Ø29 Bacteriophage Structural Proteins

By G. ALVAREZ, E. SALAS, NORMA PÉREZ
AND J. E. CELIS*

Departamento de Bioquímica y Química Facultad de Medicina,
Universidad de Chile, Casilla, 6671 Santiago-4, Chile

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SUMMARY

The proteins from bacteriophage Ø29 have been dissociated with 1 % SDS and 2 % 2-mercaptoethanol and have been examined by SDS-acrylamide gel electrophoresis. Seven different polypeptides bands have been detected in the purified virus. One of the bands (b1) is frequently detected in different positions suggesting that it represents an aggregate rather than a very large polypeptide. The molecular weights of the other polypeptide bands (b2, b3, b4, b5 and b6) estimated by coelectrophoresis with marker proteins are 93,000; 75,000; 65,000; 53,000; 41,000 and 30,000. Protein bands b2, b3 and b6 compose 80 % of the bacteriophage. The six structural polypeptides described here could account for approximately 60 % of the information contained in the virus DNA.

INTRODUCTION

Bacteriophage Ø29 was originally isolated from the soil by Reilly (1965). The hexagonal head of the bacteriophage with an overall diameter of 315 Å and a length of 415 Å has delicate projections of approximately 140 Å in length (Anderson, Hickman & Reilly, 1966). The bacteriophage tail is about 325 Å in length and its proximal portion has a neck substructure composed of an upper and a lower collar. Twelve tail appendages are attached to the lower collar.

In spite of its great morphological complexity, the bacteriophage contains a relatively small double helix DNA of a mol. wt of 11 × 10^6 (Anderson et al. 1966). This means that the DNA of bacteriophage Ø29 has enough information to code for about 20 proteins of an average molecular weight of 30,000. This relatively small number of proteins specified by the bacteriophage makes it a very promising subject for the study of virus morphogenesis.

In the present study we have analysed the virus protein sub-unit composition of purified Ø29 bacteriophage by means of SDS-polyacrylamide gel electrophoresis.

METHODS

Bacteria and bacteriophage. Bacillus subtilis Hw+ and bacteriophage Ø29 were obtained from the laboratory of Dr Thomas W. Conway.

Media. ESMM is the minimal medium described by Spizizen (1958), supplemented with 0.1 % yeast extract and 0.02 % of bactotryptone. Soft agar for top layers, bottom agar and phage diluent were prepared according to Reilly (1965).

* Present address: Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge, England.
Bacteriophage growth and purification. Bacteriophages were grown and purified by a modification of the procedure described by Reilly (1965). One litre of ESMM inoculated with 1/10 of the volume of a logarithmically growing culture of *B. subtilis* Hw+ was grown at 37° with aeration until the cells reached a concentration of 2.5 x 10⁷ cells/ml. Bacteriophage Φ29 was added at a m.o.i. of 10 and the cells were incubated until lysis occurred (1 to 2 hr). The lysate containing about 2 to 4 x 10⁸ p.f.u./ml was clarified by centrifugation at 6000 g for 20 min in an RC-2-B Sorvall centrifuge. The supernatant was then centrifuged in a Spinco model L preparative ultracentrifuge at 78,000 g for 1 hr. The pellet was resuspended in 50 ml of phage diluent and the suspension centrifuged at 3000 g for 15 min. The supernatant was treated with lysozyme (5 μg./ml.), deoxyribonuclease 1 (0.1 μg./ml.) and pancreatic ribonuclease (1 μg./ml.), at 37° for 45 min. The suspension was then centrifuged at 78,000 g for 1 hr and the pellet resuspended slowly in 20 ml of phage diluent. The preparation was centrifuged at 3000 g for 15 min. and the supernatant was treated with 20 μg./ml. of trypsin for 1 hr at 37°. The bacteriophages were recovered by centrifugation at 78,000 g for 1 hr and they were washed twice with phage diluent. Further purification of the virus was achieved by applying concentrated purified virus to a DEAE-cellulose column (21 x 1 cm.) previously equilibrated with 0.005 M-MgSO₄ and 0.05 M-phosphate buffer pH 7.0 and afterwards washing with 30 ml. of the same solution and eluting with a linear salt gradient between 0 and 0.5 M-NaCl. The bulk of the protein and virus infectivity was eluted at NaCl concentrations of approximately 0.18 to 0.2 M-NaCl. Examination of this fraction in the electron microscope revealed morphological intact particles, phage aggregates and a few empty particles.

