Purification and Some Properties of Bacteriophage ST-1

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Bacteriophage ST-1 is shown to be a small, isometric, single-stranded deoxyribonucleic acid (SS-DNA) virus with a diameter of about 260 nm. Standard methods for growth, assay, preparation of high-titer lysates, and purification of the phage are suggested. ST-1 infects K-12 and not C strains of *Escherichia coli* and requires a divalent cation to adsorb to susceptible bacteria. Adsorption also requires an activation of the particle brought on by incubation at 37 C. The latent and eclipse periods are essentially identical (9 to 11 min) in ST-1 infections, with an average burst size about 250 phages per cell. Multiple densities of ST-1 infectivity are observed during purification in CsCl gradients. The virus recovered from different densities has the same sedimentation coefficient and, therefore, all phage containing fractions are pooled during purification. The purified ST-1 particle has a sedimentation coefficient of 121S relative to ϕ X-174 (114S) in a sucrose gradient and a molecular weight of 6.8×10^6 (as estimated from its relative sedimentation). The nucleic acid is assumed to be SS-DNA on the basis of (i) the specific incorporation of ^3H -thymine, (ii) the dependence of its UV absorption on temperature, and (iii) its reaction with formaldehyde. ST-1 SS-DNA sediments at 24.4S relative to ϕ X-174 SS-DNA (23.8S).

Bacteriophage ST-1 (5) has been described as a small, spherical virus probably similar to ϕ X-174 (see 11, 20 for review). It is unique among these small phages since it infects K-12 but not C strains of *Escherichia coli*. By acridine orange staining techniques (5), it was shown that ST-1 contains single-stranded deoxyribonucleic acid (SS-DNA). However, its physiological and biochemical properties are poorly understood, and their characterization is hampered by the low titer and unstable nature of ST-1 preparations. In this paper certain physiological properties of ST-1 have been examined. An understanding of these properties has allowed the preparation and purification of stable, high-titer lysates, which in turn has simplified the determination of certain biophysical properties of ST-1 using ϕ X-174 as a comparison standard.

MATERIALS AND METHODS

Bacteria and bacteriophage. *E. coli* W3110 (an F⁻ prototroph from R. S. Edgar) was used for ST-1 assay, growth, and lysate preparation. Unless otherwise specified it is the organism referred to when the term cells is used. *E. coli* W3110 thy⁻ was obtained from J. Cairns. Bacteriophage ST-1 was obtained from D. E. Bradley (4). ϕ X-174 and ϕ X-174 *am3* have been described (13).

Media. SB (starvation buffer) and TK broth have been described (8). TKCa broth is TK broth containing 0.0005 M CaCl₂; TKMg broth is TK broth containing 0.01 M MgCl₂. Top agar contains 5.0 g NaCl, 10 ml of 1.0 M MgCl₂, 10.0 g of tryptone (Difco), and 8.0 g of agar per liter of distilled water. Bottom agar plates were made as described (18) except that the NaOH was omitted. Tris buffer (TB) is 0.033 M Tris-hydrochloride at pH 8.1.

Special chemicals. Radioactive thymine, adenine, and the scintillation fluor, Aquasol, were obtained from New England Nuclear Corp. Polyethylene-glycol (PEG) 6000 was from Mann Research Laboratories. Optical grade CsCl was obtained from the Harshaw Chemical Co. and chloroform and formaldehyde were from the Baker Chemical Co.

Biological assays. Plaque assays for ST-1 were performed utilizing the standard soft agar overlay technique (1). Seed bacteria (*E. coli* W3110) were grown with aeration in TKMg broth to 5×10^8 to 10×10^8 cells/ml and stored on ice until used. Refrigerated seed cultures could be used over a period of a week without appreciably affecting the phage assay. Dilutions of phage or infected bacteria were made into TKMg or TKCa broth. Seed bacteria (about 0.3 ml) were added to melted top agar (2 to 3 ml) at 45 to 47 C, 0.1 to 1.0 ml of phage suspension at the appropriate dilution was added, and the entire contents was poured onto bottom agar plates. The temperature range for plaque formation is about 33 to 45 C and plates were usually incubated at 42 C for 1.5 to 2.5 h. Plaques were formed in so short a time that

