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Repair of *cis*-Platinum-DNA Adducts by ABC Excinuclease In Vivo and In Vitro

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cis-Platinum compounds, which are used in cancer chemotherapy, are thought to exert their effect by damaging DNA. It is known that this damage is partially repaired in *Escherichia coli*. Using *cis*-Pt-treated pBR322 DNA as a probe, we investigated the role of nucleotide excision repair in the removal of Pt-DNA adducts. We found that the nucleotide excision pathway was the major mechanism for repairing Pt adducts in transforming plasmid DNA but that a *recA*-dependent pathway also contributed to plasmid survival. When *cis*-Pt-damaged pBR322 was treated with the purified nucleotide excision enzyme ABC excinuclease in vitro, a fraction of the adducts was removed by the enzyme; this removal resulted in a corresponding increase in transformation efficiency.

cis-Platinum complexes are potent antitumor agents (18) that are being used in treating certain human cancers with considerable success (see reference 12 for a recent review). These agents are also toxic to *Escherichia coli* (1), and it has been demonstrated that *E. coli* cells deficient in repairing UV damage (*recA* or *uvrA*) are also deficient in repairing the damage caused by *cis*-platinum(II)diamminodichloride (*cis*-DDP), suggesting that the same repair mechanisms are acting on DNA adducts caused by both agents (1).

Although it is generally accepted that the target of the cytotoxic effects of *cis*-DDP and its second-generation derivatives, such as *cis*-1,2-diaminocyclohexyldichloroplatinum(II) [(*cis*-DACH)$PtCl_2$], is DNA, there is no consensus as to which type of DNA adduct is responsible for lethality. These drugs react mainly with N7 of guanine and produce either a monofunctional adduct or a bidentate chelate. The bidentate adducts can be in the form of intra- or interstrand cross-links (16). The fact that the *trans* isomer, which is capable of forming monoadducts as well as interstrand cross-links (16, 20) but not intrastrand cross-links, is therapeutically ineffective and that the number of interstrand cross-links does not correlate with cytotoxicity in resistant lines of mouse L1210 cells (29) suggests that interstrand cross-links are not the cytotoxic lesions. However, direct evidence for the lethality of various adducts is lacking; furthermore, it has been recently found that *cis*-Pt(II) can cross-link two adjacent Gs as well as Gs separated by a third base in the G_PN_PG sequence (5), making a direct correlation between one type of adduct and lethality more difficult. Also, most methods that aim at quantitating the various Pt(II) adducts involve harsh and sometimees lengthy treatments (4, 5, 10) which may cause secondary Pt(II) reactions, resulting in isolation and identification of adducts that are originally present in low levels or are not present at all.

We have previously demonstrated that ABC excision nuclease, which is responsible for initiating nucleotide excision repair, acts on *cis*-Pt(II)-damaged DNA in vitro, and based on findings about the action mechanism of the enzyme on UV-irradiated DNA, we have suggested that the enzyme removes Pt(II) mono-and diadducts by cutting on both sides of the damaged bases, thus producing an oligonucleotide 12 to 13 bases in length carrying the Pt adduct (26). Since these oligonucleotides can be separated from the bulk of DNA and analyzed for their adduct composition, the enzyme provides a direct way to measure the relative importance of various adducts. This paper reports the results of our initial studies with this system and reveals certain interesting aspects of *cis*-Pt(II)-DNA adducts. We found that the lethality of Pt-DNA adducts could be increased by further incubation of the complexes and that *cis*-Pt-damaged plasmid DNA was repaired primarily by nucleotide excision and secondarily by postreplication-recombination repair. However, even in cells deficient in both repair mechanisms, 1.7 adducts per molecule were required per lethal hit, suggesting either that some of the *cis*-Pt adducts are not an obstacle to plasmid DNA replication or that a third repair mechanism contributes to the removal of *cis*-Pt adducts. When *cis*-Pt-damaged DNA was treated with purified ABC excinuclease in vitro and then used to transform *E. coli recA uvrA* cells, we found that the enzyme removed the adducts from plasmid DNA and that there was a corresponding increase in its transformation efficiency. This finding strongly suggests that the majority of ABC nuclease-removed DNA adducts are lethal for DNA replication and, therefore, that analysis of the removed adducts may provide direct evidence of the identity of cytotoxic DNA adducts produced by *cis*-Pt(II) compounds.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* K-12 AB1157 (*recA*$^+$ *uvrA*$^+$), AB2463 (*recA13*), AB1886 (*uvrA6*), and CSR603 (*recA1 uvrA6*) were obtained from B. J. Bachmann, *Escherichia coli* Genetic Stock Center, Yale University, New Haven, Conn. The pBR322 plasmid (4,363 base pairs) used in our studies has been described previously (2). The cells were routinely grown in Luria broth, and pBR322 transformants were selected on Luria agar containing 20 μg of tetracycline per ml. Plasmid DNA and frozen competent cells were prepared by standard procedures. For transformation, 10 to 25 μl of DNA in Tris hydrochloride 10 mM, pH 7.4–NaCl (10 mM)–EDTA (1 mM) was added to 300 μl of competent cells. The mixture was kept on ice for 50 min and heat shocked at 42°C for 2 min; after the addition of Luria broth, the cells were incubated in a 37°C shaker for 2 h before plating onto selective

