Bacteriophage MX-1: Properties of the Phage and its Structural Proteins

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SUMMARY
Bacteriophage MX-1 is a virulent DNA phage for Myxococcus. The host range includes strains of Myxococcus xanthus, M. fulvus and M. virescens. The phage has a sedimentation coefficient ($s_{20,w}$) of 1145S and a density of 1.531 g/ml. By using SDS-polyacrylamide gel electrophoresis, 23 phage proteins with apparent mol. wt. between 10000 and 150000 were resolved. Gel filtration in the presence of non-ionic detergent partially resolved the proteins. The fraction excluded from Sephadex G-100, fraction 1, contains two glycoproteins. Fraction 1 was resolved into three fractions (1·1, 1·2 and 1·3) by chromatography on Sephadex G-200. The glycoproteins were present in fraction 1·2; all the proteins from this fraction were derived from the phage tail. Comparison of the amino-acid, hexosamine and neutral-sugar compositions of the two glycoproteins showed that they are distinct molecular species; the smaller molecule is not a subunit of the larger. The significance of these findings is discussed and compared with the proteins of the tails of T-even phage of Escherichia coli.

INTRODUCTION
Bacteriophage MX-1 is a virulent DNA phage for Myxococcus xanthus. It was first described by Burchard & Dworkin (1966), who isolated it from cow dung. It is a DNA phage broadly similar in morphology to coliphage T4. The infection of vegetative M. xanthus is typical of that of virulent DNA phage; the latent period (150 min at 30 °C) is somewhat shorter than the mean generation time of the host. The final burst size is 100 and during the latent period changes in the nuclear body of the host and polar aggregation of cytoplasmic components occur. Adsorption is predominantly associated with the host-cell polar regions (Voelz & Burchard, 1971).

The host organism is a fruiting bacterium. Within the fruiting bodies, the vegetative rods convert to spherical, phase-refractile, myxospores. Also, conversion of vegetative organism to myxospore can be induced synchronously by adding glycerol, or certain other alcohols, to exponentially growing cultures in liquid medium (Dworkin & Gibson, 1964). Burchard & Dworkin (1966) observed that if phage are added to a culture of Myxococcus xanthus committed to glycerol-induced myxospore formation, mature, resistant and phase-refractile myxospores are formed from which phage appear during germination. The infected myxospores do not contain intact phage particles and the phage genome is apparently ‘trapped’ in a way analogous to Bacillus subtilis phage φε (Sonenshein & Roscoe, 1969).

In this paper we describe further characterization of the phage and the properties of its coat proteins.
METHODS

Materials. Unless otherwise stated, bacteriological media were obtained from Laboratory Industrial and Pharmaceutical Services Ltd, London and other chemicals were obtained from BDH, Poole, Dorset and were of AnalaR grade wherever possible.

Bacteria and phage. Myxococcus xanthus strains A and B are both derived from strain FB (Dworkin, 1962). M. xanthus A was selected on the basis that it produces very few phenotypically resistant colonies when plated in the presence of excess phage (Parish, 1975).

Myxococcus fulvus M is a semi-disperse growing variant of strain Mittelamerika (Parish, 1975); Myxococcus virescens V2 was a gift from Dr H. Reichenbach.

Myxococcus xanthus and M. fulvus were grown in CT medium (1% (w/v) Bactocasitone, Difco). M. virescens was grown in CT medium supplemented with 0.5% (w/v) yeast extract. Methods for the growth and assay of the phage were those of Burchard & Dworkin (1966) except, in the case of assay using M. virescens as indicator, yeast extract (0.5%, w/v) was included in the agar underlay.

Harvesting and purification of the phage. Lysates in liquid medium were clarified by centrifuging in the 6 × 250 ml rotor of the MSE Highspeed 18 centrifuge (12000 rev/min, for 10 min at 4 °C) and phage were recovered by centrifuging in the 21 rotor of the Beckman model L ultracentrifuge (21000 rev/min, for 30 min at 4 °C). These conditions would result in total recovery of a particle with the sedimentation coefficient of the phage $s_0 = 1050$ S in this rotor at top speed for 20 min, according to the calculation of Trautman & Cowan (1968).

