Purification and Characterization of the Small Subunit of Phage T4 Terminase, gp16, Required for DNA Packaging*

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Phage T4 terminase is an enzyme that binds to the portal protein of proheads and cuts and packages concatameric DNA. The T4 terminase is composed of two subunits, gene products (gp) 16 and 17. The role of the small subunit, gp16, in T4 DNA packaging is not well characterized. We developed a new purification procedure to obtain large quantities of purified gp16 from an overexpression vector. The pure protein is found in two molecular weight forms, due to specific C-terminal truncation, displays in vitro packaging activity, and binds but does not hydrolyze ATP. gp16 forms specific oligomers, rings, and side-by-side double rings, as judged by native polyacrylamide gel electrophoresis and scanning transmission electron microscopy measurements. The single ring contains about eight monomers, and the rings have a diameter of about 8 nm with a central hole of about 2 nm. A DNA-binding helix-turn-helix motif close to the N terminus of gp16 is predicted. The oligomers do not bind to DNA, but following denaturation and renaturation in the presence of DNA, binding can be demonstrated by gel shift and filter binding assays. gp16 binds to double-stranded DNA but not single-stranded DNA, and appears to bind preferentially to a gene 16-containing DNA sequence.

Although it was first established in phage T4 that the mature DNA ends result from headful packaging, in the case of T4 the mechanism of DNA end formation in packaging is less well defined (6). In fact, the phage T4 terminase displays many of the features of other phage terminases, including the small (gp16) and large (gp17) subunit structure (1, 7). Our genetic studies implicate gp16, the small subunit of T4 terminase, in amplifying a gene 17-gene 19 fragment. This is achieved by recombination following alignment of two homologous 24-base pair segments within gene 16 and gene 19 in T4 Hp17 (amplification) mutants. A synopsis model was proposed to correlate this novel activity with the known role of gene 16 in initiation of DNA packaging and in controlling activity of gp17, the terminase large subunit. This amplification suggests DNA binding by gp16 whose sequence specificity remains to be assessed (8, 9).

ATP hydrolysis is required for phage in vitro DNA packaging (1, 10). ATP not only provides an energy source for DNA translocation into the prohead, but also acts as an allosteric effector to control terminase holoenzyme specificity (11). λ terminase has two ATP binding sites, where the high affinity site is located in the large subunit, and the low affinity site exists in the small subunit in which a weak ATPase activity was detected (12–14). It is possible that ATP binding to the terminase small subunit may serve mainly as an allosteric effector rather than in energy transduction. Thus in the Bacillus subtilis phage SPP1, the small subunit binds but does not hydrolyze ATP (15). In T4, the large subunit showed DNA-dependent ATPase activity (7), whereas gp16 did not. This is consistent with our present findings; weak ATP binding to gp16 is observed, but not ATP hydrolysis.

Many overexpressed small terminase subunits form high molecular weight species. Overexpressed λ gpNu1 was found mainly in insoluble aggregates, raising difficulties for purification as well as for the study of DNA binding. This obstacle has been overcome by a well established solubilizing method, followed by a subsequent renaturation step (16). On the other hand, overexpressed T4 gp16 (Mr = 18,387; Ref. 17) is quite soluble, although it chromatographs as a high molecular weight complex whose structural properties were not characterized (7).

In this study, a new overexpression vector for production of gp16 at a higher level was constructed and a novel purification scheme was developed to obtain higher yields of pure active gp16. This report characterizes the DNA packaging activity, ATP affinity, and DNA binding properties of gp16, as well as the structure of the monomeric protein and of two specific oligomeric assemblies that it forms in vivo and in vitro.

EXPERIMENTAL PROCEDURES

Plasmid Construction and Protein Overexpression—An XhoI-EcoRI fragment containing gene 16 and its ribosome binding site (RBS) as well as the T4 late promoter for genes 16 and 17 (7, 17) was cut from plasmid pR16, and inserted into a pET12a vector containing a φ10 T7 promoter.
to make pL16 (Fig. 1). HMS174 (DE3) was transformed with pL16 and induced at a concentration of 0.4 mM IPTG at 37°C for 4 h as described by Studier et al. (18).

