An Ultracentrifuge Study of Small Peptides and Large Fragments of T3 Bacteriophage

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SUMMARY

Treatment of T3 with various denaturing agents produced distinct components which were identified in the ultracentrifuge. Combining this analysis with electron microscopy made it possible to identify the capsid and nucleocapsid, and a fragment having an $s_{20, w} = 105 \pm 10$S. This fragment appeared spherical in the electron microscope and had dimensions between 12 nm and 16 nm. It was tentatively identified as the tail. The mol. wt. of the 105S particle and the empty head (200S) were calculated to be $2.3 \times 10^6$ and $2.7 \times 10^6$, respectively. Adding these weights to the estimated weight for DNA ($25 \times 10^6$, Lang & Coates, 1968) gave a total weight for the phage of $49 \times 10^6$, equal to the mol. wt. obtained by Swaby (1959). In addition to these large fragments, two peptides were examined, one had a mol. wt. of $9300 \pm 1400$ in 6M-GuHCl (1.5S) and appeared to be a single chain; the other, released when a suspension of phage was diluted, had a mol. wt. of less than 10000. The origins of the two peptides remain largely speculative, but in view of the marked associative properties of the 1.5S peptide and its detection only when the head was destroyed, it is likely that it was a binding fraction in the head of the phage. The second peptide may be more intimately associated with the DNA of T3.

INTRODUCTION

Bacteriophage T3 is a relatively complex virus whose mol. wt. of $49 \times 10^6$ (Swaby, 1959) is compounded from a double strand of DNA of mol. wt. approx. $25 \times 10^6$ (Lang & Coates, 1968) and protein of mol. wt. approx. $24 \times 10^6$. Electron microscopy of T3 reveals both recognisable phage particles and morphological forms which resemble empty shells (called 'ghosts' by Anderson, 1950, and Anderson, Rappaport & Mascatine, 1953). These shells can be separated from the intact phage by zonal sedimentation, and their density suggests that they contain only protein. Their continued presence shows that the exo-proteins surrounding the phage DNA form a stable aggregate. Ten proteins were identified by gel electrophoresis after using SDS and mercaptoethanol as dissociating agents (Stibenz et al. 1971). Because of the addition of mercaptoethanol the protein species identified may be individual peptide chains and not necessarily representative of the native proteins. In order to obtain a more realistic estimate of the size of the native proteins, a milder dissociating

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METHODS

Growth and purification of phage T3. Phage T3 (wild type) was grown in 1500 ml cultures of *Escherichia coli*, strain b, vigorously aerated at 37 °C. The growth medium contained per 100 ml water: proteose peptone, 1.5 g; liver digest, 0.25 g; yeast extract, 0.4 g (Oxoid Ltd., England); sodium chloride, 0.5 g (Analar, B.D.H. Ltd, England).

Maximum yield of phage was assumed to have occurred when the culture cleared; this took approx. 1 h after addition of T3. The bacterial debris was centrifuged from the clear medium at approx. 8000 rev/min for 25 min using a 6 x 250 ml rotor in a MSE 17 centrifuge (Measuring and Scientific Equipment Ltd, England). The T3 was deposited from the supernatant fluid by further sedimentation at 17000 rev/min for 2 h in a fixed angle rotor (6 x 80 ml). The phage pellets from all the tubes were combined in one tube and suspended in 10 ml of 0.1 M-tris buffer (pH 9.0) containing 0.05 M-sodium chloride and 10^-3 M-diaminoethane tetracetic acid disodium salt, ionic strength 0.05. The phage was washed once and collected by centrifuging. The suspended virus was stored in tris buffer (pH 9.0) for periods not exceeding two weeks. The yield was approx. 10 mg phage.

Chemicals. Where possible, chemicals of analytical grade were used, but recrystallization of guanidine hydrochloride (GuHCl) from 80 % (v/v) ethanol was necessary to reduce the E_280 of a 6 M solution of GuHCl to 0.1. The activity of H⁺ in the presence of high concentrations of salts (e.g. 6 M-GuHCl) may not be accurately estimated by uncorrected pH measurements, but these values are quoted to define experimental conditions.

