JOURNAL OF VIROLOGY, May 1982, p. 595–601
0022-538X/82/050595-07$02.00/0

Vol. 42, No. 2

Function of an Internal Bacteriophage T7 Core During Assembly of a T7 Procapsid

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Received 28 October 1981/Accepted 13 January 1982

A DNA-free, proteinaceous procapsid of bacteriophage T7 (capsid I) has been shown in previous studies to consist of an external, spherical shell (envelope) and an internal, cylindrical core with fibrous projections that connect the core to the envelope. To determine the role of the core in assembly of the envelope of capsid I, the kinetics of appearance of capsid I and possible intermediates in capsid I assembly (AG particles) were determined in the presence and absence of the core. For obtaining these data, agarose gel electrophoresis was used and appeared to be a technique more accurate and efficient than techniques used for obtaining similar data in the past. The results of these experiments were: (i) in the presence of the core, AG particles behaved kinetically as intermediates in the assembly of capsid I; (ii) in the absence of the core, assembly of capsid I terminated prematurely and AG particles accumulated. These and other data have been interpreted by assuming that: AG particles are breakdown products of precursors of capsid I; these precursors have uncorrected errors in the assembly of their envelopes; and a function of the core is to correct these errors.

It has been shown that proteinaceous subunits in the envelopes of spherical, viral capsids are arranged with icosahedral symmetry to minimize the free energy of the envelope (3–5, 7, 9). To determine the mechanisms by which subunits of envelopes of viral capsids assemble, attempts are made to isolate incomplete capsids from virus-infected cells. Results with bacteriophages that contain double-stranded DNA packaged in a capsid with a spherical envelope, including bacteriophages λ, T3, T7, P2, P4, and P22, indicate that a spherical, DNA-free capsid (procapsid) is assembled and undergoes structural and compositional alterations before or (and) during packaging of DNA (reviewed in references 2, 6, 10, 11, 22).

As described in the above-referenced reviews, the procapsids of bacteriophages λ, P22, T3, and T7 have internal proteins that assist assembly of their outer envelope and that in some cases are ejected from the envelope or digested before or during DNA packaging (see Fig. 2 of reference 19 for models of the bacteriophage T7 procapsid [capsid I] and the capsid to which capsid I converts [capsid II] during packaging of DNA). It has been proposed that these internal proteins, sometimes referred to collectively as a core, assist assembly of protein in the outer envelope by: (i) converting the envelope protein from a nonassociating to an associating form (P22 [8]), (ii) forcing the proteins of the envelope to the orientation needed to produce an envelope with the needed curvature (T7 [15]; P22 [8]), (iii) forming a nucleus for polymerization of other subunits of the capsid (reviewed for several bacteriophages in reference 11), or (iv) preventing host proteins from entering the capsid (6). Hypotheses (i), (ii), and (iii) predict that in the absence of the procapsid core, assembly of the procapsid either does not occur or is slower than in the presence of the core. Hypotheses (i) and (iii) also predict that in the absence of the core, proteins of the capsid envelope accumulate in unpolymerized form. Therefore, the above hypotheses can be tested by measuring the kinetics of assembly of procapsids and precursors of procapsids in the presence and absence of cores. Measurement of the kinetics of assembly of procapsids in the absence of a core protein has apparently been performed only once (for P22 [1]), and in this experiment only two times were sampled; the results suggest that procapsid assembly is slowed in the absence of a functional core. Impediments to obtaining the above data are difficulties in identifying procapsid precursors and the large investment of time and resources needed if the procedure for quantitating procapsids is (as it usually is) centrifugation.

