JOURNAL OF BACTERIOLOGY, Nov. 2003, p. 6409–6414 Vol. 185, No. 21
0021-9193/03/$08.00+0 DOI: 10.1128/JB.185.21.6409–6414.2003
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Analysis of Specific Binding Involved in Genomic Packaging of the Double-Stranded-RNA Bacteriophage φ6

Xueying Qiao, Jian Qiao, and Leonard Mindich*

Department of Microbiology, Public Health Research Institute, Newark, New Jersey 07103

Received 22 May 2003/Accepted 12 August 2003

The genomes of bacteriophage φ6 and its relatives are packaged through a mechanism that involves the recognition and translocation of the three different plus-strand transcripts of the segmented double-stranded-RNA genomes into preformed polyhedral structures called procapsids or inner cores. The packaging requires the hydrolysis of nucleoside triphosphates and takes place in the order segment S-segment M, segment L. Packaging is dependent upon unique sequences of about 200 nucleotides near the 5′ ends of plus-strand transcripts of the three genomic segments. It appears that P1 is the determinant of the RNA binding sites. Directed mutation of P1 was used to locate regions that are important for genomic packaging. Specific binding of RNA to the exterior of the procapsid was dependent upon ATP, and a region that showed a high level of cross-linking to phage-specific RNA was located. Antibodies to peptide sequences were prepared, and their abilities to bind to the exterior of procapsids were determined. Sites sensitive to trypsin and to factor Xa were determined as well.

The genomes of bacteriophage φ6 and its relatives are packaged through a mechanism that involves the recognition and translocation of the three different plus-strand transcripts of the segmented double-stranded-RNA (dsRNA) genomes into preformed polyhedral structures called procapsids or inner cores. The packaging requires the hydrolysis of nucleoside triphosphates and takes place in the order segment S-segment M-segment L (10) (Fig. 1). Minus-strand synthesis begins after the completion of plus-strand packaging (3). The packaging and replication reactions can be studied in vitro with purified components. The packaging of plus strands is dependent upon a region called the *pac* sequence and located near the 5′ ends of the molecules. This region contains approximately 200 nucleotides and has an extensive secondary structure (4). There is little or no similarity among the *pac* sequences of the three genomic segments. There is an 18-base consensus sequence at the very 5′ end of the plus strands, but part of this is expendable, without prejudice for packaging, and the consensus sequence is involved primarily in the determination of plus-strand transcription.

In this study, we prepared mutations in the 769-amino-acid-containing major structural protein of procapsid P1. Procapsids formed by the mutant proteins were tested for competence in genomic packaging. Plasmids containing cDNA copies of the entire genomic segment L were used to prepare live phage so as to test the ability of the mutated proteins to function in the viral infection pathway and to isolate suppressor mutants, if possible. The results of this study were compared with those of previous studies in which the *pac* regions of genomic segments S and M were mutated and suppressor mutations were found in protein P1.

In order to determine the parts of P1 that are accessible to the exterior, we treated procapsids with trypsin and factor Xa and located sites in intact particles that were particularly sensitive. Antibodies prepared against peptides of P1 were tested for their abilities to bind to procapsids.

RNA binding to the exterior of procapsids had been demonstrated earlier (8). We found that the binding had the same specificity as the packaging reactions (19). In the present study, we reexamined direct binding to procapsids and found that ATP is necessary for specific binding and that cross-linking of bound RNA is localized to a small region of P1.

MATERIALS AND METHODS

Bacterial strains and plasmids. *Pseudomonas syringae* strain HB10Y was the host for φ6 (23). *Escherichia coli* strain JM109 [*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* λ^- Δ(*lac-proAB*)] (F′ *traD36 proAB lacI*^{q}ΔM15) (25) was used for the propagation of all plasmids and for the production of procapsids. Epicurian Super Competent cells of *E. coli* were used for transformation after directed mutagenesis. Plasmids pLM659, pLM656, and pLM687 contain cDNA copies of genomic segments S, M, and L, respectively, in vector pT7T319U (14). Plasmid pLM1157 contains a cDNA copy of segment L with a deletion of the *Xho*I fragment from positions 545 to 5366. Its transcript is about 1.5 kb and is packaged more efficiently than the normal L segment in vitro. Other plasmids used are listed in Table 1.

