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DNA Packaging In Vitro by an Isolated Bacteriophage T7 Procapsid

WARREN E. MASKER[1]* AND PHILIP SERWER[2]

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830,[1] and Department of Biochemistry, The University of Texas Health Science Center, San Antonio, Texas 78284[2]

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The results of previous in vivo studies indicate that a DNA-free procapsid (capsid I) packages bacteriophage T7 DNA during infection of *Escherichia coli*. It was shown here that capsid I, isolated by electrophoresis in metrizamide density gradients, packaged DNA and formed infectious phage particles when incubated in vitro with extracts deficient in capsid proteins.

Procedures developed for performing DNA replication, recombination, and packaging under controlled conditions by using cell-free extracts of bacteriophage T7-infected *Escherichia coli* have proved to be useful in increasing the understanding of these processes (2, 3, 5, 8, 10). To identify those components of such extracts that are essential for DNA metabolism, the extracts can be fractionated, and nonessential components can be removed; in the case of T7, this has most successfully been performed during studies of DNA replication (8). In vitro packaging of exogenous T7 DNA can be accomplished without significant recombination with endogenous DNA by using crude extracts (3, 5). However, fractionation of these extracts has not yet been achieved. Results of in vivo studies of T7 DNA packaging indicate that T7 DNA is encapsulated by a procapsid (capsid I) which is DNA free and has an envelope that is smaller, rounder, thicker, and more electrically charged than the envelope of a whole-phage particle (9, 11, 14, 15). During packaging, capsid I is converted progressively to several forms of a second capsid (capsid II) which has a bacteriophage-like envelope (9, 13, 14, 15, 16). To test the correctness of this proposed pathway and to begin fractionation of the in vitro packaging system, it was desirable to determine whether isolated capsid I or capsid II is capable of packaging T7 DNA in vitro. A similar approach has proved to be successful in demonstrating the activity of proheads in packaging the DNA of bacteriophages λ, P22, and T3 (the latter is a phage closely related to T7) (1, 6, 7, 20); however, the failure to separate capsid I from capsid II during sucrose gradient velocity sedimentation thwarted a similar study of bacteriophage T7 (4). Recently, an effective procedure for separating T7 capsid I from capsid II by electrophoresis through metrizamide density gradients has been demonstrated (15). In this report we show that capsid I, but not capsid II, isolated in this way was able to encapsulate T7 DNA in vitro.

The product of T7 gene 9 (P9) is required for the in vivo assembly of any T7 capsids. Also, the product of T7 gene 19 (P19) is necessary for DNA packaging, but not for capsid formation (9, 18, 19). As a first step in isolating capsids, lysates were prepared (3) from *E. coli* infected with T7 that was deficient in genes 3, 5, and 6 (three genes essential to DNA synthesis and recombination [8, 10] and additionally deficient in gene 9 ($T7_{3,5,6,9}$) or gene 19 ($T7_{3,5,6,19}$). Neither lysate by itself was able to encapsulate DNA, but when equal volumes of the lysates were mixed, packaging activity was restored, suggesting that capsid proteins (probably already assembled as capsids) from the lysate deficient in P19 were active in packaging DNA. Thus, complementation assays performed under our experimental conditions agreed with earlier findings (2). When the lysates were centrifuged under conditions sufficient to pellet extraneous cellular material but to retain essential phage structural components (such as capsids) in the supernatant, these low-speed extracts prepared from $T7_{3,5,6,9}$ and $T7_{3,5,6,19}$ were still able to complement one another (data not shown).

To further separate capsids from residual whole phage, nucleic acids, and other cellular material, an extract was fractionated by buoyant density sedimentation in CsCl. *E. coli* W3110 was infected with $T7_{3,5,6}$, and an extract was prepared as described above. To help locate phage-induced proteins, this extract was mixed with one-third of its volume of a similar extract labeled with ^{14}C-amino acids. After centrifugation, fractions of the CsCl gradient were examined for radioactivity and, after dialysis, for ability to restore in vitro packaging activity to an extract prepared with phage deficient in gene 9.