The pellets were resuspended in 0.1 M-NaCl, 0.01 M-KH$_2$PO$_4$/K$_2$HPO$_4$ buffer, pH 7.6, and were further purified by one of the following procedures. (a) A gradient of sucrose (15 to 40% w/v); 500 ml linear with volume in 0.1 M-NaCl, 0.01 M-KH$_2$PO$_4$/K$_2$HPO$_4$, pH 7.6, was established in the B XIV rotor of the MSE Superspeed 65 ultracentrifuge over a layer of 45% (w/v) sucrose in the same buffer. The sample (10×10 p.f.u. in 40 ml 5% w/v sucrose) was introduced over the gradient and displaced with buffer (100 ml). Centrifugation was for 1 h at 24000 rev/min and 5 °C. The gradient was pumped out with 48% (w/v) sucrose. Phage were recovered from pooled fractions by centrifuging in the rotor of the Beckman model L ultracentrifuge (30000 rev/min, at 4 °C for 15 h). (b) For preparing large batches of phage for isolation of coat proteins, phage (approx. 10$^{13}$ p.f.u.) in NaCl/phosphate buffer (100 ml were extracted with ice-cold chloroform (100 ml); the phases were separated by centrifuging and the upper phase was recovered and re-extracted with chloroform. Upper phase (15 ml) was layered over 60% (w/v) sucrose, 0.1 M-NaCl, 0.01 M-KH$_2$PO$_4$/K$_2$HPO$_4$ buffer, pH 7.6 (20 ml), in tubes for the 30 rotor of the Beckman model L ultracentrifuge and the phage were obtained as a pellet following centrifuging (30000 rev/min, at 4 °C for 15 h).

Sedimentation and buoyant density analysis. The sedimentation coefficient was determined by analytical centrifuging of suspensions of the phage in NaCl/phosphate ($E_{260}$ 0.5 to 10.0) in a single-sector cell of the AnD rotor of the Beckman model E ultracentrifuge (9945 rev/min at 20 °C); photographs of the Schlieren image were recorded at 200 s intervals. The sedimentation coefficient was extrapolated to infinite dilution. The value was corrected to $s_0$; the viscosity of the buffer was measured with an Ostwald viscometer calibrated with water; the density of the phage was determined as described in the following paragraph and that of the buffer was measured pyknometrically.

Densities of phage MX-1 and its derived particles were determined in a cell with a filled Epon centrepiece of the AnD rotor of the Beckman model E ultracentrifuge, using CsCl dissolved in NaCl/phosphate buffer with phage. The starting density was determined...
by the methods of Vinograd & Hearst (1962) the sample was centrifuged to equilibrium for 48 h at 33500 rev/min, 20 °C, and the positions of the zones were measured from the crossover points on the extrapolated baseline in photographs of the Schlieren image. Densities of the zones were measured by the isoconcentration method (Vinograd & Hearst, 1962).

For preparative centrifugation, phage suspension (approx. $10^{12}$ p.f.u.) in 0.1 M-NaCl, 0.01 M-KH$_2$PO$_4$/K$_2$HPO$_4$ buffer, pH 7.6 (350 ml) was added to CsCl (2.911 g) in a tube for the MSE Superspeed 65 ultracentrifuge and centrifuged for 48 h at 35000 rev/min and 5 °C.

Isolation of coat proteins. Proteins were extracted from phage with phenol (Alvarez et al. 1972). The proteins are denatured and dissolved in phenol; the details are based on the procedure of Parish & Kirby (1966) for isolating nucleic acids. Phenol mixture was made by liquefying phenol (1 kg) with m-cresol (110 ml) and water (140 ml); 8-hydroxyquinoline (1 g) was added to prevent autoxidation of the phenol. Phage (approx. $10^{13}$ p.f.u.) suspended in 6% (w/v) 4-aminosalicylic acid Na salt, 1% (w/v) NaCl, 1% (w/v) tri-isopropynaphthalene sulphonate Na salt (Kodak Ltd, Kirkby, Liverpool) containing 3% (v/v) phenol mixture (100 ml) was extracted by stirring for 30 min at room temperature with an equal vol. of phenol mixture. The phases were separated by centrifuging in the 6 x 250 ml rotor of the MSE High Speed 18 centrifuge (12000 rev/min, for 10 min at 5 °C). The upper phase was removed and re-extracted with 0.5 vol. phenol mixture at 60 °C for 15 min. The phases were separated as above and the phenol layers were pooled. Ethanol (3 vol.) was added and the mixture was kept at −20 °C for 15 h. The precipitated proteins were recovered by centrifuging and dissolved in either 0.01 M-KH$_2$PO$_4$/K$_2$HPO$_4$ buffer, pH 7.1, containing 2% (w/v) SDS and 2% (v/v) 2-mercaptoethanol, or 0.1 M-tris HCl, pH 8.0, containing 1% (w/v) Brij 35. In either case the proteins were dissolved by incubation at 60 °C for 10 min and the solution was dialysed against the same buffer for 15 h at room temperature.

