Toxic Effects of *cis*- and *trans*-Diamminedichloroplatinum(II) in *uvr*D mutants of *Escherichia coli*[1]

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Abstract. Excision repair has been shown to be the major pathway for repair of damage in DNA caused by *cis* and *trans*-diamminedichloridoplatinum(II) (DDP) in *Escherichia coli*. The effects of limiting excision repair on resistance to DDP was determined using three *uvr*D mutants of *E. coli* having different defects in the UvrD protein, DNA helicase II. Excision repair is limited because the UvrD protein function is necessary for catalytic activity of the UvrABC nuclease. The compound *cis*-DDP was more toxic than *trans*-DDP in decreasing survival of exposed bacteria and transforming ability of treated plasmid DNA. Repair of chromosomal DNA damaged with DDP was dependent on *uvr*D function as both compounds were more toxic to *uvr*D mutants than to isogenic wild-type bacteria. The transforming ability of plasmids treated with *trans*-DDP was also decreased further when competent bacteria were deficient in the UvrD gene function, whereas, plasmid DNA treated with *cis*-DDP had similar transforming ability in both *uvr*D mutant and wild-type cells. Repair of *cis*-DDP modified plasmid DNA in competent bacteria was inefficient and therefore did not require UvrD function.

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INTRODUCTION

The compound, *cis*-diamminedichloroplatinum(II) or cisplatin (*cis*-DDP), is a potent antitumor agent while the *trans*-isomer is ineffective in the treatment of cancer (Rosenberg et al. 1969, Roberts and Thompson 1979). Both compounds bind to DNA to form a number of adducts and adducts which are unique to *cis*-DDP are thought to be responsible for its cytotoxic and antitumor properties. The major adduct formed upon reaction of *cis*-DDP with DNA is an intrastrand crosslink between two adjacent guanines while the second most frequent adduct is an intrastrand crosslink between a 5' adenine and an adjacent guanine (Eastman 1986, Fichtinger-Schepman et al. 1985, Pinto and Lippard 1985). The isomeric *trans*-DDP is stereochemically unable to bind adjacent bases of the DNA and can not form such adducts (Stone et al. 1976, Eastman and Barry 1987). It is likely then that intrastrand crosslinks between adjacent bases in DNA are responsible for the effects of *cis*-DDP in biological systems.

In addition to its antitumor effects, *cis*-DDP is highly mutagenic in bacteria (Beck and Brubaker 1975, Beck and Fisch 1980) and is toxic to *E. coli* cells which are deficient in various DNA repair processes (Alazard et al. 1982, Beck and Brubaker 1973, Jarosik and Beck 1984, Popoff et al. 1987). The *trans*-isomer is not as mutagenic as *cis*-DDP (Beck and Brubaker 1975) and there is evidence indicating that DNA damage caused by *trans*-DDP is repaired differently than that caused by *cis*-DDP (Ciccarelli et al. 1985).

The major pathway for repair of *cis*-DDP damage in DNA of *E. coli* is excision repair (Husain et al. 1985a, Popoff et al. 1987). Excision repair is initiated by the incision reaction of the UvrABC nuclease which cuts on both the 5' and the 3' sides of damage in DNA (Sancar and Rupp 1983, Yeung et al. 1983, Van Houten 1990). The UvrA protein facilitates the formation of a complex between UvrB and damaged DNA while the UvrB and UvrC proteins perform the actual incisions (Sancar and Hearst 1993). After the incision reaction, the UvrD protein (helicase II) and DNA polymerase I catalyze excision of the oligonucleotide with platinated bases and subsequent release or turnover of the UvrB and UvrC proteins from damaged DNA (Husain et al. 1985b, Caron et al. 1985, Van Houten 1990).

Studies performed *in vitro* have demonstrated the role of excision repair in removal of intrastrand *cis*-DDP-crosslinked adjacent guanines from DNA in addition to a large number of other types of DNA damage (Husain et al. 1985a, Beck et al. 1985). In *E. coli*, the transforming ability of plasmid DNA reacted with *cis*-DDP depends on the competent bacteria having a functional UvrB (Popoff et al. 1987) and UvrA gene product (Husain et al. 1985a). This research is an extension of our previous research on the process of excision repair in mediating resistance of bacteria to DDP. The effects of mutant alleles of the *uvr*D gene on the ability of the mutant bacteria to repair damage in DNA caused by DDP was characterized because one *uvr*D mutants which had been examined previously was not appreciably sensitive to DDP. Three mutants at the *uvr*D locus were used which had different limitations in their DNA excision repair capabilities. As expected, viability of bacteria was dependent on UvrD function after treatment of the cells with *cis*- or *trans*-DDP. Transforming ability of *trans*-DDP modified plasmid DNA in competent bacteria was also dependent on UvrD function but that of *cis*-DDP modified plasmids was not. This confirmed our previous results (Popoff et al. 1987) that repair of *cis*-DDP modified plasmid DNA was different from that of *trans*-DDP modified plasmid DNA in that it was inefficient and therefore did not require UvrD function. Inefficient repair of *cis*-DDP adducts in DNA may be one of the reasons that *cis*-DDP is effective as an antitumor agent while *trans*-DDP is not.

