EFFECT OF MITOMYCIN C ON THE CAPACITY OF *E. COLI* TO SUPPORT MULTIPLICATION OF ϕA^1

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Effect of Mitomycin C treatment on the host cell capacity for multiplication of ϕA was investigated, taking advantage of Ca^{2+} -dependent transfection of the replicative-form DNA. In contrast to *uvr⁺*, *uvrD*, and *uvrF* strains, cells of *uvrA*, *uvrB*, or *uvrC* mutant sufficiently supported the viral multiplication after treatment with 50 $\mu g/ml$ of Mitomycin C. The host capacity of *uvrE* strain showed an intermediate sensitivity to Mitomycin C. Plaque yield of ϕA in *rec⁻* strains was, as in *rec⁺ uvr⁺* hosts, markedly decreased by pretreatment with Mitomycin C. In contrast with DNA polymerase II, both polymerizing activity and 5'-3' exonuclease activity of DNA polymerase I were involved in host-cell reactivation for ultraviolet-irradiated replicative form. The capacity to develop ϕA of *polA*, *polA 107*, and *resA* mutants was, like that of *pol⁺* or *polBI* strains, conspicuously decreased upon treatment with the antibiotic. Effect of several radiomimetic agents on the host capacity was compared with that of Mitomycin C. In ultraviolet sensitivity of the host capacity, a small difference was observed between *uvr⁺* bacteria and *uvrA* mutant. Infectivity yield of Mitomycin C-treated replicative-form was not particularly affected by *uvrA* mutation.

Whereas synthesis of bacterial DNA is selectively inhibited by MC² (1), DNA synthesis induced by certain bacteriophages is rather refractory to the antibiotic (2). On the other hand, multiplication and DNA synthesis of $\phi X174$ in *E. coli* C or *E. coli* 15 are severely inhibited by MC treatment (3, 4). When, however, HCR⁻ strain such as HF4704 is used as the host, multiplication of the

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² Abbreviations used: MC, Mitomycin C. RF, double-stranded replicative form DNA. SS, single-stranded viral DNA. HCR, host-cell reactivation. 4A-2Q-ran, 4-amino-2[(5-nitro-2-furyl)vinyl]quinoline. PFU, plaque-forming unit. UV, ultraviolet light.

virus proceeds normally without concomitant synthesis of host DNA (5). Taking advantage of this property of HCR⁻ hosts, isotopic labeling studies of DNA replication in infected cells have been performed with several "mild" phages such as S13, M13, fd, or λ . Regardless of practical applications, little is known about the differential action of MC on viral DNA synthesis in HCR⁺ and HCR⁻ cells. It seems apparently paradoxical that inhibition of viral growth caused by MC is counteracted by a defect in HCR function required for repair of viral as well as host DNA. In order to understand better the host functions necessary for viral replication, a survey was made on the MC effect on the growth of ϕ A in various *E. coli* mutants deficient in DNA repair. Since *E. coli* strains used were mostly resistant to ϕ A, MC-pretreated or untreated bacteria were made competent by Ca²⁺ method and then challenged by the RF-DNA. The MC effect was clearly detected even in this transfection system (6).

MATERIALS AND METHODS

Bacteria and phage. Strain KMBL 1789 *polA* 107 (7) was furnished by Dr. B. W. Glickman. HF4704 *uvrA* (8), PK1620 *recA uvrA*, and ATR15 *resA1* (9) were obtained from Dr. M. Sekiguchi. Other sources of *E. coli* used were described previously (10, 11). ϕ A is a single-stranded DNA phage immunologically related to ϕ X174 (6).

Chemicals. MC was purchased from Sankyo Co., Tokyo, nalidixic acid was a gift from Daiichi Pharmaceutical Co., Tokyo, and 4A-2Q-ran was generously supplied by Dr. K. Miura. Phenethyl alcohol was obtained from Wako Pure Chemical Ind., Ltd., Osaka.

Treatment of host cells. Bacteria were grown in nutrient broth at 37° with shaking and, when the optical density at 660 nm reached 0.5, the culture was divided into two equal portions. To the one portion, MC (final concentration 50 μ g/ml) was added and shaken further for 10 min at 37°. The cultures were chilled, centrifuged, and the collected cells were suspended in chilled 50 mM CaCl₂. Treatment with 4A-2Q-ran (5 μ g/ml) was done similarly. When bacteria were to be treated with nalidixic acid (50 μ g/ml) or phenethyl alcohol (0.3%), incubation was extended to 25 min. For UV irradiation, cells were suspended in chilled 50 mM CaCl₂ and irradiated, for the indicated time, with a 15 W Mazda germicidal lamp at a distance of 25 cm.