Directed mutagenesis. Oligonucleotides of about 30 bases (see Table 6) were prepared by MWG Biotech and used in the Quick Change procedure (Stratagene, La Jolla, Calif.), which involves PCR on a plasmid template and subsequent treatment with *Dpn*I to eliminate the template DNA. The template was usually plasmid pLM1009, which is vector pT7T319U carrying a cDNA copy of φ6 gene 1. Mutated gene 1 was then inserted into pLM1403, a plasmid containing a cDNA copy of the entire genomic segment L. The resulting plasmid could then be used for the production of procapsids or for the preparation of live phage containing mutated gene 1. The latter was accomplished by electroporating the plasmid into cells of HB10Y and then infecting them with φ1980, a derivative of φ6 that is missing genes 7, 2, and 4 (16). The virus is able to acquire the plasmid transcript as a replacement for deleted segment L.

Preparation of procapsids. Procapsids were isolated from *E. coli* JM109. They were purified by sucrose gradient centrifugation from French press lysates produced at 7,000 lb/in^2 as described by Gottlieb et al. (5). Purified procapsids were divided into aliquots and frozen at −70°C. Aliquots were thawed immediately prior to use.

In vitro synthesis of radioactive plus-sense transcripts by T7 RNA polymerase. Plasmids derived from cDNA copies of genomic segments in pT7T319U were cut with restriction endonuclease *Xba*I. The resulting 5′ overhang was removed with mung bean nuclease before transcription with T7 RNA polymerase (14). This procedure generated 3′ ends of the transcripts identical to that of the φ6 virus-

* Corresponding author. Mailing address: Public Health Research Institute, 225 Warren St., Newark, NJ 07103. Phone: (973) 854-3420. Fax: (973) 854-3421. E-mail: mindich@phri.org.

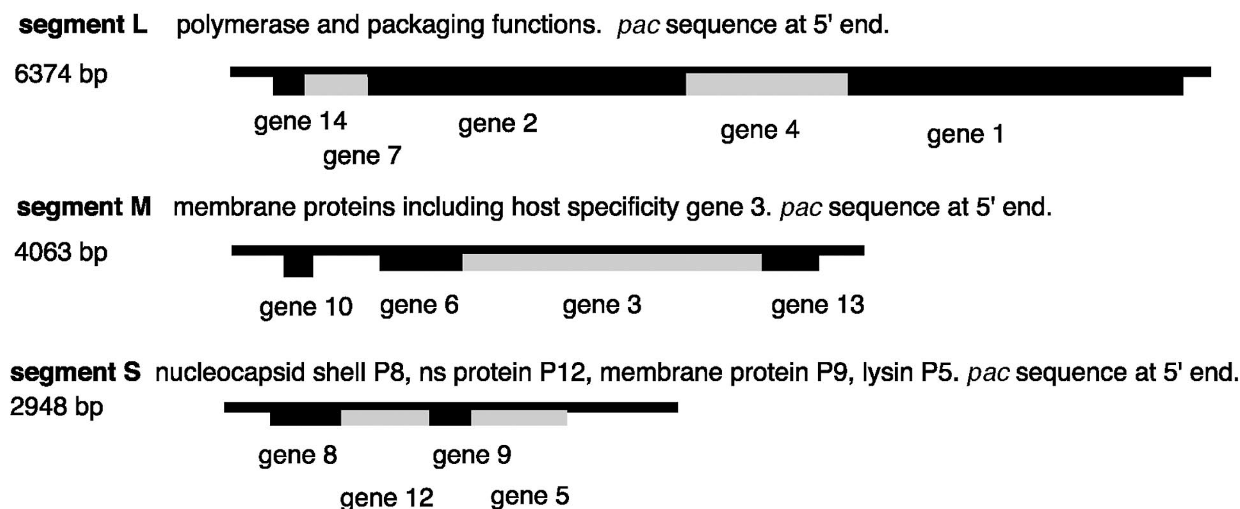

FIG. 1. Physical map of genomic segments of bacteriophage ϕ6. ns, nonstructural.

produced mRNA. The polymerase reaction mixture contained 2 mM each of UTP, ATP, GTP, and CTP and 400 μCi of α-^{32}P-labeled UTP per ml. The RNA was purified by phenol extraction prior to filtration through G-50 Sephadex spin columns (Boehringer Mannheim).