Therefore, to test the above hypotheses, it is advantageous to develop: (i) efficient (in time and cost) techniques for identifying and accurately quantitating either procapsid precursors or at least breakdown products of precursors (such precursors and their breakdown products will both be referred to as procapsid assembly

intermediates) and (ii) techniques more efficient than the techniques of centrifugation used in the past for accurately quantitating procapsids. Agarose gel electrophoresis has been demonstrated to be an accurate technique for identifying T7 capsid I, and it meets the above criteria for a technique to quantitate capsid I during kinetic labeling experiments (16, 17). In addition, agarose gel electrophoresis is used in the accompanying communication (19) to identify particles (AG particles) which are produced during assembly of capsid I and are possibly capsid I assembly intermediates (AG particles are identified by their failure to form a sharp band during agarose gel electrophoresis and their possession of several characteristics of capsid II; most AG particles are converted by trypsin to particles that migrate with capsid II during agarose gel electrophoresis). Therefore, in the present communication, the kinetics of appearance of capsid I and AG particles were determined in the presence and absence of the T7 core, using agarose gel electrophoresis to quantitate capsid I and AG particles. The results indicate that hypotheses (i) to (iii) do not accurately describe the role of the T7 core and suggest another hypothesis for the role of the T7 core during the assembly of capsid I.

MATERIALS AND METHODS

Bacteriophage and bacterial strains. Wild-type bacteriophage T7 and T7 amber mutants (20) were received from F. W. Studier. The following amber mutants were used: gene 5-28 and gene 8-11. The host for bacteriophage T7 and the nonpermissive host for amber mutants was *Escherichia coli* BB/1; the permissive host for amber mutants was *E. coli* 0–11′. Lysates of the nonpermissive host infected with a T7 amber mutant will be referred to by the number of the mutant T7 gene; particles isolated from such lysates will also be referred to by the number of the mutant gene.

Buffers and media. Standard/G buffer is 0.15 M NaCl, 0.05 M Tris-chloride, pH 7.4, 0.005 M EDTA, and 100 μg of gelatin per ml. Electrophoresis buffer is 0.05 M sodium phosphate, pH 7.4, and 0.001 M EDTA. Sample buffer is 0.005 M sodium phosphate, pH 7.4, 0.001 M EDTA, 100 μg of gelatin per ml, 4% sucrose, and 400 μg of bromophenol blue per ml. Cultures used for radiolabeling were grown in M9 medium (14).

Stocks of amber mutants. Stocks of amber mutants and wild-type phage T7 were grown and purified as previously described (19).

Kinetic radiolabeling and artificial lysis of amber mutant-infected *E. coli*. Log-phase cultures (1.25 ml) of *E. coli* BB/1 were grown in M9 medium with aeration at 30°C to 4×10^8/ml and were then infected at a multiplicity of 15. At 14 min after infection, ^{14}C-labeled algal hydrolysate (Schwarz/Mann; 12 μCi/ml) was added to the infected culture, and 1 min later 100 μl of the culture was chilled, as previously described (13); simultaneously with chilling, 15 μl of 20% Casamino Acids (freshly prepared) was diluted into the culture, and aeration was continued at 30°C. Further 100-μl samples were chilled at 15.5, 16.3, 17, 18, 20, 22.5, and 27 min after infection.

All chilled samples were centrifuged in 1-ml cellulose acetate butyrate centrifuge tubes (Sorvall) at 4°C and 11,000 rpm for 10 min. Pelleted bacteria were suspended and lysed with lysozyme and the nonionic detergent Brij 58 as previously described (13), except that volumes used were one-half those previously used.

Kinetic labeling, lysis, and prefractionation of wild-type T7-infected *E. coli*. Wild-type T7-infected *E. coli* were subjected to kinetic labeling, lysed (with lysozyme and Brij 58), and fractionated by sedimentation in a biphasic gradient of sucrose and metrizamide (sucrose-metrizamide gradient), as previously described (16).

Digestion with trypsin. Samples were digested with trypsin as previously described (19). Trypsin-induced conversion of AG particles in unfractionated or prefractionated lysates to particles comigrating with capsid II during agarose gel electrophoresis (19) was not decreased by decreasing the amount of trypsin used by a factor of 10, indicating that this conversion was at completion for all experiments reported here.

