Preparation and Characterization of Temperate, Non-inducible Bacteriophage P2 (host: *Escherichia coli*)

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**SUMMARY**

P2 phage particles contained DNA (38 %) and protein (62 %), and were assigned a particle weight of $5.8 \times 10^7$ daltons, based on the known molecular weight of P2 DNA. The extinction (1 cm.) of suspensions of $10^{11}$ particles per ml. was 0.09 at 260 nm. wavelength. In purified preparations 20 to 50 % of the particles present were not detected by biological assay. The preparations were heterogeneous in respect to heat stability. Even at relatively low temperatures, changes in light scattering accompanied heat inactivation.

**INTRODUCTION**

The bacterial virus P2 is representative of a group of temperate bacteriophages not uncommon in nature: we isolated from hospital material some 40 different temperate phages able to attack *Escherichia coli* C and found that more than a third were serologically related to P2. Phage P2 has been used extensively in genetic studies of lysogeny, thus a better knowledge of its physical—as opposed to genetic—properties has become desirable, and methods for its preparation, concentration and purification are presented here, together with data on its composition.

**METHODS**

*Biological material.* Phage P2 was originally isolated (Bertani, 1951) from the Lisbonne & Carrère lysogenic strain of *Escherichia coli*. It was studied in the electron microscope by Anderson (1960). It has equal e.o.p. on *E. coli* strain C (number 122 of National Collection of Type Cultures, London) and *Shigella dysenteriae* strain sh (Bertani, 1951) and lower e.o.p. on *E. coli* strains K-12 and B, and some *Serratia* strains (Bertani, Torheim & Laurent, 1967).

Wild type P2 is, by definition, the phage liberated by a derivative of strain sh (our collection number sh-17), made lysogenic for the original P2 isolate obtained from the Lisbonne and Carrère strain. Most of the observations to be reported were made before it was discovered (Bertani, Choe & Lindahl, 1969) that certain mutants (lg and lg cc) were gradually replacing wild type phage in our preparations. There is no evidence that this genetic heterogeneity is relevant to the conclusions to be presented, and we shall neglect it.

The bacteria used were: a line (called ‘adapted C’, our collection number C-1a) of *Escherichia coli* C, which grew better than the parental strain in simple defined media, and two streptomycin resistant derivatives (C-85 and C-1055) which were usually grown in the
presence of streptomycin (10 \( \mu g/mL \)). For details of standard phage techniques, see Adams (1959). Unless otherwise indicated, all incubation was at 37\(^\circ\).  

**Culture media and other solutions.** Unless stated otherwise compositions are given in g./L of distilled water. **LB broth:** Bacto Tryptone (Difco) or N-Z-amine type B (Sheffield Chemical), 10; NaCl, 10; yeast extract (Difco), 5; glucose, 1. pH adjusted to about 7.4 with NaOH. **LB agar** is LB broth solidified with agar, 10, and supplemented with CaCl\(_2\) (2.5 \( \times 10^{-3}\) M) before pouring. **NB broth:** Nutrient broth (Difco), 8; NaCl, 5. pH adjusted to 7.4 with NaOH. **TB broth:** Bacto Tryptone (Difco), 10; NaCl, 5. pH adjusted to 7.1 with NaOH. **Soft agar** is NB solidified with agar, 6.5. **A medium:** Consists of 9 volumes of solution I (KH\(_2\)PO\(_4\), 15; (NH\(_4\))\(_2\)SO\(_4\), 3.6; NaOH, about 2.4, to obtain pH 6.8), one volume of solution II (glucose, 100), and one volume of solution III (Casein Acid Hydrolysate, salt free, Nutritional Biochemicals Corporation, 100; NaCl, 50). To this one adds small volumes of various solutions to obtain the following final concentrations: MgSO\(_4\), \( 1.4 \times 10^{-3}\) M; CaCl\(_2\), \( 10^{-4}\) to \( 5 \times 10^{-5}\) M (i.e. short of precipitation); FeCl\(_3\), \( 6 \times 10^{-6}\) M (this last being replaceable by a mixture of trace metals as given by Lwoff, Kaplan & Ritz (1954). Poor phage yields were sometimes obtained when using other commercially available casein hydrolysate preparations. **KP:** KH\(_2\)PO\(_4\), 14; K\(_2\)HPO\(_4\), 18 (pH 6.8) (2 M in phosphate). **SSC:** 0.15 M NaCl, 0.015 M Na\(_2\) citrate (pH 7.0). **NaCl. KP:** NaCl, 8; KH\(_2\)PO\(_4\), 1.4; K\(_2\)HPO\(_4\), 1.8. **ABI:** KH\(_2\)PO\(_4\), 2; K\(_2\)HPO\(_4\), 7; NaCl, 10 (pH 7.0). **Other chemicals.** Pancreatic deoxyribonuclease I, pancreatic ribonuclease, \( \alpha \)-chymotrypsin, trypsin, pepsin, wheat germ lipase, pan-protease (all from Worthington Biochem. Corp.), pronase (Calbiochem).  