RNA polymerase reaction conditions. Conditions for minus-strand synthesis were similar to those reported previously (6), except that the pH of the reaction buffer was 8.9 (3). Equimolar amounts of each segment were used, and the total RNA concentration in a 12.5-μl reaction volume was about 120 μg/ml. The reaction was stopped by adding 3× sample buffer (22) and EDTA to final concentrations of 10 mM. The reaction products were analyzed with 1 μg of carrier RNA on 1% agarose gels containing 0.1% sodium dodecyl sulfate in 0.5× Tris-borate-EDTA buffer; the samples were subsequently dried, and the ^{32}P-labeled RNA was visualized after autoradiography with a Cronex enhancing screen.

Packaging reaction conditions. Frozen purified procapsid preparations were thawed and incubated for 60 min at 28°C in a 12.5-μl packaging reaction mixture consisting of 50 mM Tris-Cl (pH 8.9), 3 or 4 mM $MgCl_2$, 100 mM ammonium acetate, 20 mM NaCl, 5 mM KCl, 5 mM dithiothreitol, 0.1 mM disodium EDTA, 1 mM ATP, 100 ng of Macaloid, 5% polyethylene glycol 4000, and about 150 ng of ^{32}P-UTP-labeled single-stranded ϕ6 RNA for each segment. Approximately 1 μg of procapsid was used per reaction. The samples were then treated with 10 U of RNase I (Promega) (9) and incubated for 30 min at 28°C. Ten microliters of stop solution (3× sample buffer [22] and 25 mM EDTA) was added, and the samples were heated at 85°C for 5 min. The samples were then electrophoresed in 1.5 or 2% agarose gels as described above.

Specific binding of RNA to procapsids. Procapsids were incubated in a mixture similar to that used for genomic packaging. Approximately 1.5 μg of procapsid and 0.2 μg of ^{32}P-labeled RNA were incubated at 28°C for 60 min in a volume of 12.5 μl. ATP was used at 5 mM, and magnesium was used at 3 mM. The samples were then filtered through Millipore HAWP02500 paper and washed with 50 mM Tris (pH 8.9)–100 mM ammonium acetate–3 mM magnesium chloride. The filters were assayed by scintillation counting. Transcripts were prepared from plasmids pLM1772, pLM1773, pLM1454, pLM786, and pLM794. None of these transcripts are packaged because they have deletions in the region 5′ to the *pac* sequences or, in the case of pLM1454, the *pac* sequence is absent. However, the transcript of pLM1772 competes for the packaging of segment S, and that of pLM794 competes for the packaging of segment M. The transcripts of pLM1773 and pLM786 have larger deletions and do not compete for packaging, although they have the *pac* sequences of segments S and M, respectively.

Cross-linking of RNA to procapsids. Procapsids were incubated with labeled RNA as described for the binding assay, except that the RNA synthesis mixture included α-^{32}P-labeled CTP at 0.1 mM and the other nucleotides at 1 mM, including 4-thio-UTP instead of UTP. The samples were incubated for 30 min at 28°C and then irradiated for 30 min with 360-nm-wavelength light. The samples were then treated with RNase A, mixed with cracking buffer (22), boiled for 2 min, and applied to acrylamide gels. The location of cross-linked RNA on protein P1 was determined by first locating the labeled fragment resulting from factor Xa treatment and trypsin digestion. Final location was done by cleaving at cysteine residues with 2-nitro-5-thiocyanobenzoic acid (TNCBA) (24). Cutting was done with 200 mM Tris (pH 9)–8 M urea and 10 mg of TNCBA per ml. The samples were incubated for several days at 37°C and then analyzed by electrophoresis and autoradiography. Radioactivity was not reduced by the loss of the N-terminal peptide that extended to cysteine at position 98 but was completely reduced by the loss of the peptide from positions 98 to 155.

Treatment of procapsids with antibodies. Antibodies against synthetic peptides were prepared from rabbits (see Table 5) by Research Genetics and Sigma. Magnetic beads coated with protein A were purchased from Dynal A.S., Oslo, Norway. The beads were incubated with antibodies and washed. The antibody-coated beads were incubated with procapsids, washed with 0.1 M sodium phosphate buffer (pH 8.1), collected, and treated with cracking buffer to isolate the bound proteins. The proteins were analyzed by acrylamide electrophoresis and staining. The locations of the binding sites of the polyclonal and monoclonal antibodies were determined by analysis of Western gels with cell extracts containing truncated molecules of protein P1. The truncated molecules were expressed by *E. coli* cells containing plasmids that had 3′-terminal deletions in gene 1.

