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The role of the excision-repair enzymes in mutation-induction by *cis*-$Pt(NH_3)_2Cl_2$

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ABSTRACT

Mutation induction by cis-$Pt(NH_3)_2Cl_2$ (cisplatin) has been shown to be absent in E.coli strains carrying a deletion of the uvrB gene (1). This suggested that excision-repair, which is normally thought to be error-free, is involved in mutation induction with cisplatin. Here, the role of the excision repair enzymes UvrA, UvrB and UvrC is investigated using E.coli strains with different repair capacities. It is shown that cisplatin induced mutagenesis is dependent both on UvrA and UvrB but not on UvrC. Of the UvrB enzyme the N-terminal 113 aminoacids are sufficient for mutation induction by cisplatin.

INTRODUCTION

The antitumor compound cis-$Pt(NH_3)_2Cl_2$ induces several different types of DNA-damage in treated cells. Among the induced lesions are monofunctional adducts, DNA-protein crosslinks and inter- and intrastrand crosslinks (2). Intrastrand crosslinks can be formed between two adjacent guanines but also on two guanines separated by a third base or between an adenine and an adjacent guanine (3). It has been proposed that these intrastrand crosslinks are the biologically important lesions (4). In E.coli it has been shown that the GG-intrastrand crosslink is the most abundant lesion in DNA (60%) after treatment with cisplatin (3). Beck et al. (5) have demonstrated that the E.coli excision repair system, the UvrABC-excinuclease, can act on GG-crosslinks by incising the 8^{th} phosphodiester bond at the 5'-site and the 4^{th} phosphodiester bond at the 3'-site of the lesion. Therefore GG-Pt intrastrand crosslinks seem to be recognized and repaired in the same general way as the DNA adducts produced by UV-light (6), psoralen (7), N-AAAF (8) and benzo [α] pyrene (9).

Whether the other Pt-DNA adducts are also repaired by the UvrABC excinuclease in a similar fashion is not known, although some cutting by the enzymes at AG-adducts has been reported (5). However, the relative importance of the different Pt-DNA adducts for mutation- induction is not yet known.

Using the LacI system (10) we have demonstrated (1) that GXG-sequences are hotspots for the induction of base-pair substitutions. This was attributed to the occurrence of intrastrand crosslinks at two guanines separated by a third base. Using other systems, in which the specificity of mutation induction in plasmid DNA by *in vitro* treatment with cisplatin was determined, evidence for mutation induction at AG-sequences was also found (11). Although the GG-Pt adduct is probably very important for the lethal effect of the compound (2), so far no evidence has been obtained for a major role in mutation induction.

In an earlier report (1) it was shown that mutation induction by cisplatin was dependent upon the presence of the UvrB gene product since mutation induction was absent in cells carrying a deletion of the *uvrB* gene. This finding indicated a role for the excision repair system, which is normally error-free, in mutation induction with cisplatin. Therefore we examined the role of the three different repair enzymes UvrA, UvrB and UvrC involved in excision repair.

MATERIALS AND METHODS

Strains

The strains used in this study are listed in Table 1. The Δ*uvrC* strain CS4926 was constructed as follows. Strain KA1140 carries a Tn10 insertion in the *uvrC* gene at approximately 2/3 of the gene. By P1*vir* mediated transduction this transposon insertion was transferred to GM-1, using tetracycline resistance as selection. This strain was made Cm^R Tc^R using a P1*vir* lysate grown on strain UD-1, which carries an insertion of the *cam* gene from Tn9 in the 24 kD open reading frame that precedes the *uvrC* gene on the chromosome (12). From this strain Tc^S clones were isolated using fusaric acid selection and tested for simultaneous loss of the Cm-resistance. In this way clones carrying chromosomal deletions from the 24 kD open reading frame in front of the *uvrC* gene up to at least 2/3 of the *uvrC* gene can be isolated. The *uvrB5* and *uvrA6* mutations were transferred to GM-1 from strains AB1885 and AB1886 (13) respectively.

Media

Tryptone broth medium contained 1% tryptone and 0.5% yeast extract (Difco) supplemented with 0.8% NaCL.P-gal plates contained phenyl-β-D-galactoside (Sigma Chemical Co.) at 75 μg/ml in minimal medium (Vogel-Bonner) as described (15) and supplemented when required with amino acids at 50 μg/ml, thiamine at 5 μg/ml and biotine at 0.5 μg/ml.

