Replication and Packaging of Choleraophage φ149 DNA

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The intracellular replication of the circularly permuted DNA of choleraophage φ149 involves a concatemeric DNA structure with a size equivalent to six genome lengths. The synthesis of both monomeric and concatemeric DNAs during replication of φ149 occurred in the cytoplasm. The concatemers served as the substrate for the synthesis of mature phage DNA, which was eventually packaged by a headful mechanism starting from a unique pac site in the concatemeric DNA. Packaging of DNA into phage heads involved binding of concatemeric DNA to the cell membrane. A scheme involving sequential packaging of five headfuls proceeding in the counterclockwise direction from the pac site is proposed. After infection under high-phosphate conditions, the concatemeric DNA intermediates were not formed, although synthesis of monomeric molecules was unaffected.

MATERIALS AND METHODS

Bacteria and bacteriophage. V. cholerae Ogawa 154, the universal host for the propagation of choleraophages, was used for phage propagation, and the hypertoxigenic strain V. cholerae Inaba 569B was used as the host for phage infection studies. Bacteriophage φ149, belonging to group IV, was obtained from the Cholera Research Centre, Calcutta, India. The efficiencies of plating of phage φ149 on V. cholerae 154 and V. cholerae 569B cells were not significantly different. Cultures were maintained as described previously (9, 18). Cell and phage growths were assayed as CFU and PFU, respectively, on nutrient agar plates. High-titered phage stock and 32P-labeled phage were prepared by infecting V. cholerae 154 cells with φ149 as described previously (17).

Media and buffers. The media used for growing bacteria were prepared as described previously (2, 6, 17). The Tris-Casamino Acid-glucose medium containing 0.04% K2HPO4 used for phage growth will be referred to as LP medium, whereas the Tris-Casamino Acid-glucose medium containing 0.4% KH2PO4 used for cell growth will be termed HP medium. The dilution buffer used contained 50 mM Tris hydrochloride (pH 7.5) and 5 mM MgCl2. Label-terminating buffer (LTB) contained 50 mM Tris hydrochloride (pH 7.5), 5 mM MgCl2, 2 mM NaN3, and 3 mg of thymidine per ml.

Isolation of φ149 DNA. DNA was isolated from purified phage by extraction with phenol saturated with 10 mM Tris–1 mM EDTA buffer (pH 8.0). The aqueous phase was reextracted with a 1:1 mixture of phenol and chloroform and finally with chloroform. The aqueous phase was extensively dialyzed against 10 mM Tris–1 mM EDTA (pH 8.0) buffer.

Preparation of infected cell lysates. Cells in the logarithmic phase of growth (2 × 108 to 3 × 108 CFU/ml) in HP medium were infected with phage φ149 at a multiplicity of infection (MOI) of 10. Two minutes were allowed for adsorption, after which the infected cells were washed and suspended in LP medium. At different times during infection, samples of the infected culture were labeled with 20 μCi of [3H]thymidine (specific activity, 18.8 Ci/mmole; Bhabha Atomic Research Centre, Trombay, India) per ml for the desired length of time. Labeling was terminated by adding an equal volume of

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label-terminating buffer, and the sample was sedimented (10,000 × g, 10 min, 4°C) and then lysed by suspension in a solution (1/20 the culture volume) containing 0.01 M Tris hydrochloride (pH 7.9)-0.01 M EDTA and 1% sodium dodecyl sulfate or 2% Sarkosyl NL97. The suspension was incubated for 30 min at 37°C before being layered on neutral 5 to 20% (wt/vol) sucrose gradients.

Transfer of infected cultures from LP to HP medium. Cells in the midlogarithmic phase of growth were infected with φ149 in LP medium at a MOI of 10. After 15 min at 37°C, KH₂PO₄ was added to the LP medium to give a final concentration of 0.4% phosphate. The pH of the medium was adjusted to 7.5 with NaOH. The culture was then incubated at 37°C, and at different times samples were withdrawn and labeled with [³²P]labeled thymidine for 2 min.