Sperm whale myoglobin (Miles Serevac (Pty.) Ltd, England), used as a standard at various stages in the analyses, was purified by chromatography on a 0.3 x 0.01 m column of G-50 Sephadex (Pharmacia Ltd, Sweden). The Sephadex was equilibrated with the buffer solution (0.05 M-phosphate, pH 7.0 or 0.1 M-tris, pH 9.0) and the protein eluted at 4 °C with the same buffer. The eluate was collected in 5 ml samples and the sample from the centre of the peak (measured at 280 nm) taken for analysis.

Analytical ultracentrifuge experiments

Apparatus. A Beckman model E analytical ultracentrifuge fitted with a temperature control unit was used. The runs were carried out at about 20 °C using 12 m/m Kel-f centrepieces. Velocity experiments employed Schlieren optics on solutions having concentrations between 0.05 % and 0.3 % (w/v). Since fragments remaining after treatment showed a wide range of sedimentation rates, it was necessary to examine every solution at several rotor speeds. Mol. wt. estimations were carried out by sedimentation-equilibrium using absorption optics; records of extinction at varying radii were collected through a computer system programmed to optimise for high ratios of signal-to-noise (Spragg, 1967; Spragg & Goodman, 1969). Monochromatic light at 280 nm was produced by a monochromator (using a 250 W Xenon light source) having a band-width of approx. 10 nm.

Analyses and corrections

Velocity runs. These measurements were taken from photographs using a conventional travelling microscope. Sedimentation coefficients were calculated from the slopes of ln r versus time (r = radius of centroid of boundary). A least-squares method was used to estimate the slopes from measurements in which the centroid of the boundary was taken
as being the centres of the Schlieren peaks. Statistical tests showed that a straight line was generally the best fitting model for the records. The calculated coefficients were corrected for density and viscosity of the solvent relative to water (Schachman, 1959) assuming that the partial specific volume of the macromolecule was unaffected by the solvent. The densities and relative viscosities of the solvents were measured both at 20 °C and at the temperature of the experiment using conventional pycnometers and viscometers.

**Sedimentation equilibrium.** Assuming the measured extinction \( (E) \) is proportional to concentration of the protein \( (C_2) \), then the apparent mol. wt. \( (M) \) can be calculated from equation (1),

\[
\frac{\delta \ln E}{\delta (r^2)} = \frac{\omega^2 M (\partial p/\partial C_2)_p}{2RT},
\]

where \( R \) and \( T \) have the usual connotations, \( p \) the density of the system and \( \omega \) the velocity of the rotor. The subscript \( p \) indicates constant chemical potential for the diffusible components of the solution (Cassassa & Eisenberg, 1964). In dilute buffer solutions and at equilibrium between solvent and solution \( (\partial p/\partial C_2)_p \approx (1 - \bar{v} \rho) \), where \( \bar{v} \) is the partial specific volume of the macromolecule. From this approximation the apparent mol. wt. are usually estimated. In the presence of 6 M-GuHCl preferential interactions between the protein and GuHCl lead to non-ideality which reduces the slope \( \delta \ln E/\delta (r^2) \). The extent of this interaction is difficult to evaluate in a system containing macromolecules of mol. wt. less than 10000 (as in the present work). Because the molecule passed through most cellophane membranes, and because it aggregated, dialysis to equilibrium before analysis was impossible. Instead, an empirical method was adopted where the mol. wt. of sperm whale myoglobin was determined under conditions identical to those used for the unknown molecule. The mol. wt. of this standard was calculated from these results using the accepted partial specific volume for myoglobin \( (0.743, \text{Theorell, 1934}) \) and then an empirical correction factor was calculated by comparison of this estimated weight with the accepted mol. wt. of 17830 (Edmunson & Hirs, 1962).

The slope, \( \delta \ln E/\delta (r^2) \), was estimated by a non-linear regression analysis using a weighting factor calculated in the recognized manner for logarithmic transformations (Wilkinson, 1961), in this case proportional to \( E^2 \). The rotor speeds were adjusted to ensure that the ratio between the extinction at the bottom of the cell and that at the meniscus never exceeded four.