Preparative fractionation of proteins. A column (70 x 1.8 cm) of Sephadex G-100 was pre-equilibrated with 0.1 M-tris HCl, pH 8.0, 1% (w/v) Brij 35. The column was loaded with proteins dissolved in the same buffer (approx. 5 mg in 2 ml) and eluted with 300 ml (flow rate 20 ml/h at temperature). The extinction of the eluate was monitored at 257 nm and 8 ml fractions were collected. Samples were concentrated by ultrafiltration using an Amicon PM10 membrane. Protein was precipitated at −20 °C overnight by adding an equal vol. of acetone.

Further fractionation was performed with a column (72 x 2 cm) of Sephadex G-200 eluted as above.

Analysis of proteins. Isoelectric points were determined in the LKB 8100-10 isoelectric focusing column using a sample protein dissolved in tris-Brij buffer and ampholines to establish a pH gradient of 3.0 to 10.0.

Mol. wt. were determined by polyacrylamide-gel electrophoresis in the presence of SDS by the method of Weber & Osborn (1969). The gels were calibrated by using bovine serum albumin, ovalbumin, liver alcohol dehydrogenase, muscle creatine phosphokinase, trypsin, hen egg-white lysozyme, bovine pancreatic ribonuclease and cytochrome c (Sigma) as standards (Bryce & Crichton, 1971). Gels were fixed in 12% (w/v) trichloroacetic acid overnight and were stained for 5 to 8 h in a solution made by dissolving 125 mg Coomassie brilliant blue in 454 ml 50% (v/v) aqueous methanol and then adding 46 ml glacial acetic acid. The gels were destained in 10% (v/v) acetic acid at 37 °C. Glycoprotein was stained by the periodic acid-Schiff’s method (PAS) of Grossman & Neville (1971).

Preparative gel electrophoresis was performed on an E-C 4700 vertical gel cell (E-C Apparatus Corp, Philadelphia, U.S.A.). The gel was 3 mm thick and a layer of protein solution was placed on top of the gel. The gel was of 5% polyacrylamide. Other conditions
were as in the method of Weber & Osborn (1969). After electrophoresis, a vertical slice was
cut from the centre of the slab and stained with PAS reagent. Horizontal slices were cut out
corresponding to the stained zones and proteins were eluted by incubation for 18 h at room
temperature in 0.2 M-NaHCO₃, 1% (w/v) SDS.

For amino acid analysis, protein was hydrolysed in 6 M-HCl at 110 °C for different periods
of time and the hydrolysates were analysed using the Biocal/LKB BC-200 analyser. Aspar-
tate and alanine were regarded as invariant; serine and threonine values were extrapolated
to zero-time; 24 h values were used for tyrosine and 96 h values for other hydrophobic
amino acids. Cysteine residues were assayed using protein reduced and carboxymethylated
prior to hydrolysis (Hassall & Soutar, 1974). For analyses of the glycoprotein bands eluted
from electrophoresis gels, and for fractions of the tryptic digest, hydrolysis was for 10 h.

For certain experiments the products of hydrolysis, following digestion of the unmodified
protein for 24 h, were analysed by ascending chromatography on Whatman No. 1 paper.
The chromatogram was developed with a mixture of butan-1-ol, pyridine and water
(6:4:3 by vol.). Papers were sprayed either with ninhydrin to locate amino acids, or with the
Elson-Morgan reagent (Hunt, 1969) to locate amino sugars.

Tryptic digests were obtained by treating reduced carboxymethylated proteins with
TPCK-trypsin (Smyth, 1967) and analysed by two-dimensional chromatography and
electrophoresis by the methods of Katz, Dreyer & Anfinsen (1959).

