A Lack of Inhibitory Action of Bacteriophage T4 Ghosts in the presence of EDTA

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SUMMARY

Adsorption of bacteriophage T4 ghosts on to Escherichia coli cells has been known to cause a dramatic change in the cellular metabolism, the effect being similar to that of colicin K. It is shown here that the inhibitory activity of ghosts is not expressed either at 0 °C in the ordinary media (like colicin K) or even at 30 °C in a medium containing EDTA, in contrast to colicin K.

Since the process of sheath contraction of T4 phage is blocked by EDTA, it is suggested that the adsorbed ghosts may not exert their inhibitory activity until sheath extraction occurs.

It is known that the attachment of T-even bacteriophage ghosts to the surface of Escherichia coli cells causes an immediate inhibition of cellular metabolism, leading to cell death. This inhibition occurs in a drastic way, and in many respects, the mode of its action resembles that of certain kinds of colicins, such as colicin K (cf. Okamoto, 1973). The cellular disturbance caused by these inhibitors through the bacterial membrane is a phenomenon of great interest, yet in neither case has the mechanism of inhibition been clarified.

Silver, Levine & Spielman (1968) reported that when cells were infected with phage T4 at 25 °C, intracellularly accumulated ^24K ion leaked out into the medium, while if the infection was done at 4 °C, no such leakage occurred. Since the phage induced leakage is triggered by the action of phage coat protein (N. Saijo & K. Okamoto, unpublished data), it would appear that the expression of the coat protein is somehow depressed at 4 °C. In fact, Winkler & Duckworth (1971) observed that at lower temperatures, the inhibitory action of T4 ghosts was less marked as compared with that at higher temperatures. In the case of colicin K, a lack of inhibition at a low temperature was similarly reported (Wendt, 1970). More recent studies (Okamoto, 1975) on the mechanism of colicin K action indicate that metabolic energy and 'heat' are required for the expression of its inhibitory action after adsorption on to cells. The present paper deals with the question of whether these two factors (heat and metabolic energy) are the only requirements for expression of the inhibitory action of phage T4 ghosts.

Bacterial strain and medium used (medium E), preparation and titration of T4 ghosts and colicin K, and determination of ghost inhibition by measuring the uptake of [14C]-labelled thiomethyl-β-galactoside (TMG), have been described previously (Okamoto, 1973, 1975).

As Fig. 1 a shows, when Escherichia coli cells were infected with T4 ghosts at 0 °C, the cellular activity of TMG uptake was not inhibited at least for the first 30 min, after which a gradual inhibition occurred. It was confirmed that under these conditions, the adsorption of ghosts occurred at only a slightly reduced rate as compared to that at 30 °C. At the same multiplicity of ghost infection, a complete inhibition was obtained within 2 min after infection at 30 °C (Fig. 1 b). A lack of inhibition at 0 °C is, therefore, a feature common to both colicin K and phage ghosts (Okamoto, 1975). However, when EDTA was added to the culture which had been infected with T4 ghosts at 0 °C, the [14C]-TMG uptake continued without
Fig. 1. The effect of temperature and EDTA on the inhibitory action of T4 ghosts and colicin K. E. coli cells were mixed with T4 ghosts (24 inhibition units/cell) or colicin K (30 inhibition units/cell) at 0 °C. The uptake of [14C]-TMG was then examined either at 0 °C or 30 °C: (a) at 0 °C; (b) at 30 °C; (c) at 30 °C in the presence of EDTA (8 mM). In the case of (b) and (c), [14C]-TMG was added at 5 min after transferring to 30 °C. A sample (0.1 ml) at each point contained 2.5 x 10^7 cells, and 60 nc = 26 nmol of [14C]-TMG. ○—○, control (untreated); •—•, T4 ghost-treated; ■—■, colicin K-treated.

Table 1. Expression of the inhibitory action of adsorbed ghosts after heat treatment in the presence of Mg++

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment of cell-ghost complex*</th>
<th>Uptake of [14C]-TMG (ct/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>1581</td>
</tr>
<tr>
<td>2</td>
<td>+ Mg (50 mM)</td>
<td>1630</td>
</tr>
<tr>
<td>3</td>
<td>+ Mg (50 mM), 37 °C, 2 min</td>
<td>76</td>
</tr>
</tbody>
</table>

* T4 ghosts (70 inhibition units/cell) were adsorbed to E. coli cells at 0 °C for 10 min in the presence of EDTA (2.3 mM). The cell-ghost complex was collected by centrifuging, resuspended in medium E lacking EDTA, and subjected to the treatments as shown. 0.1 ml (2 x 10^7 cells) was used for [14C]-TMG uptake (0 °C, 2 h). Final concentration of TMG: 0.6 μc = 0.26 μmol/ml.

any interruption even after the culture was transferred to 30 °C (Fig. 1 c). The presence of EDTA at this concentration did not cause a considerable effect on the TMG uptake per se. However, it should be emphasized that in the presence of EDTA, the inhibitory effect of colicin K on cells is completely blocked (Fig. 1 c). Thus, it may be presumed that the adsorbed ghosts require divalent cations for the expression of their inhibitory action, in contrast to the case of colicin K.

Although it was confirmed (data not shown) that EDTA neither inhibits the adsorption of ghosts to the cells, nor inactivates ghosts themselves, there still remains the possibility that, once adsorbed, ghosts might be inactivated (irreversibly) or detached from the cells by the presence of EDTA, thereby giving an apparent failure of inhibition. To test this possibility, the following experiment was carried out. Cells were allowed to adsorb ghosts at 0 °C in the presence of EDTA for 10 min, and then the unadsorbed ghosts were removed by centrifuging at 0 °C. The cell-ghost complex collected was resuspended in a chilled medium lacking EDTA, and the portions of this cell suspension were examined for
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[I^4C]-TMG uptake ability at 0 °C (2 h), without any treatment, with Mg++ added, or after 37 °C treatment (2 min) in the presence of Mg++. The result (Table 1) shows that the ghosts adsorbed in the presence of EDTA still retain the capacity of inhibiting cells if they were subjected to heat treatment in the presence of Mg++. The results reported here indicate that the mere attaching of ghosts to the cell surface would probably not be sufficient to kill the cells even under conditions where metabolic energy and heat are supplied. In fact, King (1968) demonstrated that T4 mutants, defective in gene 11 or 12 (defect in tail sheath function), cannot kill host bacteria after adsorption, although the adsorption of these phage followed almost the same kinetics as that of wild-type phage. On the other hand, it was shown (Kozloff & Lute, 1959) that for the contraction of the tail sheath of T-even bacteriophage, divalent cations are required and that EDTA therefore blocks the process of sheath contraction. In view of these facts, the present finding that T4 ghosts cannot exert the inhibitory activity in the presence of EDTA may be interpreted to mean that the contraction of the tail sheath might be necessary for expression of the inhibitory action of T4 ghosts after their adsorption onto the cells.

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