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# Gene Expression Caused by Alkylating Agents and *cis*-Diamminedichloroplatinum(II) in *Escherichia coli*[1]

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## ABSTRACT

**Previous work has demonstrated heterogeneous effects of methylating agents on induction of DNA damage inducible genes in *Escherichia coli*. These studies employed *E. coli* mutants that have fusions of the *lac* operon to genes induced by treatment with sublethal levels of alkylating agents. These mutants were selected from random insertions of the Mu-dI (Ap$^r$ *lac*) phage by screening for induction of β-galactosidase activity in the presence of methylmethanesulfonate or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. The current report extends these findings by analyzing gene expression caused by mechlorethamine, chloroethylnitrosoureas and *cis*-diamminedichloroplatinum(II) (*cis*-DDP). The results demonstrate heterogeneous effects by these agents on gene expression. While 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea induces *alkA*, other nitrosoureas, mechlorethamine, and *cis*-DDP do not cause expression of this gene. Further, while all nitrosoureas caused expression of *aidC*, mechlorethamine and *cis*-DDP did not. Lastly, *cis*-DDP caused marked expression of a *sulA* fusion mutant while not inducing any of the other *E. coli* fusion mutants.**

## INTRODUCTION

When *Escherichia coli* cells are treated with simple alkylating agents, three independently regulated genes or sets of genes are induced: the SOS response, the adaptive response to alkylation damage, and the *aidC* gene. The SOS response includes at least 17 chromosomal genes, all of which are repressed by the *LexA* gene product. Induction of this response occurs when RecA protein is activated by DNA damage. Once activated, RecA protein stimulates cleavage of LexA protein. This cleavage appears to be autolytic and results in destruction of the repressor function of LexA protein and induction of the genes it controls (1). The SOS response is induced by a wide variety of DNA-damaging agents including UV light, cross-linking agents, and many agents that produce adducts in DNA (2).

The adaptive response is induced by methylation damage to DNA and by some ethylating agents (3–4). Four genes arranged in three transcriptional units constitute the adaptive response to alkylation damage; the *ada-alkB* operon, *alkA*, and *aidB*. These genes are regulated by the Ada protein. Ada protein repairs $O^6$-methylguanine, $O^4$-methylthymine, and methylphosphotriesters by transferring the methyl group from the lesion to one of two methyl acceptor sites present in the Ada protein, one that accepts methyl groups removed from methylated bases and a second that accepts methyl groups removed from methylphosphotriesters (3–4). It is also a regulatory protein that controls the expression of the adaptive response in a positive fashion (3–4). The regulatory function of Ada protein is activated when its methylphosphotriester acceptor site is occupied. This methylated form of Ada protein binds to a sequence adjacent to the promoters of the genes it controls and serves as a transcriptional activator, thus inducing the adaptive response genes.

Previous studies have shown that monoadduct lesions produced by chloroethylating agents appear to serve as substrates for the adaptive response repair enzymes when this is tested *in vitro*, but it is not clear from this study if these types of lesions also serve as inducers of the adaptive response (5). Two results also suggest that cytotoxic DNA lesions caused by BCNU[3] are not substrates for the repair enzyme of the adaptive response; *ada* mutants of *E. coli* are not more sensitive, nor are cells that express the adaptive response repair proteins at elevated levels more resistant to lethal effects of this agent (6–7). These findings also raise the issue of whether this agent fails to induce the adaptive response in addition to causing DNA lesions not readily repaired by this mechanism in *E. coli*.

The *aidC* gene is poorly understood. It is not part of either the adaptive response or the SOS response. The regulatory pathway leading to *aidC* induction is not known, nor is *aidC* function understood. However, *aidC* is induced by several different methylating, ethylating, and propylating agents.[4] *aidC* regulation is complicated by the result that induction not only requires alkylation, but is also dependent upon the state of aeration. Induction of *aidC* requires anaerobic conditions and alkylation treatment (8). In contrast to *aidC*, induction of the SOS and adaptive responses does not require anaerobic conditions.

Previous comparisons of the inducing capabilities of the alkylating agents MNU, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and streptozotocin have demonstrated that the induction capabilities of methylating agents are heterogeneous (8). For example, streptozotocin, which resembles MNU structurally, differs from MNU in that it fails to induce *aidC*. Both agents, however, are effective inducers of *ada* and the genes it regulates.

In this study we use fusions of the *lac* operon to several different genes in order to monitor the induction of the three alkylation inducible responses by nitrosourea and platinum compounds. A fusion to *alkA* was used to monitor induction of the adaptive response, a *sulA* fusion was used to monitor induction of the SOS response, and an *aidC* fusion was used to monitor induction of this gene.