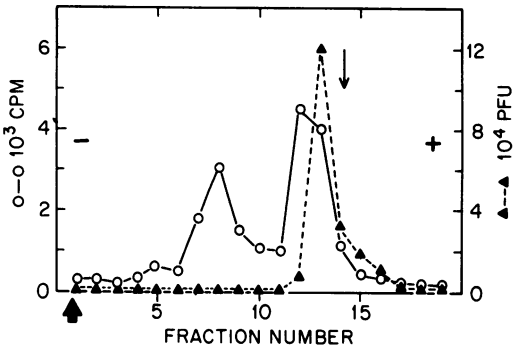

FIG. 1. Isolation of T7 proheads by electrophoresis through metrizamide gradients. *E. coli* W3110 incubated at 30°C in M9 medium with 4 μg of thymine per ml and 0.4% (wt/vol) glucose was grown to a density of 2.5×10^8 cells per ml, and T7 phage carrying amber mutations in genes 3, 5, and 6 were introduced at a multiplicity of infection of three. After 10 min of incubation, ^{14}C-amino acids (New England Nuclear Corp.) were introduced at 0.1 μCi/ml. The infection was allowed to proceed for an additional 8.5 min before the phage-infected cells were harvested and frozen as previously described (3). These cells were treated with lysozyme to prepare a lysate and mixed with a 3× volume of lysate prepared from unlabeled phage-infected cells that had been prepared in L-broth as previously described (3). The mixture was blended briefly in a Vortex mixer and spun at 0°C in a Spinco type 40 rotor at 15,000 rpm (18,000 × *g*) for 20 min. The supernatant was removed and mixed with enough low-salt T7 diluent (2) to give a weight of 5.0 g. This was added to 2.0 g of solid CsCl, and the resulting mixture was spun at 4°C in a Spinco SW50.1 rotor at 27,000 rpm for 20 h. Fractions were collected, and the acid-precipitable radioactivity in a 20-μl portion of each sample was determined. The remainder of each fraction was dialyzed against low-salt T7 diluent to remove CsCl. Each fraction was then centrifuged at 1,500 rpm for 7 min at 0°C in an International PR6 centrifuge with a type 253 rotor. The supernatants were recovered, and a 5-μl portion of selected fractions was mixed with 15 μl of a "receptor" extract prepared as described above except that the T7 phage carried amber mutations in genes 3, 5, 6, and 9. The mixture was incubated in a standard 30-μl DNA-packaging reaction (3) with 1.0 nmol (nucleotide phosphorous) of highly purified mature wild-type T7 DNA. The yield of resulting phage was determined by using strain W3110 as indicator bacteria. Portions (0.1 ml) of those fractions of the CsCl gradient which were able to complement the gene 9 deficiency were layered onto 3-ml linear gradients of 1.040 to 1.150 g of metrizamide per cm^3 poured in cylindrical glass tubes with inner diameter of 0.55 cm. Electrophoresis was performed as previously described (15) at 20°C with a constant current of 12.2 mA/cm^2 for 15 h with a Buchler tube gel apparatus and a buffer consisting of 0.05 M sodium phosphate (pH 7.4), 1 mM $MgSO_4$, and 1 mM 2-mercaptoethanol. Fractions were removed manually from the top of the gradients, and the radioactivity present in 20-μl portions of each fraction was determined. A 5-μl portion of each fraction was mixed with 15 μl of a receptor extract prepared from $T7_{3,5,6,9}$-infected cells and was incubated in the standard packaging reaction as described above. The capsids were found to be stable to storage in liquid N_2, but in this experiment freshly prepared capsids were used. The figure shows profiles of ^{14}C radioactivity as well as the number of PFU of wild-type T7 phage recovered from each reaction. In this experiment 130 cpm represents approximately 1 μg of protein. The arrow indicates the approximate position of bromophenol blue tracking dye run in a parallel gradient. The arrow head indicates the origin of electrophoresis. If we assume that the protein content of a T7 phage is primarily due to the head protein, then each reaction contains about 10^{10} phage equivalents of capsid I.

The fractions that were able to complement the P9 deficiency formed a peak at a density of 1.28 g/cm^3 (data not shown), the density of capsid I (11). Controls performed with similar gradients confirmed that the fractions recovered from the CsCl gradient could not package DNA unless they were mixed with extracts from $T7_{3,5,6,9}$-infected cells and that the fractions containing the peak of complementation activity did not contain infective whole-phage particles (data not shown). Particles recovered from the CsCl gradient were dialyzed and subjected to electrophoresis in a metrizamide density gradient. The metrizamide gradient was fractionated, and the radioactivity of a sample of each fraction was determined (Fig. 1). The profile of radioactivity had two peaks; the peak formed by the more rapidly migrating particle was at the position expected for capsid I, and the other peak was at the position of capsid II. Electron microscopy performed on samples recovered from metrizamide gradients in experiments similar to this one confirmed the identity of the radioactive peaks as capsid I and capsid II (Table 1). An analysis by agarose gel electrophoresis was carried out on samples recovered from the experiment shown in Fig. 1 after the samples had been cross-linked with glutaraldehyde to prevent possible degradation of capsid I to capsid II, which was sometimes observed in preliminary experiments (Fig. 2). Treatment with glutaraldehyde increases the electrophoretic mobility of capsid I and II (17), but the capsid types are easily identified in Fig. 2, and these data also confirm the identification of the peaks of radioactivity seen in Fig. 1. The diverse particles which migrated more slowly than did capsid II in Fig. 2, but which comigrated with capsid II in the metrizamide gradient, have been observed previously (18). Buoyant density measurements in metrizamide gradients (13) indicated that both previously described (13) forms of capsid II were present in samples recovered from the experiment shown in Fig. 1 (data not shown). In