Small columns of liquid (3 mm or less) were used in these experiments, and the over-speeding technique of Richards, Teller & Schachman (1968) was employed to reduce the time to reach equilibrium. Tests using myoglobin showed that equilibrium was achieved within 2 h of reaching final speed as judged by the fact that the mol. wt. of myoglobin did not increase between that time and 17 h later.

**Density sedimentation.** A simple separation of protein from nucleic acid was all that was required from this technique, hence a single step in density was generated by layering the solution to be analysed on 3 to 5 ml 45% \((w/v)\) CsCl (density of approx. 1.4 g/ml at 20 °C) (Analar), in a 6 ml centrifuge tube which was centrifuged in a swing-out rotor on an MSE 75 centrifuge at 60000 rev/min for approx. 1 h. Two 0.1 ml samples were taken, one from the top 0.5 cm and the other from the centre of the tube. Where bands of molecules could be seen, these were sucked into the pipette in place of sampling empirically.

**Electron microscopy.** All the studies were made with a Phillips E.M. 200 electron microscope using standard negative staining techniques. Its magnification had previously been calibrated using platinum phthalocyanin crystals as an internal marker.
Estimation of radioactivity. The contamination of the protein by nucleic acid after denaturation in 6 m-GuHCl was estimated by growing *Escherichia coli* in a medium containing 1 mCi [³²P] (Radiochemical Centre, Amersham, England, as phosphoric acid and neutralized with NaOH before use), before infecting with T3. 0.3 ml samples were assayed for [³²P] by scintillation counting in a 10 ml mixture of 2,5-diphenyloxazole (PPO) dissolved in 2:1 (v/v) of toluene and Triton X-100 (Patterson & Greene, 1965). The determinations were made on duplicate samples using an Intertechnique S 140 liquid scintillation spectrometer, and the results were corrected for quenching using previously calibrated quenching curves (Barnett, 1972). Difficulties incurred in maintaining a single phase for the mixture of [³²P] chemicals, 6 m-GuHCl and scintillation fluid were overcome by adding a quantity of water equal to the volume of 6 m-GuHCl solution.

From a typical experiment in which the concentration of [³²P] in the growth media was 484 x 10⁵ d/s a solution of T₃ was obtained which had 6.76 x 10⁶ d/s (3.3 ml of a 0.2 % (w/v)

Preparation of fragments from phage. Several reagents were chosen because of their known properties in dissociating particles. Ethanolamine has been used in work with TMV (Leberman, 1968), lithium chloride dissociates phage lambda (Dyson, 1966; Dyson & Van Holde, 1967), urea and GuHCl are effective denaturing agents for proteins (Tanford, 1968, 1970), and SDS is used widely in combination with gel-electrophoresis to study the subunits of particles and macromolecules (see for example the study of T₃ by Stibenz *et al.* 1971).

Preliminary experiments showed that 6 m-GuHCl, pH 9.0, in tris buffer (0.01 M) was the most suitable reagent for dissociating phage particles. The undissociated particles remaining after the treatment were removed by centrifuging at 45000 rev/min for 2 h in a swing-out rotor; this treatment removed from the top half of the tube all molecules having sedimentation coefficients greater than 2S.

Light extinction measurements. Spectra from 200 nm to 800 nm were recorded on a Unicam S.P. 800 spectrophotometer (Unicam Instruments Ltd, England); extinctions at fixed wavelengths were measured on a Unicam S.P. 500 spectrophotometer. In general the measurements were made using cuvettes having 10 mm path length and solvent blanks.

The concentration of phage was calculated from the $E_{260}$ in a 10 nm cuvette assuming a 1 % (w/v) solution had an extinction of 84.2 (Spragg & Rankin, 1967), or the number of particles were determined assuming 10¹² particles/ml had an $E_{260}$ of 1.18 (Anderson & Cline, 1967; Simon & Anderson, 1967).