Purification Scheme—IPTG induced HMS174 (DE3) bacteria containing pL16 from 2 liters of Luria Broth were collected by centrifugation at 4,221 × g for 15 min and resuspended in buffer A (20 mM Tris-HCl (pH 7.0), 1 mM EDTA, 0.5 mM DTT, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF)). The suspension was then disrupted in a French press at a pressure of 20,000 lb. After centrifuging down the cell debris at 26,890 × g for 30 min, 5% (final concentration) streptomycin sulfate was added to the 30-ml supernatant. The supernatant was stirred for 20 min at 4°C and centrifuged again at 26,890 × g for 30 min. Solid ammonium sulfate was slowly added to the streptomycin supernatant to reach 50% final concentration at 4°C. After sitting on ice for 1 h, the insoluble material was collected by centrifuging at 26,890 × g for 1 h and was resuspended in 15 ml of buffer B (20 mM Tris-HCl (pH 7.0), 0.5 mM DTT, and 0.1 mM PMSF). Solid urea was added to reach a final concentration of 6 M after overnight dialysis against buffer B. The urea-deNatured sample was loaded into a 10-ml Bio-Rad ceramic hydroxyapatite column, which was equilibrated with buffer C (buffer B + 6 M urea). After extensively washing with buffer C, a 0–10 mM sodium phosphate (pH 7.0) gradient in buffer C was developed. A Bio-Rad High Q column (5-ml cartridge) was equilibrated with buffer Q (50 mM Tris-HCl (pH 7.5), 0.5 mM DTT, 0.1 mM ATP, 1 mM EDTA, 5 mM MgCl2, and 0.1 mM PMSF). The hydroxyapatite peak fractions were free from urea gradually by dialysis against buffer Q containing, successively, 3, 1.5, 0.75, and 0.5 M urea. The renatured protein was loaded onto the High Q column, and a 0.10–1 M NaCl gradient was applied. The fractions containing gp16, as judged by SD-SAGE, were collected and rechromatographed on the High Q column to remove high molecular weight contaminants. Except for the room temperature hydroxyapatite column, the remainder of the purification was performed at 4°C on a Bio-Rad Econo System.

Preparation of Anti-gp16 Antiserum and Immunoprecipitation—100 μg of gp16 was eluted from the gel according to Hager and Burgess (19). 20–30 μg of the eluted gp16 in phosphate-buffered saline was emulsified with an equal volume of Freund's complete adjuvant and injected into the rabbit subcutaneously. 20 μg of eluted gp16 in Freund's incomplete adjuvant was injected subcutaneously 3 weeks later. A subsequent booster, using 10 μg of gp16, was given similarly to the first one. Four days later, the antiserum was obtained and was able to interact with gp16 as judged by Western blotting. The preimmune serum was harvested prior to the first antigen injection from the same rabbit. 85-Labeled late protein lysates from T4 wild type and mutants grown in the absence of IPTG and IPTG were obtained according to Vanderslice and Yegian (20), except the labeling time was from 10–30 min after the first infection. 10 μCi/ml [35S]methionine (850 Ci/mmol) was added to the overexpression strain after a 10-min induction. A labeled gp16-containing bacterial lysate was harvested 50 min after the addition of the isotope at 37°C. Labeled bacterial extracts were prepared immediately after centrifugation by boiling in SDS-polyacrylamide gel electrophoresis running buffer and 15,000 rpm from each lysate was used to do immunoprecipitation with anti-gp16 antiserum according to the procedure of McNicol et al. (21).

ATP-phoaffinity Labeling and ATPase Assay—20 μl of gp16 in 50 mM Tris-HCl (pH 7.4), 10% glycerol, 6 mM MgCl2, 5 mM DTT, 5 μM ATP, and 0.5 μM ATP was added to the overexpression strain after an aluminum block on ice and irradiated with a General Electric G15T8 15-watt germicidal UV lamp at a distance of 8 cm for 20 min. 1 μl of 50 mM ATP was added to stop the reaction. After the addition of 10 μg of gp16 as carrier protein, 200 μl of 20% trichloroacetic acid was added and a precipitate was allowed to form on ice for 1 h. After spinning at 23,500 × g for 1 h, the pellet was washed with 1 ml of 20% trichloroacetic acid followed by acetone. Then it was subjected to SDS-PAGE, dried, and autoradiographed (22). ATPase assays were carried out by chromatography on polyethyleneimine-cellulose, followed by autoradiography according to Debreceni et al. (23).