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medium. The transformation frequency varied linearly with the amount of DNA used in our experiments (<100 ng), and it ranged from about 1×10^5 transformants per μg for strains AB2463 and CSR603 to 4×10^5 transformants per μg for strains AB1157 and AB1886. Competency showed some variability from batch to batch, and therefore, all points of a plasmid survival experiment were obtained with the same batch of competent cells.

Preparation of radiolabeled (*cis*-DACH)$PtCl_2$. [4,5-^{3}H]*cis*-1,2-diaminocyclohexane was obtained from the Radiosynthesis Laboratory of Research Triangle Institute, Research Triangle Park, N.C. The compound was prepared by catalytic reduction of *cis*-δ^4-1,2-diaminocyclohexane with tritium gas over 10% palladium per liter. [4,5-^{3}H]*cis*-1,2-diaminocyclohexanedichloroplatinum(II) [^{3}H(*cis*-DACH)$PtCl_2$] was prepared by reacting the [4,5-^{3}H]*cis*-diaminocyclohexane with K_2PtCl_4 for 3 h in water at room temperature. The final compound was purified by recrystallization from water and dried in a vacuum at 80°C. The final specific activity was 267 mCi/mmol, and purity was greater than 95% as judged by reverse-phase high-pressure liquid chromatography (I. Husain, S. G. Chaney, and A. Sancar, manuscript in preparation).

Preparation of ABC excinuclease. The subunits UvrA, UvrB, and UvrC were prepared, by a modification of the method of Sancar and Rupp (26), to apparent homogeneity as tested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Our purified A and C subunits contained some nonspecific endonuclease activity as measured by the incision assay (see below) of superhelical DNA; however, when the excision assay was used as the criterion for purity (see below), none of the subunits removed *cis*-Pt adducts from the DNA. The adducts were removed, however, when all three subunits were present in the reaction mixture simultaneously.

Treatment of pBR322 with ^{3}H(*cis*-DACH)$PtCl_2$ in vitro. ^{3}H(*cis*-DACH)$PtCl_2$ was dissolved in 0.15 M NaCl and added to pBR322 to obtain final concentrations of 14 μg/ml for DNA and 50 μM for the drug in a reaction mixture that contained Tris hydrochloride (1 mM, pH 7.4), NaCl (25 mM), and EDTA (0.1 mM). The mixture was incubated at 23°C, samples were taken at time intervals, and the reaction was stopped by adding NaCl to a concentration of 0.5 M. The unincorporated drug was removed by dialyzing the samples at 4°C against 0.5 M NaCl for 4 h followed by overnight dialysis at 4°C against Tris hydrochloride (10 mM, pH 7.4)–NaCl (10 mM)–EDTA (1 mM). The average number of adducts per plasmid molecule was determined by measuring the radioactivity in each sample with liquid scintillation counter (LKB Instruments, Inc., Rockville, Md.) and measuring the plasmid concentration by optical density with an extinction coefficient of 20 M^{-1} cm^{-1} at 260 nm and an M_r of 2.88×10^6 for pBR322. One reason for using (*cis*-DACH)$PtCl_2$ in these studies was that it can be radiolabeled to form a relatively stable, isotope-containing compound, unlike *cis*-dichlorodiamine platinum. The adducts of the latter must be quantitated with atomic absorption spectrometry whose sensitivity is about an order of magnitude lower than that of our method.

Repair of *cis*-platinum-damaged plasmid in vivo and in vitro. To determine the effects of various *E. coli* DNA repair systems on plasmid DNA, *E. coli* strains of different repair capabilities were transformed with pBR322 containing 1 to 10 (*cis*-DACH)Pt-DNA adducts per molecule, and the survival of transforming efficiency relative to untreated DNA was calculated. The survivals reported are the averages obtained from three experiments. For each point, the variation was less than 10% of the mean.

