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Enhancement of Excision Repair of Cisplatin-DNA Adducts by Cell-Free Extract from a Cisplatin-Resistant Rat Cell Line

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ABSTRACT. To characterize the enhanced repair synthesis of defined DNA lesions, oligodeoxyribonucleotides were synthesized and inserted into plasmid DNA. The inserted plasmid DNA was treated with *cis*-diamminedichloroplatinum(II) (cisplatin) and subjected to *in vitro* DNA repair assay with soluble extract from the rat liver cell line Ac2F. All cisplatin adducts tested stimulated DNA repair synthesis. Moreover, two cisplatin-resistant cell lines, Ac2F-CR4 and Ac2F-CR10, were established by stepwise exposure of Ac2F cells to this drug. The DNA repair synthesis was enhanced 3- to 4-fold in the extract from cisplatin-resistant Ac2F cells relative to that from Ac2F cells. Such repair synthesis was suppressed by the specific DNA polymerase inhibitor aphidicolin. The results of the present study suggested that the enhanced repair activity induced by a cisplatin adduct can be detected by *in vitro* DNA repair assay with soluble cell extract. BIOCHEM PHARMACOL **57**;12: 1415–1422, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. cisplatin; cisplatin-resistant cell; host-cell reactivation; excision repair; cell-free repair; aphidicolin

Although *cis*-diamminedichloroplatinum(II) (cisplatin) is one of the most commonly used antitumor drugs, the development of resistance to this drug is a major limitation in clinical use. One of the major mechanisms of such acquired resistance has been suggested to be increased DNA repair activity [1–12]. The repair activity has been monitored by various assay techniques such as adduct removal using HPLC, unscheduled DNA synthesis, and host-cell reactivation of damaged plasmid DNA. On the other hand, to investigate the factors involved in DNA excision repair, Wood *et al.* [13] first developed an assay system with soluble extracts from human lymphoid cells. This system demonstrated that these extracts perform repair synthesis in plasmid DNA containing UV photoproducts, thymine-psoralen monoadducts, cisplatin cross-links, and acetylaminofluorene adducts [13–15]. Using such a human cell-free system, an asymmetric pattern of dual incision during nucleotide excision repair has been found for several DNA lesions [16–19]. With a host-reactivation assay, we have shown that the DNA repair activity of Ac2F cells is enhanced by exposing the cells to a DNA-damaging agent, cisplatin [20]. In the present study, we characterized the enhanced repair of DNA molecules containing a single defined cisplatin lesion using a rat cell-free system.

MATERIALS AND METHODS

Cell Lines and Culture

The rat-liver cell line Ac2F was provided by the Japanese Cancer Research Resources Bank and maintained as a monolayer in MEM§ (Gibco)/10% FBS (M.A. Bioproducts)/0.006% kanamycin in a humidified atmosphere of 5% CO_2 at 37°. The cisplatin-resistant cell lines Ac2F-CR4 and Ac2F-CR10 were selected and established by exposing Ac2F cells to the drug at concentrations up to 60 μM. These cell lines were maintained as described above and harvested in the conventional manner.

Cytotoxicity Assay

Each of the Ac2F cell lines was plated in MEM/10% FBS at high density (1×10^6 cells/100-mm culture dish: Falcon) and incubated for 48 hr to reach confluency. At this stage, the cells were treated with various concentrations of cisplatin for 24 hr and washed with PBS. The washed cells were trypsinized, and living cells were counted by trypan blue staining.

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§ *Abbreviations*: MEM, minimal essential medium; FBS, fetal bovine serum; *cis*-DDP-pGL3, cisplatin-damaged pGL3; LC_{50}, concentration lethal to 50% of cells; and CAT, chloramphenicol acetyltransferase.

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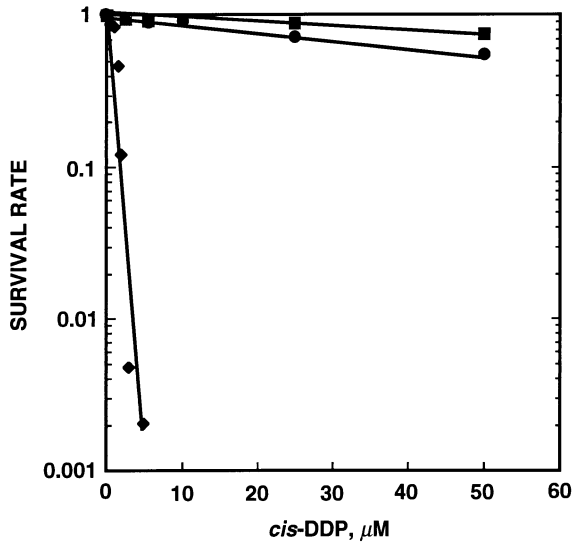

FIG. 1. Survival of Ac2F, Ac2F-CR4 and Ac2F-CR10 cells. Cells (10^6/100-mm culture dish) were incubated for 48 hr at 37° and treated with various concentrations of cisplatin (*cis*-DDP) for 24 hr, as described in the text. The results are expressed as values relative to those without this drug. Key: ◆, Ac2F; ●, Ac2F-CR4; ■, Ac2F-CR10.