Radioactive bacteriophages. [¹⁴C]-labelled bacteriophages were prepared by incubating 500 ml. quantities of infected cultures in ESMM with a mixture of 15 [¹⁴C]-labelled amino acids (50 μc., New England Nuclear) added 15 min. after infection. Radioactive virus was purified as described above.

Bacteriophage dissociation. 50 ml. of purified bacteriophage at a concentration of 5 x 10¹² p.f.u./ml. were treated with one vol. of water-saturated phenol for 5 min. at 37°. The phenol suspension was precipitated with 3 vol. absolute ethanol. After 12 hr at -20° the DNA was recovered by means of a glass rod and the protein precipitate was collected by centrifugation at 10,000 g for 20 min. The protein pellet was washed once with a small amount of cold ethanol. The protein was dissolved in a solution containing 1 % sodium dodecyl sulfate (SDS), 2 % 2-mercaptoethanol and 0.01 M-phosphate pH 7.1. The sample was then dialyzed overnight against 200 vol. of the same solution. In cases in which the phenol extraction was avoided, the bacteriophages (pelleted) were directly taken up in the SDS+2-mercaptoethanol solution and were extensively dialysed against the same solution.

Polyacrylamide gel electrophoresis: SDS gels. SDS-polyacrylamide gel electrophoresis was performed according to the procedure of Maizel (1969). 5 % acrylamide gels contained in 10 ml.: 0, 5 g. acrylamide; 16, 7 mg. N, N-methylene bisacrylamide; 2 μl. N, N, N', N'-tetraethylmethylenediamine; 10 mg. SDS; 50 mg. ammonium persulfate and 0.1 ml. 1 M-phosphate pH 7.1. The upper gel buffer contained 0.01 M-phosphate pH 7.1, 2 % 2-mercaptoethanol and 0.4 % SDS.

Virus protein treated as described above was further dissociated by heating 3 min. in a boiling water bath before electrophoresis. In some cases, the virus proteins were pre-treated with 2-mercaptoethanol (2 %) before heating and electrophoresis.

Samples were applied in 10 % glycerol and no sample or stacking gels were used. Electrophoresis was carried out at 6 ma/gel long enough to allow the tracking dye (bromophenol blue) to move 7 cm. from the origin. The gels were removed from the glass tubes, shaken
for 4 days with several changes of 10% TCA, and stained with Coomassie brilliant blue as described by Weber & Osborn (1969). Gels were scanned in a Carl Zeiss densitometer.

**Urea-SDS gels:** 8 m-urea gels containing 0.1% SDS were prepared as described by Jovin, Chrambach & Naughton, 1964. The upper gel buffer contained 8 m-urea, 0.4% SDS and 2% 2-mercaptoethanol. The lower buffer contained 8 m-urea and 2% 2-mercaptoethanol. The samples in 8 m-urea, 1% SDS and 2% 2-mercaptoethanol were applied to the gels in 10% sucrose. Electrophoresis was carried out at 3 mA/tube until the tracking dye had reached 7 cm. from the origin. The gels were washed and stained as described above.

**Determination of radioactivity.** When gels were assayed for radioactivity, they were sliced into 1 mm. slices with a razor blade. The slices were transferred to counting vials containing 0.1 ml. 30% H₂O₂ (Bachrach, Reuven & Friedman, 1970). The gels were left at 50° for 3 hr and 10 ml. of toluene scintillation liquid was added to each vial.

**Protein determination.** Protein was determined according to the procedure of Lowry et al. (1951) with bovine serum albumin as the standard.

**Chemicals.** L-amino acid [¹⁴C]-mixture (NEN-445) was purchased from New England Nuclear Corporation. Trypsin, rennin, RNAse, lysozyme, lactic dehydrogenase, catalase and bovine serum albumin have been obtained from Sigma Chemical Company. Yeast alcoholic dehydrogenase was purchased from Worthington.

Urea (Merck) and SDS were recrystallized as described by Burgess (1969). All other chemicals were obtained from commercial sources and were reagent grade.

**RESULTS**

**Number of proteins in Ø29 bacteriophage**

Fig. 1 illustrates the electrophoretic patterns in 5% acrylamide gels of bacteriophage preparations dissociated with 1% SDS and 2% 2-mercaptoethanol with and without pre-treatment with phenol. Fig. 1 A corresponds to the sample treated with phenol and presents five distinct bands (b₁, b₂, b₃, b₄ and b₅) and a minor one which is barely detectable and which has been designated b₆. In later experiments this band will become more evident. The pattern in (B) shows the proteins obtained without phenol extraction. Although the sample examined in this case was smaller, the first five bands are clearly revealed.

