The Mechanism of Inactivation of Bacteriophage ϕ X174 by Anthracycline Antibiotics

Akira TANAKA, Junji MORITA and Tohru KOMANO

Laboratory of Biochemistry, Department of Agricultural Chemistry,
Kyoto University, Kyoto 606, Japan

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The interaction of some anthracycline antibiotics (adriamycin, daunomycin, aclacinomycin-A) with bacteriophage ϕ X174 was investigated. Adriamycin and daunomycin inactivated the infectivity of both free ϕ X174 phage and naked single-stranded ϕ X174 DNA without DNA strand scission, but aclacinomycin-A did not show this action. The phage inactivation reaction was reversibly inhibited by superoxide dismutase, catalase or other oxygen radical scavengers. The inactivation of ϕ X174 by adriamycin and aclacinomycin-A was stimulated by the addition of Cu^{2+} , while the ϕ X174 inactivation by daunomycin was inhibited by the addition of Cu^{2+} . The ϕ X174 inactivation by adriamycin and aclacinomycin-A in the presence of Cu^{2+} was caused by degradation of DNA, and this inactivation reaction was inhibited irreversibly by oxygen radical scavengers. These results indicate that anthracycline antibiotics bind to ϕ X174 DNA in the form of free radicals and that during the auto-oxidation of these antibiotics in the presence of Cu^{2+} , oxygen radicals were generated to cause the degradation of ϕ X174 DNA.

Daunomycin (daunorubicin)¹⁾ and adriamycin (doxorubicin),²⁾ antibiotics of the anthracycline group, are known for their strong antitumor activities. They are structurally similar, but differ from each other in a hydroxyl group at C-14 of the tetracycline moiety. Aclacinomycin-A,³⁾ isolated from a culture of *Streptomyces galilaeus* MA144-M1, has an antitumor activity against various experimental tumors with low cardiac toxicity.⁴⁾

It has been reported that daunomycin inhibits the multiplication of DNA viruses such as the herpes simplex and vaccinia viruses, and bacteriophages of the T series.⁵⁾ We also reported that daunomycin and adriamycin inactivated the infectivity of free ϕ X174 phage containing single-stranded DNA, but aclacinomycin-A did not.⁶⁾

The antitumor activity of the anthracycline antibiotics is considered to be due to intercalation between adjacent nucleotides in the DNA strand. The intercalation resulted in significant alternations in the secondary structure and in reducing the template activity for RNA polymerase or DNA polymerase.⁷⁾ It has

been recently reported that reduced forms of adriamycin, daunomycin and aclacinomycin-A caused single-stranded scission in phage PM2 DNA.^{8,9)}

In this paper, we report the effect of anthracycline antibiotics on bacteriophage ϕ X174 and its DNA. Adriamycin and daunomycin inactivated ϕ X174 and its naked DNA by the formation of a DNA-drug complex without DNA strand scission. Adriamycin and aclacinomycin-A inactivated ϕ X174 with DNA strand scission in the presence of Cu^{2+} .

MATERIALS AND METHODS

Chemicals. Daunomycin hydrochloride and adriamycin hydrochloride were supplied by Farmitalia, Milan, Italy. Aclacinomycin-A hydrochloride was generously given by Sanraku Ocean Co., Ltd. Superoxide dismutase (EC 1.15.1.1) and catalase (EC 1.11.1.6) were purchased from Sigma Chemical Co. Redistilled water was used throughout the experiments.

Phages and bacteria. Bacteriophage ϕ X174, and ³²P- or ¹⁴C-thymidine-labeled ϕ X174am3 were prepared as described earlier.¹⁰⁾ *Escherichia coli* C_N was used as the

indicator bacteria for wild type ϕ X174 and *E. coli* C_x for ϕ X174am3.

DNA extraction. ϕ X174 DNA was extracted from phage particles by the hot phenol method¹¹⁾ and precipitated with two volumes of ethanol overnight at -40°C . The precipitates were collected by centrifugation and resuspended in $0.1\times\text{SSC}$ (Standard Saline-Citrate solution; 0.15 M NaCl - 0.015 M sodium citrate, pH 7.0).