Preparation of cis*-DDP-pGL3 for Luciferase Activity Assay*

pGL3-Control vector (pGL3) (Promega) was used as a luciferase cDNA expression vector for the assay. C*is*-DDP-pGL3 was prepared essentially according to the method of Husain *et al.* [21]. The plasmid DNA (1.4 mg) was dissolved in 1 mL of 25 mM NaCl/1 mM Tris-HCl (pH 7.4)/0.1 mM EDTA containing 25 μM cisplatin at 37° for 5 hr. The incubated material was extracted with an equal volume of 1 mM Tris-HCl (pH 8.0)/0.1 mM EDTA saturated phenol/chloroform (1:1, v/v). The aqueous phase was subjected to ethanol precipitation, and the precipitate was washed with 70% ethanol and used as *cis*-DDP-pGL3.

TABLE 1. Luciferase activities in Ac2F and cisplatin-resistant Ac2F cells for pGL3 or *cis*-DDP-pGL3

Cell	Light intensity	
	pGL3 (fold)*	*cis*-DDP-pGL3 (fold)*
Ac2F	1774 ± 96	173 ± 18 (1)
Ac2F-CR4	1875 ± 80 (1.06 ± 0.05)	472 ± 38 (2.73 ± 0.22)
Ac2F-CR10	1776 ± 53 (1.00 ± 0.03)	512 ± 33 (2.96 ± 0.19)

Ac2F, Ac2F-CR4 and Ac2F-CR10 cells were transfected with pGL3 or *cis*-DDP-pGL3 containing pRL-SV40 vector and incubated at 37° for 44 hr. The luciferase activity of the incubated cell lysate was determined with a Dual Luciferase Assay system kit. The activity of the cotransfected pRL-SV40 reporter vector provides an internal control. The results are the mean of 3 samples with SD.

*These values are expressed relative to those of Ac2F cells.

Luciferase Activity Assay

Exponentially growing Ac2F, Ac2F-CR4 and Ac2F-CR10 cells were collected and washed with PBS. The washed cells were suspended in PBS (10^7 cells/mL), and 0.5 mL of the suspension containing 0.5 μg of pRL-SV40 vector (Promega) as an internal control was mixed with 20 μg of pGL3 or *cis*-DDP-pGL3 in a disposable electroporation chamber (Electroporator II, Invitrogen). The mixture was chilled on ice for 10 min prior to delivery of a single electrical pulse at 330 V and a capacitance of 1000 μF. After a further 10 min on ice, 1 mL of pre-warmed medium was added to the chamber. Then the mixture was transferred to a culture dish (Falcon) containing 9 mL of pre-warmed medium and incubated under 5% CO_2 at 37° for 20 hr. The incubated cells were washed with PBS and further incubated in 10 mL of fresh medium for 24 hr. The lysate of the incubated cells was prepared, and the luciferase activity of the lysate was determined with a Dual Luciferase Assay System kit (Promega), according to the manufacturer's instructions. The activity of the co-transfected pRL-SV40 reporter vector was used as an internal control to normalize experimental values. The light intensity was measured with a Micro

d(GTG) 54-mer

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5'CTAGATATATATATATATATATATATATATGTGTATATATATATATATATATATATAT 3'
   3'TATATATATATATATATATATATACACATATATATATATATATATATATAGATC 5'
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d(GG) 53-mer

```
5'CTAGATATATATATATATATATATATATGGTATATATATATATATATATATAT 3'
   3' TATATATATATATATATATATATACCATATATATATATATATATATAGATC 5'
```

d(GC) 54-mer

```
5'CTAGATATATATATATATATATATATATGCATATATATATATATATATATATAT 3'
   3'TATATATATATATATATATATATACGTATATATATATATATATATATAGATC 5'
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d(G) 52-mer

```
5'CTAGATATATATATATATATATATATATGTATATATATATATATATATATAT 3'
   3'TATATATATATATATATATATATACATATATATATATATATATATAGATC 5'
```

d(AT) 54-mer

