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# Stability and In Vitro DNA Packaging of Bacteriophages: Effects of Dextrans, Sugars, and Polyols

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Attempts were made to increase the efficiency of infectious particle formation during the in vitro assembly of bacteriophage T7 from procapsids and DNA. It was found that dextrans and some smaller, related compounds (sucrose and sorbitol) increase this efficiency by a factor of 8 to 50. Dextrans also inhibited elevated temperature-induced emptying of DNA from bacteriophages T7, P22, and T4, suggesting that the stimulation of assembly is caused, at least in part, by the stabilization of packaged DNA in capsids. The data indicated that the sugars and polyols can slow DNA emptying from bacteriophages at elevated temperature whether they permeate the bacteriophage capsid or not. In contrast, the data suggested that permeation of some particle, probably a capsid, results in inhibition of in vitro T7 assembly.

All double-stranded DNA bacteriophages that have been studied assemble in vivo a DNA-free protein capsid (procapsid). The procapsid subsequently draws DNA into its interior (packaging; reviewed in reference 9). Packaging of DNA into procapsids can be performed in cell-free extracts (e.g., T7 [14, 18], P22 [21], P2 [22], T4 [6], λ [4], T3 [20], and φ29 [5]). However, except in the case of φ29, the amount of exogenous DNA packaged in vitro is usually less than 1%. To optimally use in vitro procedures to analyze DNA packaging, it is desirable to increase the efficiency of packaging. The observation that the T7 procapsid converts to a capsid less permeable than other T7 capsids at the initiation of in vivo packaging (25) suggested to us the possibility that capsid-excluded molecules assist T7 DNA packaging. In preliminary experiments, it was found that dextrans, compounds probably big enough not to enter bacteriophage capsids (see reference 19), stabilized bacteriophages and dramatically increased the efficiency of T7 DNA packaging. Therefore, a detailed study was made of the effects of dextrans, sugars, and polyols on (i) the stability of bacteriophages, and (ii) the efficiency of T7 in vitro DNA packaging. The results are reported here.

## MATERIALS AND METHODS

**Bacteriophage and bacterial strains.** Wild-type bacteriophage T4D was obtained from the California Institute of Technology collection of bacteriophages (referred to as T4). Bacteriophage P22 with an amber mutation in gene 13 (lysis; referred to as P22) and P22 missing tail spikes, from a lysate of a nonpermissive host infected with P22 having an additional amber mutation in gene 9 (referred to as $9^-$ P22) (7), were received purified from J. King. Wild-type bacteriophage T7 was received from F. W. Studier; T7 with an amber mutation in genes 3, 5, and 6 (referred to as $T7_{3,5,6}$) has been previously described (14). The host for T4 was *Escherichia coli* BB/1, which was also the host for T7 unless otherwise indicated. The host for P22 was *Salmonella typhimurium* DB7704.

**Preparation of bacteriophages.** Growth and purification of the T7 used to make extracts for in vitro assembly were performed as described previously (14); for other experiments, this was done as described by P. Serwer (24). Growth and purification of T4 were performed as described in reference 26. All P22 and $9^-$ P22 used were from the samples originally received.

**Buffers.** Bacteriophages at concentrations above 100 μg/cm$^3$ were stored in Tris-Mg buffer (0.20 M NaCl, 0.01 M Tris-hydrochloride [pH 7.4], 0.001 M MgCl); at lower concentrations, bacteriophages were stored in Tris-Mg-G buffer (Tris-Mg buffer with 100 μg of gelatin per cm$^3$). Inactivation of bacteriophages by elevated temperature was performed in either NPE buffer (0.10 M NaCl, 0.01 M sodium phosphate [pH 7.4], 0.001 M EDTA) or NPE-G buffer (NPE buffer with 100 μg of gelatin per cm$^3$). Inactivation of bacteriophages by osmotic shock was performed in NPM-G buffer (0.2 M NaCl, 0.01 M sodium phosphate [pH 7.4], 0.001 M $MgCl_2$, 100 μg of gelatin per cm$^3$). If not otherwise indicated, dilutions for assays of infectivity were made in T7 buffer (12); in some experiments low-salt diluent (13) was used. Electrophoresis buffer was 0.05 M sodium phosphate (pH 7.4)–0.001 M $MgCl_2$; sample buffer for electrophoresis was 0.005 M sodium phosphate (pH 7.4)–0.02 M $MgCl_2$–400 μg of bromophenol blue per cm$^3$–50 μg of DNase I per cm$^3$, with an additional component to increase density as described below. Dextrans added to some buffers were purchased from Pharmacia Fine Chemicals. The percent-

