Effect of Oxidized Polyamines on the Infectivity of ϕ X174 DNA

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Oxidized spermine and oxidized spermidine inhibited markedly the infectivity of the 6M-urea treated ϕ X174 particle, whereas they did not inactivate the infectivity of the untreated phage particle. They also markedly inhibited the infectivity of ϕ X174 DNA, while ϕ X174 RF I DNA was less sensitive to these reagents. These facts suggested that oxidized polyamines could react with phage DNA.

The possible reasons of the insensitivity of phage ϕ X174 particle and less sensitivity of ϕ X174 RF I DNA to these reagents were discussed.

Oxidized polyamines, the oxidized products of naturally occurring spermine and spermidine by beef plasma amine oxidase, have been shown to be toxic for some bacteriophages.^{1~3)} Oxidized spermine forms a complex with DNA of phage T₅, and the complex is injected into the host cells on infection.⁴⁾ The oxidized spermine also forms a complex with DNA of phage λ , and whereas the complex is injected into host cells, it does not form the mature phages.⁵⁾

When phages ϕ X174 and T₄, insensitive to oxidized spermine, are dialyzed exhaustively, these phages become susceptible to the reagent.⁵⁾ On the contrary, phages P465 and T₇, which are inactivated markedly by oxidized

spermine, become insensitive by the pretreatment with spermine.⁵⁾ These facts suggest that the oxidized polyamines compete with polyamines in reaction with phage DNA.

It is expected that the toxicity of oxidized polyamines is caused primarily by their binding to phage DNA. Effects of oxidized polyamines on the infectivity of urea-treated ϕ X174 particles and ϕ X174 DNA were therefore examined.

The present work deals with the inactivation of urea-treated ϕ X174 particles, infectious ϕ X174 DNA (single stranded circular DNA), and its replicative form DNA (RF I: double stranded closed circular DNA) by oxidized spermine, oxidized spermidine and oxidized N-n-butyl propan-1,3-diamine.

MATERIALS AND METHODS

Media. TKB medium was described by Denhardt and Sinsheimer.⁶⁾ TPG-CA medium was described by Lindqvist and Sinsheimer⁷⁾ in which casamino

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3) T. Oki, H. Kawasaki, K. Ogata, H. Yamada, I. Tomida, T. Morino and H. Fukami, *Agr. Biol. Chem.*, **32**, 1849 (1968).

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5) T. Oki, H. Kawasaki, K. Ogata, H. Yamada, I. Tomida, T. Morino and H. Fukami, *Agr. Biol. Chem.*, **33**, 994 (1969).

6) D. T. Denhardt and R. L. Sinsheimer, *J. Mol. Biol.*, **12**, 641 (1965).

7) B. H. Lindqvist and R. L. Sinsheimer, *ibid.*, **28**, 87 (1967).

acid was used instead of amino acid mixture. 3XD, PA and PAM media were described by Guthrie and Shinsheimer.⁸⁾ Dilution buffer contained 0.05 M-tris and 0.01 M-sodium borate (pH 8.0).

Bacteria and phages. *E. coli* K12W6 grown in 3XD medium was used for the preparation of spheroplasts. *E. coli* HF4714 grown in TKB medium was used for the titration of the phage. *E. coli* C_N grown in TPG medium enriched with casamino acid was used for the preparation of phage ϕ X174 am 3 and RF I.

ϕ X174 am 3 was purified by the method of Sedat and Sinsheimer⁹⁾ and was used throughout the experiments.

Enzymes and chemicals. Crystalline egg white lysozyme was purchased from Sigma Chemical Company. Highly purified amine oxidase was prepared from beef plasma by the method of Yamada and Yasunobu.¹⁰⁾

Spermine tetrahydrochloride and spermidine trihydrochloride were the products of Sigma Chemical Company.

Oxidized spermine, oxidized spermidine and oxidized N-n-butyl propan-1,3-diamine were prepared by the treatment with beef plasma amine oxidase as described previously.³⁾

Spheroplast. Spheroplasts were prepared from *E. coli* K12W6 by the modified method of Guthrie and Sinsheimer.⁸⁾ Cells grown to 5×10^8 /ml in 30 ml of 3XD medium were centrifuged and the pellet was suspended in 0.35 ml of 1.5M-sucrose. The following solutions were added in order with gentle mixing after each: 0.26 ml of 20% gelatin solution (sterilized), 0.2 ml of 2 mg/ml lysozyme solution, 0.4 ml of 4% EDTA and 10 ml of PA medium. The mixture was incubated at room temperature for 2 hr, to which was added 0.5 ml of 50% MgSO₄ solution containing 10% sucrose.