the bacterial lawn was very light and the plaques were very faint. To see and count plaques more easily, plates were placed at room temperature after the 42 C incubation period to allow the bacterial lawn to grow more turbid without increasing plaque size (since ST-1 plaque formation is inhibited below 30 to 33 C). The time allowed for solidification of top agar at room temperature prior to incubation at 42 C should be kept to a minimum (less than 10 min) so as to prevent a reduction in the efficiency of plaque formation.

Infective centers were assayed by merely plating the appropriate dilutions of the infected culture in the ST-1 plaque assay.

Free phages in the infected culture were measured by diluting 1:100 into TKMg or TKCa broth saturated with CHCl_3 to kill infected cells. After mixing vigorously, the samples were allowed to stand undisturbed for 20 min. Appropriate dilutions (at least 1:10) of the aqueous phase were then plated in the ST-1 plaque assay. CHCl_3 does not release intracellular phage particles, at least not $\phi\text{X-174 } am3$ particles (13).

Both intracellular and free phage should be detected by the lysozyme-EDTA-SDS or lysozyme-EDTA-freeze-thaw assays: 0.1 ml of a sample was diluted into 0.8 ml of TB containing 0.1 ml of lysozyme solution (2 mg/ml in water) at 4 C. After 5 min, 1.0 ml of EDTA (0.8% in 0.033 M Tris, pH 8.1) was added. Ten to 15 min later the samples were either frozen and thawed 3 to 6 times or 0.5 ml of the solution was mixed with 0.5 ml of SDS (0.1% in 0.05 M Tris, pH 7.0) at room temperature for 10 min. Appropriate dilutions were then plated in the ST-1 plaque assay. This treatment did not decrease the EOP of free phage.

Measurement of eclipse. A phage particle is said to have eclipsed when it interacts with a susceptible bacterial cell so that the virus is rendered incapable of initiating another infection if the cell is destroyed and the phage particle or its remains are eluted from the cellular debris. Whether just adsorption or both adsorption and eclipse are measured depends on whether the phage are eluted from the destroyed cell. In this paper eclipse is defined operationally as the loss of free phage which occurs as a function of time of mixing with cells when either the CHCl_3 or the lysozyme-EDTA-SDS assays are used.

Starvation synchrony. Starvation synchrony was essentially as described (8) except that in some cases the cells were washed by filtration rather than centrifugation. It should be mentioned that a complication was observed with starved cells. At MOI greater than about 2 only 20% or so of the viable cells were converted to infective centers capable of plaque formation. This effect was not observed with starved cells at lower MOI (less than 1) or with growing cells even at higher MOI.

Assays of $\phi\text{X-174}$ and $\phi\text{X-174 } am3$ have been described (13).

Supernatant lysates. Cells were grown with aeration in TKMg broth to about 4×10^8 to 6×10^8 cells/ml and infected with ST-1 at an MOI of about 0.05. Under these conditions the burst size of ST-1 was about 100 to 200 phages per cell and two phage growth cycles were required to lyse all the bacteria

(the MOI for the final growth cycle which lyses the bacteria was 5 to 10). Visible lysis usually began 40 to 50 min after infection. The cell density was followed with a spectrophotometer and the lysate was harvested (cooled to about 0 to 4 C) when the cell density had decreased to about 50% of the maximum value (optical density at 660 nm OD_{660} of about 1.2 down to about 0.6). If lysis was allowed to continue much past 50% of the maximum cell density, the phage titer dropped off quite rapidly (up to 20-fold) presumably due to absorption and eclipse on cellular debris. Neither RNase nor DNase (1 $\mu\text{g/ml}$ each) had any effect on ST-1 phage production. The lysate was cleared of cellular debris by low-speed centrifugation and the supernatant, which contained up to 100% of the phage detectable in the mass lysate, was refrigerated and stored for future use. The titers of supernatant lysates prepared in this manner ranged from 10^{11} to 4×10^{11} PFU/ml and remained fairly constant for periods of at least one year. Large lysates of 10 liters were prepared in this way by using a New Brunswick fermentor. If the phage were to be used to infect cultures in SB or defined media, they were either diluted 10^{-4} or more into the appropriate media, or precipitated with PEG (see below) and resuspended in the media of choice.