Amino acid analyses. Protein samples were freed from salt by 2 h dialysis against an excess of distilled water. If necessary the protein was precipitated by addition of an excess of pure ethanol, and the precipitate was centrifuged at low speed and finally washed with 50 % (w/v) ethanol. 0.3 mg samples were added with 0.1 μM-norleucine (an internal standard) to 1 ml of distilled concentrated HCl. This was hydrolysed *in vacuo* for approx. 48 h at 105 °C and then neutralized. The amino acids were estimated using ion-exchange chromatography and an automatic monitoring system.

Preliminary experiments established that the optimum time for hydrolysis lay between 48 h and 72 h. The results were not corrected for losses of the hydroxy-amino acids during the hydrolysis.

Dialysis. Dialysis was carried out using small-bore Visking tubing which had been boiled in distilled water for at least 2 h to extract water soluble impurities from the cellulose.
Table 1. Summary of the sedimentation rates of components produced by treatments of T3-phage with denaturing reagents*

<table>
<thead>
<tr>
<th>Sedimentation rate</th>
<th>Reagent...conc. (m)...</th>
<th>Ethano-</th>
<th>LiCl</th>
<th>LiCl</th>
<th>LiCl</th>
<th>GuHCl</th>
<th>GuHCl</th>
<th>GuHCl</th>
<th>SDS</th>
<th>Tris</th>
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<tr>
<td>(S)</td>
<td>pH...</td>
<td>10.8</td>
<td>4.5</td>
<td>10.0</td>
<td>4.0</td>
<td>6.5</td>
<td>5.9</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>475</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>370</td>
<td>+</td>
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<td>+</td>
<td>-</td>
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<td>-</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
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<td>20</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* See text for identification of the components; with one exception the suspension of the virus was treated at room temperature (20 °C) for periods up to 5 h; however, in the case of 5 M-LiCl the treatment was for 5 min at 46 °C. The error coefficient for $s_{20,w}$ increased progressively from ±2 % at 475S to ±14 % at 15S. + indicates presence; however, no quantitative estimates of relative concentrations were obtained in the analyses.

RESULTS

Dissociation of the particles

Untreated preparations of phage in tris buffer consistently gave two boundaries, one having an $s_{20,w}$ value of 475 ± 10S, identified as phage particles (Swaby, 1959); the other had a $s_{20,w} = 200 ± 10S$ and it was identified by microscopy, after isolation on a density gradient, as the ‘ghosts’ (Morrod, 1969).

Following treatment of phage preparations with various denaturing agents, up to seven components (including ‘ghosts’) could be recognized in the ultracentrifuge (Table 1). Splitting the phage often produced a fraction having an $s_{20,w}$ of 105 ± 10S which had a density less than 1.4 g/ml. The fragment having an $s_{20,w}$ of 20 ± 6S was identified as DNA since it had a density greater than 1.4 g/ml and its sedimentation coefficient was similar to that reported for DNA from this phage (Bendet, 1962; Lang & Coates, 1968).

Other components having $s_{20,w}$ values intermediary between 30S and 70S were discovered not only in the presence of the two mild denaturing agents, ethanolamine and lithium chloride (3.5 M), but also with SDS. Their structures and origins are uncertain but they may be aggregates of several phage components. It was surprising to discover stable components in the presence of 0.05 m-SDS (pH 9.0) at 20 °C, having $s_{20,w}$ values of 370, 200, 105, 60 and 1.5S. These products were not further examined because of the strong binding of SDS to the proteins and difficulties in measuring molecular parameters in this solvent (Barnett & Spragg, 1971).

Concentrations of GuHCl below 1.0 M did not affect the sedimentation rate of the virus (475S). In 1 M-GuHCl only one boundary was detected having a sedimentation rate less than the virus particle by about 100S. With GuHCl concentrations between 1 M and 3 M at pH 6.0, and with 5 M-LiCl at pH 3.5, a white precipitate formed. The quantity of this precipitate was reduced when 0.1 M-mercaptoethanol was added to the reagents; but this addition caused the formation of multiple boundaries in the ultracentrifuge making interpretation of records unreliable. From qualitative examination of the Schlieren peaks it seemed probable that the precipitate was formed from the component having an $s_{20,w}$ of 105 ± 10S. Examination of this precipitate in the electron microscope showed many particles...
having shapes which resembled either true or oblate spheroids and having diameters of approx. 12 nm. At higher magnifications some of these particles were observed to have a central hole with an approximate diam. of 4 nm (Morrod, 1969).

