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# POST REPLICATION REPAIR IN AN EXCISION DEFECTIVE STRAIN OF *ESCHERICHIA COLI* FOLLOWING TREATMENT WITH *CIS* DICHLORODIAMMINEPLATINUM(II)

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SUMMARY : The size of the DNA synthetized after treatment of an excision defective *E. coli* strain with *cis*-dichlorodiammineplatinum(II) (*cis*-PDD) was examinated using sedimentation in alkaline sucrose gradients. DNA synthetized during a 10 minutes pulse after treatment with *cis*-PDD sediments with a molecular weight lower than control DNA from untreated cells. Post treatment incubation of the cells leads to an increase in the sedimentation rate of this DNA which approaches that of normal DNA. This last process is partially abolished in a uvr B5 rec B21 double mutant.

These results suggest that single strand breaks or gaps are produced during treatment and are filled in during further reincubation as part of a post replication repair process.

## INTRODUCTION

According to numerous observations, when *E. coli* bacteria are defective in the excision of pyrimidine dimers, DNA can be replicated past these lesions by a process called post replication recombination or daughter strand gap repair (1, 2). This allows replication of DNA containing unexcised lesions. The widely accepted hypothesis implies that gaps in the daughter strands exist opposite dimers. The repair occurs by sister strand exchange which insert parental undamaged DNA in each gap and the resulting discontinuities in the template strand are filled in by repair synthesis (3). It is assumed that after irradiation the transient accumulation of DNA of lower molecular weight than control followed by an increase in size of that DNA during post incubation is an evidence of post replication repair. A similar process can take place when cells are treated with several cytotoxic agents introducing monoadducts or interstrand cross-links in the DNA (4). It has been shown that the antitumor drug *cis*-dichlorodiammineplatinum(II) (*cis*-PDD) binds to DNA and inhibit DNA synthesis

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in bacteria (5, 6, 7). This inhibition is enhanced in excision deficient strains and is correlated with the toxic effect of this compound (8). Although the exact structures of the different DNA lesions are still imprecise, there is evidence that some of them are processed by classical excision repair and/or post replication repair mecanisms (5, 7). Since this last process has not been firmly established in bacteria treated with *cis*-PDD, we have examined the size of the DNA synthetized after treatment of an excision defective *E. coli* strain. Changes in molecular weight of DNA during subsequent post incubation periods were also analyzed. Our data clearly indicate that single strand gaps or breaks appeared after treatment with *cis*-PDD and that rejoining occurs during post treatment incubation as part of a post replication repair like process.

## MATERIALS AND METHODS

*E. coli* AB 2500 uvr A6 a thymine requiring strain derived from the parent AB 1886 (9) was provided by M. Defais. IC 20 ($F^-$ thr thi lac $supE^+$ $str^R$ rec B21 uvr B5) was obtained from M. Blanco. These strains were grown in M63 medium (10) containing 0.2 % glucose, 0,25 % casamino acids and 2 µg/ml thymidine. Log-phase cultures were treated for 1 hour with various amounts of *cis*-PDD or were UV irradiated. The washed cells were then labelled with [$^3$H]thymidine (50 or 100 µci/ml) during 5 or 10 minutes. Bacteria were collected and kept in ice or transfered in a non radioactive pre warmed medium for 60 minutes or less. Medium transfer was done by membrane filtration and the cells were washed several times with the appropriate medium. Spheroplasts formation, lysis and alkaline sucrose centrifugation were done according to Rupp and Howard Flanders (1). Gradients were formed by 3 cycles of freezing and thawing as described by Baxter-Gabbard (11),using a 10 % sucrose solution containing 0.7 M NaCl, 0.3 M NaOH and 1 mM EDTA. Recovery from the gradients was greater than 70 % and was not affected by treatment with *cis*-PDD. [$^3$H]labelled λ cI857 S7 DNA was used as a reference with a molecular weight of $15.10^6$ daltons (12) and run in a separate tube in each experiment. The molecular weight of the DNA strands was estimated by comparing their sedimentation distances (central peak fraction) with that of λ DNA using the calculation described by Abelson and Thomas (13). [Methyl $^3$H]thymidine (50 Ci/mmole) was obtained from the Commissariat à l'Energie Atomique, Saclay, France. Platinum compounds were synthetized in our laboratory following published methods (14). DNA for platinum determinations was purified according to Ljungquist and Bukhari (15). The amount of platinum covalently bound to this DNA was measured with a Perkin Elmer Atomic Absorption Spectrometer model 603.

