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Effects of *cis*-Diamminedichloroplatinum(II) on *Escherichia coli* and Bacteriophage Systems

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The effects of *cis*-diamminedichloroplatinum(II) (cisplatin) on *Escherichia coli* cells and bacteriophages were investigated. The bacteriocidal effect of cisplatin was stronger on *uvrA* or *recA* mutants than on wild type cells. The drug, like UV, induced prophage development in lysogenic bacteria. Host cell reactivation of α3 replicative form (RF) I DNA treated with cisplatin *in vitro* was more efficient in wild type or *recA* cells than in *uvrA* host. When wild type cells were exposed to cisplatin, decay of the host's capacity to sustain the viral multiplication proceeded nearly in parallel with the loss of colony-forming ability, whereas the capacity of *uvrA* mutant was much more resistant to the drug, as compared with the viability. In the DNA preparation from cisplatin-treated α3-infected wild type cells, RF II was deficient, but the RF I molecules extracted from the cells were moderately infective. The microvirid gene A protein, required for RF I→RF II conversion, was hardly detectable in wild type cells exposed to cisplatin. The possible relationship between *uvr*$^+$-dependent repair and synthesis of the viral protein is discussed.

Key words: bacteriophage, cisplatin, *Escherichia coli*, nucleotide excision repair, *uvrA*.

Cisplatin has been widely used for chemotherapy of head, neck, gastrointestinal, lung, and ovarian cancers (*1*). The therapeutic effect of cisplatin is based on its covalent binding to DNA to form a spectrum of adducts: 1,2-intrastrand *cis*-[$Pt(NH_3)_2d(GpG)$] and *cis*-[$Pt(NH_3)_2d(ApG)$] cross-links account for 65 and 25%, respectively, whereas interstrand adducts represent about 1% of the total adducts (*2, 3*). Besides its mutagenicity (*4-6*), cisplatin exerts its cytotoxicity through blocking DNA synthesis (*7-9*), by forming intrastrand cross-links. In addition, it has been suggested that interstrand cross-links may also play a role in determining the cytotoxicity of platinum compounds (*10*). Thus, the target of cisplatin action is DNA, and repair function for cisplatin-induced damage is essential for cell survival.

Cell survival assay has demonstrated that *Escherichia coli* cells defective in repair of UV damage are also deficient in repairing the damage caused by cisplatin, suggesting the involvement of the same mechanism in the repair of DNA lesions caused by both agents (*11, 12*). In a cell-free system, the UvrABC endonuclease from *E. coli* is capable of initiating the repair of a wide range of DNA damage, by incision of the damaged strand on both sides of the lesion (*13-15*). Moreover, covalently closed-circular DNAs from bacteriophages and plasmids have been used as probes to elucidate the mechanism of interaction of cisplatin with DNA. Some transfection studies have indicated that the majority of the lesions caused by cisplatin are, like those induced by UV (*16*), repaired by a system dependent on host *uvrA*, *uvrB*, or *uvrC* functions (*17-19*). The nucleotide excision repair (NER) system, thus, plays an important role in repairing DNA lesions caused by cisplatin (*20*). Other cellular responses to DNA damage are the mechanisms regulated by the RecA protein. The *recA*-dependent recombinational repair or trans-lesion bypass of UV- or cisplatin-damaged DNA is more important than *uvrA* function, in terms of bacterial survival (*11*). The influence of cisplatin on bacterial and viral multiplication, however, has not been extensively studied as yet.

Lindqvist and Sinsheimer reported previously that upon treatment with mitomycin C (MC), an alkylating agent that cross-links to DNA bifunctionally, cellular capacity to support the growth of a single-stranded (ss) DNA phage ϕX174 is impaired in the wild type (wt) *E. coli*, but not in a mutant strain deficient in host cell reactivation (HCR) (*21*). Subsequent studies have demonstrated the MC-resistant host capacity in *uvrA*, *uvrB*, or *uvrC* cells, but not in *rec* mutants, for microvirid replicative form (RF) DNA (*22, 23*). Although this phenomenon has been applied for preferential labeling of certain phage DNA in *hcr*$^-$ cells, the mechanism remains to be elucidated in detail. Thus, the biological and physical properties of the RF molecules synthesized in MC-treated HCR-proficient cells remain to be examined. In addition, the mechanism of inhibition of

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Abbreviations: cisplatin, *cis*-diamminedichloroplatinum(II); CFU, colony-forming unit; ds, double-stranded; HCR, host cell reactivation; MC, mitomycin C; NER, nucleotide excision repair; OD_{660}, optical density at 660 nm; PFU, plaque-forming unit; PS, physiological saline; RF, replicative form; ss, single-stranded; TE, 10 mM Tris·HCl-1 mM EDTA buffer, pH 7.5; W reactivation, Weigle reactivation; wt, wild type.

the progeny RF replication in the treated cells is totally unknown.