Electrophoresis in agarose gels. Samples were brought to 13 μl with standard buffer and were diluted with 17 μl of sample buffer. Of this mixture, 20 μl was layered beneath 4 to 6 mm of electrophoresis buffer in sample wells of a 0.9% ME agarose (Marine Colloids, Rockland, Maine) slab gel in electrophoresis buffer, and electrophoresis was performed as previously described (19) for 16 h. After electrophoresis, gels were dried under vacuum and subjected to autoradiography.

Densitometry. The amount of ^{14}C in selected regions of gels was determined by densitometric scanning of autoradiograms (an R and D densitometer of Helena Laboratories, Inc., Beaumont, Tex., was used) and converting areas thus obtained to counts per minute, using an empirically determined calibration factor.

RESULTS

Infections with T7 amber mutants. To measure the kinetics of appearance of capsid I and AG particles, the amount of radiolabel in these particles is measured as a function of time during kinetic labeling (pulse-chase) experiments. Agarose gel electrophoresis of unfractionated lysates can be used to quantitate ^{14}C in capsid I and also in those AG particles which are converted by trypsin to particles migrating with capsid II (19) (all references to AG particles made below will refer only to those AG particles converted by trypsin to particles migrating with capsid II during agarose gel electrophoresis). Advantageous aspects of agarose gel electrophoresis of lysates without prefractionation by centrifugation are: (i) avoidance of the possible selective loss of particles during a procedure that includes prefractionation by centrifugation and (ii) requirement for 1/10 to 1/100 the time and cost of a procedure which includes centrifugation. However, if ^{14}C-labeled capsid II is

present in lysates, these particles, because they comigrate with trypsinized AG particles, will decrease the accuracy with which the amount of ^{14}C in AG particles can be measured; the presence of ^{14}C-labeled phage T7 and T7 capsid-DNA complexes would further decrease the accuracy of such measurements (17, 18).

Therefore, to measure the kinetics of appearance of capsid I and AG particles in the presence of the T7 core, *5am* T7 were used to infect a nonpermissive host (no DNA synthesized [21]) because capsid I, but neither capsid II nor phage T7 nor capsid-DNA complexes, is produced during such an infection (16, 19, 21; P. Serwer and R. H. Watson, unpublished data). To measure kinetics of appearance of AG particles and capsid I in the absence of a functional core, an *8am* mutant was used to infect the nonpermissive host because an *8am* mutant is the only one of the core-defective T7 mutants that makes no capsid II, capsid-DNA complexes, or bacteriophage T7 during such an infection (12, 19; Serwer and Watson, unpublished data). However, the *8am* mutant makes some capsid I in a nonpermissive host; the envelope of *8am* capsid I is indistinguishable from the envelope of wild-type capsid I by electron microscopy (12) and agarose gel electrophoresis (19).

A log-phase culture of *E. coli* BB/1 was infected with the *5am* mutant, and a second portion of the same culture was infected with the *8am* mutant at 30°C. Kinetic labeling (with ^{14}C-labeled amino acids) of both cultures was performed as described in the Materials and Methods; samples taken at eight times after the start of labeling were subjected to agarose gel electrophoresis after treatment with trypsin and without treatment with trypsin.

No band at the position of capsid II was present in any of the untrypsinized *5am* lysates, as expected (Fig. 1a, channels 3–10). In the trypsinized *5am* lysates (Fig. 1a, channels 11–18), a band at the position of capsid II was observed; this band is formed by AG particles (19). In Fig. 1a, ^{14}C appeared in AG particles earlier than it appeared in capsid I; the amount of ^{14}C in AG particles became maximal at 16.3 to 17 min after infection and subsequently decreased (shown quantitatively in Fig. 2). These kinetics suggest that the AG particles are capsid I assembly intermediates.

Initially, cells infected with the *8am* mutant (core negative) incorporated ^{14}C into capsid I and AG particles at roughly the same rate as the cells infected with the *5am* mutant (core positive). However, in contrast to results obtained with the *5am* mutant, with the *8am* mutant entry of ^{14}C into capsid I ceased by 1.3 min after termination of labeling with ^{14}C (Fig. 1b and 2). The premature cessation of the entry of ^{14}C into capsid I during infection with the *8am* mutant was accompanied by an accumulation of ^{14}C in AG particles, an accumulation not seen during infection with the *5am* mutant.