Infectious DNA and transfection. The SS and RF DNA of ϕ A and ϕ X174, nonlabeled or labeled with ¹⁴C-thymine, were prepared as described previously (10). Bacteria were made competent by CaCl₂ method as described elsewhere (12). For transfection assay, 0.1 ml of the cell suspension (OD₆₆₀=15) in chilled 50 mM CaCl₂ was mixed with 0.05 ml of infectious DNA in 50 mM Tris-HCl, pH 7.5, and kept at 0° for 20 min. Without heat pulse, the infected cells were diluted with chilled 50 mM CaCl₂ and plated with the indicator bacteria (strain C), at least in

duplicate. Omission of a heat-pulse step simplified the assay method without reducing infectivity yield of the DNA.

MC treatment of RF. ϕ A RF labeled with ^{14}C -thymine was mixed at room temperature with 50 $\mu\text{g}/\text{ml}$ of MC and 200 $\mu\text{g}/\text{ml}$ of sodium hydrosulfite in a total volume of 0.5 ml. Q gas was bubbled through all the components and the reaction mixture. Control mixture without MC was run in parallel. After 5 min, the mixture was passed through a CPG-10 column (0.5×26 cm), using 50 mM Tris-HCl, pH 7.5, and 10 drops of the filtrate were collected per fraction. Peak of the labeled RF appeared at fraction 14 and MC (detected by bacteriocidal activity) was eluted around fraction 25.

RESULTS

MC action on viral growth

Infectivity yield of ϕ A or ϕ X174 RF in *E. coli* C was greatly reduced by

Fig. 1. Effect of MC on the growth of ϕ A in *E. coli* C.

Log phase cells of *E. coli* C *uvr*⁺ in broth were infected with ϕ A at a multiplicity of 0.1. At the indicated time, aliquot of the culture was transferred to a broth containing 50 $\mu\text{g}/\text{ml}$ of MC and shaken at 37°. Mature phage yield was determined 40 min after infection (●). In parallel, amount of intracellular phage was determined immediately before addition of MC (○).

incubating the bacteria with 50 $\mu\text{g/ml}$ of MC for 10 min, before Ca^{2+} treatment or after completion of RF uptake. Similar inhibition of the viral growth was observed when SS molecule was used as the transfecting DNA. In normal cell-whole virus system, MC exerts its inhibitory effect even in the late eclipse phase (Fig. 1). These results suggest that replication of progeny RF, and not parental RF formation, is blocked by the antibiotic.

Host capacity of uvr mutants treated with MC

As several *uvr* functions are known to participate in HCR process of UV-irradiated ϕA RF (10, 11), effect of MC on the capacity to support ϕA growth was investigated first with various *uvr* mutants. In *uvr*⁺ strains, the host capacity (expressed by the relative yield of transfectants) was generally reduced to less than 10^{-3} by pretreatment with 50 $\mu\text{g/ml}$ of MC. On the other hand, *uvrA*, *B* or *C* mutants of both N and AB series retained nearly normal capacity to propagate ϕA , after MC treatment (Table 1). While *uvrD* function was involved in HCR,

Table 1. Effect of MC treatment on the host capacity of *uvr* strains.

Strain	Plaque yield (PFU/ml)		
	Untreated	MC-treated	MC-treated/ Untreated
W3623 <i>uvr</i> ⁺	1.6×10^5	$< 1.0 \times 10^1$	6.2×10^{-5}
N17-9 <i>uvrA</i>	2.1×10^5	7.4×10^4	3.5×10^{-1}
N3-1 <i>uvrB</i>	1.8×10^4	1.3×10^4	7.2×10^{-1}
N17-7 <i>uvrC</i>	6.0×10^3	4.8×10^3	8.0×10^{-1}
N14-4 <i>uvrD</i>	1.3×10^4	$< 1.0 \times 10^1$	$< 7.7 \times 10^{-4}$
AB1157 <i>uvr</i> ⁺	2.3×10^5	$< 1.0 \times 10^2$	$< 4.4 \times 10^{-4}$
AB1886 <i>uvrA</i>	5.1×10^4	3.4×10^4	6.7×10^{-1}
AB1885 <i>uvrB</i>	2.3×10^5	1.3×10^5	5.7×10^{-1}
AB1884 <i>uvrC</i>	8.9×10^5	3.4×10^5	3.8×10^{-1}
CT-2 <i>uvr</i> ⁺	2.6×10^6	8.0×10^1	3.1×10^{-5}
HF4704 <i>uvrA</i>	9.3×10^5	5.0×10^5	5.4×10^{-1}
ES108 <i>mutU4(uvrE)</i>	3.3×10^5	2.8×10^4	8.5×10^{-2}
PC1249 <i>uvrF</i>	5.9×10^5	$< 1.0 \times 10^1$	$< 1.7 \times 10^{-5}$

