Assembly-associated structural changes of bacteriophage T7 capsids

Detection by use of a protein-specific probe

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ABSTRACT To detect changes in capsid structure that occur when a preassembled bacteriophage T7 capsid both packages and cleaves to mature-size longer (concatemeric) DNA, the kinetics and thermodynamics are determined here for the binding of the protein-specific probe, 1,1′-bi(4-anilino)naphthalene-5,5′-di-sulfonic acid (bis-ANS), to bacteriophage T7, a T7 DNA deletion (8.4%) mutant, and a DNA-free T7 capsid (metrizamide low density capsid II) known to be a DNA packaging intermediate that has a permeability barrier not present in a related capsid (metrizamide high density capsid II). Initially, some binding to either bacteriophage or metrizamide low density capsid II occurs too rapidly to quantify (phase 1, duration <10 s). Subsequent binding (phase 2) occurs with first-order kinetics. Only the phase 1 binding occurs for metrizamide high density capsid II. These observations, together with both the kinetics of the quenching by ethidium of bound bis-ANS fluorescence and the nature of bis-ANS-induced protein alterations, are explained by the hypothesis that the phase 2 binding occurs at internal sites. The number of these internal sites increases as the density of the packaged DNA decreases. The accompanying change in structure is potentially the signal for initiating cleavage of a concatemer. Evidence for the following was also obtained: (*a*) a previously undetected packaging-associated change in the conformation of the major protein of the outer capsid shell and (*b*) partitioning by a permeability barrier of the interior of the T7 capsid.

INTRODUCTION

Bacteriophage T7, like several other double-stranded DNA bacteriophages, consists of both a protein capsid and DNA packaged within the capsid. During assembly of these bacteriophages in infected cells, a DNA-free procapsid (called capsid I for T7) initially forms and, subsequently, packages DNA; the procapsid has an outer shell that differs in structure from that of the mature bacteriophage capsid (reviewed in Casjens, 1985; Black, 1989; Serwer, 1989). During packaging, T7 capsid I converts to a capsid (capsid II) that has an outer shell indistinguishable (thus far) from the outer shell of the mature T7 capsid. Two capsid II particles have been isolated: capsid II impermeable to metrizamide (metrizamide low density [MLD][1] capsid II) and capsid II permeable to metrizamide (metrizamide high density [MHD] capsid II). Both are in the T7 DNA packaging pathway (Serwer, 1980). The normal DNA substrate for packaging is an end-to-end multimer (concatemer); the concatemer is cut to mature size (39.936 kilobase pairs) (Dunn and Studier, 1983) during packaging (reviewed in Serwer, 1989; Chung et al., 1990). The mature T7 capsid consists of a T = 7 icosahedral outer shell (radius = 30 nm), an externally projecting tail, and an internally projecting cylinder; a sixfold symmetric ring (connector) joins the cylinder, outer shell, and tail (reviewed in Steven and Trus, 1986) (see Fig. 1 *a*). Only the outer shell, inner cylinder, and connector are present in MLD capsid II (Serwer, 1980) (see Fig. 1 *b*). The packaged, wild-type T7 DNA occupies about one-half of the space in the mature capsid (Stroud et al., 1981; Rontó et al., 1983). A connector is present in all double-stranded DNA bacteriophages (reviewed in Bazinet and King, 1985; Carrascosa, 1986; Kellenberger, 1990). Evidence that the different connectors have at least one function in common includes both similarity of structure (Carrascosa, 1986; Casjens et al., 1992) and, in the case of bacteriophages ϕ29 and λ, interchangeability of the connectors (Donate et al., 1990). In the case of bacteriophage P22, one of the connector's functions is to regulate the cleavage of concatemers that occurs at the end of packaging (Casjens et al., 1992).

For determining the mechanism by which capsids both package and cut DNA, structural transitions of capsids are probed. 1,1′-bi[4-anilino]-naphthalene-5,5′-disulfonic acid (bis-ANS) is a protein-specific probe for a hydrophobic environment (Farris et al., 1978). Both bis-ANS and structurally related probes have been used to detect variation in the conformation of proteins (Wang and Edelman, 1971; Farris et al., 1978; O'Brien and Gennis, 1979; Horowitz and Criscimagna, 1985; Dunker et al., 1991; Javor et al., 1991). In the case of bacteriophage T4, bis-ANS binding sites are present, but none are on the outer surface of the capsid (Griess et al., 1991). In the present study, the structure of both bacteriophage T7 and T7 MLD capsid II have been probed by use of bis-ANS. To determine effects of DNA packing density, the structure of a T7 deletion mutant has also been probed. Implications are discussed for understanding the mechanisms of T7 DNA cutting and packaging.