```
5'CTAGATATATATATATATATATATATATATATATATATATATATATATATATAT 3'
   3'TATATATATATATATATATATATATATATATATATATATATATATATAGATC 5'
```

FIG. 2. Duplex oligodeoxyribonucleotides used in this study. The 52- to 54-mer oligonucleotides, in which GTG, GG, GC, or G was positioned at the middle of the AT-oligonucleotide, were synthesized. These duplex oligonucleotides were inserted into the *Xba*I site of the plasmid pGEM4. Platination with this drug was capable of forming a single 1,3-intrastrand, a single 1,2-intrastrand, or a single 1,2-interstrand cross-link and a single mono-G adduct, at the sequences of GTG, GG, GC, and mono-G, respectively, in the duplex oligonucleotide. Duplex AT-oligonucleotide was used as a control.

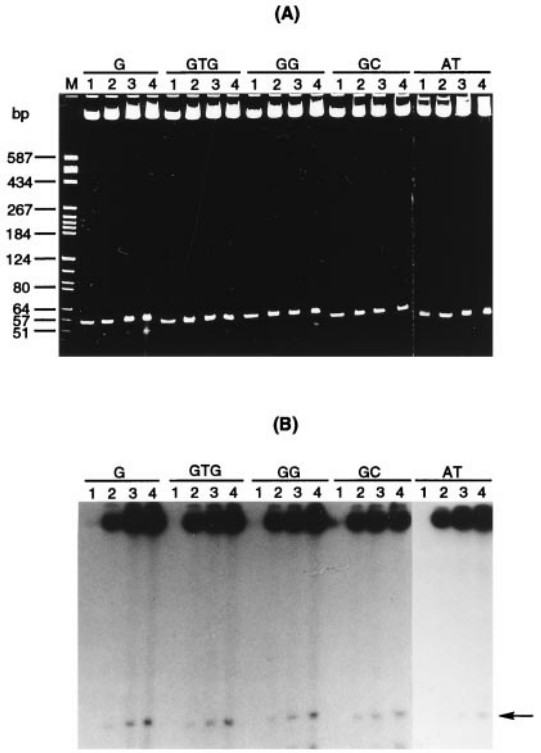

FIG. 3. Repair synthesis of cisplatin-treated duplex oligodeoxyribonucleotide in soluble extract of Ac2F cells. Plasmid containing the duplex oligonucleotide was treated with 1 μM cisplatin and subjected to the cell-free repair assay, as described in the text. (A) Gel electrophoretic profiles of *Xba*I digests. The gel was stained with ethidium bromide. (B) Autoradiograph of (A). Lane M, DNA size markers (pBR322/*Hae*III digest); lanes 1–4, with 0, 20, 40 and 80 μg proteins, respectively. G, GTG, GG, GC, and AT are d(G), d(GTG), d(GG), d(GC), and d(AT), respectively, as shown in Fig. 2. The arrow shows the position of the duplex oligonucleotides released by *Xba*I digestion of the plasmid.

Lumino Reader (Corona, MRL-100). The DNA content was estimated as 50 μg/A_{260}.

Preparation of Cisplatin-Damaged Plasmid DNA for Cell-free Repair Assay

Five kinds of oligodeoxyribonucleotides were synthesized by the cyanoethyl phosphoramidite method using an Applied Biosystems model 380B DNA synthesizer. After annealing the complementary oligonucleotide, each of the duplex oligonucleotides was inserted into the *Xba*I site of the plasmid pGEM4 (Promega). The inserted plasmids were transformed and propagated in *Escherichia coli* (DH5α). Then these cells were subjected to alkaline lysis. The plasmid DNAs were purified from the lysate by ethidium bromide/CsCl ultracentrifugation [22] and subjected to sequence analysis. Cisplatin-damaged plasmid DNA was prepared according to the method described above.

Quantification of DNA–Platinum Adducts in Plasmids

Cis-DDP-pGL3 or cisplatin-damaged plasmid DNA was dissolved in 1 M HCl. The DNA solution was injected into a pyrolytic graphite cuvette (Hitachi, model 170-5101) and subjected to flameless atomic absorption spectrophotometry (Hitachi, model 180–300). A standard platinum solution (Wako Pure Chemical Industries, Co.) was used for calibration. The level of platination of the DNA was represented as the number of platinum adducts per plasmid.

Cell-free Repair Assay

The standard reaction mixture (20 μL) for cell-free DNA repair was composed of 40 mM creatine phosphate, 45 mM Tris-HCl (pH 7.8), 7.5 mM $MgCl_2$, 0.9 mM dithiothreitol,

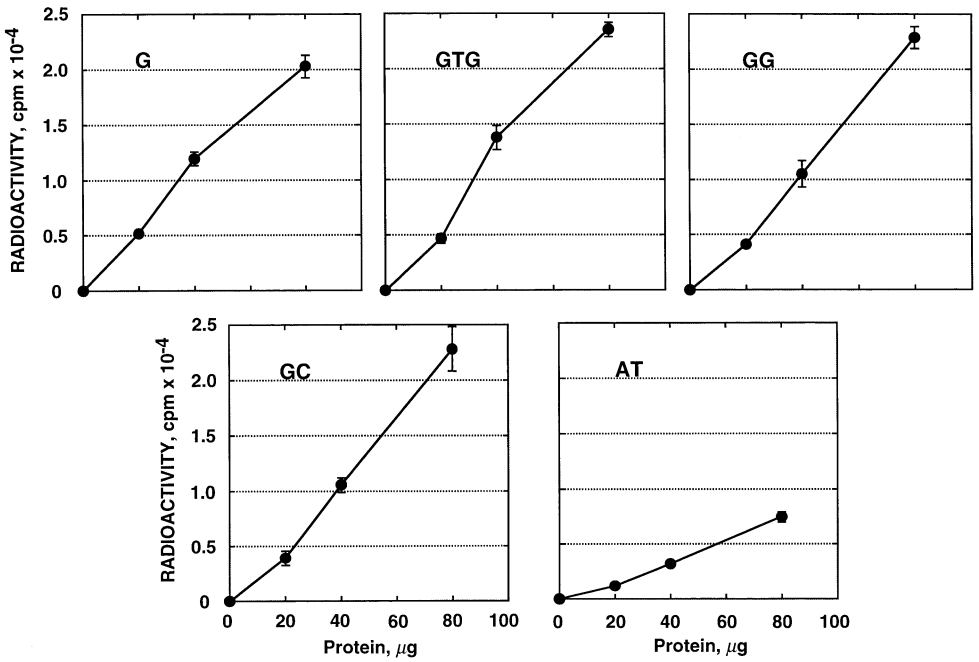

