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Influence of Temperature on Platinum Binding to DNA, Cell Killing, and Mutation Induction in *Escherichia coli* K-12 Cells Treated with *cis*-Diamminedichloroplatinum(II)[1]

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ABSTRACT

Cell killing and specificity of mutation induction by treatment of *Escherichia coli* K-12 cells with *cis*-$Pt(NH_3)_2Cl_2$ at various temperatures have been studied. Survival experiments show that the cell killing by *cis*-$Pt(NH_3)_2Cl_2$ is enhanced with increasing temperature. This effect is explained by an increase in the amount of platinum bound to the DNA. The binding is the same in repair-proficient and in repair-deficient cells.

However, the mutation induction in the *lacI* gene is much more strongly enhanced than could be expected from the increased platinum binding. This phenomenon is attributed to a different distribution of the induced lesions by *cis*-$Pt(NH_3)_2Cl_2$ at various temperatures and not to an aberrant excision repair. Analysis of the induced *lacI* mutants revealed an increase in the percentage of nonsense mutants at higher temperature. Among the nonsense mutations, base-pair substitutions at GAG and particularly at GCG sequences are enhanced by the increasing temperature. The results are in agreement with our hypothesis that local denaturation of DNA, known to be promoted at higher temperature, is necessary for the formation of intrastrand cross-links at two guanine bases separated by a third base.

INTRODUCTION

cis-DDP[3] has been shown to exhibit anti-tumor activity, most probably due to the interaction with DNA (10). This interaction can lead to the formation of inter- and intrastrand cross-links, which presumably block DNA replication (5, 6). Recently (2), we described the results of an investigation on the specificity of *cis*-DDP-induced mutagenesis in *Escherichia coli*, using the *lacI* system developed by Coulondre and Miller (3, 4) and Miller *et al.* (9). This system allows for the detection of 72 independent transition and transversion events, leading to amber (TAG) or ochre (TAA) codons in the known base sequence of the *lacI* gene of *E. coli*. Determination of sites where the nonsense mutations are preferentially induced ("hot spots") may provide evidence for the interaction of a mutagenic agent with certain nucleotide sequences in the DNA. The previous study revealed the presence of hot spots for *cis*-DDP-induced base-pair substitutions at sites where the guanine that is substituted is part of a GAG or GCG sequence. It was proposed that lesions leading to this type of mutations consist of intrastrand cross-links formed by *cis*-DDP between 2 guanines separated by a third base. Recently, Raman studies also have shown that the 2 guanines involved in intrastrand cross-links with *cis*-DDP are not necessary adjacent bases (1). Furthermore, we demonstrated that, in *E. coli*, the repair of platinum-DNA lesions leading to base-pair substitutions is dependent both on excision repair and the *recA* gene product.

The cytotoxic effect of *cis*-DDP towards CHO cells is strongly potentiated by hyperthermia (8). This effect could only partially be attributed to the increased amount of interstrand DNA cross-links. However, if the formation of intrastrand cross-links by *cis*-DDP at GAG and GCG bases is also involved, it would not be surprising if this process is stimulated at higher temperature because of the necessity of local denaturation of the DNA.

The present study describes the influence of the temperature on platinum binding to DNA, cell killing, and mutation induction in *E. coli* K-12 cells by treatment with *cis*-DDP. In addition, the influence of the temperature on the excision repair of platinum-DNA lesions will be described.

MATERIALS AND METHODS

Platinum Treatment of the Cells. Exponentially growing cells were harvested, suspended in buffer solution (9.5 mM citric acid-0.7 mM $MgSO_4$-37.0 mM $KHPO_4$-16.7 mM $Na(NH_4)HPO_4$, pH 7.0; Vogel-Bonner), and incubated for 60 min at the required temperature. Subsequently, freshly prepared solutions of *cis*-DDP in dimethylformamide were added. After incubation for 120 min in the dark, the cells were spun down and washed with ice-cold buffer.