The smallest component liberated by any reagent had an $s_{20,w}$ of $1.5 \pm 0.2$ S at concentrations of approx. 0.05 g/ml, and this component was examined in more detail.

**Preparation and purity of 1.5S protein**

6 M-GuHCl (pH 9.0) was used to prepare the protein and the larger fragments were removed by sedimentation; however, this procedure does not guarantee removal of all nucleic acids. A useful criterion of purity in routine preparations of protein is to measure the ratio $E_{280}/E_{260}$ ($R$); unfortunately this is a relatively insensitive criterion if the extinction coefficients of the constituents are modified by the chemical environment. Measuring the $^{32}$P present at each stage of the preparation is not open to this criticism; hence, combining the radioactivity analyses with the absorbance measurements in preliminary experiments provided a calibration of $R$.

Centrifuging $[^{32}$P]-labelled T3 in 6 M-GuHCl for 20 h typically reduced the radioactivity from 658 d/s/ml in the 6 M-GuHCl to approx. 40 d/s/ml in the supernatant solution. The results from three separate experiments were similar, giving proportional decreases to approx. 6% of the original radioactivity. The proportion of $[^{32}$P] to protein (estimated as the ratio d/s/$E_{280}$) decreased from 865 d/s to approx. 43 d/s in these experiments, showing that the fractionation caused a drop in contamination to approx. 5% of the original. The $R$ for these preparations was approx. 1.13, but absolute concentrations of DNA cannot be calculated from the extinctions because of uncertainties in the coefficients thereof.

Short periods of dialysis reduced $R$, but since protein was lost in this procedure it was of doubtful value as a preparative method. The results from the dialysis did confirm, however, that the contaminating nucleic acids were of relatively small size and possibly degraded fragments of the original DNA.

**Estimation of the mol. wt. of the 1.5S subunit**

Equilibrium sedimentation was employed to estimate the mol. wt. of the subunit in 6 M-GuHCl, pH 9.0. Attempts to reduce the concentration of GuHCl in the buffer solution before making the measurements caused noticeable aggregation of the protein, even in 5 M-GuHCl. Measuring the mol. wt. of several preparations of protein, having $E_{280}$ values of less than 0.7 ($R$ ranging from 1.1 to 1.2) in 6 M-GuHCl (pH 9.0), at increasing rotor speeds showed that the weight average mol. wt. decreased with increasing speed to reach a relatively constant value above 17250 rev/min. The mean mol. wt. from seven independent preparations run at speeds between 17250 rev/min and 25980 rev/min was 8600 ± 1300, calculated using a $\bar{v} = 0.73$ cm$^3$/g. These records showed no significant curvature for the relationship between $\ln E$ and $r^2$ (eqn. 1) for the range of radii taken for analysis.

Measurements of the weight average mol. wt. of sperm whale myoglobin under identical conditions (0.03 %, w/v, solution in 6 M-GuHCl, pH 9.0) gave a mean mol. wt. over three experiments of 16400 ± 600. This value is 8 % lower than the accepted mol. wt. of 17830 for myoglobin (Edmunson & Hirs, 1962). Consistent with our observations for myoglobin, Lee & Timasheff (1974) observed a decrease in partial specific volumes when a number of proteins were added to 6 M-GuHCl without dialysis.