STEM Microscopy—Analysis of oligomers of gp16 was performed at Brookhaven National Laboratory using the STEM facility. Freeze-dried specimens for mass analysis were prepared by the wet film technique (24). Briefly, samples in solution were deposited on thin carbon film, where ultraviolet light with controlled internal control was deposited on them. The samples were extensively washed with 20 mM ammonium acetate before being freeze-dried overnight. Stained specimens were prepared similarly except that the final wash was stain, which in this case was 2% osmium tetroxide (Novanov, Nano-probes, Inc., Stony Brook, NY), and the samples were air-dried.

Mass analysis of unstained freeze-dried specimens is possible because the number of scattered electrons collected by the annular detectors in the dark field mode is directly proportional to the mass thickness. An automated computer program (AutoMass) was developed by J. Wall to analyze the digital STEM data. By subtracting the background of the thin carbon supporting film, using an appropriate calibration (either determined from the control TMV present, or using the microsphere calibration), the sum of scattered electrons over the area of an individual particle gives its molecular weight. The AutoMass program selects TMV segments and particles to measure which fit a model (models) whose parameters have been chosen. Two different models were used to select the larger oligomer forms (see Table II).

DNA Probes and Gel Shift Binding Assay—A 24-base ssDNA oligonucleotide sequence (5′-GAAGCTCATGATCCTGCCTGTCAGAAG-3′) was synthesized (Biopolymer, Inc.) and annealed at 50°C at Baltimore Medical School. 1 pmol of DNA was labeled by phosphorylation with bacteriophage T4 polynucleotide kinase (25). The radiolabeled fragment was purified by Ultrafine-MC filter units, 5,000 nominal molecular weight limitation low binding-regenerated cellulose membrane (Millipore), spun at 6,500 rpm (microcentrifuge) for 1 h. 100 μl of Triis-EDTA (TE) buffer was added, and the polynucleotide was spun for another 1 h to wash free of radioactive mononucleotide. The probe was resuspended in 30 μl of TE (pH 8.0). A 215-base pair PCR product, made from pL16 with primer 22 (5′-GAATCCGCACTGATCATGATCCTGCCTGTCAGAAG-3′) and primer 4 (5′-AGATTTATCATCATCATCT3′), which corresponds to the 3′ end of gene 16 (8), was isolated from 4% FMC Nusieve GTG agarose run in 1× Tris-acetate-EDTA (TAE) buffer using a Qiaex gel extraction kit from Qiagen Co. The DNA fragment was labeled by T4 polynucleotide kinase (25) and was purified as the above except using Ultrafine-MC filter units, 10,000 nominal molecular weight limitation low binding-regenerated cellulose membrane. The labeled DNAs (8 × 106–8 × 107 μm ssDNA; 2.5 × 10−12 μm 215-base pair dsDNA fragment) were mixed with 2.7 × 10−12 μm denatured gp16 in 100 μl (final volume) of buffer Q with 2 μM urea and renatured by gradually dialyzing away the urea from buffer containing successive urea concentrations of 1, 0.5, 0.25, 0.125, 0.0125, and 0 M. The renatured gp16 with or without the addition of anti-gp16 antiserum was run on an 10% native polyacrylamide gel in 1× TAE in the presence of ATP and Mg2+. The gel was dried and subjected to autoradiography.

