# Tail Components of T2 Bacteriophage. I. Properties of the Isolated Contractile Tail Sheath*

NILIMA SARKAR,† SATYAPRIYA SARKAR,‡ AND L. M. KOZLOFF#

*From the Department of Biochemistry, University of Chicago, Chicago, Illinois*

*Received November 19, 1963*

The contractile sheath protein of the tail of T2L bacteriophage has been isolated by centrifugation from proteolytic digests of the protein coat of the virus particle. As judged by sedimentation patterns, light-scattering measurements, electrophoresis, electron micrographs, and amino acid analysis, the preparations were free of contaminants and contained only completely contracted tail sheaths. Tail sheaths have a molecular weight of $8.0 \times 10^6$ calculated from light scattering. The sheaths are composed largely of amino acids, and also contain a small amount of hexosamine but no other carbohydrate. Based on the minimum integral number of histidine residues, 144 per sheath, tail proteins appear to consist of 144 subunits, each of molecular weight of 55,500. In neutral buffers tail sheaths had a $s^\circ_{20,w}$ of 118 S. The addition of various substances, particularly nucleotides, converted the sheaths into a form with a markedly lower sedimentation rate. After removal of the nucleotides by dialysis, the sedimentation rate again was 118 S. ATP was the most effective nucleotide in producing a lowering of the sedimentation coefficient. Electron micrographs of sheaths after incubation with ATP showed that the contracted sheaths had partially relaxed. With 0.02 M ATP, for example, the observed change in axial ratio was proportional to that expected from the 18% decrease in sedimentation coefficient. Evidence has also been obtained that the single histidine residue in the subunits is probably involved in binding the ATP.

The T-even bacteriophages (T2, T4, T6) are known to have a complex tail structure consisting of several distinct proteins. Kozloff and Lute (1959) demonstrated that the major component of the tail of T2 bacteriophage was a contractile protein and that contraction was necessary for successful infection of the host cell. Brenner *et al.* (1959) later isolated this protein from T2 in the contracted state and studied some of its properties. They also presented electron micrographs of the purified protein and observed occasional helical stretched forms. Since this protein surrounds another tail constituent, the tail core, it has been called the *tail sheath* (Brenner *et al.*, 1959). The tail core has now been isolated and its properties are described in an accompanying paper (Sarkar *et al.*, 1964).

Analysis of T2 preparations has shown that there are 140 molecules of nucleoside triphosphates (Wahl and Kozloff, 1962) and 140 atoms of $Ca^{2+}$ (Kozloff and Lute, 1960) per virus particle. These workers suggested that one nucleoside triphosphate and one calcium ion are bound to each protein subunit of the tail sheath. T2 particles possess phosphatase activity (Dukes and Kozloff, 1959) and upon interaction with cell walls there is the release of ADP and $P_i$. However, since sheath contraction can also occur under certain circumstances with the release of unhydrolyzed nucleotides, it was proposed that the function of the nucleoside triphosphates was to relax or extend the tail protein rather than to serve as source of energy for contraction (Kozloff and Lute, 1959; Wahl and Kozloff, 1962).

Very recently Tikhonenko and Polglazov (1963) have partially purified tail sheaths containing tail cores from T2 after degradation with KOH at *p*H 11.8. The sheath preparation not only still possessed adenosine triphosphatase activity but contained almost all the enzymatic activity of the original phage preparation.

In the present study the tail-contractile protein has been isolated in the pure state. The physical and chemical properties of this protein such as molecular weight, amino acid composition, and sedimentation behavior have been investigated. The effect of the various substances, especially nucleoside triphosphates, on the relaxation of the contractile protein have been studied by following its sedimentation behavior and by observation with the electron microscope.

## EXPERIMENTAL PROCEDURE

*Preparation of Phage Stock.*—T2L phage was grown and purified as previously described (Kozloff and Lute, 1959). Protein coats or "ghosts" of T2, free of DNA, were prepared by the osmotic shock method of Herriott and Barlow (1957). The concentration of T2 "ghosts" was determined by comparing the protein content of intact phage preparations and "ghost" preparations and by assuming that intact phage and "ghosts" contain the same amount of protein (Kozloff and Lute, 1959). The value of $1.8 \times 10^{-11}$ $\mu$g of phosphorus per T2 was used (Wahl and Kozloff, 1962) to determine the number of phage particles.

*Preparation of Contractile Tail Protein.*—With some modifications the method of Brenner *et al.* (1959) for the preparation and purification of the contractile protein from whole T2L was used. They have reported the isolation of this tail protein from purified phage after precipitation with glycine-HCl buffer at *p*H 2. In our hands deoxyribonuclease treatment did not completely remove the DNA in these precipi-

* Aided by grants from the National Foundation, the John A. Hartford Foundation, Inc., the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago, and a U. S. Public Health Service grant (GM-K3-179-C4B).

† Postdoctoral trainee on a Biochemistry Training Program (5 T 1 GM 424) sponsored by the National Institutes of Health, Division of General Medical Sciences, U. S. Public Health Service. Current address: Dept. of Microbiology, Tufts University Medical School, Boston, Mass.

‡ Postdoctoral trainee on a Biochemistry Training Program (5 T 1 GM 424) sponsored by the National Institutes of Health, Division of General Medical Sciences, U. S. Public Health Service. Current address: Department of Biochemistry, Tufts University Medical School, Boston, Mass.