Tryptic digests were fractionated by chromatography on Dowex 50 × 4 using a column
60 × 0.6 cm. The column was eluted with pyridine acetic acid mixtures at 55 °C using the
methods of Schroder (1967) except that the pH gradient was 2.1 to 5.0. Peptides were de-
tected in the eluate by fluorimetric assay of derivatives following reaction with fluorescamine
(Nakai, Lai & Horecker, 1974).

N-terminal amino acids were determined by two dimensional thin-layer chromatography
of hydrolysates of dansyl derivatives (Gray, 1967).

Total neutral sugars, hexosamines, fucose and sialic acid were determined in protein
hydrolysates by the colorimetric procedures of Spiro (1966). Neutral sugars and hexosamines
were determined quantitatively by gas–liquid chromatography. Protein samples in 0.2 M-
ammonium bicarbonate and added mannitol (an internal standard) were lyophilized and
dried in vacuo over P₂O₅ and were treated successively with methanolic HCl, Ag₂CO₃,
Ac₂O and Me₃SiCl (Chambers & Clamp, 1971) and were injected into a column of 3% SE 30
stationary phase supported on Gas Chrom. The chromatogram was developed with
nitrogen using a 120 to 200 °C temperature programme (Bhatti, Chambers & Clamp, 1970).

Isolation of tail sheaths. Ghosts were prepared from phage MX-1 by osmotic shock and
digestion with DNase, following the procedure devised for coliphage T2 by Herriott &
Barlow (1957). The tail sheaths were prepared from the ghosts by proteolysis following the
procedure devised for coliphage T2 by Sarkar, Sarkar & Kozloff (1964), except that the two
final centrifugations (in 0.13 M-NH₄OAc) were omitted. Phage, ghosts and tail sheaths were
prepared for electron microscopy by negative staining with uranyl acetate. The samples were
examined in the Phillips EM 300 microscope.
RESULTS

Host range

Burchard & Dworkin (1966) found that three strains of Myxococcus xanthus were hosts to the phage but that M. fulvus MF and also strains of Cytophaga and Sporocytophaga were not. In addition to M. xanthus, we find M. fulvus M and M. virescens V2 are hosts. In every case the phage form clear plaques. There is variation of e.o.p. when the phage is plated on different hosts. The plaques are formed on M. xanthus A with 45 to 55% the efficiency of either M. xanthus B or M. fulvus M. As the difference is consistently found irrespective of the previous host (strain A, B or M) we conclude that the difference is probably not due to restriction but may reflect a difference in phage receptors in strain A. In the case of M. virescens V2 the e.o.p. is significantly lower than on M. xanthus B (approx. 7%). In this case the efficiency can be increased by using phage whose last host was M. virescens V2 and the effect is apparently due to restriction (D. W. Morris & J. H. Parish, unpublished data).

Sedimentation and buoyant density of the phage

From measurements in the analytical ultracentrifuge, the sedimentation coefficient ($s_{20,w}$) of the phage is 1145S. By using large-scale rate-zonal centrifuging through sucrose gradients in the zonal ultracentrifuge rotor, we have developed a simple procedure for purification of the phage. An example of such a purification is shown in Fig. 1.

Using phage purified by method (b), we determined the buoyant densities of phage particles. The preparation resolved into peaks of densities 1.546 and 1.531 g/ml. Preparative centrifuging in this gradient revealed that infectious phage particles were associated with the component of density 1.531 g/ml (Fig. 2). However, the peak of infectivity is sharper than that of optically absorbing material, and the recovery of infectious particles from the gradient was extremely low (approx. 1%) presumably due to osmotic shock of the phage upon dilution. Moreover, electron microscopic examination of the particles from this zone revealed...
that the population was heterogeneous and contained some phage without tails; approx. 20% of the phage had empty heads. The particles from the non-infective peak consisted exclusively of particles without tails. The proportion of phage without tails in the preparation was less than 1% prior to centrifuging and we therefore conclude that loss of tails from some particles is the result of treatment with CsCl.

**Fractionation of structural proteins**

The coat proteins of the phage are insoluble in buffer except in the presence of detergent. We did not succeed in separating proteins and DNA by any single-phase aqueous reagent including treatment with SDS and EDTA at 60°C. Protein extracted with phenol (see Methods) is denatured but can be solubilized in detergent.