In this study the effect of cisplatin was investigated on *E. coli* cells and their phage systems, with special reference to the mechanism of reduction of the host capacity in *uvr*$^+$ cells for microvirid phages. In the NER-proficient cisplatin-treated host, infecting ss phage DNA is converted to covalently closed RF I, but the next step (nicking of RF I to RF II) is impaired by deficiency in viral gene A protein.

MATERIALS AND METHODS

Bacteria, Phages, and DNA—The strains of *E. coli* used were C, C (λh), H502 *uvrA*, *thy*, and CK111 *recA*. These were from our laboratory stock. ss DNA phages α3, ϕX174, and ϕX174 am 3 (an amber mutant in *gene E*) were used. ss DNA of α3 was extracted from purified phage particles by the phenol method. Double-stranded (ds) RF I DNA of α3 or ϕX174 was extracted from the phage-infected *E. coli* C cells, and purified by CsCl equilibrium centrifugation (*24*). Infectivity of the DNA and host capacity of *E. coli* cells were assayed by use of the $CaCl_2$ method as described previously (*25*). In brief, bacteria grown in nutrient broth at 37°C with shaking were treated with and suspended in chilled 50 mM $CaCl_2$ at $OD_{660}=15$. For transfection assay, 0.1 ml of the competent cell suspension in chilled 50 mM $CaCl_2$ was mixed with 0.05 ml of viral DNA in 10 mM Tris·HCl-1 mM EDTA buffer, pH 7.5 (TE) and kept at 0°C for 20 min. The infected cells were diluted with chilled 50 mM $CaCl_2$ and plated with the indicator bacteria.

Chemicals—Cisplatin was kindly provided by Bristol-Myers Squibb, Tokyo, and was dissolved in physiological saline (PS). Restriction endonuclease *Sph*I and β-agarase I were purchased from Nippon Gene, Toyama.

λ *Phage Inducibility*—After treatment of the lysogen, *E. coli* C (λh), with cisplatin or UV, induced cells and free phage were titrated as described (*26*), using strain C as the indicator.

Cisplatin Treatment of RF—α3 RF I (5 μg/ml in 10 mM Tris·HCl, pH 7.4, 10 mM NaCl, 1 mM EDTA) was incubated with cisplatin (15 μg/ml) at 30°C for the indicated time, and then the DNA was washed with TE four times, using a spin column (Ultrafree C3plus, Nihon Millipore, Tokyo) under the conditions recommended by the manufacturer. A control mixture with PS was run in parallel. Finally, the DNA was dissolved in TE.

Preparation of RF I from Phage-Infected Bacteria Pretreated with Cisplatin—Bacteria were grown in nutrient broth at 37°C with shaking and, when the OD_{660} reached 0.2, the culture was divided into two equal portions. To one portion, 200 μg/ml of cisplatin was added and shaking was continued for further 2 h at 37°C. Then, the cells were collected, washed, centrifuged, suspended in nutrient broth, and infected with α3 phage (at a multiplicity of infection of 10). After addition of $CaCl_2$ to 5 mM, the culture was incubated at 37°C for 30 min, centrifuged, and washed three times with 50 mM Tris·HCl, 5 mM EDTA, pH 8.0. The washed bacterial pellet was resuspended in 50 mM Tris·HCl (pH 8.0) containing 10 mM EDTA. Triton X 100 (final concentration; 1%) and lysozyme (final concentration; 0.86 mg/ml) were added and the mixture was incubated for 30 min at 0°C. After further treatment with 100 μg/ml of proteinase K at 0°C for 30 min, NaCl was added to 1 M and the lysate was kept at 0°C for 2 h. The lysate was centrifuged at 15,000 rpm for 30 min at 0°C, and the supernatant was treated with phenol and chloroform. RF DNA was collected by ethanol precipitation, then dissolved in TE, and residual RNA was removed by RNase treatment. The RF sample was subjected to electrophoresis using 0.9% low-melting agarose gel. The gel was photographed, and the covalently closed RF I band was cut out and eluted according to the β-agarase I method under the conditions recommended by the manufacturer. The DNA was dissolved in TE and stored at −20°C. Nicked RF II molecules formed in untreated control cells were not detected in the cisplatin-treated host.