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MATERIALS AND METHODS

Survival Assays

Bacteria of relevant genotypes were obtained from colleagues at Yale University, University of Georgia, and University of North Carolina at Chapel Hill (Table 1). Methods for cultivation of bacteria and purification of plasmid pBR322 DNA were as described previously by Popoff et al. (1987). Stocks of bacterial strains were kept at -20° C in Luria-Bertani Broth (LB) (Maniatis et al. 1982) containing 15% glycerol and were used to obtain cultures for all experiments. Transposon Tn5 was maintained in strain SK3451 by supplementing LB media with kanamycin (25 μg/ml).

Experiments measuring survival of logarithmically growing cells of *E. coli* treated with DDP (Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD) were performed as described previously by Popoff et al. (1987). Bacteria were grown in LB at 37° C with shaking in a New Brunswick Model G76 gyrotory water bath shaker. A culture of logarithmically growing cells was subdivided upon reaching an absorbance at 550 nm of 0.20 and treated with DDP. Stock solutions of DDP (250-500 μg/ml) were made by dissolving compounds in sterile saline, and were either used the day of the experiment or kept frozen at -20° C for no more than two weeks. Colony forming units were determined periodically by diluting bacteria with sterile saline and plating on LB solidified with 1.5% agar.

Sensitivity of bacteria to UV light was determined by plating 10^2-10^4 cells of a log phase culture and irradiating the plates with a 30 Watt G.E. germicidal lamp at a distance of 50 cm (1.2 Joules/sec/M^2) for 0-60 sec. After irradiation, plates were kept in the dark to prevent photoreactivation and were incubated overnight at 37° C.

Transformation Assays

Plasmid pBR322 DNA (10 μg) was treated with 2 μg DDP in 200 μl of buffer containing 10 mM Tris·HCl, 1 mM EDTA, 10 mM NaCl, pH 7.4 (TEN 7.4) for 20-60 min at 37° C. Removal of unbound platinum by spot dialysis and the use of atomic absorption spectrophotometry to determine the amount of platinum bound to DNA were as described previously by Popoff et al. (1987). Cells were made competent as described previously (Popoff et al. 1987) and were transformed with DDP modified DNA or untreated DNA as a control. Transformants were selected by plating bacteria on LB agar (Maniatis et al. 1982) with ampicillin (50 μg/ml). The number of viable bacteria was determined by diluting bacteria and plating samples on LB agar. Transformation frequencies (number of transformants per viable cell per μg DNA) are the averages of three experiments. The average transformation frequency of each isogenic wild-type parent and mutant strain with untreated plasmid DNA was 2.0 x 10^{-3} for AB1157 (*uvr*$^+$), 3.0 x 10^{-4} for JC8471 (*uvr*D252); 1.5 x 10^{-4} for W3623 (*uvr*$^+$), 2.0 x 10^{-4} for N14-4 (*uvr*D3); 1.4 x 10^{-3} for SK707 (*uvr*D$^+$), and 1.1 x 10^{-3} for SK3451 (*uvr*D254::Tn5). Relative frequency of transformation is the ratio of the average transformation frequency obtained with DDP treated plasmids to that obtained with untreated plasmid DNA. A lethal dose of compound is defined as the number of platinum molecules bound to plasmid DNA which reduce its transformation frequency to 37% that of untreated DNA.

RESULTS

Effects of *cis*- and *trans*-DDP on Bacterial Survival

The compound *cis*-DDP was more toxic to *uvr*$^+$ and *uvr*D mutant bacteria than *trans*-DDP as measured by

TABLE 1

Bacterial strains.