We observed also in one experiment an increase in mol. wt. to 9200 when the 1.5S protein was first dialysed against 6 M-GuHCl for 4 h. We therefore conclude that the effect of the interaction between the virus protein and GuHCl was to reduce the apparent mol. wt.
Components of T3

Table 2. Amino acid composition of the 1·5 S subunit prepared by denaturation of T3 phage particles with 6 m-GuHCl in 0·01 m-tris buffer (pH 9·0)*

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Composition (molar %)</th>
<th>No. of residues in 1·5S unit (fractional)</th>
<th>(integer)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>T3</td>
<td>1·5S</td>
<td></td>
</tr>
<tr>
<td>LYS</td>
<td>6·36</td>
<td>5·9</td>
<td>4·9</td>
</tr>
<tr>
<td>HIS</td>
<td>1·66</td>
<td>1·1</td>
<td>1·0</td>
</tr>
<tr>
<td>ARG</td>
<td>7·35</td>
<td>6·1</td>
<td>5·1</td>
</tr>
<tr>
<td>ASP</td>
<td>11·74</td>
<td>9·5</td>
<td>7·9</td>
</tr>
<tr>
<td>GLU</td>
<td>10·74</td>
<td>8·9</td>
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</tr>
<tr>
<td>THR</td>
<td>5·33</td>
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</tr>
<tr>
<td>SER</td>
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<td>6·7</td>
</tr>
<tr>
<td>PRO</td>
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</tr>
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<td>GLY</td>
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</tr>
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<td>9·40</td>
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<td>11·2</td>
</tr>
<tr>
<td>CYS</td>
<td>--</td>
<td>1·3</td>
<td>1·1</td>
</tr>
<tr>
<td>VAL</td>
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<td>7·0</td>
<td>5·8</td>
</tr>
<tr>
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<td>4·3</td>
<td>3·6</td>
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<tr>
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<td>8·0</td>
<td>6·7</td>
</tr>
<tr>
<td>TYR</td>
<td>4·24</td>
<td>2·7</td>
<td>2·3</td>
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<tr>
<td>PHE</td>
<td>3·62</td>
<td>2·5</td>
<td>2·1</td>
</tr>
</tbody>
</table>

* The composition of whole T3 particles is taken from the mean of results of Fraser & Jerrel (1953) and Knight (1954). The integer numbers of residues in the 1·5 S protein were calculated assuming a single histidine residue. Minimum mol. wt. calculated from integer values for residues is 8860.

Through reduction of the partial specific volume and distortion of the concentration profile in the centrifuge. Hence, increasing the mean weight average mol. wt. for the protein from T3 by 8% gave a final value of 9300 ± 1400 for the subunit.

Irreversible aggregation of the 1·5 S protein was found to occur when solutions were stored in 6 m-GuHCl (pH 9·0) at both 4 °C and 20 °C. In one experiment the average mol. wt. increased from approx. 12000 after 24 h to approx. 16000 after 144 h storage at 20 °C. In another experiment the mol. wt. increased from 10000 to 23000 during storage for 720 h at 4 °C. Indications were found from the relationships between ln E and r² that the stored solutions were heterogeneous.

Amino acid composition of the 1·5 S subunit

Three samples of the subunit were collected after dissociating the phage with 6 m-GuHCl (pH 9·0) by centrifuging and subsequent precipitation. These were analysed separately for their amino acid composition to give the mean results shown in Table 2. For most of the residues the results of the three analyses agreed within the expected errors of the analysis; however, there were large variations between samples in the values obtained for cysteine, and it is probable that more than one residue of cysteine may be present in the protein. For this reason histidine was chosen as the reference amino acid when calculating the number of residues in the chain. A value of 8860 was calculated from the amino acid composition (excluding contributions from the tryptophane) which may be compared with 9300 estimated from the centrifuge. Comparing the present analysis with that given by Fraser & Jerrel (1953) and Knight (1954) for whole T3 showed that the 1·5 S subunit differed in composition from the complete particle. This suggests the 1·5 S subunit did not contribute a major part to the total phage proteins.

Adding the proportional contributions of partial specific volumes of individual amino
acids (Cohn & Edsall, 1950) gave an approximate partial specific volume for the protein of 0.73 cm³/g. This value was used in the calculations of the mol. wt.