The premature cessation of the entry of ^{14}C-labeled protein into capsid I during infection with the *8am* mutant could be caused by either (i) a decrease in the capability of the infected cell for assembling capsid I with increasing time or (ii) the presence of two pathways for assembling capsid I, one in which capsid I is assembled more rapidly than in the other, and the requirement for a functional core in only the pathway in which capsid I is assembled more slowly. If possibility (ii) is correct, it must also be assumed that no ^{14}C-labeled protein can enter the pathway of more rapid capsid I assembly later than 1.3 min after completion of labeling.

To test the possibility of a time-dependent cessation in the capability of *8am*-infected cells for assembling capsid I, the experiment of Fig. 1 was performed with labeling starting at 19 instead of 14 min after infection. The amount of ^{14}C that entered *8am* capsid I was 1.5 times the amount that entered in the experiment of Fig. 1, and a premature termination of the synthesis of capsid I occurred at 1.3 min after the completion of labeling. Thus, it is concluded that the premature cessation of the assembly of capsid I does not result from a decrease in the capability of the infected cell for assembling capsid I. It is likely, therefore, that possibility (ii) above is correct.

Infection with wild-type T7. Although, as described above, kinetic labeling of wild-type T7-infected *E. coli* does not yield data as accurate as data obtained with the above mutants, a kinetic labeling experiment was also performed with wild-type T7-infected cells. To remove interference of phage T7 and capsid-DNA complexes, determination of the amount of ^{14}C in AG particles was performed with lysates prefractionated by sedimentation in sucrose-metrizamide gradients, as described in Materials and Methods. The amount of ^{14}C in AG particles was determined by subjecting ^{14}C-labeled particles cosedimenting with capsid II to agarose gel electrophoresis with and without prior treatment with trypsin, and subtracting the amount of ^{14}C migrating as capsid II in the untrypsinized sample from the amount of ^{14}C migrating as capsid II in the trypsinized sample.

Figure 3 shows the results of kinetic labeling of wild-type T7-infected *E. coli*. As found with *5am* T7-infected *E. coli* in Fig. 2, the kinetics of appearance of AG particles suggested that most of the AG particles were intermediates in capsid I assembly. However, at the later times in Fig. 3, ^{14}C left AG particles less rapidly than it left capsid I. Although the reason for this effect is not known, it could result from the production of