Strain	Relevant genotype	Other chromosomal markers	Source	Reference
AB1157	*uvr*$^+$	*thi*1, *thr*1, *leu* B6, *pro* A2, *thy*1, *his* G4, *arg* E3, *lac* Y1, *gal* K2, *ara* 14, *xyl* 5, *mtl*1, *tsx* 33, *str* 31, *sup* E44.	B. J. Bachmann[a]	d
JC8471	*uvr*D252	Same as AB1157, also *rps* L31	B. J. Bachmann	d
SK707	*uvr*$^+$	*lac* BK1, *mal* A1, *mtl*1, *xyl* 7, *arg* H1, *his* G4, *ind* 188, *met* E46, *sup* E44.	S. R. Kushner[b]	
SK3451	*uvr*D254::Tn5	Same as SK707	A. Sancar[c]	e
W3623	*uvr*$^+$	*trp* 56, *gal* T23	B. J. Bachmann	f
N14-4	*uvr*D3	Same as W3623, also *rps* L178	B. J. Bachmann	f

[a] *E. coli* Genetic Stock Center, School of Medicine, Yale University.
[b] Department of Molecular and Population Genetics, University of Georgia.
[c] Department of Biochemistry, University of North Carolina, Chapel Hill.
[d] Horii and Clark 1973.
[e] Maples and Kushner 1982.
[f] Ogawa *et al.* 1968.

their effects on colony forming ability of treated bacteria (Fig. 1). Three different *uvr*D mutants (*uvr*D3, *uvr*D252, *uvr*D254::Tn5) varied in their ability to survive exposure to DDP, but each mutant exhibited an increase in sensitivity when compared to its parent strain upon exposure to either isomer of DDP or to UV light (Fig. 1, Table 2). Colony forming ability in cultures of cells bearing the *uvr*D3 mutation (strain N14-4), Uvr^+ cells of the isogenic strain W3623, and Uvr^+ cells of strain AB1157 did not decrease upon exposure of the bacteria to *cis*-DDP (Fig. 1-a). These cells underwent one or two divisions during the first hour of exposure to 30 μg *cis*-DDP per ml LB and thereafter, there was no change in the number of colony forming units for 2 hr. Inhibition of cell division was indicated by the appearance of filamentous cells (Table 2) and was responsible for the lack of increases in the number of colony forming units. Inhibition of cell division which results in filamentous growth is one of the SOS responses to DNA damage in *E. coli* (Walker 1984).

Repair-proficient cells of strain SK707 were more sensitive to *cis*-DDP than those of the other Uvr^+ strains or the *uvr*D3 mutant strain (Fig. 1-a). Colony forming units increased during the first hour that cells of this strain were treated with *cis*-DDP, and survival decreased to 37% after an additional 90 min exposure to *cis*-DDP. The reason for enhanced sensitivity of SK707 cells to *cis*-DDP is unknown but they were not appreciably more sensitive to *trans*-DDP (Fig. 1-b).

Cells of mutant strains JC8471 (*uvr*D252) and SK3451 (*uvr*D254::Tn5) were more sensitive to *cis*-DDP than the *uvr*D3 mutants. Survival in these cell populations was reduced to 37% upon exposure to *cis*-DDP for 30 and 90 minutes, respectively. This was a greater than five-fold increase in sensitivity (ratio of times of exposure resulting in decrease of colony forming ability to 37%, parent strain relative to mutant strain) for strain SK3451 compared to its parent strain SK707 and a greater than two-fold increase in sensitivity for strain JC8471 compared to its parent strain AB1157 (Table 2). Cell mass increased in all cultures in the presence of either platinum compound as indicated by turbidity measurements (data not shown).

Because *trans*-DDP has a lower toxicity than the *cis*-isomer, cells were treated with a two fold higher concentration of *trans*-DDP (60 μg/ml). Repair-proficient cells and *uvr*D3 mutants were most resistant to treatment with *trans*-DDP; after addition of *trans*-DDP there was