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[1] *Abbreviations used in this paper:* bis-ANS, 1,1′-bi(4-anilino)naphthalene-5,5′-di-sulfonic acid; K_d, dissociation constant; MHD, metrizamide high density; MLD, metrizamide low density; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Tris, tris(hydroxymethyl)-aminomethane.

MATERIALS AND METHODS

Bacteriophage and capsids

Wild-type bacteriophage T7 (T7wt) and an 8.4% T7 deletion mutant, C5-LG3 (T7-C5,LG3) (Studier, 1973*a*) were received from Dr. F. W.

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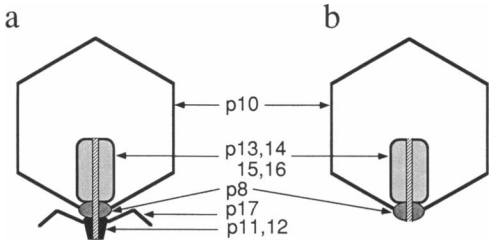

FIGURE 1 The structure of capsids. The structure of (*a*) the capsid component of T7wt and T7-C5,LG3 and (*b*) MLD capsid II are shown. These structures are derived from electron micrographs of negatively stained specimens (reviewed for T7wt in Steven and Trus, 1986; data for MLD capsid II are in Serwer, 1980). The T7 connector is formed by p8.

Studier. The host for both bacteriophages was *Escherichia coli* BB/1. Bacteriophages were grown in 2 × LB medium (20 g Bacto tryptone, 10 g yeast extract, 5 g NaCl in 1,000 ml H_2O) and purified by centrifugation in cesium chloride density gradients (Serwer, 1980). By use of procedures described in Serwer (1980), capsid II from T7-C5,LG3-infected cells was fractionated by centrifugation in cesium chloride gradients, followed by rate zonal centrifugation in a sucrose gradient and then by buoyant density centrifugation in a metrizamide density gradient. MHD capsid II was preparatively separated from MLD capsid II during this latter centrifugation. To further purify MLD capsid II, buoyant density centrifugation in a cesium chloride density gradient was repeated. This final step removed comparatively small amounts of MLD capsid II that had some subgenomic, packaged DNA. By ethidium staining of MLD capsid II after nondenaturing agarose gel electrophoresis, <0.01% of the mass of the MLD capsid II used here was nucleic acid. T7 capsids and mature bacteriophage were dialyzed against tris(hydroxymethyl)-aminomethane (Tris)/Mg buffer: 0.5 NaCl, 0.01 Tris-Cl, pH 7.4, 0.001 $MgCl_2$. Molar concentrations of T7wt were determined by use of absorbance at 260 nm and a molar extinction coefficient of 1.5 nM^{-1} cm^{-1} (Bancroft and Freifelder, 1970). After correcting the molar concentration by 8.4%, the same procedure was used for T7-C5,LG3. Molar concentration of MLD capsid II was determined by comparison of the amount of major capsid protein, p10 (T7 proteins will be indicated by p, followed by the number of the protein's gene [Dunn and Studier, 1983]), with the amount of p10 in a preparation of a known concentration of T7wt. The amount of p10 was quantified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (see below), followed by staining with Coomassie blue (Serwer, 1980) and video densitometry (Griess and Serwer, 1992). The amount of p10 per capsid was assumed to be the same in DNA-free capsids as it is in the mature bacteriophage.

Quantification of the binding of bis-ANS

Binding of bis-ANS to T7 proteins was quantified by the fluorescence enhancement induced by binding. As previously found for bacteriophage T4 (Griess et al., 1991), no binding was observed to either denatured or native T7 DNA prepared by phenol extraction. A recording fluorescence spectrometer (model 500C; SLM Instruments, Inc., American Instrument Co., Urbana, IL) was used for all measurements of fluorescence intensity, I. This instrument (*a*) had a thermostated cell holder accurate to ±0.5°C, (*b*) had a 300-W xenon lamp and slit set for a band width = 5 nm, and (*c*) was controlled by SPF-500C software, version 2.4, obtained from SLM Instruments. Unless otherwise indicated, to minimize exposure of the sample to the light beam of the spectrometer, the advanced features menu of the software was used to open the shutter for 0.5 s only, whenever a reading was taken. The wavelength of excitation was 400 nm; the wavelength of emission was 500 nm (see Griess et al., 1991). The operating program both digitized and stored measurements in data files that were subsequently transferred to text files. The text files were processed by use of a spreadsheet program (Cricket Graph; Computer Associates, San Jose, CA). A Macintosh IIci microcomputer (Apple Computer, Inc., Cupertino, CA) was used to process data. All graphs were obtained directly from the microcomputer by use of a laser printer.