# Recipient of Public Health Service Research career award (1-K6-GM-179) from the National Institutes of Health, Division of General Medical Sciences. On leave until September, 1964 at the Dept. of Biology, University of California, San Diego, La Jolla, Calif.

tates. The contractile protein, isolated from such preparations, was contaminated with DNA, as shown by a strong absorbance at 260 mμ and by the P content. A phage "ghost" solution (50–60 ml containing 5–8 × $10^{12}$ "ghosts"/ml), free of DNA, was therefore chosen as the starting material. To this was added an equal volume of 0.2 M glycine-HCl buffer, *p*H 2, and the *p*H was readjusted to 2 and the solution was left at 2° overnight to complete the precipitation. The precipitate, obtained by centrifuging the solution at 20,000 × *g* for 15 minutes, was washed once in cold distilled water, resuspended in 0.1 M Tris buffer, *p*H 7.5, containing 5 × $10^{-3}$ M $Mg^{2+}$, and was incubated at 37° for 1 hour with 20 μg deoxyribonuclease/ml to remove any last traces of DNA. The flocculent protein was again sedimented by centrifuging at 20,000 × *g* for 15 minutes and the precipitate was washed twice with 2% ammonium bicarbonate solution, *p*H 8.0. The pellet was homogenized by hand in 40 ml of 2% $NH_4HCO_3$ in a glass-Teflon tissue grinder and was incubated with 40 μg of crystalline trypsin per ml at 37° for 1 hour. A rapid clearing of the suspension took place indicating that the phage-head protein was being digested. The solution was then further incubated for 1 hour with 50 μg/ml of chymotrypsin to complete the digestion of the phage-head protein. After removing any denatured material by centrifuging at 10,000 × *g* for 10 minutes, the supernatant solution was centrifuged at 100,000 × *g* for 2 hours in the Spinco Model L preparative ultracentrifuge. The pellet obtained was allowed to leach out in 0.13 M ammonium acetate, *p*H 7.0, and the contractile protein was then purified by two cycles of low (10,000 × *g* for 15 minutes) and high (100,000 × *g* for 2 hours) speed centrifugation. (The supernatant after the initial high-speed centrifugation was saved and used for the isolation of tail cores (Sarkar *et al.*, 1964). The final solution in 0.13 M ammonium acetate was exhaustively dialyzed against 0.1 M NaCl containing 1 × $10^{-3}$ M $Mg^{2+}$, *p*H 7.0. Finally the dialyzed solution was centrifuged at 10,000 × *g* for 10 minutes to remove any denatured material. Calculated on the initial numbers of "ghosts" and the weight of the sheath the yield of purified contractile sheath protein ranged from 30 to 50% in various preparations.

*Ultracentrifugation and Electrophoresis Studies.*—Sedimentation velocity studies of the purified contractile protein were made with the Spinco Model E analytical ultracentrifuge at 15° using schlieren optics. The speed and solvent routinely used were 17,980 rpm and 0.1 M NaCl, buffered to *p*H 7.0 with 0.01 M Tris, respectively. The protein was always equilibrated against the solvent by dialysis. For sedimentation studies at alkaline *p*H a standard single-sector Kel-F centerpiece was employed instead of the regular aluminum centerpiece. Various nucleotides used in the sedimentation studies were neutralized to *p*H 7.0 with Tris immediately before an appropriate aliquot, 10–50 μl, was added to the protein solution to give the desired final concentration of the nucleotide. After incubation at room temperature for 30 minutes the solution was examined in the ultracentrifuge. The calculated sedimentation coefficients were corrected to the standard state of water at 20°. The partial specific volume of the contractile protein, as calculated from its amino acid composition, was 0.721 ml/g. Moving-boundary electrophoresis was carried out in a standard Tiselius cell in a Spinco Model H electrophoresis apparatus.

*Light-Scattering Measurements.*—The contractile protein solution was examined in an Aminco light-scattering microphotometer using a cylindrical cell at 436 mμ. The instrument was calibrated with Ludox (colloidal silica) at 436 mμ (Goring *et al.*, 1957). Dust-free solutions of the contractile protein were obtained by repeated cycles of low and high speed centrifugation using 0.13 M ammonium acetate, *p*H 7.0, to dissolve the protein. The solvent was cleaned by repeated filtration with Seitz pressure-filtration apparatus using fine filter pads with a pore size of 0.1 μ. The protein solution, prior to use, was centrifuged at 30,000 × *g* for 15 minutes in the preparative ultracentrifuge to remove any dust particles. As in the customary procedure the top fraction of the solution was transferred to a dust-free flask. Different aliquots of this solution (2.7 mg/ml) were added to clean solvent in the scattering cell to give a range of concentrations. Reduced intensities, $R_\phi$, at various concentrations were obtained at angles, $\phi$, between 30° and 135° to the incident beam. The Brice-Phoenix differential refractometer was used for the direct measurement of $dN/dc$, the difference in refractive index between a dilute protein solution and its solvent (Brice and Halwer, 1951). The value was 0.1905 at 436 mμ.

*Amino Acid Analysis.*—The amino acid composition of the contractile protein was determined by the method of Moore and Stein (1954) using the Technicon amino acid analyzer. Hydrolysis was conducted at 110° in 6 N HCl in sealed tubes using 3–4 mg of samples of purified contractile sheath prepared from different batches. Both 24- and 70-hour hydrolysis times were used in order to correct for losses. The amide nitrogen of the protein was determined independently by a modification of the method of Laki *et al.* (1954) and Hirs *et al.* (1954). The Conway microdiffusion technique was employed. A solution of 5–6 mg of the protein in 1.5 ml of 6 N $H_2SO_4$ was heated for 16 hours in a 2-ml stoppered volumetric flask at 100°. This concentration and time of heating were necessary to dissolve the sheath protein completely. After cooling the volume was made up to 2 ml with deionized water and the $NH_3$ was determined in the usual way. Standardization with 0.01 N $(NH_4)_2SO_4$ solution gave 99.5–100% recovery in the diffusion units. Control experiments with crystalline pancreatic ribonuclease (Nutritional Biochemicals Corp.) showed that there was no loss of the amide nitrogen during hydrolysis and subsequent diffusion process.

