Isolation and Characterization of Polysheaths, Phage Tail-like Defective Bacteriophages of *Alcaligenes eutrophus* H 16

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**SUMMARY**

Polysheaths were spontaneously formed inside cells of the hydrogen bacterium *Alcaligenes eutrophus* H 16. These particles are long tube-like structures of 24 nm diam. belonging to the phage tail-like defective bacteriophages (Lotz, 1976). In mid log-phase fermenter-grown cells, polysheaths were observed in about 20% of all cells sectioned. Evidence is provided for an inhibition of cell fission by polysheaths. Polysheaths were isolated by differential centrifugation and precipitation techniques using PEG and antibodies. The morphology of polysheaths was investigated electron microscopically by negative staining, ultrathin sectioning and metal shadowing. A surface lattice of the polysheath was derived from light optical diffraction data. The particles were also characterized by their biochemical and biophysical features: mol. wt. of the subunit determined by SDS-gel electrophoresis (58,000), amino acid composition, isoelectric point (4.4), u.v. absorbance spectrum indicating the absence of nucleic acid, buoyant density (1.258), and stability against denaturants and proteolytic enzymes.

**INTRODUCTION**

During ultrastructural investigations on gram-negative hydrogen bacteria, long tube-like structures were detected in *Alcaligenes eutrophus* H 16 (Walther-Mauruschat et al. 1977). Their dimensions and appearance inside the cells fulfilled the criteria stated by Lotz (1976) for a special type of defective bacteriophages, polysheaths. This term was first used by Kellenberger & Boy de la Tour (1964) to describe aberrant forms of phage T4 in *Escherichia coli*. Except for their length, the structures resembled those of contracted sheaths of T-even bacteriophages (Moody, 1967). Similar structures have been found in a variety of bacteria (see Lotz, 1976). Gumpert & Taubeneck (1968) related these structures to the phenomenon of 'defective lysogeny': bacteria producing polysheaths are believed to have once harboured a complete temperate prophage. In the course of time, the genetic information for the phage has decreased to such an extent that the information for the sheaths is the only structural information left.

Polysheaths were classified by Lotz (1976) as phage tail-like defective bacteriophages together with rhapsodosomes and particular bacteriocins like pyocin R. The relationship of the latter two groups to complete phages was established by morphological as well as by biochemical, physicochemical and immunological properties (Amako *et al.* 1970; Ito &...
Kageyama, 1970; Delk & Dekker, 1972; Yui-Furihata, 1972). Up to the present time information about polysheaths has been mainly confined to morphological features.

In the present study, isolation of polysheaths was achieved, thus fulfilling the basic requirement forphysicochemical and biochemical investigations. Moreover some information about the production of polysheaths inside the bacterial cells will be given as well as a detailed description of their morphology. The results obtained provide further evidence for the viral origin of polysheaths.

**METHODS**

**Bacterial strains and growth conditions.** The organisms used were Alcaligenes eutrophus H 16 (ATCC 17699, DSM 428) and the mutant PHB-4 (DSM 541) derived from the wild-type strain and unable to accumulate poly-β-hydroxybutyric acid. Cells were grown at 30 °C in the minimal salts medium of Schlegel et al. (1961), supplemented with 0.5% fructose for heterotrophic growth. Large-scale cultivation of the bacteria was carried out in a 10 l fermenter (Biosat, Braun Melsungen, Germany) at 600 rev/min. The gas flow was 5 l of air/min for heterotrophic growth, or 0.5 l/min of a gas mixture (80% H2, 10% O2, 10% CO2) for autotrophic growth. The fermenter was inoculated with exponentially growing cells, giving a starting absorbance at 436 nm of less than 1. At an absorbance of 2.5 to 3 the culture was drained off on to ice and harvested by continuous flow centrifugation.

**Determination of growth parameters.** The absorbance was measured at 436 nm. Protein was determined according to Schmidt et al. (1963). The membrane filter technique as described by Stouthamer (1969) was used for dry wt. determination. Cell counting was performed in a Neubauer counting chamber.