Detection of ϕX174 Gene A Protein in the Infected Bacteria—Before infection, cisplatin-treated (or untreated) cells were incubated in M9 medium without sulfate, for 15 min at 37°C. [^{35}S]Methionine (70 μCi/ml) (Daiichi Kagaku Yakuhin, Tokyo) was added together with ϕX174 am 3 (at a multiplicity of infection of 10) and the incubation was continued at 37°C. After 50 min, an excess of unlabeled methionine was added and incubation was continued for 10 min. The cells were collected, washed and suspended in TE at 2×10^{10}/ml. Lysozyme (100 μg/ml) was added and the mixture was incubated on ice for 30 min and then mixed with 1/10 volume of 10% Triton X 100. After 10 min, NaCl was added to 1 M and kept at 0°C for 1 h. The clear viscous lysate was centrifuged at $100,000\times g$ for 1 h, and the supernatant was dialyzed against 10 mM Tris·HCl, pH 7.5, with a centrifugation column (Ultrafree CL, Nihon Millipore, Tokyo) under the conditions recommended by the manufacturer. The proteins were subjected to 10% SDS-PAGE and the bands on the gel were analyzed using a BAS-1500 (Fuji Film, Tokyo).

RESULTS

In *E. coli*, repair of DNA damaged by UV irradiation or MC treatment is carried out by the NER and Rec systems. These radiomimetic agents induce prophage development in lysogenic bacteria. Moreover, the host NER system is required for repair of small DNA phages exposed to UV or MC. This repair, or HCR, is enhanced by moderate exposure of host cells to UV or MC [Weigle (W) reactivation]. Because the target of cisplatin action is DNA, we first studied whether this platinum compound exerted similar effects on *E. coli* cells.

Effect of Cisplatin on Bacterial Multiplication and Prophage Development—The influence of cisplatin on cellular growth and viability was studied using wt, *uvrA*, and *recA* strains of *E. coli*. As shown in Fig. 1A, sensitivity to this drug was considerably higher in cells of H502 *uvrA* and CK111 *recA* than in strain C wt. Qualitatively similar results were obtained for UV sensitivity (data not shown). Cisplatin forms covalent adducts with the bases of DNA, and the result that H502 and CK111 were far more sensitive than strain C indicates involvement of *uvr* and *rec* functions in repair of the adducts. On the other hand, increase in cell mass (mainly protein) continued in *uvrA* or *recA* cells, as in wt bacteria, in the presence of cisplatin (Fig. 2B), at least at moderate doses. This result suggests that cisplatin preferentially inhibits synthesis of DNA rather than that of cellular protein or RNA.

Cells of *E. coli* C (λ h) were incubated with or without 50 μg/ml of cisplatin and the number of plaque-forming units (PFU) was determined directly (induced cell assay) or after chloroform treatment (free phage assay). Within 1 or 2 h after cisplatin treatment, λ phage induction took place as revealed by a marked increase in the number of the induced cells or the titer of free phage (Fig. 1C). Replacement of the indicator strain C with H502 did not alter the plaque yield. A similar profile of prophage induction was observed upon UV irradiation (data not shown).

Host Cell Reactivation of α3 RF I Treated with Cisplatin—The RF I DNA was incubated with 15 μg/ml of cispla-

