Disruption of T-even Bacteriophages by Dimethyl Sulfoxide

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Dimethyl sulfoxide (DMSO) disrupted T-even bacteriophages as well as λ bacteriophage. The component substructures of T2L, T4B01, or T6, in particular heads, were readily isolated after treatment with 67% DMSO (v/v). In contrast, concentrations of DMSO above 50% not only separated heads from tails of bacteriophage λ but led to degradation of the λ heads. Examination of the isolated free heads of T-even bacteriophage indicated that a distinct neck substructure was attached to one apex of the head. On some free tails a similar neck substructure was also found at the proximal end of the sheath. The dimensions of this neck substructure were found to be about 130 by 180 Å; by virtue of its size and morphological attachment to the free heads, it was concluded that this was a distinct substructure and not an extension of the tail tube.

A major obstacle to studying the physical and chemical properties of the various substructures of T-even bacteriophage has been the absence of reliable and gentle methods for disrupting the phage without degrading the substructures. The tail components were obtained by Brenner et al. (4) by precipitation at pH 2, followed by homogenization of the precipitate and proteolytic enzyme treatment; recently, Poglazov et al. (20) claimed that brief treatment of T2 bacteriophage at pH 12 separates the heads from the tail components. Both of these procedures have disadvantages, since denaturing conditions were necessary for disruption and, in the case of the pH 12 treatment (20), increasing the pH to 12.6 led to dissociation of the head membranes into subunits. Ideally, the method of choice in disrupting bacteriophages into component substructures would be one in which neither denaturation nor degradation took place.

The elegant assembly experiments of Edgar and Wood (11) and Edgar (*personal communication*), in which T4 amber phage substructures could be assembled *in vitro*, suggested that some substructures, such as head to tail, are not necessarily covalently linked. This implied to us the possibility that some organic solvents could disrupt such weak bonds without denaturing the substructures. In a recent symposium, MacGregor (18) and Rammner and Zaffaroni (21) reviewed the wondrous properties of dimethyl sulfoxide (DMSO). DMSO is a dipolar aprotic solvent, completely miscible with water, which forms stronger hydrogen bonds with water than does

water itself and is thought consequently to form 2:1 association complexes with water. The formation of the hydrated complex is accompanied by considerable evolution of heat. So long as temperature is controlled, DMSO has little permanent effect on such proteins as β -galactosidase (12) and lysozyme (21) but can denature proteins and nucleic acids (16) at higher temperatures by virtue of its interference with hydrogen bond formation.

In the present investigation, it was found that DMSO at 67% (v/v) disrupted T-even bacteriophage into its component substructures without appreciable denaturation or degradation. In addition, in studying isolated heads of T-even bacteriophage, a neck substructure, not previously reported, has been observed.

MATERIALS AND METHODS

Bacteriophage growth conditions and purification. Bacteriophages T2L, T4B01 (an osmotic shock-resistant mutant of T4B obtained from Bruce Ames), and T6 (obtained from M. Jesaitis) were grown and assayed under standard conditions and purified by differential centrifugation. Protein coats or ghosts of T2L and T6 were prepared by the high salt osmotic shock method of Herriot and Barlow (13), and ghosts of T4B01 were prepared by the addition of 2 g of sucrose per ml of whole phage followed by a 20-fold rapid dilution with demineralized water or 0.10 N NaCl at 25°C. These ghost particles were collected by centrifugation and suspended in a solution containing 1.0 molal CsCl in 90% D₂O (density, 1.25 g/ml) at 4°C. The remaining whole phage particles (density, 1.51 g/ml) could then be quantitatively removed by

centrifugation (5) with little loss of the ghost particles (density, 1.29 g/ml). The supernatant solution containing ghost particles was diluted sixfold with buffer consisting of 0.15 N NaCl, 0.001 M MgCl₂, and 0.001 N phosphate buffer, pH 7.3 (phage diluent), to reduce the solution density, and was then purified by centrifugation. By this procedure, pure preparations of ghosts were obtained, even of T4B01. [With the sucrose procedure, T4B01 yields about 10% unghosted particles (7).] Whole phage and ghosts were stored in phage diluent at 4 C.

Bacteriophage λ was grown by mitomycin C induction (17) of *Escherichia coli* K-12 (λ); it was purified by differential centrifugation, followed by purification in a CsCl density gradient to remove debris, and stored in diluent (8).

Disruption procedure. DMSO was obtained from Fisher Scientific Co. As indicated previously, the formation of hydrates by DMSO is an exothermic reaction. Consequently, DMSO was added slowly, with gentle mixing, to the phage preparation in an ice bath and incubated for 10 min. As a precaution against possible surface denaturation, 10 mg of bovine serum albumin was added per ml of phage. For examination in the electron microscope, the phage mixture was diluted 12-fold with phage diluent to break up the association complexes with water and to prevent heat denaturation while handling. Separation of the substructures could be achieved by either differential centrifugation of the 12-fold diluted mixture or by sucrose gradient centrifugation. To minimize dilution and to break up the association complexes, the mixture was dialyzed against phage diluent prior to sucrose gradient centrifugation.