DNA Filter Binding Assay—Plasmids pL16 and pET11a were radioactively labeled by nick translation (Amersham Corp.). Membrane filters (HATF) from Millipore were soaked in soaking buffer (50 mM Tris-HCl (pH 7.5), 0.5 mM DTT, 3 mM ATP, and 1.5 mM MgCl2) for 30 min. gp16 was concentrated by 5% trichloroacetic acid precipitation on ice, washed twice with 200 μl of 50 mM Tris-HCl, pH 8, to 10 mg/ml in 6 μl urea in Q buffer. Aliquots of the 6 μl urea-dissolved protein were pipetted into a series of microcentrifuge tubes. The protein was then gradually renatured by adding aliquots (sequential additions of the original volume) of the Q buffer to dilute the urea. When the protein was suspended in 2 μl urea, 5 fmol of DNA probe (3000–5000 cpm) was added to each reaction tube in the final volume of 20 μl. By adding serial amounts of Q buffer, the protein and probe were renatured gradually by diluting the urea concentration to 1, 0.5, and 0.25 M at room temperature. The samples were then allowed to sit at room temperature overnight, and 1 ml of Q buffer was added the next day, mixed, and the mixture slowly filtered through the membrane at a speed of 2 ml/min on a Millipore filtration manifold. The membranes were washed once with 1 ml of 50 mM NaCl in 50 mM Tris-HCl (pH 7.5) and 2.5 mM EDTA. The membranes were air-dried and subjected to scintillation counting.

Protein and DNA Gel Analysis—A 12.5% SDS-polyacrylamide gel was used for the separation of the gp16 monomer, and an 8% native polyacrylamide gel was used for the DNA binding (band shift) measurements and for characterization of the oligomeric complexes. Polyacrylamide gels were run by standard methods (25). Electrophoretography of SDS-PAGE for N-terminal sequencing was according to Bio-Rad, and N-terminal sequencing was done by the Macromolecular Resources Lab at the 4° C State University. C-terminal sequencing of the purified gp16 (Q2, Table I) and of the two proteins separated by SDS-PAGE and blotted to Teflon supports (26) was kindly performed by Dr. Jerome Bailey, Hewlett-Packard Co. Mass spectrometry was carried out on fraction Q2 at the Hewlett-Packard Co., Palo Alto, Calif. Protein concentration was determined by the Bradford assay (Bio-Rad).

Other Materials and Methods—The in vitro T4 DNA packaging assay was according to Black (27) using gene 16amN87-amN67 restriction mutant-infected bacterial extracts containing –2 × 109 phageheads.
**RESULTS**

New Purification Procedures—pL16 has three ATG and RBS sites (Fig. 1); one in the NdeI site 5' to the gene 16 coding region, a second at the beginning of gene 16, and a third within gene 16 which initiates translation of gene 17 at the five-codon, out-of-frame overlapping region between genes 16 and 17. The first 105 base pairs of gene 17 remain in the pL16 plasmid. A truncated peptide is expected from the first ATG, since there are two downstream stop codons before the second gene 16 ATG site. Therefore, the overexpressed gp16 is expected to be synthesized only from the RBS and ATG site for gene 16. Therefore, the overexpressed gp16 is expected to be a truncated peptide from the first ATG, since there are two downstream stop codons before the second gene 16 ATG site. Therefore, the overexpressed gp16 is expected to be synthesized only from the RBS and ATG site for gene 16. Therefore, the overexpressed gp16 is expected to be synthesized only from the RBS and ATG site for gene 16.

The purified gp16 appeared as a closely migrating doublet band upon SDS-polyacrylamide gel electrophoresis (Fig. 3B), containing gene 16 and overlapping gene 17 with their ribosome binding sites inserted into pET12a. The purified protein is highly active, since a half-maximal yield of phage is reached at a concentration of the purified gp16 of about 5.5 x 10^-2 μM (0.05 μg/ml). However, the enzymatic activity measured in the lower concentration range is not linear with respect to the amount of protein added. Probably, multiple copies of gp16 are required to form an active packaging gp16-gp17 complex, which occurs below the 0.01 μg (1.1 x 10^-2 μM) level and contributes to a threshold stage (Fig. 4, inset). When gp16 and gp17 are co-expressed, gp17 and gp16 are found to be associated during purification, although the association appears to be weak (7). ATP-photophosphorylation Labeling and ATPase Activity—ATP was UV-cross-linked to gp16 (22), and the labeling was prevented by the presence of 50 (and 500) μM non-radioactive ATP (Fig. 5, A and B). Without UV irradiation, ATP was not able to cross-link to gp16 (data not shown). We observed that the lower gp16 band (the major form) bound ATP less well than the upper band. Unexpectedly, a 50-kDa band appeared in the protein-stained gel as well as the autoradiogram. Since the band also appeared in the UV-irradiated sample containing protein without the addition of any ATP, but was dependent upon UV illumination.