**Lysis of cells.** Cells (PHB-4 exclusively) were disintegrated immediately after harvesting, basically according to the method of Osborn & Munson (1974). About 5 g wet unwashed cells were suspended in 100 ml 0.01 M-tris-HCl, pH 7.8 containing 0.75 M-sucrose (0 to 4 °C). Lysozyme (0.4 g/ml) was added and the mixture stirred for 3 to 5 min at 0 °C. Then the suspension was gradually diluted with 2 vol. of ice-cold distilled water over a period of 10 min and centrifuged for 15 min at 10000 g. After the pellet was resuspended in a small vol. of buffer (0.01 M-Na-phosphate, pH 7), it was syringed into a large vol. of the same buffer, which was stirred vigorously. Some crystals of DNase and MgSO4 were added to the lysate, which was incubated at room temperature until viscosity had disappeared. The lysate was then centrifuged for 15 min at 7000 g. The turbid supernatant was cleared by an additional centrifugation (15 min, 20000 g).

**Purification of polysheaths.** Polysheaths were separated from the cleared supernatant of the lysate by precipitation with polyethylene glycol 6000 (PEG) according to Yamamoto et al. (1970). The optimal concentration was found to be 3%. The flocculent precipitate was collected by centrifuging for 15 min at 15000 g. The pellet was resuspended in 0.1 M-Na-phosphate buffer, pH 7, and adjusted to an A450 (10 mm light path) of 0.4 to 0.5. The suspension was warmed to 37 °C and anti-H 16 rabbit serum was added (10 μl serum per ml suspension) and the mixture was incubated at 37 °C for 30 min. (The rabbits were immunized against flagellated cells of A. eutrophus H 16, which were killed by formaldehyde prior to injection. The serum containing anti-H 16 antibodies was obtained from the rabbit blood by differential centrifugation and used without further purification.) The resulting precipitate was separated as carefully as possible by centrifugation (two runs in a SW-rotor, no. 8570, Heraeus-Christ, Osterode, Germany, at 20000 g for 20 min). Polysheaths were removed from the supernatant by a second PEG treatment. The resulting pellet was resuspended in 0.1 M-Na-phosphate buffer, pH 9.2, and subjected to high-speed
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Centrifugation (1.5 h at 100,000 g). Sedimented polysheaths were resuspended in a small vol. of the alkaline phosphate buffer.

**Protein determination.** Protein was determined according to Lowry et al. (1951).

**SDS-gel electrophoresis.** For SDS-gel electrophoresis the method described by Weber et al. (1972) was used. Staining of gels for glycoproteins was done according to Glossman & Neville (1971). Ovalbumin was used as a positive control.

**Isoelectric focusing.** The isoelectric point was determined in an electrofocusing column (type LKB-8100, LKB, Bromma, Sweden) employing an ampholine-pH-gradient of 3.5 to 10 (LKB application note no. 219, 1976). The fractions were examined for their pH, their absorbance at 280 nm and for polysheaths in the electron microscope.

**Isopycnic centrifugation.** The buoyant density of polysheaths was determined by isopycnic centrifugation in a pre-formed CsCl gradient (2 ml of 0.21 g/ml on 2 ml of 0.54 g/ml). One ml of sample (0.3 mg protein/ml) was layered on top. The solvent was 0.05 M-Na-phosphate buffer, pH 9.2. Centrifugation took place in a SW 65 Ti rotor (Spinco L2 65B, Beckman Instruments, Munich, Germany) at 65,000 g and 25 °C for 21 h. The density of the fractions was calculated from their refractive index by the formula

\[ \rho^25 = 10.2402 \eta_0^2 - 12.6483 \]

for \( \rho = 1.0 \) to 1.38 (Weigle et al. 1959). The fractions were diluted with water and subsequently were examined for their absorbance at 280 nm and for polysheaths in the electron microscope.

**Ultraviolet absorption spectrum.** The u.v. absorption spectrum was recorded in a DMR 10 double beam spectrophotometer (Zeiss, Oberkochen, Germany). The spectrum was corrected according to Bonhoeffer & Schachmann (1960).

**Amino acid analysis.** Amino acid analysis was performed by the method of Moore & Stein (1963) using an automatic Biotronic analyser (Wiss. Geräte GmbH, Munich, Germany). The sample was hydrolysed in 6 N-HCl at 110 °C for 20 h in vacuum-sealed tubes.

**Dissociation of polysheaths.** Detergents, acetic acid and NaOH were added directly to the polysheath solution. All other chemicals were applied by dialysis. The resulting effects were studied qualitatively by electron microscopy (negative staining).