The platinated DNA was repaired in vitro by incubating it with ABC excinuclease in a 50-μl reaction mixture containing Tris hydrochloride (50 mM, pH 7.5), KCl (100 mM), $MgCl_2$ (10 mM), ATP (2 mM), dithiothreitol (5 mM), bovine serum albumin (100 μg/ml), pBR322 DNA (0.27 pmol [containing an average of either 12.5 or 10.7 adducts per molecule]) and UvrA, B, and C proteins at 0.44, 0.6, and 0.6 pmol, respectively. The reaction mixtures were incubated at 23°C for 3, 7, and 30 min, and the reaction was stopped by adding EDTA to a concentration of 20 mM. The reaction products were analyzed on agarose gels and by transformation. To analyze the DNA for its adduct content, sodium dodecyl sulfate, bromophenol blue, and glycerol were added to the samples to final concentrations of 0.5, 0.05, and 10%, respectively, and the samples were heated at 65°C for 5 min and loaded onto a 1% agarose gel. After the run, the gel was stained with ethidium bromide, the bands corresponding to superhelical and open circular DNA were cut out, and the radioactivity in each band was determined as described previously (23). From the decrease in total radioactivity in superhelical plus open circular DNA, the remaining average number of adducts per DNA molecule was calculated. We named this assay the excision assay to differentiate it from the incision assay which we have used previously and which relies on the fraction of superhelical DNA remaining in the reaction mixture to quantitate the number of cuts made by ABC excinuclease (23). Although the incision assay is more sensitive, it is not reliable when more than five cuts per molecule are made (less than 0.67% of DNA is superhelical); also, it is subject to interference by nonspecific endonucleases. The excision assay, on the other hand, is specific for ABC nuclease and can reliably measure the removal of any number of adducts, provided that the adducts are made with a compound of relatively high specific activity. To measure the biological repair of ABC excinuclease-treated DNA, we used the transformation assay as follows: the enzyme-treated samples were extracted with phenol, and the DNA was precipitated with ethanol and then suspended in Tris (10 mM, pH 7.4)–NaCl (10 mM)–EDTA (1 mM); 50 to 80 ng of this suspension was used for transforming *E. coli* CSR603 (*recA uvrA*). From the increase in transforming efficiency as a result of nuclease treatment and the known survival curve of pBR322 on this strain, the number of adducts repaired was calculated.

RESULTS

Formation of DNA adducts by (*cis*-DACH)$PtCl_2$ in vitro. (*cis*-DACH)$PtCl_2$ is a second-generation antineoplastic platinum compound which is effective against the mouse L1210/DDP cell line that is resistant to the traditional chemotherapeutic Pt compound *cis*-$Pt(NH_3)_2Cl_2$; therefore, (*cis*-DACH)$PtCl_2$ may be advantageous in cancer chemotherapy over the diaminodichloro- form (3). The cause of the differential effects of the two drugs is not known. However, it has been reported that they have similar reactivities (16), since the carrier ligand (diaminocyclohexyl or NH_3) has little effect on the leaving ligand (Cl in both cases). To test the reactivity of (*cis*-DACH)$PtCl_2$ towards DNA in vitro, we incubated the radiolabeled drug with pBR322 and measured the adduct formation as a function of time (Fig. 1). After an inital lag of about 10 min, adducts were formed at a rate of about 0.12 adduct per min under our experimental conditions. The initial lag was probably due to the time required

for the formation of aquated forms of the drug, as it is known that these are the reactive species (16). The kinetics shown in Fig. 1 are similar to those we obtained with *cis*-$Pt(NH_3)_2Cl_2$, and in both cases, the addition of NaCl to a concentration of 0.5 M completely stopped the reaction without an effect on the adducts already formed (data not shown).