age of dextran, sugar, and polyol solutions is given as weight per final volume.

**Osmotic pressure of buffers used.** By lowering the vapor pressure of water, any solute in an aqueous solution will produce an osmotic pressure if this solution is equilibrated across a solute-impermeable membrane with a solution missing the solute (8). The equilibrium pressure thus generated (P) is used to characterize the solution (3). The P values of glucose and sucrose solutions used are from Jones (11). The P values of NaCl solutions were calculated from osmotic coefficients given by Stokes and Levien (30). When two solutes were present, P values were assumed to be additive. The difference between the P values of two solutions is indicated by $\Delta P$.

**Assays for infectivity.** All assays for infectivity were made by using standard procedures (1). Agar for the assays of T7 and P22 contained T broth (32); agar for the assay of T4 contained EHA medium (31).

**Packaging of T7 DNA in vitro.** To prepare an extract for the packaging of T7 DNA in vitro, *E. coli* W3110 was infected at 30°C with $T7_{3,5,6}$ (multiplicity of infection, 3), and at 18.5 min after infection, the cells were lysed as previously described (14). The lysate was clarified by centrifugation at 18,000 × *g* for 20 min and used as follows to package DNA and complete the subsequent assembly steps necessary for an infective particle. To 20 μl of clarified extract was added 0.93 nmol (nucleotide phosphorus equivalent) of DNA from mature T7 ($7.0 \times 10^9$ phage equivalents per reaction), 9 mM $MgCl_2$, 11 mM Tris-hydrochloride (pH 7.5; in addition to the $MgCl_2$ and Tris in the extract), 3.5 mM 2-mercaptoethanol, 3.5 mM spermidine, and various final concentrations of dextran; the final volume was 30 μl. After incubation for 60 min at 30°C, the reactions were terminated by dilution with ice-cold low-salt diluent, and the number of infective particles was determined by plating on *E. coli* W3110. Each reaction was performed three times, and platings were performed in duplicate; the average of the six titers determined was used as the yield of the reaction.

**Inactivation by elevated temperature.** Unless otherwise indicated, inactivation of bacteriophages with elevated temperature was performed by preheating 500 μl NPE-G buffer to the temperature of inactivation for 2 to 5 min. A 5-μl sample of the bacteriophage to be inactivated was diluted from Tris-Mg-G buffer into the preheated buffer, and incubation was continued at the temperature indicated. At various times after the start of exposure to the elevated temperature, samples of the heated mixture were quenched by 1:100 dilution into T7 buffer at room temperature and subsequently assayed for infectivity.

**Detection of the emptying of DNA from capsids.** To detect emptying of DNA from capsids at elevated temperatures, the following procedure was used. A 20-μl portion of a heated sample was diluted with an equal volume of sample buffer (DNase in sample buffer digests emptied, but not packaged, DNA), and 20 μl of this mixture was layered in one of 30 sample wells in a 0.9% horizontal agarose (ME agarose; Marine Colloids) slab gel, cast in electrophoresis buffer, and submerged beneath electrophoresis buffer. Electrophoresis was performed at 0.96 ± 0.08 V/cm at room temperature (25 ± 3°C) as previously described (27). After electrophoresis, the gel was treated with 10% acetic acid to burst bacteriophages and, therefore, to increase subsequent staining of DNA as previously described (27). Subsequently, the gel was neutralized (27) with electrophoresis buffer; DNA in the gel was stained overnight in 1 μg of ethidium bromide per $cm^3$–0.001 M sodium EDTA (pH 7.4). The gel was illuminated from the top with a UV Transilluminator (peak wavelength, 302 nm; Ultraviolet Products, Inc.) and was photographed as previously described (27); the contrast was reversed before printing. Subsequently, to determine the position and amount of capsids in the gel, the gel was stained with Coomassie brilliant blue and photographed as previously described (28).