FIG. 4. Incorporation of radioactive nucleotide into cisplatin-treated duplex oligodeoxyribonucleotide in soluble extract of Ac2F cells. The radioactivity of the duplex oligonucleotide was quantified as described in the text. G, GTG, GG, GC, and AT are d(G), d(GTG), d(GG), d(GC), and d(AT), respectively, as shown in Fig. 2. The results are representative of 3 experiments and are expressed as the means of 3 samples with SD.

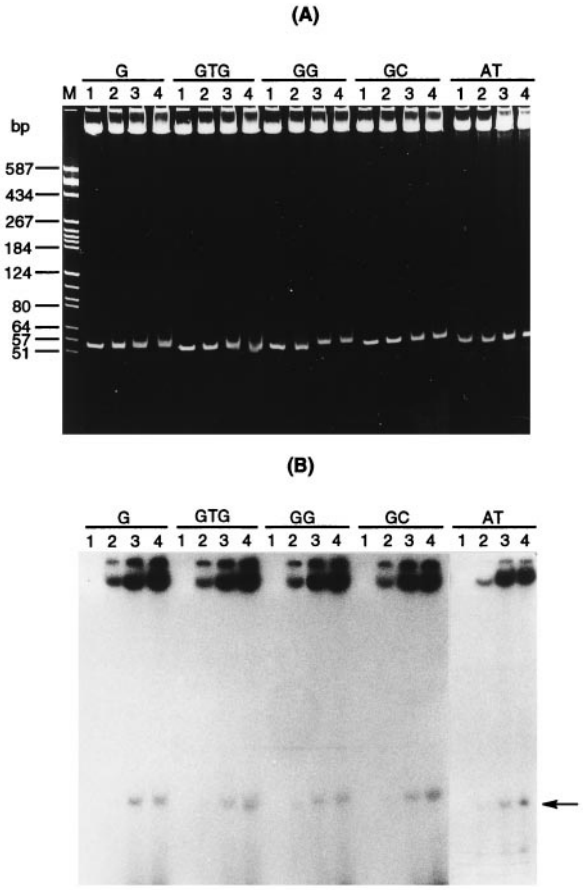

FIG. 5. Repair synthesis of duplex oligodeoxyribonucleotide treated with various concentrations of cisplatin. Plasmid containing the duplex oligonucleotide was treated with cisplatin and subjected to the cell-free repair assay, as described in the text. (A) Gel electrophoretic profiles of *Xba*I digests. The gel was stained with ethidium bromide. (B) Autoradiograph of (A). Lane M, DNA size markers (pBR322/*Hae*III digest); lanes 1–4, with 0, 0.1, 0.5 and 1.0 μM cisplatin, respectively. G, GTG, GG, GC, and AT are d(G), d(GTG), d(GG), d(GC), and d(AT), respectively, as shown in Fig. 2. The arrow shows the position of the duplex oligonucleotides released by *Xba*I digestion of the plasmid.

2 mM ATP, dCTP, dGTP, and dTTP (10 μM each), 2 μCi [α-^{32}P]dATP, phosphocreatine kinase (Sigma Type I, 2.5 μg), 60 mM KCl, 0.4 mM EDTA, 3.4% glycerol, bovine serum albumin (7.2 μg), whole-cell extract (typically 80 μg of protein), and plasmid DNA (2.5 μg). Whole-cell extract was prepared according to the method of Wood *et al.* [13]. The reaction was carried out at 30° for 5.5 hr and stopped by the addition of EDTA to a final concentration of 25 mM. The reaction mixture was extracted twice with an equal volume of phenol/chloroform (1:1, v/v). The aqueous phase was subjected to ethanol precipitation, and the precipitate was digested with *Xba*I in a 20-μL reaction solution. Digestion was stopped by the addition of a mixture (5 μL) of 0.25% bromophenol blue, 2.5% sodium dodecyl sulfate and 10% glycerol. The digest was electrophoresed on a slab-gel of 6% polyacrylamide and stained with ethidium bromide. Autoradiography was performed at −80° with Kodak X-Omat film. A Fujix BAS 2000 Bio-imaging Analyzer was also used to quantify the incorporation of the radioactive nucleotide into DNA. This assay was also performed in the presence of aphidicolin. The protein content was determined according to the method of Lowry *et al.* [23].

RESULTS

Cisplatin-Resistant Cell Lines

Ac2F cells were exposed to a stepwise increase in cisplatin concentration over several months. The resulting two cisplatin-resistant cell lines were designated Ac2F-CR4 and Ac2F-CR10. These cell lines were maintained in culture medium containing 60 μM cisplatin. Figure 1 shows the survival rate of each cell line in the presence of this drug. The LC_{50} after a 24-hr incubation was approximately 1 μM for Ac2F and more than 50 μM for Ac2F-CR4 or Ac2F-CR10. The survival rate of resistant cells was shown to be over 50-fold higher than that of the parental cell line, Ac2F.

