JOURNAL OF VIROLOGY, July 1982, p. 365-367
0022-538X/82/070365-03$02.00/0

Vol. 43, No. 1

In Vitro Packaging of Bacteriophage T7 DNA Requires ATP

WARREN E. MASKER

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

Received 28 December 1981/Accepted 4 March 1982

Removal of nucleoside triphosphates from extracts prepared from bacteriophage T7-infected *Escherichia coli* results in a stringent requirement for added ATP to form infective phage particles by in vitro packaging of bacteriophage T7 DNA. Optimal packaging efficiency was achieved at a concentration of about 1.25 mM. Other nucleoside triphosphates could be substituted for ATP, but none of the common nucleoside triphosphates was as effective as ATP in promoting in vitro encapsulation.

Although double-stranded DNA bacteriophages have served as the system of choice in many recent studies of DNA metabolism, the details of the mechanism(s) by which the various phages encapsulate their DNA remain obscure (6). Bacteriophage T7 provides an especially interesting system because of the wealth of genetic and biochemical information accumulated with this phage (8, 23) and because of the availability of in vitro systems which facilitate study of DNA metabolic processes (16, 18). In vitro packaging of bacteriophage T7 DNA has also been described (10, 12). Initially, encapsulation of exogenously provided T7 DNA was achieved by recombination with endogenous DNA present in the crude extracts used in the packaging reaction (19). It has also been possible, however, to achieve in vitro encapsulation of highly purified exogenous DNA without any apparent recombination during the packaging reaction itself (12, 14). This system shows a linear relationship between yield of viable phage and DNA concentration, and it produces phage with an efficiency of 10^{-4} to 10^{-5} viable T7 phage per genome equivalent of input DNA. Higher efficiencies can be attained when fast-sedimenting, concatemer-like DNA structures are used in place of genomic-length DNA as the substrate (14). Initial observations of in vitro packaging, performed using crude lysates, indicated maximum packaging efficiency in reactions containing Mg^{2+} and spermidine (12). We were, however, unable to determine any need for exogenously provided ATP. Since ATP requirements had been demonstrated with other packaging systems (2, 6), including the closely related phage T3 (7), and since the question of a possible energy source to drive the encapsulation process is of some importance in understanding the molecular mechanism of phage DNA packaging, we have refined our system for T7 DNA packaging and readdressed the question of whether encapsulation requires ATP.

Suppressor-free *Escherichia coli* W3110 was infected with T7 carrying amber mutations in genes 3, 5, and 6, three genes required for efficient phage DNA replication and recombination (8, 22, 23). The phage-infected cells were gently lysed, and an extract was prepared by centrifuging the lysate and recovering the supernatant. Nucleoside triphosphates (NTPs) were removed by sedimenting the supernatant through "minicolumns" of Sephadex G-50. These extracts, as well as the crude extracts, were used in the standard packaging reaction to encapsulate genome-length T7 wild-type DNA in the presence of 1 mM added ATP, 1 mM adenosine 5'-*O*-(3-thiotriphosphate) (ATP[γS]), or no additional triphosphate. The data in Table 1 verify that the crude extract (which may already contain NTPs) showed no requirement for additional ATP. The encapsulation process was, however, severely retarded when ATP[γS] was included during incubation. Removal of NTPs with Sephadex caused a pronounced dependence upon an exogenous source of ATP. In some experiments a lower packaging efficiency was found after extracts had been passed through Sephadex, but the dependence on ATP was still pronounced, and ATP[γS] was found to inhibit the packaging reaction.

The effect of ATP concentration on packaging capability is shown in Fig. 1. It was found that production of viable phage was greatest at an ATP concentration of about 1.25 mM; higher ATP levels caused a reduction in packaging efficiency. The possibility that other NTPs might effectively serve as energy sources during encapsulation was also considered. Table 2 shows that at a fixed concentration of 1.25 mM, each of the eight deoxyribo- and ribonucleoside triphosphates conferred significant potential for in vitro

TABLE 1. ATP dependence of in vitro packaging[a]

Extract	ATP	PFU
Standard	None	3.7×10^4
Standard	ATP	2.5×10^4
Standard	ATP[γS]	$<1 \times 10^2$
Sephadex	None	$<1 \times 10^2$
Sephadex	ATP	4.4×10^4
Sephadex	ATP[γS]	$<1 \times 10^2$

