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INDUCTION OF recA PROTEIN IN *ESCHERICHIA COLI* BY THREE PLATINUM(II) COMPOUNDS

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The extent of induction of recA protein in *Escherichia coli* after treatment by *cis*-$Pt(NH_3)_2Cl_2$, *trans*-$Pt(NH_3)_2Cl_2$ and $[Pt[(HNCH_2CH_2)_2NH]Cl]Cl$ has been monitored via a 2 site immunoradiometric assay. The kinetics of induction are presented and, for an equal amount of platinum fixed on the DNA, the maximum relative amplification was found to be 22 : 5 : 2 respectively for the three platinum(II) compounds. A good correlation seems to exist between the extent of recA protein induction by these compounds and their previously reported mutagenic and antitumoral properties.

INTRODUCTION

Platinum compounds covalently bind to nucleophilic molecules in cells, among which DNA seems to be the ultimate target (for review see ref. 1). *Cis*-PDD, a derivative used in cancer chemotherapy, has been shown to induce filamentous growth (2) and prophage induction (3) in *Escherichia coli*. These functions are some of the so-called SOS functions (4) which are expressed when DNA is damaged and/or the replication fork is blocked (for review see ref. 5, 6). SOS functions are under the control of *lexA* and *recA* genes. The product of the *recA* gene is a 37800 dalton protein. RecA protein is regulated by the *lexA* gene product and amplified to high levels during induction of the SOS functions. This protein can now be easily quantified by a 2 site IRMA test (7).

Platinum(II) compounds have been divided into three classes based on the perturbations of DNA secondary structure induced by their binding to DNA

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Abbreviations : IRMA : immunoradiometric assay. *cis*-PDD : *cis*-$Pt(NH_3)_2Cl_2$; *trans*-PDD : *trans*-$Pt(NH_3)_2Cl_2$; [Pt(dien)Cl]Cl : $[Pt[(HNCH_2CH_2)_2NH]Cl]Cl$.

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in vitro (8). This paper compares the efficiency of recA protein induction in *Escherichia coli* treated with the three representative compounds, *cis*-PDD, *trans*-PDD and [Pt(dien)Cl]Cl.

MATERIALS AND METHODS

Drugs and buffers : Platinum compounds were a generous gift of Dr. J.P. Macquet. Solutions were freshly prepared in 0.9 % KCl and stored in the dark. IRMA-A buffer contained 10 mM barbital, 0.5 M NaCl, 0.02 % NaN_3, 0.1 % bovine serum albumin and 0.1 % calf serum, adjusted to pH 7.3. IRMA-B buffer was the IRMA-A buffer without serum albumin and calf serum.

Experimental procedure : *Escherichia coli*, AB1157 strain, was grown in M 63 medium (9) supplemented with 0.2 % glucose and 0.5 % casamino acids (Difco). An overnight culture was diluted and grown for at least one hour at 37°C with aeration to a concentration of 2-3 X 10^7 bacteria per ml. The drugs were then added at appropriate concentrations and 5 to 20 ml were withdrawn and centrifuged at indicated times after treatment. The pellet was resuspended in IRMA-B buffer, kept on ice and sonicated three times for thirty seconds (50 watts) with a Branson Sonic Power company sonicator. The sonicated suspension was centrifuged for 15 min at 10000 rpm, and the supernatant was used for protein mesurement by the Lowry procedure (10) and for recA protein determination.

Analysis of recA protein : RecA protein was determined by a 2 site IRMA test as described by Miles et al.(11), modified by Mirault et al.(12) and which is described elsewhere (7). Briefly, the experiment was performed in polystyrene tubes which had been coated with cold antibodies (0.5 ml) against recA protein. Cell extracts were added (20, 50 and 100 µl) and the volume was adjusted to 0.5 ml with IRMA-A buffer. The tubes were incubated for 4 hours at 37°C and rinsed once with 0.6 ml IRMA-A buffer. I^{125} labeled antibodies in 0.5 ml IRMA-A buffer were added and kept at room temperature for 12-15 hours. The tubes were then rinsed twice with 0.6 ml IRMA-A buffer. The radioactivity was determined with a Packard 5110 auto-gamma counter and the recA protein concentration was standardized to the total soluble protein content. Each value represents the mean of two or three independent experiments.