Kinetics of binding

To measure the dependence on time, t, of I at any given t (I_t), a solution of either bacteriophage or capsid was diluted by 100 to 200 times into a solution of bis-ANS in Tris/Mg buffer. Subsequently, values of I_t were obtained for the times indicated. The initial value of I_t is called I_0; the equilibrium value is called I_∞. To determine whether binding was first order, a semilogarithmic plot of $(I_\infty - I_t)/(I_\infty - I_0)$ versus t was made; first-order kinetics are indicated by linearity of this plot (Cantor and Schimmel, 1980). The slope is the first-order rate constant, k^*. This procedure was checked by the method of Kezdy and Swinbourne (described in Moore and Pearson, 1981, and applied in Griess et al., 1991). When appropriate, this latter method can be used to represent data as the superposition of more than one first-order reaction.

Binding at equilibrium

For the binding at equilibrium of bis-ANS to a specified (in the Results) class of sites, both the dissociation constant, K_d, and the number, N, of binding sites per bacteriophage were determined at 25°C, by use of a double reciprocal plot of $\Psi P_0/I_f$ vs. $1/D_0$ (Griess et al., 1991). The definitions of these latter symbols are as follows: D_0, molar concentration of bis-ANS; I_f, the equilibrium I for the class of sites involved; P_0, molar concentration of either bacteriophage or capsid; and Ψ, the I induced per mole of bound bis-ANS. Values of K_d are independent of Ψ, but values of N are not. The value of Ψ was determined from the limiting change in I value obtained when a known concentration of bis-ANS was saturated with protein (see also Griess et al., 1991). Because of background from light scattering, intact bacteriophage could not be used for this purpose. Instead, MHD capsid II was used.

Kinetics of quenching

Ethidium quenched the fluorescence induced by binding of bis-ANS to T7 proteins. To measure the kinetics of quenching, initially a sample was equilibrated with 25 μM bis-ANS. Subsequently, ethidium was diluted 33 times into the equilibrated sample; I_t vs. t was determined for bis-ANS by use of the procedure described previously. To determine whether quenching was first order, a semilogarithmic plot of $(I_t - I_\infty)/(I_0 - I_\infty)$ vs. t was made (slope = k_q^*). The Kezdy-Swinbourne analysis was used for analysis of kinetics that were comprised of first-order quenching of more than one site. The subscript of a rate constant will be used to designate the sites quenched. For example, the sites quenched with a k_q^* of k_{q1}^* will be called q1 sites.

SDS-PAGE

SDS-PAGE in a 12.5% polyacrylamide gel was performed by use of procedures described in Studier (1973*b*). Proteins were stained by use of Coomassie blue (Serwer, 1980).

Agarose gel electrophoresis of undenatured capsids

Intact capsids were subjected to electrophoresis at 1 V/cm for 18 h at room temperature through a 0.9% agarose gel (Seakem LE agarose; FMC Bioproducts, Rockland, ME) in 0.05 M sodium phosphate, pH 7.4, 0.001 M $MgCl_2$. The gel was stained with Coomassie blue, by use of procedures previously described (Serwer, 1980).

RESULTS

Binding of bis-ANS

Initially, attempts were made to use continuous monitoring of fluorescence enhancement to determine the ki-

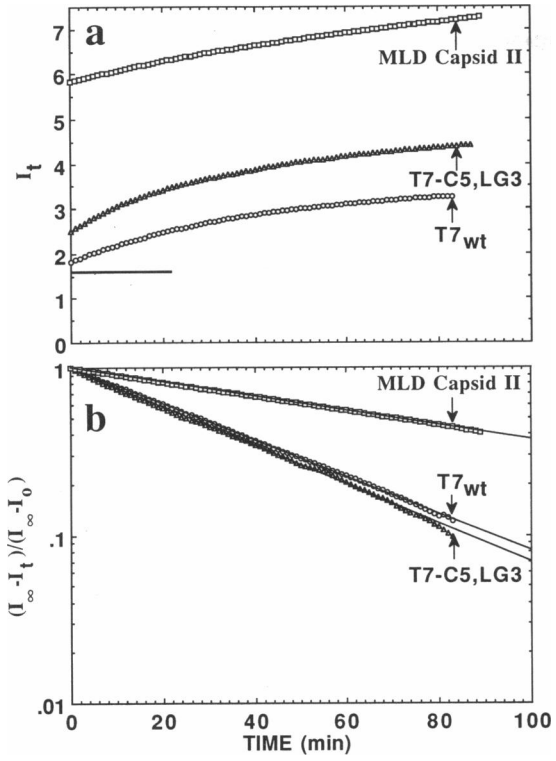

FIGURE 2 Fluorescence as a function of time. (*a*) After addition to 25 μM bis-ANS, values of I_t vs t were determined for the particles (9.2×10^{-4} μM) indicated in the figure. (*b*) A semilogarithmic $(I_\infty - I_t)/(I_\infty - I_0)$ vs t plot was constructed from the phase 2 binding in *a*. The horizontal bar indicates the base line.