The tryptophan content of the contractile-sheath protein was determined from the ultraviolet-absorption spectrum of the intact protein in 0.1 N NaOH according to the method of Goodwin and Morton (1946). The optical densities of alkaline solutions of sheath, 0.5–0.6 mg/ml at 280 and 294.4 mμ, were read from the absorbancy curve obtained with a Perkin-Elmer recording spectrophotometer and a cell of 1-cm light path. The presence of tryptophan in the contractile tail sheath was also confirmed by the appearance of a characteristic 340-mμ fluorescence peak when solutions of sheaths were activated in an Aminco-Bowman spectrophotometer (Teale, 1960).

*Chemical Methods.*—Phosphorus was determined by the procedure of Bartlett (1959), and protein was measured by the modified biuret method of Westley and Lambeth (1960) using bovine serum albumin, dried to constant weight over $P_2O_5$, as the standard. A correction factor of 1.10 equal to the ratio, weight of average amino acid in bovine serum albumin per apparent weight of average amino acid in contractile sheath, was used to compensate for the difference in the biuret color yields from the same amount of the two proteins. This correction was necessary because the contractile-sheath protein is relatively poorer in the heavier amino acid residues, and therefore the average weight of the amino acid residue in sheath

is only 106.4 as compared to the corresponding value of 117.2 in bovine serum albumin. If the acetylated hexosamines bound to sheath protein interfered in this method the correction factor of 1.10 would be only 1–2% too high and the calculated recovery of amino acids would be 1–2% too low.

Sheaths were analyzed for carbohydrate by both the indole and tryptophan reactions described by Dische (1955). Two methods were used to determine hexosamine, the variation of the Elson-Morgan method described by Winzler (1955) and the method given by Dische (1955) which involves deamination followed by the reaction with indole-HCl.

*Preparation of Pauly's Reagent.*—Diazotized sulfanilic acid (Pauly's reagent) was prepared by a modification of the method described by Fraenkel-Conrat (1957). To a chilled saturated solution of 21 mmoles of sulfanilic acid in concentrated HCl was slowly added 0.7 g of $NaNO_2$ at 0° with stirring. After 30 minutes the solution was filtered through glass wool; the clear filtrate was treated with cold acetone to 98% saturation. The white precipitate of diazotized sulfanilic acid was purified by reprecipitation from ice-cold water with acetone, followed by filtration and repeated washing with acetone. The dry powder was stored at −10° in a desiccator. The recovery was 85%.

*Other Chemicals.*—Crystalline trypsin, chymotrypsin and deoxyribonuclease were obtained from the Nutritional Biochemicals Corp. The various purine and pyrimidine nucleotides were obtained from Pabst Laboratories except adenosine-3′,5′-cyclic phosphate which was purchased from California Corp. for Biochemical Research.

*Electron Microscopy.*—Electron micrographs were taken with an RCA EMU-3 electron microscope using the phosphotungstic acid negative staining method of Brenner and Horne (1959). Sheaths were incubated with ATP for 1 hour and then small drops were placed on carbon grids. The sheaths were washed with water and then stained with 1% phosphotungstic acid. Fixation with 2% formol was found to distort the sheaths and so unfixed preparations were used.

## Results

*Analytical Ultracentrifugation.*—Single sharp symmetrical peaks were observed in sedimentation patterns of contractile protein under almost all conditions studied. (Fig. 1 and Table I). Sedimentation coefficients for the contractile protein were determined at concentrations of 0.62, 0.5, 0.4, 0.3, 0.15, and 0.06% in 0.1 M NaCl containing 0.01 M Tris, *p*H 7.0 (ionic strength 0.1). The sedimentation coefficients were found to have a small dependence on concentration (Fig. 2). A 10-fold decrease in concentration from 0.6 to 0.06% caused only a 2.6% increase in $s_{20,w}$ values. When extrapolated to zero concentration, the sedimentation coefficient, $s^\circ_{20,w}$ was calculated as 118 S. This is in reasonable agreement with the $s^\circ_{20,w}$ value of 114 S, reported for the T2L contractile protein isolated by Brenner *et al.* (1959), although the exact conditions used in their work were not given.

The sedimentation rate of the isolated tail sheath was measured after various treatments which either cause relaxation of the contracted tail of phage particles or inhibit the contraction of the normally extended tail (Kozloff and Lute, 1959). The protein should sediment at a slower rate if relaxation takes place. From the results in Table I it can be seen that the protein sedimented at a slower rate in the presence of

TABLE I
SEDIMENTATION PROPERTIES OF T2L CONTRACTILE PROTEIN

| Conditions[a] | $s_{20,w}$ (S) |
|---|---|
| Purified contractile protein | 118 |
| + 0.01 M EDTA | 108 |
| + 0.01 M ATP | 102 |
| + 0.01 M ATP and subsequent dialysis | 117 |
| + 0.01 M ATP + 0.01 M EDTA | 102 |
| + *p*H 11.0 buffer | 102 |
| + *p*H 11.0 buffer and subsequent dialysis | 118 |
| + *p*H 11.0 buffer + 0.01 M ATP | 94 |

[a] Normally 0.1 ionic strength, 0.01 M Tris buffer, *p*H 7.0.