**Phage titrations** were routinely made on LB agar plates with 2.5 ml. soft agar, using undiluted cultures of strain c-85, grown overnight in NB or TB broth and aerated by bubbling for 2 to 4 hr before use. We know now that larger plaques and higher titres are obtained with undiluted, unaerated cultures of strain c-1055, grown overnight in LB broth, and that still higher titres can be obtained more laboriously by preadsorbing the phage for 8 to 10 min. to bacteria of strain c-1a, concentrated by centrifugation to \( 2 \times 10^8 \)/ml. from an exponentially growing culture in LB broth with added \( 2.5 \times 10^{-3}\) M-CaCl\(_2\) (adsorption mixture: 0.25 ml. concentrated bacteria and 0.1 ml. phage).  

The efficiency of this last method was compared with that of the first on 10 different phage preparations. It was on the average 1.7 times higher (range 1.3 to 2.2), independently of whether the preparations contained the Ig mutants or not, and of whether they had been purified in CsCl density gradients or not.  

**Preparation of high titre, large volume lysates.** The following method gave lysates of pure P2 wild type having at least \( 2 \times 10^{10}\) p.f.u./ml. and of P2 Ig or Ig cc with at least \( 8 \times 10^{10}\) p.f.u./ml. The average for a series of lysates of the latter type was \( 1.2 \times 10^{11}\) p.f.u./ml.  

A culture was prepared by inoculating 500 ml. of medium A in a Fernbach flask (or other wide bottom container of about 3 l. capacity) on a reciprocating shaker with 20 ml. of an unaerated, overnight culture of strain c-1a also in medium A. When the culture reached a titre of 8 to \( 9 \times 10^8\) colony forming elements per ml., corresponding to \( E_{590}\) between 2.5 and 2.8, it was infected with phage at an input ratio p.f.u./bacteria of 0.2 to 0.3. The bacterial concentration at the time of infection was critical. After infection, the rate of shaking was increased. In addition, a gentle flow of sterile air into the flask was established. The rate of oxygen uptake under these
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2.03 conditions, measured by the sulphite method (see Meynell & Meynell, 1965), was about $3.5 \times 10^{-4}$ moles O$_2$.min$^{-1}$.l$^{-1}$.

After 60 to 65 min. from the time of infection (that is, after only one cycle of phage reproduction had taken place and the majority of bacteria present had been infected), 40 ml of KP were added in order to block readsoption of the phage produced. After another 60 to 65 min. the occurrence of bacterial lysis was indicated by viscosity of the culture and the presence of clumps of stringy material. The flask was removed from the shaker and 0.5 to 1 mg. of deoxyribonuclease was added. In 10 to 20 min. the preparation became visibly less viscous and could be centrifuged (45 min. at 1500g) to remove surviving cells and debris.

Concentration of phage by precipitation with methanol. For reasons unknown the phage was unstable in a lysate prepared as described, and immediate concentration and purification was desirable. The lysate was chilled at 4$^\circ$ and a volume of cold methanol equal to 35% of the lysate was added with constant stirring. The mixture was left standing at 4$^\circ$. Flocculation and precipitation occurred after 1 to 4 days. When the sediment was sufficiently compact, the supernatant fluid was sucked off. The sediment was resuspended in 100 ml. of 0.03 M-EDTA (pH 7.0) in 0.8 M NaCl, trying to dissolve clumps as well as possible. The suspension was centrifuged (45 min. at 6000 g) and the supernatant fluid, containing at least 50% of the original phage activity, was collected.