DNA Isolation. *E. coli* cells were lysed with sodium triisopropylnaphthalenesulfonate (5 g)-2-butanol (30 ml)-sodium 4-aminosalicylate (30 g)-NaCl (5 g)-water (470 ml) followed by deproteination with phenol-*m*-cresol-8-hydroxyquinoline according to methods described by Kirby and Cook (7). DNA was precipitated from the aqueous phase, made 3% with respect to NaCl by addition of 2 volumes of 2-ethoxyethanol, and dissolved in 0.01 M phosphate buffer, pH 7.0. The solution was treated for 15 min at 37° with RNase, and the DNA was precipitated again. Finally, the DNA was washed twice with ethanol and ether, dried quickly, and dissolved in phosphate buffer.

Platinum Measurements. The amount of platinum bound to DNA was determined by AAS with a Perkin-Elmer Model 4000 atomic absorption spectrophotometer equipped with a HGA-500 graphite furnace and an AS-40 autosampling system. K_2PtCl_6 solutions were used for calibration.

Survival and Mutagenesis. After *cis*-DDP treatment of the *E. coli* cells, appropriate dilutions were plated on tryptone-broth agar for determination of the survival, and undiluted aliquots were plated on phenyl-β-D-galactoside plates to select for *lacI* mutants. Details have been described previously (2).

***lacI* System.** Materials and techniques used in the *lacI* system were as described by Coulondre and Miller (3, 4) and by Miller *et al.* (9), with the exception that the cells were plated directly after treatment with *cis*-DDP. We have shown before that the direct plating does not influence the mutation induction by *cis*-DDP (2). In this system, muta-

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[3] The abbreviations used are: *cis*-DDP, *cis*-diamminedichloroplatinum(II); CHO, Chinese hamster ovary; AAS, atomic absorption spectroscopy.

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tions in the *lacI* gene, which is located on a F′ episome, are screened for nonsense mutations. For that purpose, the mutated F *lacI* episomes are conjugated into several suppressor strains, which allows the detection of different amber and ochre mutations. Subsequently, the positions of the nonsense mutations in the *lacI* gene are determined by conjugation of the F′ episomes into strains carrying *lacI* deletions of various length. Dependent on the position of the mutations, *lacI*$^+$ recombinants can be formed or not. In this way, 72 independent mutations can be detected.

Strains. *E. coli* K-12 strains carrying deletions in the *lacI* gene and the suppressor strains used in the *lacI* system were kindly provided by J. H. Miller and have been described (9). GM-1 – (F *lacI*^{Q}*L8*, *pro/ara*, Δ*lac-pro*, *thi*) was used as repair-proficient *E. coli* K-12 strain. The excision repair-deficient strain was GM-1 (Δ*chlA-uvrB-bioFCD*) (2).

RESULTS

Relation between Cell Killing and Platinum Binding at Various Temperatures. In Chart 1, the survival curves of wild-type *E. coli* cells treated with *cis*-DDP at 25°, 32°, and 40° are given. They show that the survival of *E. coli* cells is much lower at higher temperature. To investigate whether this enhanced killing is due to an increased binding of the platinum compound to the DNA, the amounts of platinum were determined with AAS. In Chart 2, the results are given for *E. coli* cells treated with *cis*-DDP (50 μg/ml) at 32°, 36°, and 42°. The amount of platinum bound to the DNA is the same in wild-type cells and *uvrB*-deficient cells and shows a linear increase with temperature. A doubling in the platinum binding is achieved upon a temperature shift of about 8°, which is in accordance with the survival data shown in Chart 1.

Effect of Temperature on Mutation Induction. To examine the effects of temperature on mutation induction by *cis*-DDP, repair-proficient *E. coli* cells were treated at 25°, 32°, and 40° and *lacI* mutants were selected. Chart 3 shows that the frequency of mutation induction is strongly increased at 40° compared with 32° and 25°. The ratio between the frequencies of mutation induction at 40° and 32° is 7. However, on the basis of the platinum-binding data, only an increase with a factor of 2 could be expected. This result can be explained