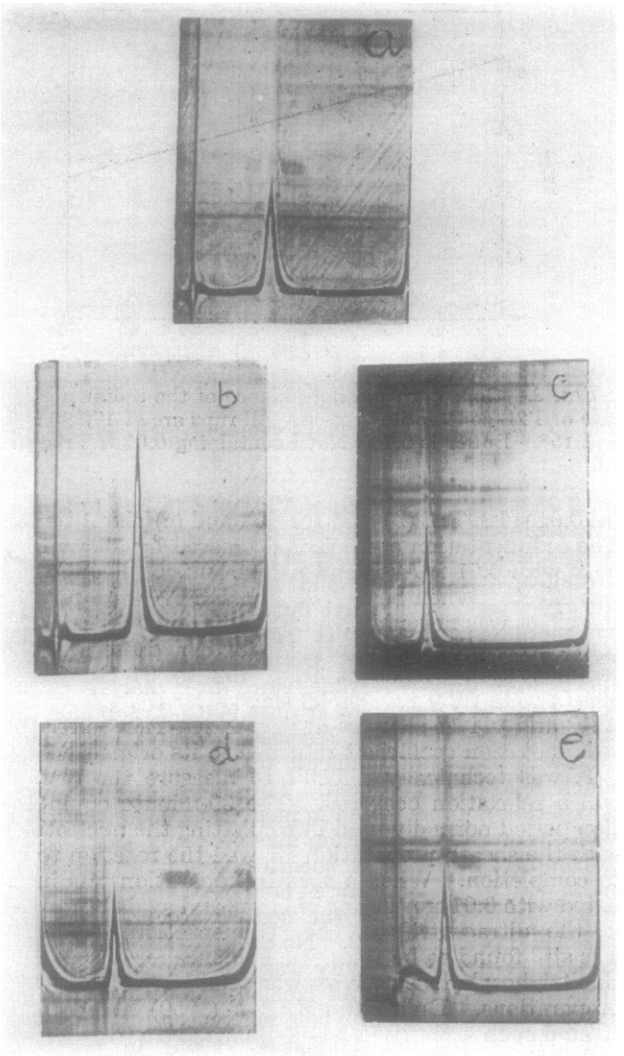


FIG. 1.—Sedimentation patterns of contractile tail sheaths of T2L under various conditions. (a) In 0.13 M ammonium acetate, *p*H 7.0. Concentration 2.7 mg/ml, speed 15,220 rpm, bar angle 60°, temperature 11°; (b) in 0.01 M EDTA and 0.08 M $NH_4Cl$ containing 0.01 M Tris, *p*H 7.0. Concentration 3 mg/ml, speed 17,980 rpm, bar angle 50°, temperature 15°; (c) in 0.15 M Tris, *p*H 11.0. Concentration 2.5 mg/ml, speed 17,980 rpm, temperature 15°, bar angle 50°; (d) in 0.1 M NaCl, containing 0.01 M Tris, *p*H 7.0 and 0.01 M ATP. Concentration 2 mg/ml, speed 17,980 rpm, temperature 15°, bar angle of 45°; (e) in 0.1 M NaCl, containing 0.01 M Tris, *p*H 7.0 and 0.02 M ATP. Concentration 2.5 mg/ml, speed 17,980 rpm, temperature 15°, bar angle of 45°. Note second slow peak in (e) caused by the high ATP concentration.

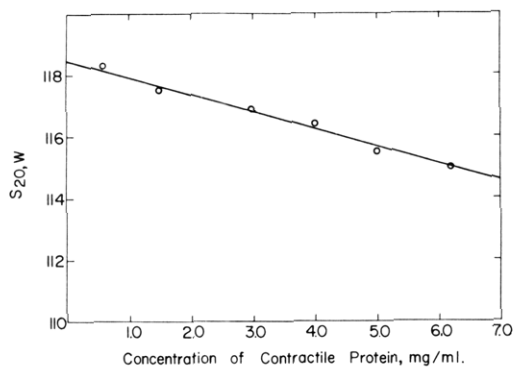


FIG. 2.—Concentration dependence of the sedimentation rate of T2L contractile protein. All runs are at 17,980 rpm and 15°. Buffer is 0.1 M NaCl containing 0.01 M Tris, *p*H 7.0.

(a) 0.01 M EDTA, (b) 0.01 M ATP, and (c) *p*H 11 buffer. The sedimentation coefficient decreased by about 15% both with 0.01 M ATP and *p*H 11 buffer. EDTA had less effect. When both ATP and EDTA were present the effect was not additive but addition of ATP to the contractile protein at *p*H 11 further decreased the sedimentation coefficient from 102 to 94 S. These effects were fully reversible, since after dialysis of the ATP and *p*H 11 samples against NaCl-Tris buffer the sedimentation coefficient returned to its original value.

It was technically difficult to measure the rate at which relaxation occurred. The 30-minute equilibration period normally used in incubating the nucleotides with the sheath preparation allowed the reaction to go to completion. When a sheath preparation was incubated with 0.01 M ATP for 24 hours and again examined in the ultracentrifuge, the sedimentation coefficient was still found to be 102. No evidence for any adenosine triphosphatase activity was found in these sheath preparations. Dukes and Kozloff (1959) had demonstrated such activity in whole phage and phage ghosts and Tikhoneko and Polglasov (1963) in partially purified sheath preparations.

The specificity of the effect of nucleotides on the sedimentation rate of the contractile sheath was examined for a variety of nucleotides all at a concentration of 0.01 M. As shown in Table II, ATP is the most

TABLE II
EFFECT OF VARIOUS NUCLEOTIDES ON SEDIMENTATION OF T2L CONTRACTILE PROTEIN

| Conditions[a] | $s_{20,w}$ (S) |
|---|---|
| Purified contractile protein | 118 |
| + 0.02 M ATP | 97 |
| + 0.01 M ATP | 102 |
| + 0.001 M ATP | 116 |
| + 0.01 M d-ATP | 108 |
| + 0.01 M ADP | 111 |
| + 0.01 M 5'-AMP | 111 |
| + 0.01 M 3',5' cyclic AMP | 114 |
| + 0.01 M ITP | 108 |
| + 0.01 M GTP | 112 |
| + 0.01 M UTP | 114 |
| + 0.01 M CTP | 112 |
| + 0.01 M Na pyrophosphate | 114 |

[a] Normally 0.0 ionic strength, 0.01 M Tris buffer, *p*H 7.0.

effective in decreasing the sedimentation rate. ITP and d-ATP exhibit about 60% of the effect shown by ATP. ADP, 5'-AMP, GTP, and CTP are still less effective, while 3',5'-cyclic AMP and UTP and even

