Structural Damage of Bacteriophage T1 by Surface Inactivation

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SUMMARY

The structure of bacteriophage T1 was examined after inactivation by rotating the virus suspension in a rotating bulb or by bubbling gas through the suspension. After inactivation the [3H]-label of the nucleic acid was located in CsCl and sucrose gradients at the site of free DNA. The small fraction of phage which is resistant to surface inactivation had the same sedimentation coefficient and temperature sensitivity as the control phage, indicating that resistance is not due to agglomerate formation. No morphological differences between phage particles before and after inactivation could be demonstrated by electron microscopy.

INTRODUCTION

The viability of a virus suspension can be lost by shaking or as a result of bubbling with gas (McLimans, 1947; Adams, 1948; Trouwborst, de Jong & Winkler, 1972). Both methods produce a large air/water interface and inactivation has been ascribed to unbalanced forces at the freshly created interface (Adams, 1948). Inactivation of virus might occur by damage to structures required for adsorption or by other structural changes. In this report studies with phage T1 are described which attempt to analyse this inactivation process. As reported previously (Trouwborst et al., 1972), a small fraction of a T1 phage population was found to be resistant to this type of inactivation. The sedimentation properties of this resistant fraction were examined for aggregate formation which might account for its increased resistance to surface inactivation.

METHODS

Growth of bacteriophage. Bacteriophage T1 was propagated on Escherichia coli B as follows: (a) for labelling with [14C]: an overnight culture of E. coli B was diluted 1:30 and incubated at 37 °C for 2 h. The bacterial cells were centrifuged and the pellet was suspended in 20 ml of synthetic medium (SM) which contained in 1 l H2O: 60 g KH2PO4, 60 g K2HPO4, 20 g NH4Cl, 0·5 g MgSO4, 0·05 g FeSO4, 5·6 g NaCl, 0·02 m-glucose, 0·001 m-CaCl2 and 25 mg/ml of adenine, cytosine, guanine and thymine, pH 6·8. A [14C]-labelled purified protein hydrolysate was added to a concentration of 1 μCi/ml and phage T1 was added to a final concentration of 108 p.f.u./ml. After incubation of this suspension for 3 h at 37 °C, the bacterial debris was removed by sedimentation and the phage suspension was purified. (b) [3H]-labelled DNA: a 16 h culture of E. coli B in SM without nucleotides was diluted 1:30 in SM. After 1·5 h of growth at 37 °C 4 μCi/ml of [3H]-thymidine was added. After

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incubation for half an hour phage was added to a final titre of $10^8$ p.f.u./ml. After 2 h at 37 °C the lysate was centrifuged and the phage suspension was purified.

*Purification of bacteriophage T1.* Bacteriophage T1 was sedimented by centrifuging at 60000 g (19000 rev/min) for 3 h in a 6 x 250 ml rotor (MSE-65). The sediment was resuspended in the synthetic medium and was further purified by sedimentation in a CsCl gradient using the two layer method of Brunck & Leick (1969). The lower layer contained 1.8 ml phage suspension to which 2.7 g solid CsCl was added. The upper layer consisted of 2.5 ml phage suspension and 0.8 g CsCl. Sedimentation was performed in a 3 x 5 ml rotor (SW 40, MSE-65) at 32000 rev/min (100000 g) at 10 °C for 24 h. After sedimentation the fractions containing phage were dialysed against 0.1 M-NaCl.

*Determination of radioactivity.* Radioactivity in aqueous samples of $[^{14}C]$- and $[^{3}H]$-labelled phage was counted with a liquid scintillation counter (Nuclear Chicago, Mark II) in a toluene-Triton X-100 scintillation liquid mixture.

*Gradient sedimentation.* CsCl gradient sedimentation was performed as described under purification. Sucrose gradient sedimentation was performed in a linear 5 to 20% sucrose gradient in phosphate-buffered saline (PBS) for 2.5 h at 16000 rev/min in a SW 25 rotor (Spinco, Beckman).

*Surface inactivation.* Surface inactivation was induced by aeration or rotation of the virus suspension. For aeration, an air stream was led through a capillary tube with a diam. of 0.65 cm at a flow rate of 4.7 l/min. The end of the tube was fixed at a distance of 0.2 cm above the bottom of a cylindrical flask ($\phi = 2.5$ cm) filled with 10 ml of virus suspension and incubated in a waterbath of 20 °C. Virus suspensions (10 ml) were rotated at 70 rev/min in a round bottom flask held at an angle of 30° from horizontal and maintained at a temperature of 20 °C.

*Determination of viable phage.* Phage samples were titrated for viable phage according to the agar layer method of Adams (1966) with *Escherichia coli B* as an indicator strain.
Surface inactivation of bacteriophage T₁

Fig. 2. CsCl gradient analysis of [²H]-labelled DNA from phage T₁. (a) O - - - O, p.f.u.; O--O, radioactivity of non-inactivated phage. (b) O - - - O, p.f.u.; O--O, radioactivity of surface inactivated phage. (c) × - ×, radioactivity of heat inactivated phage. ••••, × - ×, index of refraction (n).
Fig. 3. Analysis of samples of [3H]-labelled DNA from phage T2 by sucrose gradient sedimentation. (a) ○—○, d/min; ○—○, p.f.u. of non-inactivated phage. (b) ○—○, d/min; ○—○, p.f.u. of surface inactivated phage; (c) ○—○, d/min of heat inactivated phage. •—•, percentage sucrose in gradient.
Surface inactivation of bacteriophage \( T_1 \)

Table 1. Heat inactivation of phage \( T_1 \)*

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>( \log N_t/N_0 )</th>
<th>Control</th>
<th>s.i.r.</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>-1.45</td>
<td>-1.64</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>-2.00</td>
<td>-2.59</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>-2.29</td>
<td>-2.59</td>
<td></td>
</tr>
</tbody>
</table>

* Samples of untreated \( T_1 \) phage (control) and the fraction resistant to surface inactivation (s.i.r.) were suspended in \( 1 \) m-NaCl, incubated at 65 °C and assayed for surviving infectivity.