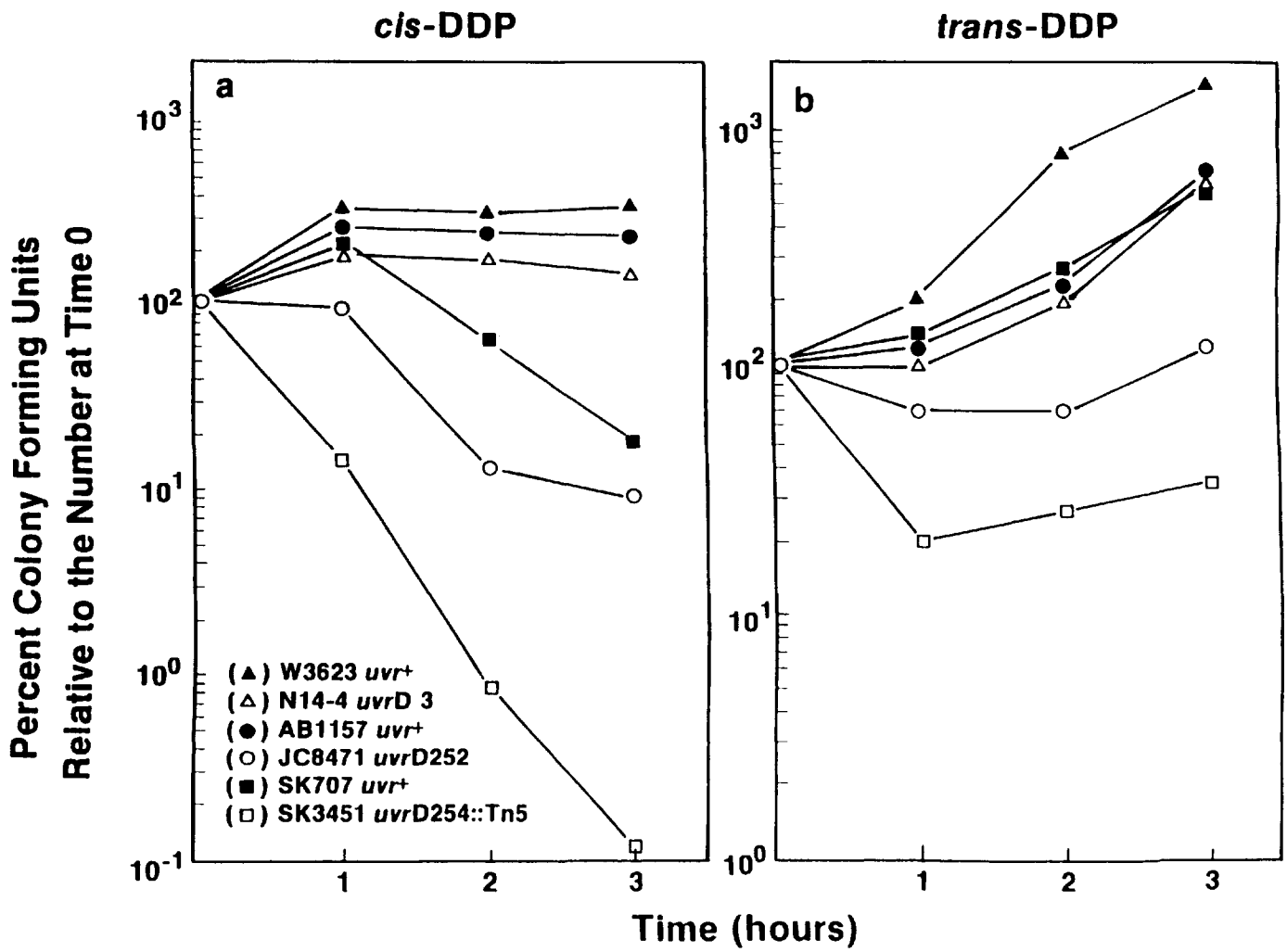

FIGURE 1. Survival (colony forming ability) of *uvr*D mutants and wild-type *E. coli* when cells were grown in 30 μg *cis*-DDP (Panel a) or 60 μg *trans*-DDP (Panel b) per ml LB broth. Symbols for strains of *E. coli* are indicated. The same symbol is used for isogenic bacteria; closed symbols are used for wild-type bacteria and open symbols for *uvr*D mutants.

TABLE 2

Effects of DDP and UV on Wild-type and uvrD *Mutants of* Escherichia coli.

Strain	Exposure time to reduce viability to e^{-1} (37%)[a]		Filamentation[b]	
	cis-DDP min	UV sec	*cis*-DDP	*trans*-DDP
AB1157 (*uvr*$^+$)	>180	25	++	-
JC8471 (*uvr*D252)	90	4	++	+
SK707 (*uvr*$^+$)	150	13	++	-
SK3451 (*uvr*D254::Tn5)	30	3	++	+
W3623 (*uvr*$^+$)	>180	25	++	-
N14-4 (*uvr*D3)	>180	12	++	-

[a]A culture of logarithmically growing cells was subdivided upon reaching an absorbance at 550 nm of 0.20 and treated with no DDP, 30 μg *cis*-DDP/ml or 60 μg *trans*-DDP/ml. Bacteria were UV irradiated with a germicidal lamp at 1.2 Joules/sec/M^2. Viability was monitored by plating and the time of exposure reducing colony forming units to 37% is given.