[a] Bacterial strain W3110 was infected with T7 having amber mutations in genes 3, 5, and 6 (*am*29, *am*28, and *am*147 from F. W. Studier [22]) for 18.5 min at 30°C. The cells were then harvested and frozen in liquid N_2 as previously described (12). The phage-infected cells were thawed and lysed with 1 mg of lysozyme per ml for 2 min at 30°C before being spun at 15,000 rpm for 20 min at 0°C. A portion of the resulting supernatant was retained as the "standard" extract shown in the table. A 0.25-ml portion of the remainder of the supernatant was layered upon a 0.25-ml (packed volume) column of Sephadex G-50 prepared in a 0.5-ml microcentrifuge tube. This minicolumn was prepared by adding 0.5 ml of a 50% (vol/vol) slurry of Sephadex G-50 in 10 mM Tris-hydrochloride (pH 7.5)–1 mM EDTA to a microcentrifuge tube packed with a small amount of glass wool and punctured through the bottom with a 24-gauge hypodermic needle (5). The tube was placed inside a 1.5-ml microcentrifuge tube and spun at 1,000 rpm for 30 s at 4°C in a model 273 swinging bucket rotor in an International PR6 centrifuge. The column was washed three times with ice-cold low-salt T7 diluent (8) before 0.25 ml of the sample was layered and spun in the same way. The pass-through was collected and used as the "Sephadex" extract shown in the table. In vitro packaging containing 2.8×10^9 phage equivalents of genome-length T7 wild-type DNA was performed as previously described (12), except that 1 mM ATP (Schwarz/Mann) or ATP[γS] (Boehringer Mannheim Corp.) was included where indicated. Reactions were performed in triplicate, and a duplicate determination of wild-type phage yield was made using strain W3110 as an indicator. Values shown are averages of these measurements.

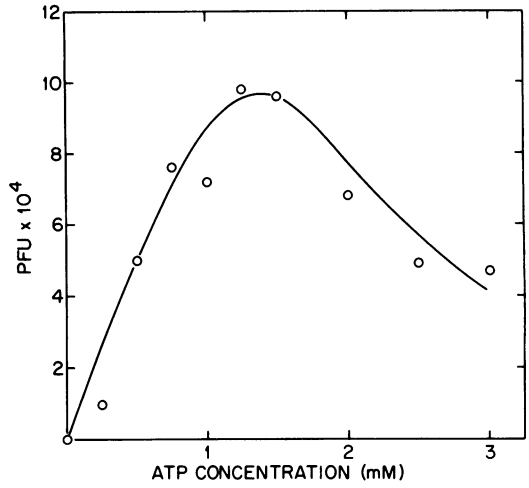

FIG. 1. Effect of ATP concentration on packaging capability. In vitro packaging reactions were performed using an extract sedimented through Sephadex as described in Table 1, footnote *a*. The reactions each contained 3.5×10^9 phage equivalents (0.46 nmol of nucleotide phosphorus) of wild-type T7 DNA and the indicated concentration of ATP. The figure shows the average yield of viable wild-type T7 phage.

packaging. It is apparent, however, that the highest level of phage production was achieved with ATP.

Presumably the condensation of T7 DNA into a tightly packed configuration during encapsulation is ultimately responsible for the observed dependence upon an energy source. It is not clear, however, at what stage of the packaging process the ATP is required. Since the experiments reported here were performed with T7 mutants deficient in T7 DNA polymerase and since deoxynucleoside triphosphates were removed from the reaction, it is unlikely that DNA replication, known to be ATP dependent, is directly involved in packaging. T7 codes for an ATP-dependent ligase (1). However, this enzyme is nonessential, and T7 mutants deficient in polynucleotide ligase are able to grow by using the *E. coli* (NAD-dependent) ligase (13). Experiments using a modified version of the system described here also showed ATP-dependent packaging performed by extracts prepared

TABLE 2. Effect of common NTPs on in vitro packaging[a]

NTP	Fraction of +ATP control
ATP	1.0
UTP	0.63
CTP	0.41
dATP	0.38
dCTP	0.28
dGTP	0.19
TTP	0.13
GTP	0.05
None	2×10^{-5}

[a] In vitro packaging reactions were performed as described in Table 1, footnote *a*, using an extract passed through Sephadex. The reactions contained 3.5×10^9 phage equivalents of T7 wild-type DNA and a 1.25 mM concentration of the indicated triphosphate. The composition of the stocks of NTPs (Schwarz/Mann) was verified by chromatography on polyethyleneimine thin-layer plates (Brinkmann Instruments Inc.) as described previously (15). The values shown are the average of two separate experiments in which each reaction was performed in triplicate. Values shown are relative to the reaction containing ATP. The average yield of phage for the +ATP reaction was 7.5×10^5 PFU; the packaging efficiency was 2.1×10^{-4}.

with a T7 ligase deletion mutant (data not shown). Thus, it is also unlikely that the ATP dependence observed with the T7 packaging system is entirely due to direct involvement of the ligase of the phage. Various models have been proposed to account for T7 DNA encapsulation (6, 9, 17, 20, 21). One of these suggests that physical expansion of a capsid attached to DNA could cause the capsid to engorge the DNA, possibly as the result of a pressure differential (20, 21). It is possible that alteration and expansion of the capsid might be promoted by an ATP-dependent process as yet undefined. Alternatively, ATP could be used to drive the DNA into the phage head in a more direct way. There are already several examples of ATP-driven DNA conformational proteins within *E. coli* and its phages (3, 4). The T7 gene 4 product, known to be a potent ATPase (11), is probably not responsible for the dependence we observe, since T7 DNA has been successfully packaged using extracts deficient in the gene 4 product (18). Recently, Hendrix proposed a model in which a protein pump, using ATP as an energy source, is imagined to twist the DNA into the unfilled capsid by rotation between the capsid and a collar which surrounds the double-stranded DNA molecule (9). Although our observation of an energy requirement for encapsulation of T7 DNA is compatible with such a protein pump model, a thorough understanding of the molecular mechanism of maturation and packaging must await further refinements of the in vitro packaging system to allow fractionation into the system's component parts.