netics with which bis-ANS bound to bacteriophage T7. This procedure had been used previously in a study of bacteriophage T4 (Griess et al., 1991). As found with T4, incubation of T7 with bis-ANS resulted in a binding-induced increase in the fluorescence of bis-ANS. However, after equilibration of bis-ANS with bacteriophage T7, exposure to the light of a fluorometer caused a progressive decrease in fluorescence that had not been observed for T4. Production of this decrease in the fluorescence required simultaneous exposure to both light and bis-ANS. The decrease was unaltered when 0.3 M β-mercaptoethanol was added. When pulsed exposure to light was used during fluorometry (Materials and Methods), the decrease in fluorescence observed by use of a continuous light source was eliminated (data not shown). In addition, pulsed fluorometry caused no change in the infectivity of either T7wt or T7-C5,LG3; it also did not alter the mobility of MLD capsid II during agarose gel electrophoresis (data not shown).

When I_t was measured for T7wt by use of pulsed fluorometry, the first measurement performed revealed an increase in I (phase 1 binding). This increase was followed by an additional progressive increase in I_t (phase 2 binding; Fig. 2 *a*, T7wt). The initial increase (to be called I_0) occurred during a time $\leq$ 10 s, i.e., 10 s is the minimum time required to take the first reading. After the first reading, 5 min were required before I_t doubled. Thus, the initial binding of bis-ANS occurred more rapidly than subsequent binding. The speed of the initial binding has not yet been determined. When DNA was expelled from either T7wt or T7-C5,LG3 by raising temperature to 65°C for 30 min in Tris/Mg buffer, subsequently all binding of bis-ANS was phase 1 binding. The following observations indicate that the observed phase 1 binding of bis-ANS to intact T7wt is not the result of binding to contaminating burst bacteriophage: (*a*) electron microscopy by use of negative staining (Steven and Trus, 1986) reveals that <0.5% of the unheated bacteriophage are burst and (*b*) binding of ethidium to intact T7wt occurs without the phase 1 that is expected (Griess et al., 1986) if this DNA-specific probe bound to DNA expelled from a T7 capsid (data not shown).

When a semilogarithmic plot was made of $(I_\infty - I_t)/(I_\infty - I_0)$ vs. t for the phase 2 binding to T7wt, the result was a straight line (Fig. 2 *b*, T7wt). Thus, the phase 2 binding of bis-ANS to T7wt was first order.

When I_t was determined for the binding of bis-ANS to T7-C5,LG3, both I_0 and I_∞ were found to be higher than those of T7wt (Fig. 2 *a;* T7-C5,LG3). However, within experimental error, the value of k^* was the same for T7-C5,LG3 as it was for T7wt (Fig. 2 *b;* Table 1).

When I_t was determined for the binding of bis-ANS to MLD capsid II, I_0 and I_∞ became even higher than they were for T7-C5,LG3 (Fig. 2 *a*). In addition, k^* was significantly lower (Fig. 2 *b;* Table 1). Unlike MLD capsid II, MHD capsid II had no phase 2 binding; all detected binding to MHD capsid II was phase 1 binding (not shown).

For T7wt, T7-C5,LG3, and MLD capsid II, k^* was determined as a function of the concentration of bis-ANS. For concentrations between 15 and 120 μM bis-ANS, k^* did not vary significantly. For less concentrated bis-ANS solutions, the experimental error progressively increased (±50% at 5 μM), but no significant change in k^* was observed (data not shown). These results are in contrast to a significant dependence of k^* on bis-ANS concentration for bacteriophage T4 (Griess et al., 1991). Also in contrast to observations made for T4 was the observation that adenosine triphosphate (ATP) had no effect on the T7 k^* for concentrations as high as 1 mM ATP (data not shown).