[b]Filamentation was observed microscopically after growth of bacteria in LB broth containing DDP for 3 hr at 37° C: (++) filamentous cells approximately 5-10 times longer than untreated control cells; (+) elongated cells twice the length of control cells; (-) cells equal in size to the control cells.

an initial 1 hr lag followed by increases in colony forming units (Fig. 1-b). Increases in the number of colony forming units were most inhibited when cultures of *uvr*D252 and *uvr*D254::Tn5 cells were treated with the *trans*-compound in comparison to the wild-type isogenic cells. Repair-proficient cells did not become filamentous, but the sensitive *uvr*D252 and *uvr*D254::Tn5 mutants appeared slightly elongated after exposure to *trans*-DDP for 3 hr indicating that damage in their DNA caused by *trans*-DDP had caused induction of the SOS response (Table 2).

Effects of DDP in Transformation Assays

The relative frequencies of transformation of competent bacteria with DDP-modified plasmids decreased as the numbers of platinum adducts per molecule of pBR322 DNA increased (Fig. 2). Relative frequency of transformation was defined as the ratio of the average transformation frequency for competent cells transformed with DDP treated plasmids to the average transformation frequency obtained with untreated control plasmids.

Modification of plasmid DNA with *trans*-DDP was far less toxic than *cis*-DDP modification in transformation assays (Fig. 2). The transformation frequency was reduced to 80% for DNA molecules with 14 bound *trans*-DDP adducts, and to 37% (a lethal dose) for molecules with two bound *cis*-DDP adducts in repair-proficient bacteria. The transforming ability of plasmids treated with *trans*-DDP was decreased further when competent bacteria were deficient in the UvrD gene function, whereas, plasmid DNA treated with *cis*-DDP had similar transforming ability in both *uvr*D mutant and wild-type cells. Six adducts in *trans*-DDP modified plasmids reduced the relative frequency of transformation of the most sensitive *uvr*D mutant (SK3451::Tn5) to 37% whereas 8-14 adducts were not lethal and only reduced the transformation frequency of repair proficient cells to about 70% that obtained with untreated plasmid DNA.

DISCUSSION

The pleiotropic nature of the UvrD gene product has been noted before and results in a diversity of phenotypes among *uvr*D mutant isolates (Washburn and Kushner 1993, Yamamota et al. 1988, Kuemmerle and Masker 1980, Zieg et al. 1978). Biochemical defects in Helicase II caused by each of the *uvr*D mutations caused enhanced lethality of *cis*-DDP and *trans*-DDP in the three *uvr*D strains and thus affected UvrD functions needed for effective removal of DDP adducts in DNA. The UvrD protein coded by the *uvr*D3 allele has lysine at position 387 rather than glutamic acid and is thought to have increased binding to single stranded DNA (Yamamota et al. 1988). Although the *uvr*D3 allele confers exceptional sensitivity to methyl methanesulfonate (Washburn and Kushner 1991), it conferred only slight sensitivity to DDP.

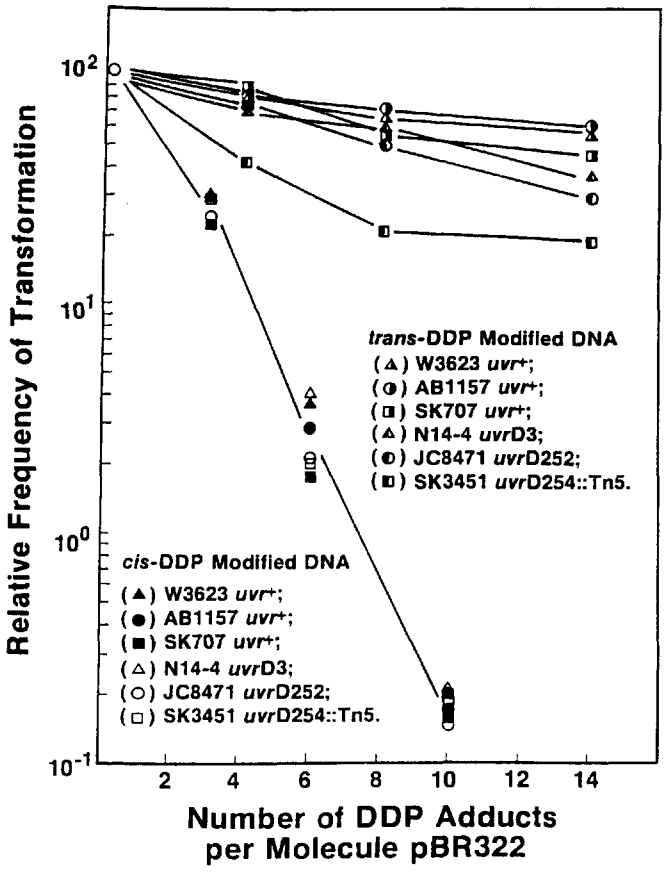

FIGURE 2. Relative frequency of transformation of competent cells of *E. coli* with pBR322 DNA modified with increasing amounts of *cis*-DDP or *trans*-DDP. Symbols for strains of bacteria are indicated. The same symbol is used for isogenic bacteria. Closed symbols designate wild-type and open symbols the isogenic *uvr*D mutant bacteria treated with *cis*-DDP. Right side closed symbols designate wild-type and left side closed the isogenic *uvr*D mutant bacteria treated with *trans*-DDP.