Proteins were extracted, and fractionated by gel-exclusion chromatography on Sephadex G-100 in the presence of the non-ionic detergent, Brij 35. The excluded fraction (fraction 1) was re-run on Sephadex G-200. It resolved into three fractions, 1-1 (excluded), 1-2 and 1-3. Examples of these fractionations are shown in Fig. 3.

The proteins were analysed by isoelectric focusing. Unfractionated protein produced a broad, unresolved profile with a maximum at pH 6.85; fraction 1 produced a sharp zone with an isoelectric point of 7.65 pH units, together with unresolved material with a maximum at pH 4.

**Tail sheaths**

The preparation of tail sheaths was followed by examining phage under the electron microscope. Representative pictures are shown in Fig. 4. Phage and ghosts with tails in both extended and contracted configurations were seen. Isolated tail sheaths have a geometry similar to those from phage T2 (Sarkar et al. 1964). In certain fields, we found sheaths showing end-to-end aggregation. Such aggregates were never seen in preparations of intact phage or ghosts. The aggregation of isolated sheaths to form chains has been described for coliphage T4 by Moody (1967), who refers to the structures as ‘polysheaths’.
Electrophoretic analysis of proteins

The proteins of phage MX-1 (Fig. 5), fractions 1, 1·1, 1·2, 1·3 and proteins derived from tail sheaths were analysed by SDS-polyacrylamide gel electrophoresis and were classified according to their apparent mol. wt. and also their ability to stain with PAS reagent (Table I). The unambiguous identification of certain bands in the independent analyses was not possible. Moreover P15 is probably a mixture and both G1 and G2 are mixtures of glycoprotein with other components as, in fraction 1·3, proteins (not detected with PAS reagent) are present in the position of G1 and G2. The apparent absence of P16 from fraction 1, but presence in fraction 1·1, implies that the protein is either present in such small amounts that its detection in fraction 1 was not possible or that degradation or aggregation occurred during the subfractionation. With the exception of P2, P3 and P13 all the proteins of the tail sheaths occur in fractions 1·2 and 1·3 and conversely, all the proteins of fractions 1·2 and 1·3 except P19 are derived from the sheath. The low apparent mol. wt. of G1 in tail sheaths suggests that degradation occurred, probably during proteolysis involved in isolating the
Fig. 4. Electron micrographs of phage MX-I and derived particles. (a) Purified phage preparation showing tails in extended and contracted configurations together with one ghost. (b) Preparation of ghosts showing also some intact phage. (c) Ghosts showing tails in extended and contracted configurations. The panel contains images at identical magnifications from two fields. (d) Preparation of contract sheaths. In this field, most of the sheaths are seen in side view. (e) Contract sheaths seen in side view and end view. The panel contains two regions from the same field. (f) Polysheath seen in preparation of phage tail sheaths.
Fig. 5. Electrophoresis of MX-I proteins in the presence of SDS. The gels were 10% polyacrylamide; details are given in Methods. Gels were stained with (a) Coomassie blue and (b) PAS reagent and scanned in a Unicam SP 1800 spectrophotometer fitted with a gel-scanning attachment. The traces show extinction at (a) 555 nm and (b) 620 nm. The bands are identified by the numbers in Table 1, but the letter P has here been omitted from protein bands. In certain cases, bands which could be resolved by eye and measured are not resolved by the gel scanner. In particular, 17 and 18 resolve clearly and can be identified in fractions (see Table 1).

tail sheaths. The large peaks comprising P11, P12 and P18, which are absent from the tail sheaths, are probably capsid proteins of the head.

In the light of the difference in electrophoretic mobility between components G1 and G2 and the lack of resolution of fraction I on Sephadex G-100, we examined whether G2 might be a subunit of G1. One procedure involves plotting mobilities as a function of polyacrylamide concentration; subunits of multimeric proteins are expected to show mobilities extrapolated to zero gel concentration identical with the extrapolated mobilities of the multimers (Banker & Cotman, 1972). In the case of G1 and G2 the dependence on gel concentration was non-linear and an evaluation of extrapolated mobilities was not possible. The apparent mol. wt. of G2 increased with decreasing gel concentration (16000 in 5% polyacrylamide) in a manner typical of glycoproteins. The apparent mol. wt. of G1 decreased
Table 1. Fractionations of MX-I proteins by SDS-polyacrylamide gel electrophoresis using 10% gels