Quenching of bis-ANS fluorescence

To help determine whether the biphasic bis-ANS binding kinetics were caused by a difference in access to bis-ANS binding sites, the kinetics were determined for the quenching of bound bis-ANS fluorescence by ethidium. As found for the binding of bis-ANS, the quenching kinetics were biphasic for T7wt, T7-C5,LG3, and MLD capsid II. The first phase occurred too rapidly to measure; the second phase occurred over a period of hours (Fig. 3 *a*). For both MHD capsid II and bacteriophage from which DNA had been expelled, only the first phase

TABLE 1 **Equilibrium and kinetic constants**

Particle	k^* (min^{-1})* ±15%	K_d (μM) ±15%		N ±20%		k^*_{q1} (min^{-1})‡ ±10%	k^*_{q2} (min^{-1})‡ ±15%
		Phase 1	Phase 2	Phase 1	Phase 2		
T7wt	0.025	5.3	3.3	4	47	0.15	0.042
T7-C5,LG3	0.027	5.2	3.0	10	71	0.30	0.040
MLD capsid II	0.010	70.4	4.6	201	132	§	0.037
MHD capsid II	§	7.0	§	50	§	§	§

* For determining k^*, the concentration of bis-ANS was 25 μM.
‡ For quenching of bis-ANS fluorescence, the concentration of ethidium was 30 μM.
§ Kinetic component was not observed.

was observed (data not shown). When the slower phase of quenching was analyzed for both T7wt and T7-C5,LG3, this phase could be represented by two first-order components, rate constants = k^*_{q1} and k^*_{q2}. Plots for these two components are separately shown in Fig. 3 *b* (q1 and q2 regions, respectively). The k^*_{q1} for T7-C5,LG3 was higher than the k^*_{q1} for T7wt (Fig. 3 *b*, q1 region, T7wt and T7-C5,LG3; Table 1). No significant difference was found between the k^*_{q2} values for T7wt and T7-C5,LG3 (Fig. 3 *b*, q2 region; T7wt and T7-C5,LG3).

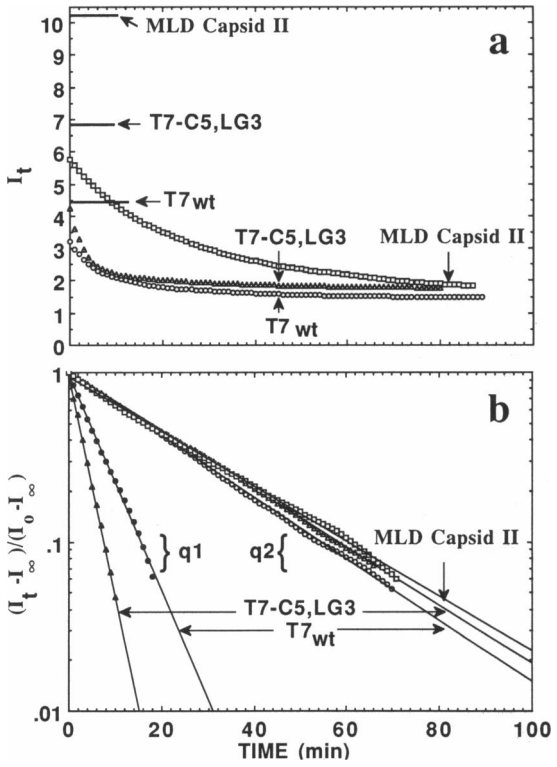

FIGURE 3 Quenching as a function of time. (*a*) After addition of 30 μM ethidium bromide to particles (9.2×10^{-4} μM) pre-equilibrated with 25 μM bis-ANS, values of I_t vs t were determined for the particles indicated in the figure. The I before addition of ethidium is separately indicated for the different particles by the horizontal bars (labeled with the name of the particle). (*b*) The following semilogarithmic $(I_t - I_\infty)/(I_0 - I_\infty)$ vs t plots were extracted from the phase 2 quenching in *a* for both T7wt and T7-C5,LG3: q1, a comparatively rapid first order binding; q2, a slower first order binding. The plot for MLD capsid II exhibited simple first order binding characterized by a single k^*.

In contrast to the results obtained for T7wt and T7-C5,LG3, for MLD capsid II, phase 2 of the quenching kinetics consisted of only one first order component. This component had a k^*_q not significantly different from the k^*_{q2} of both T7wt and T7-C5,LG3 (Fig. 3 *b*, q2 region; MLD capsid II; Table 1).