RESULTS AND DISCUSSION

The kinetics of induction of recA protein in wild type bacteria after treatment with *cis*-PDD, *trans*-PDD and [Pt(dien)Cl]Cl are shown in fig. 1. Cells were treated with a 30 µM solution of each drug, a concentration which causes a low cytotoxicity (13). The basal level of recA protein was quite stable in uninduced bacteria (about 1500 molecules/cell) and corresponded to the level previously determined by polyacrylamide gel electrophoresis analysis (14). The three compounds provoked an increase in the amount of recA protein. Only a slight but significant induction could be detected with [Pt(dien)Cl]Cl, the amplification was more pronounced with *trans*-PDD and *cis*-PDD was the most efficient in inducing recA protein ; however, in the case of *cis*-PDD a lag period was observed in the induction process and the

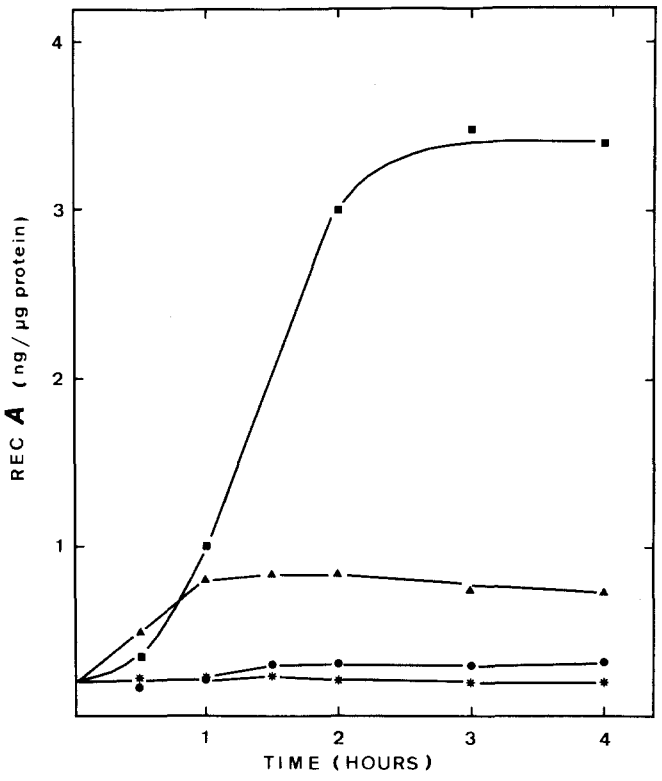

Fig. 1. Relative content of recA protein *versus* time after treatment with ■ *cis*-PDD, ▲ *trans*-PDD and ● [Pt(dien)Cl]Cl ; ✱ control without drug. Cells were grown up to 2-3 X 10^7 per ml before adding 30 µM drugs. The abscissa is the time after treatment.

maximum level was reached later (2 hours) than for the *trans*-PDD (1 hour). Slower induction of recA protein by the *cis* isomer was also obtained with a 10 µM concentration (data not shown). This finding might be explained by the faster binding of *trans*-PDD to DNA (15).

After two hours in the presence of these drugs, bacteria maintained a stable level of recA protein. The plateau was still observed after six hours of culture in exponential growth, in the presence of 5 or 10 µM of *cis*-PDD (data not shown). In contrast, when bacteria grown in the presence of 30 µM *cis*-PDD were washed after one or two hours of culture and incubated in drug-free medium, the amount of recA protein reached a maximum and then decreased (fig 2). Similar curves with a maximum level of recA protein induction followed by a decrease have been found after irradiation of bacteria by UV-light (Salles and Paoletti, in preparation).

