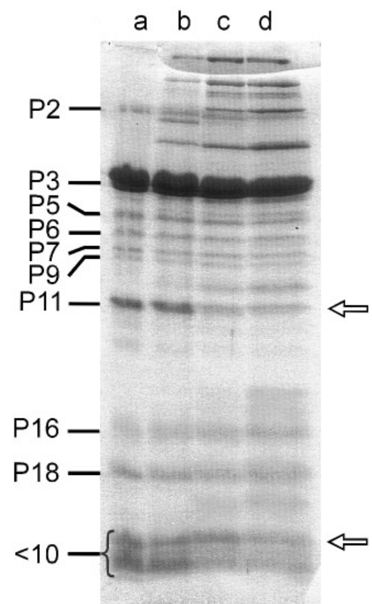

FIG. 1. SDS–PAGE of purified PRD1 particles produced in different GroE host strains, lane a. *S. typhimurium* DS88, a control, lane b. *E. coli* DW720(pLM2), *wt* GroEL/ES, lane c. *E. coli* DW717 *groEL59*(pLM2), lane d. *E. coli* DW719 *groES619* (pLM2). The PRD1 proteins are indicated at the left. The arrows indicate the positions of reduced protein band intensities.

DS88 strain (*27*). This was due to the very low yield of particles produced in the *E. coli* strains and the presence of flagellar proteins. The contaminant protein bands, however, did not prevent us from observing differences between samples purified from *groE* mutant hosts and those purified from the *wt* host (see arrows in Fig. 1). Intensity was clearly diminished in at least two positions in the PRD1 protein pattern. The amount of protein P11, which is located between the membrane and the capsid and causes the membrane to aggregate (*15, 28*) was reduced to about one-fifth of the *wt* level. In addition the signal was much weaker at the position of a number of small membrane bound proteins with molecular weights below 10 kDa (*15, 29*).

The influence of *groE* mutant hosts on the multimerization and aggregativity of the PRD1 capsid proteins was studied by SDS–PAGE and Western blot analysis of the infected cells. Mutant strains were grown at +37° and infected with wild-type PRD1. The cells were collected at 10-min intervals and lysed by sonication. The P3 and P5 homomultimers are dissociated only after boiling (*15, 27*). The migration of unboiled capsid proteins in SDS–PAGE reveals their multimeric status. Two different protein gel samples in the standard sample buffer (*25*) were prepared in each case: one was boiled before SDS–PAGE, and the other one was not. The protein bands were identified by Western blot analysis using P3-specific antibody N180 (*30*) and polyclonal anti-P5 serum (*31*). Multimers of the capsid proteins P3 and P5 could be detected in the absence of functional GroEL or GroES, but the amount of monomeric protein increased at the late stages of infection in $GroEL^{-}/ES^{-}$ hosts (shown for P3 in Fig. 2). In another experiment the distribution of the capsid proteins between the aggregative and soluble fraction of disrupted cells was investigated. The proportion of the aggregative P3 and P5 was found to be increased compared to the wild-type situation, and practically all P3 and P5 in the aggregated fractions of the mutant infections was in a monomeric form as analyzed by boiling/nonboiling SDS–PAGE (not shown).

We showed here that both GroEL and GroES are essential for PRD1 assembly. As bacteriophage T4 needs only the GroEL counterpart of the GroEL/GroES complex and encodes a functional analogue, gp31, to GroES cochaperonin (*32*), we tested whether the multimeric (unpublished results) PRD1 assembly factor P17 could also substitute for GroES. The results from the ATPase assays carried out as described earlier (*33*), cosedimentation and complementation tests with GroEL and P17 did not support this hypothesis (not shown).

There seems to be several processes in PRD1 assembly that depend on GroEL and GroES. The correct folding and the following trimerization of capsid proteins P3 and P5 does not occur efficiently if either of the *groE* genes is deficient. Also the assembly of protein P11 and small membrane protein(s) to the particle is affected in the *groE*

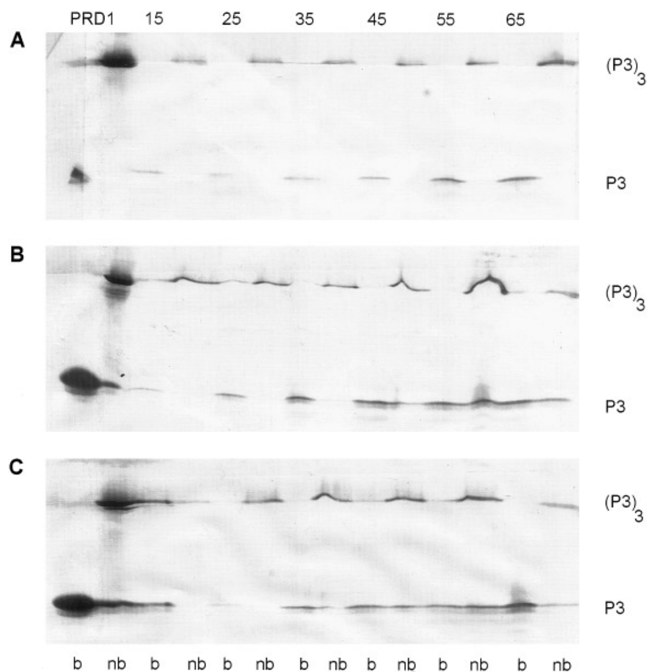

FIG. 2. Western blots detecting P3 monomers and multimers in PRD1-infected cells collected 15, 25, 35, 45, 55, and 65 min postinfection. Two samples from each time point were analyzed by SDS–PAGE: one was boiled (b) and the other was not boiled (nb). Three different strains were used as hosts. (A) DW720(pLM2) (B) DW717(pLM2) *groEL59* (C) DW719(pLM2) *groES619*.

hosts. The reduction of the PRD1 titer in the absence of GroEL/GroES was much more severe than the effect seen on particle formation. The misfolding of the capsid proteins reduce the particle formation, whereas the reduction of protein P11 in the formed particle affects the infectivity (*34*). This explains the observed differences in the titers and numbers of particles detected. It is intriguing that GroEL/ES is involved in the folding/assembly pathway of the viral membrane proteins.