Luciferase Activity Assay

To understand the molecular mechanism that reactivates cisplatin-damaged plasmid DNA, a luciferase activity assay was performed with cisplatin-treated pGL3 plasmid containing the luciferase gene (*cis*-DDP-pGL3). As shown in Table 1, the luciferase activity was approximately the same among all the cell lines transfected with untreated pGL3. However, when the cisplatin-resistant cell lines were transfected with *cis*-DDP-pGL3 (62 platinum adducts/plasmid) that had luciferase activity equivalent to about 10% of that of untreated pGL3 in Ac2F, the level of the activity of the resistant cells was about 3-fold higher in the resistant cells than that in Ac2F. These results suggested that the repair activity for *cis*-DDP-pGL3 was enhanced in the cisplatin-resistant cell lines.

Repair Activity Assay

To determine the substrate specificity for a cell-free repair assay, 52- to 54-bp oligodeoxyribonucleotides containing single GG, GTG, GC, or G were synthesized (Fig. 2) and inserted into pGEM4. Platination formed a single 1,2-intrastrand, a single 1,3-intrastrand, or a single 1,2-interstrand cross-link or a single mono-G adduct at the sequences GG, GTG, GC, or mono-G, respectively, in duplex oligonucleotides. The *cis*-DDP-damaged plasmid DNAs were incubated in the standard repair reaction solution and digested with *Xba*I to recover the oligonucleotide fragments (Figs. 3 and 4). This cell-free repair assay gave fairly reproducible results. The extraction protocols of DNA for each sample were identical, and the final DNA contents were similar among the reaction mixtures (Fig. 3A). When pGEM4 containing each of the duplex oligonucleotides was incubated with the soluble extract from Ac2F cells, incorporation of the radioactivity increased with increasing amounts of cell extract as compared with

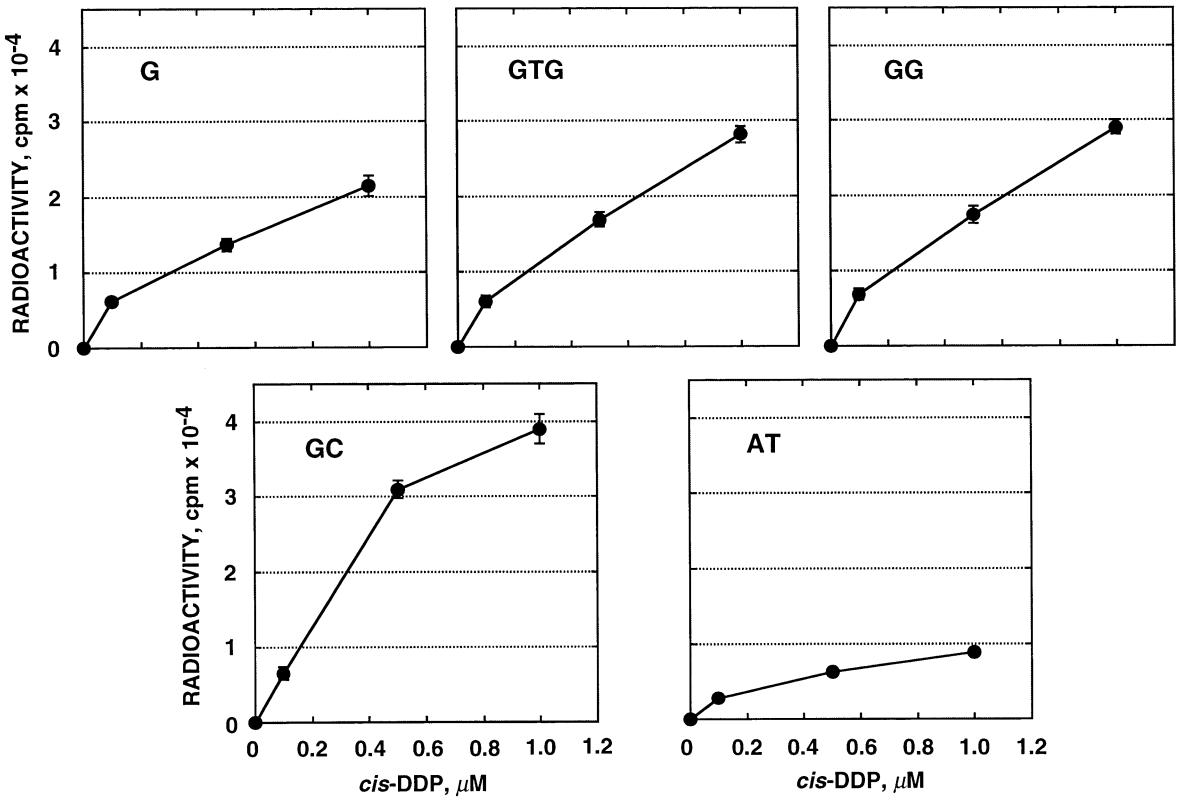

FIG. 6. Incorporation of radioactive nucleotide into duplex oligodeoxyribonucleotide treated with various concentrations of cisplatin (*cis*-DDP). The radioactivity of the duplex oligonucleotide was quantified as described in the text. G, GTG, GG, GC, and AT are d(G), d(GTG), d(GG), d(GC), and d(AT), respectively, as shown in Fig. 2. The results are representative of 3 experiments and are expressed as the mean of 3 samples with SD.

the control (AT-fragment) (Fig. 3B). The two bands around the origin were due to the incorporation of radioactivity into the aggregate and the *Xba*I-digested plasmids, respectively. Figure 4 shows the radioactivities of GG, GTG, GC, G, and AT fragments. The degree of incorporation into each of the fragments was proportional to the protein concentration in the range of 20–80 μg protein per 20-μL reaction mixture. The low level of incorporation into the AT fragment seemed to be dependent on the G residue at the *Xba*I recognition site of the fragment. Moreover, the incorporation of the radioactivity was enhanced with