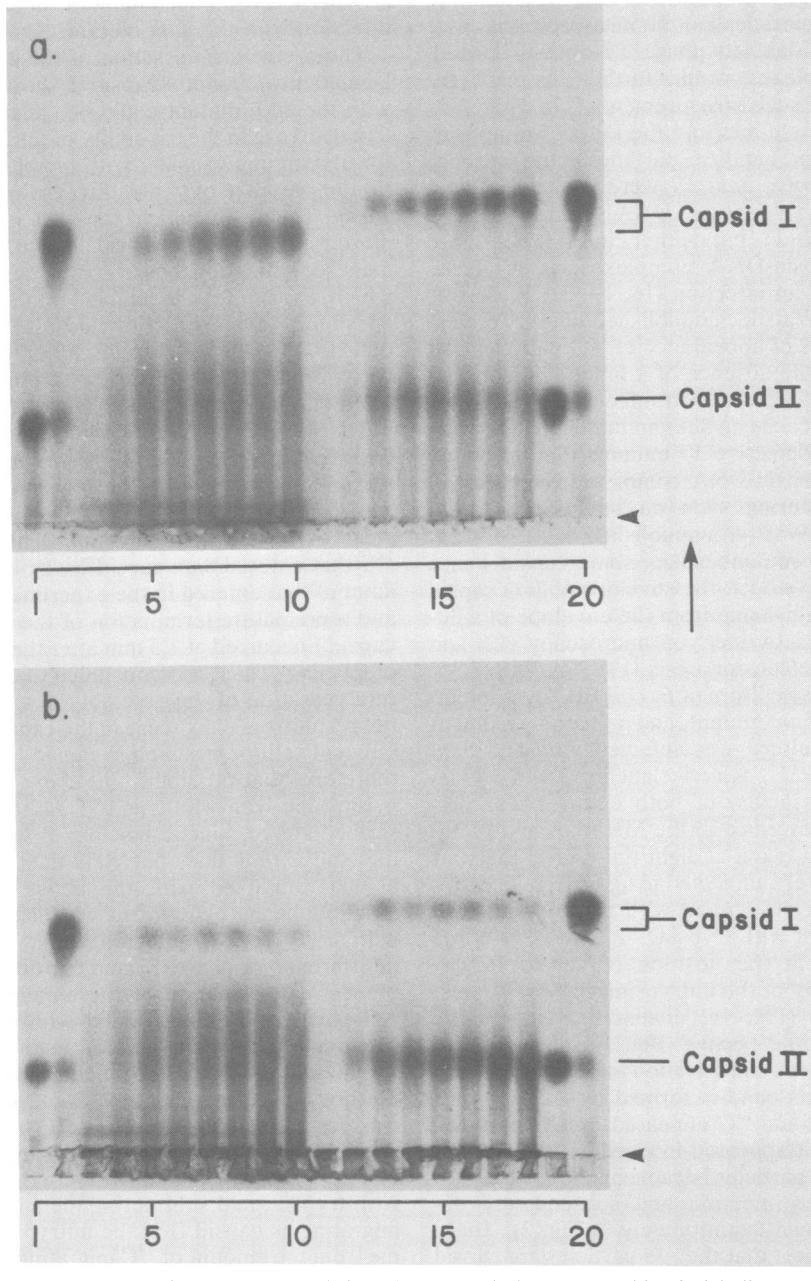

FIG. 1. Kinetic labeling of 5*am*- and 8*am*-infected nonpermissive host. A kinetic labeling experiment was performed, and infected bacteria were lysed as described in the text, using as the infecting bacteriophage: (a) 5*am* T7 and (b) 8*am* T7. The same culture of *E. coli* BB/1 was used for both mutants. A portion of each lysate was digested with trypsin, as described in the text. Trypsinized and equal untrypsinized portions of the lysates were subjected to electrophoresis, as were capsid I and capsid II isolated by sedimentation (16), as described in the text. The channels have, respectively: (1) capsid II; (2) capsid I; (3–10) untrypsinized samples taken at 15, 15.5, 16.3, 17, 18, 20, 22.5, and 27 min after infection; (11–18) the same samples as in channels 3 through 10, but with trypsinization; (19) trypsinized capsid II; (20) trypsinized capsid I.

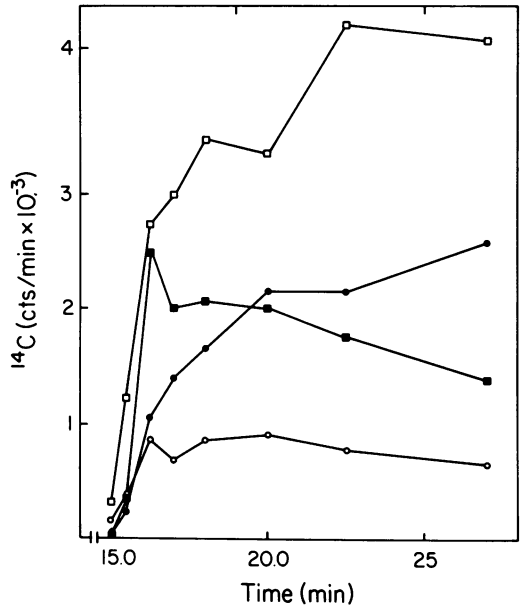

FIG. 2. Kinetics of appearance of capsid I and AG particles after infection with *5am* and *8am* T7. Amounts of ^{14}C in capsid I were determined by densitometry of profiles of untrypsinized lysates in Fig. 1; amounts of ^{14}C in those AG particles converted by trypsin to particles comigrating during agarose gel electrophoresis with capsid II were determined by densitometry of profiles of trypsinized lysates in Fig. 1. An empirical calibration factor was used to convert integrated optical densities to counts per minute. Plotted as a function of time after infection are the amounts of ^{14}C in: (●) *5am* capsid I; (○) *8am* capsid I; (■) *5am* AG particles; (□) *8am* AG particles.