Reaction of ϕ X174 particles or DNA with anthracycline antibiotics. The reaction mixture (1.0 ml) contained 0.35 mM anthracycline antibiotics, 3×10^8 plaque-forming units (pfu) of ϕ X174 in 0.05 M Tris-HCl buffer (pH 8.1) and additional agents when necessary. The reaction was carried out for 180 min at 37°C with gentle shaking. An aliquot (0.1 ml) was withdrawn and diluted 100-fold with ice-cold 0.05 M Tris-HCl buffer (pH 8.1) to stop the reaction. The infectivity of the phage was assayed by the agar overlayer method of Adams.¹²⁾ Survival ratio (%) was calculated from the ratio of the number of plaque-forming units at 180 min to that at zero time.

When ϕ X174 DNA was treated with anthracyclines, 1×10^9 pfu equivalent of ^{32}P -labeled ϕ X174am3 DNA was incubated with 0.35 mM drugs in 0.05 M Tris-HCl buffer (pH 8.1) for 180 min at 37°C . The DNA was precipitated with ethanol at -40°C to stop the reaction and to remove unreacted anthracycline antibiotics. The precipitates were collected and resuspended in $0.1\times\text{SSC}$ and analyzed by sucrose gradient sedimentation.

Alkaline sucrose gradient sedimentation analysis. ϕ X174 DNA was layered on a 4.5 ml 5 to 20% (w/v) alkaline sucrose gradient in 0.05 M NaCl , 3 mM EDTA , 0.3 M NaOH and 0.1% Sarkosyl. The gradient was centrifuged for 7 hr at 38,000 rev/min in a Hitachi RPS40T-2 rotor. After fractionation, the radioactivity was counted in Triton X-100-toluene scintillation fluid with a Beckman liquid scintillation counter.¹³⁾

Transfection assay. The biological activity of phage DNA was examined by the calcium-dependent transfection system as described earlier.¹⁴⁾

RESULTS

Kinetics of inactivation of ϕ X174 by anthracycline antibiotics

Figure 1 shows the inactivation curves of ϕ X174 with adriamycin, daunomycin and aclacinomycin-A in the absence or presence of Cu^{2+} . Adriamycin and daunomycin inactivated ϕ X174 in the absence of Cu^{2+} . The phage inactivation by adriamycin was stimulated by Cu^{2+} . Aclacinomycin-A alone did not

FIG. 1. Inactivation of ϕ X174 by Anthracycline Antibiotics.

ϕ X174 (3×10^8 pfu/ml) was incubated at 37°C with 0.35 mM anthracycline antibiotics in the presence or the absence of 0.2 mM CuCl_2 . At the indicated times, an aliquot (0.1 ml) was withdrawn, diluted with ice-cold 0.05 M Tris-HCl buffer (pH 8.1) and assayed for infectious phages by the agar overlayer method. Survival ratio (%) is the ratio of the number of plaque-forming units at the indicated time to that at zero time. $\circ$, aclacinomycin-A; $\bullet$, aclacinomycin-A and CuCl_2 ; $\square$, daunomycin; $\blacksquare$, daunomycin and CuCl_2 ; $\triangle$, adriamycin; $\blacktriangle$, adriamycin and CuCl_2 ; $\times$, CuCl_2 .

affect the infectivity of ϕ X174, but it inactivated ϕ X174 in the presence of Cu^{2+} . The phage inactivation by daunomycin, on the contrary, was inhibited by Cu^{2+} .

Effect of pH on phage inactivation reactions

The phage inactivation reactions by anthracycline antibiotics depended on pH (Fig. 2). The degree of phage inactivation by adriamycin increased with decreasing pH below 8.0. Aclacinomycin-A did not inactivate ϕ X174 at pHs above 8.0, although it slightly affected the infectivity of ϕ X174 at pHs below 8.0. Daunomycin inactivated ϕ X174 infectively between pH 7.6 and 8.2.

Effect of metal ions

As shown in Figure 1, Cu^{2+} greatly affects ϕ X174 inactivation by anthracycline anti-

FIG. 2. pH Dependence of ϕ X174 Inactivation by Anthracycline Antibiotics.

The reaction was carried out for 180 min at 37°C in 0.05 M Tris-HCl buffer at an appropriate pH. Survival ratio (%) is the ratio of the number of plaque-forming units in the presence of 0.35 mM anthracycline antibiotics to that in the absence of antibiotics. ○, aclinomycin-A; □, daunomycin; △, adriamycin.

FIG. 3. Effect of Cu^{2+} on Inactivation of ϕ X174 by Anthracycline Antibiotics.