## RESULT AND DISCUSSION

The sedimentation profiles of the DNA synthetized during a 10 minutes pulse after UV irradiation with a dose of $6J/m^2$ (about 1 dimer per 11 X $10^6$ daltons) and after treatment by increasing concentrations of *cis*-PDD are shown in figure 1. Untreated control DNA sedimented with the position of the peak fraction

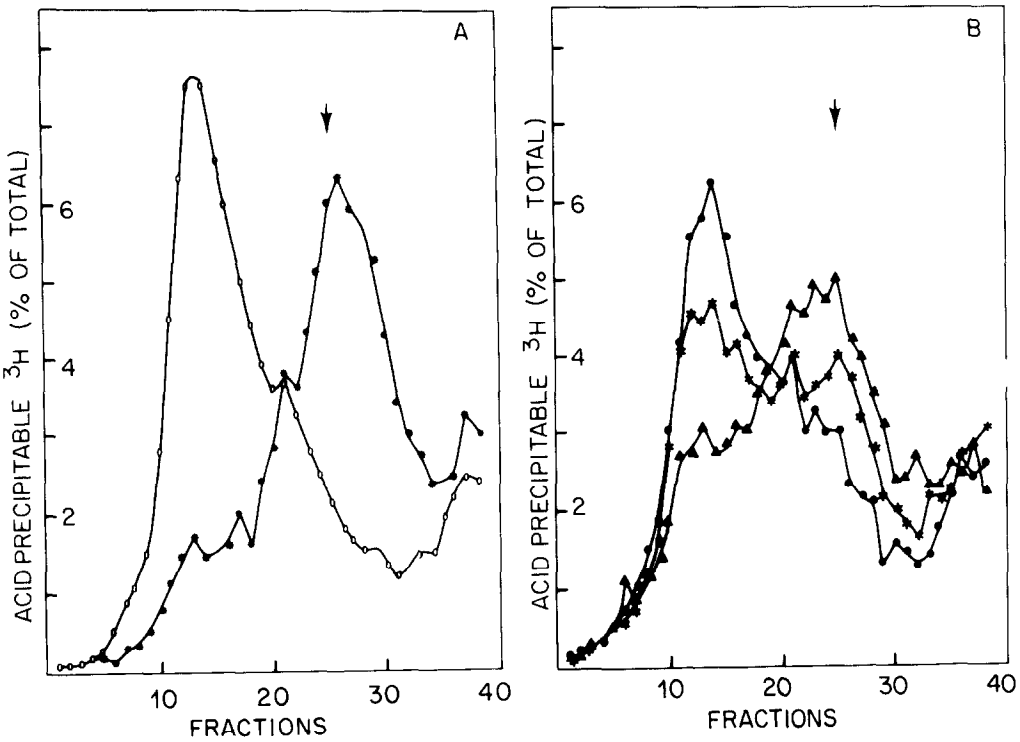


Figure 1 : Alcaline sucrose gradient sedimentation profiles of DNA from *E. coli* AB 2500 synthetized after exposure to UV and various concentrations of *cis*-PDD. (A) Untreated control, 5 minutes of incubation with [$^3$H] thymidine ( o ) and exposure to 6 J/m$^2$ of UV light followed by a 10 minutes incubation with [$^3$H] thymidine ( * ) (B) Effect of increasing concentrations of *cis*-PDD on sedimentation rates. After exposure to 5 ( ● ), 15 ( * ) and 30 ( ▲ ) µg of this compound per ml during 1 hour, cells were incubated in M63 medium containing [$^3$H]thymidine for 10 minutes and lysed as described by Rupp and Howard Flanders (1). The position of λ DNA is indicated by the arrow. Fraction 1 is at the bottom of the gradient.

corresponding to a molecular weight of about $10^8$ daltons. After irradiation the DNA sedimented more slowly with a molecular weight of about 12 X $10^6$ daltons which is in good agreement with the average distance between dimers. DNA from cells treated with *cis*-PDD has a reduced rate of sedimentation. The average size of this DNA decreases as the treatment dose increases. A molecular weight of about 28 X $10^6$ daltons has been estimated for DNA from cells treated with 30 µg/ml of *cis*-PDD (9 X $10^{-4}$ Pt per DNA nucleotide). This effect which is similar to that observed with increasing fluence of UV light (1) indicates that treatment of *E. coli* with *cis*-PDD leads to the rapid appearance of single strand breaks or alkali labile sites in the DNA. No accumulation of high mole-