**Fig. 2.** The protein profiles on SDS-polyacrylamide gel of steps in the purification. Lane 1, protein size markers. Lane 2, the cell lysate without IPTG induction. The induced lysate is shown in lane 3. gp16 was overexpressed in the range of 5–10% of the total protein. The streptomycin sulfate precipitate, which was used in subsequent purification steps, is shown in lane 4. Lane 5, the ammonium sulfate precipitate. Hydroxyapatite, Q1, and Q2 fractions (Table I) are shown in lanes 6–8, respectively.
irradiation, it could be due to cross-linking of protein monomers to form multimers via tyrosine residues, which are in proximity in the gp16 oligomer. ATPase activity was also measured in the presence and absence of dsDNA and of 1% Sarkosyl to dissociate the oligomers; however, no ATP hydrolysis was detected even following overnight incubation with 10 μg of gp16 (data not shown). Of course, a gp16-associated ATPase activity could appear when the protein is assembled with gp17 and proheads.

The Structure of the gp16 Oligomers—The purified gp16 fractions were run on an 8% native polyacrylamide gel. gp16 migrated as two sharp high molecular weight complexes toward the top of the gel, C1 and C2, with little or no detectable gp16 monomer (Fig. 6). The oligomers reformed from 6 M urea, and were stable in 1 M NaCl, although Sarkosyl treatment released some monomer (Fig. 6, lane 9). The molecular weight of the lower major band was judged from the polyacrylamide migration compared to standards to be in the mass range of 150–200 kDa. This measurement is consistent with the STEM measurement of the complexes (Table II).

Fraction 19 from the 8% native acrylamide gel was used to prepare specimens for the STEM. Masses were determined on unstained, freeze-dried specimens, such as shown in Fig. 7A. The summary of the mass measurements is shown in Table II. A histogram of the mass measurements (Fig. 8) shows that the mass distribution over 680 particles is bimodal, suggesting that the sample contained at least two populations of particles. One appeared quite round, possibly with a hole in the center, indicated by single-headed arrows in Fig. 7. The other was more rectangular and is indicated by double-headed arrows. The mass determination suggests that the round particles are an oligomer of approximately eight copies of gp16, whereas the other form appears to be approximately a dimer of that.

While unstained freeze-dried specimens are necessary for mass measurements, fine structural details are often blurred because of radiation damage in the electron beam. Stained specimens were prepared from the same fraction 19. The hope was to see some symmetry in the round particles to better determine their oligomeric state. Fig. 7B shows the stained

| Fraction | Protein amounts | Total activity | Specific activity | Yields | Purification |
|----------|----------------|----------------|------------------|--------|--------------|
| 1. Crude extract | 984 | 8.0 × 10^11 | 8.1 × 10^6 | 100 | 1 |
| 2. Ammonium sulfate | 42 | 8.8 × 10^10 | 2.1 × 10^6 | 11 | 2.6 |
| 3. Hydroxyapatite | 20.4 | (0) | (0) | (0) | (0) |
| 4. Renaturation | 20.4 | 3.9 × 10^11 | 1.9 × 10^7 | 48.8 | 23.5 |
| 5. High Q (Q1) | 13.2 | 3.3 × 10^11 | 2.5 × 10^7 | 41.2 | 30.9 |
| 6. High Q (Q2) | 9.8 | 3.0 × 10^11 | 3.1 × 10^7 | 37.5 | 38.3 |

Fractions are as described in Fig. 1B.

Unit is a plaque forming unit assayed as defined under "Materials and methods".
gp16 Is Required for DNA Packaging

FIG. 5. ATP-photoaffinity labeling of gp16. A, the Coomassie Brilliant Blue R-250 stained gel, which shows the position of the gp16 doublet following SDS-polyacrylamide gel electrophoresis; B, the autoradiogram of A. Lane 1, the protein size markers. Lanes 2 and 3 represent 10 and 20 min of UV incubation time, respectively. Lanes 4 and 5 show the results of the UV incubation carried out in the presence of 50 and 500 μM nonradioactive ATP.