I thank Marilyn Maupin for excellent technical assistance.

This research was supported by Public Health Service research grant GM-28113 from the National Institute of General Medical Sciences and by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corp.

LITERATURE CITED

1. **Becker, A., G. Lyn, M. Gefter, and J. Hurwitz.** 1967. The enzymatic repair of DNA. II. Characterization of phage-induced sealase. Proc. Natl. Acad. Sci. U.S.A. **58**:1996–2003.
2. **Black, L. W.** 1981. *In vitro* packaging of bacteriophage T4 DNA. Virology **113**:336–344.
3. **Champoux, J. J.** 1978. Proteins that affect DNA conformation. Annu. Rev. Biochem. **47**:449–479.
4. **Cozzarelli, N.** 1980. DNA topoisomerases. Cell **22**:327–328.
5. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacteria genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Earnshaw, W. C., and S. R. Casjens.** 1980. DNA packaging by the double-stranded DNA bacteriophages. Cell **21**:319–331.
7. **Fujisawa, H., J. Miyazaki, and T. Minagawa.** 1978. In vitro packaging of phage T3 DNA. Virology **87**:394–400.
8. **Hausmann, R.** 1977. Bacteriophage T7 genetics. Curr. Top. Microbiol. Immunol. **75**:77–110.
9. **Hendrix, R. W.** 1978. Symmetry mismatch and DNA packaging in large bacteriophages. Proc. Natl. Acad. Sci. U.S.A. **75**:4779–4783.
10. **Kerr, C., and P. D. Sadowski.** 1974. Packaging and maturation of DNA of bacteriophage T7 *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **71**:3545–3549.
11. **Kolodner, R., and C. C. Richardson.** 1977. Replication of duplex DNA by bacteriophage T7 DNA polymerase and gene 4 protein is accompanied by hydrolysis of nucleoside 5'-triphosphates. Proc. Natl. Acad. Sci. U.S.A. **74**:1525–1529.
12. **Kuemmerle, N. B., and W. E. Masker.** 1977. In vitro packaging of UV radiation-damaged DNA from bacteriophage T7. J. Virol. **23**:509–516.
13. **Masamune, Y., G. D. Frenkel, and C. C. Richardson.** 1971. A mutant of bacteriophage T7 deficient in polynucleotide ligase. J. Biol. Chem. **246**:6874–6879.
14. **Masker, W. E., N. B. Keummerle, and D. P. Allison.** 1978. In vitro packaging of bacteriophage T7 DNA synthesized in vitro. J. Virol. **27**:149–163.
15. **Randerath, K., and E. Randerath.** 1964. Ion-exchange chromatography of nucleotides on poly-ethyleneimine)-cellulose thin layers. J. Chromatogr. **16**:111–125.
16. **Richardson, C. C., M. J. Engler, R. Kolodner, J. E. LeClerc, D. Richardson, L. J. Romano, and F. Tamanoi.** 1979. Replication of bacteriophage T7 DNA by purified proteins. Cold Spring Harbor Symp. Quant. Biol. **43**:427–440.
17. **Roeder, G. S., and P. D. Sadowski.** 1977. Bacteriophage T7 morphogenesis: phage-related particles in cells infected with wild-type and mature T7 phage. Virology **76**:263–285.
18. **Roeder, G. S., and P. D. Sadowski.** 1979. Pathways of recombination of bacteriophage T7 DNA *in vitro*. Cold Spring Harbor Symp. Quant. Biol. **43**:1023–1032.
19. **Sadowski, P. D., and D. Vetter.** 1976. Genetic recombination of bacteriophage T7 DNA *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **73**:692–696.
20. **Serwer, P.** 1980. A metrizamide-ion permeable capsid in the DNA packaging pathway of bacteriophage T7. J. Mol. Biol. **138**:65–91.
21. **Serwer, P., and R. H. Watson.** 1981. Capsid-DNA complexes in the DNA packaging pathway of bacteriophage T7: characterization of the capsids bound to monomeric and concatemeric DNA. Virology **108**:164–176.
22. **Studier, F. W.** 1969. The genetics and physiology of bacteriophage T7. Virology **39**:562–574.
23. **Studier, F. W.** 1972. Bacteriophage T7. Science **176**:367–376.