In all cases analysed, we obtained well separated bands and no detectable protein remained at the origin. Band b₅ is the major bacteriophage protein and is quite diffuse in the trailing edge suggesting that it might be composed of more than one polypeptide. The migration of bands b₂, b₃, b₄ and b₅ is very reproducible from experiment to experiment. None of these bands could be eliminated by more extensive purification of the virus or by increasing concentrations of SDS and 2-mercaptoethanol. On the other hand, band b₁ showed very variable migrations from preparation to preparation. Pre-treatment of the sample with higher concentrations of 2-mercaptoethanol either eliminated or greatly reduced the intensity of this band while not affecting the other bands (Fig. 2) which strongly suggests that b₁ represents an aggregate rather than a very large virus polypeptide.

Although the stained gels present well-separated discrete bands, it was of interest to analyze the number of bands and their relative proportion in gels sliced and counted in scintillation fluid as described by Bachrach et al. (1970). Seven polypeptides could be resolved using this technique (Fig. 3). Besides the six polypeptides already described, it is possible to see a seventh polypeptide (designated b₅₋₄) which appears as a shoulder in the trailing edge of band b₅. This method also provides us with a good estimate of the relative amount of labelled protein in each of the seven bands since the bacteriophage was labelled
Fig. 1. Electrophoresis of the $\Phi 29$ proteins in SDS-acrylamide gel. (A) about 250 $\mu$g. of bacteriophage protein obtained after phenol-SDS and 2-mercaptoethanol treatment of the $\Phi 29$ virus were analysed in 5% acrylamide gels containing SDS (Methods). The gel was stained with Coomassie brilliant blue as described by Weber & Osborn (1967) and was scanned with a Carl Zeiss densitometer. (B) approximately 200 $\mu$g. of virus protein obtained by the SDS-2-mercaptoethanol treatment purified bacteriophage were analysed in 5% acrylamide gels containing SDS. The gel was stained and scanned as above.

with a mixture of 15 [14C]-amino acids. Table 1 summarizes the relative amount of the different structural proteins as determined from the experiment in Fig. 3. Protein $b_4$ is the major virus component and together with bands $b_2$ and $b_6$ accounts for approximately 80% of the total virus protein. Even though bands $b_3$ and $b_5$ are present in smaller amounts, their relative proportions remain constant from preparation to preparation. It is interesting to mention that there is a discrepancy between the intensity of staining and the apparent amount of radioactivity present in bands 5 and 6. Since we could not rule out the possibility that the six bands ($b_2$, $b_3$, $b_{3-4}$, $b_4$, $b_5$ and $b_6$) represent mixtures of different polypeptides rather than single polypeptides, samples of unlabelled bacteriophages were subjected to poly-
Fig. 2. Comparison of electrophoretic patterns of Φ29 proteins dissociated with 1% SDS in the presence of different concentrations of 2-mercaptoethanol. The sample applied to the gel was about 200 μg of protein. The protein in gel (A) was dissociated with 1% SDS and 2% 2-mercaptoethanol as described in Methods. The protein in gel (B) was pre-treated with 2-mercaptoethanol (2%) before electrophoresis.

acrylamide gel electrophoresis in the presence of 8 M. urea, SDS and 2-mercaptoethanol (Fig. 4). In this different electrophoretic condition, it is not possible to assign the correspondence with the bands obtained in the previous gels. However, again six polypeptides of varying intensities are obtained. The result strengthens our belief that we are accounting for all the major polypeptides present in the virus.

**Molecular weight determination of Φ29 proteins**

The molecular weights of the six virus proteins (b2, b3, b3−4, b4, b5 and b6) were determined using co-electrophoresis with marker proteins of known molecular weight (Weber & Osborn, 1969; Shapiro, Vinuela & Maizel, 1970). In all cases, the values are based on relative migration in gel with respect to trypsin. The result of this experiment is also shown in Table 1. The molecular weight of phage proteins b2 and b6 (93,000 and 75,000) are more subject to error since no marker proteins between 70,000 and 136,000 molecular weight were used. The estimated molecular weights of the three major proteins (b2, b4 and b6) are 93,000, 53,000 and 30,000 respectively. The molecular weight of b3−4 (65,000) has been estimated with respect to the mobility of bands 3 and 4 (Fig. 3) and therefore its value is less reliable.
Fig. 3. Polyacrylamide gel electrophoresis of proteins isolated from [14C]-labelled Φ 29 bacteriophage. The radioactive bacteriophage was dissociated as described in Figure 2B. About 10,000 counts/min. of radioactive proteins were subjected to SDS-acrylamide gel electrophoresis. Gel slices of approximately 1 mm. were treated and counted as described in Methods.