TABLE 1. Capsid content of metrizamide gradient[a]

Fraction no.	All capsids	Capsid I	Capsid II	Polycapsids[b]	PFU × 10^{-2}
Load[c]	109	43	28	38	
6	61	0	57	4	1.0
7	217	0	170	47	1.5
8	106	1	63	42	1.1
9	106	1	78	27	2.6
11	202	193	7	2	37.2
12	277	258	18[d]	1	137.3
13	226	194	28[d]	4	66.8

[a] A portion taken from fractions from a metrizamide gradient like the one shown in Fig. 1 was deposited on grids, stained with uranyl acetate, and examined under the electron microscope (10). The table shows the number of each type of particle observed and the number of phage produced by in vitro packaging with 5 μl of the sample (3). Fractions designated by larger numbers are farther from the origin.

[b] Polycapsids have been previously described (12). Most polycapsids seen here appeared to be 2 to 20 times the size of capsid II and therefore were smaller than some polycapsids previously described (12).

[c] Load, Material initially layered onto the metrizamide gradient.

[d] In other experiments (including the experiments shown in Fig. 1 and 2) the amount of capsid II relative to capsid I was 15 to 30 times less than that found here.

some respects these findings are surprising, since earlier studies (9, 18) indicate the absence of capsid II in cells infected with $T7_5$. Although it is possible that the capsid II particles were formed by in vitro degradation of capsid I during preparation of the extract, it seems more likely that removal of the P3 and P6 nucleases in $T7_{3,5,6}$-infected cells reduces the degradation of input T7 DNA, resulting in some initiation of DNA packaging and formation of capsid II.

A sample of each fraction from the metrizamide gradient was mixed with a "receptor" extract prepared from $T7_{3,5,6,9}$-infected cells and assayed for ability to package wild-type T7 DNA. There was single peak of complementation activity at one fraction farther from the origin of electrophoresis than the peak of radioactivity identified as capsid I (Fig. 1, triangles). No detectable packaging complementation activity was associated with the peak formed by capsid II. Experiments such as that shown in Fig. 1 were also performed by using cells infected with wild-type T7. Although the radioactive profiles showed generally poorer resolution between the capsid I and capsid II regions of the metrizamide gradient, a single peak of P9 complementation activity again coincided with the region of the gradient where capsid I migrates (data not shown). We concluded that capsid I (but not capsid II) is active in packaging T7 DNA in this in vitro system.

The data described above are most easily explained by assuming that intact capsid I packages DNA, but it is also possible that capsid I disassembles into subunits in the packaging extract and then reassembles around the DNA. To test for this possibility, the effect of the concentration of capsid I on packaging efficiency was measured. Fractions containing gene 9-complementing activity recovered from the metrizamide gradient were pooled and dialyzed to remove metrizamide. (Metrizamide was found to reduce packaging efficiency by as much as two orders of magnitude.) Packaging reactions were performed in which the concentration of capsid I was varied, and the yield of infective wild-type phage was measured (Fig. 3). The linear rise in titer as a function of capsid amount at the lower capsid concentrations indicates that the capsids