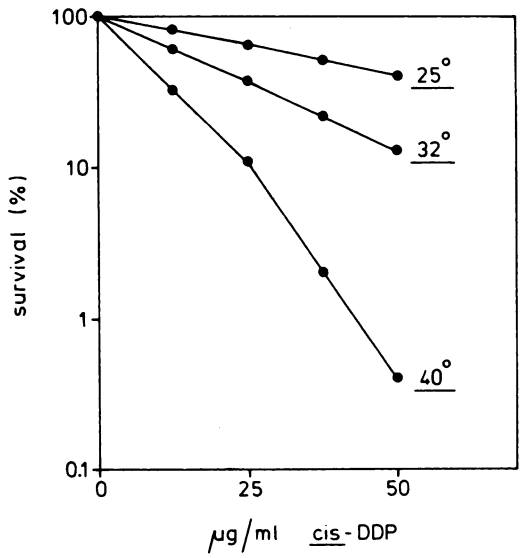

Chart 1. Survival curves for repair-proficient *E. coli* cells upon treatment with *cis*-DDP at various temperatures.

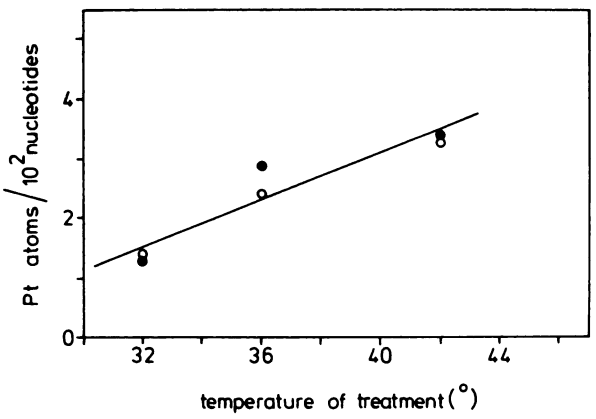

Chart 2. Platinum binding to DNA isolated from repair-deficient *uvrB* cells (●) and repair-proficient cells (○) after treatment with *cis*-DDP at 50 μg/ml in buffer for 2 hr at various temperatures.

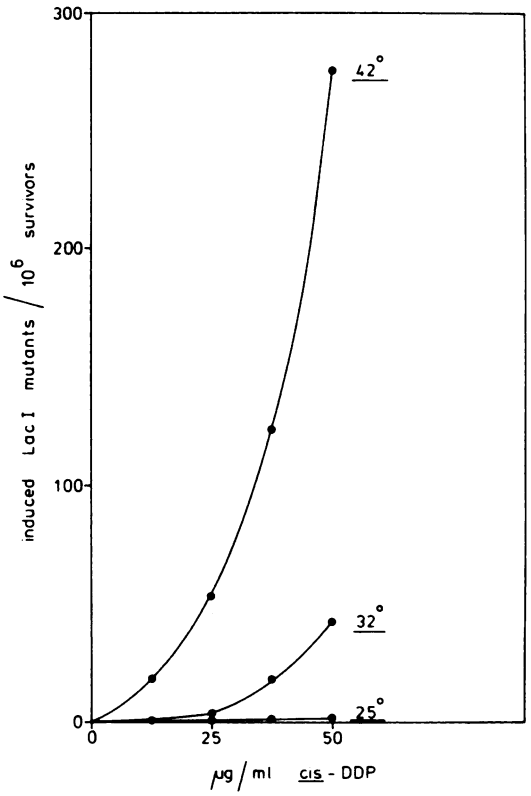

Chart 3. Induction of *lacI* mutants upon treatment of repair-proficient *E. coli* cells with *cis*-DDP at various temperatures.

either by a more mutagenic excision repair at higher temperature or by a change in the distribution of the type of lesions induced by the platinum compound.