increasing cisplatin concentration as compared with the control (AT fragment) (Fig. 5B). Figure 6 shows that the incorporation was 2- to 5-fold higher in all the fragments than that in the AT-fragment. As the efficiency of platination of each fragment was not determined, the efficiency of repair could not be estimated. With this cell-free repair assay, repair synthesis was measured by independent extracts from the parental and resistant cells (Fig. 7). Both cisplatin-resistant cell lines showed enhanced specific incorporation of the radioactive nucleotides. Similarly, the enhancement of repair was observed in the oligonucleotides containing cisplatin adducts by calculating the ratio of the incorporation of radioactivity into the fragment (Fig. 8). Moreover, the specific inhibitor of DNA polymerases α, δ, and ϵ, aphidicolin, showed a concentration-dependent capacity to inhibit DNA repair synthesis. Of these enzymes, DNA polymerases δ and ϵ have been reported to be involved in nucleotide excision repair. Figure 9 shows that the repair synthesis in Ac2F and the cisplatin-resistant cell lines was inhibited in the presence of 10 and 50 μM aphidicolin.

DISCUSSION

The transient expression of transfected DNA has been used to study the effects of DNA damage on transcription and to determine the ability of recipient cells to repair lesions. Several investigators have shown that ultraviolet light- or cisplatin-damaged DNA inhibits expression of a reporter gene transfected into cells [8–11, 20, 24, 25]. In the present study, we performed the luciferase activity assay to investigate the DNA repair capacity of Ac2F cell lines. Two cisplatin-resistant cell lines, Ac2F-CR4 and Ac2F-CR10, were selected from Ac2F cells under continuous exposure to cisplatin with stepwise increases in concentration over many months. The survival rates of the cell lines were over 50-fold higher than that of the parental cell line (Fig. 1). To assay the cytotoxicity, we adopted the trypan blue dye exclusion method. Although it has been reported that this method tends to overestimate viability, more than 90% of the cells that excluded trypan blue proved to be capable of

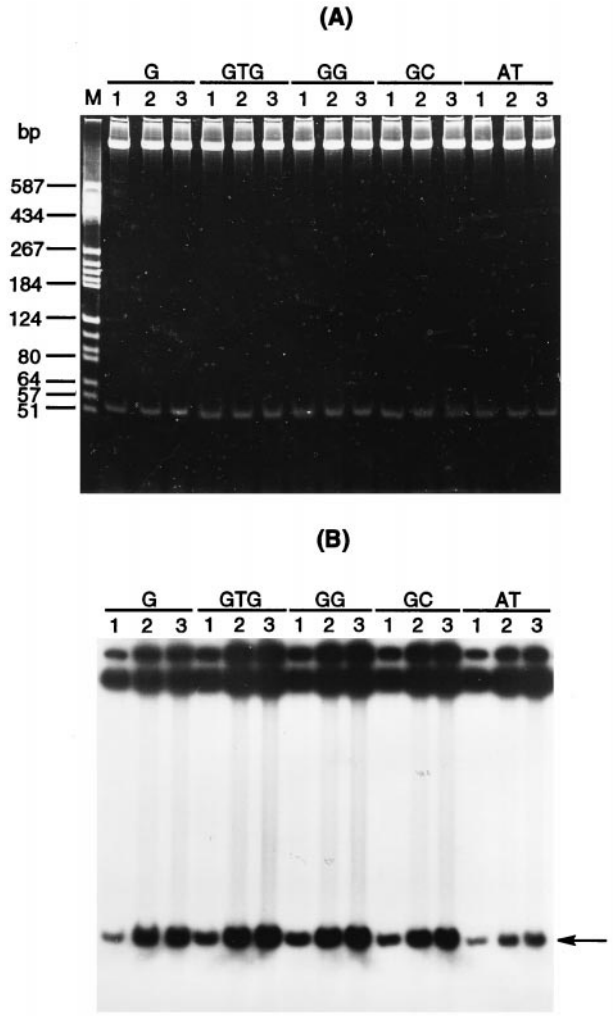

FIG. 7. Repair synthesis of cisplatin-treated duplex oligodeoxyribonucleotides in soluble extracts of Ac2F and cisplatin-resistant Ac2F cells. Plasmid containing the duplex oligonucleotides was treated with 1 μM cisplatin and subjected to the cell-free repair assay with soluble extracts of Ac2F and cisplatin-resistant Ac2F cells. (A) Gel electrophoretic profiles of *Xba*I digests. The gel was stained with ethidium bromide. (B) Autoradiograph of (A). Lane M, DNA size markers (pBR322/*Hae*III digest); lanes 1–3, with extracts of Ac2F, Ac2F-CR4, and Ac2F-CR10 cells, respectively. G, GTG, GG, GC, and AT are d(G), d(GTG), d(GG), and d(AT), respectively, as shown in Fig. 2. The arrow shows the position of the duplex oligonucleotides released by *Xba*I digestion of the plasmid.