Preparation of radioactive phage. *E. coli* W3110 thy^- was grown in TKMg to about 5×10^8 cells/ml, and ST-1 (MOI of 0.05) and ^3H -thymine to 20 μCi were added per ml. The lysates were then treated in the same manner as described for supernatant lysates.

^{14}C -adenine labeled $\phi\text{X-174 } am3$ preparation was as previously described (6). ^{14}C -thymine labeled $\phi\text{X-174 } am3$ were prepared in a similar manner except that *E. coli* HF4704 (14) was used as the host cell and ^{14}C -thymine was substituted for ^{14}C -adenine.

Radioactivity determinations. Samples were placed in scintillation vials and 10 ml of Aquasol scintillation fluid was added. The radioactivity was then determined in a Packard Tri-Carb liquid scintillation counter.

PEG has been described (23). NaCl was first added and then PEG was added to a final concentration of 0.5 M and 10%, respectively. When the resuspended pellet was highly concentrated relative to the original phage suspension, a second PEG precipitation and resuspension to the same volume was performed to remove more of the PEG, since the volume of 10% PEG remaining from the first precipitation was often as great or greater than the volume of resuspending medium. Phage recovery up to 100% has been obtained.

Equilibrium CsCl density gradients. CsCl was added to the ST-1 sample (usually in TB, borate or TKMg) to a mean density of about 1.41 g/cm³ (0.625 g of CsCl per g of sample). Centrifugation was in the SW 50.1 rotor at 37,000 rpm for 24 to 28 h in the Spinco model L2-65B ultracentrifuge.

Analytical ultracentrifugation. A Spinco Model E analytical ultracentrifuge (equipped with electronic speed control, temperature control, UV monochromator and UV scanner) was used for all centrifugations. A double-sectored cell with charcoal-filled Epon centerpiece, sapphire windows, and wide window holders (for use with the UV scanner) was used in the AN-H

rotor. All sedimentations of phage particles were carried out at 20 C and 18,000 rpm. For phage DNA, sedimentations were done at 44,000 rpm and 20 C. Sedimentation coefficients were calculated from the movement of the middle of the sedimenting boundary as a function of time.

DNA extraction. A room temperature phenol extraction (19) was used for all ST-1 DNA extractions. In some cases standard saline citrate (SSC, 0.15 M NaCl, and 0.015 M NaCitrate) replaced borate buffer as the aqueous phase.

RESULTS

Description. ST-1 is a small, spherical virus with an average diameter of 260 nm (5; Fig. 1). ST-1 infects both male and female strains of *E. coli* K-12 but not strains B, C, 15, or a male derivative of strain C. The lower temperature

limit for plaque formation is about 33 C with the upper limit as high as the host *E. coli* will grow.

Adsorption requires a divalent cation. Studies were conducted to determine the conditions required for adsorption and eclipse of supernatant ST-1 (see Materials and Methods). Upon addition of ST-1 to starved cells in the absence of divalent cations no eclipse was observed until Ca was added (Fig. 2). When Mg replaced Ca the rate of eclipse was about 10 times slower in SB at 0.01 M divalent cation (either Mg or Ca; results not shown). At 30 min after adding phage to a culture lacking divalent cations (Fig. 2) a sample was taken and centrifuged to remove cells. All of the phage particles remained in the supernatant, showing that

FIG. 1. Electronmicrograph of ST-1. An ST-1 preparation purified by two CsCl gradients was dialyzed into TB, and then negatively stained (12). $\times 225,300$.