**Enzyme treatment.** Pronase P: 1 mg/ml, pH 7.4, 37 °C, 60 min; proteinase K: 1 mg/ml, pH 8, 37 °C, 60 min; trypsin: 1 mg/ml, pH 8, 25 °C, 60 min; DNase and RNase: 1 µg/ml, pH 7 to 7.8, 25 °C, 24 h. The concentration of polysheaths was about 0.1 mg/ml. After enzyme treatment the samples were examined in the electron microscope.

**Electron microscopy.** Negative staining was performed by the method of Valentine et al. (1968) with 4% uranyl acetate. In order to look inside 'whole' negatively stained cells (Fig. 1), exponentially growing cells were treated with 1% SDS for 3 to 5 min at room temperature. The viscous solution was then diluted 1:10 with Na-phosphate buffer, pH 7. Some DNase and MgSO4 crystals were added. After the viscosity had disappeared, the sample was centrifuged at 4000 g for 10 min. The pellet was resuspended and negatively stained. The 'sandwich' technique (Fig. 3) was performed according to Beuscher et al. (1974).

**Chemical fixation and ultramicrotomy.** Cell samples were washed once with 0.05 M-K-phosphate buffer, pH 6.8. The cell pellets were directly frozen in the centrifuge tubes at -18 °C. Freezing and thawing were repeated twice. The thawed pellet was suspended in 10 ml of phosphate buffer. A double fixation with glutaraldehyde and OsO4, according to Behn & Arnold (1974) was followed by dehydration in a graded series of acetone solvents. A block-staining with uranyl acetate was included during the dehydration procedure. Cells were embedded in Spurr's (1969) 'low-viscosity medium'. Ultrathin sectioning was
performed as described previously (Walther-Mauruschat et al. 1977). The sections were post-stained with lead citrate. Thin sections were evaluated directly in the electron microscope. For each value, about 300 sectioned cells were examined for polysheaths.

**Metal shadow casting.** Polysheaths were mounted on carbon films using the diffusion technique described above for negative staining, but omitting the stain. A Pt-C shadowing was applied at an angle of 30 to 55°.

Electron micrographs were taken with a Philips EM 301 microscope (Philips, Eindhoven, The Netherlands). Magnifications were calibrated by means of a cross-lined grating replica.

**Optical diffraction and reconstruction.** Slides of electron micrographs showing the structural periodicities at a high resolution (magnification in the EM: ×57000 to ×71000; on the slides: ×45000) were used for optical diffraction.

The diffractometer (Spindler und Hoyer, Göttingen, Germany) was equipped with a helium–neon laser and a built-in reconstruction device. The camera length L was calibrated with a line grating.

Optical reconstruction and filtering was performed by the method of Klug & De Rosier (1966). Filtering masks were produced photographically as described by Horne & Markham (1973) for small diffraction patterns. For one-sided reconstructions, the intensity of the central beam was reduced by a copper gauze of 50% transmission (Hert, Munich, Germany). Merkham rotation was performed as described by Markham et al. (1963).

**Chemicals.** Uranyl acetate was obtained from Fluka (Buchs, Switzerland). Resin compounds, glutaraldehyde, PEG 6000, detergents, bovine serum albumin and components for SDS-gel electrophoresis were purchased from Serva (Heidelberg, Germany). Enzymes were from Boehringer (Mannheim, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany). Rabbit serum containing antibodies against *A. eutrophus* H16 was a gift from Dr Bernhard Schink, Institut für Mikrobiologie der Universität Göttingen.

**RESULTS**

**Morphology**

Polysheaths were the only virus-like particles occurring in the hydrogen bacterium *Alcaligenes eutrophus* H16 during growth. They were hollow cylinders (Fig. 1, 2) with an outer diam. of 24 nm (min 21.8; max 26.4). The diameter of the inner channel was 7 nm. In negatively stained, isolated polysheaths the inner channel seemed to be constricted about every 110 nm (Fig. 2). The length of the particles varied considerably. They could be as long as a whole cell, i.e. about 2 μm (Fig. 1, 2). Short pieces of polysheaths standing upright on the supporting film displayed a 12-fold rotation symmetry when viewed from above (Fig. 4). Cross-sections of polysheaths showed the same symmetry (Fig. 5). Using the ‘sandwich’-technique polysheaths obviously became flattened between the two carbon films (Fig. 3), as their diam. in this case was about half of their circumference (75 nm). Six longitudinal rows running parallel to the axis could be discerned. Thus a total of twelve rows is most likely present.