attachment 24 hr later. Table 1 shows that although there were no differences in transcriptional activity among the cell lines containing cisplatin-undamaged plasmid pGL3, the cells transfected with *cis*-DDP-pGL3 (62 platinum adducts/plasmid) had luciferase activity equivalent to about 10% of that of undamaged pGL3. In contrast, the level of luciferase activity in Ac2F-CR4 or Ac2F-CR10 cells was about 3-fold higher than that in Ac2F cells. These results suggested that the repair activity for *cis*-DDP-pGL3 was enhanced in cisplatin-resistant cells. Using the CAT assay, Sheibani *et al.* reported that cisplatin-resistant L1210 cells are capable of enhancing the capacity for repair of cisplatin-induced intrastrand cross-links [8]. Chao *et al.* [10] reported that the expression of *cis*-DDP-damaged CAT gene is enhanced by about 3-fold in cisplatin-resistant HeLa cells. In the present study, we employed firefly luciferase cDNA as a reporter, as it is 2–3 orders of magnitude more sensitive than the CAT assay, allowing the detection of $1–3 \times 10^5$ luciferase molecules [20, 26]. Using this method, we showed that the excision repair activity for *cis*-DDP-pGL3 was reactivated and enhanced by about 3-fold in cisplatin-resistant Ac2F cell lines.

Cisplatin is an important antitumor drug that reacts with DNA to form intrastrand and interstrand cross-links that are removed by nucleotide excision repair with varying efficiencies. Of such cross-links, the incision reaction has been examined using a closed circular DNA substrate containing a single 1,3-intrastrand d(GpTpG)-cisplatin cross-link at a specific site [19]. With DNA molecules containing a single defined lesion at a specific site, we have shown that during *in vitro* repair reactions in the Ac2F cell extract, several types of cisplatin lesions stimulated DNA repair activity at the sites of the adducts. In addition, DNA repair activity induced by the adducts was shown to be 3- to 4-fold higher in the soluble extracts from cisplatin-resistant Ac2F cells than in that from Ac2F cells. These results suggested that the enhanced DNA repair activity induced by cisplatin adducts can be detected by *in vitro* repair assay with soluble cell extract. It has been reported that 1,2-intrastrand cisplatin diadducts are poorly repaired by human cell extract *in vitro*, and among minor lesions interstrand cisplatin cross-links represent a major lesion contributing to the repair synthesis signal in the *in vitro* assay [12, 27]. On the other hand, DNA polymerases α, δ, and ϵ were shown to be involved in DNA repair [28–31]. The specific inhibitor of DNA polymerase α, aphidicolin, was shown to have a concentration-dependent capacity to inhibit DNA repair activity [3]. Ali-Osman *et al.* [11] reported that there was no significant increase in DNA polymerase α activity in cells of a human malignant glioma following *in vivo* cisplatin therapy and that the increased tumor DNA polymerase β and DNA ligase activities may be associated with the increased DNA repair. In the present study, enhanced repair synthesis induced by cisplatin adducts was shown to be inhibited by aphidicolin. This indicated that aphidicolin-sensitive DNA polymerases participated in repair synthesis not only in cisplatin-resistant cell lines but also in Ac2F cells.