**Osmotic shock.** Bacteriophages T2, T4, and T6 are inactivated by dilution from a concentrated solution (preshock buffer) to a less concentrated solution (dilution shock buffer) of some solutes, if the difference in concentration is large enough. Because the extent of inactivation increases as the speed of dilution increases, it is assumed that: (i) the solute penetrated the bacteriophage, and (ii) exit of the solute from the bacteriophage is slow enough so that internal osmotic pressure inactivates the bacteriophage, if the dilution rate is rapid enough (referred to as osmotic shock) (2, 3, 15, 16). Bacteriophage T4 is inactivated by rapid dilution from preshock buffers with NaCl, if $\Delta P$ is greater than approximately 75 atm (1 atm = 0.0057 kPa) (2). It is predicted, therefore, that lowering $\Delta P$ by dissolving a non-osmotic shock-producing solute in dilution shock buffer will prevent inactivation. Sucrose does not cause osmotic shock of bacteriophage T4 (see Table 3). As expected, progressively increasing the amount of sucrose in dilution shock buffer progressively inhibited osmotic shock inactivation of T4 (Table 1). Except for a slight, but reproducible, 10 to 15% inactivation (possibly caused by low-molecular-weight contaminants), dextran 10 in preshock buffer also did not inactivate T4 (see Table 3). As predicted, dextran 10, like sucrose, inhibited the osmotic shock inactivation of T4; the data suggest that 20 to 40% dextran 10 solutions have P values that are roughly the same as those of 20 to 40% sucrose solutions (Table 1). The data of LeNeveu et al. (17) suggest that these values of P are reasonable (such concentrated dextran solutions are nonideal). The above data indicate that dextran inhibition of rapid-dilution inactivation is a substitute for the dilution rate dependence of inactivation as a criterion for determining whether osmotic shock is occurring. The former criterion has been used in experiments reported here (see Table 3).

For inactivation by osmotic shock, bacteriophages were first diluted by at least a factor of 100 into preshock buffer. After remaining in preshock buffer for 25 min, the sample was then rapidly diluted at 25°C into dilution shock buffer. The rapid dilution was made with an automatic pipettor (Pipetman) and took less than 1 s. Each experiment was performed three times, and subsequently, two independent infectivity assays were made of each. The mean and the standard deviation of the six platings is reported.

## RESULTS

**Stimulation of T7 assembly in vitro.** Dextran 10 increased the efficiency of bacteriophage T7 infectious particle formation during in vitro assembly of T7 from procapsids and DNA. With

TABLE 1. Inhibition of osmotically induced inactivation of bacteriophage T4

| Preshock buffer (solute in NPM-G) | Dilution shock buffer (solute in NPM-G) | ΔP (atm)[a] | Titer (×10⁷)[b] | Fraction not inactivated |
|---|---|---|---|---|
| 0 | 0 | 0 | 51.3 ± 3.9 | 1.0 |
| 2.8 M NaCl | 0 | 144 | 1.21 ± 0.1 | 0.02 |
| 2.8 M NaCl | 0.15 M Sucrose (5%) | 140 | 3.22 ± 1.3 | 0.06 |
| 2.8 M NaCl | 0.29 M Sucrose (10%) | 136 | 3.43 ± 1.3 | 0.07 |
| 2.8 M NaCl | 0.58 M Sucrose (20%) | 128 | 9.42 ± 1.6 | 0.18 |
| 2.8 M NaCl | 1.2 M Sucrose (40%) | 103 | 39.9 ± 2.4 | 0.78 |
| 2.8 M NaCl | 1.8 M Sucrose (60%) | 61 | 42.9 ± 2.9 | 0.84 |
| 2.8 M NaCl | 5% Dextran 10 | | 4.41 ± 0.74 | 0.086 |
| 2.8 M NaCl | 10% Dextran 10 | | 7.44 ± 0.53 | 0.15 |
| 2.8 M NaCl | 20% Dextran 10 | | 12.7 ± 1.4 | 0.25 |
| 2.8 M NaCl | 40% Dextran 10 | | 28.8 ± 2.5 | 0.56 |

[a] Values of ΔP were determined as described in the text.