Sucrose gradient centrifugation. Infected cell lysates were analyzed by velocity sedimentation in either neutral or alkaline 5 to 20% (wt/vol) sucrose gradients in a Sorvall AH650 rotor at 30,000 rpm for 150 min at 15°C. Fractions were collected, precipitated with trichloroacetic acid, filtered, washed, dried, and assayed for radioactivity as described previously (5). The molecular weights of the replicative DNA intermediates were estimated from distances sedimented, with labeled φ149 DNA as a marker (1, 3).

Analysis of the fast-sedimenting complex. Cells infected with [³²P]labeled φ149 (specific activity, 4 × 10⁸ PFU/cpm) at a MOI of 10 in LP medium were centrifuged (10,000 × g for 5 min) after 2 min at 4°C, washed with cold medium, and resuspended in fresh prewarmed medium. At different times, samples were withdrawn and labeled with 20 μCi of [³²P]labeled thymidine per ml. Labeling was terminated by addition of an equal volume of ice-cold label-terminating buffer. The cells were sedimented (10,000 × g, 10 min, 4°C), suspended in cold 0.01 M Tris hydrochloride (pH 7.6)-0.01 M EDTA buffer, and gently lysed by three to four cycles of freezing in liquid nitrogen and thawing at 40°C. The infected cell lysates were layered on a 4.7-ml 5 to 20% (wt/vol) neutral sucrose gradient formed over a shelf of 1.3 g of CsCl per ml in 40% sucrose. The gradients were centrifuged for 2 h at 30,000 rpm at 15°C in a Sorvall OTD50 ultracentrifuge with the AH650 rotor. Fractions were collected, washed, and dried, and radioactivity was counted.

Deproteinization of the fast-sedimenting complex. For further analysis of the DNA in the fast- and free-sedimenting fractions, samples were pooled from several gradients, dialyzed against 0.01 M Tris hydrochloride (pH 7.5)-0.001 M EDTA-0.1 M NaCl buffer to remove sucrose and deproteinized as follows. Sodium dodecyl sulfate was added to each sample to a final concentration of 1%. The samples were incubated for 1 h at 37°C, extracted with 0.01 M Tris-0.01 M EDTA (pH 8.0) buffer-saturated phenol, and precipitated with ethanol. The precipitate was suspended in 0.01 M Tris-0.01 M EDTA buffer and analyzed by centrifugation through 5 to 20% (wt/vol) neutral or alkaline sucrose gradients.

Nuclease digestion and ligation. The restriction endonucleases BamHI and BglII and T4 DNA ligase were obtained from Boehringer Mannheim Biochemicals, and the nuclease Bal31 was obtained from Bethesda Research Laboratories, Inc. Digestion of purified φ149 DNA with these enzymes was carried out as recommended by the manufacturer. All digests were heated at 65°C for 5 min and quenched in ice to melt small hydrogen-bonded overlaps before being loaded on gels. Digestion with restriction enzymes after Bal31 treatment of the DNA was carried out by the method of Maniatis et al. (10). One unit of Bal31 was added per 5 μg of DNA at different times during incubation, and the reaction was stopped by adding ice-cold ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N''-tetraacetic acid (EGTA) to a final concentration of 0.2 M. The samples were diluted to reduce the concentration of NaCl before further digestion with restriction enzyme. For the ligation reaction, 0.1 U of T4 DNA ligase was used per μg of DNA. The reaction was carried out at 12°C for 4 h.

Gel electrophoresis. Analysis of the restriction fragments was done in agarose (0.8%) horizontal slab gels as described previously (5).