The phage was further concentrated and purified by several cycles of differential centrifugation at 4$^\circ$, resuspending the high speed pellets in SSC or other defined medium. For each high speed centrifugation (angle rotor, 2 hr at 18,000g), about 80% of the phage activity was recovered in the resuspended pellet, and less than 10% was not recoverable in either supernatant or pellet.

Phage concentration by means of polyethylene glycol. Although all the observations to be reported here concerned P2 preparations concentrated by methanol, we found that the technique of virus concentration with polyethylene glycol (Leberman, 1966) could be applied with success also to P2. It was unnecessary to centrifuge the lysate before applying the polyethylene glycol, and therefore large volumes could be processed. Following the deoxyribonuclease treatment, 25 g. NaCl (dissolved in as little water as possible) were added to each l. of lysate, mixing in the cold, followed by 200 ml. of a 30% (w/w) solution of polyethylene glycol (mol. wt 6000 to 7000) also in water. After 2 to 4 days storage at 4$^\circ$, precipitation was practically complete. The supernatant fluid was withdrawn by suction. The sediment was resuspended in a small volume of SSC, and centrifuged at low speed. The supernatant fluid was kept and the pellet was re-extracted once or twice in the same way. The recovery of phage activity approached 100%. The phage was then purified in a CsCl density gradient, as differential centrifugation alone was inefficient in removing completely the polyethylene glycol.

Light scattering of phage suspensions at 90$^\circ$ from the incident light ($\lambda = 546$ nm., narrow band, obtained by a filter combination Corning 1-60 with Wratten 58) was measured in the Fluoromicro-photometer of American Instrument Co. The sample was placed in a cylindrical glass cuvette of 1 cm. diameter in a thermostatted holder. The intensity of the scattered light—given in the relative instrument units—was corrected by subtracting the intensity produced by the suspension medium alone. Under these conditions, water and SSC gave an intensity reading of 0.03 units, and a
1% colloidal suspension of silica in water (Ludox LS, DuPont) gave an (uncorrected) intensity of about 0.06 units.

Extinction \( (E) \) of phage preparations and of bacteria was measured in a Zeiss spectrophotometer PMQ II with thermostatted holder in quartz cuvettes of 1 cm depth.

Total phosphorus was determined according to Fiske and Subba Row's method (Leloir & Cardini, 1957) reading \( E_{660} \).

DNA was determined by a modification of the Dische (1955) diphenylamine reaction, which measures the deoxyribose residues attached to purines. The reaction tubes were incubated overnight at 37\(^\circ\) instead of for a shorter period in boiling water. Estimates were obtained both from \( E_{660} \) and from the difference between \( E_{660} \) and \( E_{490} \), and averaged. Deoxyguanosine (Sigma), dissolved in SSC, was used as the standard. \( E \) was not strictly proportional to the concentration of deoxyguanosine above \( 6 \times 10^{-5} \) M. DNA amounts were calculated throughout as the Na salt of DNA containing the four normal bases in equimolar proportions, assuming that all and only the purine deoxyribose reacted like free deoxyguanosine.

Phage preparations in SSC were analysed for DNA without any previous fractionation to remove the protein, since preliminary trials had shown that preparations which had been frozen and thawed many times did not react differently from the untreated preparations. This treatment inactivated the phage and set free the phage DNA, as indicated by the increased viscosity of the preparation. Furthermore, in reconstruction experiments, addition of albumin to DNA (10 to 1, respectively, by weight) did not affect the reaction, provided the mixture was not, as in Burton's (1956) procedure, treated with 0.5 N-perchloric acid.

Protein was determined by the procedure of Lowry et al. (1951), reading \( E_{13700} \). Sterile bovine albumin solution (Armour Pharmaceutical Company) was used as the standard. Different proteins may give different intensities per unit weight in this reaction. DNA, at least up to a concentration of \( 5 \times 10^{-5} \) g./ml of analysed sample, gave no reading in this reaction. As with DNA determinations, the phage was not extracted in any way before the reaction. No difference was observed between whole phage and phage frozen and thawed several times.