FIG. 6. gp16 forms stable oligomers (C1 and C2) as judged by native polyacrylamide gel electrophoresis. Lanes 1 and 2, protein size markers. Fractions 15–20 (Q2) are shown in lanes 3–7, respectively. Lane 8, gp16 renatured from 6 M urea. Lane 9, partial dissociation of the oligomer into monomeric form (M) in the presence of Sarkosyl.

specimens. The single arrows point to round particles that are indeed rings. What was surprising was the structure of the larger particles, indicated by double-headed arrows, which appear to be two joined, interlocked, or side-by-side rings. The single rings can be seen in Fig. 7C. They are rings with a diameter of approximately 8 nm and a hole of approximately 2 nm diameter. There is no obvious symmetry. In stain, both forms appear slightly irregular and variable, which is what was seen in their mass measurements.

DNA Binding Activity—Modified DNA filter binding and band shift assays using a denaturation and renaturation process demonstrated that gp16 binds to dsDNA. A 215-base pair DNA fragment corresponding to the 3′ end of gene 16 was used as a probe in a modified gel band shift assay (see “Experimental Procedures”), which showed a band shift only after gp16 in the presence of the dsDNA probe underwent denaturation and renaturation, as compared to the reaction with the oligomer (Fig. 9B, lanes 2 versus 1). In addition, a minor, slower migrating band (x in Fig. 9B) always appears in the free dsDNA probe. Loop formation after denaturation and renaturation or bending could explain the appearance of this minor component and its absence in lanes 2–4 (Fig. 9B) could be explained by greater accessibility to contaminants in the purified gp16 or antisera. gp16 binding to DNA was enhanced by the presence of the gp16 antiserum, which contributed to a band supershift (Fig. 9B, lane 4) that was absent when using the preimmune serum (Fig. 9B, lane 3). In contrast, a ssDNA did not show any evidence of gp16 interaction using the same procedures (Fig. 9A), suggesting specificity for double-stranded DNA. The band shift assay is not quantitative, and use of other DNA fragments that did not correspond to gene 16 sequences also showed band shifts under these assay conditions (data not shown).

When quantitative DNA filter binding assays were carried out, the results supported the band shift assays in showing binding of gp16 to DNA. By this method of detection, binding also requires the denaturation and renaturation procedure. Binding to pL16 is stronger than to pET12a, suggesting preferential binding to a gene 16 sequence (Fig. 10). Under such conditions, the binding occurs only when the molar ratio of protein:probe is over 10,000 and a critical protein concentration is achieved. A comparable requirement for high molar ratios of protein to DNA to observe DNA binding has also been observed for other terminase small subunit proteins (see “Discussion”).

At its N-terminal end, gp16 contains a predicted helix-turn-helix (H-T-H) motif, similar in structure and location to the putative DNA binding motifs of gpNu1 and gp1, the small subunits of λ and SP1 terminases, respectively. The gp16 sequence has the characteristic signature pivot residues of a H-T-H structure, Ser3, Gly11, and Ile17. This region of gp16 is predicted to be α-helical (28). In addition, 60% homology with H-T-H residues in other proteins in addition to conservative replacements at other positions are observed (Table III).

The DNA binding domains from λ and other proteins were determined by crystallography or predicted by computer homology search. Comparison of gpNu1 predicted an N-terminal DNA binding domain (29). A DNA binding motif of gp1 in phage SP1 is well characterized, and the sequence also shows some similarities to gpNu1 (15). Therefore, all three comparable molecular weight terminase proteins contain the binding motif in the same N-terminal region, an unusual location among H-T-H proteins.
**DISCUSSION**

T4 terminase is an enzyme that displays DNA-dependent ATPase, DNA packaging, and endonuclease activities. The active holoenzyme did not bind to single-stranded or double-stranded DNA columns during purification, suggesting that other factors are required to activate DNA binding *in vivo* (7). The individual subunits display different activities. The large subunit gene apparently is associated with nonspecific endonuclease activity and is toxic to the host cells when overexpressed alone (30). Gene 16 reduced gene 17 toxicity and allowed its overexpression in a ApL promoter-containing plasmid (7). Possibly, gp16 modulates gp17 to produce a more specific regulated endonuclease activity following assembly into holoenzyme, as gpNu1 does with gpA in λ (31).