At high concentrations of ghosts, 5×10^{13} to 10×10^{13} /ml, the 67% (v/v) DMSO-phage mixture was quite viscous, probably because of complexing with water. It was therefore necessary to remove the DMSO prior to sucrose gradient centrifugation. If the mixture was layered directly onto a precooled sucrose gradient solution (20 to 45% sucrose, 0.3 N NaCl), the mixture sedimented directly to the bottom of the tube. However, after overnight dialysis in the cold, the substructures sedimented according to their individual properties.

Electron microscopy. Electron micrographs of the phage preparations were made by use of the phosphotungstic acid-negative staining method (3) with either an RCA-EMU 3G or 4 electron microscope fitted with a modified grid cap to enhance contrast (14). Final results were recorded on Dupont Cronar Ortho 708 Litho A film at a magnification of about 15,000 and were photographically enlarged four to five times.

RESULTS

Initially, whole bacteriophage at (10^{12} to 2×10^{12} /ml) were mixed with increasingly higher concentrations of DMSO. After incubation at 0 C and slow dilution with phage diluent, it was observed that the treatment with higher concentrations of DMSO led to loss of deoxyribonucleic acid (DNA) into the medium, presumably owing

to osmotic shock. The infectivity of the phage decreased gradually until the DMSO concentration exceeded 50% (Table 1). Bacteriophage λ , a phage noted for its resistance to osmotic shock (10), was similarly treated and was found to be more resistant to DMSO. Above 50%, however, all phages examined displayed a dramatic loss of plaque-forming ability. At 67% DMSO, electron microscopy revealed that the phages had been disrupted into their component substructures. At 50% DMSO, little or no such effect was detected; although many phage heads were empty, they were still attached to their tail substructures. Although the effects of DMSO were apparent, the presence of DNA in the medium interfered with electron microscopy and necessitated the use of ghost particles to demonstrate the results of DMSO treatment.

Figure 1 shows a representative field of T4B01 ghosts before treatment with DMSO. Many of the ghosts have visible tail collars, and all but a few have their heads attached to the tail substructures. The inserts illustrate the few free tails in the field; above the sheath, there is a knobby substructure which has been thought to be part of the tail tube (2). This structure has a lateral dimension of 120 to 140 A, whereas Simon and Anderson (*personal communication*) indicated that the tail tube has a lateral dimension of only 80 A. As shown below, this substructure is often attached to free heads.

Treating such ghost particles of T2L, T4B01, or T6 with 67% DMSO leads to disruption of the phage into their component substructures. Figure 2 shows T4B01 ghosts after treatment but without purification. Comparison with Fig. 1 indicates clearly that the phage has been disrupted. Figure 2 shows many free heads, free contracted tail sheaths, and free tail tubes still attached to tail plates, which in turn have tail fibers attached. It would seem that DMSO separates the tail tube from the head, and the sheath contracts and slides off the tail tube.

TABLE 1. *Percentage survival of bacteriophage in DMSO^a*

Bacteriophage	DMSO (% v/v)			
	0	25	50	67
T2L or T6	100	100	7	$<1 \times 10^{-2}$
T4B01	100	87	11	$<1 \times 10^{-2}$
λ	100	93	56	3×10^{-3}

^a Dimethyl sulfoxide was added slowly to bacteriophage at 10^{12} /ml, in the cold. After 10 min of incubation, the phage were diluted and plated on sensitive bacteria.

FIG. 1. *T4B01* ghosts. The few free tails in the field have a knobby substructure at the proximal end, to which the tail collar is attached.

FIG. 2. *T4B01* ghosts after treatment with 67% DMSO (v/v). The phage-DMSO mixture was diluted 12-fold with phage diluent and examined directly. Note that the ghosts have been disrupted and that all substructures are visible.

Some of the free heads appear to have tail sheaths still attached. We feel that this may be an artifact due to drying effects on the electron microscope grids, since onefold and twofold purification by centrifugation greatly decreases the number of tail sheaths and tubes in the preparation. That is, if the treated ghosts are centrifuged at $25,000 \times g$ at 4 C for 1 hr and the resultant pellets examined, increased numbers of free heads are obtained. Figure 3 shows T2L after one such purification, and Fig. 4 shows T6 after two such purifications. Alternatively, these phage substructures could be separated by centrifugation in a sucrose density gradient (*unpublished data*). After centrifugation at 23,000 rev/min in a Spinco 25SW rotor at 4 C for 3 hr, the heads were clearly separated from the tail components. In general, we estimated that treatment of the T-even bacteriophages with 67% DMSO disrupted about 95% of the particles into their component substructures. While it was difficult to measure the recovery of each of the substructures, in terms of total amount of material, at least 90% of the mass was reisolated.

The inserts in Fig. 2, 3, and 4 are enlargements of some of the free heads in the respective fields. It can be observed that the knobby substructure seen in Fig. 1 is attached to one apex of the head. In fact, in phage which have tail collars (19), T4BO1 and T6, the collar is still attached to this substructure. T2L, which does not have a collar (9), has this substructure, which we call the neck, attached to the head. Fragments of sheath may still be attached to some of the neck substructures, and this may indicate that some sheaths have been partially degraded.