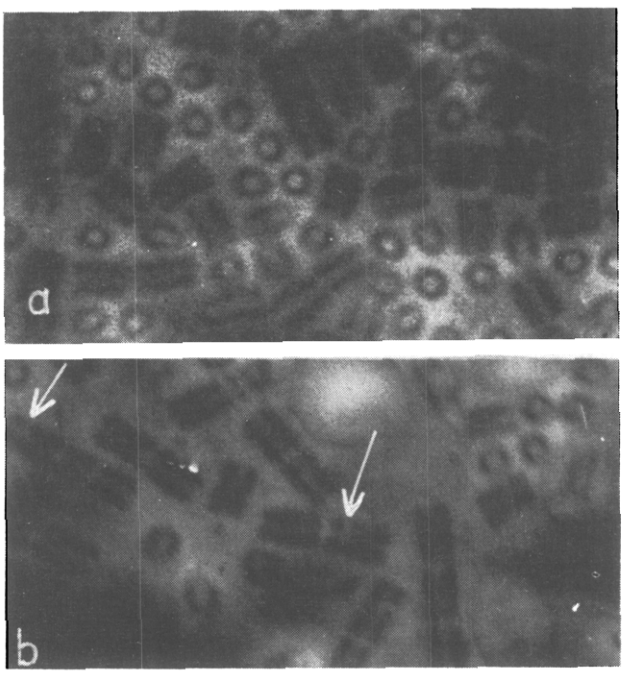


FIG. 3.—Effect of ATP on the isolated contractile sheath protein from T2 phage. (a) Normal untreated sheaths, axial ratio 1.4 (240,000 ×). (b) Sheaths after incubation with 0.02 M ATP at *p*H 7.0 (270,000 ×). Note that the axial ratio has increased to 1.6 and that numerous gaps in the cylinders (see arrows) have appeared.

pyrophosphate had a weak but detectable activity. Using a wide range of concentrations of ATP it was observed that the highest concentration used, 0.02 M, the sedimentation rate was lowered by 18%, while with 0.01 M ATP the sedimentation constant was only 14% lower. At still lower concentrations of ATP, such as $10^{-3}$ and $10^{-4}$, the effect almost disappeared. In the presence of 0.02 M ATP the sedimentation diagram showed an interesting feature (Fig. 1e). In addition to the main peak with $s_{20,w}$ of 97 S, a second slow component appears as a diffuse peak and hardly moves from the meniscus. Upon dialysis against buffer both slow components disappeared and only a single sharp peak with $s_{20,w}$ of 118 S was observed. This indicated that some of the sheaths, as a result of elongation by ATP, are being converted into a still more asymmetric form. This second peak was not observed with concentrations of ATP lower than 0.02 M.

*Electron Microscopy of Sheaths.*—Sheath preparations were examined in the electron microscope and no extraneous material was observed (Fig. 3a). Sheaths consist of hollow cylinders approximately 350 A by 250 A with a center hole about 100 A in diameter (Kozloff and Lute, 1959; Brenner *et al.*, 1959). The axial ratio of over 100 untreated sheaths was measured and found to be 1.4 ± 0.05. Observations on the configurational changes induced by ATP were complicated by the failure to find a fixative which did not distort the normal sheaths. Even with this limitation it was found that ATP did cause the sheaths to elongate or relax. After incubation with 0.01 M ATP the axial ratio increased to 1.5 ± .05 (average of 60 measurements); while with 0.02 M ATP (Fig. 3b) the axial ratio increased to 1.6 ± 0.05 (average of 50 measurements). Since the decrease in $s_{20,w}$ value is presumably due to a change in the frictional coefficient, it should be inversely proportional to the increase in the observed axial ratio. A decrease in the $s_{20,w}$ from 118 to 97 S with 0.02 M ATP, for example, would be expected from an increase in the

axial ratio from 1.4 to 1.7. The observed increase from 1.4 to 1.6 (especially with the use of unfixed preparations) agrees with the sedimentation behavior.

The electron micrographs also offer an explanation for the additional slower diffuse peak observed with 0.02 M ATP. This concentration of ATP causes apparent defects or gaps in many sheaths (Fig. 3b). Since this change is also reversible it would appear that these gaps are probably the expanded helical regions observed by Brenner *et al.* (1959). Fresh preparations of sheaths contain very few particles containing such regions, but upon prolonged storage for 3 months numerous altered particles were formed even in the absence of ATP.

*Molecular Weight by Light Scattering.*—The molecular weight of the protein was determined from the scattering envelopes recorded over the angular range of 30–135° for each of the five concentrations. At concentrations above 6 mg/ml the contractile protein showed a tendency to aggregate. This was shown by an occasional shoulder or small fast-moving peak in the sedimentation pattern. These forms disappeared upon dilution. The sedimentation and light-scattering studies were therefore made with sufficiently dilute solutions of concentrations 2–2.7 mg/ml and the purity was checked by ultracentrifugation. The weight-average molecular weight $M_w$ was obtained by the usual Zimm double extrapolation method (Zimm, 1948).

One of our three Zimm diagrams is shown in Figure 4. Most weight is assigned to the points taken at intermediate angles. The points at very low angles are included to indicate that the preparations were relatively free from contaminating large particles or aggregates. A weight-average molecular weight of 8.0 ($\pm$ 0.2) $\times$ $10^6$ based on three experiments was obtained for the contractile protein. This value is in disagreement with that of 12 $\times$ $10^6$ reported by Brenner *et al.* (1959) as a preliminary measurement.