FIG. 2. Adsorption and eclipse in SB (no divalent cations). Cells starved in SB lacking divalent cations were divided into 20-ml portions at 37 C. CaCl_2 was added to 0.01 M to one culture; no CaCl_2 was added to the other culture. Both cultures were infected with ST-1 at an MOI of about 0.1 and samples were taken and assayed for uneclipsed phage (CHCl_3 assay). At 30 min after adding phage, the culture without CaCl_2 was treated as follows: (i) 10 ml was centrifuged to remove cells (see text) and (ii) CaCl_2 was added to 0.01 M to the remaining 10 ml and samples were taken for an additional 30 min.

ST-1 requires a divalent cation to adsorb to cells. It is not known if subsequent steps leading to eclipse also require the presence of a divalent cation.

Adsorption requires a temperature-induced activation. It was noticed that phage incubated at 37 C for 30 min with cells in the absence of Ca (Fig. 2) seemed to eclipse faster (upon addition of Ca) than when no time was allowed for phage to pre-mix with the cells at 37 C. Since adsorption to cells did not occur in the absence of Ca, this pre-mixing effect had to occur before the phage were associated with the cells. It was thought that the more rapid eclipse might be due to an activation of the phage caused by the incubation at 37 C. To test this hypothesis phage were incubated at 37 C for 30 min in the absence of divalent cations and then used to infect starved cells; phage which had not been preincubated at 37 C were used to infect a parallel culture. The heat-activated phage began to eclipse immediately, but there

was about a 2-min delay before eclipse occurred when the phage had not been activated. However, once eclipse began, the rate appeared to be the same in both cases (results not shown).

The requirement for temperature-induced activation is easily illustrated if the infection is done at 25 to 26 C. At this temperature no activation of the phage occurs and only phage previously activated are capable of adsorption and eclipse (Fig. 3). Essentially 100% of the input nonactivated phage remained in the supernatant after centrifugation to pellet cells indicating that the activation process is required for adsorption (Fig. 3). The activation process is not readily reversible (at least not in TK broth or TB). However, the activated phage are not stable and the infectivity titer of an activated ST-1 preparation decays with a half-life of about 15 h (H. R. Patel, personal communication).

ST-1 growth parameters. A single cycle of ST-1 phage production in starvation synchronized cells had a minimum latent period of 10 to 11 min and a burst size of 200 to 400 phage per cell (Fig. 4). Since unsynchronized infections with activated phage also had a minimum latent period of 10 to 11 min, starvation synchrony seemed to function as it was intended, allowing phage entry but no further development until dilution into growth conditions. The apparent doubling of infective centers was quite reproducible and appeared to occur as a function of the infective process. It is most probably the result of the EOP of infected cells being greater than the EOP of free phage by a factor of about 2. Since a UV inactivation curve of ST-1 yielded no shoulder and extrapolated back to 1 (not 2) phage at zero time, it would appear that the doubling of infective centers upon infection is probably not due to an aggregation of the phage with the process of heat activation or infection releasing a hypothetical phage pair so that each could initiate a separate infection.

There is no detectable buildup of intracellular phage prior to cellular lysis. However, lysis of the infected cells did occur (Fig. 5). Since both OD_{660} and phage titer extrapolated to about the same time of 10 min (the minimum latent period) it appears that phage production and release are initiated at the same time as cellular lysis. Furthermore, the two curves intersect when both have values of 50% of the maximum change, indicating that when 50% of the cells have lysed, 50% of the final phage yield has been released. In other words, the kinetics of phage production and release parallel the kinetics of cellular lysis. The decrease in phage titer in the mass lysate (i.e., no dilution as in Fig. 5)