<table>
<thead>
<tr>
<th>Protein*</th>
<th>Relative mobility</th>
<th>Total protein</th>
<th>Fraction 1</th>
<th>Fraction 1'1</th>
<th>Fraction 1'2</th>
<th>Fraction 1'3</th>
<th>Tail sheaths</th>
</tr>
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<tbody>
<tr>
<td>P¹</td>
<td>0.04</td>
<td>147</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P²</td>
<td>0.05</td>
<td>145</td>
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<td></td>
</tr>
<tr>
<td>P³</td>
<td>0.07</td>
<td>134</td>
<td>137</td>
<td>130</td>
<td>130</td>
<td>140</td>
<td>126</td>
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<tr>
<td>P₄, P₅, P₆</td>
<td>0.09, 0.10, 0.11</td>
<td>121, 119, 113</td>
<td>117</td>
<td>116</td>
<td>126, 117</td>
<td>126</td>
<td>114</td>
</tr>
<tr>
<td>P⁷</td>
<td>0.13</td>
<td>106</td>
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<tr>
<td>P₈</td>
<td>0.14</td>
<td>104</td>
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<tr>
<td>P₉, P₁₀, P₁₁</td>
<td>0.15, 0.16, 0.17</td>
<td>101, 95, 93</td>
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<td>99</td>
<td>97</td>
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<td>100</td>
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<tr>
<td>P₁₂</td>
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<td>P₁₄</td>
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<td>P₁₅</td>
<td>0.24</td>
<td>73</td>
<td>75</td>
<td>74-76†</td>
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<tr>
<td>P₁₆</td>
<td>0.26</td>
<td>68</td>
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</tr>
<tr>
<td>G¹</td>
<td>0.31</td>
<td>61</td>
<td>60</td>
<td></td>
<td>57</td>
<td>56‡</td>
<td>52</td>
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<tr>
<td>P₁₇</td>
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<tr>
<td>G₂</td>
<td>0.82</td>
<td>10</td>
<td>10</td>
<td>10‡</td>
<td></td>
<td>10</td>
<td>10‡</td>
</tr>
</tbody>
</table>

* Proteins (P₁, P₂, etc.) and glycoproteins (G¹ and G₂) are in the order of their electrophoretic mobilities relative to bromophenol blue, and apparent mol. wt. in total, unfractinated protein. In certain cases (for example, P₄, P₅ and P₆) the bands were so close together that unambiguous identification in independent analyses was not possible and such proteins are placed in the same horizontal line. In other cases analysis of fractions resolved components that appeared as single bands in the total phage protein (see below).

† Three bands.
‡ Not stained by PAS reagent.

Table 2. Analyses of acid hydrolysates of fraction 1*

<table>
<thead>
<tr>
<th></th>
<th>9.3</th>
<th>Ile</th>
<th>4.6</th>
</tr>
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<tbody>
<tr>
<td>CMC</td>
<td>9.0</td>
<td>Leu</td>
<td>8.0</td>
</tr>
<tr>
<td>Asx</td>
<td>5.2</td>
<td>Tyr</td>
<td>1.4</td>
</tr>
<tr>
<td>Thr</td>
<td>6.5</td>
<td>Phe</td>
<td>4.3</td>
</tr>
<tr>
<td>Ser</td>
<td>6.0</td>
<td>GlcNH₂</td>
<td>2.3</td>
</tr>
<tr>
<td>Glx</td>
<td>2.9</td>
<td>Lys</td>
<td>4.8</td>
</tr>
<tr>
<td>Pro</td>
<td>10-1</td>
<td>GalNH₂/ManNH₂</td>
<td>2-3</td>
</tr>
<tr>
<td>Gly</td>
<td>10-35</td>
<td>His</td>
<td>2.7</td>
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<tr>
<td>Ala</td>
<td>7-3</td>
<td>Arg</td>
<td>3.7</td>
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<tr>
<td>Met</td>
<td>0.5</td>
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</tr>
</tbody>
</table>

* Components are recorded as molar percentages of all components. Cysteine was measured in a separate experiment (see Methods). Components are listed in elution order.
† Abbreviations: CMC-Cys, carboxymethyl cysteine; GlcNH₂, glucosamine; GalNH₂/ManNH₂, galactosamine/mannosamine (unresolved).