a comparatively small amount of AG particles after assembly of capsid I.

DISCUSSION

The data presented here indicate that there are two pathways for the assembly of capsid I: (i) a core-independent pathway in which AG particles are not an assembly intermediate and (ii) a core-dependent pathway in which assembly of capsid I is slower than in the core-independent pathway and in which AG particles are an assembly intermediate. Furthermore, in the absence of a functional core, AG particles accumulate, apparently as abortive end products of assembly. If so, two questions concerning assembly of capsid I can be asked: How much has the structure of AG particles changed during cellular lysis and subsequent fractionation? What is the function of the core in the pathway of slower capsid I assembly?

Postlysis changes in AG particles. It has been shown that *9am* T7 lysates and *10am* T7 lysates contain no AG particles or capsids of any recognizable kind (19). This suggests that P9 and P10, the two proteins of the capsid I envelope (14), must bind to each other before either of these two proteins can assemble in a capsid-like complex. The final product of assembly, capsid I, also has P9 and P10, apparently bound to each other. Unless P9 and P10 disassociate and then reassociate during assembly, an unlikely occurrence, both of these proteins must be present in all intermediates in capsid I assembly. Therefore, it is likely that AG particles (capsid II-like particles without P9), shown here to be intermediates in capsid I assembly, are a breakdown product of a capsid I-like particle (AG* particle) that has P9; these ideas are illustrated in Fig. 4.

Possible function of the T7 core during capsid I assembly. The presence of an apparently unaltered pathway of more rapid capsid I assembly in a nonpermissive host infected with the *8am* mutant indicates that the core does not affect the rate of capsid I assembly in this pathway. The data in Fig. 2 also suggest that in the absence of the core the rate of AG* particle assembly is not

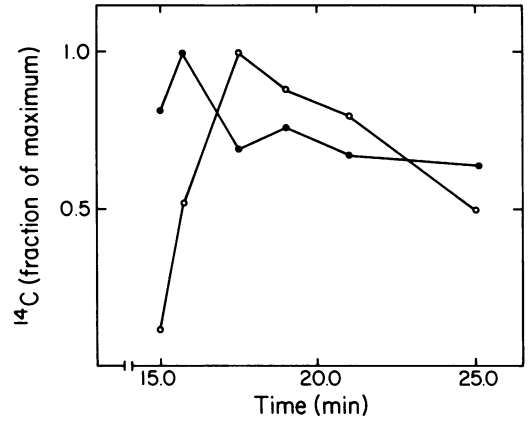

FIG. 3. Kinetics of appearance of capsid I and AG particles after infection with wild-type T7. Kinetic labeling of wild-type T7-infected *E. coli* and fractionation of lysates by sedimentation were performed as described in Materials and Methods. The amount of ^{14}C in capsid I was determined by agarose gel electrophoresis of unfractionated lysates and densitometry of the resultant profiles. The amount of ^{14}C in AG particles was determined by subjecting samples from the capsid II regions of gradients (used to prefractionate the lysates) to agarose gel electrophoresis, with and without digestion with trypsin, and then subtracting the amount of ^{14}C migrating as capsid II without trypsinization from the amount of ^{14}C migrating as capsid II after trypsinization. The ratio of the amount of ^{14}C to the maximal amount is plotted as a function of time. Symbols: (●) AG particles, maximal amount of ^{14}C = 51,101 cpm; (○) capsid I, maximal amount of ^{14}C = 122,305 cpm. The samples used in this experiment are the same as the samples used in reference 16.

