PHAGE INACTIVATION BY ACLACINOMYCIN 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 ϕX174, containing single-stranded circular DNA, was inactivated by anthracycline antibiotics (daunomycin, adriamycin and aclacinomycin A).[4,5] Aclacinomycin A showed little inactivation activity against ϕX174, unless Cu^{2+} was present. Therefore, we reinvestigated the interaction of aclacinomycin A with bacteriophage λ, 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 S1 (decinerulosylaclacinomycin A) and MA144 T1 (aklavin), and aklavinone[2] were generously supplied by Sanraku Ocean Co., Ltd. Bacteriophages ϕX174 and λ were prepared as described earlier.[6,7] *Escherichia coli* C_N 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.[3] Agarose gel electrophoresis of ϕX174 single-stranded DNA was carried out as reported earlier.[9]

Table 1. Inactivation of phage ϕX174 by aclacinomycins.

Aclacinomycins	Phage survival (%)	
	$-CuCl_2$	$+CuCl_2$
Aclacinomycin A	90.9	20.9
Aclacinomycin B	64.6	35.7
MA144 M1	75.6	62.5
MA144 N1	60.6	34.3
MA144 S1	65.5	63.3
MA144 T1	61.5	50.7
MA144 L1	84.5	10.4
MA144 K1	68.0	16.9
Aklavinone	64.9	77.9

Phage ϕX174 (3×10^3 plaque-forming units/ml) was incubated with 1 mM antibiotic for 180 minutes at 37°C in 50 mM Tris-HCl buffer (pH 8.1) in the presence or absence of 0.6 mM $CuCl_2$. Phage survival (%) is the ratio of the number of plaque-forming units at 180 minutes to that at zero time.

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 ϕX174 infectivity. However, in the presence of Cu^{2+}, there was an enhancement of phage inactivation with the exception of aklavinone, the aglycone of aclacinomycin A. This stimulatory effect of Cu^{2+} 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 electrophoreic 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 Cu^{2+}. 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 Cu^{2+}.

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 $CuCl_2$.

A and M: Drug-free control, B: aclacinomycin A, C: aclacinomycin B, D: MA144 M1, E: MA144 L1, F: MA144 K1, G: aclacinomycin A+$CuCl_2$, H: aclacinomycin B+$CuCl_2$, I: MA144 M1+$CuCl_2$, J: MA144 L1+$CuCl_2$, K: MA144 K1+$CuCl_2$, L: $CuCl_2$.

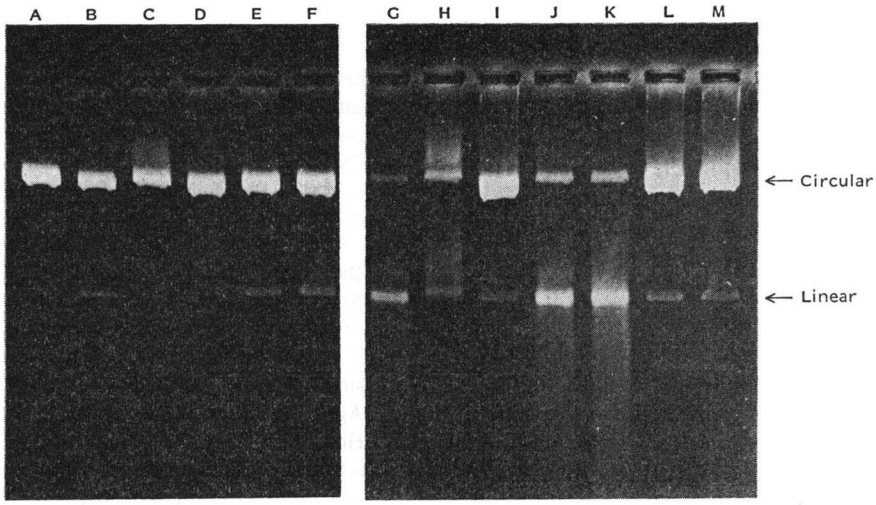

Table 2. Inactivation of phage λ by aclacinomycins and effects of metal ions.

Aclacinomycins	Phage survival (%)			
	Antibiotic**	+$CuCl_2$*	+$FeCl_2$*	+$MgCl_2$*
Aclacinomycin A	1.8	0.5	3.5	20.5
Aclacinomycin B	15.2	4.6	23.5	31.1
MA144 M1	8.8	1.1	11.5	42.3
MA144 N1	17.7	5.0	18.7	38.8
MA144 S1	1.0	0.2	3.5	6.3
MA144 T1	19.3	14.3	32.6	32.0
MA144 L1	6.3	1.3	9.3	37.3
MA144 K1	0.6	0.3	0.4	1.3
Aklavinone	77.2	63.8	87.0	100.0

Phage λ (3×10^8 p.f.u./ml) was incubated with 50 μM of an aclacinomycin in the presence* or absence** of metal ions (50 μM of $CuCl_2$ and $FeCl_2$, 10 mM $MgCl_2$) 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, Cu^{2+} stimulated the inactivation of phage λ by aclacinomycins while the other metals showed no effect except for high concentration (10 mM) of $MgCl_2$, which showed an inhibitory effect on phage λ inactivation.

In summary, aclacinomycin A and its analogues inactivated bacteriophage λ more effectively than

ϕX174. The relationship between chemical structure of aclacinomycin A and its analogues, and antiphage activity obtained was as follows: 1) Aclacinomycins with disaccharides (MA144 Sl) and trisaccharides (aclacinomycin A and MA-144 Kl) were more active than monosaccharides (MA144 Tl). 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*-Demethyl-aclacinomycin A (MA144 Kl) posesses more potent antiphage activity than *N*-monomethyl-aclacinomycin A (MA144 Ll) or aclacinomycin A. UMEZAWA *et al.* reported that aclacinomycin A was nonmutagenic in the AMES' test, but its derivative *N*-demethylaclacinomycin A was mutagenic.[10] 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.[11,12] 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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