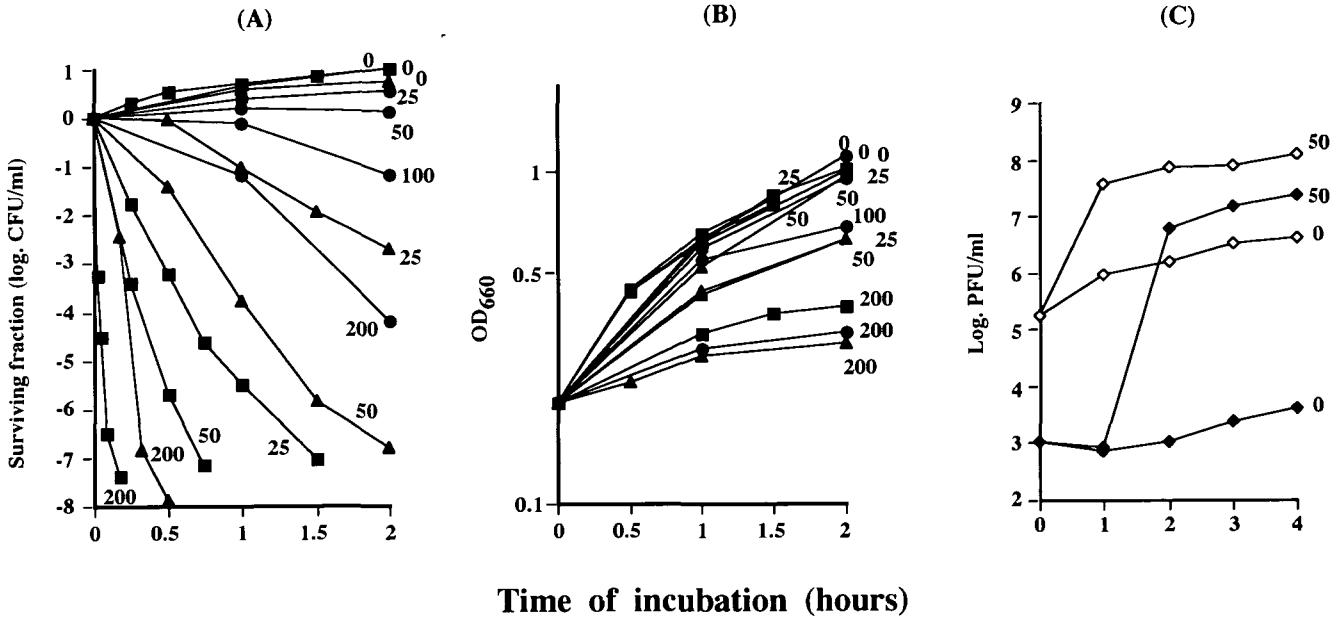

Fig. 1. **Effects of cisplatin on multiplication of *E. coli* and prophage development.** Cells of strain C wt, H502 *uvrA*, CK111 *recA*, or C (λ h) were grown at 37°C in nutrient broth containing the indicated amount (μg/ml) of cisplatin, and viability (A), turbidity (B), and prophage development (C) were followed. (A) and (B): ●, wt; ▲, *uvrA*; ■, *recA*. (C): ◇, induced C (λ h) cells; ◆, free λ h phage.