Fig. 4. Polyacrylamide gel electrophoresis of Φ 29 proteins in 8 M-urea and 0.1% SDS. About 200 μg. of virus protein obtained by dissociating the phage particle with 8 M-urea, 1% SDS and 2% 2-mercaptoethanol (Methods) were analysed.

Table 1

<table>
<thead>
<tr>
<th>Band number</th>
<th>% of total protein in phage*</th>
<th>Molecular weight estimated from SDS-acrylamide gel electrophoresis†</th>
</tr>
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<tr>
<td>b₁</td>
<td>4.3</td>
<td>Not determined</td>
</tr>
<tr>
<td>b₂</td>
<td>10.9</td>
<td>93,000 ± 4000</td>
</tr>
<tr>
<td>b₃</td>
<td>6.3</td>
<td>75,000 ± 2000</td>
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<tr>
<td>b₄₋₅</td>
<td>1.4</td>
<td>65,000 ± 2000</td>
</tr>
<tr>
<td>b₆</td>
<td>6.2</td>
<td>53,000 ± 2000</td>
</tr>
<tr>
<td>b₇</td>
<td>5.1</td>
<td>41,000 ± 2000</td>
</tr>
<tr>
<td>b₈</td>
<td>10.0</td>
<td>30,000 ± 2000</td>
</tr>
</tbody>
</table>

* Values are based on [14C] recovered in each of the radioactive bands in Fig. 3.
† Molecular weights were estimated from gels stained with Coomassie brilliant blue. To trypsin we assigned a mobility equal to 1.
‡ The size of protein b₄₋₅ was calculated from its mobility with respect to bands b₃ and b₄ (Fig. 3).

DISCUSSION

At least six different protein components can be separated from bacteriophage Φ 29 solubilized by treatment with either phenol-SDS-2-mercaptoethanol or SDS-2-mercaptoethanol. About 80% of the virus protein is accounted for by three polypeptides (b₂, b₄ and b₆) of estimated molecular weights of 93,000, 53,000 and 30,000. Two minor components which are, nevertheless, reproducibly present under a variety of conditions have also been detected. Their estimated molecular weights are 75,000 and 41,000 respectively, and they
account for approximately 11.8% of the Ø29 protein content. Another protein band (b2–4) has been detected in gels which have been sliced and counted in scintillation fluid. This protein band has an estimated molecular weight of 65,000 and it is present in relatively very small amounts (approximately 1%).

We have also detected a minor protein component (b4) whose amount and relative migration varies from preparation to preparation and thus seems to represent an aggregate rather than a very large virus polypeptide.

Although we obtained well-separated proteins (with the exception of protein band b2–4) by SDS-polyacrylamide gel electrophoresis, we could not rule out the possibility that some of the protein bands represent an aggregate of more than one polypeptide. However, the finding that electrophoresis of the solubilized proteins in 8 M-urea, 1% SDS and 2% mercaptoethanol yielded no additional bands makes this possibility unlikely. We also cannot rule out the possibility of the existence of additional polypeptides that represent minor phage components. These may be detected by analysis of separated purified phage structures.

We have not yet made any attempt to purify the different virus structures.

The sum of the estimated molecular weights of the six polypeptides (b2, b3, b2–4, b4, b5, and b6) is 357,000. Since the bacteriophage DNA, 11 × 10^6 (Anderson et al. 1966), has enough information to code for approximately 611,000 daltons of protein, there are still 254,000 daltons of protein which could correspond to non-structural proteins or structural proteins which we have not been able to detect under our conditions.

Recently, Hagen, Zeece & Anderson, 1971, have mapped ts mutants of bacteriophage Ø29 in 13 complementation groups widely distributed along the genome. They suggest that the complex phage structure would require several proteins that are involved in the virus assembly process. It is possible, therefore, that some of the information not accounted for by our analysis is related to this type of protein.

We have initiated experiments in order to separate each one of the virus structural proteins with the aim of characterizing them with respect to their function, physical properties, and their amino acid composition.

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REFERENCES


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