Greater sensitivities to both platinum compounds were conferred by the *uvr*D252 and *uvr*D254::Tn5 mutations. The *uvr*D252 allele does not confer the mutator phenotype as observed for null mutations and thus mutants have the necessary UvrD activities to be proficient in mismatch repair. The *uvr*D252 mutation is a base substitution in the region coding for the ATP binding domain of the UvrD protein and causes a glycine-to-aspartic-acid change at amino acid 30 (Washburn and Kushner 1993). The *uvr*D252 encoded enzyme has reduced levels of ATPase activity and decreased ability to unwind DNA containing nicks in comparison to wild-type DNA helicase II. This reduction in its unwinding activity is thought to cause its decreased ability to facilitate release of the UvrC and the incised oligonucleotide containing damaged bases. The insertion of Tn5 into the *uvr*D gene results in a truncated UvrD protein which would be expected to have no UvrD activities. Mutants having deletion-insertion null mutations in the *uvr*D gene are viable but are deficient in mismatch repair, deficient in excision repair and have high levels of genetic recombination (Washburn and Kushner 1991). As expected then, the mutation *uvr*D255::Tn5 conferred the greatest sensitivity to the platinum compounds.

Treatment with *cis*-DDP was more detrimental to plasmid function than treatment with *trans*-DDP. The toxicity of *cis*-DDP adducts for plasmid inactivation in transformation assays was also higher than that reported by Husain et al. (1985a) for *cis*-1,2-diaminocyclohexyldichloro-platinum(II). This difference is due to the particular carrier ligands of these two compounds which gives them slightly different properties in biological systems. The relative frequency of transformation decreased to 37% at two *cis*-DDP adducts per molecule pBR322 DNA for all *uvr*D isolates as well as wild-type isogenic strains. Thus two adducts constituted a lethal dose indicating that repair of cis-DDP adducts in plasmid DNA was very inefficient. The *uvr*D mutants were as proficient as wild-type bacteria in the repair of *cis*-DDP adducts in plasmid DNA indicating that turnover of repair enzymes did not enhance repair of *cis*-DDP adducts in plasmid DNA.

We think that the difference in repair requirements for plasmid DNA treated with *cis*-DDP in comparison to DNA treated with *trans*-DDP is due to inefficient incision reactions at *cis*-DDP-DNA adducts by the excision repair nuclease. Recently it has been shown that the UvrABC nuclease is not efficient in making incisions in DNA at localized *cis*-DDP-GG adducts *in vitro* (Page et al. 1990, Visse et al 1994). Although the formation of preincision complexes on linear DNA containing *cis*-DDP-intrastrand crosslinks was fast the actual rate of incision was slow (Visse et al. 1994). Intrastrand crosslinks at adjacent guanines comprise 65% of all adducts caused by *cis*-DDP (Eastman 1986) and can not be formed by *trans*-DDP (Stone et al. 1976). If the incision reactions performed by the UvrB and UvrC proteins are slow at *cis*-DDP-DNA adducts, then the repair complex would be likely to encounter a DNA replication complex and repair of the adduct might be exacerbated. In such cases, the incision reactions might be aborted or the adducts processed in a different fashion eliminating the need for UvrD function. Repair of the adducts in plasmid DNA caused by *trans*-DDP was more efficient than for those caused by *cis*-DDP as indicated by decreased toxicity of *trans*-DDP. These results indicate that efficient repair of *trans*-DDP adducts in plasmid DNA does occur and therefore requires UvrD function for catalytic activity of the UvrABC nuclease.