ϕ X174 (3×10^8 pfu/ml) was incubated with 0.35 mM anthracycline antibiotics for 180 min at 37°C in 0.05 M Tris-HCl buffer (pH 8.1) in the presence of the indicated concentrations of CuCl_2 . ○, aclinomycin-A; □, daunomycin; △, adriamycin.

biotics. ϕ X174 particles were treated with 0.35 mM antibiotics in the presence of various concentrations of CuCl_2 (Fig. 3). Low concentrations of CuCl_2 up to $1 \mu\text{M}$ did not affect the phage inactivation by anthracycline anti-

TABLE I. EFFECTS OF METAL IONS ON INACTIVATION OF ϕ X174 BY ANTHRACYCLINE ANTIBIOTICS

ϕ X174 (3×10^8 pfu/ml) was incubated with 0.35 mM anthracycline antibiotics in the presence of 0.1 mM metal ions in 0.05 M Tris-HCl buffer (pH 8.1) for 180 min at 37°C. Survival ratio (%) is the ratio of the number of plaque-forming units in the presence of antibiotics (None*), or antibiotics and metal ions to that of a drug-free control. Metal ions did not affect the infectivity of ϕ X174 at this concentration.

Metals (0.1 mM)	Survival ratio (%)		
	Adriamycin	Aclacinomycin-A	Daunomycin
None*	48.4	82.4	20.8
CuCl_2	2.4	28.5	47.1
FeCl_3	54.6	91.5	47.5
FeSO_4	26.4	67.2	22.8
MgSO_4	56.2	90.0	14.4
CaCl_2	40.8	97.5	23.8
MnCl_2	40.8	91.5	25.2
ZnCl_2	38.3	78.1	21.6

biotics. Higher concentrations than $1 \mu\text{M}$ of CuCl_2 stimulated the phage inactivation by adriamycin and aclinomycin-A. The stimulatory effect of Cu^{2+} on the reaction of adriamycin was greater than that of aclinomycin-A. The phage inactivation by daunomycin was inhibited by the addition of Cu^{2+} at concentrations above 0.05 mM.

The effect of various metal ions on ϕ X174 inactivation by anthracycline antibiotics is shown in Table I. Only Cu^{2+} strongly stimulated the phage inactivation by adriamycin and aclinomycin-A. Fe^{2+} had little potency for stimulating the phage inactivation by both antibiotics. Other metal ions such as Mg^{2+} , Mn^{2+} and Zn^{2+} did not promote the phage inactivating ability of adriamycin and aclinomycin-A. Cu^{2+} and Fe^{3+} inhibited the phage inactivation by daunomycin at the concentration of 0.1 mM. High concentrations of Fe^{2+} , Mg^{2+} and EDTA ($1 \sim 10$ mM) also inhibited the phage inactivation of daunomycin. This inhibitory effect of metal ions and EDTA was specific for daunomycin-induced phage inactivation.

Reversibility of inactivation of ϕ X174 by daunomycin

As described above, higher concentrations than 1 mM EDTA and MgSO_4 inhibited the ϕ X174 inactivation by daunomycin. When ϕ X174 was incubated for 180 min with daunomycin, EDTA or MgSO_4 was added to the reaction mixture (Fig. 4). The survival of

ϕ X174 was restored immediately after the addition of EDTA or Mg^{2+} . When the reaction mixture was incubated for an additional 60 min in the presence of EDTA or Mg^{2+} , ϕ X174 was no longer inactivated. Cu^{2+} also inhibited this phage inactivation reaction in a similar manner (data not shown). These results indicate that the phage inactivation by daunomycin is reversible.

FIG. 4. Reversibility of ϕ X174 Inactivation by Daunomycin.

ϕ X174 (3×10^8 pfu/ml) was incubated with 0.35 mM daunomycin at 37°C for 180 min, and 10 mM MgSO_4 or 10 mM EDTA was added to the reaction mixture. The reaction mixture was incubated for an additional 60 min, and at the indicated times, an aliquot (0.1 ml) was withdrawn, diluted with ice-cold 0.05 M Tris-HCl buffer (pH 8.1) and assayed for infectious phages. □, ϕ X174 inactivation curve with daunomycin; ●, addition of MgSO_4 ; ○, addition of EDTA. The arrow indicates the time when MgSO_4 or EDTA was added to the reaction mixture.