RESULTS

Pulse-labeled intracellular DNA after φ149 infection. The synthesis of phage-specific DNA was examined by infecting V. cholerae 569B cells with phage φ149 at a MOI of 10 in LP medium and pulse-labeling the replicating DNA with [³²P]thymidine for 2 min at different times during infection. At the end of the labeling period, the cells were lysed and the crude lysates were analyzed by velocity sedimentation through neutral sucrose gradients as described in Materials and Methods. The incorporation of [³²P]thymidine into phage DNA could be detected between 5 and 7 min after infection (Fig. 1a). Since the host DNA synthesis is shut off by this time and more than 60% of the cellular DNA is degraded (17), synthesis of host DNA did not complicate the sucrose sedimentation profile of φ149-specific DNA even at early times during infection. Up to about 20 min after infection most of the pulse-labeled DNA cosedimented with [³²P]labeled φ149 DNA used as a marker (Fig. 1b). After 25 min of infection, the nascent DNA sedimented faster than the mature phage DNA (Fig. 1c). This is consistent with the fact that φ149 DNA, being circularly permuted (19), has terminal redundancy and can form concatemeric structures during replication. With φ149 DNA as a marker, the size of the concatemeric DNA was estimated to be about six genome-equivalent lengths. At later times during infection the nascent DNA molecules were resolved into two peaks, one sedimenting as concatemeric DNA and the other cosedimenting with the marker phage φ149 DNA (Fig. 1d).

Role of DNA replicative intermediates. To examine whether the concatemeric DNA molecules (Fig. 1c) serve as precursors for the synthesis of mature phage DNA, the kinetics of [³²P]thymidine incorporation into different phage-specific DNA components were examined. Ten minutes after infection, V. cholerae cells were pulse-labeled for 5 min with [³²P]thymidine, and then the label was chased by suspending infected cells in unlabeled medium. To measure the distribution of the label at the end of the pulse, a sample of infected culture was removed and lysed and the lysate was analyzed by sedimentation in sucrose gradients.

At the start of the chase, the pulse-labeled DNA cosedimented with the marker phage DNA (Fig. 2a). During subsequent times, part of the pulse-label sedimented faster (Fig. 2b), and by 20 min of the chase more than 60% of the pulse-label was resolved at the position of the concatemeric DNA intermediate (Fig. 2c). At later times during infection the pulse-label appeared primarily in two peaks, one sedimenting with the marker phage DNA and the other sedimenting faster than the mature phage DNA but slower than the concatemeric DNA molecules (Fig. 2d). This result shows that the concatemeric structures formed during replication of φ149 DNA are cleaved into mature phage DNA which are packaged into phage heads like most circularly permuted DNA phages (5, 8, 16, 21).
Fate of parental DNA and site of new synthesis. To monitor the fate of the parental phage DNA during infection, cells were infected with 32P-labeled phage at a MOI of 10, washed to remove unadsorbed phage, and suspended in LP medium. At different times, infected cells were pulse-labeled for 2 min with 3H-thymidine and lysed gently by freezing and thawing, and the lysates were analyzed in neutral sucrose gradients. This enabled the determination of the distribution of parental 32P-labeled and newly synthesized 3H-labeled phage DNA.

Upon infection, about 70% of the injected φ149 DNA became associated with the bacterial membrane, as evidenced by rapid sedimentation of the DNA in sucrose gradients (Fig. 3a and b). At later times, 20 to 30% of the parental label was released from the fast-sedimenting complex (Fig. 3c), and by 45 min after infection about 20% of the acid-precipitable radioactivity of the parental label was lost (Fig. 3d). About 15% of the parental label could be recovered in the progeny phage, as measured by the acid-precipitable radioactivity in the cell-free supernatant of the infected culture. This is consistent with the fact that φ149 primarily utilizes DNA degradation products for its own DNA synthesis (17).