Table 1.
Strains used in this study.

strain	Genotype/Phenoptype	Source
GM-1	F'lacI^{Q}L8 pro/ara, Δ(lac-pro), thi	Miller (10)
KMBL3838	GM-1 but Δ(chlA-uvrB-bioFCD)	Brouwer (1)
KMBL5068	GM-1 but uvrB5 (from AB1885)	This work
KMBL5075	GM-1 but uvrA6 (from AB1886)	This work
CS4926	GM-1 but ΔuvrC	This work
KMBL5081	KMBL3838 carrying plasmidpBL01	Backendorf(14)
KMBL5079	KMBL3838 but uvrA6	This work

Treatment with cis-$Pt(NH_3)_2Cl_2$

Exponentially growing cells in tryptone broth medium were harvested and resuspended in Vogel-Bonner buffer and incubated for 60' at 37°C. Freshly prepared cisplatin solutions were added and the incubation at 37°C was prolonged for 2 hrs. After centrifugation the cells were washed and resuspended in ice-cold buffer. Appropriate dilutions of the cells were spread on tryptone broth plates and P-gal plates to determine respectively the survival and the mutation induction in the lacI gene. The calculations of the induced mutation frequencies were as described previously (1).

RESULTS

In repair proficient wild type E.coli cells cisplatin is mutagenic as measured by its mutation induction in the lacI gene. In cells from strain KMBL3838, carrying a deletion over the uvrB gene, cis- $Pt(NH_3)_2Cl_2$ does not induce mutagenesis. When, however, cells carry a uvrB5 point mutation (strain KMBL5068), mutation induction by cisplatin does occur (see Fig.1). The uvrB5 mutation abolishes excision- repair of UV-lesions and therefore renders the cells sensitive to UV- light (13). Cells carrying a uvrB5 mutation are also very sensitive to cisplatin. In fact all the excision repair mutants that are used in this study (uvrA6, ΔuvrB, uvrB5 and Δ uvrC) show the same sensitivity towards cisplatin (see Fig.2). Apparently, for survival the complete excision repair system is required. Results from this laboratory (14) have shown that

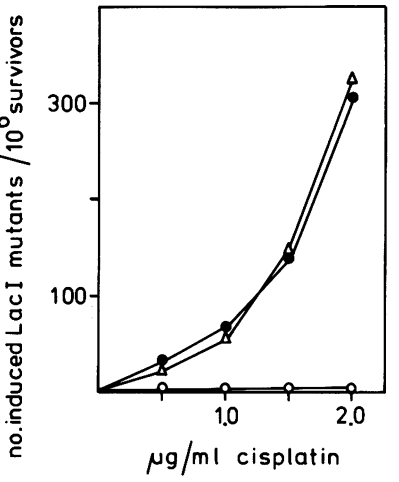

Fig.1. Induction of lacI mutants after treatment with cisplatin. Strain KMBL3838 (uvrB, o), strain KMBL5068 (uvrB5, •), strain KMBL5081 (Δ uvrB + plasmid pBL01 uvrB5, Δ).

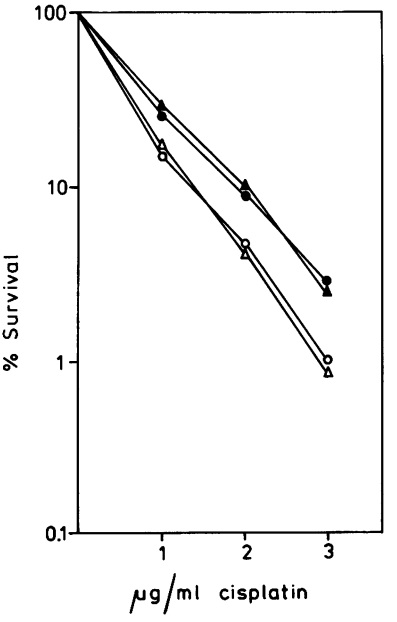

Fig.2. Survival curves for cells from strain: KMBL3838 (ΔuvrB, o) KMBL5068 (uvrB5, •) KMBL5075 (uvrA6, Δ) KMBL4926 (ΔuvrC, ▲). The survival for repair proficient cells from strain GM-1 at 3 µg/ml cisplatin was 95%.