Mutation Induction and Excision Repair at Various Temperatures. It has been shown earlier that mutation induction in *E. coli* by *cis*-DDP is dependent upon the excision repair process (2). To investigate whether the strong increase in mutation induction at a higher temperature is due to a more mutagenic excision repair, the temperature effects on this repair process were studied. Therefore, the procedure of the platinum treatment of the cells needs to be separated from the repair process of the induced lesions. We obtained such a separation by introducing excision repair in repair-deficient

ΔUvrB cells at a given time and temperature by means of infection with λ-phage carrying the bacterial *uvrB* gene. As depicted in Chart 4, the survival of the *cis*-DDP-treated UvrB cells is increased to nearly the same level as that of repair-proficient cells by the phage-mediated excision repair. When *cis*-DDP treatment is performed at 37° and followed by excision repair at 32°, 37°, or 42°, no difference in survival or mutation induction could be observed (data not shown). However, when the mutagenic treatment is done at 32°, 37°, or 42° and excision repair is allowed to take place at 37°, then the differences in survival (Chart 4) and mutation induction (Chart 5) are observed. These experiments prove that the strongly increased mutation induction observed after treatment with *cis*-DDP at high temperature is not due to a more mutagenic excision repair. Therefore, the increased mutation induction must be attributed to a change in the distribution of the different platinum-DNA lesions.

Influence of the Temperature on the Specificity of Mutation Induction. To investigate whether the observed increase in mutation induction by *cis*-DDP at elevated temperatures is accompanied by a change in mutation specificity, mutants collected after treatment with *cis*-DDP (50 μg/ml) at 33°, 35°, 39°, and 41° were screened for nonsense mutations using the *lacI* system. An increasing percentage of amber and ochre mutants was found: 3.0% at 33° to 7.2% at 35°, 14.3% at 39°, and 15.6% at 41°. Apparently, the lesion responsible for the induction of nonsense mutants becomes more abundant when the temperature of the platinum treatment is raised. Previously, we demonstrated that 68% of all base-pair substitutions which lead to nonsense mutations are induced at GAG or GCG base sequences after treatment of the cells with *cis*-DDP at 37° (2). The occurrence of hot spots for mutation at GAG and GCG sequences was explained by proposing the formation of intrastrand cross-links by *cis*-DDP on 2 guanines separated by a third base. The preference for mutation induction at these sites is increased with the temperature: 50% at 33°; 72% at 39°; and 81% at 41° (Table 1, Column 4). Furthermore, Columns 2 and 3 show that the percentage at GAG sites remains almost constant whereas the percentage at GCG sites is strongly increased at higher temperature. These results will be discussed in relation to the mechanism for the formation of the proposed intrastrand cross-links.

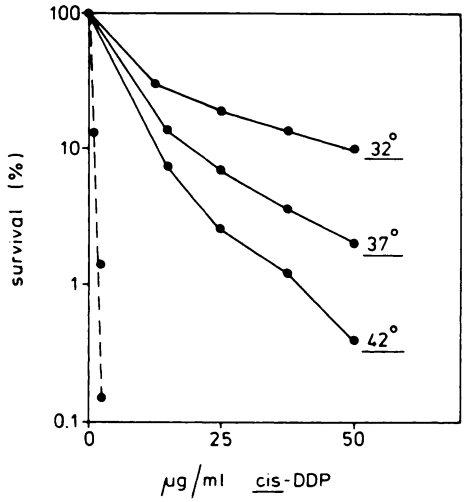

Chart 4. Survival curves for repair deficient Δ*uvrB* (λ*ind*⁻) *E. coli* cells after treatment with *cis*-DDP at various temperatures and subsequent infection with λ*uvrB* (multiplicity of infection, 5) and incubation at 37°. The Δ*uvrB* cells were made lysogenic for λ phage to prevent lytic growth of the infecting λ*uvrB*. The prophage carries an *ind* mutation to prevent induction by the platinum treatment. For comparison, the survival curve for Δ*uvrB* (λ*ind*) *E. coli* cells treated at 37° without subsequent infection with λ*uvrB* is given (- - - -).