Table 2. Loss of viable phage and of \([\text{14C}]\)-label during aeration of the virus suspension in \( 1 \) m-NaCl*

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>( \log N_t/N_0 )</th>
<th>p.f.u.</th>
<th>( \log N_t/N_0 )</th>
<th>Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>-1.42</td>
<td>-0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>-1.95</td>
<td>-0.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>-2.95</td>
<td>-0.75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The original phage was diluted \( 1:10^4 \) in \( 1 \) m-NaCl.

**Electron microscopic preparations.** Samples of inactivated phage were dialysed against \( 0.1 \) m NH\(_4\) acetate with \( 0.01 \) % (w/v) peptone. Negative contrast staining was performed with \( 3 \) % (w/v) aqueous phosphotungstic acid (PTA), adjusted to pH 5.2 with potassium hydroxide. Equal parts of phage suspension, negative stain and a \( 0.01 \) % (w/v) aqueous egg albumin solution (to facilitate spreading) were mixed. A drop of the mixture was applied to a formvar-carbon coated grid, and after a few seconds the excess fluid was blotted away with a piece of filter paper. The specimens were examined in a Philips EM 200 electron microscope at 80 kV and photographed at instrumental magnifications from \( \times 20000 \) up to \( \times 95000 \).

**RESULTS**

Inactivation of phage \( T_1 \) during rotation of the \([\text{3H}]\)-labelled virus suspension in the rotating bulb is shown in Fig. 1. After 60 min of inactivation, \( 1 \) ml of the suspension was analysed: (a) by sedimentation to isopycnic equilibrium in a CsCl gradient; (b) by sedimentation through a sucrose gradient. In two other tubes, a sample of the control (uninactivated) phage and a sample of heat inactivated phage (\( 1 \) h at 75 °C) were analysed in the same run. As shown in Fig. 2a, b, the \([\text{3H}]\)-label of the inactivated phage was found at a different density from that of the control phage. The peak of the \([\text{3H}]\)-label (DNA) coincided with that of a heated phage suspension (Fig. 2c) and it is known that heat inactivation of phage \( T_1 \) releases the DNA from the coat protein (Bresler et al. 1967). Sucrose gradient analysis confirmed that the \([\text{3H}]\)-label of the phage inactivated by rotation was separated from the active phage peak (Fig. 3a, b) and that the position of the peak, in the gradient coincided with that of free DNA (Fig. 3c).

As shown in Fig. 1, the inactivation rate slowed down after inactivation had reached a level of \( 10^{-4} \), leaving a more resistant surviving fraction. This resistance could be the result of agglomerate formation. As demonstrated in the sucrose gradient (Fig. 3a, b) the surviving fraction of phage had the same sedimentation rate as the untreated control, suggesting that no agglomerate was present. The heat sensitivity of the population surviving rotation (Table 1) was not different from that of the untreated phage.
Loss of viable virus during rotation might be a result of adsorption on the air/water interface as well as on the glass/solution interface (Trouwborst et al. 1974). Therefore, the glass/solution interface was reduced in an aeration experiment, in which air was bubbled through a virus suspension with $^{14}$C-labelled coat protein. As shown in Table 2, the loss of $^{14}$C-label, due to adsorption to the glass surface was much less than the loss of viable phage. Consequently inactivation seemed to occur mainly at the air/water interface.

Inactivation of phage might be caused by breakage of the binding between tail and head.
Surface inactivation of bacteriophage \( T_1 \)

In order to investigate this problem, the inactivated phage suspension was examined in the electron microscope after inactivation by aeration. \([^{14}\text{C}]\)-labelled phage \( T_1 \) was diluted 1:100 in 1 M-NaCl, and just before aeration and after 60 min of aeration a sample was taken for electron microscopic observation. The original phage titre was \( 1.9 \times 10^{10} \) p.f.u./ml and the radioactivity was \( 3.413 \) d/min/ml. After 60 min of aeration the phage titre dropped to \( 3.3 \times 10^8 \) (reduction factor: 57.6) and the radioactivity dropped to 761 d/min (reduction factor: 4.5). As shown in the electron micrographs (Fig. 4), the tail was connected to the phage head in all cases. No other differences could be observed between the treated and the untreated phages. It was, however, not possible in these morphological studies to detect whether or not nucleic acid was present in the phage heads of both treated and untreated phages.

**DISCUSSION**

After inactivation of virus particles by rotation or aeration, the DNA-bound \([^{3}\text{H}]\)-label was found by CsCl and sucrose gradient analysis to have the properties of free DNA. This suggests that during inactivation the nucleic acid is released from the coat protein.

Inactivation might occur at the air/water interface or at the glass/solution interface. The second possibility cannot be excluded but the aeration experiments with a small glass surface showed that a large fraction of phage was inactivated at the air/water interface.

The electron micrographs showed that the protein coat had kept its morphological integrity, suggesting that release of nucleic acid might have occurred through the tail. Adsorption to an interface might trigger the ejection of DNA and seems to parallel the effect of adsorption to the host surface.

**REFERENCES**


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