Survival of (*cis*-DACH)$PtCl_2$-damaged plasmid. The effect of Pt-base adducts on DNA replication in *E. coli* was studied by the standard *E. coli* transformation assay. When *E. coli* CSR603 (*recA uvrA*), which is deficient in the two major repair pathways acting on Pt adducts (1), was the host, the results shown in Fig. 2 were obtained. If the DNA was used immediately after the removal of unincorporated drug, two adducts per molecule were required per lethal hit. However, if the DNA, after the removal of unincorporated drug by dialysis, was incubated at 37°C for 30 h before transformation, 1.7 adducts per molecule constituted a lethal hit. These results led to the following conclusion. First, since 1.7 adducts per molecule appeared to be the minimum number corresponding to one lethal hit, either *E. coli* possessed a third repair mechanism to eliminate at least certain types of Pt adducts or its replication machinery could bypass about 50% of Pt adducts. Second, some nonlethal Pt adducts were converted to lethal ones upon further incubation at 37°C. It has been observed by Roberts and Frieldos (15) that incubation of platinated DNA at 37°C for 24 h leads to an increase in the frequency of interstrand cross-links from 0.66 to 3%. However, this observation cannot completely account for our results, because even if we assume that every interstrand cross-link was a lethal adduct and that 3% of the adducts became interstrand cross-links after incubation at 37°C, this would have reduced the number of adducts per lethal hit from 2 to 1.94, which is higher than the number we obtained experimentally. Therefore, we favor the interpretation that a substantial fraction of Pt-DNA adducts formed under our incubation conditions were monoadducts, that prolonged incubation of monoadducts led to formation of intra- as well as interstrand cross-links, and that the increase in frequency of intrastrand cross-links was the predominant contributor to increased lethality.

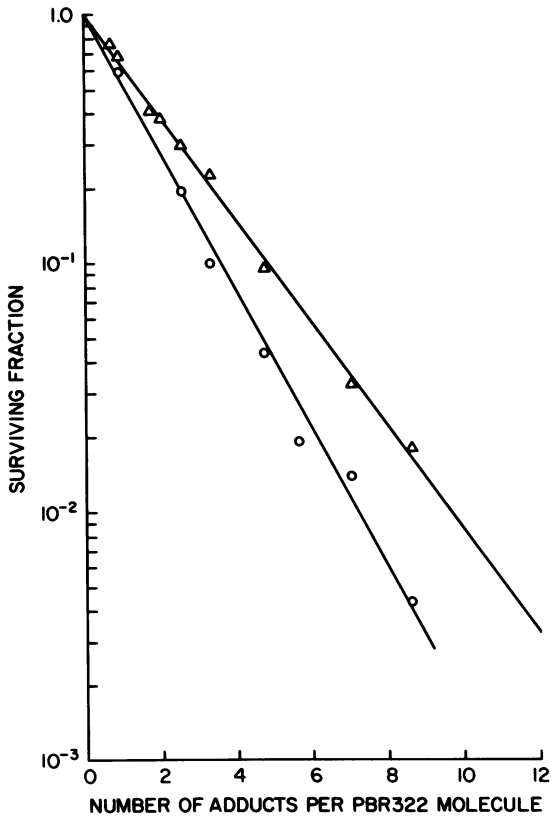

FIG. 2. Survival of transforming efficiency of (*cis*-DACH)$Pt(Cl)_2$-damaged pBR32 on an *E. coli* CSR603 (*recA uvrA*) host. Symbols: △, the plasmid was used immediately after the removal of the unincorporated drug by dialysis at 4°C; ○, after the removal of unincorporated drug, the DNA was incubated at 37°C for 30 h and then used for transformation.

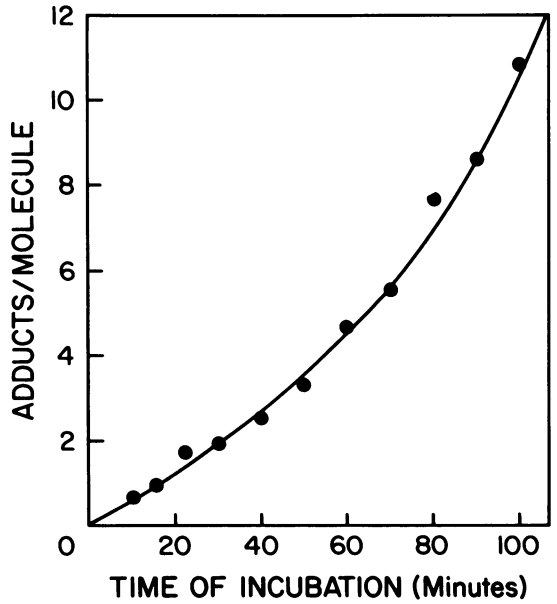

FIG. 1. Kinetics of incorporation of [4,5-^{3}H](*cis*-DACH)$Pt(Cl)_2$ into superhelical pBR322. The drug-to-nucleotide ratio in the reaction mixture was 2.38. The reaction was conducted at 23°C; samples were taken at time intervals, and the adduct formation was measured as described in the text.