**Release of protein by dilution of T3 phage with tris buffer**

Proteins which associate to form aggregates through weak bonds can often be induced to separate into monomers by diluting the system. This is true for TMV where raising the pH and diluting the virus leads to disassembly of the particle to yield the monomer (Ansevin & Lauffer, 1959). Diluting suspensions of T3 containing between 0.03 % and 0.05 % (w/w) phage by approx. 1:5 (v/v) with tris buffer (pH 9:0) released a small protein. This molecule was relatively stable in the buffer and did not aggregate noticeably at the ionic strength of the buffer (0.1). The extinction ratio (R) of the supernatant solution after centrifuging the diluted phage was higher than that of the protein prepared by disaggregation in 6 M-GuHCl and varied between 1.3 and 1.5 for four experiments. This indicated a relatively impure preparation of protein. Estimates of the weight average mol. wt. of several preparations having E₂₈₀ measurements of between 0.1 and 0.3, gave a mean of 10770 ± 1240. In all the ultracentrifuge experiments there were positive indications of heterogeneity, but the nature of the contamination was not explored.

When a concentrated suspension of T3 was centrifuged at 17150 rev/min in an analytical cell, the supernatant solution gave an E₂₈₀ of 0.8 (determined in the centrifuge) and at equilibrium the solute was found to have a weight average mol. wt. of 6300 ± 2000. The low mol. wt. was caused by a preponderance of small absorbing species (possibly nucleic acid residues) which were subsequently reduced by dialysis of the supernatant solution against an excess volume of tris buffer, leading to a decrease in R. This dialysis also reduced the level of proteins making it impossible to estimate the mol. wt. of the residue.

**DISCUSSION**

The results presented here have shown the presence of two peptides in T3 too small to be detected by Stibenz et al. (1971). One had a mol. wt. of 9300 ± 1400 and the second less than 10000. The marked dissimilarity in the aggregation characteristics between these two sub-units and the fact that one is produced by dilution of the phage, suggest they are from different parts of the phage. The ultracentrifuge analyses in a variety of reagents show that T3 and ‘ghost’ particles not only consistently produced a sticky 1.5 S protein but also a 105 S fragment. This large fragment must be formed from a well-defined structural feature of the phage which has a diam. of approx. 12 nm; hence, assuming a density 1.3 g/ml, the calculated mol. wt. would be approx. 2.3 × 10⁶. A similar calculation for the ‘ghost’ particle (S₂₀,₅ 200 S, density 1.3 g/ml and diam. 47 nm) gave a mol. wt. of approx. 21.7 × 10⁶, showing that the smaller structure was not a ‘ghost’ which had collapsed but was a separate structural unit. Though normally the tail remains attached to the empty head after injection of DNA into *Escherichia coli*, artificial ejection of DNA in media produces a high proportion of tail-less ‘ghosts’, suggesting that the tail may be a stable body having fragile attachments to the head. Comparing the approximate dimensions of the 105 S particle with measurements of the tail for the whole phage suggested that this 105 S fragment could be either part or complete tail sections. It is interesting that adding the approximate estimate of the mol. wt. of this particle to that of the ‘ghost’ (see above) gave a total mol. wt. of approx. 24 × 10⁶. This, when added to the mol. wt. of DNA (25 × 10⁶, Lang & Coates, 1968) gave a total mol. wt. of T3 of approx. 49 × 10⁶ and equal to the value estimated by Swaby (1959).
Components of T3

It seems likely that the 1.5S subunit comes from the head of the phage, and its strong tendency to associate with itself suggests it may act as a binding agent for other proteins. The relative differences between the amino acid composition of the whole phage compared with the 1.5S sub-unit suggest it is not a major component. Another small protein of similar mass and having considerably less tendency to self-association was identified. This second peptide appears to be produced by dilution of the phage and is also present in purified preparations. We suggest it may be produced during ejection of the nucleic acid, and consequent production of ghosts. It may have a role similar to that of the polyamines in phage T4 (Showe & Black, 1973). These are thought to aid the folding of DNA within the head of T4 through ionic interactions (Bancroft, Hills & Markham, 1967; Hohn, 1969).

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