DNA base analysis was performed according to Wyatt (1955). Whole phage, purified by centrifugation, or DNA extracted from it by the phenol method (Mandell & Hershey, 1960), and precipitated with ethanol, was hydrolysed with perchloric acid. The bases were separated by two-dimensional paper chromatography, first descending in isopropanol + HCl and then ascending in isopropanol + NH\(_3\). Material containing about 100 \( \mu \)g. of nucleobases was applied to the paper. Only four ultraviolet absorbing spots were observed in each case. They were cut out, eluted with 0.1 N-HCl, and identified by their spectra and \( R_f \) values. The extinction coefficients given by Wyatt (1955) were used to calculate the amount present in each spot.

Nitrogen was kindly determined for us by the Kjeldahl method by Ing. E. Avots of the Department of Medical Chemistry.

Preparative density gradient equilibrium centrifugation in CsCl was according to Weigle, Meselson & Paigen (1959).

Sedimentation coefficients were kindly determined for us by Ing. H. Persson and Prof. T. Laurent of Uppsala University in a Spinco analytical ultracentrifuge, at phage concentrations between \( 3 \times 10^{-2} \) and \( 1.7 \times 10^{-4} \) g./ml., in 0.15 M-NaCl, at 10\(^\circ\), using ultraviolet optics. They were independent of concentration in that range.
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RESULTS

Analysis of P2 bacteriophage

Analytical data for nine preparations of P2 phage concentrated by methanol precipitation and purified by centrifugation are summarized in Table I. The original values for total P, DNA, protein and $E_{600}^{1cm}$ in the individual preparations were highly correlated with each other indicating that the composition of the purified preparations was reproducible and that the main variable was the efficiency of plaque formation, i.e. the proportion of inactive particles present in the various preparations. The P content observed (9·24 % of the DNA content, on the average) corresponded well with the theoretical value (9·36 %) for the Na salt of DNA. There was thus no evidence

Table I. Average analytical data for purified preparations of phage P2

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Number of preparations analysed</th>
<th>Phosphorus*</th>
<th>DNA*</th>
<th>Protein*</th>
<th>Extinction†</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Purified by differential centrifugation (3 to 5 cycles) in SSC.</td>
<td>6</td>
<td>1·14±0·20</td>
<td>1·23±0·16</td>
<td>2·95±0·15</td>
<td>2·92±0·30</td>
</tr>
<tr>
<td>(B) As above (2 to 3 cycles), then banded in CsCl and transferred to SSC, either by dialysis or by repeated centrifugations.</td>
<td>3</td>
<td>0·81±0·05</td>
<td>0·88±0·07</td>
<td>1·39±0·07</td>
<td>2·33±0·09</td>
</tr>
</tbody>
</table>

* Total P/p.f.u. in units of 10$^{-17}$ g. DNA (as the Na salt)/p.f.u. and protein (as albumin)/p.f.u. in units of 10$^{-16}$ g. For each preparation one p.f.u. titre obtained with indicator C-85 soon after the last step in the preparation was used throughout in the calculations as though it were a constant for that preparation. Averages± standard deviations are given. The values for individual preparations were themselves averages of 2 to 4 analyses each.

† $E_{600}^{1cm}$ divided by p.f.u./ml. and multiplied by 10$^{12}$. Not corrected for scattering.

for any large fraction of P not in the DNA of the phage. All four analytical values for three preparations purified by equilibrium centrifugation in CsCl density gradients were significantly lower than the corresponding values for the other six preparations, even though the density gradient step involved additional handling which might tend to reduce the biological activity of the preparation. The proportionality between the four analytical data remained essentially constant however for the two groups of preparations. The difference could be explained if the CsCl density gradient step resolved clumps of phage particles, thus increasing the biological activity of the preparation, or if it removed impurities of relatively small mass that were tightly adsorbed to the phage making it unable to form plaques.

A preparation (containing 8·4 x 10$^{-18}$ g. P, 8·4 x 10$^{-17}$ g. DNA and 1·5 x 10$^{-16}$ g. protein/p.f.u.) was analysed twice for total N, and an average of 3·30 x 10$^{-17}$ g./p.f.u. was found. Of this, 1·3 x 10$^{-17}$ g./p.f.u. was accounted for by DNA (assuming equimolarity of the nucleobases). If all the remaining N were in protein, the N content of the phage proteins would have been 14 % by weight.