At early times during infection (up to about 10 min) most of the newly synthesized DNA sedimented freely at the position of the mature φ149 marker DNA (Fig. 3a). At about 30 min after infection, although most of the newly synthesized DNA was sedimenting freely, the molecules were heavier than the monomeric units (Fig. 3b). At times when the synthesis of concatameric molecules was predominant (35 min after infection), almost 50% of the newly synthesized DNA was recovered in a fast-sedimenting complex (Fig. 3c). During subsequent times most of the newly synthesized DNA sedimented freely as a broad peak (Fig. 3d).

To analyze which DNA components were present in the fast- and free-sedimenting material, for each of the gradients in Fig. 3, the fast- and the free-sedimenting materials were pooled from several gradients, deproteinized, and sedimented in neutral sucrose gradients.

The free-sedimenting material of Fig. 3a consisted of monomeric units, as evidenced by its cosedimentation with marker phage DNA (Fig. 4a). Both the fast-sedimenting (Fig. 4b) and free-sedimenting (Fig. 4c) materials of Fig. 3c represented concatameric DNA molecules with a size equiv-
These multiple as a concatemeric associated free-sedimenting DNA low-molecular-weight the DNA (Fig. 4). DNA were synthesized in the gradient and solved in the nicks during concatemeric membrane-associated length which are repairable by DNA ligase (19). To examine when during infection the nicks are introduced in the DNA, the monomeric DNA molecules (Fig. 4a) and the free and membrane-associated concatemeric DNA (Fig. 4b and c) were analyzed by sedimentation in alkaline sucrose gradients (Fig. 5). In contrast to mature phage DNA (Fig. 5a), the newly synthesized monomeric DNA molecules were resolved in the gradient as a sharp peak (Fig. 5b). Whereas the free-sedimenting concatemeric DNA was resolved in the gradient as a broad peak sedimenting faster than monomeric DNA (Fig. 5d), the membrane-associated concatemeric DNA showed, along with the heavier peak, multiple peaks in the low-molecular-weight region of the gradient (Fig. 5c). These multiple peaks resembled the sedimentation profile of mature φ149 DNA in alkaline sucrose gradients (Fig. 5a; 19). These results suggest that the nicks in the mature phage DNA are introduced during packaging of membrane-associated concatemeric DNA into the phage head.

Abortive φ149 replication in HP medium. When φ149 infection was carried out in media containing phosphate ions at concentrations of about 0.1%, phage growth was completely inhibited (17). In the present study, although no functional phage replication occurred in HP medium, host macromolecular synthesis was shut off and the cell DNA was degraded in a manner similar to that observed during infection in LP medium. Since no difference was observed in the association of the infecting phage DNA with the bacterial membrane for infections under HP and LP conditions (data not shown), the extent of replication of φ149 DNA was examined by infecting cells in HP medium. At 15 min after infection, infected cells were pulse-labeled for 5 min with [3H]thymidine. At this end of this period the infected cells were washed, a part of the sample was lysed, and the lysate was analyzed in a neutral sucrose gradient to resolve the phage replicative DNA intermediates. The rest of the infected cells were suspended in fresh HP medium, and the pulse-label was chased. At different times during the chase, samples were removed, lysed, and analyzed by velocity sedimentation. At the start of the chase most of the newly synthesized DNA cosedimented with the marker φ149 DNA (Fig. 6a) in a manner similar to that observed for infection in LP medium (Fig. 2a). No difference in the uptake of labeled thymidine was observed for infection in HP and LP media at this time of infection. However, at 35 min after infection, when more than 60% of the pulse-label in LP medium was converted to a rapidly sedimenting concatemeric DNA structure (Fig. 2c), that in HP medium retained the monomeric configuration (Fig. 6c). At times up to 60 min after infection, examined in the present study, no change in the sedimentation pattern of the pulse-labeled DNA was observed for infection in HP medium (Fig. 6d). Thus, the conversion of the monomer to a concatemeric
structure, which serves as the precursor for the synthesis of mature phage DNA, did not occur when infection was carried out in HP medium.