Equilibrium binding

To obtain values of N and K_d for only those sites bound during the kinetic phase 1 (phase 1 sites), background-corrected I_0 has been used as the I_f for these sites. A double-reciprocal plot for phase 1 sites (Fig. 4 *a*) revealed that N increased in the order T7wt < T7-C5,LG3 < MLD capsid II (Table 1). The value of N for MLD capsid II was 20-fold higher than it was for T7-C5,LG3. For phase 1 sites, the K_d observed for T7wt was the same as that observed for T7-C5,LG3; however, the K_d observed for MLD capsid II was 13-fold larger (Table 1). Values of N and K_d for MHD capsid II are also in Table 1. However, MHD (but not MLD) capsid II appears to lose internal proteins during purification (Serwer, 1980); thus, N for MHD capsid II will not be further used.

To obtain values of N and K_d for only those sites bound during the kinetic phase 2 (phase 2 sites), $I_\infty - I_0$ has been used as the I_f for these sites. The double reciprocal plot for phase 2 sites reveals that N again increased in the order T7wt < T7-C5,LG3 < MLD capsid II. With the exception of the phase 1 sites for MLD capsid II, values of K_d were within a factor of 2.3 of each other. The phase 1 sites for MLD capsid II were also more numerous than any other type of site. These observations are interpreted in the Discussion.

Bis-ANS-induced alteration of proteins

Although initially a hindrance to quantifying bis-ANS binding, the alterations caused by illumination of a T7wt-bis-ANS complex could help identify bis-ANS-bound proteins. Thus, as a function of time of exposure

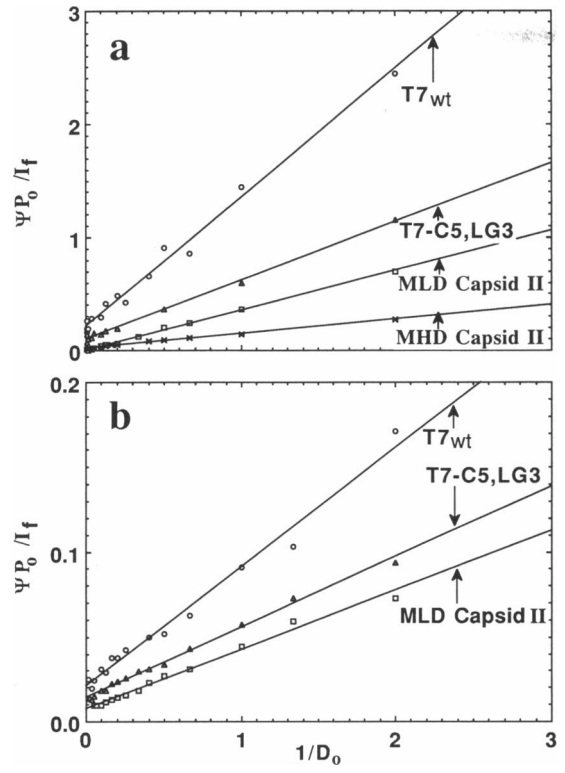

FIGURE 4 Equilibrium binding of bis-ANS. Double reciprocal plots of the equilibrium binding of bis-ANS are shown for the particles indicated in the figure. (*a*) Phase 1 binding; (*b*) phase 2 binding. Each point is the average of two to three independent measurements.

to the fluorometer's light beam in the presence of 100 μM bis-ANS, the proteins of T7wt were analyzed by use of SDS-PAGE. This analysis revealed alteration that progressively occurred in the following proteins: p8, p15, p16 (Fig. 5 *a;* time [h] of exposure to light is at the top of a lane). Initially, the primary alteration appeared to be lowering of mobility (altered proteins are indicated by an asterisk in Fig. 5). However, as time increased above 1.5–2.0 h, the amounts of recognizable p8, p15, and p16 decreased. This decrease was accompanied by an increase in the amount of protein at the electrophoretic origin (arrowheads in Fig. 5 *a*), as though p8, p15, and p16 had aggregated. The alteration was initially seen for both p8 and p15 and subsequently for p16. Both p15 and p16 are internal proteins; p8 is the protein that forms the connector (Fig. 1). The percentage loss of bis-ANS fluorescence (indicated at the bottom of a lane in Fig. 5) is best correlated with alteration of p16. No evidence was observed for alteration of either p10 (the major outer shell protein) or proteins of the T7 tail, p12 and p17 (Fig. 5) or other internal proteins, p13, and p14 (not shown). When the experiment of Fig. 5 was repeated for MLD capsid II, again p8, p15, and p16 were the only proteins altered (data not shown). Light-induced alterations that require the presence of other dyes have been observed (reviewed in Bryant and King, 1984). These alterations are usually caused by singlet oxygen (Matheson and Lee, 1979).