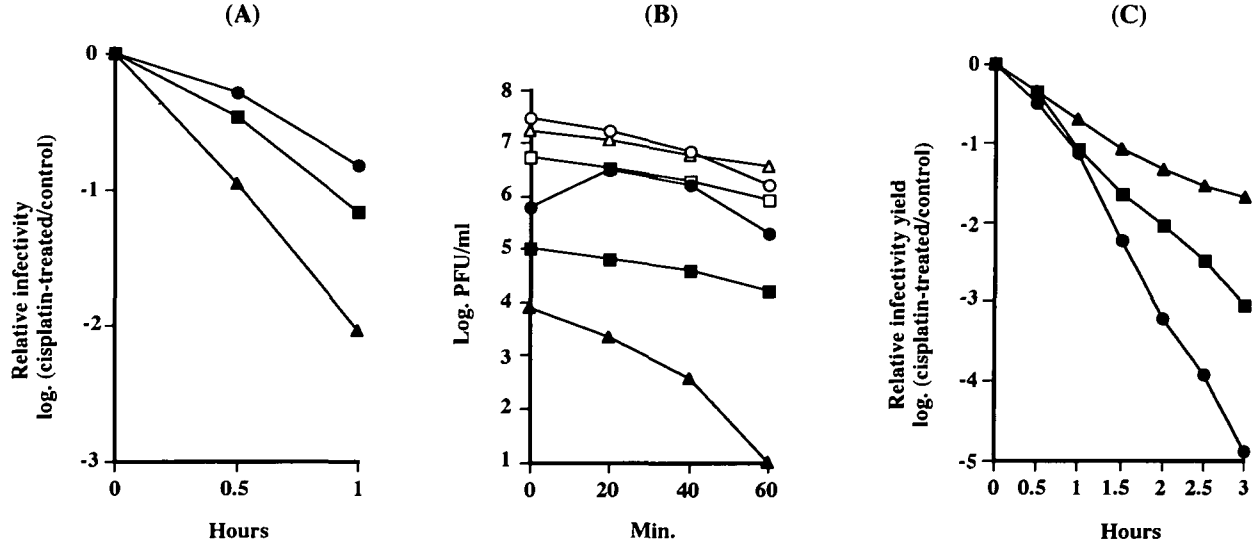

Fig. 2. **Host cell reactivation (A), Weigle reactivation (B), and host capacity (C) of *E. coli* cells for cisplatin-treated or untreated α3 RF I.** (A) The RF I DNA was incubated *in vitro* with 15 μg/ml of cisplatin at 30°C in 10 mM Tris-HCl, pH 7.4 containing 10 mM NaCl, and 1 mM EDTA for the indicated time and transfected into $CaCl_2$-treated wt (●), *uvrA* (▲), or *recA* (■) cells. (B) Each *E. coli* strain was treated with 200 μg/ml of cisplatin for the indicated time and their capacity to reactivate the RF I, incubated with (filled symbols) or without (open symbols) 15 μg/ml of cisplatin for 1 h, was determined. ○, ●: wt; △, ▲: *uvrA*; □, ■: *recA*. (C) Host capacity of cisplatin-treated cells for α3 RF I. Bacteria of wt (●), *uvrA* (▲), or *recA* (■) were incubated with 200 μg/ml of cisplatin at 37°C and their capacity to support α3 RF I transfection was periodically determined by means of the $CaCl_2$ method.

tin at 37°C and an aliquot was periodically removed, freed from unreacted drug, and assayed for infectivity on wt, *uvrA*, or *recA* cells. Reduction in the transfectivity was approximately proportional to the reaction time with cisplatin. These DNAs, after reaction with the anticancer drug, exhibited reduced transfectivity in *uvrA* mutant cells, compared with wt or *recA* bacteria (Fig. 2A). These results indicated that the host *uvrA* gene product was involved in the repair of cisplatin-induced damage in the viral DNA.

Although it has been reported that cisplatin is detoxified by thiols (*27*), cysteine, dithiothreitol, or β-mercaptoethanol was ineffective in recovery of the transfectivity (data not shown).

When the cisplatin-treated RF I was transfected into wt, *uvrA*, or *recA* cells treated with cisplatin, W reactivation was detected only in wt cells: the surviving fraction of the cisplatin-treated RF was significantly increased in the host cells pretreated with a moderate dose of cisplatin (Fig. 2B).

Host Capacity of Cisplatin-Treated Cells for α3 RF I—Cells of wt, *uvrA*, and *recA* were treated with 200 μg/ml of cisplatin and their capacity to support α3 RF I transfection was determined by the $CaCl_2$ method. When the bacteria exposed to cisplatin were made competent with chilled 50 mM $CaCl_2$ and infected with α3 RF I DNA, the yield of the transfectants was distinctly reduced, depending on the genotype, as well as the drug-treatment time. As shown in Fig. 2C, the host capacity to grow α3 was more resistant to cisplatin in *uvrA* bacteria than in wt or *recA* cells. In addition, a significant, albeit small, difference was observed between wt and *recA* strains. When the cells of *E. coli* wt were exposed to cisplatin, decay of the host capacity proceeded nearly in parallel with the loss of colony-forming ability, whereas the host capacity of the *uvrA* mutant was much more resistant to the drug than the colony-forming ability was. The present results are qualitatively similar to those for MC (*22, 23*), which cross-links complementary strands of DNA, as regards the host capacity to support the growth of microvirid phages.