Urea treatment. The phage spikes of ϕ X174 were removed by the modified method of Edgell *et al.*¹¹⁾ Three volumes of 8M-urea in 0.01 M-tris buffer (pH 8.0) were added to 1 volume of the phage solution,

the titer of which was adjusted to approximately 3×10^9 infective units per ml. The mixture was incubated at 30°C for 3 hr. This was dialyzed overnight against 100 volumes of cold 0.1 M-NaCl and then against fresh 100 volumes of the same solution for an additional 5 hr.

Phage DNA and RF I. Phage ϕ X174 DNA was extracted from purified phage particles by the hot phenol method.⁸⁾

RF I DNA was isolated and purified by the method of Komano and Sinsheimer.¹²⁾

Infectivity assay. The infectivity assay of the DNA was performed by the reported procedure.⁸⁾ The infectivity assay of urea-treated ϕ X174 particle was also performed by the same procedure, in which 0.4 ml of urea-treated phage particle solution was used instead of DNA solution.

RESULTS AND DISCUSSION

When phage T₆, a titer of 10^8 p.f.u., was treated with 0.6 mM of oxidized spermine or oxidized spermidine at 30°C for 90 min, survival titer was usually less than 100. Phage ϕ X174 was, on the other hand, unaffected under the same conditions.

When 6M-urea treated ϕ X174 particles were incubated with spheroplasts without pre-treatment with oxidized polyamines, the yield of mature phage was about 1×10^4 p.f.u., while incubated with host bacteria there was no detectable amounts of phage production.¹³⁾

Effect of oxidized spermine and oxidized spermidine concentrations on the inactivation of infectivity of 6M-urea treated ϕ X174 particles was examined. The 6M-urea treated phage particles (1.4×10^8 p.f.u. phage equivalents) pre-treated with the same volume of various concentrations of oxidized spermine or oxidized spermidine for 5 min at 30°C was incubated with spheroplasts and continued to incubate for 2 hr in PAM medium. As shown

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11) M. H. Edgell, C. A. Huchison and R. L. Sinsheimer, *J. Mol. Biol.*, **42**, 547 (1969).

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13) K. Nishimura, T. Komano, H. Yamada and H. Fukami, unpublished data.

FIG. 1. Effects of Oxidized Spermine and Oxidized Spermidine Concentrations on the Infectivity of 6M-urea Treated ϕ X174.

An amount of 0.4 ml of spheroplasts solution was incubated with 0.4 ml of the 6M-urea treated ϕ X174 (7.0×10^7 /ml phage equivalents) at 30°C for 15 min which had been treated with oxidized spermine (●—●) or oxidized spermidine (○—○) in the dilution buffer, pH 7.0, at 30°C for 5 min. To the infected spheroplast solution were added 3.2 ml of PAM medium and the mixture was incubated at 30°C for 2 hr.

in Fig. 1, the infectivity of 6M-urea treated phage particles decreased markedly even when they were treated with 0.001 mM of oxidized spermine or 0.01 mM of oxidized spermidine for 5 min.

When phage ϕ X174 (1.4×10^8 p.f.u.) was treated with 4M-urea and dialyzed against NaCl solution according to the procedure of Edgell *et al.*, it was still infectious to host bacteria (5×10^4 p.f.u.) but not to spheroplasts and was unaffected even on the treatment with 0.6 mM of oxidized polyamines. It seemed that oxidized polyamines could not penetrate into the 4M-urea treated phage particle, as the spikes were not removed completely or the conjugation of spikes to the phage particle was reversible so that they reconstructed under the condition used in the treatment with oxidized polyamines.

The fact that the infectivity of the phage particles treated with 6M-urea but not with 4M-urea was decreased markedly by oxidized polyamines might indicate that the spikes were removed from the phage particles as described by Edgell *et al.*: these reagents might enter into the phage particles through the parts to which the spikes had been attached and reacted with the phage DNA and then the particles might be inactivated.