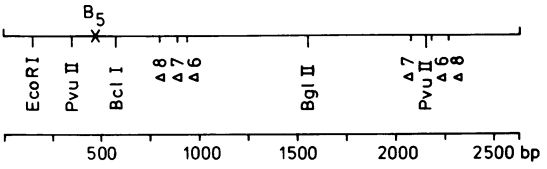

Fig.3. Schematic representation of the uvrB gene. Some restriction sites have been indicated. The position of the uvrB5 mutation is shown by an asterisk. Both endpoints of the Bal31 generated deletions that are present in the plasmids pBL06, pBL07 and pBL08 are indicated.

the UvrB5 mutant has a -1 frame shift at position 338 in the uvrB gene. As a result of this frameshift, translation of the uvrB5 messenger will terminate at the first stop codon in the -1 reading frame which is a UGA-opal codon at position 469, producing a truncated protein of 18 kD comprising the 113 N-terminal amino acids of the wild type UvrB gene product and a 43 amino acids long tail coded for by the -1 frame. Frame shift mutations are in general not leaky. Therefore mutation induction by cisplatin in the UvrB5 mutant is probably mediated by the truncated UvrB5 product. Apparently only the 113 terminal amino acids are required for mutation induction with cisplatin. Alternatively, a gene close to the uvrB gene and therefore absent in the deletion UvrB strain, but present in the UvrB5 strain could be responsible for mutation induction by cisplatin. However, cells having the deletion uvrB but carrying the plasmid pBL01 (strain KMBL5081) on which the uvrB5 allele is situated (14) are mutable with cisplatin (see Fig.1). Therefore it seems that the UvrB5 product itself is active in mutation induction with cisplatin.

The truncated UvrB5 protein has an artificial tail of 43 amino acids. We have investigated whether this tail is important for the mutation induction in the UvrB5 mutant using plasmids carrying uvrB genes with internal deletions generated by the action of the enzyme Bal31. The endpoints of the deletions were mapped and for three of the obtained plasmids (pBL06, pBL07 and pBL08) these endpoints are shown in figure 3. Cells with a uvrB deletion in the chromosome but carrying one of these plasmids, are mutable with cisplatin (Fig.4) to the same extent as the UvrB5 strain. Therefore mutation induction in cells carrying the uvrB5 mutation is due to a function of the UvrB product that is present in the 113 amino acid N-terminal part of the protein.

We have investigated whether the UvrC gene product that is essential for

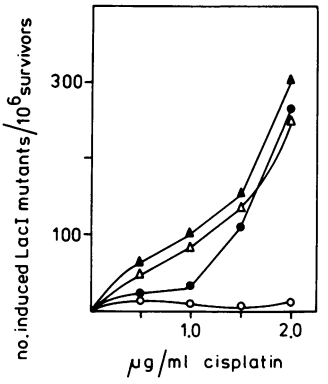

Fig.4. Induction of lacI mutants after treatment with cisplatin of cells from strain KMBL3838 (uvrB) carrying the vector plasmid pACYC177 (o) or plasmids pBL06 (●); pBL07 (△) or pBL08 (▲).

the incision events in excision repair is also necessary for mutation induction with cisplatin. For this purpose we constructed strain CS4926 that carries a deletion of the uvrC gene as described in the Materials and Methods section. It appears that this strain is normally mutable with cisplatin (see Fig.5) showing that the UvrC gene product, and therefore incision as occurs in excision repair, is not required for mutation induction.

The role of the UvrA gene product was investigated using a strain carrying the uvrA6 mutation (KMBL5075). This strain shows a strongly reduced

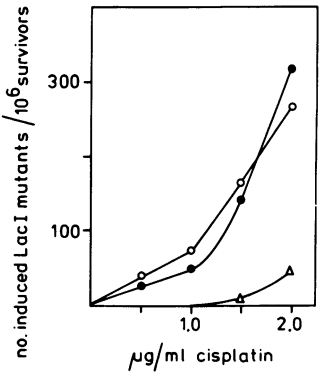

Fig.5. Induction of lacI mutants after treatment with cisplatin of cells from strain KMBL5068 (uvrB5, o), strain CS4926 (ΔuvrC, ●) or strain KMBL5075 (uvrA6, △).

mutability by cisplatin at high dose (see Fig.5). This low level of mutation induction could be due to a residual activitity of UvrA in the UvrA6 mutant or alternatively the UvrB protein could function in mutation induction by cisplatin in the absence of UvrA at low efficiency. We also constructed a strain (KMBL5079) that carries both the uvrA6 and the deletion uvrB mutation. As expected this strain is non-mutable by cisplatin. If the cells of this strain are transformed with plasmid pBL01, which carries the uvrB5 allele, the same reduced level of mutation induction is found as in the UvrA6 strain (data not shown). Apparently the UvrB5 product is dependent upon the presence of a functional UvrA product for mutation induction to occur.