Characterization of α3 DNA Synthesized in Cells Exposed to Cisplatin—Upon infection, microvirid ss DNA is first converted to RF I by host enzymes. This parental RF I is then nicked by phage gene A protein, to yield RF II having a 3′-OH end, which serves as the primer for subsequent replication. In Fig. 3A, the electrophoretic profile of α3 DNA extracted from the phage-infected wt cells treated with 200 μg/ml of cisplatin for 2 h is shown, together with that of the untreated control sample. (In Fig. 3A, a considerable amount of host DNA was left, because of impracticability of alkaline lysis method for detection of RF II molecules.) In the DNA preparation from the cisplatin-treated host, the presence of RF I was evident, but RF II was not detected, whereas in the control (cisplatin non-treated) DNA, both forms were clearly distinguished. These results indicate that in wt bacteria treated with cisplatin, conversion of the phage ss DNA into ds RF is not blocked, whereas subsequent conversion into RF II by phage gene A protein is inhibited. After electrophoresis, the α3 RF I from the cisplatin-treated cells was eluted and nuclease sensitivity was tested using *Sph*I. This restriction enzyme recognizes GCATG ↓ C, and intact α3 RF I has three such sites (*28*). Interstrand cross-linking might occur between this region and the complementary strand. The 5′ nucleotides preceding this sequence were Ap (817th and 2775th nucleotides of α3 DNA) or Gp (3133rd nucleotide of α3 DNA), forming potential intrastrand cross-linking loci, *cis*-[$Pt(NH_3)_2$d(ApG)] or *cis*-[$Pt(NH_3)_2$d(GpG)]. As shown in Fig. 3B, α3 RF I from the cisplatin-treated cells was cleaved by this enzyme at one site at least, and converted to linear RF III DNA. In addition, two fragments (1,958 and 3,771 bp) were detected, as in the cisplatin-untreated control DNA subjected to the enzyme digestion.

TABLE I. **Infectivity of the RF molecules extracted from α3-infected cells of *E. coli* (wt) preincubated with or without 200 μg/ml of cisplatin.** The RF I and RF II were purified by agarose gel electrophoresis and their infectivity was determined by the $CaCl_2$ method, using cisplatin-untreated (I) or treated (II) wt and *uvrA* cells.

Exp.	Recipient strain	Infectivity (per μg/ml)			B/A
		RF I from cisplatin-untreated cells (A)	RF II from untreated cells	RF I from cisplatin-treated cells (B)	
I	C(*uvr*$^+$)	6.2×10^6	5.3×10^6	5.4×10^5	8.7×10^{-2}
	H502(*uvrA*)	1.7×10^6	1.5×10^6	9.0×10^2	5.3×10^{-4}
II	C(*uvr*$^+$)	7.9×10^4	—	1.9×10^4	2.4×10^{-1}
	H502(*uvrA*)	8.5×10^4	—	6.0×10^1	7.1×10^{-4}

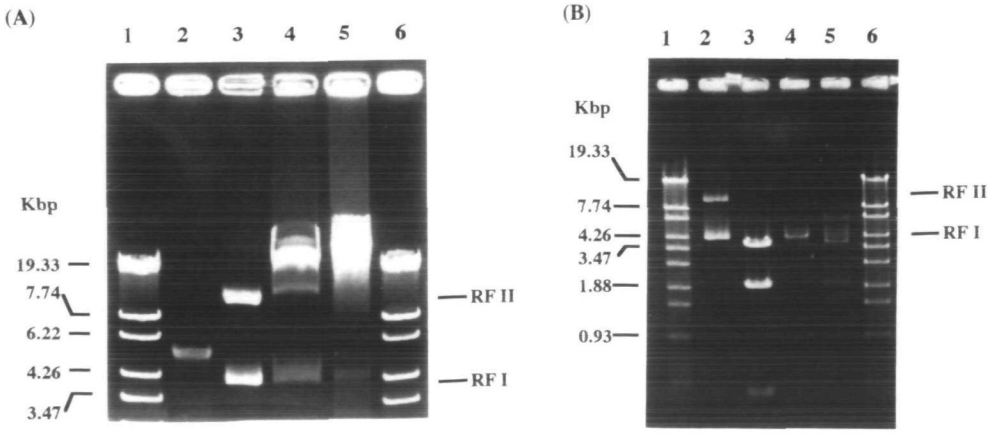

Fig. 3. **Electrophoretic pattern of α3 DNA extracted from the phage-infected wt cells treated with or without 200 μg/ml of cisplatin for 2 h (A), and the profile of the α3 RF I subjected to *Sph*I digestion (B).** (A) Lanes 1 and 6, markers; lane 2, ss DNA; lane 3, RF I; lane 4, α3 DNA isolated from cisplatin-untreated cells; lane 5, α3 DNA isolated from cisplatin-treated cells. (B) Lanes 1 and 6, markers; lane 2, RF I; lane 3, α3 RF I cleaved with *Sph*I; lane 4, α3 RF I isolated from cisplatin-treated cells; lane 5, α3 RF I isolated from cisplatin-treated cells and then cleaved with *Sph*I.