ACKNOWLEDGMENTS

We are grateful to Ms. Marja-Leena Perälä for her excellent technical assistance. This investigation was supported by a research grant from the Finnish Academy of Sciences (D.H.B.).

REFERENCES

1. Casjens, S., and Hendrix, R., *in* "The Bacteriophages" (R. Calendar, Ed.), Vol. 1, pp. 15–91. Plenum, New York, 1988.
2. Hendrix, R. W., and Garcea, R. L., *Semin. Virol.* **5**, 15–26 (1994).
3. Olsen, R. H., Siak, J., and Gray, R. H., *J. Virol.* **14**, 689–699 (1974).
4. Lyra, C., Savilahti, H., and Bamford, D. H., *Mol. Gen. Genet.* **228**, 65–69 (1991).
5. Bamford, D. H., and Mindich, L., *J. Virol.* **44**, 1031–1038 (1982).
6. Bamford, J. K. H., Hänninen, A.-L., Pakula, T. M., Ojala, P. M., Kalkkinen, N., Frilander, M., and Bamford, D. H., *Virology* **183**, 658–676 (1991).
7. Bamford, D. H., and Mindich, L., *J. Virol.* **50**, 309–315 (1984).
8. Savilahti, H., and Bamford, D. H., *Gene* **49**, 199–205 (1986).

9. Savilahti, H., Caldentey, J., Lundström, K., Syväoja, J. E., and Bamford, D. H., *J. Biol. Chem.* **266,** 18737–18744 (1991).
10. Caldentey, J., Blanco, L., Bamford, D. H., and Salas, M., *Nucleic Acids Res.* **21,** 3725–3730 (1993).
11. Davis, T. N., Muller, E. D., and Cronan, J. E. Jr., *Virology* **120,** 287–306 (1982).
12. Butcher, S. J., Bamford, D. H., and Fuller, S. D., *EMBO J.* **14,** 6078–6086 (1995).
13. Tuma, R., Bamford, J. K. H., Bamford, D. H., Russell, M. P., and Thomas, G. J. Jr., *J. Mol. Biol.* **257,** 87–101 (1996).
14. Tuma, R., Bamford, J. K. H., Bamford, D. H., and Thomas, G. J. Jr., *J. Mol. Biol.* **257,** 102–115 (1996).
15. Mindich, L., Bamford, D. H., McGraw, T., and Mackenzie, G., *J. Virol.* **44,** 1021–1030 (1982).
16. Bamford, D. H., Caldentey, J., and Bamford, J. K. H., *Adv. Virus Res.* **45,** 281–319 (1995).
17. Georgopoulos, C., and Welch, W. J., *Annu. Rev. Cell Biol.* **9,** 601–634 (1993).
18. Craig, E. A., Weissman, J. S., and Horwich, A. L., *Cell* **78,** 365–372 (1994).
19. Georgopoulos, C. P., Hendrix, R. W., Casjens, S. R., and Kaiser, A. D., *J. Mol. Biol.* **76,** 45–60 (1973).
20. Martin, J., Langer, T., Boteva, R., Schramel, A., Horwich, A. L., and Hartl, F. U., *Nature* **352,** 36–42 (1991).
21. Weissman, J. S., Kashi, Y., Fenton, W. A., and Horwich, A. L., *Cell* **78,** 693–702 (1994).
22. Mindich, L., Cohen, J., and Weisburd, M., *J. Bacteriol.* **126,** 177–182 (1976).
23. Bartolomé, B., Jubete, Y., Martínez, E., and de la Cruz, F., *Gene* **102,** 75–78 (1991).
24. Fayet, O., Louarn, J.-M., and Georgopoulos, C., *Mol. Gen. Genet.* **202,** 435–445 (1986).
25. Olkkonen, V. M., and Bamford, D. H., *Virology* **171,** 229–238 (1989).
26. Walin, L., Tuma, R., Thomas, G. J. Jr., and Bamford, D. H., *Virology* **201,** 1–7 (1994).
27. Bamford, J. K. H., and Bamford, D. H., *Virology* **177,** 445–451 (1990).
28. Luo, C., Hantula, J., Tichelaar, W., and Bamford, D. H., *Virology* **194,** 570–575 (1993).
29. Caldentey, J., Luo, C., and Bamford, D. H., *Virology* **194,** 557–563 (1993).
30. Bamford, J. K. H., Luo, C., Juuti, J. T., Olkkonen, V. M., and Bamford, D. H., *Virology* **197,** 652–658 (1993).
31. Hänninen, A.-L., Bamford, J. K. H., and Bamford, D. H., *Virology* **227,** 198–206 (1996).
32. van der Vies, S. M., Gatenby, A. A., and Georgopoulos, C., *Nature* **368,** 654–656 (1994).
33. Viitanen, P. V., Lubben, T. H., Goloubinoff, P., O'Keefe, D. P., and Lorimer, G. H., *Biochemistry* **29,** 5665–5671 (1990).
34. Mindich, L., Bamford, D. H., Goldthwaite, C., Laverty, M., and Mackenzie, G., *J. Virol.* **44,** 1013–1020 (1982).