*Amino Acid Analysis.*—Quantitative amino acid analysis was carried out with four different preparations of purified contractile sheath, two of them in duplicate (Table III). Corrections for decomposition of threonine and of serine, as extrapolated from the difference in the values of 24- and 70-hour hydrolysates, assuming each decomposition to follow first-order kinetics (Hirs *et al.*, 1954), were very small. Apparently the threonine and serine residues in T2L sheaths are fairly resistant to decomposition which normally takes place to the extent of 8 and 12%, respectively, under the usual hydrolytic conditions (Ramachandran, 1958). For valine, isoleucine, and leucine the values after 70 hours of hydrolysis increased by 17.5, 18, and 23%, respectively, over those for the shorter period. These figures were therefore accepted for these three amino acids. The other amino acids did not change significantly during hydrolysis. Since some of the amide $NH_3$ was usually lost during removal of HCl from the hydrolysate by vacuum evaporation, the values of amide $NH_3$ reported were obtained by the procedure described earlier. The average recovery was 92–93.5% of the initial weight of the sheaths. For comparison the amino acid composition calculated from the data of Brenner *et al.* (1959) is also given in Table III.

Anderson (1963) has recently shown by high-resolution electron microscopy that the tail of T2 is composed of 144 subunits. From the molecular weight of intact contractile sheath of 8 $\times$ $10^6$ and the number of subunits of 144 it may be calculated that the molecular weight of each subunit should be 55,500. In good agreement with this value, it was found that there was one histidine residue per 55,500 mw unit.

TABLE III
AMINO ACID COMPOSITION OF T2L CONTRACTILE SHEATH

| Amino Acid | Number of Amino Acid Residues for Molecular Weight of 55,500 | | Residues to Nearest Integer | |
|---|---|---|---|---|
| | Residues | | Found | Brenner *et al.* (1959) |
| Aspartic acid | 58.6 | ± 1.4[a] | 59 | 53 |
| Glutamic acid | 42.2 | ± 1.3 | 42 | 37 |
| Threonine[b] | 40.6 | ± 1.9 | 41 | 36 |
| Serine[b] | 35.1 | ± 1.7 | 35 | 30 |
| Proline | 19.6 | ± 0.9 | 20 | 14 |
| Glycine | 37.7 | ± 1.5 | 38 | 36 |
| Alanine | 50.7 | ± 1.7 | 51 | 47 |
| Valine[c] | 30.9 | ± 1.0 | 31 | 31 |
| Isoleucine[c] | 30.7 | ± 0.9 | 31 | 32 |
| Leucine[c] | 29.6 | ± 1.2 | 30 | 29 |
| Tyrosine | 18.7 | ± 0.8 | 19 | 15 |
| Phenylalanine | 17.6 | ± 0.9 | 18 | 16 |
| Lysine | 18.1 | ± 0.7 | 18 | 24 |
| Arginine | 25.6 | ± 0.4 | 26 | 18 |
| Histidine | 1.08 | ± 0.02 | 1 | 2 |
| Methionine | 4.28 | ± 0.1 | 4 | 3 |
| Half-cystine | 2.27 | ± 0.1 | 2 | 1 |
| Tryptophan[d] | 7.75 | ± 0.2 | 8 | [e] |
| Amide $NH_3$ | 44.9 | ± 1.5 | 45[f] | 63[f] |
| Total [g] | | | 474 | 424 |

[a] The variation is expressed as the average of the deviations from the mean. [b] Extrapolated values, corrected for losses during hydrolysis. [c] Values calculated from the data of 70-hour hydrolysates to correct for incomplete hydrolysis. [d] The concentration of tryptophan is calculated from the ultraviolet spectra (Goodwin and Morton, 1946). Tyrosine calculated from the spectra gave a value of 17.5 ± 1.1 residues per mole. [e] Not determined by Brenner *et al.* (1959). [f] Our values obtained by the direct determination of amide nitrogen by Conway method but Brenner *et al.* (1959) do not give procedure. [g] The amide groups are not included in the summation of the amino acid residues. The molecular weight calculated on the basis of the integral number of residues is 51,600, including the terminal molecule of water and the amide groups. Calculated from the data of Brenner *et al.* (1959), the molecular weight is 45,300.

The number of amino acid residues in each subunit was therefore calculated on the basis of this weight. Each subunit of the contractile protein thus appears to consist of 474 amino acid residues. The molecular weight calculated on the basis of this amino acid composition is 51,600 and amounts to 93% of that expected.

An estimate of the nature and quality of the charge of the contractile protein at *p*H 7 may be made from the amino acid analysis. Our results indicate that each subunit has 12 negative charges at *p*H 7.0. On the other hand, from the data of Brenner *et al.* (1959), each subunit should have 15 positive charges at this *p*H and these workers reported that sheaths do move toward the cathode.

Electrophoretic analysis of contractile-sheath solution in potassium phosphate buffer, 0.15 ionic strength, *p*H 7.0, showed only one schlieren peak using a potential gradient of 5.06 v/cm. The mobility, as determined from the ascending side, was $-13.1 \times 10^{-5}$ $cm^2$/sec. This indicated that the tail sheaths were negatively charged at *p*H 7.0 and agreed well with the net charge predicted by our determination of the amino acid composition.

*Hexosamine Content of Sheath Protein.*—Since the amino acid content of the isolated sheaths only accounted for 93% of the total weight, sheaths were analyzed for carbohydrate. Essentially negative reac-

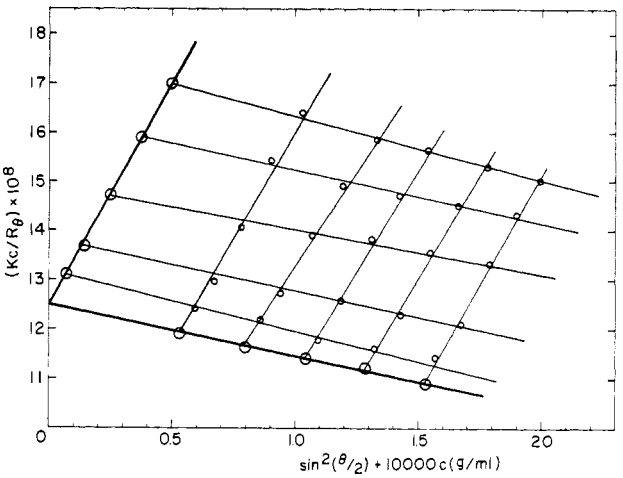


FIG. 4.—Zimm plot for contractile tail sheath of T2L in 0.13 M ammonium acetate, *p*H 7.0. The molecular weight is the reciprocal of the intercept of the ordinate, i.e., $8 \times 10^6$.