In all three *uvr*D isolates, the function of the UvrD helicase II activity was needed for repair of *cis*-DDP adducts in chromosomal DNA but not for *cis*-DDP adducts in plasmid DNA. This may be due to amplification of the differences in the sensitivities of the *uvr*D mutants in comparison to wild-type bacteria as the SOS response is induced in bacteria treated with *cis*-DDP; SOS induction causes inhibition of DNA replication allowing time for repair and increased synthesis of many repair enzymes including the *uvr*B and *uvr*D gene product (Walker 1984). This was not the case when competent bacteria were transformed with *cis*-DDP modified plasmid DNA, SOS induction was not observed. This conclusion is strengthened by the observation that SOS induction in competent bacteria enhances repair of *cis*-DDP damaged plasmid DNA but not that of *trans*-DDP (Popoff et al. 1987). Another reason for these differences in repair may be that the spectrum of adducts formed in plasmid DNA during *in vitro* treatment with *cis*-DDP is different from the spectrum formed *in vivo* in chromosomal DNA of treated bacteria.

In summary, a functional Helicase II protein is required for repair of *cis*-DDP adducts in chromosomal but not plasmid DNA. This is most likely resultant from amplification of the *uvr*D defect as SOS repair is induced in *cis*-DDP treated cells causing inhibition of DNA replication and enhanced synthesis of repair enzymes (*uvr*B and *uvr*D gene products included). Repair of *cis*-DDP damaged plasmid DNA in SOS uninduced cells is inefficient or slow, few adducts are actually excised and the UvrD gene product is therefore not needed for turnover of Uvr proteins to enhance repair. Repair of *trans*-DDP-modified plasmid DNA occurs more frequently than that of *cis*-DDP-modified DNA and requires the Helicase II function for catalytic activity of DNA repair complexes. More efficient removal of *trans*-DDP adducts from DNA relative to that of *cis*-DDP adducts has previously been shown to occur also in eucaryotic CV-1 cells (Ciccarelli et al. 1985). Further comparative studies on repair of *cis*- and *trans*-DDP adducts might shed light on the reason for their different repair requirements and different anti-tumor properties.

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LITERATURE CITED

Alazard, R., M. Germanier, and N. P. Johnson 1982 Mechanism of toxicity of platinum(II) compounds in repair-deficient strains of *Escherichia coli*. Mutat. Res. 93: 327-337.

Beck, D. J. and R. R. Brubaker 1973 Effect of *cis*-platinum (II)diamminodichloride on wild type and deoxyribonucleic acid repair-deficient mutants of *Escherichia coli*. J. Bacteriol. 116: 1247-1252.

___ and R. R. Brubaker 1975 Mutagenic properties of *cis*-platinum(II)diamminodichloride in *Escherichia coli*. Mutat. Res. 27: 181-189.

___ and J. E. Fisch 1980 Mutagenicity of platinum coordination complexes in *Salmonella typhimurium*. Mutat. Res. 77: 45-54.

___, S. Popoff, A. Sancar, and W. D. Rupp 1985 Reactions of the UVRABC excision nuclease with DNA damaged by diamminedichloroplatinum(II). Nuc. Acids Res. 13: 7395-7412.

Caron, P. R., S. R. Kushner, and L. Grossman 1985 Involvement of helicase II (*uvrD* gene product) and DNA polymerase I in excision mediated by the UvrABC protein complex. Proc. Natl. Acad. Sci. U.S.A. 82: 4925-4929.

Ciccarelli, R B., M. J. Solomon, A. Varshavsky, and S. J. Lippard 1985 *In vivo* effects of *cis*- and *trans*-diamminedichloroplatinum(II) on SV40 chromosomes: Differential repair, DNA-protein cross-linking, and inhibition of replication. Biochemistry 24: 7533-7540.

Eastman, A. 1986 Reevaluation of interaction of *cis*-dichloro(ethylenediamine)platinum(II) with DNA. Biochemistry 25: 3912-3915.

___ and M. A. Barry 1987 Interaction of *trans*-diamminedichloroplatinum(II) with DNA: Formation of monofunctional adducts and their reaction with glutathione. Biochemistry 26: 3303-3307.

Fichtinger-Schepman, A. M. J., J. L. van der Veer, J. H. J. den Hartog, P. H. M. Lohman, and J. Reedijk 1985 Adducts of the antitumor drug *cis*-diamminedichloroplatinum(II) with DNA: formation, identification, and quantitation. Biochemistry. 24: 707-713.

Horii, Z. I. and A. J. Clark 1973 Genetic analysis of the *recF* pathway to genetic recombination in *Escherichia coli* K12: Isolation and characterization of mutants. J. Mol. Biol. 80: 327-344.

Husain, I., S. G. Chaney, and A. Sancar 1985a Repair of *cis*-platinum-DNA adducts by ABC excinuclease in vivo and in vitro. J. Bacteriol. 163: 817-823.