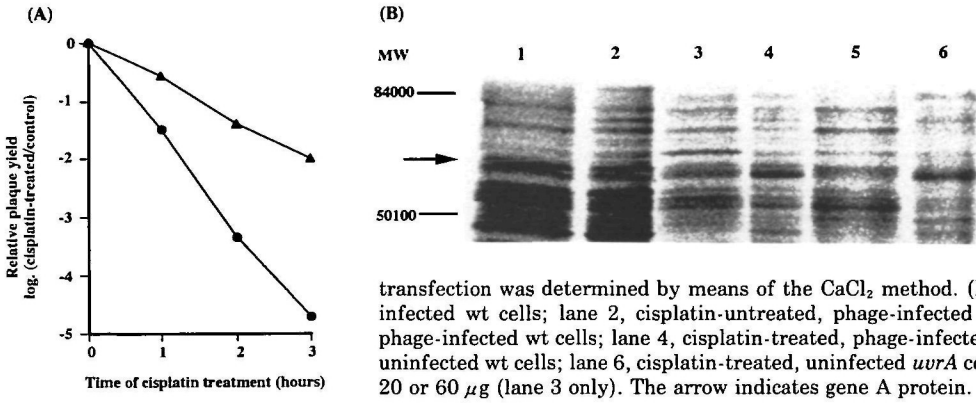

Fig. 4. **Host capacity of cisplatin-treated cells for ϕX174 RF I (A) and SDS-PAGE patterns of proteins extracted from the phage-infected cells treated with 200 μg/ml of cisplatin for 2 h (B).** (A) Cells of wt (●) and *uvrA* (▲) were incubated with 200 μg/ml of cisplatin at 37°C, and their capacity to support ϕX174 RF I transfection was determined by means of the $CaCl_2$ method. (B) Lane 1, cisplatin-untreated, phage-infected wt cells; lane 2, cisplatin-untreated, phage-infected *uvrA* cells; lane 3, cisplatin-treated, phage-infected wt cells; lane 4, cisplatin-treated, phage-infected *uvrA* cells; lane 5, cisplatin-treated, uninfected wt cells; lane 6, cisplatin-treated, uninfected *uvrA* cells. The amount of protein applied was 20 or 60 μg (lane 3 only). The arrow indicates gene A protein.

Infectivity of RF I Extracted from α3-Infected Cells Pre-incubated with or without Cisplatin—Transfectivity of RF I treated with cisplatin *in vitro* was distinctly decreased in *uvrA* cells, as mentioned above. When α3 phage was used to infect cisplatin-pretreated and washed bacteria, the plaque yield was lower in the wt host than the *uvrA* strain (data not shown), but at least RF I was detectable in the wt cells, as stated above. Owing to the extremely simplified conditions, the results obtained *in vitro* might not reflect changes in the RF molecules synthesized in cells preexposed to cisplatin. Therefore, the infectivity of RF I and RF II (detected only in untreated control cells) extracted at 30 min (cisplatin-treated cells) or at 18 min post-infection (untreated cells) was determined, using wt and *uvrA* strains as the recipient cells. As shown in Table I, the infectivity of RF I extracted from the cisplatin-pretreated cells was somewhat low on *uvr*$^+$ strain and markedly reduced on *uvrA* host. This result is similar to that obtained with the RF subjected to cisplatin treatment *in vitro*. When host cells pre-treated with 200 μg/ml of cisplatin for 1.5 h were used in transfection, the relative infectivity of RF I extracted from cells exposed to cisplatin was increased with *uvr*$^+$ strain, but not *uvrA* bacteria, suggesting the occurrence of W reactivation (Table I). As stated above (Fig. 2B), W reactivation was evidently detected in the wt host, but not in *uvrA* or *recA* strain, subjected to mild treatment with cisplatin.