Effects of nucleotide excision and postreplication-recombination repair pathways on plasmid survival. The use of plasmids offers certain advantages in probing cellular DNA repair systems compared with direct treatment of cells or an infecting phage with the damaging agents in that any biological consequence of the treatment is a result of DNA damage only and reflects the ability of the cell to deal with that damage. The *E. coli* transformation assay with plasmid DNA has been used to probe the repair of this bacterium for acting on DNA damaged by various agents (13, 17, 22, 24, 25, 27, 30). The survival of (*cis*-DACH)$PtCl_2$-damaged pBR322 on *E. coli* hosts with various repair capabilities is shown in Fig. 3. In all of these cases, DNA that had been incubated at 37°C for 30 h after drug treatment was used to eliminate a possible change in adduct composition because of prolonged storage in a refrigerator. Indeed, the same survival curve was obtained for our tester strain, CSR603, over the 3-week period in which these experiments were conducted. We found D_{37} of 1.7, 2.6, 4.8, and 5.5 adducts per molecule for CSR603 (*recA uvrA*), AB1886 (*recA*$^+$ *uvrA*), AB2463 (*recA uvrA*$^+$), and AB1157 (*recA*$^+$ *uvrA*$^+$), respectively (Fig. 3). The last number is in reasonable agreement with the finding that five *cis*-Pt adducts per phage particle

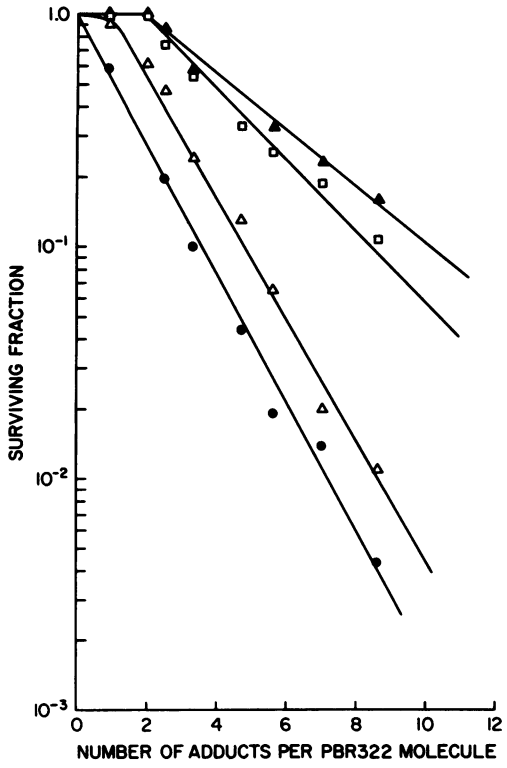

FIG. 3. Survival of a (*cis*-DACH)Pt-adducted pBR322 plasmid on *E. coli* strains of various repair capabilities. The data points for CSR603 are taken from Fig. 2. The transformation experiments in all cases were carried out with DNAs that were incubated at 37°C for 30 h after the incorporation of the drug into the plasmid. Symbols: ●, CSR603 (*recA uvrA*); △, AB1886 (*recA*$^+$ *uvrA*); □, AB2463 (*recA uvrA*$^+$); ▲, AB1157 (*recA*$^+$ *uvrA*$^+$).

(four of which were with the nucleic acid) constituted a lethal hit for the T7 phage (28). From these data it seems clear that the main repair system removing Pt adducts from DNA was the nucleotide excision repair. However, it also seems clear that the RecA$^+$ phenotype contributed significantly to plasmid survival. Similar effects have been observed with plasmids damaged by UV (17) and *N*-acetoxy-*N*-2-acetylaminofluorene (N-AAAF) (27), but not with those damaged by benzo(a)pyrene-7,8-dihydrodiol-9,10-oxide (BPDE) (13). We compared the survival of plasmid-containing Pt adducts in *E. coli* strains of different repair capabilities with survival rates reported in the literature for other DNA damaging agents (Table 1). As is apparent from Table 1, *cis*-Pt adducts were more like UV- and BPDE-produced adducts with regard to their lethalities, and they differed significantly from N-AAAF adducts, which are removed very efficiently by ABC excinuclease (7, 27, 30). Whether the differences among the lethalities of BPDE, UV, and *cis*-Pt adducts are real or an artifact due to the different sensitivities of the methods used to determine the number of adducts remains to be seen. However, it is clear from this table that N-AAAF adducts are far less lethal than the other three.