Another preparation (containing 1·1 x 10$^{-17}$ g. P/p.f.u.) was suspended in a 2 % solution of the volatile salt ammonium acetate and dried to constant weight, first at 55° and then at 110°. The dry weight was found to be between 3·1 and 32·x 10$^{-16}$ g./
p.f.u. The DNA of this preparation (calculated from the P content) would thus account for 34 to 37% of the dry weight. This proportion could also be estimated independently from the analytical data of Table 1, on the assumption that the phage consisted exclusively of DNA and protein: the average value found, 38% (range 35 to 42%), was in satisfactory agreement, and justified the use of albumin as the standard in measuring phage protein. Such a composition would predict for P2 phage a buoyant density in CsCl solution of 1.445 (see Weigle et al. 1959), assuming 1.71 and 1.32 for the densities of DNA and protein, respectively. Three determinations of the buoyant density of P2 in CsCl density gradient by drop collection gave values in the range 1.434 to 1.447. More precise determinations and an analysis of buoyant density heterogeneity of P2 preparations have been made by D. H. Walker (in preparation).

The sedimentation coefficients $S_{20,w}$ for two purified P2 preparations (one also banded in CsCl) were found to be 280 and 285 s.

Table 2. Base analysis of DNA from phage P2

<table>
<thead>
<tr>
<th>Molar ratios*</th>
<th>Extracted DNA</th>
<th>Whole phage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/T</td>
<td>1.08</td>
<td>1.07</td>
</tr>
<tr>
<td>G/C</td>
<td>1.00</td>
<td>1.04</td>
</tr>
<tr>
<td>A+G+C+T</td>
<td>1.04</td>
<td>1.06</td>
</tr>
<tr>
<td>A+T/G+C</td>
<td>0.99</td>
<td>1.01</td>
</tr>
<tr>
<td>Recovery†</td>
<td>0.70</td>
<td>0.85</td>
</tr>
</tbody>
</table>

* The identification of the four bases adenine, guanine, thymine, and cytosine (A, G, T and C) was based on the $R_f$ values in isopropanol+HCl and in isopropanol+NH₄, and the extinction ratios ($E_{260}$/$E_{280}$, $E_{260}$/$E_{320}$) observed for each spot. These values were very similar in the two analyses and are given here (in the order stated above) only for the extracted DNA. For A: 0.38, 0.47, 0.74, 0.38. For G: 0.27, 0.19, 1.38, 0.83. For T: 0.80, 0.65, 0.76, 0.58. For C: 0.50, 0.37, 0.46, 1.56.
† Total moles of nucleobase detected per mole of deoxyribose in the sample, as measured by the diphenylamine reaction.

**Base composition of P2 DNA**

It is known that the base composition of P2 DNA is approximately equimolar according to buoyant density measurements in CsCl gradients (Bertani et al. 1967). We analysed the base composition of P2 DNA following hydrolysis of both whole phage and P2 DNA extracted with perchloric acid (Table 2). Although the recoveries were not high, the data showed that the bulk of the nitrogen bases of P2 DNA consisted of the four normal bases, guanine, adenine, cytosine and thymine, and was consistent with an equimolar base composition and a double-stranded, complementary structure for P2 DNA.

A similar analysis was performed following hydrolysis in 6N-HCl (Hershey, Dixon & Chase, 1955). Although hydrolysis of the pyrimidine nucleotides appeared to be incomplete, the same four bases could be demonstrated.

**Ultraviolet absorption spectra**

The u.v. absorption spectra of purified P2 preparations were typical of nucleoproteins (Fig. 1). The difference between preparations banded or not in CsCl density gradient was very small and only suggestive of a slightly stronger scattering in the latter.