[b] Titers were determined after rapid dilution from the preshock buffer into the dilution shock buffer indicated, performed as described in the text.

increasing amounts of dextran 10, the efficiency of DNA packaging increased until a maximum efficiency of 0.3% phage production per genome equivalent of DNA was reached at a concentration of about 14% dextran 10 (Fig. 1). Higher concentrations of dextran reduced the efficiency increase. Several repetitions of this experiment verified the stimulatory effect of including dextran in the reaction mixtures but showed optimal dextran concentrations that varied in a range between 5 and 15%. The maximum stimulation due to dextran varied from 5- to 50-fold. These variations were probably caused by an uncontrollable lack of uniformity in extracts used for DNA packaging; as previously noted (14), these extracts exhibit somewhat variable packaging efficiencies without dextran added. Dextran 40 and dextran 70 also increased the efficiency of in vitro T7 DNA packaging, and the stimulation was approximately equal to that found with dextran 10 at a given dextran concentration (data not shown).

When sucrose or sorbitol (5%) was substituted for dextran, 8- to 20-fold increases in efficiency were observed. In contrast, 5% glycerol reduced the yield to undetectable levels (at least a $10^4$-fold decrease). Glycerol does not inactivate T7 (see below), indicating that it is some step in in vitro assembly that is inhibited by glycerol.

Although the viscosity of 20 to 40% dextran 10 is high enough to be perceived during pipetting, it does not cause any significant experimental problems. However, 20 to 40% dextran 40 and dextran 70 are viscous enough to be difficult to handle and pipet accurately. Therefore, dextran 10 is preferred for use during in vitro assembly.

**Prevention of the emptying of DNA from capsids due to temperature inactivation.** The above compounds might have been stimulating in vitro T7 assembly by stabilizing the packaged state of DNA during or after entry into the capsid. Because elevated temperature-induced inactivation of mature bacteriophage T7 is accompanied by emptying of DNA from the T7 capsid (24), stabilizing of the packaged state of T7 DNA should result in decreased inactivation of T7 at elevated temperatures. It was found that dextran 10 did decrease the elevated temperature-induced inactivation of bacteriophages T7, P22, and T4 (Table 2). One explanation for these results is that dextran 10 was inhibiting emptying of DNA from all three bacteriophages.

To determine whether dextran 10 was inhibiting the emptying of DNA at elevated tempera-

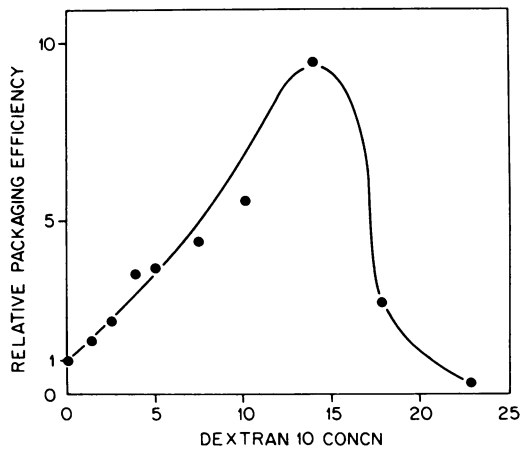


FIG. 1. Effect of dextran 10 on the efficiency of T7 DNA packaging in vitro. The yield of DNA packaging reactions in the presence of several concentrations of dextran 10 was determined, as described in the text. The ratio of this yield to the yield of a reaction performed with no dextran 10 is plotted. In the reaction with no dextran 10, 0.03% of the added DNA was packaged into infectious particles.