It is known that if infection by phage φ149 is carried out in LP medium for 15 min and the infected cells are then transferred to HP medium, the infection becomes phosphate insensitive (17). To examine the replication of phage DNA after the transfer from LP to HP medium, cells were infected with φ149 in LP medium and at 15 min after infection labeled with [3H]thymidine for 5 min. At the end of the labeling period, the cells were washed and suspended in unlabeled HP medium, and the pulse-label was chased. At the end of the pulse, most of the newly synthesized DNA sedimented as monomeric units (Fig. 7a). After 20 min of chase, the pulse-labeled DNA sedimented as concatemers (Fig. 7b), and at later times most of the label sedimented as monomeric units (Fig. 7c), a pattern similar to that observed for infection in LP medium (Fig. 2). In view of the fact that for infection in HP medium all the phage-coded early proteins are made except a 64,000-dalton (Da) species (17), it seems that this protein might be essential for the formation of concatemers and the synthesis of this protein is phosphate repressible.

Packaging of phage DNA. For circularly permuted DNA phages, concatemers of phage DNA are generally the substrate for packaging of DNA into phage heads (4, 14, 21). Furthermore, packaging by a headful mechanism can initiate either from a unique site or from any part of the concatemeric DNA. The extent of permutation in a DNA population depends on which of the two possibilities occurs in the packaging process, in addition to the length of the concatemer and the size of the terminal redundancy (12, 21, 22). For restricted permutation in the DNA, packaging normally starts from a unique site known as the pac site (22). Since the permutation in the phage φ149 DNA is restricted (19), the following experiments were performed to investigate the site in φ149 DNA from which the packaging initiates.

FIG. 4. Velocity sedimentation analysis of deproteinized fast- and free-sedimenting fractions from the gradients for which profiles are shown in Fig. 3a and c. Free-sedimenting fractions at 5 (a) and 35 (b) min after infection and fast-sedimenting complex after 35 min of infection (c) are shown.

FIG. 5. Velocity sedimentation analysis of deproteinized fast- and free-sedimenting fractions from the gradients for which profiles are shown in Fig. 3a and c in alkaline 5 to 20% (wt/vol) sucrose gradients. (a) 32P-labeled φ149 DNA; (b and d) free-sedimenting fractions at 5 and 35 min after infection, respectively; (c) fast-sedimenting complex after 35 min of infection. Sedimentation is from right to left.

FIG. 6. Velocity sedimentation analysis of cells infected with φ149 in HP medium. The infected cells were pulse-labeled for 5 min with 25 μCi of [3H]thymidine per ml at 15 min after infection (a) and chased for 10 (b), 20 (c), and 40 (d) min in HP medium. The cell lysates were analyzed in 5 to 20% neutral sucrose gradients. The sedimentation is from right to left.
It was reported previously that upon complete digestion of φ149 DNA with the enzyme BamHI five fragments were produced and the intensity of fragment C was greater than that of the other fragments. The linkages between these fragments are known (19). When the phage DNA was treated with the enzyme Bal31 for different times and then digested with BamHI, none of the fragments disappeared completely. However, the intensity of fragments A1 and C was reduced more than that of the other fragments (data not shown). Thus, for most molecules fragments A1 and C should be at the ends. In other words, packaging from the concatemeric structure initiates from a site at or near fragment A1 or C. In circularly permuted DNA any sequence between two restriction sites can either stay intact or be interrupted by molecular ends arising from headful packaging. If the packaging initiates at or near the BamHI site bounding fragment C of φ149 DNA, BamHI fragment C should occur twice in the first headful and in all subsequent molecules at least one copy of this fragment should be present since the terminal redundancy in the φ149 DNA is 10 to 12%. Thus, the intensity of BamHI fragment C should be more in a complete digest relative to that of other fragments; this was found to be true (19).