DISCUSSION

MLD capsid II is known (Serwer, 1980) to have a permeability barrier that MHD capsid II does not have. Therefore, for the phase 2 binding that exists for MLD (but not MHD) capsid II, binding is assumed to occur only after bis-ANS passes the outer surface of MLD capsid II (i.e., the sites are internal). By this hypothesis, the phase 1 sites are probably external. However, if the interior of MLD capsid II was compartmentalized by a permeability barrier, some of the phase 1 sites could be structurally internal. In support of the hypothesis that the phase 2 binding sites are internal, ethidium-induced quenching of MLD capsid II-bound bis-ANS fluorescence also had a phase 2 that was not present for MHD capsid II.

In analogy with the observations made for MLD capsid II, both T7wt and T7-C5,LG3 had both phase 2 bis-ANS binding and phase 2 quenching that were absent when the bacteriophage was permeabilized by expelling its DNA. Thus, for both T7wt and T7-C5,LG3, the sites bound by bis-ANS in phase 2 will be assumed to be internal. Values of N for both phase 1 and phase 2 binding were higher for T7-C5,LG3 than they were for T7wt. Because DNA packing density is the only known difference between T7wt and T7-C5,LG3, the lower DNA packing density of T7-C5,LG3 is assumed to be the cause of the higher N values of T7-C5,LG3. Thus, the bis-ANS is reporting on a DNA packing density-dependent change in conformation in T7 capsid proteins. Based on N values for T7wt and T7-C5,LG3, internal protein undergoes most, possibly all, of the change reported. This idea is in agreement with the observation that the bis-ANS, light-altered proteins (Fig. 5) are proteins of the T7 connector-cylinder. The comparatively small amount of phase 1 binding for intact bacteriophage is explained by binding to regions of the connector that are external. In the case of bacteriophage P22, the evidence indicates that the connector is part of the gauge that both determines how much DNA is packaged and initiates the cleavage of a concatemer to package a "headfull" of DNA (Casjens et al., 1992). Although cutting of a T7 concatemer requires nucleotide sequence specificity (Dunn and Studier, 1983) not present for P22 (Casjens and Hayden, 1988), during in vitro T7 DNA packaging the T7 capsid is capable of both packaging and sizing nonhomologous DNA by a "headfull" mechanism (Son and Serwer, 1992). Because of the apparent similarity in the function of bacteriophage connectors (see Introduction), the assumption is made that the T7 connector, like the P22 connector, is part of the gauge that determines when a headfull of DNA is packaged. Thus, the changes in N value associated with deletion of 8.4% of T7 DNA are potentially secondary effects of changes that evolved to cleave a concatemer at the con-