Bacteria preincubated with or without MC were treated with CaCl_2 challenged with ϕA RF and then plaque yield was determined by infective center assay. The number of transfecting cells represents relative host capacity for ϕA .

the capacity of N14-4 *uvrD* was no more resistant to MC than were *uvr*⁺ bacteria. MC treatment significantly reduced the yield of transfectants in ES108 *mutU4* (= *uvrE*), but the decrement was much less than in *uvr*⁺ strains. HCR ability of *uvrF* mutant was as high as that of *uvr*⁺ cells and the host capacity of *uvrF* strain was quite sensitive to MC.

Host capacity of rec mutants treated with MC

Recombination deficient strains are generally sensitive to UV irradiation

Table 2. Effect of MC treatment on the host capacity of *rec* strains.

Strain	Plaque yield (PFU/ml)		
	Untreated	MC-treated	MC-treated/ Untreated
AB2463 <i>recA</i>	2.1×10^5	$< 1.0 \times 10^1$	4.8×10^{-5}
PK1620 <i>recA uvrA</i>	9.1×10^5	3.5×10^5	3.8×10^{-1}
AB2470 <i>recB</i>	6.6×10^5	1.5×10^2	2.3×10^{-4}
JC6720 <i>recBC</i>	8.9×10^5	4.5×10^2	5.0×10^{-4}
JC5183 <i>recBC sbcA</i>	2.0×10^6	1.3×10^3	6.5×10^{-4}
JC7623 <i>recBC sbcB</i>	2.5×10^5	4.0×10^1	1.6×10^{-4}
JC8287 <i>recBC xonA</i>	2.8×10^4	3.6×10^2	1.4×10^{-2}
PC0297 <i>recG</i>	2.8×10^5	1.6×10^2	5.7×10^{-4}
PC1250 <i>recH</i>	2.2×10^5	6.0×10^1	2.7×10^{-4}

The conditions are the same as in Table 1.

Fig. 2. HCR of UV-irradiated RF in Ca^{2+} -treated cells.

ϕ A RF was UV-irradiated with a 15 W germicidal lamp at 25 cm, in 50 mM Tris-HCl, pH 7.5, and the infectivity was determined on Ca^{2+} -treated cells of strain C *uvr*⁺ (●); P3478 *polA1* (○); N611 *polA* (□); ATR15 *resA1* (△); HMS85 *polB1* (■); JC8287 *recBC xonA* (▲); KMBL1789 *polA 107* (◐); and N17-7 *uvrC* (×).

but retain HCR ability to UV-irradiated phages. As seen in Table 2, most *rec* strains tested were deprived of the host capacity by the MC treatment. However, the host capacity of *recA uvrA* double mutant was, like *rec⁺ uvrA* strains, rather resistant to the antibiotic. The ability of JC8287 *recBC xonA* to support ϕ A growth was somewhat less affected by MC as compared with wild type or *recBC sbcB* bacteria. HCR function of the *recBC xonA* strain is normal (Fig. 2) and significance of the small decrease in MC sensitivity is unknown.

Effect of MC on the host capacity of pol, res, or rep mutants

It has been demonstrated that UV-irradiated ϕ A RF exhibits lower survival in spheroplasts of *polA* mutants (10). As shown in Fig. 2, decreased HCR ability of *polA* mutants is more clearly detectable in Ca^{2+} -dependent transfection system. Recently, HEIJNEKER *et al.* (13) and GLICKMAN *et al.* (14) have suggested that 5'-3' exonuclease activity of DNA polymerase I is deficient in *polA 107*. HCR activity of *polA 107* and *resA1* is also compared in Fig. 2. While survival of UV-irradiated ϕ A RF was as low in *resA1* mutant as in *polA* strains, HCR activity was less affected by *polA 107* mutation.