Effect of radical scavengers and enzymes

To determine whether oxygen radicals are involved in the inactivation of ϕ X174 by anthracycline antibiotics, the effect of various radical scavengers and enzymes was examined. The results are shown in Table II. All reactions were inhibited by catalase which removes hydrogen peroxide and by tiron, a scavenger for the superoxide anion. Superoxide dismutase inhibited the phage inactivations except for the reaction of adriamycin in the presence of Cu^{2+} . Each inactivation reaction was inhibited by one or two hydroxyl radical scavengers such as sodium benzoate, D-mannitol and KI, although hydroxyl radical scavengers which could inhibit all reactions were not found. 1,4-Diazabicyclo-[2,2,2]octane (DABCO) also inhibited some phage inactivation reactions. These results

TABLE II. EFFECTS OF RADICAL SCAVENGERS ON INACTIVATION OF ϕ X174 BY ANTHRACYCLINE ANTIBIOTICS

The reaction conditions were identical to those for Table I, except that the radical scavenger was added instead of metal ions. Radical scavengers used at these concentrations did not affect the infectivity of ϕ X174. Percentage inhibition was computed from the following equation, $(S_p - S_a)/(100 - S_a) \times 100$ (%), where S_p = the phage survival in the presence of radical scavenger and S_a = the phage survival in the absence of radical scavenger (drug only).

Scavengers	Concentrations	% Inhibition			
		ADM ^a	ADM + Cu^{2+}	ACM-A ^b + Cu^{2+}	DM ^c
Catalase	100 $\mu\text{g/ml}$	45.7	87.4	52.7	85.0
S.O.D. ^d	100 $\mu\text{g/ml}$	58.6	0	31.6	59.4
Tiron ^e	100 mM	20.0	55.3	63.4	81.0
AET ^f	10 mM	0	27.2	0	73.0
Sodium benzoate	100 mM	0	2.6	0	100
D-Mannitol	100 mM	27.6	0	90.7	0
KI	1 mM	34.3	0	82.5	0
DABCO ^g	100 mM	33.7	37.6	0	100

^a Adriamycin. ^b Aclacinomycin-A. ^c Daunomycin. ^d Superoxide dismutase. ^e 1,2-Dihydroxybenzene-3,5-disulfonic acid. ^f 2-Aminoethylisothiuronium bromide HBr. ^g 1,4-Diazabicyclo[2,2,2]octane.

TABLE III. REVERSAL EFFECTS OF RADICAL
SCAVENGERS ON ϕ X174 INACTIVATION
BY ANTHRACYCLINE ANTIBIOTICS

ϕ X174 (3×10^8 pfu/ml) was incubated with 0.35 mM anthracycline antibiotics in the presence or absence of 0.2 mM CuCl_2 in 0.05 M Tris-HCl buffer (pH 8.1) for 180 min at 37°C (None*) and then the radical scavenger was added to the reaction mixture. Survival was assayed immediately after the radical scavenger was added,^a and after further incubation for 30 min.^b Abbreviations are as in Table II.

Antibiotics	Radical scavengers	Survival ratio (%)	
		0 min ^a	30 min ^b
Daunomycin	None*	19.6	18.6
	Catalase (100 $\mu\text{g/ml}$)	34.3	70.8
	S.O.D. (100 $\mu\text{g/ml}$)	21.0	94.4
	DABCO (100 mM)	63.5	91.4
	AET (10 mM)	44.6	71.7
	Sodium benzoate (100 mM)	45.1	100
Adriamycin	None*	58.3	54.4
	Catalase (100 $\mu\text{g/ml}$)	74.3	57.7
	S.O.D. (100 $\mu\text{g/ml}$)	81.4	85.7
Aclacinomycin-A and CuCl_2	None*	13.7	11.2
	Catalase (100 $\mu\text{g/ml}$)	12.5	16.3
	S.O.D. (100 $\mu\text{g/ml}$)	17.6	8.2
Adriamycin and CuCl_2	None*	0.19	0.17
	Catalase (100 $\mu\text{g/ml}$)	0.23	0.19
	S.O.D. (100 $\mu\text{g/ml}$)	0.21	0.05

indicate that some free oxygen radicals participate in the anthracycline-induced ϕ X174 inactivation.

To examine the inhibitory effect of radical scavengers in more detail, radical scavengers or enzyme were added to ϕ X174 inactivated by anthracycline antibiotics. After the addition of radical scavengers, the reaction mixture was incubated for 30 min and the survival of ϕ X174 was assayed (Table III). The inhibition of ϕ X174 by daunomycin or adriamycin was reversed immediately when radical scavengers were added, and the restoration was also clearly observed at 30 min after addition of scavengers. But ϕ X174 inactivated by adriamycin or aclacinomycin-A in the presence of CuCl_2 did not recover on the addition of catalase,

superoxide dismutase and other radical scavengers. When the reaction mixture was incubated for an additional 30 min, the survival of ϕ X174 was not restored at all. The phage inactivation reaction of adriamycin is also partially reversible. But adriamycin and aclacinomycin-A in the presence of Cu^{2+} inactivated ϕ X174 irreversibly.