Repair of (*cis*-DACH)PtCl$_2$-damaged DNA in vitro. The results reported above indicate that ABC excinuclease is involved in repairing *cis*-Pt-DNA adducts. If this indeed is the case, then treatment of platinated DNA with ABC excinuclease in vitro should result in the removal of the adducts from the plasmid and in a corresponding increase in the transforming efficiency of the damaged DNA. To repair the *cis*-Pt-damaged DNA in vitro, we incubated the modified DNA (12.5 or 10.7 Pt adducts per pBR322 molecule) with the enzyme for various time periods. At the end of each period, part of the reaction mixture was analyzed to measure the number of adducts removed, and the other part was tested for biological activity by transforming *E. coli* CSR603 (*recA uvrA*) to tetracycline resistance. We plotted the number of adducts removed as measured by the transformation assay against that measured by the excision assay (Fig. 4). As is

TABLE 1. Survival of plasmids damaged by various agents in *E. coli* strains of different repair capabilities

Phenotype of *E.coli* K-12	No. of adducts/lethal hit[a]			
	BPDE[b]	UV[c]	*cis*-Pt[d]	N-AAAF[e]
RecA$^+$ UvrA$^+$	3	5.54	5.5	20
RecA$^-$ UvrA$^+$	3	3.75	4.8	18
RecA$^+$ UvrA$^-$	1	1.48	2.6	5
RecA$^-$ UvrA$^-$		1.09	1.7	2

[a] A lethal hit per molecule reduces the transformation efficiency of plasmid to 37% as predicted from Poisson distribution of adducts.
[b] From reference 13. The plasmid used was pKO482, a 4.2-kilobase-pair derivative of pBR322.
[c] From reference 17. The plasmid used was NTP16, an 8.8-kilobase-pair nonconjugative plasmid.
[d] This study.
[e] From the data in references 7 and 27. In reference 27, the plasmid was pKO482, and in reference 7, the plasmid was pBR322. Tang et al. (30) reported less than two N-AAAF adducts per molecule per lethal hit in *uvrA* cells with the φX174 transfection assay.

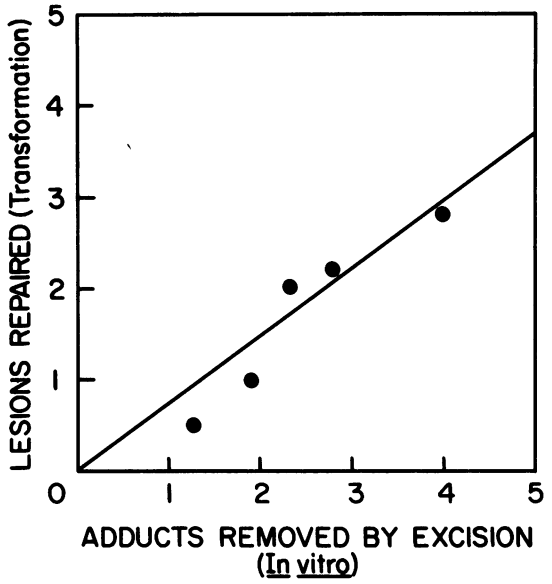

FIG. 4. Removal of *cis*-Pt adducts from DNA results in biological activation of transforming DNA. pBR322 DNA containing *cis*-Pt adducts was incubated with ABC excinuclease; the numbers of adducts removed and the numbers of lesions repaired at various time points were determined by the exicision assay and transformation assay, respectively. The data points in this figure were obtained from two representative experiments with plasmid DNAs that originally contained 12.5 to 10.7 adducts per molecule. ABC excinuclease had no effect on the transforming efficiency of undamaged DNA (data not shown).

apparent from Fig. 4, there was a good correlation between the results obtained with the two assays. The activity measured by the transformation assay was consistently 20 to 25% lower than that measured by the excision assay. We think this is because the gapped DNA generated by ABC nuclease (a 12- to 13-nucleotide gap per adduct removed) was more susceptible to endonuclease attack upon uptake than was superhelical or nicked DNA. Whatever the cause for the relatively small discrepancy between the two assays, it seems clear from our results that platinated plasmid DNA could be repaired by ABC excinuclease in vitro.

DISCUSSION

In this paper we report our investigation of the production of DNA adducts by (*cis*-DACH)$PtCl_2$ in vitro with pBR322 DNA and the repair of these adducts in *E. coli* in vivo and by ABC excinuclease in vitro. Our main findings and conclusions can be summarized as follows.