Figure 5 is a schematic representation of the effects of DMSO on the T-even bacteriophage. As indicated, the neck substructure may be attached either to the tail tube and sheath or to the free head. Its dimensions are about 130 by 180 Å, although its length is uncertain. The knobs observed when attached to the tail are shown wedged into the head and may be the means by which head and tail are attached. It is interesting that, in the assembly scheme of Edgar and Wood (*personal communication*), genes 13 and 14 must operate on heads which appear normal but which cannot assemble to tails. The product of these genes may be this neck substructure.

Since DMSO seemed to disrupt T-even bacteriophage in a manner reminiscent of assembly, we also examined phage λ , in which assembly has also been demonstrated (23). It was found that λ phage was more resistant to osmotic shock (Table 1) but that plaque-forming ability decreased dramatically at 67% DMSO. Such results were verified by examining the particles in

the electron microscope. In Fig. 6, there is little difference between λ phage at 0% and at 50% DMSO. There are numerous ghost particles in the field (the ghost particles arose after the CsCl gradient purification) but few free tails or heads.

At 67% DMSO, many more free tails are visible (Fig. 7) but there did not seem to be as many free heads. At 80% DMSO (Fig. 7), there are even fewer free heads but many free tails. It appears that DMSO does separate λ tails from heads but that it also degrades the λ heads. This may simply indicate that λ heads are readily dissociated; in fact, treatment with CuCl_2 also tends to dissociate λ heads (22).

DISCUSSION

By means of a simple organic solvent, DMSO, we have been able to show that T2L, T4BO1, T6, and λ bacteriophage are disrupted into their component substructures. In the case of λ , only heads and tails were found after treatment with 67% DMSO. However, DMSO tended to dissociate the λ heads, making isolation of heads alone difficult. The T-even phage separated into heads, tail sheaths, and a complex of tubes, plates, and fibers. All these substructures appeared stable after treatment. In fact, the ability to collect heads by centrifugation, resuspend them, and recentrifuge them, can be taken as evidence that the substructures are not denatured. The pellets of heads alone resuspend readily, and no tendency to aggregate in the electron microscope grids was observed. When temperature was not rigidly controlled during incubation in DMSO, the parts tended to clump and their pellets could not be resuspended. Thus, a simple, reliable method for obtaining substructures of T-even phage is now available. By use of this method, it may be possible to reexamine some of the substructures obtained previously by treatments which may have caused some denaturation (4, 20).

It is of interest that λ heads were fairly unstable in 67 to 80% DMSO, whereas T-even phage heads were quite stable and did not dissociate. Bacteriophage λ heads are obviously isometric in shape (2, 8) and are thought to have an icosahedral structure. On the other hand, T-even phage heads are not isometric (2, 6). To make use of the advantages of icosahedral symmetry, models for T-even phage heads have been proposed (2, 19) which essentially are distortions of icosahedral symmetry. Since λ and T-even heads differ greatly in their stability to some reagents, it is possible that this reflects a difference in their symmetry relations. It should be pointed out that other models of T-even head structure are

FIG. 3. T2L ghosts after treatment with 67% DMSO. After 12-fold dilution with phage diluent, the heads were collected by centrifugation and then examined. Note that fewer free heads appear to have tail components attached than in Fig. 2.

FIG. 4. T6 ghosts after treatment with 67% DMSO. The treated ghost heads were purified twice by centrifugation, and it can be seen that practically all the heads are free of tail sheath. In the inserts in Fig. 2, 3, and 4, it can be seen that a distinct neck substructure, about 130 by 180 Å, is attached to the head.

FIG. 5. Schematic diagram of the attachment sites of the neck substructure. This substructure can be found attached either to the head or the tail. It may be attached to both the sheath and the tail tube.

not ruled out; in fact, one has already been proposed (6) which accounts for some physico-chemical properties of the head protein subunits.

The characteristics of virus disruption by DMSO suggests a mechanism for its action. As indicated, DMSO forms 2:1 association complexes with water and can cause reversible configurational changes in some proteins (21). The fact that at least 67% DMSO was required strongly suggests that it was necessary to complex all the water in the system to allow disruption. It may be that there is a hydration mantle surrounding the substructures, and removal of such a mantle weakens the bonds linking substructures. Or, the DMSO may simply have competed with weak coordination bonds between substructures. Further work with other organic solvents may be necessary to distinguish between these possibilities.

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FIG. 6. Bacteriophage λ . (A) Without treatment with DMSO. (B) After treatment with 50% DMSO. Note that some of the ghost heads have what appears to be a central core, which may be related to previous observations (8, 15). The phage were examined without purification after treatment with DMSO in both Fig. 6 and Fig. 7.

FIG. 7. Bacteriophage λ after treatment with (A) 67% DMSO and (B) 80% DMSO. Free tails are present in both fields but the heads appear to be disintegrating.

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