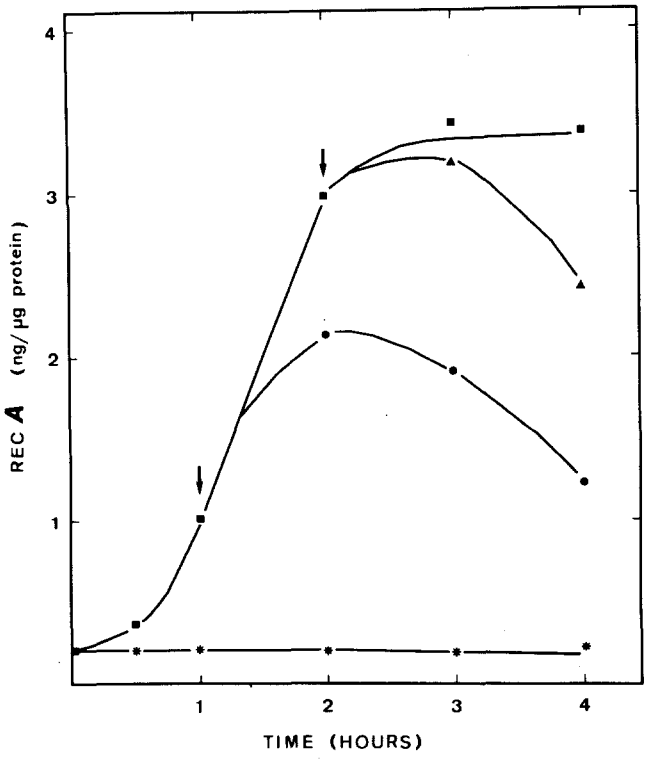

Fig. 2. Kinetics of recA protein induction after treatment with 30 µM *cis*-PDD. ■ bacteria were grown in the presence of *cis*-PDD ; ✱ control without drug in the medium. Samples were centrifuged and resuspended in prewarmed drug free medium after 1 hour (●) or 2 hours (▲) in the presence of *cis*-PDD. The arrows indicate the time at which the samples were washed.

RecA protein induction was determined as a function of the number of platinum per DNA-nucleotide in order to take into account the differences in penetration and/or reactivity of each drug (fig 3). RecA protein concentration was determined after 2 hours treatment at which time the plateau of induction had been reached for all concentrations of compounds. The amount of platinum per nucleotide was calculated from the drug concentrations and from the data given by ref. 13. After treatment with *cis* and *trans*-PDD, the concentration of recA protein increased as more platinum bound on the DNA and reached a plateau with *trans*-PDD but not with *cis*-PDD. The latter exhibited a"two-hit" response curve which may correspond to the formation of two distinct platinum-DNA lesions with different efficiencies for the induction of recA protein. This hypothesis implies the formation of two distinct adducts, one of which is relatively more abundant at low levels of DNA binding.

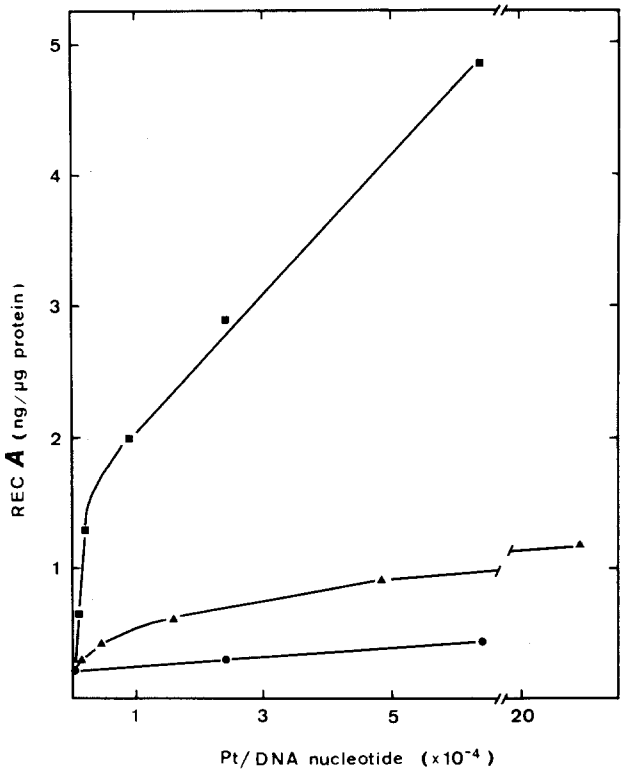

Fig. 3. Relative content of recA protein *versus* platinum per DNA-nucleotide ; the amount of platinum-DNA adduct was calculated from ref. 13. About 2 X 10^7 bacteria per ml were incubated with the drugs for two hours. ■ *cis*-PDD ; ▲ *trans*-PDD ; ● [Pt(dien)Cl]Cl.

For the maximum level of DNA binding observed, the relative amplification of recA protein after treatment with *cis*-PDD, *trans*-PDD and [Pt(dien)Cl]Cl were respectively 22 : 5 : 2 fold (fig. 3). The increased amount of recA protein induced after *cis*-PDD treatment is in the range of that observed after UV induction of recA protein (Salles and Paoletti, in preparation).