On average, five to six polysheaths were observed inside the cells, occurring as bundles parallel to the cell’s longitudinal axis (Fig. 1). In dividing bacteria these bundles stretched from one daughter cell into the other across the region of the septum (Fig. 6).

As revealed by optical diffraction (Klug & Berger, 1964), the subunits of H16-polysheaths are helically arranged (Fig. 7, 8). The different diffraction spots could be interpreted by applying reconstruction techniques combined with optical filtering (Klug & De Rosier, 1966). This technique enabled us to get one-sided images of polysheaths (Fig. 9a, b). The
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Fig. 1. Bundle of polysheaths (arrow) in a 'transparent' *Alcaligenes eutrophus* H 16 cell. The cell was treated with detergent prior to negative staining.

Fig. 2. Negatively stained preparation of isolated polysheaths. Arrowheads indicate apparent 'constrictions' of the inner channel.

Fig. 3. Negatively stained polysheath lying between two layers of carbon film ('sandwich'). A region is marked where six longitudinal rows of particles can be discerned.

Fig. 4. Short piece of polysheath standing upright on the carbon film displaying a 12-fold rotation symmetry.

Fig. 5. (a) Cross-sectioned bundle of five polysheaths. (b) Higher magnification of the particle indicated in (a). (c) to (e) Analysis of the particle shown in (b) by the Markham rotation technique revealing a 12-fold rotation symmetry.
results were checked by an appropriate model (Fig. 10, 11): the diffraction pattern of the model picture was identical to the diffraction pattern of the original micrograph (Fig. 7, 8). The following surface lattice was deduced from the diffraction pattern and rotation symmetry parameters (Fig. 15).

The centres of the subunits are to be imagined as being situated on the intersection points of two helices. Twelve large-scale helices of pitch angle $\alpha = 86^\circ$ and an axial repeat of 80 nm wind around a cylinder at a diffraction radius of 10.9 nm. They are intersected by small-scale helices of pitch angle $\beta = 30^\circ$ and an axial repeat of 4 nm. The small helices give rise to the most prominent diffraction spots on layer lines of 1/4 nm$^{-1}$ spacing. The diffraction spots arising from the large-scale helices appear on layer lines close to the equatorial reflections. The dimensions of the unit cell were calculated to be 6.23 nm ($x$) and 3.73 nm ($y$). The tilting of the large-scale helices as well as their left-handedness was concluded from metal shadowed specimens (Fig. 12) and their diffraction patterns, respectively (Fig. 13). Bacteriophage T4, which is known to be right-handed (Moody, 1967), was taken as a control (Fig. 14). The tilt of the large-scale helices is concealed in negatively stained specimens (Fig. 3, 7). In this case, both sides of the particle contribute to the total image contrast (Klug & Berger, 1964). The large-scale helices from the back and the front are superimposed, thus simulating a parallelism with the polysheath's axis. This effect may also be responsible for the 'constrictions' of the central cavity mentioned above. There would be no explanation for this effect if the large-scale helices had no pitch angle.

**Formation of polysheaths**

Polysheaths were produced spontaneously in *A. eutrophus* grown in a fermenter, under autotrophic and heterotrophic growth conditions (Fig. 16, 17). Around the middle of the log phase, polysheaths were detected in as much as 20% of all cells sectioned. Other growth parameters, i.e. absorbance, dry weight, protein, and cell count, were not influenced. From the total cell count/ml and from the percentage of cells containing polysheaths, the 'absolute' number of cells with polysheaths/ml was calculated. In contrast to the relative number of cells with polysheaths this value remained constant between the maximum formation of polysheaths and the stationary phase (Fig. 17). Free polysheaths were never obtained in the supernatants of the cultures.

These findings suggest that the production of polysheaths is paralleled by the loss of ability for complete cell fission. This point will be discussed later.

During the development of polysheaths, special structures like those found in
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Fig. 7. High-resolution micrograph of a negatively stained polysheath.

Fig. 8. Light optical transform of the region marked in Fig. 7. Layer lines at a spacing of \( 1/4 \text{ nm}^{-1} \) are indicated. The scale is the same as in Fig. 12 and 14.