At wave lengths 320 nm. or greater, true absorption for protein or DNA is negli-
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gible, and $E$ values in this range are often used to calculate by extrapolation the contribution to $E$ of intrinsic scattering of particles at the lower wave lengths (see Jagger, 1967). For this purpose, the relationship of log. $E$ to log. $\lambda$ must be linear. This was not the case for P2 preparations in the $\lambda$ range 320 to 600 nm. In the shorter range 320 to 450 nm, a straight line of slope $-3.6$ could be approximated. On this basis, for an observed ratio $E_{260}^{\text{cm}}/E_{190}^{\text{cm}}$ of 0.06 (Fig. 1), scattering was calculated to contribute 14% of $E_{260}^{\text{cm}}$ and 16% of $E_{190}^{\text{cm}}$. The corrected $E_{260}^{\text{cm}}$ of P2 preparations was then interpreted in terms of DNA and protein content with reasonable results. The expected true absorbance at 280 nm (specific absorbance data of Felsenfeld & Hirschman, 1965) due to the DNA (from Table 1) was subtracted. The residual $E_{260}^{\text{cm}}$ divided by the observed protein content (from Table 1) gave a specific absorbance of 1.7 for a 1 mg./ml. solution of phage protein. This value was well within the range 1.2 ± 0.7 of specific absorbance values given for a number of proteins (Long, 1961, p. 82).

![Absorption spectra of purified P2 phage suspensions in SSC, not corrected for scattering.](image)

**Fig. 1.** Absorption spectra of purified P2 phage suspensions in SSC, not corrected for scattering. (○), Average values for the three preparations under B, Table 1; (□), average values for four preparations in group A, Table 1. $E_{260}$ values were set equal to 1.

**Stability**

Concentrated suspensions of purified phage P2 in SSC were fairly stable upon storage at 4°C. Their plaque-forming activity decayed at a rate of 3 to 5%/month, assuming first order kinetics. Similar rates of inactivation were observed when citrate was replaced by small amounts of nutrient broth, or for suspensions in NaCl-KP, or in Davis medium (Sasaki & Bertani, 1965) (without trace metals or glucose). Diluted phage suspensions in SSC appeared to be initially less stable upon storage, but approached in the end the decay rate of concentrated suspensions. Stability upon cold
storage was definitely lower in pure NaCl solutions than in SSC. Dilution of phage into distilled water, even at low temperature, produced variable but often strong inactivation of the phage.

Heat inactivation

At higher temperatures survival of P2 activity did not follow first order kinetics, but gave upwards concave survival curves in semilogarithmic plot, suggesting heterogeneity in the phage population. At 45° survival in SSC was, for example, 10 to 20 % after 15 min., 2 to 5 % after 2 hr, 0.1 % after 2 days. Attempts to isolate more heat resistant mutant lines of P2 failed, and we suspect that the bulk of this heterogeneity is phenotypic, which is not unusual in bacteriophages (‘phenocopies’ in Adams, 1959). Heterogeneity was a stable property, at least at low temperature; samples of P2 in SSC, held at 45° for 4 hr, stored at 4° for up to 50 days, and then exposed again to 45°, were inactivated in the same way as if the exposure to 45° had not been interrupted.

Phage survival at 45° in 0.15 M-NaCl was similar to that in SSC, but less reproducible. Addition of divalent cations (5 x 10^-4 to 10^-2 M), as for other viruses, increased stability at high temperatures. Higher survivals than in SSC were obtained at 45° also for suspensions in 2 % ammonium acetate, or 10 x more concentrated SSC, or NaCl. KP. In cold storage however these suspension media were not better than SSC, and large initial losses of activity were often observed as an immediate result of dilution from SSC into some of these media.

Similar heterogeneity of resistance to heat was observed for suspensions of P2
exposed to still higher temperatures. For example, at 60 ° in 0.14 M-NaCl, inactivation down to a survival of about 10⁻⁴ was almost immediate, whereas after a 5 hr exposure the survival was still as high as 10⁻⁶ to 3 x 10⁻⁵. Although the final inactivation rate was affected by the phage concentration in the suspension, this effect was not sufficient to explain the shape of the survival curves.