Considering the length of the concatemeric DNA which serves as the substrate for packaging to be six genome-equivalent lengths, a packaging scheme for the five headfuls obtained from each concatamer was constructed by assuming that the pac site is at or near BamHI fragment C (Fig. 8). To confirm this packaging scheme, the effect of BglII digestion on Bal31-treated φ149 DNA was examined to determine whether the perturbation in the fragmentation pattern could be accounted for by the proposed packaging model. φ149 DNA was treated with Bal31 for different times and then digested completely with the enzyme BglII. None of the BglII fragments constitutes part of a fragment (19) (Fig. 9, lane a), and hence the size of φ149 DNA estimated from BglII digestion was taken as that of the unit set of φ149 genes. When Bal31-treated φ149 DNA was digested with BglII, the intensities of fragments A, B, E, F, H, I, K, and M were reduced relative to those of the other fragments or were diffuse (Fig. 9, lanes b to f) and fragments C, D, P, and O were not affected. These observations support the present scheme, because most of the fragments whose intensities were reduced are at the terminii of one or another of the mature phage DNA molecules. The fragments which were not affected are never at the end of a molecule. However, the intensity of fragment L, which was supposed to be reduced, remained apparently unaffected. This could have been due to accumulation of parts of other fragments. Although BglII fragment G disappeared within the first few seconds of Bal31 treatment, this fragment does not represent the pac site for the following reasons. First, if BglII fragment G is the pac site the invariance of the intensity of BamHI fragment A2 after Bal31 digestion of the φ149 DNA cannot be explained.

**FIG. 7.** Velocity sedimentation analysis of φ149-infected cells after transfer from LP to HP medium. Cells in the logarithmic phase of growth were infected with φ149 at a MOI of 10 in LP medium and pulse-labeled for 5 min at 10 min after infection (a). The infected cells were transferred to HP medium, and the pulse-label was chased for 20 (b) and 40 (c) min. The cell lysates were analyzed in 5 to 20% neutral sucrose gradients. The arrow indicates the position of marker φ149 DNA. The sedimentation is from right to left.

**FIG. 8.** Map of BamHI (inner circle) and BglII (outer circle) cleavage sites and headful maturation cleavages in φ149 DNA. The physical maps are drawn to scale (innermost circle). A segment of a concatamer is shown as a spiral wound around the circular physical maps. Headful packaging of DNA initiates at the site designated pac and continues sequentially in the counterclockwise direction. The terminal redundancy in φ149 DNA was considered to be 12% of the length of a unit set of genes. The different headfuls are indicated by Roman numerals. The shaded areas represent the sequences in φ149 DNA which never constitute the terminal regions of any DNA molecules.
Second, if the packaging starts from a site on BglII fragment G, then in the complete digest of φ149 DNA with BamHI a part of fragment A2 will appear once in every five headfuls. Such a faint band was not observed. Third, if the packaging initiates at or near BglII fragment G, the intensity of this fragment should be more in the complete digest relative to those of other fragments; this was not true. φ149 DNA has single-strand interruptions along its length, and these interruptions have been mapped in BamHI fragment A (22). It is likely that BglII fragment G, overlapping BamHI fragment A2, carries these interruptions. Although the φ149 DNA was ligated before treatment with Bal31, some of the nicks might have escaped ligation and thus have been susceptible to Bal31 treatment. This might have been the reason for the rapid disappearance of this fragment after Bal31 treatment of φ149 DNA. The extent of permutation in φ149 DNA was estimated from this scheme to be 65%.