Analysis of ϕ X174 DNA by sucrose density gradient sedimentation

To examine the target molecules in phage particles inactivated by anthracycline antibiotics, viral DNA was extracted from ^{14}C -thymidine labeled ϕ X174 particles treated with anthracycline antibiotics and analyzed by alkaline sucrose sedimentation. As shown in Fig. 5 (a), (c) and (e) the DNA from ϕ X174 treated with antibiotics in the absence of Cu^{2+} sedimented as a single peak at the position of closed circular DNA and DNA strand scission was not observed. When ϕ X174 was treated with aclacinomycin-A in the presence of Cu^{2+} , half of the extracted DNA was detected as linear DNA (Fig. 5, b). The DNA in the ϕ X174 particles inactivated by adriamycin and Cu^{2+} sedimented as a broad peak having a sedimentation coefficient of 10S (Fig. 5, d). Adriamycin showed more marked capacity for DNA cleavage than aclacinomycin-A. The results indicate that adriamycin and aclacinomycin-A in the presence of Cu^{2+} cleave ϕ X174 single-stranded DNA in the virion. Neutral sucrose gradient sedimentation analysis confirmed that inactivated phage particles were not degraded and retained the DNA within coat proteins (data not shown). Adsorption experiments revealed that ϕ X174 treated with anthracycline antibiotics can adsorb normally to host cells (data not shown). These results suggest that anthracycline antibiotics do not damage ϕ X174 coat proteins although it can not be excluded that a minor modification occurred in the coat proteins.

Interaction of anthracycline antibiotics with isolated ϕ X174 DNA

The above results revealed that the target

FIG. 5. Alkaline Sucrose Gradient Sedimentation Analysis of ϕ X174 DNA Extracted from Inactivated Phage Particles.

^{14}C -Thymidine labeled ϕ X174 *am3* (2×10^{10} pfu/ml) was treated with 0.35 mM anthracycline antibiotics for 180 min at 37°C in 0.05 M Tris-HCl buffer (pH 8.1). Deproteinized DNA was sedimented through a 4.5 ml 5 to 20% (w/v) alkaline sucrose gradient for 7 hr at 38,000 rev/min in a Hitachi RPS40T-2 rotor at 4°C . After fractionation, the radioactivity was measured. Arrows indicate the position of ϕ X174 closed circular DNA. (a) aclacinomycin-A; (b) aclacinomycin-A and 0.2 mM CuCl_2 ; (c) adriamycin; (d) adriamycin and 0.2 mM CuCl_2 ; (e) daunomycin. Survival ratios in this experiment were (a) 100%, (b) 47.8%, (c) 20.3%, (d) 7.8% and (e) 12.1%, respectively.

FIG. 6. Inactivation of the Biological Activity of ϕ X174 DNA by Anthracycline Antibiotics.

Isolated ϕ X174 *am3* DNA (1×10^{11} plaque-forming unit equivalents) was treated with various concentrations of anthracycline antibiotics in 0.05 M Tris-HCl buffer (pH 8.1) for 180 min at 37°C . An aliquot was withdrawn, diluted with the same buffer and assayed for the infectivity with calcium-treated *E. coli* Cx cells. $\circ$, aclacinomycin-A; $\square$, daunomycin; $\triangle$, adriamycin.

molecule of the phage inactivation by anthracyclines in the presence of Cu^{2+} is DNA in the virion. But adriamycin and daunomycin inactivate ϕ X174 without DNA strand scission. To examine whether or not anthracycline antibiotics directly affect ϕ X174 DNA, isolated single-stranded DNA was exposed to these drugs. This DNA was assayed for the biological activity by the transfection assay method and analyzed by alkaline sucrose gradient sedimentation. As shown in Fig. 6, the transfection ability of ϕ X174 DNA was reduced by adriamycin and daunomycin, but not by aclacinomycin-A. Adriamycin inactivated ϕ X174 DNA more effectively than daunomycin. Each inactivated DNA sedimented as a single peak at the position of closed circular DNA and nicked DNA was not observed (data not shown). It is considered that the effect of these antibiotics on the transfection ability of ϕ X174 DNA was not caused by DNA strand scission but by another type of DNA damage, for example, the binding to single-stranded DNA.