tions (<0.05% by weight) were obtained with the indole and tryptophan reactions which all carbohydrates except hexosamines give. However, sheaths were found to contain significant amounts of hexosamine. The Elson-Morgan reaction was positive and gave a value of 1.2% by weight using acetylglucosamine as the standard. The Dische procedure (1955) involving deamination followed by heating with indole HCl was also positive and gave a hexosamine content about 50% of that found with the Elson-Morgan reaction. Similar low values with indole-HCl reaction as compared to the Elson-Morgan value have been found with various substances containing hexosamine linked to other sugars or other substances. In view of the lability of hexosamine when linked to other components, it seems likely that the 1.2% hexosamine found after hydrolysis in HCl must represent a minimal value. During the HCl hydrolysis which liberates the hexosamine there is considerable opportunity for reactions with various amino acids of the sheath protein and the free sugar to occur. These observations suggest that a considerable fraction of the 7% unknown material of the sheath is due to the hexosamines and the loss of some amino acids. On the assumption that the hexosamine is an integral part of the sheath subunit there would be a minimum of 3 hexosamine residues per subunit.

*Effect of an Imidazole Reagent on Relaxation by ATP.*—Since there is only one histidine residue per subunit, the possibility was considered that histidine plays a specific role in the binding of ATP. Tail-sheath protein in 0.1 M NaCl containing 0.01 M Tris, *p*H 7.5, was incubated with 100 μg/ml of diazobenzene sulfonic acid (Pauly's reagent) at 25° for 2 hours to block the imidazole group. The sheaths were then examined in the ultracentrifuge in the presence and absence of 0.01 M ATP (Table IV).

TABLE IV

EFFECT OF AN IMIDAZOLE REAGENT ON SEDIMENTATION PROPERTIES OF SHEATHS

| Treatment | $s_{20,w}$ (S) |
|---|---|
| (1) Purified sheaths at *p*H 7.5 | 118 |
| (2) + Pauly's reagent at *p*H 7.5 | 118 |
| (3) + Pauly's reagent + 0.01 M ATP at *p*H 7.5 | 111 |
| (4) + Pauly's reagent + 0.01 M ATP at *p*H 7.5 + subsequent dialysis | 118 |
| (5) Dialyzed sheaths from (4) + 0.01 M ATP at *p*H 7.0 | 110 |

[a] Pauly's reagent is diazobenzenesulfonic acid. The sheaths were incubated for 2 hours at 25° with 0.1 mg/ml reagent in buffer at *p*H 7.5.

Excess Pauly's reagent was removed by dialysis against buffer leaving a slightly yellow protein, and the effect of ATP was again studied with similar results. Pauly's reagent effectively abolished the specific effect of the adenosine portion of the ATP. ATP was no more effective than any other nucleoside triphosphate or even pyrophosphate in decreasing the sedimentation rate and it seems likely that the imidazole moiety may be involved in the binding of the adenine portion of the ATP.

## DISCUSSION

Sedimentation patterns, light-scattering measurements, and electron micrographs of the isolated material clearly indicate that the preparation studied contained monodisperse contractile tail sheaths and strongly suggest that there was no significant contamination with other macromolecular components. The molecular weight of 8.0 (± 0.2) × $10^6$ obtained by light scattering for tail sheaths is in agreement with the dimensions of tail sheaths reported by Brenner *et al.* (1959). According to them, the volume of the contractile sheath (either relaxed or contracted) is 1.3–1.4 × $10^7$ $A^3$. A molecular weight of 7.8 × $10^6$ can then be calculated for the sheaths making the usual assumption that protein-bound water occupies 25% of the volume and the protein has a density of 1.3 g/$cm^3$ (Wahl and Kozloff, 1962).

The tail sheath of T2 apparently consists of about 144 subunits, each of mw about 55,500. The number of subunits that can be counted on the T2 tail by high-resolution electron microscopy is 144 (Anderson, 1963). A minimum molecular weight based on the 144 histidine residues per tail sheath also suggests that there are 144 subunits, each of mw 55,500. The estimate of 54,000 as the molecular weight of each subunit by Brenner *et al.* (1959) agrees fortuitously with that found here. They thought that there were 2 histidine residues per subunit even though they were able to identify only one histidine-containing peptide after trypsin digestion. The cause for differences in amino acid composition found by Brenner *et al.* from that found here is not clear although it should be pointed out that their results account for only 82% of the weight of the sheath.

The question whether all the subunits in tail sheath are identical or even whether they are composed of smaller units is not settled. All attempts to dissociate sheaths by treatment with mild acid, alkali, urea, or sodium dodecylsulfate were unsuccessful.

The most remarkable property of the isolated contractile-sheath protein is the maintenance of its capacity, although to a quite limited degree, to undergo relaxation. Kozloff and Lute (1959) previously reported that the contractile property of the tail of intact T2 bears some similarities to those of other contractile systems. In particular they showed that EDTA, which can actually cause a relaxation of contracted muscle fibers (Tonomura *et al.*, 1957; Watanabe and Sleator, 1957), inhibited contraction of the proximal tail of T2, and that high concentrations of ATP caused 60% relaxation of tail protein after the contraction resulting from reacting with host-cell walls. The present experiments clearly indicate that the isolated contracted tail sheaths similarly undergo a partial relaxation when treated with the above reagents and

support the earlier proposal that contraction is spontaneous but that relaxation requires the energy involved in the binding of ATP to the protein.