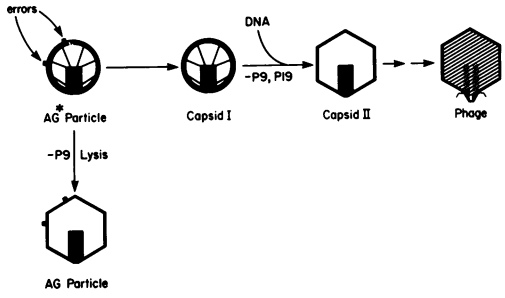

FIG. 4. Pathway of slower capsid I assembly. The pathway of slower capsid I assembly, as deduced in the text, is illustrated. The models of capsids I and II were deduced from observations in references 14 and 15 (see also reference 19).

decreased; the only process in capsid I assembly whose rate appears to be decreased by the absence of the core is the conversion of AG* particles (capsid I-like particles) to capsid I. Therefore, hypotheses (i), (ii), and (iii) mentioned at the beginning all appear to be incorrect for the assembly of T7 capsid I. (The data presented here are not a test of hypothesis [iv].)

Knowledge of the difference in structure between capsid I and AG* particles should provide clues to the function of the T7 core during capsid I assembly. Because the AG* particles have not yet been isolated, deduction of this difference in structure must be made from the properties of their breakdown products, AG particles. The only property of AG particles that appears to provide a clue is the sensitivity to trypsin of a small percentage of P10 molecules in AG particles and the lack of this trypsin-sensitive P10 in capsid II (19). This suggests that the trypsin-digestible molecules of P10 are incorrectly assembled in AG and presumably AG* particles. Therefore, it is proposed here that: (i) the function of the T7 core during capsid I assembly is to correct errors in the assembly of the capsid I envelope and (ii) AG* particles are particles that have uncorrected errors in the assembly of their envelopes (error correction hypothesis) (see Fig. 4). The error correction hypothesis explains particles of capsid I assembled in the pathway of more rapid assembly as particles which, by chance, did not make errors during assembly of their envelopes and, therefore, did not need the core for assembly. This hypothesis explains the conversion of AG* particles to capsid I in the pathway of slower assembly as a process of error correction, possibly performed by proteolysis of incorrectly assembled P10 and (or) P9.

The data that now exist suggest some aspects of how a process of error correction might work. The internal core of capsid I has been shown to consist of: (i) a cylinder connected at its base to the capsid I envelope and (ii) fibers connecting the cylinder to the capsid I envelope (14, 15; see Fig. 4). The fibers may be designed to fit the envelope in complementary fashion (the binding of the fibers to the envelope would be analogous to the fitting together of two separately assembled halves of a jigsaw puzzle). If the correct binding of envelope and fibers does not occur after assembly of the capsid I envelope, an error correction process is activated.

Some core-deficient particles of capsid I package DNA and the particles of bacteriophage formed are noninfectious (12). Therefore, a core-promoted correction of errors in the assembly of the capsid I envelope may increase the efficiency of production of infective particles by reducing the probability of the packaging of DNA by core-deficient particles. This would occur because a particle with a defective core would not be able to correct errors in the assembly of its envelope, and the resultant particles with uncorrected assembly errors in their envelopes would be incompetent to package DNA.

Other capsid I assembly intermediates. Intermediates in capsid I assembly less assembled than AG* particles must exist, but have not been analyzed in the present study. In a previous study (16), it was shown that unassembled T7 capsid proteins sediment either more slowly than capsids (i.e., with sedimentation coefficients less than 20) or more rapidly; the more rapidly sedimenting capsid proteins are bound to particles that have properties of membranes of *E. coli*. Further studies of capsid I assembly intermediates less completely assembled than AG particles are in progress.

ACKNOWLEDGMENTS

For technical assistance we thank Elena S. T. Moreno. For secretarial assistance we thank Brenda Moylan.

Support was received from the National Institutes of Health (Public Health Service grant AI 16117).

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