TABLE 2. Inhibition by dextran 10 of elevated temperature-induced inactivation of bacteriophages

| Bacteriophage | Temperature (°C)[a] | Time[b] (min) | % Dextran 10 | Fraction surviving |
|---|---|---|---|---|
| T7 | 51 | 14 | 0 | 0.017 |
| T7 | 51 | 14 | 20 | 0.29 |
| T7 | 51 | 14 | 40 | 0.61 |
| P22 | 57 | 36 | 0 | 0.021 |
| P22 | 57 | 36 | 20 | 0.13 |
| P22 | 57 | 36 | 40 | 0.55 |
| T4 | 57 | 36 | 0 | 0.047 |
| T4 | 57 | 36 | 20 | 0.29 |
| T4 | 57 | 36 | 40 | 0.41 |

[a] Temperature at which inactivation was conducted ± 0.2°C.
[b] Time of inactivation.

ture, the DNA packaged in capsids was observed as a function of time during incubation. Observation of packaged DNA was performed by staining capsid-associated DNA with ethidium bromide after fractionation of capsids by agarose gel electrophoresis. After incubation of 9⁻ P22 at 57°C, a progressive decrease in the DNA staining intensity occurred (Fig. 2a; 0%, lanes 1 to 5). The integrated intensity of protein staining did not significantly decrease (determined by densitometry of bands in Fig. 2b; 0%, lanes 1 to 5), although the band formed by capsids increased in width during incubation (Fig. 2b; 0%, lanes 1 to 5). These observations indicate that DNA was progressively emptying from the 9⁻ P22 capsid during the incubation. Bands formed by P22 were broader than bands formed by 9⁻ P22, suggesting electrophoretic heterogeneity of P22 (not shown). It was for this reason that 9⁻ P22 was used instead of P22 in the experiments reported here.

To determine the effect of dextran 10 on the emptying of DNA from the P22 capsid at 57°C, the experiment of Fig. 2a, at 0%, in lanes 1 to 5, was repeated in buffer containing 5, 10, 20, and 40% dextran 10. As shown in Fig. 2a, dextran 10 inhibited emptying of DNA from the P22 capsid; inhibition was almost complete in 40% dextran 10 and progressively decreased with decreasing concentrations of dextran 10. At any percentage, dextran 40 was approximately as effective as dextran 10 at inhibiting the emptying of DNA from 9⁻ P22 at 57°C (not shown). In addition, the smaller, but chemically related, compounds glycerol and ribitol inhibited emptying of DNA from 9⁻ P22. At any percentage, these compounds were also approximately as effective as dextran 10 at inhibiting emptying of DNA from 9⁻ P22 (data not shown).

Experiments similar to the experiment shown in Fig. 2 were also performed with T4 and T7 (with and without tail fibres [27]). It was found that the amount of DNA migrating with capsids during agarose gel electrophoresis decreased during inactivation at elevated temperatures. However, it was also found that the amount of protein at the capsid position decreased (not shown). The missing protein was found close to the origin of electrophoresis and may have aggregated or adhered to the gel (see reference 27). By electron microscopy it was shown that DNA is released from T4 (data not shown) and T7 (24) during inactivation at elevated temperatures. As found for bacteriophage P22, dextrans 10 and 40 inhibited the decrease in intensity of DNA stained capsid bands after incubation of bacteriophages T4 and T7 at elevated temperature (data not shown), presumably by preventing emptying of DNA from these capsids. Thus, inclusion of dextrans in buffers appears to stabilize DNA packaged in all three of the above bacteriophages. In contrast with the results for P22, glycerol (20 to 40%) did not have a significant effect on the decrease in intensity of DNA stained capsid bands after incubation of T7 at elevated temperatures. Glycerol also did not significantly alter the loss of T7 titer at elevated temperatures (data not shown).