FIG. 3. Eclipse at 25 to 26 C requires temperature activation. *E. coli* W3110 *thy*⁻ was grown at 37 C in TK broth to 3.8×10^8 cells/ml (OD_{550} 0.6). The culture was then diluted by addition of 1 volume of TK broth at room temperature, and $MgCl_2$ and $CaCl_2$ were added to 10^{-2} and 10^{-3} M, respectively. Two 5-ml portions of this culture were then incubated at 25 to 26 C in a shaking water bath for 20 min. At this time one culture (A) was infected with 0.25 ml of activated ST-1 (a supernatant lysate in TK broth at about 4×10^8 PFU/ml was incubated at 37 C for 30 min and then iced for 5 min before infection); the other culture (B) was infected with 0.25 ml of nonactivated ST-1 (same as activated except iced with no 37 C incubation). Samples were taken from both cultures for assay of uneclipsed phage ($CHCl_3$ assay). The same samples assayed without $CHCl_3$ (both A and B cultures) gave essentially the same results as the $CHCl_3$ -treated samples from culture B. At 20 min after infection both cultures were centrifuged (10,000 rpm in Sorvall RC2B in SS-34 rotor for 10 min at 4 C) to pellet cells and attached phage. The supernatants were then assayed: culture A supernatant was at 6×10^4 PFU/ml (<1%), 2×10^4 PFU/ml of which were $CHCl_3$ resistant. Culture B supernatant was at 1.1×10^5 PFU/ml (essentially 100%) with or without $CHCl_3$ assay.

after it reached a maximum at 25 min was probably caused by adsorption and eclipse on cells or cellular debris.

Multiple densities in $CsCl_2$. During the purification by $CsCl_2$ density gradient centrifugation, ST-1 banded at several densities (3 to 5) in the same gradient. The different peaks of ST-1

infectivity were both coincident and reasonably proportional to the UV adsorbance, suggesting that each density species was infective to roughly the same degree. Upon examination in the electron microscope each band appeared the same, containing only ST-1 particles with all the particles appearing identical. All the density species seemed to have the same S-value (results not shown) and therefore all the species were combined during purification.

Sedimentation coefficient of the particle.

The $s_{20,w}$ of the particle was estimated from its sedimentation relative to ϕX -174 in sucrose gradients (16; Fig. 6). Upon sedimenting five different ST-1 preparations an average $s_{20,w}$ of 121 was observed.

Some of the ST-1 preparations exhibited a slower sedimenting component of about 40S.

FIG. 4. Starvation synchronized, single-step growth curve of ST-1 at 37 C. Cells starved in SB at 0.01 M $MgCl_2$ (filtration rather than centrifugation was used to remove the cells from TKCa broth and to wash them free of nutrients, see Materials and Methods) were infected with ST-1 at an MOI of about 0.1 and 10 min were allowed for adsorption and eclipse. The infected cells were then diluted 10^{-4} into TKCa broth at 37 C to initiate the infection process. Samples were taken and assayed for infective centers (I.C.), free phage ($CHCl_3$), and intracellular phage (lysosome-EDTA SDS).

FIG. 5. Kinetics of cellular lysis and phage release. Cells were grown to 2.3×10^8 per ml in TKMg broth at 37°C and infected with ST-1 at an MOI of about 3. Samples were taken at the times indicated and assayed for infective centers and absorbance at 660 nm. The data are plotted as the ratio of the absorbance (or infective centers) at time t to the maximum absorbance (or infective center) reached.

This component was not infective and its significance is not understood.

Molecular weight. The molecular weight of the ST-1 phage particle can be estimated from its sedimentation coefficient and from the molecular weight and sedimentation coefficient of ϕ X-174 (16), provided that it is assumed that (i) both ϕ X and ST-1 are spherical phages, (ii) both have the same partial specific volumes, and (iii) ϕ X-174 has $s_{20,w}$ of 114 and a molecular weight of 6.2×10^6 (19); $S_1/S_2 = [M_1/M_2]^{2/3}$. Using an S-value of 121 for ST-1 yields a molecular weight of 6.8×10^6 by this method.

Nucleic acid of ST-1. Incorporated ^3H -thymine coincided with ST-1 infectivity in both CsCl density gradients and sucrose velocity gradients. Since thymine is a specific precursor to DNA, its incorporation into the ST-1 particle strongly suggests that the phage nucleic acid is DNA, and in all further discussions the nucleic acid of ST-1 will be assumed to be DNA.