Effect of incubation time of 6M-urea treated phage particles with 0.001 mM of oxidized spermine and oxidized spermidine on the infectivity was investigated. The results are shown in Fig. 2. Incubating with 0.001 mM of oxidized spermine, the infectivity of the urea-treated phage particles was reduced with logarithmic linearity up to 5 min. On the other hand, the same concentration of oxidized spermidine gave no effects on the infectivity.

Effect of oxidized spermine and oxidized

FIG. 2. Effects of Incubation Time of 6M-urea Treated ϕ X174 with Oxidized Spermine and Oxidized Spermidine on the Infectivity.

Conditions of the infection were the same as described in Fig. 1. An amount of 0.2 ml of the 6M-urea treated ϕ X174 (1.4×10^8 p.f.u. phage equivalents) was treated with the same volume of 0.002 mM of oxidized spermine (●—●) or oxidized spermidine (○—○) at 30°C.

FIG. 3. Effects of Oxidized Spermine and Oxidized Spermidine Concentrations on the Infectivity of ϕ X174 DNA.

An amount of 0.4 ml of spheroplasts was incubated with 0.4 ml of ϕ X174 DNA solution (2×10^9 particles) at 30°C for 15 min which had been treated with oxidized spermine (●—●) or oxidized spermidine (○—○) in the dilution buffer, pH 7.0, at 30°C for 5 min. 3.2 ml of PAM medium were added to the infected spheroplast solution and the mixture was incubated at 30°C for 2 hr.

spermidine concentrations on the infectivity of ϕ X174 DNA was examined. The results are shown in Fig. 3. The infectivity of ϕ X174 DNA decreased markedly even when the DNA was treated with 0.1 mM of oxidized spermidine or 0.01 mM of oxidized spermine for 5 min. This indicates that ϕ X174 DNA interacts sufficiently with oxidized spermine and oxidized spermidine.

Effect of incubation time of ϕ X174 DNA and RF I DNA with 0.1 mM of oxidized spermidine and oxidized N-*n*-butyl propan-1,3-diamine on the infectivity was examined. These reagents markedly decreased the infectivity of ϕ X174 DNA but not that of RF I DNA, as shown in Fig. 4. The less sensitivity of RF I DNA to oxidized spermidine and oxidized N-*n*-butyl propan-1,3-diamine might be due to the double stranded structure of the DNA.

FIG. 4. Effects of Incubation Time of ϕ X174 DNA and RF I DNA with Oxidized Spermidine and Oxidized N-*n*-Butyl Propan-1,3-diamine on the Infectivity.

Conditions of the infection were the same as described in Fig. 3. An amount of 0.2 ml of ϕ X174 DNA solution (which gave 2×10^9 phage particles; —) and RF I DNA solution (1×10^{10} single strand equivalents; ----) were treated with 0.2 mM of oxidized spermidine (○) or oxidized N-*n*-butyl propan-1,3-diamine (●).

Phage ϕ X174 particle was not inactivated by oxidized polyamines, but fully dialyzed phage was inactivated significantly, and the infectivity of the urea-treated phage particles as well as that of the purified DNA were inhibited markedly. These facts suggested that ϕ X174 DNA as well as DNA's of susceptible phages, T₈⁴¹ and λ ⁵¹ could react with oxidized polyamines. The reason of insensitivity of phage ϕ X174 particle to oxidized polyamines might be considered that the reagents could not penetrate through the coat protein or competed with polyamine(s) in phage particle, so that they could not react with phage DNA.

Effect of hydroxylamine on the infectivity of phage S13 DNA and its RF has been reported.¹⁴⁾ The RF is fairly stable on the

14) E. S. Tessman, *J. Mol. Biol.*, **17**, 218 (1966).

treatment with the reagent, while SI3 DNA is very sensitive. The inactivation of the infectivity of ϕ X174 DNA and RF I by hydroxylamine was therefore tested. The reagent inactivated the ϕ X174 DNA but not RF I. Moreover, glyoxal gave similar curves in inactivation of ϕ X174 DNA and RF I.¹⁵⁾ These reagents are known to react with specific bases in nucleic acid. Bachrach *et al.* described that guanine is the most probable base

to react with oxidized polyamines.¹⁶⁾ It is, therefore, speculated that a loss of infectivity is caused by the binding of these oxidized polyamines to DNA. In DNA molecule, bases modified with them may inhibit the activity of replication and transcription.

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