A mechanism can be proposed to account for the increase in axial ratio by the nucleotides and particularly by pH 11 buffer. All active compounds would tend to increase the net negative charge on the sheath. Since the subunits are arranged helically the increase in charge would cause repulsion and an increase in the distance between subunits or a lengthening of the molecule. Although it cannot be concluded that this is the only factor involved in the relaxation of this protein (especially since the addition of various substances to the isolated sheath only increased the axial ratio from 1.4 to 1.6 while the completely relaxed protein has a ratio of 4.8), it should be emphasized that the addition of ATP to phage after interaction with cell walls (Kozloff and Lute, 1959) was much more effective and caused an increase in the axial ratio of the sheath protein from 1.4 to 2.8.

ACKNOWLEDGMENT

We gratefully appreciate the advice of Dr. Arthur Vies of the Department of Biochemistry of Northwestern University in the light-scattering measurement.

REFERENCES

Anderson, T. F. (1963), *Symp. Fundamental Cancer Res. 17th*, Abstracts, p. 8.
Bartlett, G. R. (1959), *J. Biol. Chem. 234*, 466
Brenner, S., and Horne, R. W. (1959), *Biochim. Biophys. Acta 34*, 103
Brenner, S., Streisinger, G., Horne, R. W., Champe, S. P., Barnett, L., Benzer, S., and Rees, M. W. (1959), *J. Mol. Biol. 1*, 281
Brice, B. A., and Halwer, M. (1951), *J. Opt. Soc. Am. 41*, 1033.
Dische, Z. (1955), *Methods Biochem. Analy. 2*, 313.
Dukes, P. D., and Kozloff, L. M. (1959), *J. Biol. Chem. 234*, 534.
Fraenkel-Conrat, H. (1957), *Methods Enzymol. 4*, 261.
Goodwin, T. W., and Morton, R. A. (1946), *Biochem. J. 40*, 628.
Goring, D. A. I., Senez, M., Melanson, B., and Hugue, M. M. (1957), *J. Colloid. Sci. 12*, 412.
Herriott, R. M., and Barlow, J. L. (1957), *J. Gen. Physiol. 40*, 809.
Hirs, C. H. W., Stein, W. H., and Moore, S. (1954), *J. Biol. Chem. 211*, 941.
Kozloff, L. M., and Lute, M. (1959), *J. Biol. Chem. 234*, 539.
Kozloff, L. M., and Lute, M. (1960), *Biochim. Biophys. Acta 37*, 420
Laki, K., Kominz, D. R., Symonds, P., Lorand, L., and Seegers, W. H. (1954), *Arch. Biochem. Biophys. 49*, 276.
Moore, S., and Stein, W. H. (1954), *J. Biol. Chem. 211*, 941.
Ramachandran, L. K. (1958), *Virology 5*, 244.
Sarkar, S., Sarkar, N., and Kozloff, L. M. (1964), *Biochemistry 3*, 517 (this issue).
Teale, F. W. J. (1960), *Biochem. J. 76*, 381.
Tonomura, Y., Matsumiya, H., and Kitagawa, S. (1957), *Biochim. Biophys. Acta 24*, 569.
Wahl, R., and Kozloff, L. M. (1962), *J. Biol. Chem. 237*, 1953.
Watanabe, S., and Sleator, W., Jr. (1957), *Arch. Biochem. Biophys. 68*, 81.
Westley, J., and Lambeth, J. (1960), *Biochim. Biophys. Acta 40*, 364.
Winzler, R. J. (1955), *Methods Biochem. Analy. 2*, 279.
Zimm, B. H. (1948), *J. Polymer Sci. 14*, 29.

# Tail Components of T2 Bacteriophage. II. Properties of the Isolated Tail Cores*

SATYAPRIYA SARKAR,† NILIMA SARKAR,‡ AND L. M. KOZLOFF#

*From the Department of Biochemistry, University of Chicago, Chicago, Illinois*

*Received November 19, 1963*

Highly purified and homogeneous preparations of tail cores of T2L bacteriophage have been obtained by centrifugation of proteolytic digests of denatured virus protein coats. The cores do not neutralize anti-T2 serum nor do they clump host cells. The isolated tail cores have sedimentation and diffusion coefficients of 11.6 S and 21.8 $\times 10^{-8}$ cm$^2$/sec, respectively. The molecular weight of the individual cores is 487,000, all of which can be accounted for as amino acids. A model for the structure of the tail core has been proposed based upon the amino acid analysis and the fact that cores are stiff, long, hollow rods. Each core appears to be a hollow five-stranded cable made up of five identical polypeptide chains or subunits.

Bacteriophage T2 has a complex tail structure with a number of distinct components (Kellenberger and Arber, 1955; Williams and Fraser, 1956). The main component of the tail is a contractile protein (Kozloff and Lute, 1959) which has been termed the *contractile-sheath protein* (Brenner *et al.*, 1959) because it surrounds an inner component known as the *tail core*.

Some observations on the formation of tail cores in the infected cell have been reported by Kellenberger and Séchaud (1957) which indicated that they are formed independently of the whole phage. Brenner and his colleagues (1959) in their investigation of the structural components of bacteriophage obtained electron micrographs showing the cores to be stiff hollow

* Aided by grants from the National Foundation, the John A. Hartford Foundation, Inc., the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago, and a U. S. Public Health Service grant (GM-K3-179-C4B).

† Postdoctoral trainee on a Biochemistry Training Program (5 T 1 GM 424) sponsored by the National Institutes of Health, Division of General Medical Sciences, U. S. Public Health Service. Current address: Dept. of Biochemistry, Tufts University Medical School, Boston, Mass.

‡ Postdoctoral trainee on a Biochemistry Training Program (5 T 1 GM 424) sponsored by the National Institutes of Health, Division of General Medical Sciences, U. S. Public Health Service. Current address: Department of Microbiology, Tufts University Medical School, Boston, Mass.

# Recipient of Public Health Service Research Career Award (1-K6-GM-179) from the National Institutes of Health, Division of General Medical Sciences. On leave until September, 1964 at the Dept. of Biology, University of California, San Diego, La Jolla, Calif.