The analysis of ninhydrin-positive components in acid hydrolysates of fraction 1 is summarized in Table 2. In addition to amino acids, the hydrolysates contained hexosamines (see Table 2) and two unidentified substances, X and Y. Substance X eluted from the amino acid

(26000 in 5% polyacrylamide). The explanation for this behaviour is unknown but has been observed for certain peptidoglycans (Sergest & Jackson, 1972).

Amino acids and sugars in the proteins of fraction 1
Table 3. Qualitative summary of ninhydrin-positive components in peptide fractions derived from tryptic digests of reduced and carboxymethylated fraction I*

<table>
<thead>
<tr>
<th>Peptide fraction</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Met</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tyr</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phe</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GlcNH₂†</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GalNH₂/ManNH₂†</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lys</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>His</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arg</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* The peaks (A to F) are in increasing order of elution from a column of Dowex 50 × 4 (see Methods). All the peaks contained, in addition to the components shown, Asx, Thr, Ser, Glx, Gly, Ala, Val, Ile, Leu.
† Abbreviations: see Table 2.

analyser before aspartic acid in a position close to that of 3-hydroxyproline. The ratio of $E_{470}/E_{540}$ for the ninhydrin derivative was high, also suggestive of a proline derivative. However, samples enriched with authentic hydroxyproline showed a new peak and we regard X as an artefact. Substance Y eluted between lysine and arginine but separable from ammonia. This position suggests a modified lysine.

This method would not detect tryptophan; however, there is no evidence for tryptophan residues in fraction I. Tryptic maps of fraction I resolved 52 peptide components, none of which stained with the Ehrlich reagent in a manner characteristic of tryptophan peptides.

The presence of hexosamines in fraction I was independently confirmed by analysing hydrolysates by paper chromatography and spraying the paper with Elson-Morgan reagent. The presence of amino sugars in 0.7 mg of fraction I was clearly demonstrated. No amino sugars were detectable in 1.4 mg of total MX-I protein.

From these data we conclude that essentially all the proteins containing hexosamine in MX-I phage are present in fraction I. We confirmed that the hexosamines are covalently bound to the polypeptide chains by analysing a tryptic digest. The peptides were fractionated by column chromatography. The compositions of six separate fractions from the column are summarized in Table 3. The complexity of amino acid components suggests that each of the peaks are mixtures of peptides. The presence of hexosamines in four separate peptide fractions (A, B, C, D) indicates that these sugars are specifically attached to regions of the polypeptide backbones of fraction I components and are not derived from non-covalently bound carbohydrate.

Colorimetric analysis of neutral sugars in fraction I revealed the presence of fucose and aldohexoses. Sialic acid was not detectable. The ratio of fucose to aldohexose was of the order of 2:1; the precise ratio could not be calculated as the colour response of the reagents depends on the ratio of (galactose + glucose)/(mannose).

Glycoproteins G1 and G2 were recovered from preparative SDS-polyacrylamide gel electrophoresis. They were analysed for ninhydrin-positive acid hydrolysis products (amino acid analyser), neutral sugars (gas–liquid chromatography) and for amino terminal amino acid. The results are summarized in Table 4. The amino acid compositions are not recorded; the data of Table 1 suggest that both G1 and G2 are mixtures of proteins. In addition to the neutral sugars recorded in Table 4, three unidentified peaks were found in gas–liquid
Table 4. Summary of N-termini, amino sugars and neutral sugars in glycoproteins GI and G2*

<table>
<thead>
<tr>
<th>Amino terminus</th>
<th>Fraction 1 mixture</th>
<th>GI Ser</th>
<th>G2 Ser</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosamine</td>
<td>1.2</td>
<td>1.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Galactosamine</td>
<td>2.3†</td>
<td>2.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Mannosamine</td>
<td>†</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>—</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Glucose</td>
<td>±†</td>
<td>13.4</td>
<td>6.6</td>
</tr>
<tr>
<td>Mannose</td>
<td>.‡</td>
<td>ND</td>
<td>2.9</td>
</tr>
<tr>
<td>Galactose</td>
<td>.‡</td>
<td>ND</td>
<td>1.4</td>
</tr>
<tr>
<td>Fucose</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

* Glucosamine was worked out as a molar percentage of total ninhydrin-positive material from the amino acid analyser. Other sugar components were calculated from ratios of derivative peaks from gas-liquid chromatography. The numbers refer to molar percentages of total ninhydrin positive material; + and – refer to results of qualitative tests; ND: not detected, NT: not tested.