RESULTS

Accessibility of P1 to proteases. There is one factor Xa (11)-sensitive site in P1, and it can be cleaved in intact procapsids, suggesting that it is near the exterior of the procapsid. This site is IDGR starting at position Ile663. Limited trypsin digestion of procapsids produced a cut at position Arg276 (Fig. 2).

Specific binding and cross-linking of RNA to procapsids. RNA was previously reported to bind to procapsids (8). The binding was ϕ6 specific but did not differentiate among the

TABLE 1. Plasmids used in this study

Plasmid	Vector	Comment[a]
pLM659	pT7T319U	cDNA of segment S
pLM656	pT7T319U	cDNA of segment M
pLM682	pT7T319U	cDNA of segment L
pLM1157	pT7T319U	cDNA of segment L with deletion
pLM1403	pT7T319U	cDNA of segment L for constructions
pLM1009	pT7T319U	cDNA of gene 1
pLM1772	pT7T319U	Segment S del 11–32; competes with wild-type segment S
pLM1773	pT7T319U	Segment S del 11–43; poor competitor
pLM1454	pT7T319U	No *pac* sequence
pLM786	pT7T319U	Segment M del 23–70; poor competitor
pLM794	pT7T319U	Segment M del 11–43; competes with wild-type segment M

[a] Segments with deletions are indicated as follows: Segment S del 11–32 indicates segment S with a deletion of positions 11 to 32.

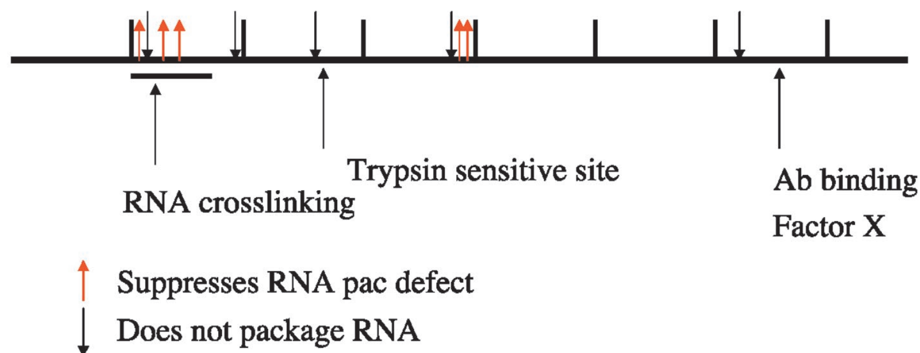

FIG. 2. Distribution on procapsid major structural protein P1 of the RNA cross-linking site and the sites for protease sensitivity, antibody binding, mutations that do not package RNA, and mutations that suppress RNA *pac* defects.

three segments. A problem in studying binding is that, in the presence of ATP, plus strands are transported into procapsids. We had dealt with that problem in a previous study by constructing plus-strand molecules that had deletions between the 18-base consensus sequences at the 5′ ends and the *pac* sequences, which are about 50 bases away. A deletion of nucleotides 11 to 32 in segment S resulted in a transcript of pLM1772 that inhibited the packaging of normal plus strands of segment S (19) but that was not itself packaged (Table 1). In the present study, we found that the binding of φ6 RNA to procapsids is specific in the presence of ATP (Table 2). In the absence of ATP, binding is nonspecific, and heterologous RNA can bind with a greater affinity than φ6 RNA. However, in the presence of 1 or 5 mM ATP, the nonspecific binding is greatly reduced and the binding of the transcript of pLM1772 clearly is preferred (Table 2). This binding is subject to competition by a cold transcript of the same plasmid but not by the transcript of pLM1454, which lacks both the 5′-terminal consensus sequence and the *pac* sequence (Table 3). ADP does not replace ATP for the promotion of specific binding (Table 2), and 5′-adenylimido diphosphate (AMP-PNP) and βγ-methylene adenosine triphosphate (AMP-PCP) have little effect. GTP does, however, substitute for ATP. The transcript of pLM1773, which has a slightly larger deletion of nucleotides 11 to 43 in the plus strand of segment S, binds to a lesser extent than that of pLM1772. The transcript of pLM1773 does not compete for normal packaging (19). The transcript of pLM794, which contains the plus strand of segment M with a deletion of nucleotides 11 to 43 and which competes for the packaging of segment M, does not compete effectively with that of pLM1772 and does not bind to procapsids effectively in the presence of ATP. These findings are consistent with the finding that empty procapsids package plus strands of segment S before those of segment M. The transcript of pLM786, which contains the plus

TABLE 2. Binding of RNA to procapsids

Transcript	Comment[a]	ATP	Counts bound (cpm)	% of total counts
pLM1772	Segment S del 11–32	−	45,692	32
	Segment S del 11–32	+	7,680	5.4
	Segment S del 11–32	− (ADP)	47,533	34
pLM1773	Segment S del 11–43	−	36,480	24
	Segment S del 11–43	+	2,627	1.8
pLM1454	Segment No *pac*	−	27,129	21
	Segment No *pac*	+	556	0.4
	Segment No *pac*	− (ADP)	27,913	21

[a] See Table 1, footnote *a*.