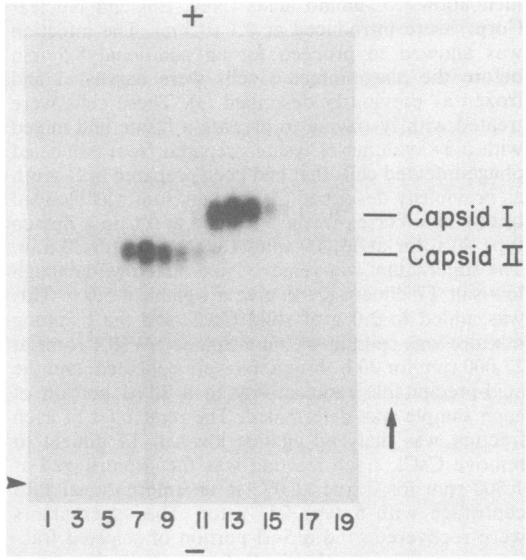

FIG. 2. Agarose gel electrophoresis of fractionated T7 capsids. Portions of fractions collected from the metrizamide gradient shown in Fig. 1 were fixed with glutaraldehyde at pH 7.4 as previously described (11). Glutaraldehyde fixation was necessary to prevent degradation of capsid I to capsid II during shipment of particles from Oak Ridge, Tenn. (where the metrizamide gradient electrophoresis was performed), to San Antonio, Tex. (where the agarose gel electrophoresis was performed). The samples (undialyzed) were subjected to electrophoresis on a 0.90% ME agarose (Marine Colloids) slab gel with 0.05 M sodium phosphate (pH 7.4)–0.001 M $MgCl_2$ as the electrophoresis buffer as previously described (18). The gel was dried under a vacuum and subjected to autoradiography. The numbers indicate the fractions of the metrizamide gradient shown in Fig. 1 from which the samples were taken. The arrowhead indicates the origin of electrophoresis; the arrow indicates the direction of electrophoresis.

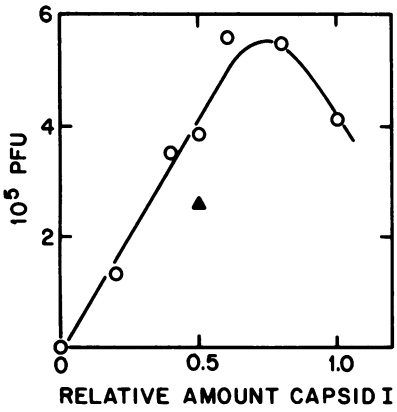

FIG. 3. Effect of capsid I concentration. Fractions recovered after electrophoresis through metrizamide produced a profile similar to that shown in Fig. 1. Fractions from the capsid I region were pooled and dialyzed overnight against low-salt T7 diluent. Reaction mixtures including 10 μl of a receptor extract prepared from $T7_{3,5,6,9}$-infected cells, 1.0 nmol of wild-type T7 DNA, and 10 μl of a mixture of capsid I and low-salt T7 diluent were incubated in the standard in vitro packaging reaction and the yield of wild-type phage was determined. Symbols: ○, phage production (in PFU) as a function of the relative amount of capsid I included in the reaction; △, phage yield when 5 μl of the capsid I preparation was mixed with 5 μl of a dialyzed capsid II preparation recovered from the same metrizamide gradient.

did not reassemble during the packaging reaction. At higher capsid concentrations the yield of infective phage diminished. Controls in which capsid II was added to an equal amount (determined by radioactive protein) of capsid I showed a 30% loss in phage yield (Fig. 3, triangle), suggesting that the even smaller amounts of capsid II which contaminate capsid I (Fig. 2) are not the cause of the inhibition of T7 assembly. However, other (unidentified) particles partially copurifying with capsid I may be responsible for this inhibition. Another possibility is that at the higher concentrations aggregation of capsid I caused the decrease in phage yield. Finally, it must be kept in mind that the assay used detects only the completion of the encapsulation process and the resultant production of infectious phage. If higher procapsid concentrations resulted in a larger amount of DNA being partially encapsulated, a net decrease in viable phage might still be observed.

The slight noncoincidence of the peak of radioactivity and the peak of P9 complementing activity (Fig. 1) suggests heterogeneity of capsid I particles. The relatively low efficiency of conversion of capsid I to T7 phage also supports the possibility that a subspecies of capsid I is responsible for in vitro packaging. Moreover, the distance of the capsid I band from the gel origin (Fig. 2) increased as a function of the distance migrated by capsid I in the metrizamide gradient (Fig. 1) from which samples were taken. The 4 to 6% difference between the distances traveled by the most and least rapidly migrating capsid I was also found when agarose gel electrophoresis was performed without prefixation with glutaraldehyde (data not shown). These data agree with previously described (18) indications of heterogeneity in capsid I preparations. Of course, the nonlinear relationship between capsid I concentration and phage production (Fig. 3) must be considered, and it cannot be firmly concluded that the observed heterogeneity of capsid I (Fig. 2) is the cause of the noncoincidence of peaks in Fig. 1.

In vivo studies of T7 DNA packaging suggest that capsid I converts to capsid II after binding DNA and that some (probably most) DNA is packaged after this conversion (9, 16). If so, all capsid II particles isolated here had been released from DNA to which they were bound before lysis. Our results indicate that during or after release from DNA, capsid II loses its capacity to continue DNA packaging in the extracts used here. This might be explained by the irreversible loss of a protein(s) necessary for the initial binding of DNA to capsid I. A candidate for such a protein is P19, a protein present in capsid I, but not in capsid II, and necessary for the efficient conversion of capsid I to capsid II in vivo (9).

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