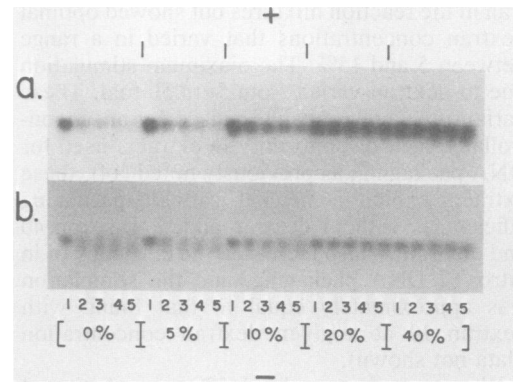


FIG. 2. Effect of dextrans on emptying of DNA during incubation of 9⁻ P22 at an elevated temperature. To 13-μl amounts of NPE buffer with the indicated final concentrations (%) of dextran 10 was added 2.0 μl of 9⁻ P22 (0.8 mg/cm³) in Tris-Mg buffer. Each of these mixtures was incubated for various times at 57°C. After incubation, 15 μl of sample buffer was added to each mixture, and analysis by agarose gel electrophoresis was conducted followed by staining with ethidium bromide (a) and Coomassie brilliant blue (b). The times of incubation at 57°C were: 1, 0 min; 2, 3 min; 3, 6 min; 4, 12 min; and 5, 24 min. The photographs in (a) and (b) are of the same gel for each respective dextran 10 concentration. Sample buffer contained 20% dextran 10 for mixtures heated in the absence of dextran; other sample buffers contained no dextran 10. The arrow indicates the direction of electrophoresis. The origin of electrophoresis is not shown.

TABLE 3. Capacity of compounds in preshock buffer for osmotically induced inactivation of bacteriophages during rapid dilution[a]

| Bacteriophage | Preshock buffer[b] | ΔP (atm)[c] | Titre (×10⁷)[d] | Fraction not inactivated |
|---|---|---|---|---|
| T4 | 0 | 0 | 84.6 ± 4.6 | 1.0 |
| | 2.8 M NaCl | 144 | 2.88 ± 0.58 | 0.034 |
| | 2.3 M Glucose | 75 | 73.3 ± 8.2 | 0.87 |
| | 1.8 M Sucrose | 75 | 80.5 ± 4.7 | 0.95 |
| | 40% Dextran 10 | —[e] | 73.7 ± 4.2 | 0.87 |
| T7 | 0 | 0 | 53.9 ± 3.0 | 1.0 |
| | 2.8 M NaCl | 144 | 52.7 ± 3.7 | 0.98 |
| | 2.3 M Glucose | 75 | 44.0 ± 2.6 | 0.82 |
| | 1.8 M Sucrose | 75 | 1.3 ± 0.3 | 0.024 |
| | 40% Dextran 10 | — | 49.1 ± 4.7 | 0.91 |
| P22 | 0 | 0 | 111 ± 5.8 | 1.0 |
| | 2.8 M NaCl | 144 | 111 ± 5.0 | 1.0 |
| | 2.3 M Glucose | 75 | 15.5 ± 0.5 | 0.14 |
| | 1.8 M Sucrose | 75 | 41.7 ± 2.9 | 0.38 |
| | 40% Dextran 10 | — | 103 ± 7.3 | 0.93 |

[a] Rapid dilution was made into NPM-G buffer, as described in the text.
[b] Component added to NPM-G buffer.
[c] Values of ΔP were determined as described in the text.
[d] In all cases of inactivation, it was shown that inactivation is prevented either by slow dilution with NPM-G buffer or by dilution into a dilution shock buffer with a high P (Table 1). Therefore, inactivation is caused by osmotic shock and not by toxicity of the compound in preshock buffer.
[e] —, Not known.