Fig. 9. (a), (b) One-sided images of a polysheath obtained by reconstruction and optical filtering.

Fig. 10. Photograph of a model of a polysheath.

Fig. 11. Optical transform of the model shown in Fig. 10. The scale is the same as in Fig. 13. A reciprocal lattice is superimposed.

Fig. 12. Metal shadowed polysheaths. The left-hand particle shows a nearly axial ‘striation’ (compare with Fig. 9a and b).

Fig. 13. Optical transform of Fig. 12. The ‘striation’ of the particle gives rise to the two diffraction spots close to the equatorial line. The location of the spots indicates left-handed helices (see Results).

Fig. 14. Metal shadowed bacteriophage T4. In contrast to the polysheath its helices are right-handed (arrows).
Chromobacterium violaceum (Rucinsky & Cota-Robles, 1973) or in Serratia marcescens (Acker, 1976) were never observed.

The formation of polysheaths was never found in cell cultures grown other than in a fermenter. Even mitomycin C did not enhance the formation of polysheaths, as is the case for many other defective bacteriophages (Mayr-Harting et al. 1972).

Isolation, biochemical and physicochemical properties

The following features of polysheaths complicated their purification: (i) as far as it is known, they lack any measurable biological activity; (ii) they are not released into the supernatant by their hosts; (iii) due to their variable lengths, they lack a uniform particle weight; (iv) their density does not differ from that of other cell proteins; (v) they tend to stick to a variety of materials (DEAE-cellulose, Sephadex, membrane filter, paper filters, etc.) as well as to each other.

The main features of the purification procedure were as follows: (1) a special lysozyme technique avoiding the generation of numerous small membrane and cell wall vesicles; (2) a combination of highly specific precipitation techniques and differential centrifugation. Rabbit antibodies against flagellated A. eutrophus H16 served to clear the polysheath preparation of cell wall vesicles (LPS) and flagella. About 1 mg of polysheaths could be isolated from 12 g wet cells (81 culture at an absorbance of 2·5).

Isolated polysheaths (Fig. 18) gave a single band in SDS-gel electrophoresis, corresponding to a subunit mol. wt. of 58000 (Fig. 19). Staining for glycoproteins was negative. Migration properties in gels of different porosities were normal according to Maizel (1971).
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The isoelectric point of polysheaths was 4.4 and they banded at a density of 1.2858 g/ml in a CsCl gradient. The u.v. absorption spectrum did not show a maximum at 280 nm and a minimum at 260 nm (Fig. 20, ---), unless it was corrected for scattered light according to Bonhoeffer & Schachmann (1960; Fig. 20, ----). Scattering of light, caused by the length of the particles, normally obscured the absorption spectrum. The turbidity of concentrated polysheath solutions is most likely due to this effect. Thus banded polysheaths were easily detected with the naked eye in density gradients or isoelectric focussing columns. Reduction of the average particle length by NaOH (see below) resulted in a faint maximum at 280 nm (Fig. 20, . . . .).

The results of the amino acid analysis are shown in Table 1. The abundance of glutamic and aspartic acid agrees well with the acid isoelectric point.

Polysheaths from A. eutrophus proved to be extraordinarily resistant against denaturants (Table 2) and degrading enzymes. They withstood treatment with trypsin, pronase P, proteinase K, DNase, and RNase without any observable ultrastructural change. Treatment with either sodium hydroxide or guanidine hydrochloride broke polysheaths into short pieces. The polysheaths showed random breakage as demonstrated by the variation in particle length. Sodium hydroxide and guanidine hydrochloride were able to destroy voluminous aggregations which formed during the concentration of polysheaths.

All attempts at reassembly of dissociated polysheaths using well-known procedures.
Fig. 17. Formation of polysheaths in the mutant PHB-4 of H16. The cells were grown autotrophically with hydrogen, oxygen and carbon dioxide in a fermenter (○—○, absorbance at 436 nm; △—△, cell count/ml; □—□ percent cells with polysheaths; ■—■, cells with polysheaths/ml.)

Fig. 18. Isolated polysheaths seen by negative staining.

Fig. 19. SDS-polyacrylamide gel electrophoresis of isolated polysheaths. About 10 μg of protein were applied to the gel containing 7.5 % acrylamide.
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Fig. 20. U.v. absorbance spectrum of polysheaths. (—, polysheaths, in neutral buffer; . . . . ., in 0.1 M-NaOH; ———, spectrum corrected for scattered light according to Bonhoeffer & Schachmann, 1960).