It has been shown that various cisplatin damage-recognition proteins are present in mammalian cell nuclei and may be involved in the mechanism of DNA repair [32–39]. DNA-damaging agents such as cisplatin and ultraviolet light have been demonstrated to be capable of inducing cellular factors responsible for enhancement of DNA repair activity [11, 40]. These proteins may be involved in initial recognition of damaged DNA sites as a part of the repair event or may be a part of the cellular response to stress. Binding of the proteins to damaged DNA may represent recognition of a disruption in the DNA duplex rather than

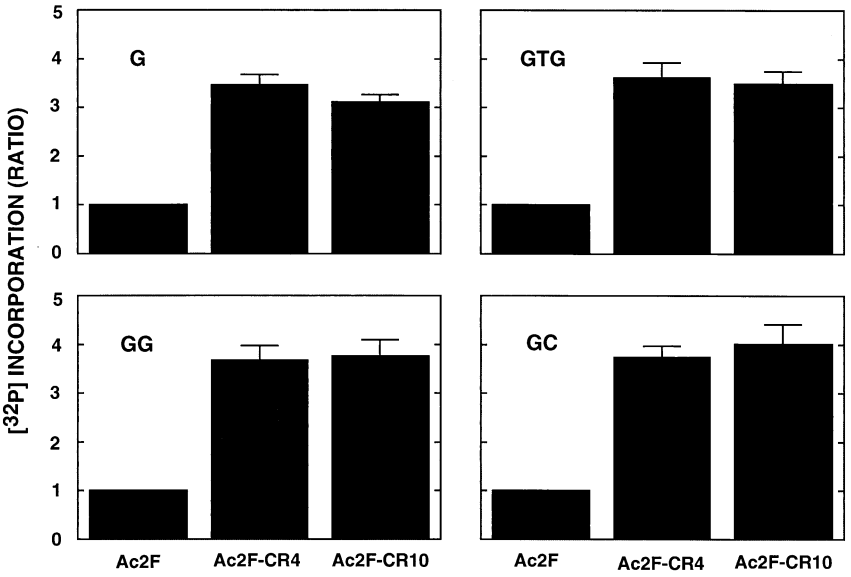

FIG. 8. Incorporation of radioactive nucleotide into cisplatin-treated duplex oligodeoxyribonucleotide in each of soluble extracts of Ac2F, Ac2F-CR4, and Ac2F-CR10 cells. The radioactivity of the duplex oligonucleotide was quantified as described in the text. G, GTG, GG, and GC are d(G), d(GTG), d(GG), and d(GC), respectively, as shown in Fig. 2. The results are representative of 3 experiments and are expressed as the mean values of 3 samples relative to those of Ac2F cells with SD.

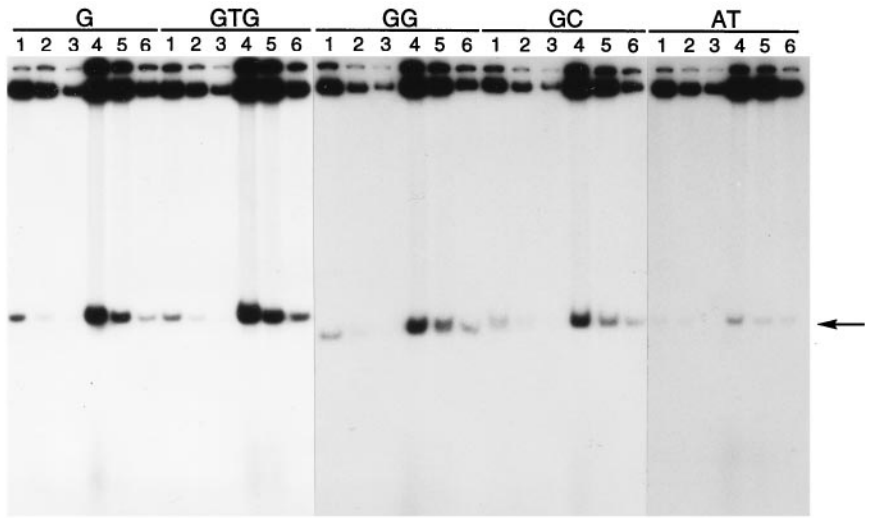

FIG. 9. Inhibition of repair synthesis in each of soluble extracts of Ac2F and cisplatin-resistant Ac2F cells with aphidicolin. Plasmid containing the duplex oligonucleotide was treated with 1 μM cisplatin and subjected to the cell-free repair assay. Lanes 1–3, with soluble extracts of Ac2F cells containing 0, 10, and 50 μmol aphidicolin in the reaction mixture, respectively; lanes 4–6, with soluble extracts of Ac2F-CR10 cells containing 0, 10 and 50 μmol aphidicolin in the reaction mixture, respectively. G, GTG, GG, GC and AT are d(G), d(GTG), d(GG), d(GC), and d(AT), respectively, as shown in Fig. 2. The arrow shows the position of the duplex oligonucleotides released by *Xba*I digestion of the plasmid.

the adduct itself [34]. Our previous studies have suggested that an endo-exonuclease activity endogenous to rat liver nuclei is involved in incision/excision of UV-damaged DNA for repair synthesis [41, 42], and we have shown that this nuclease activity and selective binding of a 23-kDa nuclear protein to cisplatin-damaged DNA are enhanced markedly in Ac2F cells exposed to cisplatin [43, 44]. Thus, these results could allow complementation experiments to characterize some cellular factors involved in further enhancement of repair synthesis in cisplatin-resistant cells. To understand the resistance to cisplatin in Ac2F cells, we currently are performing molecular cloning of such DNA binding proteins.

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