(i) We used ^3H-labeled (267 mCi/mmol) (*cis*-DACH)$PtCl_2$ in our experiments to monitor the reactivity of this drug with DNA in vitro. The use of such a highly radioactive compound enabled us to detect the formation of as little as one adduct per 4,400 base pairs with reasonable accuracy. This level of detection is far superior to the conventional atomic absorption spectrometry used in *cis*-Pt studies. Under our experimental conditions, (*cis*-DACH)$PtCl_2$, after an initial lag, produced DNA adducts at a constant rate over the duration of the experiment; this rate was similar to the rate obtained with *cis*-$Pt(NH_3)_2Cl_2$.

(ii) With plasmid DNA treated in vitro with *cis*-Pt compounds, it should be possible to determine the relative importance of various adducts in cytotoxicity and to identify the DNA repair pathways involved in removing these adducts. We found that 5.5 *cis*-Pt adducts per transforming DNA molecule constituted a lethal hit in wild-type *E. coli*. This is in reasonable agreement with the four adducts per molecule per lethal hit found with T7 phage infection (27) but is considerably lower than the about 20 adducts per molecule per lethal hit that was found by the transfection assay with lambda DNA (6). It is possible that in the latter experimental system, the multiplicity of infection was higher than in the other two experimental setups, which may allow extensive recombination and high survival by what might be called a marker rescue mechanism. In *E. coli* mutants deficient in excision repair, 2.6 adducts per molecule constituted a lethal hit, indicating that nucleotide excision repair is the major pathway for removing platinum adducts from transforming plasmid DNA. This finding is in line with the fact that *uvrA*$^-$ cells are more sensitive to *cis*-DDP (1) and that *cis*-DDP-treated DNA is cut by ABC excinuclease in vitro (26), but it differs from the results obtained by Munchausen with the *Haemophilus influenzae* transformation assay (14). In that system it was found that transforming DNA treated with *cis*-Pt had the same survival on Uvr$^+$ and Uvr$^-$ cells. This is not surprising because the two systems are different in two important aspects. First, transformation in *H. influenzae* involves incorporation of single-stranded DNA into the host chromosome, whereas the *E. coli* plasmid transformation assay depends on establishment of a second replicon in the host cells. Second, although it is well established that *E. coli* ABC excinuclease acts on a wide variety of nucleotide adducts, it is not clear at present whether the *H. influenzae* nuclease encoded by the *uvr-1* and *uvr-2* genes acts on adducts other than pyrimidine dimers.

Cells carrying mutations at the *recA* locus repaired the *cis*-Pt-damaged plasmid less efficiently than did their *recA*$^+$ counterparts whether the latter cells were *uvr*$^+$ or not, indicating that *recA* participants in repair pathway that, although inefficient, is *uvrA*$^+$ independent. Whether this repair pathway functions by the classical postreplication-recombination mechanism (21) is debatable. Similar relatively small effects of *recA* on plasmid survival have been observed for UV-damaged or N-AAAF-treated DNAs (17, 27). The plasmid concentrations used in all of these experiments appear to be too low to give rise to double transformants, which might easily explain the *recA* effect (11, 31). However, the usual means of estimating double-transformation frequency may not actually reflect the fraction of cells that take up more than one DNA molecule. With a method developed by Goodgal and Herriott (8), it has been determined that in *E. coli* cells rendered competent by the Mandel-Higa $CaCl_2$ method, only 0.26% of the cells are competent to be transformed (31). If we assume that only these cells take up DNA, then under our experimental conditions the ratio of pBR322 to competent cells is about 6×10^4. As previously argued (31), an average of 6×10^4 molecules of pBR322 per competent cell may need to be taken up for transformation to be established. If such is the case, then there would be plenty of opportunities for recombinational repair of transforming plasmids. However, such reasoning fails to explain the fact that *recA* had no effect on the survival of BPDE-damaged plasmids (13). It is formally possible that the UvrABC- and RecA-dependent pathways operate on different specific classes of Pt adducts of equal lethality but that one type of adduct (repaired by the *recA* pathway) is formed to a lesser extent compared with the adducts repaired by the ABC excinuclease. Further investigation is needed to clarify this point, but it is worth mentioning that of the DNA adducts tested by the *E. coli* transformation assay, those produced by BPDE seem to be the most lethal even in wild-type cells (three adducts per molecule per lethal hit).