Despite minimal sequence homology, phage terminases often display similar organization of structural and functional domains. We observed that the intact gp16 binds ATP more strongly than the truncated form, which implies an effect of the truncation on the ATP interaction site. The ATP reactive site seems unlikely because a serine-protease inhibitor did not prevent formation of the truncated gp16. Moreover, these two proteins are also synthesized in similar proportions during a transcriptional round of T4 infection analyzed without incubation of the extract as determined by immunoprecipitation (Fig. 3C). The interaction domain between gp16 and gp17 has not been identified. However, in phage λ, the C terminus of the small subunit interacts with the N terminus of the large subunit (33). It could be that in T4 synthesis of the major truncated gp16 component accounts for the weak association to gp17 during the purification of the holoenzyme if only the minor full-length gp16 binds to gp17 (7). However, the biological roles of the two gp16 proteins *in vivo* remain to be tested.

Our present biochemical analysis of gp16 is compatible with the genetic evidence suggesting a DNA binding role of this component of the T4 terminase (8). Most of the terminase small subunits are DNA-binding proteins as is predicted from extensive genetic analysis (GpNu1 in λ, gp19 in T3, and gp1 in SPP1) (cf. Ref. 1). As already discussed, several small terminase subunits, including gp16, contain a predicted helix-turn-helix DNA binding motif. However, in order to show DNA binding, a large excess of each protein has generally been required in the binding reaction. In our DNA binding assay, we also used a very high molar ratio of protein:DNA (>10,000-fold), and denaturation and renaturation together with the DNA were also required to observe binding in the gel band shift and filter bind-
ing assays. A threshold concentration of protein was also required for the filter binding, comparable to that observed for the SPP1 gp1 binding (34). In the case of SPP1, other factors were not required for DNA binding. In the case of phage λ, other DNA-binding proteins (IHF or HU) are required for binding (35). From our analysis, the gp16 oligomer does not bind DNA and the tendency of gp16 to oligomerize is very strong. We suppose that other factors are also required to observe the T4 small subunit DNA binding in vivo; however, urea denaturation allows a specific nucleoprotein complex to form in their absence.

The native polyacrylamide gel and STEM analysis shows that the gp16 oligomer, far from being a nonspecific aggregate, consists of specific ring and double ring structures. The STEM measurements show that the oligomer is an ~8-nm ring with a ~2-nm central hole, with approximately eight subunits, on average, per single ring. In fact, this is the first close look at the structure of a terminase subunit. A comparable ring-like structure also appeared in the terminase small subunit of the B. subtilis SPP1 phage, although only single rings estimated to be decamers were observed (34). A number of possible gp16 interactions might account for the formation of the ring doublet. This structure should be relatively stable, since it apparently survives gel electrophoresis. One possibility based on a single type of gp16 self-association is that the rings are helical, and that the ring doublet is a flattened two-turn helix, i.e. the rings and double rings are actually washers and double washers, where the latter has spread out due to weak stacking. How the T4 ring structures correlate with terminase function and DNA binding are interesting questions. We speculate that gp16 forms an analogous nucleoprotein complex with the DNA. The formation of ring dimers could correspond to the postulated synopsis of two homologous DNA fragments, serving either as a packaging signal or triggering the recombination event in vivo probably in conjunction with other host or phage accessory proteins (8).

Previous studies indicating that gp16 was not only involved in DNA packaging but also in the sequence-specific in vivo recombination of two homologous sequences, which results in the formation of multiple copies of terminase gene 17 and adjacent genes (8), suggested that the T4 terminase subunit recognizes a pac-like site for preferential packaging. In this work we did observe preferential binding to gene 16-containing plasmid DNA. Deletion of the pac-like sequence in gene 16-containing plasmid constructions also resulted in substantial decreases in T4 transduction of these plasmid DNAs. Taken together with the DNA binding studies reported in the present study, it appears that the small terminase subunit does bind preferentially to a sequence in its structural gene. Studies are under way to determine the extent of this binding specificity and the identification of other accessory factors required for this binding in vivo.

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