ST-1 DNA was found to sediment with an

$s_{20,w}$ of 24.4 in a sucrose gradient (0.2 M NaCl - 0.02 M NaCitrate, Fig. 7) relative to ϕ X-174 DNA ($s_{20,w}$ of 23.8; 7, 19). Assuming the conformation of ϕ X and ST-1 DNA to be similar under the same ionic conditions, the molecular weight of the ST-1 DNA is probably slightly

FIG. 6. Sedimentation of ST-1 relative to ϕ X-174. An ST-1 supernatant lysate was diluted 10^{-4} into SB and combined with a similarly diluted ϕ X-174 lysate (about 10^7 PFU/ml of each phage). A 0.1-ml amount of this mixture was layered onto a 5 to 20% sucrose (in TB) gradient and spun at 37,000 rpm for 45 min (SW 50.1 rotor in Spinco Model L2-65B). Three-drop fractions were collected directly into 20-mm test tubes containing 10 ml of TKCa broth, and each fraction was assayed for ϕ X and ST-1 infectivity.

FIG. 7. Sedimentation of ST-1 DNA relative to ϕ X-174 am3 DNA. ST-1 DNA was phenol extracted from a CsCl purified ^3H -thymine labeled phage preparation. A similar phenol extraction was performed on a ^{14}C -thymine labeled ϕ X-174 am3 preparation. A 0.2-ml amount of ST-1 DNA and 0.1 ml of ϕ X-174 am3 DNA were mixed and then layered onto a preformed 5 to 20% (wt/vol) sucrose gradient containing 0.2 M NaCl-0.02 M NaCitrate. The gradient was then centrifuged for 6 h at 37,000 rpm in the SW 50.1 rotor of the Spinco Model L2-65B preparative ultracentrifuge. Three-drop fractions were collected directly into scintillation vials and the radioactivity was determined.

larger than the 1.7×10^6 reported for ϕ X DNA. In an analytical sedimentation (Fig. 8a) ST-1 DNA sedimented as a single moving boundary and had an $s_{20,w}$ of 24.4 in 0.2 M NaCl-0.02 M sodium citrate.

As expected for SS-DNA (19) the UV absorption of ST-1 DNA was very dependent on temperature over a wide range (Fig. 9). In contrast, the UV absorption of double-stranded DNA did not show a temperature dependence except over a very narrow range of elevated temperatures.

Another indication that ST-1 DNA is single-stranded comes from its ability to react with formaldehyde. SS-DNA but not DS-DNA will react with formaldehyde at low temperature and sufficiently high ionic strength (19). The reaction causes a change in the UV absorption pattern of SS-DNA producing both an overall increase in UV absorption and a shift in the maximum UV absorption towards higher wave lengths. Both the ST-1 DNA and the intact phage particle reacted with formaldehyde giving similar UV adsorption scans (Fig. 10).

Reaction with formaldehyde increases the molecular domain of SS-DNA by destroying its intramolecular hydrogen bonding capabilities and extending the molecule. This extension can be measured as a decrease in sedimentation rate of SS-DNA. ϕ X-174 DNA is not only SS-DNA, it is also circular, although, a proportion of the molecules are linear counterparts of the same molecular weight. The extension of the ϕ X DNA molecule caused by reaction with formaldehyde allows the circular molecule to be distinguished from the linear molecules by sedimentation (9). Since the circular molecule cannot extend its molecular domain to the same extent as the linear molecule, the circular molecule sediments faster. Reaction with formaldehyde (1.8 to 1.9%) decreased the sedimentation rate of ST-1 DNA (Fig. 8b). Two sedimenting components were observed with $s_{20,obs}$ of 16.6 and 15.4. It is assumed that the 16.6S component is circular and the 15.4S component linear. However, further experimentation is needed to establish this assumption. Under similar conditions two sedimentation constants of 15.8 and 13.9 are observed for ϕ X DNA (19). The linear form of ϕ X-174 DNA has been assumed to be a broken DNA molecule, i.e., a whole molecule but with a single, covalent break per molecule (9).