While UV- and *cis*-Pt-produced DNA adducts have similar lethalities in various *E. coli* strains, N-AAAF-DNA adducts seem to be less lethal in all strains tested. The large number of adducts required to assure lethality in UvrA$^+$ strains (27, 30) is consistent with the idea that these latter adducts are removed more efficiently by ABC excinuclease. However, we believe that the adducts are partly removed by spontaneous depurination as well, as it is known that the major N-AAAF adduct, G-C8-AAF, weakens the glycosidic bond of G (32). This might also explain why two or more N-AAAF adducts are required per lethal hit in *recA*$^-$ UvrA$^-$ cells (7, 27). However, spontaneous depurination cannot be the explanation for the fact that two adducts per molecule in freshly treated plasmids and 1.7 adducts per molecule in posttreatment-incubated plasmids are needed for a lethal hit by (*cis*-DACH)$PtCl_2$, since the major *cis*-Pt adducts, the Pt-G-N7 coordination complexes (mono- or bifunctional) appear to strengthen the glycosyl bond (9, 32). It is conceivable that approximately 50% of *cis*-Pt adducts formed in vitro are monoadducts that either remain so after cellular uptake or more likely form DNA-Pt-protein cross-links and that the monoadducts and perhaps the DNA-Pt-protein cross-links are removed by a DNA glycosylase specific for these *cis*-Pt adducts. With respect to monoadducts, current chemical estimates (4, 5) are much lower than our model suggests, but all of the analyses to isolate Pt base adducts involve lengthy incubations which may lead to underestimation of monoadducts. Numerous in vitro and in vivo experiments have demonstrated that rapid formation of DNA-platinum adducts which can be subsequently converted to

cross-links (15, 29, 35). Thiourea rescue experiments suggest that the majority of the platinum adducts formed in short incubations may be monoadducts, with conversion of the monoadducts to both intra- and interstrand cross-links occurring over a period of hours (34). This is consistent with our finding that incubation of our platinated DNA at 37°C increased the lethality of the adducts. However, even after 30 h of incubation at 37°C, we found 41% of the *cis*-Pt adducts to be nonlethal. It is possible that even after such a treatment, 41% of the Pt-DNA adducts are still in the monofunctional form and are rapidly removed by a hypothetical glycosylase or are converted to the nonlethal DNA-Pt-protein cross-links upon entering the cell. The DNA-Pt-protein cross-links appear to be a major form of platinum adducts (5, 35) and have apparently little or no toxicity (35).

(iii) (*cis*-DACH)$PtCl_2$-damaged DNA can be efficiently repaired in vitro by ABC excinuclease. Even though previous studies have demonstrated that purified ABC excinuclease cuts *cis*-platinum-damaged DNA (26), this is the first demonstration that cutting resulted in the removal of *cis*-Pt adducts from DNA and in repair, in a biological sense, since the transforming efficiency of the nuclease-treated DNA was increased. The removal of *cis*-Pt adducts from DNA led to a parallel decrease in the average number of lethal hits remaining per molecule. However, the repair measured by the increase in transformation efficiency corresponded to about 75% of that measured biochemically by our excision assay. We believe that this discrepancy is due to the reduced transforming efficiency of gapped DNA as the removal of *cis*-Pt DNA adducts creates 12- to 13-nucleotide-long gaps which are likely to be more susceptible to attack by nonspecific endonucleases. However, we cannot exclude the possibility that ABC excinuclease has a slight preference in removing nonlethal Pt adducts. Analysis of the adduct composition in total and excised DNA should help in clarifying this point.

Finally, although the results presented in this paper, together with those obtained by Van Houten and Masker (30a) with benzo[a]pyrene-damaged T7 phage DNA and partially purified cell extracts of Uvr^+ cells, may be considered as the reconstitution of *E. coli* nucleotide excision repair in vitro, it should be pointed out that in our experiments the plasmid DNA, after ABC excinuclease digestion, was deproteinized by either heating in the presence of sodium dodecyl sulfate or, phenol extraction before analysis on agarose gels or by transformation. Thus, even though it is clear that the enzyme excises an oligonucleotide carrying the damaged nucleotide(s), it remains to be seen whether it dissociates from its cutting site (33) at a physiological rate or whether additional factors (*uvrD* helicase, DNA polymerase) are required to facilitate the dissociation. Preliminary experiments indicate that *cis*-Pt-DNA adducts are removed by ABC excinuclease only stoichiometrically and that additions of DNA polymerase I and helicase II (*uvrD* protein) to the reaction mixture promote the turnover of the excision nuclease.

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