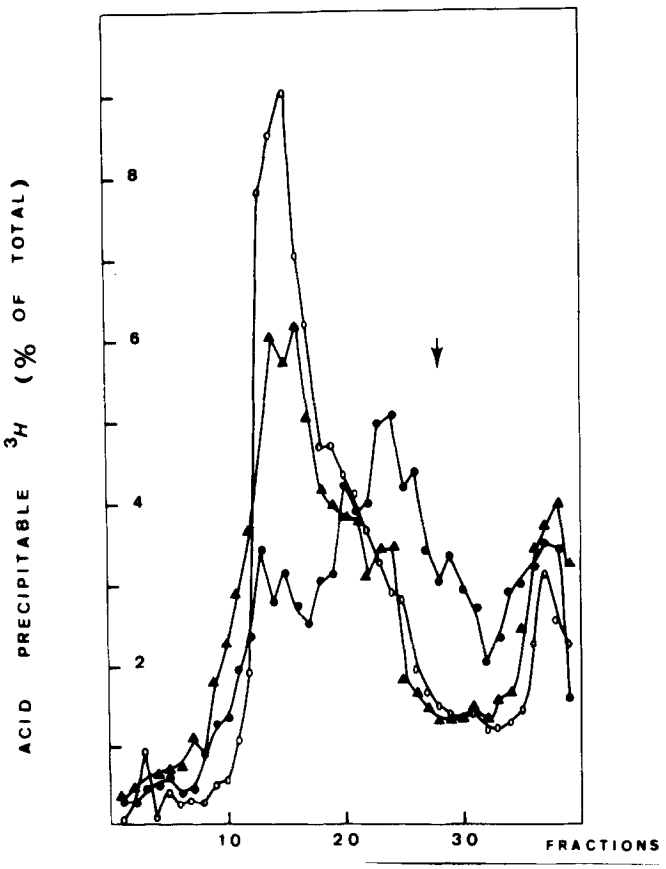


Figure 2 : Effect of post incubation on sedimentation rate in alkali of DNA labelled after treatment with *cis*-PDD. *E. coli* AB 2500 cells were treated for 1 hour with 30 µg/ml *cis*-PDD and incubated in M63 medium containing [$^3$H] thymidine for 10 minutes. Bacteria were analysed immediately ( ● ) or reincubated in non radioactive medium for 60 minutes ( ▲ ). The third profile corresponds to an untreated control that was incubated with [$^3$H]thymidine for 5 minutes ( ○ ). The procedures for lysis and sedimentation were as in Figure 1.

cular weight DNA sedimenting faster than control DNA was detected which suggest that few if any DNA cross link lesions occurred.

When cells were exposed to 30 µg/ml of *cis*-PDD during one hour and further post incubated for one hour in a non radioactive medium the sedimentation profile indicated an increase in size to that of DNA synthetized in non treated cells (figure 2). This phenomenon has been described in detail in UV irradiated cells and interpreted as evidence of post replication repair (1). A similar process might occur after *cis*-PDD treatment in bacteria. The same sedimentation profile was obtained when the post treatment period is reduced to 10 minutes (data not shown) indicating a very efficient process. Experiments simi-

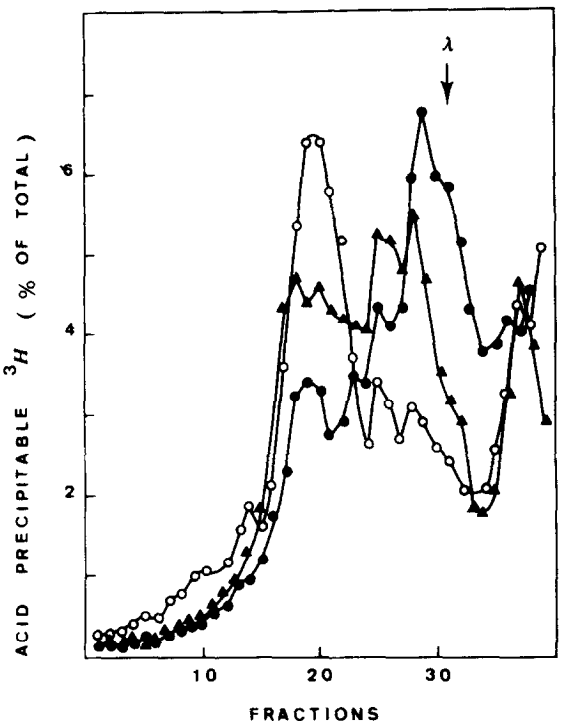


Figure 3 : Alkaline sucrose sedimentation profiles of acid precipitable DNA from *E. coli* strain IC 20 (uvr B5 rec B21). Conditions are those described in Figure 2.

lar to those described in Figure 2 were performed using a uvr B5 rec B21 strain in which the rejoining of DNA fragments during post UV irradiation incubation is partially abolished (16). As shown in Figure 3 there is a decrease in size of the DNA synthetized after treatment with *cis*-PDD but an incomplete conversion to fragments of normal size during subsequent post incubation. Our data indicate that in the absence of incision enzymes, platinum lesions lead to transient daughter strand gaps or breaks in the DNA which are filled in during subsequent incubation periods. Since this process is abolished in rec B21 cells it might reflect a mechanism analogous to post replication repair.