**DISCUSSION**

Identification of the concatemeric DNA intermediate during φ149 replication is consistent with the fact that φ149 DNA is circularly permuted (19). For infection under HP conditions, no concatemeric DNA was formed, although synthesis of monomeric molecules was unaffected. It has been reported that for infection under HP conditions all the phage-specific early proteins are made except a DNA-binding protein with a molecular weight of 64,500 (17). Furthermore, none of the 23 phage-coded late proteins are synthesized for infection in HP medium. The present study showed that if the 64,500-Da protein was allowed to be synthesized by infecting cells in LP medium before switching to HP medium, the concatemeric DNA intermediate was formed and could subsequently be chased to mature phage DNA. It thus appears that the 64,500-Da early protein might play an essential role in the formation of the concatemeric DNA intermediate, like the products of genes 6 of T3 (8), 3, 5, and 4 of T1 (7), 46 and 47 of T4 (15), and 6 of T7 (11). The products of all of these genes have exonuclease activity (14–16, 23). However, whether these gene products are directly responsible for concatemer formation or for just stabilizing the concatemeric structures is not known. Whereas for T7, the latter view is favored (11), for T3 the former is more likely (8). By using the genomic library of φ149 DNA, constructed by cloning HindIII restriction fragments into pBR322, the DNA fragment coding for the 64,500-Da protein has been identified (R. Chowdhury, S. Biswas, and J. Das, unpublished observation). Characterization of the protein coded by this fragment will allow selection between the possibilities for φ149 infection. Interestingly enough, for φ149 infection of el tor cells, which is a biotype of classical vibrios and a nonpermissive host for all group IV choloraphages, the concatemeric DNA intermediate is formed but cannot be chased to mature DNA. Further, the concatemeric DNA molecules formed are unstable (R. Chowdhury, S. Biswas, and J. Das, unpublished observation), suggesting that besides the 64,500-Da protein, a cell-coded gene product might also be involved in stabilizing the concatemeric DNA intermediate.

It has been reported that when cells infected in LP medium are transferred to HP medium 15 min after infection, i.e., when all the early proteins are made, all the phage-coded late gene products are synthesized (17). The present study showed that upon such transfer the concatemeric DNA was formed. Whether the concatemeric DNA is necessary to switch on the late transcripts is not known. Parenthetically, throughout the process of infection in HP medium, monomeric DNA molecules were synthesized but none of the late proteins were made. This is in contrast to what was reported for the transcription of late genes during T4 infection. It has been shown that T4 DNA replication and switching on of the late genes are coupled (15, 20). The late proteins are made even before the synthesis of concatemeric DNA intermediates.

The results presented in this report show that phagespecific DNA synthesis takes place primarily in the cytoplasm. The membrane attachment of newly synthesized phage DNA at times when phage heads could be seen in thin sections of infected cells under the electron microscope suggests that membrane association of the concatemeric DNA might be a necessary step for packaging of DNA into phage heads. No single-strand breaks were detected in the newly synthesized φ149 DNA; therefore, the nicks in the mature phage DNA might be introduced during packaging at the membrane site. Since these nicks are present in defined genetic loci (19), it seems that the binding of the concatemeric DNA to the membrane site before packaging involves a unique conformation of the DNA so that DNA ligase does not have access to the nicks during packaging.

The packaging of φ149 DNA into phage heads starts from a unique site. This is not surprising since the permutation in φ149 DNA is restricted (19). The pac site is located on or near BamHI fragment C, and based on the analysis of the intensity of fragments produced by restriction enzyme digestion after exonuclease treatment, the direction of packaging was assigned as counterclockwise. A concatemeric DNA with a size equivalent to six genome lengths can produce five headful lengths of DNA. The packaging scheme of five headfuls from a concatemeric DNA shows that although the permutation in φ149 DNA is restricted, there should be no faint bands in the complete digest of the DNA with the

**FIG. 9.** Digestion of φ149 DNA with BglII after Bal31 treatment. φ149 DNA (2 μg) was incubated with 0.4 U of Bal31 at 30°C, and samples were removed at 15 (lane b), 30 (lane c), 45 (lane d), 60 (lane e), and 90 (lane f) s. Reactions were stopped, and the DNA was digested with the restriction enzyme and analyzed by electrophoresis in a 0.8% agarose slab gel as described in the text. Lane a, Untreated φ149 DNA digested with BglII.
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LITERATURE CITED