TABLE 3. Competition with binding of RNA to procapsids with ATP

Labeled transcript	Competing transcript (20-fold)	Comment regarding competitor[a]	Counts bound (cpm)	% of value obtained with no competition
pLM1772			6,226	100
	pLM1772	Segment S del 11–32	367	6
	pLM1773	Segment S del 11–43	2,790	45
	pLM786	Segment M del 23–70	8,651	139
	pLM794	Segment M del 11–43	8,252	133
	pLM1454	Segment No *pac*	7,889	127
pLM1773			3,458	100
	pLM1772	Segment S del 11–32	239	7
	pLM1773	Segment S del 11–43	745	22
	pLM794	Segment M del 11–43	1,544	45
	pLM1454	Segment No *pac*	3,072	89
pLM794			1,089	100
	pLM1772	Segment S del 11–32	192	18
	pLM1773	Segment S del 11–43	267	25
	pLM794	Segment M del 11–43	247	23
	pLM1454	Segment No *pac*	1,426	131

[a] See Table 1, footnote *a*.

TABLE 4. Binding of RNA to procapsids with a suppressor mutation in P1 (E390A)

Labeled transcript	ATP	Competing transcript (20-fold)	Counts bound (cpm)
pLM1772 (S*pac*)	−		38,280
	+		21,847
	+	pLM1772	1,338
	+	pLM794	7,770
	+	pLM1454	15,330
pLM794 (M*pac*)	−		17,602
	+		13,405
	+	pLM1772	6,894
	+	pLM794	833
	+	pLM1454	9,377

strand of segment M with a larger deletion before the *pac* sequence, is also a poor competitor for that of pLM1772.

In a previous study, we found that a change in the *pac* sequence of segment S could be suppressed by a mutation in gene 1 that resulted in the amino acid change E390A. Procapsids that contain this change are able to package the plus strand of segment S with the change GC99AU; however, packaging of the plus strand of segment S was found to be competed with by the plus strand of segment M. The binding properties of segments S and M (Table 4) were found to be similar to the properties found previously for packaging. These results reinforce the idea that the properties of binding of RNA to the procapsid exterior are identical to the properties of packaging.

Cross-linking of RNA to P1. Transcripts of plasmid pLM1772 were prepared with α-^{32}P-labeled CTP at 0.1 mM and the other nucleotides at 1 mM, including 4-thio-UTP instead of UTP. This RNA was found to bind to procapsids and to cross-link easily with 360-nm UV light, although cross-linking occurred even in the absence of UV treatment. After treatment with RNase A, cross-linking was found for proteins P1, P2, P4, and P7, although P1 cross-linking was the most intense. Cross-linking to P4 could be reduced by competition with tRNA. The location of the cross-linked RNA on P1 was first determined by treatment with factor Xa, which showed that the label was located on the first 666 amino acids. Trypsin digestion indicated that the label was located on the first 276 amino acids of P1. Since P1 contains three cysteines and they are located near the N terminus of the protein, we used cleavage with TNCBA (21, 24). The cysteines are located at positions 98, 155, and 189. The cross-linked RNA was found to be located on the fragment between positions 98 and 155 (Fig. 2).

Accessibility of P1 in procapsids to antibodies. Antibodies were prepared against peptide sequences in P1 (Table 5). Although all showed significant activity in Western analysis, none showed binding to intact procapsids. A polyclonal antibody against φ6 and monoclonal antibodies against P1 reacted with procapsids, and the dominant antigen site for both was found to be at positions 663 to 671, superimposable on the factor Xa-sensitive site. It is noteworthy that mutations in that region have no effect on plaque formation or genomic packaging (Fig. 2).