In order to clarify whether the MC sensitivity was affected by any polymerizing and/or exonucleolytic activities of DNA polymerase I, the host capacity of several *pol* or *res* mutants was compared before or after the MC treatment. The data presented in Table 3 indicate that genetic deficiency in DNA polymerase I does not render the host capacity of these cells resistant to MC. HCR ability for UV-irradiated ϕ A RF was not affected at all by *polB1* mutation (Fig. 2) and pretreatment with MC greatly reduced the host capacity of the mutant devoid of DNA polymerase II. On the other hand, ϕ A as well as ϕ X174 cannot grow in *rep* mutant of *E. coli* (11, 15). Pretreatment of *rep* strain with MC did not restore the host capacity at all.

Table 3. Effect of MC treatment on the host capacity of *pol*, *res* and *rep* strains.

Strain	Plaque yield (PFU/ml)		
	Untreated	MC-treated	MC-treated/ Untreated
W3110 wild	3.3×10^3	$< 5.0 \times 10^0$	$< 1.6 \times 10^{-3}$
P3478 <i>polA1</i>	3.0×10^3	$< 5.0 \times 10^0$	$< 1.7 \times 10^{-3}$
N611 <i>polA</i>	1.5×10^5	$< 1.0 \times 10^1$	$< 6.6 \times 10^{-5}$
ATR15 <i>resA1</i>	1.6×10^5	$< 2.0 \times 10^1$	$< 1.4 \times 10^{-4}$
KMBL1789 <i>polA 107</i>	1.2×10^5	2.3×10^2	1.9×10^{-3}
HMS85 <i>polB1</i>	1.8×10^4	$< 1.0 \times 10^1$	$< 5.6 \times 10^{-4}$
D32 <i>rep3</i>	0	0	—

The conditions are the same as in Table 1.

Effect of several radiomimetic agents on the host capacity

In *E. coli*, MC selectively inhibits DNA synthesis, causes degradation of DNA, induces λ phage development and exerts a mutagenic action. These properties are to a certain extent shared with UV, nitrofuran compounds, or nalidixic acid. Therefore, effect of these compounds on the host capacity of *uvr*⁺ and *uvrA* strains was subsequently examined. Although neither DNA degradation nor induction of lysogenic phage was observed with phenethyl alcohol, this DNA-synthesis inhibitor was also tested. As shown in Fig. 3, the host capacity of *uvr*⁺ bacteria is somewhat more sensitive than that of *uvrA* mutant to UV. The difference be-

Fig. 3. UV sensitivity of the host capacity for ϕ A RF.

Cells of *E. coli* C *uvr*⁺ (●) or HF4704 *uvrA* (○) were irradiated with UV for the indicated time and the capacity of the bacteria was determined by Ca²⁺-dependent transfection.

Table 4. Capacity of *uvr*⁺ and *uvrA* hosts treated with agent affecting DNA synthesis.

Treatment with	Relative plaque yield on	
	CT-2 <i>uvr</i> ⁺	HF4704 <i>uvrA</i>
None (untreated control)	1 (3.2×10^6 PFU/ml)	1 (2.8×10^6 PFU/ml)
4A-2Q-ran ^a (5 μ g/ml)	5.5×10^{-3}	1.0×10^{-2}
Nalidixic acid ^b (50 μ g/ml)	2.2×10^{-1}	4.0×10^{-2}
Phenethyl alcohol ^b (0.3%)	5.3×10^{-2}	6.7×10^{-2}

^a Growing culture was treated for 10 min at 37°.

^b Growing culture was treated for 25 min at 37°.

tween the two strains was, however, rather small as compared with a marked difference in their MC sensitivity. In addition, the capacity of *uvrA* strain was as sensitive as that of *uvr*⁺ cells to pretreatment with 4A-2Q-ran, nalidixic acid, or phenethyl alcohol (Table 4).

Host capacity for MC-treated RF

Although natural form is quite inert, chemically or enzymically reduced MC reacts with DNA and cross-links its complementary strands (16). In the present experiments, amount of activated MC remaining in washed cells seems to be small, and yet it may be sufficient for cross-linking of RF DNA. The reduced infectivity in MC-treated *uvr*⁺ strains may then be derived from break down of cross-linked RF rather than inactivation of host functions. In order to exclude the possibility, ϕ A RF was reacted with activated MC *in vitro*, and infectivity of the treated RF was compared with that of untreated control on *uvr*⁺ bacteria and *uvrA* mutant. As seen in Table 5, the host capacity for MC-treated RF was not significantly different between *uvr*⁺ and *uvrA* strains.

Table 5. Capacity of *uvr*⁺ and *uvrA* hosts for ϕ A RF treated with activated MC.