**Penetration of bacteriophage capsids.** To help determine whether compounds that stabilize bacteriophages enter the bacteriophage, the ability of the compounds to cause osmotic shock inactivation was determined. If a compound in preshock buffer causes osmotic shock inactivation of a bacteriophage during rapid dilution, it must enter the bacteriophage capsid. However, if the bacteriophage is not osmotically inactivated by a compound, even at ΔP values known to be large enough to cause inactivation by other compounds, there are two possibilities: (i) the compound does not enter the bacteriophage, or (ii) the compound does enter, but inactivation by osmotic shock does not occur. Possibility (ii) would occur if the compound either stabilizes the bacteriophage capsid or diffuses out of the capsid rapidly enough so that lethal osmotic pressure does not develop. In the case of bacteriophages T7 and P22, sucrose in preshock buffer caused osmotic shock inactivation during rapid dilution at a ΔP of 75 atm (Table 3), indicating that sucrose penetrated both of these bacteriophages. It is presumed that compounds smaller than sucrose also penetrate these bacteriophages, even though smaller compounds may cause comparatively little or no osmotic shock during rapid dilution at comparable ΔP values (T7 with NaCl or glucose, P22 with NaCl; Table 3). Thus, presumably glycerol and ribitol entered P22 and T7 during incubation at elevated temperatures (above).

Dextran 10 in preshock buffer did not inactivate P22 and T7, and sucrose did not inactivate bacteriophage T4 during rapid dilution (Table 3). For reasons described above, it cannot be rigorously concluded that these compounds do not enter the bacteriophages which they do not inactivate. However, the comparatively large size of dextrans 10, 40, and 70 suggest their nonpenetration into bacteriophage capsids.

## DISCUSSION

Dextrans were found to: (i) protect bacteriophages from osmotic shock, (ii) increase the efficiency of the in vitro assembly of infective bacteriophage T7 from DNA-free procapsids and DNA, and (iii) protect bacteriophages from inactivation at elevated temperatures. The data obtained indicate that protection at elevated temperatures was accompanied by inhibition of the emptying of DNA from capsids. These observations suggest the use of dextrans, or possibly other related compounds, for stabilizing either fragile viruses or related particles during isolation. The use of dextrans to increase the in vitro assembly efficiencies of bacteriophages other than T7 may also be possible (in vitro assembly of P22 is stimulated by dextrans; S. Rajalakshmi and P. Serwer, unpublished data).

Some carbohydrates and polyols smaller than dextrans also have the above effects. However, if the compound is small enough, in vitro assembly efficiency is decreased (i.e., glycerol inhibited T7 DNA packaging).

There are at least two mechanisms by which dextrans and the smaller compounds could cause the above effects: stabilization of a protein(s) (see references 10 and 29) or creation of a $\Delta P$ across bacteriophage capsids (higher P outside). It is also possible that both of these mechanisms are operative. A possible cause of the glycerol inhibition of in vitro assembly [effect (ii), above] is penetration of glycerol into a capsid that cannot be penetrated by the larger, chemically similar compounds. If so, the glycerol inhibition of DNA packaging can be interpreted by postulating that a $\Delta P$ across the capsid is necessary for DNA packaging.

The prevention of elevated temperature-induced DNA emptying from the P22 capsid [effect (iii)] by glycerol, a compound shown to penetrate P22 bacteriophage, might indicate that maintenance of a $\Delta P$ across bacteriophage capsids is not necessary for effect (iii). The glycerol may, however, be partially excluded from the P22 capsid by the bound water of hydration of packaged DNA. Water inside of bacteriophage T7 is bound tighty enough to partially exclude at least one capsid-permeable solute, sodium iothalamate (23). Such a partial exclusion would result in a $\Delta P$ across the bacteriophage that would be induced even by capsid-permeable solutes. The packing densities of P22 and T4 DNAs are comparable to the packing density of T7 DNA (9), suggesting that a similar exclusion occurs for P22 and T4.

Knowledge of the mechanism by which dextrans increase the efficiency of in vitro assembly may assist in developing an understanding of mechanisms of assembly. Because dextrans stabilized the packaged state of DNA, it seems likely that they stimulate in vitro assembly, at least in part, by either increasing the speed of DNA packaging or stabilizing packaged DNA to facilitate tail addition. However, initiation of DNA packaging, assembly of the tail, and possibly other steps in assembly may also be stimulated by dextrans and related compounds. Attempts are currently being made to physically measure the transformations occurring during in vitro DNA packaging (initiation and entry). When this can be done, the effects of dextrans and other compounds on these transformations will be determined directly.

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