Preparation of mutations in gene 1, coding for the major structural protein of the procapsid. Directed mutagenesis was used to change amino acids in P1. The targeted sites were generally charged, hydrophobic, or in the vicinity of suppressor mutations that allowed packaging of plus strands with changes in their *pac* sequences. The changes are listed in Table 6, along with the suppressor mutations found previously. The suppressor mutations are designated S*pac* or M*pac*. Mutated gene 1 was tested in two ways. In both situations, the mutated gene was reincorporated into plasmids containing cDNA copies of the entire genomic segment L. These plasmids were then used to direct the synthesis of procapsids in *E. coli* and to produce live phage by transcript acquisition with φ1980, a derivative of φ6 that has a deletion of genes 7, 2, and 4. It was therefore possible to assess the ability of mutated P1 to participate in in vitro genomic packaging and to determine the plaque-forming ability of the virus with a P1 mutation. In addition, it was possible to screen for suppressor mutations that compensate for the amino acid changes in P1.

About half the mutants were unable to package measurable amounts of RNA in vitro or to produce plaques (Table 6 and Fig. 2). A few of the mutants, particularly near the C terminus, were able to package RNA in vitro and to form plaques. Mutants that either did not appear to form plaques or had poor plaque-forming ability produced suppressor mutations that resulted in good plaque formation. The suppressor mutations were not necessarily near the original mutations. A number of procapsids with packaging defects due to amino acid changes were also tested for the binding of segment S plus strands. Those with the substitutions WR103VA and RR617AA were unable to bind RNA in the presence of ATP, while procapsids with the substitution R385A, which resulted in poor packaging, showed poor RNA binding. Similar results were obtained with cross-linking of RNA, in that the two procapsids that showed no packaging did not show significant cross-linking, while the one that showed poor packaging did cross-link to RNA but at a low level compared to that seen with the wild type.

TABLE 5. Properties of antibodies against protein P1

Peptide against which antibody was tested or antibody	First amino acid	Western analysis result	Binding to procapsids	Source
NPEIWRKLTAYITGS	99	+	No	Research Genetics
LEQLRTLAPSEHELF	133	++	No	Research Genetics
PKELDPSARLRNTNC	266	+	No	Sigma
PFKLRPINETTSYIC	330	++	No	Sigma
LANNSNQRFLDVEPG	378	+++	No	Research Genetics
LGQRRERVRILKPTV	614	+	No	Research Genetics
Complete anti-φ6 antibody, Ile663–Ala671	663	++	Yes	Public Health Research Institute or University of Nebraska
Antibody 1K$_3$, Ile663–Ala671	663	++	Yes	D. H. Bamford (15)

TABLE 6. Packaging behavior of mutants of structural protein P1

Oligonucleotides used	Base changes		Amino acid changes	Packaging	Plaque formation	Second-site revertants	Suppressor(s)
	Wild type	Change					
572 and 573	TGGCGCAAG	GTGGCCAAG	WR103VA	No	No		
574 and 575	CGTCGTATG	GCTGCTATG	RR196AA	No	Poor	Yes	T316I, A402T
601 and 602	GAGCTCGAC	GCGCTCGCC	ELD268ALA	No	No		
599 and 600	CGTTTGCGC	GCTTTGGCC	RLR274ALA	No; no procapsids	No		
505 and 506	CAGCGTTTC	CGGGCTTTC	QR384RA		No		
473 and 474	CGTTTCCTG	AAGTTCCTG	R385K	Yes	Yes		
475 and 476	CGTTTCCTG	GCTTTCCTG	R385A	Poor	No		
576 and 577	TACTACGCG	GCCGCGGCG	YY486AA		Poor		
578 and 579	CTGAGCCGT	CGGCGGCGT	EP544AA	No procapsids	Poor	EA normal	
500 and 501	AAACGCTAC	GCAGCCTAC	KR600AA	Yes	Poor	Yes	E590K
498 and 499	CGTCGCGAA	GCTGCCGAA	RR617AA	No	Poor	Yes	Y486C, Y486S
502 and 503	CGTCGCACG	GCTGCCACG	RR651AA	Yes	Yes		
562 and 563	CGTCGTATG	GCTGCTATG	RR666AA	Yes	Yes		
566 and 567	CAAAACGCT	GCGGCCGCT	QN669AA	Yes	Yes		
M*pac*		GC142AU		Yes	Yes		E101G
M*pac*		GC142AU		Yes	Yes		E133G
S*pac*		GC99AU		Yes	Yes		E142G
M*pac*		GC142AU		Yes	Yes		Q384K
M*pac*		GC142AU		Yes	Yes		Q384R
S*pac*		GC99AU		Yes	Yes		E390A