We have compared the dose response of the induction of recA protein with λ prophage induction (18), inhibition of DNA synthesis (13) and mutagenicity (18, 19, 20) after treatment with these three drugs (table 1). The largest response was observed for *cis*-PDD. However there is no correlation between a high level of recA protein and prophage λ induction as seen after treatment with [Pt(dien)Cl]Cl. The fact that the amount of recA protein is independent of the efficiency of promoting prophage induction has been previously reported (16, 17). In addition, recA protein can be induced to some

Table 1. Relative biological response for a given treatment dose

drugs	% prophage induction[a]	Relative inhibition of DNA synthesis[b]	His. revertants per nmole[c]	maximum induction of recA protein[e]
cis-PDD	6	6	100	4
trans-PDD	1	1	1 - 2[d]	1
[Pt(dien)Cl]Cl	5	0	2	0.4

a : data from ref. 21 ; b : data from ref. 13 ; c : data from ref. 18 ; d : P. Lecointe unpublished result ; e : values deduced from fig. 3 and standardized to *trans*-PDD.

extent without inhibition of DNA synthesis as in the case of [Pt(dien)Cl]Cl which binds to the bacterial DNA as efficiently as *cis*-PDD (13). It appears that, in addition to the number of platinum-DNA adducts, the nature of the lesions can play a role in the process of inducibility. Finally, previous studies have correlated the mutagenic properties (18, 19, 20) and antitumoral action (19, 21) of these drugs. In our studies we found that a high level of recA protein was associated with the mutagenicity of the platinum compound (table 1). Hence, additional experiments may show that recA protein determination could be used as a screening test for selecting new platinum antitumor compounds.

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REFERENCES

1. Roberts, J.J. and Thomson, A.J. (1979) in Progess in Nucleic Acid Research and Molecular Biology 22, 71-133.
2. Rosenberg, B., Renshaw, E., Van Camp, L., Hartwick, J. and Drobnik, J. (1967) J. Bacteriol. 93, 716-721.
3. Reslovà, S. (1971-72) Chem. Biol. Interactions 4, 66-70.
4. Radman, M. (1975) in Molecular Mechanism for repair of DNA, pp. 365-368, eds Hanawalt, P. and Setlow, R., Plenum, New-York.
5. Witkin, E.M. (1976) Bacteriol. Rev. 40, 869-907.
6. Defais, M., Hanawalt, P.C., Sarasin, A.R. (1981) Advances in Radiation Biology vol 10, in press.
7. Paoletti, C., Salles, B. and Giacomoni, P. Biochimie (submitted).
8. Macquet, J.P. and Butour, J.L. (1978) Biochimie 60, 901-914.
9. Miller, J.H. (1972) Cold Spring Harbor Laboratory.

10. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
11. Miles, L.E., Bicher, C.P., Eng, L.F. and Lipschitz, D.A. (1974) Analyt. Biochem. 61, 209-224.
12. Mirault, M.E., Reed, S.I. and Stark, G.R. (1974) Cold Spring Harbor. Symp. Quant. Biol. 39, 295-303.
13. Alazard, R., Germanier, M., Johnson, N.P. (1982) Mutation Res., in press.
14. Gudas, L.J. and Pardee, A.B. (1976) J. Mol. Biol. 101, 459-477.
15. Butour, J.L. and Macquet, J.P. (1978) Analyt. Biochem. 89, 22-30.
16. Roberts, J.W., Roberts, C.W. and Mount, D.W. (1977) Proc. Natl. Acad. Sci. USA 74, 2283-2287.
17. Moreau, P.L., Fanica, M., Devoret, R. (1980) Carcinogenesis 1, 837-848.
18. Lecointe, P., Macquet, J.P., Butour, J.L. and Paoletti, C. (1977) Mutation Res. 48, 139-144.
19. Beck, D.J. and Brubaker, R.R. (1975) Mutation Res. 27, 181-189.
20. Beck, D.J. and Fish, J.E. (1980) Mutation Res. 77, 45-54.
21. Lecointe, P., Macquet, J.P. and Butour, J.L. (1979) Biochem. Biophys. Res. Comm. 90, 209-213.