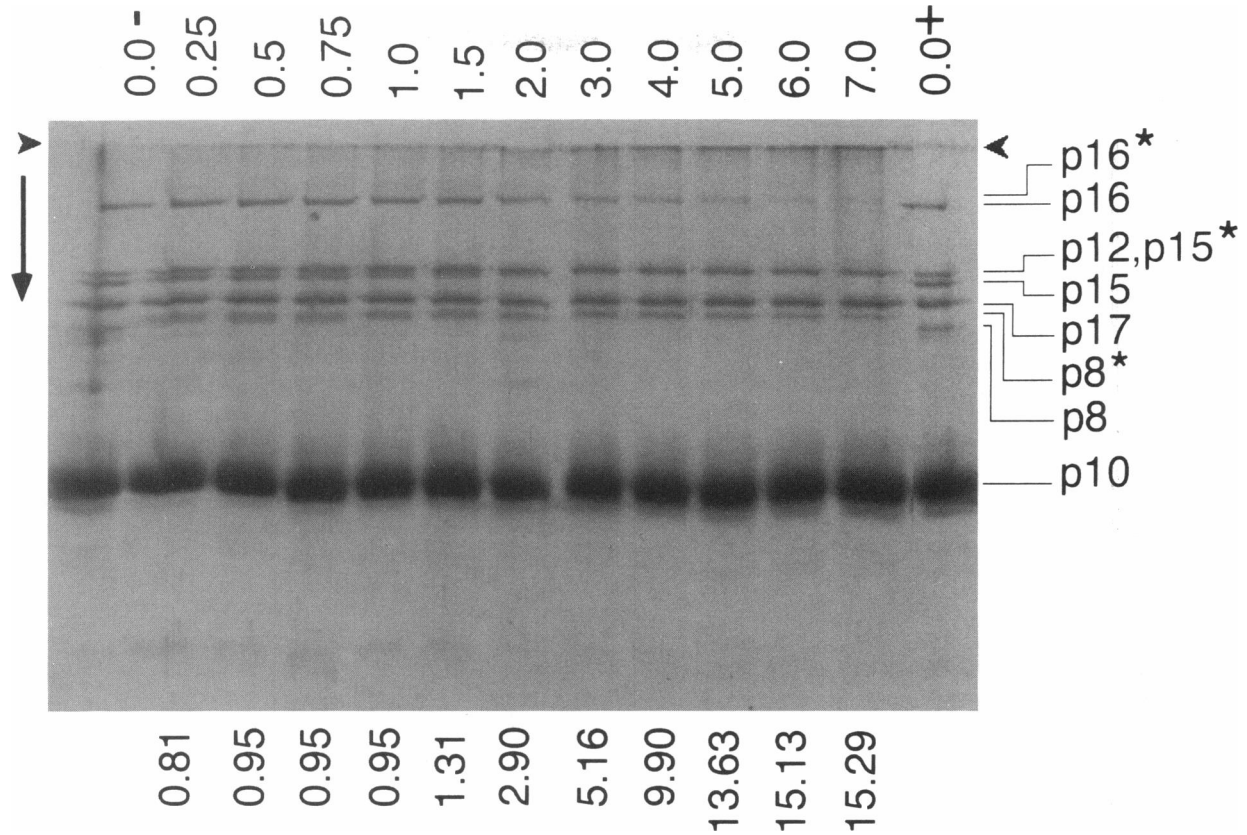

FIGURE 5 Effects of light on capsid proteins bound to bis-ANS. After addition to 100 μM bis-ANS, T7wt was illuminated in the fluorometer for the times (min) indicated at the top of a lane. At these times, I_t was measured (the percentage decrease from that at $t = 0$ is indicated at the bottom of a lane) and proteins were analyzed by SDS-PAGE, followed by staining with Coomassie blue. The interface of stacking and separating gel is indicated by arrowheads; the direction of electrophoresis is indicated by the arrow. The position of proteins is indicated at the right. An asterisk indicates altered protein. Unirradiated T7wt either treated with bis-ANS (0.0+) or not (0.0−) are the samples from time = 0.

clusion of packaging. By use of electron microscopy, flexibility has been observed for the connector of the T7-related bacteriophage, T3 (Valpuesta et al., 1992). Both the T3 connector (Valpuesta et al., 1992) and the bacteriophage ϕ29 connector (Carazo et al., 1986) have been found in two states: axial hole open and axial hole closed. Closing of the connector's axial hole is potentially the cause of the reduced permeability of MLD capsid II.

For intact bacteriophage, the sensitivity of k^*_{q1} to DNA packing density supports the assumption that, before quenching of q1 sites, ethidium passed through the packaged DNA (see also Griess et al., 1986). Conversely, the insensitivity of k^*_{q2} to DNA packing density indicates that ethidium did not pass through the packaged DNA before quenching of q2 sites. Thus, to explain these observations, the simplest hypothesis is that the q1 sites are quenched by ethidium that enters the bacteriophage through pores in the icosahedral outer p10 shell; the q2 sites are quenched by ethidium that enters the bacteriophage through the tail-connector-cylinder. In support of this hypothesis, the q1 sites are absent from (DNA-free) MLD capsid II, but the q2 sites are present. For MLD capsid II, the lost q1 sites might be converted to either q2 sites or sites of phase 1 quenching. In the latter case, ethidium would enter through the icosahedral outer shell but encounter no resistance from DNA; quenching would be too rapid to measure, even though the quenched sites were internal. However, no evidence exists to decide whether ethidium does pass through the outer shell of MLD capsid II. The q2 bis-ANS binding sites in intact bacteriophage are apparently separated by a permeability barrier from the q1 sites. The probable location of this barrier is the internal cylinder.

The total value of N for MLD capsid II is so high (332) that binding to only p8, p15, and p16 would require an average of 10 binding sites per protein. This number is more than two times higher than the highest values reported for other proteins that bind bis-ANS (for example, Farris et al., 1978; Lawson and York, 1987; Secnik et al., 1990). This observation is explained by the assumption that phase 1 binding of bis-ANS occurs on the major capsid protein, p10, of MLD (but not MHD) capsid II. This assumption is supported by the comparatively high K_d of the phase 1 sites of MLD capsid II. However, thus far, this proposed binding to p10 has not caused exposure to light to yield a protein with altered mobility during SDS-PAGE. The outer shells of MLD capsid II, bacteriophage T7, and MHD capsid II have not been distinguishable by electron microscopy

(Serwer, 1980). However, a slight difference in the migration of MHD and MLD capsid II has been observed during nondenaturing agarose gel electrophoresis (Serwer, 1980). Further investigation is being made of exactly where bis-ANS binds in the capsids of bacteriophage T7.

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