DISCUSSION

Anthracycline antibiotics inactivate the infectivity of phage ϕ X174 and its isolated DNA. The present results indicate that the target molecule of ϕ X174 inactivation by anthracyclines is phage DNA. This phage inactivation is considered to be due to transient damage of phage DNA, the formation of a DNA-drug complex. The complex is formed by reversible binding between DNA and drug free radicals produced by the reaction with oxygen, because the phage inactivation is reversibly inhibited by some oxygen radical scavengers. The phage inactivation by daunomycin is reversibly inhibited also by EDTA or metal ions. The binding of anthracycline antibiotics to double-stranded DNA has been demonstrated by several physicochemical procedures.¹⁵⁾ Calendi *et al.*¹⁶⁾ reported that a daunomycin-DNA complex is released by Mg^{2+} . Since ϕ X174 single-stranded DNA can form the helical structure in the virion or in aqueous solutions,^{17,18)} anthracyclines may bind to the helical structural part of ϕ X174 DNA. When the interaction of anthracycline antibiotics with isolated ϕ X174 DNA was examined using the technique of agarose gel electrophoresis, it was observed that daunomycin and adriamycin reduced the migration rate of ϕ X174 single-stranded DNA in agarose gel, although aclacinomycin-A did not alter the migration rate (unpublished results). This result also supports the binding of anthracycline antibiotics to ϕ X174 DNA. Daunomycin inactivated ϕ X174 particles more strongly than adriamycin. This result does not agree with the data showing that adriamycin reduced the transfection ability of ϕ X174 DNA more effectively than daunomycin. The discrepancy between these results may be due to the difference of the permeability between adriamycin and daunomycin into the ϕ X174 particle. Since aclacinomycin-A did not affect the infectivity of ϕ X174, this antibiotic can not bind ϕ X174 DNA so strongly as adriamycin and daunomycin. But aclacinomycin-A seems to bind double-stranded DNA, as it inactivated λ

phage in the absence of Cu^{2+} (unpublished results).

In the presence of Cu^{2+} , aclacinomycin-A inactivated ϕ X174. Adriamycin also inactivated ϕ X174 more effectively in the presence of Cu^{2+} than in its absence although daunomycin did not. These phage inactivations in the presence of Cu^{2+} are due to the *in situ* degradation of ϕ X174 DNA in the phage particles. Furthermore, some radical scavengers inhibit anthracycline antibiotic-induced phage inactivations in the presence of Cu^{2+} irreversibly. These results indicate that oxygen radicals such as the superoxide radical, the hydroxyl radical and singlet oxygen are involved in the induction of the cleavage of ϕ X174 DNA as well as in binding to ϕ X174 DNA. Cu^{2+} has a catalytic effect on the auto-oxidation of compounds with low redox potential,¹⁹⁾ therefore it is necessary not only for the binding to ϕ X174 DNA but also for the DNA cleavage. Cu^{2+} may make ϕ X174 DNA more prone to be attacked by oxygen radicals through DNA destabilization by the formation of complex with anthracycline antibiotics.

Lown *et al.*⁸⁾ have reported that adriamycin and daunomycin reduced by sodium borohydride induce the DNA strand scission on phage PM2 and they have concluded that this DNA cleavage is due to the attack of the hydroxyl radical produced during reduction and auto-oxidation of these anthracycline antibiotics. Someya and Tanaka⁹⁾ suggest that the free radical of anthracycline quinone (semiquinone radical) also reacts with PM2 DNA. It is considered that oxygen radicals were generated by the reduction and subsequent auto-oxidation of the quinone moiety in anthracycline. On the contrary, ϕ X174 inactivation or DNA strand scission in DNA by anthracycline antibiotics does not need reducing agents. Sodium borohydride and other reducing agents such as NADPH, $Na_2S_2O_4$ and thiol compounds exhibited no effect on ϕ X174 inactivation.⁶⁾ Our results agree with the report that oxygen radicals are produced only by the auto-oxidation of the *p*-hydroxyl group in anthracycline.²⁰⁾

The drug-DNA complex formed in the absence of Cu^{2+} may penetrate into the host cells. The drug-DNA complex itself reduces the template activity of phage DNA for DNA polymerase or RNA polymerase. In addition, if the drug-DNA complex is reduced or oxidized by intracellular enzymes in the presence of the correct amounts of Cu^{2+} , DNA strand scission may occur.

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