† Galactosamine and mannosamine were not resolved (amino acid analyser data).
‡ Galactose, mannose and galactose were not resolved (colour reactions).

Chromatograms of hydrolysates of both GI and G2. The positions suggest that these components are not amino acid derivatives. It is possible that they all derived from a single, unidentified monosaccharide, since certain sugars (including glucose) produce more than one derivative peak in this method (Chambers & Clamp, 1971). Fucose could not be measured from these chromatograms as its derivatives elute in the solvent peak. The data of Table 4 confirm that GI and G2 are distinct species and that GI is not a multimer of G2.

DISCUSSION

Phage MX-1 is shown to contain at least 23 proteins (including the glycoproteins GI and G2) from Table 1. Proteins absent from tail preparations are P1, P7, P8, P12, P15, P18, P20, P21, possibly two of the P4, P5, P6 group and two of the P9, P10, P11 group. If we regard these as head proteins, it suggests that there are 12 head proteins. The corresponding value for phage T4 is 13 (Laemmli, 1970).

Data corresponding to ours for the tail proteins of complex DNA phage are not available. The allocation of certain proteins in fractions of total protein to the tail (Table 1) suggests that despite the treatment of the tails with proteases, the structural proteins are not degraded during the isolation, with the exception of GI. Tail sheaths isolated from coliphage T2 are of similar dimensions to those of MX-1 (Sarkar et al. 1964). Although the data were obtained on the basis of numbers of histidine residues rather than electrophoretic analysis. The results of Sarkar et al. (1964) suggest that T2 sheaths contain a significantly less complex pattern than ours.

The exclusion from Sephadex G-100 of fraction 1, which contains proteins of low apparent mol. wt. (including G2) implies an anomalously high mean radius of gyration of these proteins in the presence of Brij 35.

Certain lipophilic proteins bind Triton X-100, and, by analogy, probably Brij 35 to produce anomalously high apparent mol. wt. in gel-exclusion chromatography (Helenius & Simons, 1972). We regard this mechanism as unlikely to account for the properties of fraction 1 because the analysis by Helenius & Simons (1972) suggests that it is only found among components of membranes when detergent displaces membrane lipoproteins and phospholipids. These properties are not to be expected of proteins from non-enveloped viruses.
Bacteriophage MX-1

It is more likely that the proteins of fraction 1 aggregate in Brij 35. As the proteins are predominantly derived from the tail sheaths (Table 1), it is probable that the tails never dissociate completely. Sarkar et al. (1964) were unable to dissociate T2 tail sheaths into subunits. The subfractionation of fraction 1 yields only partial separation of the components and suggests that tail-protein aggregates formed during the phenol extraction cause artefactual sorting of the proteins into incomplete sets (fractions 1.1, 1.2, 1.3; Table 1).

Component G1 (apparent mol. wt. 60000) may correspond to the main subunit protein, with ATPase activity, of the T2 sheath (mol. wt. 55000). The total hexosamine content of T2 sheath glycoprotein (1.2%; Sarkar et al. 1964) compares with 4.3% for G1. The main difference between the two is the extensive glycosylation of G1 (13.4% glucose). The other striking feature of phage MX-1 is the presence of a second glycoprotein G2. The analytical data show that G2 is not a subunit of G1. It is also unlikely that G2 is a degradation product of G1 as substantial quantities of mannose and galactose are present only in G2. It would be of interest to examine other complex DNA phage to see whether heterogeneity of glycoproteins is common.

Coliphage T4 tails are assembled at the membrane of the host (Simon, 1969). An analogous situation occurs during MX-1 formation (Voelz & Burchard, 1971). Ribosomes were found associated with forming tails. There are, however, two points of difference. In MX-1 tail assembly appears to be coordinated with attachment, as pre-formed tails are not found in infected myxococci (Voelz & Burchard, 1971) in contrast to T4 (Edgar & Wood, 1966). In MX-1 the newly formed phage (again unlike T4) do not orient radially from the membrane.

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REFERENCES


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