When a suspension of P2 in SSC was heated, E²⁵⁰ decreased rapidly and irreversibly by about 14% between 36 ° and 48 ° (Fig. 2). In the same experiment, E¹⁰⁰ decreased from 0.014 to 0.003. In a similar experiment, E¹⁰⁰ decreased from 0.92 to a final value of 0.81, whereas E¹⁰⁰ decreased from 0.055 to 0.025. At a time during the process, when E¹⁰⁰ had decreased to 0.036, which corresponds to 63% of the total change, the plaque-forming activity of the phage was measured: 70% of the original activity had been lost as a result of heating. The decrease of E was obviously caused by a decrease in light scattering (Fig. 3). It would appear that heat inactivation of P2 in SSC was accompanied by gross irreversible changes in the structure of the particles, presumably ejection of the DNA or contraction of the tail sheath or both. Formation and dissolution of clumps of phage particles could also affect scattering, but this alternative seemed unlikely because of the coincidence between phage inactivation and decrease in scattering.
Other properties

Some phages are strongly inactivated upon rapid dilution from a high to a low concentration salt solution (osmotic shock) (Anderson, 1953). Phage P2 was quite resistant to this effect. For example, rapid 200-fold dilution from 3 or 4.5 M-NaCl (in ABi medium) to plain ABi medium inactivated 99% of a preparation of phage T4, but did not affect the activity of P2.

Concentrated preparations of P2 in simple salt solutions or diluted preparations in broth or in 2 to 10% glycerol, were scarcely inactivated by a single cycle of freezing (in solid CO₂+ ethanol) and thawing, but did not survive long term storage at solid CO₂ temperature.

The activity of purified P2 preparations was unaffected over periods of a few hours by various degradative enzymes: deoxyribonuclease, ribonuclease, α-chymotrypsin, pepsin, trypsin, lipase, pronase and pan-protease.

Phage P2 was inactivated more readily than other phages when shaken with chloroform.

DISCUSSION

DNA extracted from P2 preparations consists of linear double stranded molecules with a molecular weight of 2.2 × 10⁷ daltons, as shown by sedimentation velocity (Mandel, 1967) and by electron microscopy (Inman & Bertani, 1969) using phage λ DNA as the standard (Caro, 1965). The average DNA content for all preparations described in Table 1 (1.1 × 10⁻¹⁶ g./p.f.u.) represents 6.6 × 10⁷ daltons, i.e. 3 molecules of P2 DNA/p.f.u. When reasonable corrections for e.o.p. are introduced (see below) the most likely estimate of this number becomes smaller than 2, and thus confirms the expectation that only one molecule of DNA is present in each particle. Corrections were applied for the low e.o.p. of the indicator (c-85) used, as compared with titrations by the preadsorption method (a factor of 1.3 to 2.2) and for the loss of plaque formation (5 to 15% of the titre; Bertani, 1957, 1960) due to lysogenization. The following values for DNA per biologically detectable particle were obtained: 2.7 to 4.6 × 10⁷ daltons (average of all preparations of Table 1) or 2.1 to 3.6 × 10⁷ daltons (average of preparations banded in CsCl). The case is stronger if one considers that some inactivation of the phage in the course of purification is unavoidable. Moreover, the sedimentation coefficients observed for phage P2, when compared with those established for other tadpole-shaped phages (see Pitout, Conradie & Van Rensburg, 1969) would be hardly compatible with a particle weight of 1.1 × 10⁸ daltons, as one would expect from the observed composition if P2 particles contained two DNA molecules each.

The estimate of the molecular weight of P2 DNA molecules and the data presented here can best be combined by assuming that each particle of P2 phage contains one such DNA molecule, and that 20 to 50% of the P2 particles following concentration and purification may not form plaques even under the best known titration conditions.

On this basis, a P2 particle can be characterized by the following data: DNA content (Na salt): 3.7 × 10⁻¹⁷ g. (i.e. one double stranded molecule of molecular weight 2.2 × 10⁷ daltons); P content: as expected from DNA content; protein content (Table 1, preparations banded in CsCl): 5.8 × 10⁻¹⁷ g. Other components besides DNA and
protein, if any, represent at most a few per cent of the total weight. Base composition of DNA: thymine, cytosine, guanine, adenine in equimolar proportions, as also shown by density studies (Bertani et al. 1967). Extinction at 260 nm. (1 cm. pathlength): 0.09 (uncorrected for scattering) for a suspension of 10^{11} particles per ml.

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