DISCUSSION

Bacteriophage φ6 contains three dsRNA genomic segments (20). Packaging of the genome involves serial dependent packaging of plus-strand transcripts of the segments in the order segment S-segment M-segment L (10). The specificity of packaging is determined on the exterior of the procapsid (10). Previous studies from our laboratory have implicated only protein P1, the major structural protein of the procapsid, in the specificity of RNA binding. Packaging specificity is determined by sequences of about 200 nucleotides near the 5′ ends of the plus strands (4). These *pac* sequences show a striking secondary structure (10, 17). Small changes in the *pac* sequences of φ6 have drastic effects on the packaging process (18). We have shown in this study that procapsids demonstrate specific binding of RNA in the presence of ATP. It is not clear what the role of ATP is in the binding, but there have been indications that ATP does cause conformational changes in the core of φ6 (13). In any case, the plus strand of genomic segment S binds to a limited region of P1, and this is also a region in which changes lead to a suppression of packaging defects due to changes in the RNA *pac* sequences and in which some amino acid changes lead to a loss of packaging activity. Amino acid changes that disrupt packaging were shown to reduce the binding of RNA to procapsids. At the present time, we do not know whether the changes occur in the binding sites or in regions that perturb the binding sites. It is noteworthy that binding activity is highest for the plus strand of segment S and that this binding is not competed for by the plus strand of segment M. In addition, it is not competed for by tRNA or other nonspecific RNAs. These findings are consistent with our packaging model and in contrast to the proposal by Juuti and Bamford (8) that segment specificity is determined at a stage later than initial binding. We have shown that binding specificities are markedly different depending upon the presence of ATP and that nonhydrolyzable analogs of ATP do not suffice to produce specific binding.

Packaging specificity in single-stranded RNA viruses has been shown to involve the binding of genomic RNA to sites on the capsid protein that promote the formation of a capsid around the RNA (2). The binding sites are on the interior of the capsid. DNA packaging in bacteriophages involves association of the genomic DNA with an enzyme complex called terminase (1). This complex is not a component of the mature virion. The packaging system for the *Cystoviridae* therefore is unique in that it involves selective binding of the plus-strand RNA to the exterior of the procapsid, and the available binding sites change during the packaging process. *Reoviridae* and *Totiviridae* have dsRNA genomes in capsids that show arrangements of the 120 major structural proteins that are strikingly similar to that in the *Cystoviridae*. The mechanism of packaging for the *Reoviridae* is not known, while that for the *Totiviridae* seems to follow the pattern of single-stranded RNA viruses. However, the *Totiviridae* have binding sites on the exterior of the capsid for host mRNA cap structures (12). These sites are not for packaging but for the removal of the cap structure from the host RNA. The atomic structures of the capsids of the *Totiviridae* and some of the *Reoviridae* have been determined (7, 12). Such a determination has not yet been accomplished for φ6 or its relatives.

ACKNOWLEDGMENTS

This work was supported by grant GM34352 from the National Institutes of Health.

We thank Arkady Mustaev for the gift of 4-thio-UTP and for valuable advice on the cross-linking of RNA to proteins.

REFERENCES

1. **Catalano, C. E.** 2000. The terminase enzyme from bacteriophage lambda: a DNA-packaging machine. Cell. Mol. Life Sci. **57:**128–148.
2. **Convery, M. A., S. Rowsell, N. J. Stonehouse, A. D. Ellington, I. Hirao, J. B. Murray, D. S. Peabody, S. E. Phillips, and P. G. Stockley.** 1998. Crystal structure of an RNA aptamer-protein complex at 2.8 Å resolution. Nat. Struct. Biol. **5:**133–139.
3. **Frilander, M., P. Gottlieb, J. Strassman, D. H. Bamford, and L. Mindich.** 1992. Dependence of minus-strand synthesis upon complete genomic packaging in the double-stranded-RNA bacteriophage φ6. J. Virol. **66:**5013–5017.