Table 1. Amino acid composition of polysheaths

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Molar ratio*</th>
<th>Amino acid</th>
<th>Molar ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>3.8</td>
<td>Lysine</td>
<td>2.4</td>
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<tr>
<td>Arginine</td>
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<td>Methionine</td>
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<td>Aspartic acid/asparagine</td>
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<td>Phenylalanine</td>
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<tr>
<td>Cysteine</td>
<td>0</td>
<td>Proline</td>
<td>0.5</td>
</tr>
<tr>
<td>Glutamic acid/glutamine</td>
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<td>Serine</td>
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</tr>
<tr>
<td>Glycine</td>
<td>3.5</td>
<td>Threonine</td>
<td>1.5</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.9</td>
<td>Tryptophan</td>
<td>N.D.†</td>
</tr>
<tr>
<td>Isoleucine</td>
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<td>Tyrosine</td>
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</tr>
<tr>
<td>Leucine</td>
<td>3.9</td>
<td>Valine</td>
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</tr>
</tbody>
</table>

* Relative to phenylalanine.
† Not determined.

like those of Fraenkel-Conrat (1957) and others (Ralph & Bergquist, 1967) have so far failed. Only non-specific aggregation products similar to those of Yui-Furuihata (1971) were obtained.

DISCUSSION

Polysheaths from *Alcaligenes eutrophus* H 16 resemble other polysheaths and the contracted sheaths of defective and complete T-even phages with respect to their morphological, biochemical and physicochemical properties.

The diameter of 24 nm, the 12-fold rotation symmetry and the helical arrangement of the subunits are typical for all the particles mentioned above (Lotz, 1976). The occurrence
Table 2. Influence of some denaturants on the stability of polysheaths*

<table>
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<tr>
<th>Reagent</th>
<th>Concentration</th>
<th>Temperature (°C)</th>
<th>Time</th>
<th>Effect†</th>
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<tr>
<td>Sodium dodecyl sulphate</td>
<td>2 7 25 60 min</td>
<td>2 7 100 1 min</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Urea</td>
<td>8 7·8 45 30 min</td>
<td>8 12 4 8 d</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>Guanidine hydrochloride</td>
<td>1 7 45 30 min</td>
<td>2/4 7/8 4 2 d</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>Glycine-HCl buffer</td>
<td>0·2 2 4 1 d</td>
<td>6 8 4 1 d</td>
<td>+</td>
<td>b</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>45 4·75 4 15 min</td>
<td>4 2 d</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>0·1 12·5 4 1 d</td>
<td>4 1 d</td>
<td>b</td>
<td></td>
</tr>
</tbody>
</table>

* The samples were qualitatively evaluated in the electron microscope.
† +, Complete destruction; ±, partial destruction; −, no observable effect; a, breakage into short pieces; b, same as a but pieces longer.

of polysheath bundles inside the cells has also been observed in other bacteria (van Iterson et al. 1967; Pate et al. 1967; Baechler & Berk, 1972).

The substructure of the polysheath shown in Fig. 7 seems to be intact because the diffraction spots arising from the front and the back side of the particle are placed symmetrically with regard to the meridian (Fig. 8). There is no evidence for a distortion as described for *Escherichia coli* polysheaths by Moody (1967).

The pitch angle of the large-scale helices is very large compared with that of other sheaths (Moody, 1967; Pfister, 1974; Admiraal & Mellema, 1976). This fact is probably responsible for two observations: (i) the spiral appearance of short pieces of polysheaths (Fig. 4) is not as pronounced as in T4-sheaths (Moody, 1967); (ii) polysheaths from *A. eutrophus* do not reveal a criss-cross pattern, but display regular ‘constrictions’ of the central channel. Moreover, the surface of these polysheaths appears rather smooth (Fig. 12) compared with T4 (Fig. 14) or the contracted sheath of T2 (Bayer & Remsen, 1970), suggesting closely packed non-protruding subunits. The most suitable subunit model to meet these requirements would be a flat ellipsoid (Fig. 21). Its approximate dimensions are given by the thickness of the hollow cylinder (z: 7·1 nm), by the longer side of the unit cell of the surface lattice (Fig. 15, x: 6·23 nm) and by the spacing of the small scale helices (s: 3·35 nm). The vol. of such an ellipsoid can be calculated by the formula

\[ V = \frac{4}{3} \pi x^2 z \]

to be 80·8 nm³. According to Zipper et al. (1971), 1 dalton (D) corresponds to a mol. vol. of 1·37 ± 0·12 × 10⁻³ nm³.