ϕX174 Gene A Protein in Cisplatin-Treated Cells—As mentioned above, RF II DNA was not detectable in the α3-infected wt bacteria pretreated with cisplatin, implying that viral gene A protein responsible for conversion of parental RF I into RF II (*29, 30*) was deficient in the cell. For effective detection of the gene A product, *E. coli* cells infected with lysis-defective mutant of ϕX174 were labeled with [^{35}S]methionine. Because a lysis-defective mutant of α3 was unavailable, ϕX174 am 3 strain was used for accumulation of the phage-coded protein. No difference was seen in the host capacity of cisplatin-treated *E. coli* cells between wt α3 and ϕX174 (Fig. 4A). In Fig. 4B, the SDS-PAGE patterns of ϕX174 am 3 proteins extracted from the phage-infected wt and *uvrA* cells pretreated with or without 200 μg/ml of cisplatin for 2 h are compared. The gene A protein was easily demonstrated in *uvrA* cells treated with cisplatin, upon application of 20 μg total protein, whereas in wt cells treated with cisplatin, the gene product was undetectable at the same amount of the protein, and was barely detectable when the amount of applied protein was increased to 60 μg. This result thus indicates that in the cells proficient in UV-repair, synthesis of the viral protein is considerably disturbed.

DISCUSSION

Cisplatin inhibits multiplication of *E. coli* at concentrations allowing turbidity (cell mass) increase. In addition, treatment of λ lysogen with this anticancer drug induces prophage development. These properties are shared with so-called radiomimetic agents such as UV, MC, or nalidixic acid, which preferentially inhibit cellular DNA synthesis. The bacteriocidal effect of cisplatin is potent on *uvrA* or *recA* cells deficient in DNA repair. When RF I DNA of α3 phage was reacted *in vitro* with cisplatin, its infectivity yield was markedly reduced in *uvrA* host, in comparison with wt and *recA* cells. Pre-exposure of *uvr*$^+$*rec*$^+$ host to a low concentration of cisplatin significantly increased the surviving fraction of the drug-treated RF I. This W reactivation is a manifestation of SOS induction. Dependence of the HCR on the *uvr* system has been observed in MC-treated ϕA RF as well (*22*). These results indicate that the RF DNA cross-linked with cisplatin is reactivated by the NER system, but not by the recombinational repair function. Paradoxically, bacterial *uvrA* function, required for repair of cisplatin-treated microvirid RF DNA, becomes detrimental for multiplication of the phage, when the host cell is pretreated with cisplatin. The infecting ss DNA is converted to RF I, but subsequent nicking to RF II is impaired in the pretreated *uvrA*$^+$ strain. The finding that some step, other than ssDNA→RF I conversion, is affected is consistent with the incompetence of the cisplatin-treated cells for transfection of RF I DNA.

Two possible mechanisms may be considered with regard to the deficiency in gene A-dependent RF I→RF II conversion. One is at the substrate level: the RF I in cisplatin-treated *uvr*$^+$ cells might be altered to resist nicking by the gene A protein. The other is at the enzyme level: the gene A product is deficient in the treated *uvr*$^+$ host. Changes in *Sph*I sensitivity and infectivity of the RF I extracted from the cisplatin-pretreated cells imply the occurrence of certain DNA modification(s) by the drug remaining in the bacteria. Infectivity of the modified RF I is, however, distinctly higher in *uvr*$^+$ host than in *uvrA* strain. This result is incompatible with the former possibility. On the other hand, the gene A protein was hardly detectable in the wt cells, preexposed to cisplatin and then infected with

ϕX174. Among the phage-encoded products, only the gene A protein is indispensable for RF replication. Deficiency in this nicking protein in the cisplatin-treated infected cells suggests the existence of an intimate relationship between NER and transcription or translation. Recent investigations have indeed demonstrated coupling of DNA repair with transcription (*31, 32*). It seems probable that, in *uvr*$^+$ cells, some components are used in the repair of cisplatin-induced lesions in the bacterial DNA, causing insufficiency in transcription, hence translation, of the viral nickase. Owing to deficiency in NER, *uvrA* cells might be saved from extensive mobilization of the bifunctional components, which are required, albeit in a small amount, for transcription of the microvirid genome.

It has recently been demonstrated that a mismatch repair mechanism is involved in DNA damage caused by cisplatin (*33, 34*). Although this mechanism may contribute to cisplatin resistance in *E. coli*, the infectivity of phage RF I DNA was selectively higher in *uvrA* cells than in *uvr*$^+$ bacteria pretreated with cisplatin. In addition, these *E. coli* strains were *dam*$^+$*mutHSL*$^+$, suggesting that the mismatch repair mechanism is not involved in the present system.

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