System	Plaque yield (PFU/ml) on	
	CT-2 <i>uvr</i> ⁺	HF4704 <i>uvrA</i>
Untreated control	9.8×10^5 (100) [%]	2.8×10^5 (100) [%]
Complete	9.0×10^5 (92)	2.3×10^5 (82)
Minus MC	9.5×10^5 (97)	2.7×10^5 (96)

After reaction *in vitro* with reduced MC, ϕ A RF (labeled with ¹⁴C-thymine) was reisolated by gel filtration through a CPG-10 column and the infectivity was determined. Before infectivity assay, amount of the RF samples was standardized by its radioactivity.

DISCUSSION

Repair of UV-irradiated ϕ A RF requires host gene products of *uvrA*, *uvrB*, *uvrC*, *uvrD*, *uvrE*(*mutU*), and *polA*(*resA*) (10, 11, and present data). Apparently, *uvrF* function is dispensable for HCR of the irradiated RF and MC-treated *uvrF* mutant is unable to support ϕ A multiplication. In *uvrA* or *B* mutants, repair of cellular DNA is blocked at incision process owing to the deficiency of UV-endonuclease, while defects in *uvrC* mutant is probably in subsequent excision step (17-19). Regardless of these differences, either *uvrA*, *B*, or *C* mutants sufficiently support the multiplication of ϕ A, even after severe treatment with MC. It seems noteworthy that T4 endonuclease V-dependent reactivation of UV-irradiated ϕ A or ϕ X174 RF occurs in *uvrC* mutant as well (10). MC-sensitivity of the host capacity of *uvrE* strain was higher than that of *uvrA*, *B*, or *C* strains, but considerably low compared with that of *uvr*⁺ bacteria. The role of *uvrE* gene product

in DNA repair is presently unknown. As in *uvr*⁺ cells, the capacity to support ϕ A multiplication was sensitive to MC in *uvrD* or *polAI* strains. Furthermore, MC sensitivity of the host capacity was not decreased in *resA1* or *polA 107* mutants, suggesting that neither 5'-3' exonuclease activity nor polymerizing activity of DNA polymerase I is related with the MC effect. On the other hand, HCR ability of strains such as *polB*, *recA*, *recBC*, *recG*, or *recH* was as high as that of wild type bacteria and these strains lost their capacity for ϕ A upon MC treatment.

Extensive breakdown of cellular DNA observed in MC-treated *uvr*⁺ bacteria does not occur in *uvr* mutants, which are much more sensitive to MC than are *uvr*⁺ strains (20). It has been shown that *uvr* functions are involved in the excision repair of Mitomycin-induced defect of *E. coli* DNA (17).

Occurrence of replication of ϕ A and ϕ X174 in MC-treated *uvrA*, *B*, or *C* hosts suggests the following possibilities: (i) The viral DNA may be far less cross-linked by MC owing to its small size, (ii) the cross-linked viral DNA may be repaired by host functions other than *uvr* system, and (iii) the MC-induced damage in viral DNA might be tolerated or bypassed in replication process. While the viral growth was inhibited in MC-pretreated washed *uvr*⁺ hosts, infectivity yield of ϕ A RF reacted with MC *in vitro* was not different between *uvr*⁺ bacteria and *uvrA* strain. In addition, LINDQVIST and SINSHEIMER (5) showed that growth of ϕ X174 proceeded in a HCR⁻ strain even when MC was present during the entire process of infection. These data thus suggest that viral DNA *per se* is not a direct target of the antibiotic.

Replication of minute viruses such as ϕ A or ϕ X174 depends considerably on various cellular functions coded by host DNA. However, causal relationship between DNA degradation and loss of the host capacity is not clear because cellular enzymes and proteins required for viral growth may function even after MC treatment. Breakdown of host DNA might cause some structural alterations in replication apparatus common to bacteria and virus. In connection with DNA degradation, it seems noteworthy that the capacity of *recA uvrA* mutant was, unlike *rec*⁺ or *recA* strains, not affected by MC.

Between *uvr*⁺ and *uvrA* bacteria, a difference was observed in UV sensitivity of the host capacity for ϕ A. This difference, though significant, was rather small compared with MC sensitivity. These results may suggest that more than breakdown of host DNA is involved in the MC effect. If viral DNA replication is dependent on some enzymes or sites involved in host DNA repair, MC treatment might reduce plaque yield in *uvr*⁺ bacteria, through competition between host DNA repair and viral replication.

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