___, B. Van Houten, D. C. Thomas, M. Abdel-Monem, and A. Sancar 1985b Effect of DNA polymerase I and DNA helicase II on the turnover rate of UvrABC excision nuclease. Proc. Natl. Acad. Sci. U.S.A. 82: 6774-6778.

Jarosik, G. P. and D. J. Beck 1984 The effects of *cis*-platinum-(II)diamminodichloride, UV light and *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine on *Escherichia coli*: Plasmid mediated resistance to mutagens. Chem. Biol. Interactions 51: 247-259.

Kuemmerle, N. B. and W. E. Masker 1980 Effect of the *uvrD* mutation on excision repair. J. Bacteriol. 142: 535-546.

Maniatis, T., E. F. Fritsch, and J. Sambrook 1982 Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y. p. 440.

Maples, V. F. and S. R. Kushner 1982 DNA repair in *Escherichia coli*: Identification of the *uvrD* gene product. Proc. Natl. Acad. Sci. U.S.A. 79: 5616-5620.

Ogawa, H., K. Shimada, and J. I. Tomizawa 1968 Studies on radiation-sensitive mutants of *E. coli* I. Mutants defective in the repair synthesis. Mol. Gen. Genetics 101: 227-224.

Page, J. D., I. Husain, A. Sancar, and S. G. Chaney 1990 Effect of the diaminocyclohexane carrier ligand on platinum adduct formation, repair, and lethality. Biochemistry 29: 1016-1024.

Pinto, A. L., and S. J. Lippard 1985 Binding of the antitumor drug *cis*-diamminedichloroplatinum(II) (cisplatin) to DNA. Biochim. Biophys. Acta. 780: 167-180.

Popoff, S. C., D. J. Beck, and W. D. Rupp 1987 Repair of plasmid DNA damaged in vitro with *cis*- or *trans*-diamminedichloroplatinum(II) in *Escherichia coli*. Mutation Res. 183: 129-137.

Roberts, J. J. and A. J. Thompson 1979 The mechanism of action of antitumor platinum compounds. *In:* I. W. Davidson and W. E. Cohn, eds. Prog. in Nucl. Acids Res. and Mol. Biol. 22: 71-133.

Rosenberg, B., L. VanCamp, J. E. Trosko, and V. H. Mansour 1969 Platinum compounds: A new class of potent antitumor agents. Nature 222: 385-386.

Sancar, A. and J. E. Hearst 1993 Molecular matchmakers. Science 259: 1415-1420.

___ and W. D. Rupp 1983 A novel repair enzyme: UVRABC excision nuclease of *Escherichia coli* cuts a DNA strand on both sides of the damaged region. Cell 33: 249-260.

Stone, P. J., A. D. Kelman, F. M. Sinex, M. M. Bhargava, and H. O. Halvorson 1976 Resolution of α, β and γ DNA of *Saccharomyces cerevisiae* with the antitumor drug *cis*-$Pt(NH_3)_2Cl_2$. Evidence for preferential drug binding by GpG sequences of DNA. J. Mol. Biol. 104: 793-801.

Van Houten, B. 1990 Nucleotide excision repair in *Escherichia coli*. Microbiol. Rev. 54: 18-51.

Visse, R., A. J. van Gool, G. F. Moolenaar, M. de Ruijter, and P. van de Putte 1994 The actual incision determines the efficiency of repair of cisplatin-damaged DNA by the *Escherichia coli* UvrABC endonuclease. Biochemistry 33: 1804-1811.

Walker, G. C. 1984 Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. 48: 60-93.

Washburn, B. K. and S. R. Kushner 1991 Construction and analysis of deletions in the structural gene (*uvrD*) for DNA helicase II of *Escherichia coli*. J. Bacteriol. 173: 2569-2575.

___ and S. R. Kushner 1993 Characterization of DNA Helicase II from a *uvrD*252 mutant of *Escherichia coli*. J. Bacteriol. 175: 341-350.

Yamamota, Y., M. Yabuki, and J. Furuyama 1988 Intragenic suppression in the *uvrD* gene of *Escherichia coli*. I. Temperature-sensitive *uvrD* mutations. Mutat. Res. 194: 39-48.

Yeung, A. T., W. B. Mattes, E. Y. Oh, and L. Grossman 1983 Enzymatic properties of purified *Escherichia coli* UVRABC proteins. Proc. Natl. Acad. Sci. U.S.A. 80: 6157-6161.

Zieg, J., V. F. Maples, and S. R. Kushner 1978 Recombination levels of *Escherichia coli* K-12 mutants deficient in various replication, recombination, or repair genes. J. Bacteriol. 134: 958-966.