The vol. of the model subunit calculated above, therefore, corresponds to a mol. mass of 58,978 D, which is fairly close to the value determined by SDS-gel electrophoresis (58,000). Consequently, it can be assumed that the morphological subunit as revealed in electron micrographs is composed of one polypeptide chain only.

Judging from SDS-gel electrophoresis and from electron microscopy, polysheaths from *A. eutrophus* could be isolated in the pure state. The u.v. spectrum and the buoyant density are consistent with a solely proteinacious nature of the polysheaths. Their physicochemical
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Fig. 21. Hypothetical shape of the polysheath subunit. The dimension $z$ corresponds to the thickness of the wall of the cylinder, $s$ to the spacing of the small scale helices (Fig. 15) and $x$ to the longer side of the unit cell. The $x$-axis of the ellipsoids is parallel to the small-scale helices.

data do not differ from those of other sheath proteins. Sheaths of other phages and phage-like particles are also composed of a single type of subunit, the mol. wt. being in the same range (Sarkar et al. 1964; Roslansky & Baylor, 1970; Pfister, 1974; Admiraal & Mellema, 1976). The buoyant density of polysheaths is identical with the density of purified sheaths of rhabdiosomes (Delk & Dekker, 1972; Chang & Allen, 1974). The main features of the amino acid analysis, i.e. the abundance of aspartic and glutamic acid, of glycine, alanine, leucine and valine as well as the lack of sulphur-containing and aromatic amino acids are characteristic, too, for all other sheath proteins (Lotz, 1976). The same is true for the resistance against proteolytic enzymes and denaturants (Kageyama, 1964; To et al. 1969; Delk & Dekker, 1972; Chang & Allen, 1974; Traub et al. 1974). As to their stability, polysheaths from *A. eutrophus* are more related to the contracted sheath of T4 than to the polysheaths of *E. coli* (To et al. 1969).

Occasionally, polysheaths were observed in dividing cells as shown in Fig. 6. Only breakage or division of the polysheaths would allow the cells to complete cell fission. The question of whether or not the host cells of polysheaths can divide is answered by the experiment presented in Fig. 17. There are three different possibilities for the variation of the parameter 'cells with polysheaths/ml' after the maximum of polysheath formation:

(i) if cells with polysheaths were capable of normal cell fission, the number of cells with polysheaths should increase;
(ii) if cells with polysheaths were unable to divide and if polysheaths were degraded, the number of cells with polysheaths should decrease drastically;
(iii) if cells with polysheaths were not able to divide and polysheaths were left intact, the number of cells with polysheaths should remain constant. The sharp maximum of the percentage of cells with polysheaths most likely excludes a continuous production of polysheaths. The experiment therefore clearly favours the third alternative. Polysheaths probably stay inside their hosts until natural cell death and autolysis occur. As this process takes a long time in *A. eutrophus*, the 'absolute' number of cells with polysheaths is only gradually declining. Two other observations further confirm this hypothesis: free polysheaths never appeared in the supernatant of the culture. The same amount of polysheaths was isolated from cultures with absorbances of 2.5 and 5.

Finally, one may speculate as to the significance of polysheaths for *A. eutrophus*: their synthesis means not only a waste of energy and material for the cell but also cell death.
However, one has to keep in mind that polysheaths are abundantly produced only in fermenter cultures under ideal nutritional conditions. It is conceivable that under natural conditions the genetic information for polysheaths is repressed. If, however, polysheaths originate from defective lysogeny, the cells might even benefit from them. Many lysogenic bacteria gain immunity and resistance against superinfecting phages (Barksdale & Arden, 1974). Possibly there are some more genes left from the original prophage in *A. eutrophus* in addition to those coding for the sheath. Up to now, all attempts to find virulent phages for *A. eutrophus* have failed (Auling et al. 1977), but phage tail-like particles were present in nearly all strains investigated.

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REFERENCES


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