PHAGE INACTIVATION BY ACLA Chinomicyn A AND ITS ANALOGUES

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Streptomyces galilaeus MA144-M1 produces antitumor anthracycline antibiotics aclacinomycins, A and B, and their analogues.1) Aclacinomycins consist of an aglycone (aklavinone) and sugar moiety. Aclacinomycin analogues consist of combinations of different sugars and aglycone. The structural interrelationships of these compounds have been determined by chemical and enzymatic conversions coupled with spectral interpretations.2) Among these anthracyclines, aclacinomycin A possesses a high antitumor activity against a number of animal and human tumors with low cardiac toxicity.3)

In preceding papers, we have reported that bacteriophage QX174, containing single-stranded circular DNA, was inactivated by anthracycline antibiotics (daunomycin, adriamycin and aclacinomycin A).4,5 Aclacinomycin A showed little inactivation activity against ,6X174, unless Cu2+ was present. Therefore, we reinvestigated the interaction of aclacinomycin A with bacteriophage 1., containing double-stranded linear DNA. Furthermore, we compared phage inactivation by 9 aclacinomycin analogues in relation to their structures and found that the length of the sugar chain and the amino group of the sugar moiety played significant roles in antiphage activity.

Aclacinomycin A and 8 aclacinomycin analogues; aclacinomycin B, MA144 M1 and N1 (in which terminal sugar of aclacinomycin A is converted to L-amicetose and L-rhodinose, respectively), MA144 L1 (N-monomethylaclacinomycin A), MA144 K1 (N-demethylaclacinomycin A), MA144 Si (decinerulosylaclacinomycin A) and MA144 Ti (aklavin), and aklavinone5) were generously supplied by Sanraku Ocean Co., Ltd. Bacteriophages φX174 and λ were prepared as described earlier.6,7) Escherichia coli C57 was used as the indicator bacteria for wild type φX174 and E. coli C600 for phage λ. The infectivity of phages was assayed by the double agar layer technique.8) Agarose gel electrophoresis of 6X174 single-stranded DNA was carried out as reported earlier.9) Table 1 shows the effects of aclacinomycin A and its analogues on the infectivity of phage φX174. Even high concentrations (1 mM) of aclacinomycins had only a weak effect on 6X174 infectivity. However, in the presence of Cu2+, there was an enhancement of phage inactivation with the exception of aklavinone, the aglycone of aclacinomycin A. This stimulatory effect of Cu2+ was the most marked in the cases of aclacinomycin A, and MA144 L1 and K1.

The results of phage inactivation by aclacinomycins are in good agreement with the data obtained from the agarose gel electrophoretic analysis (Fig. 1). These antibiotics alone did not cause strand scission in φX174 single-stranded DNA (Fig. 1 lanes B~F), while aclacinomycin A and B, and MA144 L1 and K1 showed DNA cleaving activity in the presence of Cu2+. The φX174 circular DNA band decreased while the linear DNA band increased. Moreover, degraded smaller fragments of φX174 DNA appeared as a smear (Fig. 1 lanes G, H, J and K). The results coincided with the degree of φX174...
Fig. 1. Induction of strand scission in φX174 DNA by aclacinomycins in the presence of Cu²⁺.

The reaction mixture (20 μl) contained 0.2 μg φX174 single-stranded DNA and 100 μM of an aclacinomycin in 50 mM Tris-HCl buffer (pH 8.1). Reactions were carried out for 180 minutes at 37°C in the presence or absence of 50 μM CuCl₂.


Table 2. Inactivation of phage λ by aclacinomycins and effects of metal ions.

<table>
<thead>
<tr>
<th>Aclacinomycins</th>
<th>Phage survival (%)</th>
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<tbody>
<tr>
<td></td>
<td>Antibiotic**</td>
<td>+CuCl₂*</td>
<td>+FeCl₂*</td>
<td>+MgCl₂*</td>
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<tr>
<td>Aclacinomycin A</td>
<td>1.8</td>
<td>0.5</td>
<td>3.5</td>
<td>20.5</td>
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<tr>
<td>Aclacinomycin B</td>
<td>15.2</td>
<td>4.6</td>
<td>23.5</td>
<td>31.1</td>
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<tr>
<td>MA144 M1</td>
<td>8.8</td>
<td>1.1</td>
<td>11.5</td>
<td>42.3</td>
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<tr>
<td>MA144 N1</td>
<td>17.7</td>
<td>5.0</td>
<td>18.7</td>
<td>38.8</td>
</tr>
<tr>
<td>MA144 S1</td>
<td>1.0</td>
<td>0.2</td>
<td>3.5</td>
<td>6.3</td>
</tr>
<tr>
<td>MA144 T1</td>
<td>19.3</td>
<td>14.3</td>
<td>32.6</td>
<td>32.0</td>
</tr>
<tr>
<td>MA144 L1</td>
<td>6.3</td>
<td>1.3</td>
<td>9.3</td>
<td>37.3</td>
</tr>
<tr>
<td>MA144 K1</td>
<td>0.6</td>
<td>0.3</td>
<td>0.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Aklavinone</td>
<td>77.2</td>
<td>63.8</td>
<td>87.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Phage λ (3×10⁶ p.f.u./ml) was incubated with 50 μM of an aclacinomycin in the presence* or absence** of metal ions (50 μM of CuCl₂ and FeCl₂, 10 mM MgCl₂) in Tris-dilution buffer (pH 7.2) for 180 minutes at 37°C. Phage survival (%) is the ratio of the number of plaque forming units at 180 minutes to that at zero time. Metal ions did not affect the infectivity of phage λ at the concentrations used.

Inactivation by aclacinomycins (Table 1), indicating that φX174 inactivation is probably due to the degradation of single-stranded DNA.

When aclacinomycin A and its analogues were reacted with bacteriophage λ, it was inactivated more markedly than φX174 (Table 2). Aclacinomycins, except for aklavinone, inactivated phage λ at a concentration of 50 μM. In particular, MA144 S1 and K1 inactivated phage λ more effectively than aclacinomycin A. In addition, of the several metal ions added to the reaction mixture, Cu²⁺ stimulated the inactivation of phage λ by aclacinomycins while the other metals showed no effect except for high concentration (10 mM) of MgCl₂, which showed an inhibitory effect on phage λ inactivation.

In summary, aclacinomycin A and its analogues inactivated bacteriophage λ more effectively than...
The relationship between chemical structure of aclacinomycin A and its analogues, and antiphage activity obtained was as follows:

1) Aclacinomycins with disaccharides (MA144 SI) and trisaccharides (aclacinomycin A and MA-144 KI) were more active than monosaccharides (MA144 TI). Aklavinone, the aglycone of aclacinomycin A, did not inactivate phage λ at all.

2) The amino group of the sugar moiety was also important for antiphage activity. N-Demethylaclacinomycin A (MA144 KI) possesses more potent antiphage activity than N-monomethylaclacinomycin A (MA144 LI) or aclacinomycin A. Umezawa et al. reported that aclacinomycin A was nonmutagenic in the Ames' test, but its derivative N-demethylaclacinomycin A was mutagenic. It is of interest to note that N-demethylation of aclacinomycin correlates with its mutagenicity and phage inactivation activity. The importance of amino sugar residues for binding to DNA was emphasized by some experiments. Therefore, it would seem that the inactivation of phage λ by aclacinomycin A and its analogues depends on their interactions with phage DNA.

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References