DISCUSSION

It has been shown that the excision-repair enzymes UvrA, UvrB and UvrC are active on cisplatin treated plasmid DNA in vitro (5) and that intra-strand crosslinks on GG sequences are repaired in generally the same way as UV-damaged DNA and several chemically induced DNA damages (6). Here we show that cells, which are deficient in excision repair due to mutations in the uvrA, uvrB or uvrC genes all have similar sensitivities for cis-$Pt(NH_3)_2Cl_2$. The great sensitivities of these cells show the importance of excision repair for survival after treatment with cisplatin. The lack of mutation induction in cells carrying a deletion uvrB could indicate that excision repair is also involved in the mutagenic process after cisplatin treatment. However, we have shown that mutation induction is dependent upon UvrA and UvrB but not on UvrC. The UvrC enzyme is essential for the incision step of the excision repair process and therefore, although UvrA and UvrB are involved, mutagenesis by cis-$Pt(NH_3)_2Cl_2$ is presumable not mediated by normal excision repair. This is further substantiated by the results with the UvrB5 mutant which shows that for mutation induction only those functions are required that are preserved in the UvrB5 part of the protein. In contrast to mutagenesis with cisplatin, mutation induction by UV-light is increased in Uvr deficient strains. UV-induced DNA damage can be bypassed by a mutagenic mechanism under SOS conditions (16). The absence of excision repair will lead to more lesions entering the replication fork and therefore to more mutations. Our results with cisplatin show that in the case of Pt-DNA damage mutagenic bypass cannot occur in the absence of the UvrA and B gene products. Apparently the N-terminal part of the UvrB product as it is present in the uvrB5 strain is sufficient for this specific interaction. The UvrB5 part of the protein contains at least two regions of interest (13): i) a site that

shows homology to the consensus for ATP-ases situated between residues 32 and 53, and ii) a sequence that shows homology to a part of the alkA gene, between aminoacids 66-84. The AlkA gene product is a glycosylase involved in the repair of alkylation damage (17). The reason for this homology between AlkA and UvrB, two enzymes that participate in basically different repair pathways, is not yet clear.

The results reported have shown that the UvrA gene product is also required for mutation induction by cisplatin. UvrA is a DNA binding protein that, together with the UvrB protein, binds specifically to DNA damage introduced by a variety of agents. For this specific binding the presence of the UvrC protein is not necessary (18). The results obtained here strongly suggest that UvrA and UvrB can also bind to the premutagenic lesions introduced by treatment with cisplatin. Since mutation induction requires the presence of UvrA and only the N-terminal part of the UvrB protein, as it is present in the UvrB5 product, the domain of UvrB involved in the interaction with UvrA is probably situated in this N-terminal part of the protein.

Several possibilities for the role of UvrA and UvrB in the process of mutation induction can be considered. Firstly, UvrA and UvrB could play a role in generating the SOS inducing signal. This seems not very likely because GalK-induction experiments using a plasmid carrying the damage inducible uvrA promoter fused to the galK gene, show that cisplatin can induce SOS in $UvrA^-$ as well as in $UvrB^-$ cells (data not shown). A second possibility could be that the binding of UvrA and UvrB is involved in a process that leads to depurination at blocked replication forks. Such apurinic sites have been proposed as common intermediates in mutagenesis by chemical agents that form bulky adducts in DNA (19). The above mentioned homology of the uvrB and alkA genes (AlkA being a glycosylase) is of interest with respect to this possibility. Thirdly, UvrA and UvrB could function in the replication of Pt-damaged sites in DNA. Binding of UvrA and UvrB to the DNA- adducts could change the conformation of the DNA surrounding the lesion in such a way that mutagenic bypass replication can occur.

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