DISCUSSION

The amount of platinum bound to the DNA as measured with AAS is increased when the *E. coli* cells are treated with *cis*-DDP at a higher temperature. This increase is directly related to the total amount of the platinum compound taken up by the cells. The survival of *E. coli* cells after treatment with *cis*-DDP at various temperatures is directly correlated with the amount of platinum bound to the DNA (Charts 1 and 2). However, from the mutagenesis data (Chart 3), it can be concluded that the mutation induction by *cis*-DDP is much more strongly enhanced at higher temperature than can be expected on the basis of the amount of DNA-bound platinum. Apparently, the platinum-DNA adducts formed at higher temperature are more effective in mutation induction. Using the λ*uvrB* complementation, we were able to prove that the increased mutation induction must be attributed to the high temperature of the platinum treatment

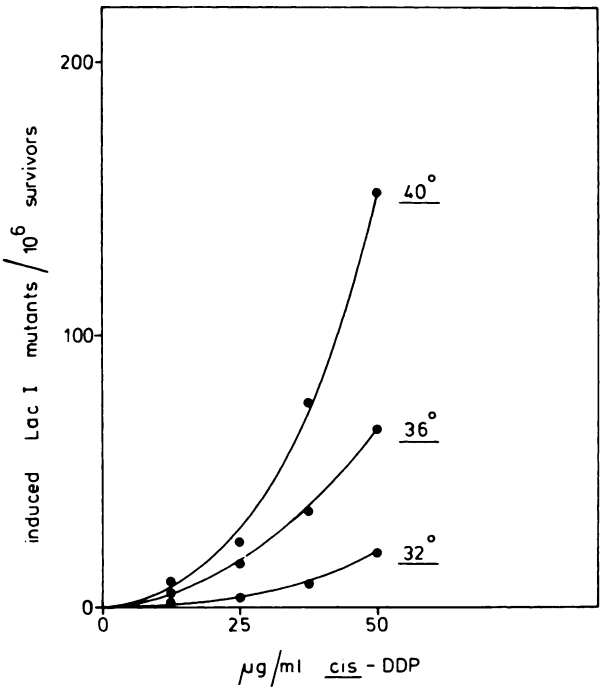

Chart 5. Induction of *lacI* mutants upon treatment of repair-deficient Δ*uvrB* (λ*ind*⁻) *E. coli* cells with *cis*-DDP at various temperatures, and subsequent infection with λ*uvrB* (multiplicity of infection, 5) and incubation at 37°.

Table 1

Percentages of mutations in GAG and GCG sequences among the lacI nonsense mutants induced by cis-DDP at various temperatures

The mutations were induced in the repair-proficient *E. coli* strain GM-1 by treatment with *cis*-DDP (50 μg/ml) in buffer for 2 hr at the indicated temperature.

	% of induced nonsense mutations		
Temperature	At GAG sites	At GCG sites	At GAG + GCG sites
33°	43	7	50
35°	44	16	60
39°	36	36	72
41°	41	40	81

and not to a changed repair mechanism. Among the increasing number of induced *lacI* mutants, the percentage of nonsense mutations at sites where the guanine is part of a GCG sequence is strongly enlarged at increasing temperature (Table 1). The increase in the number of lesions at GAG and especially at GCG sites can be explained by the effect of a higher temperature on the local denaturation of the DNA necessary to form an intrastrand cross-link. The relatively larger increase in mutation induction at GCG sites can be explained by the assumption that, at lower temperature, intrastrand cross-links at GCG sites are less frequently formed than at GAG sites because the unstacking of a GC base pair requires more energy than does the unstacking of an AT base pair.

Apparently, the intrastrand cross-links at GAG and GCG sequences are not essential for the cell-killing effect of *cis*-DDP in repair-proficient *E. coli* cells since this effect is directly correlated to the total amount of platinum bound to the DNA.

Also, in CHO cells, a hyperthermic effect on the treatment with *cis*-DDP has been described (8). Meyn *et al.* reported that the cytotoxic effect of *cis*-DDP increased by a factor of 10 as a consequence of a temperature shift from 37° to 43°, whereas the formation of interstrand cross-links increased by a factor of only 6.5. They concluded that lesions other than interstrand cross-links could be involved in the observed effect. If they are lethal in CHO cells, intrastrand cross-links on GAG and GCG sequences might account for these other lesions.

Further investigation is required to point out whether intrastrand cross-links at GAG and GCG base sequences play an important role in the antitumor activity of *cis*-DDP. If so, the temperature would be an important feature of the treatment conditions.

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