DISCUSSION

Bacteriophage ST-1 resembles ϕ X-174 in size, shape, molecular weight (as determined by sedimentation), growth parameters, and ab-

FIG. 8. Analytical sedimentation of ST-1 DNA. A 10-liter supernatant lysate of ST-1 was concentrated about 1000-fold by precipitation with PEG and purified by two CsCl density gradient centrifugations. After dialysis into TB, a phenol extraction was done. Sedimentation (see Materials and Methods) was in 0.2 M NaCl-0.02 M NaCitrate, pH 8.1 either (a) before or (b) after 24 hours' reaction with formaldehyde (1.8-1.9% in 0.2 M NaCl-0.02 M NaCitrate) at room temperature.

FIG. 9. Absorbance at 260 nm of ST-1 DNA as a function of temperature. The absorbance at 260 nm of ST-1 DNA (preparation described in Fig. 8) was recorded as a function of temperature essentially as previously described (15). Measurements were also performed on *Bacillus subtilis* DNA (kindly supplied by D. B. Yelton) as a control.

sorption and eclipse requirements. Its nucleic acid appears to be SS-DNA which sediments at a rate very close to that of ϕ X SS-DNA indicating a similar amount of genetic information. Because of these properties ST-1 is assumed to be another small, isometric, tailless, SS-DNA phage best exemplified by ϕ X-174.

Like other small bacteriophages (20), ST-1 requires divalent cations in order to adsorb to susceptible bacteria. The absorption step also requires ST-1 in an activated state induced by incubation at 37°C. ϕ X-174 exhibits a property which is at least similar to this activation property of ST-1. When ϕ X-174 is incubated at 37°C in various media, a transition occurs from a ϕ^* to a ϕ form (2). This transition probably involves a configurational change in the particle since the two forms differ in heat stability. The ϕ form is thermolabile compared to the relatively thermostable ϕ^* form. Also, the thermolabile ϕ form adsorbs to susceptible bacteria at 0 to 4°C, whereas the thermostable ϕ^* form does not. Intracellular ϕ X is in the ϕ^* form as is shown by examination of phage released from infected cells lysed artificially in the cold; the transition to the ϕ form occurs shortly after release at 37°C. Phages S-13, ϕ R, and ST-1 each exhibit this transition with the respective properties of the two forms (3). Although it is not known if small phages other than ST-1 must be in the ϕ form to adsorb at physiological temperatures, plots of adsorption and eclipse of ϕ X-174 (J. E. Newbold, Ph.D. thesis, California Institute of Technology, 1970) extrapolate to 1, indicating no requirement for heat activation. However, the ϕ X-174 used in these studies adsorbed to cells at 0 to 4°C indicating that the transition from ϕ^* to ϕ had already occurred.

FIG. 10. Reaction of ST-1 DNA with formaldehyde (1.8 to 1.9%). The ST-1 DNA preparation described in Fig. 8 was mixed with an equal volume of formaldehyde at 3.6 to 3.8% in 0.2 M NaCl-0.02 M NaCitrate at room temperature. UV absorbance was recorded as function of wave length and time on a Beckman DK-60a recording spectrophotometer.

ST-1 does not require heat activation when prepared by the borate elution technique used by Sinsheimer's group with ϕ X. It may be significant that the supernatant ST-1 preparations which were obtained in the nonactive state (ϕ^* form) were prepared in the absence of Ca.

ST-1 differs from ϕ X and other related phages in host range. Because of its *E. coli* K-12 specificity, studies on the contribution to viral replication of host cell functions involved in DNA replication, recombination, and repair synthesis could be facilitated, if ST-1 utilizes any of these functions, since the genetics of these host cell systems are much better understood in K-12 strains than in C strains. Of the *E. coli* genes known to be essential for host cell DNA synthesis, *dnaA* through *dnaG* (21), ST-1 only requires *dnaE* (J. M. Bowes, manuscript in preparation), the gene which seems to code for DNA polymerase III (10). Recent in vitro results (17, 22) indicate that ϕ X-174 requires all these *E. coli dna-ts* functions. If these results hold up in vivo then ST-1 must be considerably different than ϕ X-174, at least as regards reliance upon host cell DNA synthesis functions.

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