4. **Gottlieb, P., X. Qiao, J. Strassman, M. Frilander, and L. Mindich.** 1994. Identification of the packaging regions within the genomic RNA segments of bacteriophage φ6. Virology **200:**42–47.
5. **Gottlieb, P., J. Strassman, D. H. Bamford, and L. Mindich.** 1988. Production of a polyhedral particle in *Escherichia coli* from a cDNA copy of the large genomic segment of bacteriophage φ6. J. Virol. **62:**181–187.
6. **Gottlieb, P., J. Strassman, A. Frucht, X. Qiao, and L. Mindich.** 1991. In vitro packaging of the bacteriophage φ 6 ssRNA genomic precursors. Virology **181:**589–594.
7. **Grimes, J. M., J. N. Burroughs, P. Gouet, J. M. Diprose, R. Malby, S. Zientara, P. P. C. Mertens, and D. I. Stuart.** 1998. The atomic structure of the bluetongue virus core. Nature **395:**470–478.
8. **Juuti, J. J., and D. H. Bamford.** 1995. RNA binding, packaging and polymerase activities of the different incomplete polymerase complex particles of dsRNA bacteriophage φ 6. J. Mol. Biol. **249:**545–554.
9. **Meador, J., B. Cannon, V. J. Cannistraro, and D. Kennell.** 1990. Purification and characterization of *Escherichia coli* RNase I: comparisons with RNase M. Eur. J. Biochem. **187:**549–553.
10. **Mindich, L.** 1999. Precise packaging of the three genomic segments of the double-stranded-RNA bacteriophage φ6. Microbiol. Mol. Biol. Rev. **63:**149–160.
11. **Nagai, K., M. F. Perutz, and C. Poyart.** 1985. Oxygen binding properties of human mutant hemoglobins synthesized in Escherichia coli. Proc. Natl. Acad. Sci. USA **82:**7252–7255.
12. **Naitow, H., J. Tang, M. Canady, R. B. Wickner, and J. E. Johnson.** 2002. L-A virus at 3.4 Å resolution reveals particle architecture and mRNA decapping mechanism. Nat. Struct. Biol. **9:**725–728.
13. **Ojala, P. M., A. O. Paatero, and D. H. Bamford.** 1994. NTP binding induces conformational changes in the double-stranded RNA bacteriophage φ 6 subviral particles. Virology **205:**170–178.
14. **Olkkonen, V. M., P. Gottlieb, J. Strassman, X. Qiao, D. H. Bamford, and L. Mindich.** 1990. In vitro assembly of infectious nucleocapsids of bacteriophage φ 6: formation of a recombinant double-stranded RNA virus. Proc. Natl. Acad. Sci. USA **87:**9173–9177.
15. **Olkkonen, V. M., P. M. Pekkala, and D. H. Bamford.** 1988. Monoclonal antibodies to the major structural proteins of bacteriophage φ 6. Virology **165:**317–320.
16. **Onodera, S., X. Qiao, J. Qiao, and L. Mindich.** 1995. Acquisition of a fourth genomic segment in bacteriophage φ 6: a bacteriophage with a genome of three segments of dsRNA. Virology **212:**204–212.
17. **Pirttimaa, M. J., and D. H. Bamford.** 2000. RNA secondary structures of the bacteriophage phi6 packaging regions. RNA **6:**880–889.
18. **Qiao, J., X. Qiao, Y. Sun, and L. Mindich.** Isolation and analysis of mutants with altered packaging specificity in the double-stranded-RNA bacteriophage φ6. J. Bacteriol. **185:**4572–4577.
19. **Qiao, X., J. Qiao, and L. Mindich.** 1997. Stoichiometric packaging of the three genomic segments of dsRNA bacteriophage φ 6. Proc. Natl. Acad. Sci. USA **94:**4074–4079.
20. **Semancik, J. S., A. K. Vidaver, and J. L. Van Etten.** 1973. Characterization of a segmented double-helical RNA from bacteriophage φ 6. J. Mol. Biol. **78:**617–625.
21. **Shin, H. J., C. K. Chong, S. I. Chang, and J. D. Choi.** 2000. Structural and functional role of cysteinyl residues in tobacco acetolactate synthase. Biochem. Biophys. Res. Commun. **271:**801–806.
22. **Studier, F. W.** 1973. Analysis of bacteriophage T7 early RNA's and proteins on slab gels. J. Mol. Biol. **79:**237–248.
23. **Vidaver, A. K., R. K. Koski, and J. L. Van Etten.** 1973. Bacteriophage φ6: a lipid-containing virus of *Pseudomonas phaseolicola*. J. Virol. **11:**799–805.
24. **Wu, J., D. A. Gage, and J. T. Watson.** 1996. A strategy to locate cysteine residues in proteins by specific chemical cleavage followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Anal. Biochem. **235:**161–174.
25. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.