Analysis of the sedimentation profile obtained after treatment with 30 µg/ml of *cis*-PDD (figure 1) indicates that the estimated size of single strand fragments is not correlated with the average distance between platinum lesions. This observation may occur for a number of reasons. Platinum lesions might not be randomly distributed along the chromosome. More than one platinum atom may

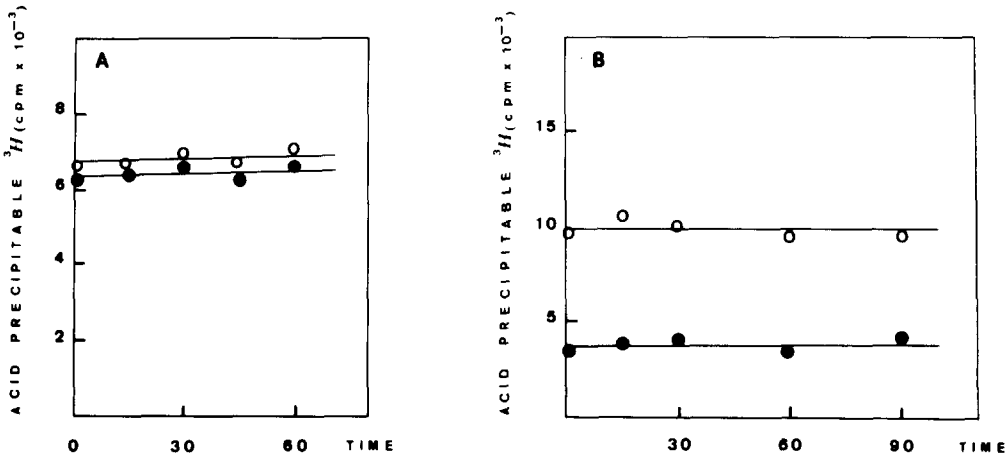


Figure 4 : Acid precipitable DNA from *E. coli* AB 2500 cells labelled before (A) and after (B) treatment with *cis*-PDD
A) log phase bacteria were uniformely labelled with [$^3$H]thymidine, (20 µci/ml) treated with 30 µg/ml of *cis*-PDD ( ● ) or not ( ○ ) and reincubated in M63 saline medium containing 2 µg/ml of non radioactive thymidine. Aliquots were removed as a function of time and the acid precipitable radioactivity determined
B) cells were treated with *cis*-PDD,( ● ) or not ( ○ ), pulse labelled for 10 minutes with [$^3$H]thymidine (50 µci/ml) and transfered to a non radioactive M63 saline medium. Aliquots were removed and assayed as above.

participate in the primary lesion leading to a gap on the opposite strand. Each lesion may not constitute an absolute block for the replication enzymes. It might also suggest that a very efficient process for circumventing or by-passing the first lesions made can take place during the treatment period. We have no evidence in favour of any of these possibilities at the present time.

Preferential degradation of a fraction of daughter strand DNA synthetized after treatment can create artifactual changes in sedimentation profiles of DNA from cells that have been reincubated. To rule out a possible degradation of the DNA the stability of the material synthetized before treatment and during the 10 minutes pulse following the addition of *cis*-PDD was investigated. As shown in figure 4 there is no significant change in the amount of acid precipitable counts from cells labelled with [$^3$H]thymidine after treatment with *cis*-PDD and reincubated in saline medium containing 2 µg/ml of non radioactive thymidine for more than one hour. Figure 4 also indicates that DNA synthetized before the addition of *cis*-PDD was not significantly degraded du-

ring the post incubation treatment. The possibility that *cis*-PDD induced lesions lead to spontaneous depurination of DNA was also considered. It has been shown by different authors that DNA modified with this compound was very stable in the absence of any incision enzymes (17, 18, 19).

The effect of [Pt(dien)Cl]Cl on the size of the DNA synthetized after treatment was also examined. This compound binds to DNA *in vivo* as effectively as *cis*-PDD but doesn't inhibit significantly the synthesis of DNA and therefore has a very low toxicity (8). DNA synthetized after treatment with this compound has the same sedimentation profile as control DNA from untreated cells (data not shown).

To our knowledge, the results of this study present the first evidence that breaks or gaps appeared in DNA after treatment with *cis*-PDD alone. The reduction in DNA size is probably due to a delayed replication as a consequence of the binding of *cis*-PDD to DNA. The conversion of this low molecular weight DNA to material of the size of the control during further post incubation suggests that a repair process similar to the so called post replication repair might occur. This hypothesis is corroborated by experiments in cells carrying rec B21 in which the low molecular weight DNA obtained after treatment is not entirely converted to a higher molecular weight form during post treatment incubation. Similar experiments made in mammalian cells revealed no accumulation of low molecular weight DNA after treatment unless caffeine is added during the labelling period (20). This observation has been interpreted by the authors as an evidence for a very efficient post replication repair system. *E. coli* cells allow a direct analysis of this process since it is possible to characterize the gapped or nicked DNA molecules which appeared as intermediates in this repair pathway.

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