Another objective was to analyze gene expression after exposure to *cis*-DDP. In human cells, excision repair is principally implicated in mediating the repair of *cis*-DDP induced DNA damage. Thus, xeroderma pigmentosum cells are more sensitive than normal fibroblasts to this agent (9). Recent evidence indicates that *cis*-DDP may also nonspecifically induce $O^6$-alkylguanine-DNA-alkytransferase in rat hepatoma cells (10).

Our prior work employing *E. coli* mutants has demonstrated that both mutagenesis and cytotoxicity by *cis*-DDP are critically

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[3] The abbreviations used are: BCNU, *N,N*-bis(2-chloroethyl)-*N*-nitrosourea; MNU, methylnitrosourea; *cis*-DDP, *cis*-diamminedichloroplatinum(II); *trans*-DDP, *trans*-diamminedichloroplatinum(II); CNU, *N*-(2-chloroethyl)-*N*-nitrosourea; MeCCNU, *N*-(2-chloroethyl)-*N'*-(4-methyl)cyclohexyl-*N*-nitrosourea; CCNU, *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea; HN2, mechlorethamine.

[4] Unpublished results.

affected by the SOS repair mechanism (11–12). Further, mismatch repair (the repair of mismatched bases or equivalents) also affects cytotoxicity by this agent (11). We wished to evaluate whether *cis*-DDP induced the expression of a gene regulated by the SOS repair mechanism and also to evaluate whether other genes induced by alkylating agents were similarly affected by *cis*-DDP and compare its effects to *trans*-DDP.

## MATERIALS AND METHODS

**Bacterial Strains.** The *E. coli* K-12 strains employed in the experiments are described in Table 1.

**Reagents.** BCNU, CNU, CCNU, and MeCCNU were obtained from Dr. V. L. Narayan (Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute). A 1 mg/ml stock solution of each drug was prepared in 100% ethanol just prior to use. *cis*-DDP and *trans*-DDP were obtained from Sigma and dissolved in dimethylfluoride at 1 mg/ml just prior to each experiment. The structures of these drugs are shown in Fig. 1.

**Induction of β-Galactosidase Activity.** Cells were grown overnight in minimal medium (E salts; glucose, 0.4%; Bacto Casamino acids, 0.2%; thiamine, 0.2 μg/ml), diluted 1:50 and regrown to $10^8$ cells/ml as determined by readings of the optical density. 1 ml of cells was incubated with the appropriate concentration of drug or no drug at 30°C for 3 h. A 3-h incubation was employed since maximal gene induction by a variety of alkylating agents occurred after this interval (13–14). Cells were incubated without aeration to allow induction of *aidC*. Other strains were treated in a similar manner in order to permit comparisons between the three types of fusion containing strains. β-Galactosidase activity was measured as previously described (15). Experiments were performed at least twice and representative results are shown.

To insure that a biologically relevant range of drug concentrations was employed, clonogenic survival was measured in wild type cells as previously described (8). Less than 30% control survival was found for wild type cells exposed to peak concentrations of the various agents under the conditions employed in inducing gene expression.

## RESULTS

Prior results demonstrate that the *alkA* fusion is induced by treatments with simple methylating agents (11–12). Its induction was examined after treatment with CNU, BCNU, CCNU, MeCCNU, and HN2 (Fig. 2). Of these agents only CCNU served as an inducing agent of the adaptive response. Similar results were also seen for a fusion in the *alkB* portion of the *ada-alkB* operon (data not shown). These results suggest that hydroxy-ethylated Ada protein stimulates transcription of the genes it regulates, and implies that the signaling lesion, presumably chloroethylphosphotriesters, is not produced by the other chloroethylating agents in sufficient amounts to cause induction of the adaptive response.

The *aidC* gene is induced by all of the nitrosoureas (see Fig. 3). HN2, a bifunctional chloroethylating agent that differs from nitrosoureas in the DNA lesions it causes, did not induce *aidC*.

Table 1 *Bacterial strains*

| Strain | Relevant genotype |
|---|---|
| MV1161[a] | Wild type |
| MV1571 | *alkA51*::Mu-dl (Ap$^r$ *lac*) |
| MV1563 | *aidB2*::Mu-dl (Ap$^r$ *lac*) |
| MV1601 | *aidD6*::Mu-dl (Ap$^r$ *lac*) |
| MV1608 | *aidC8*::Mu-dl (Ap$^r$ *lac*) |
| DM4000[b] | *sulA*::Mu-dl (Ap$^r$ *lac*B::Tn9) |

[a] All strains are derivatives of MV1161 and contain the following additional markers: *argE3 his-4 leu-6 proA2 ara-14 galK2 lacY1 mtl-1 xyl-5 thi-1 rpsL31 supE44 tsx-33 rfa-550* (14).

[b] DM4000 contains the following additional mutations: del (*lac-pro*)X111 *hisG4 argE3 thr-1 ara-14 xyl-5 mtl-1* (obtained from Dr. D. Mount) (29).

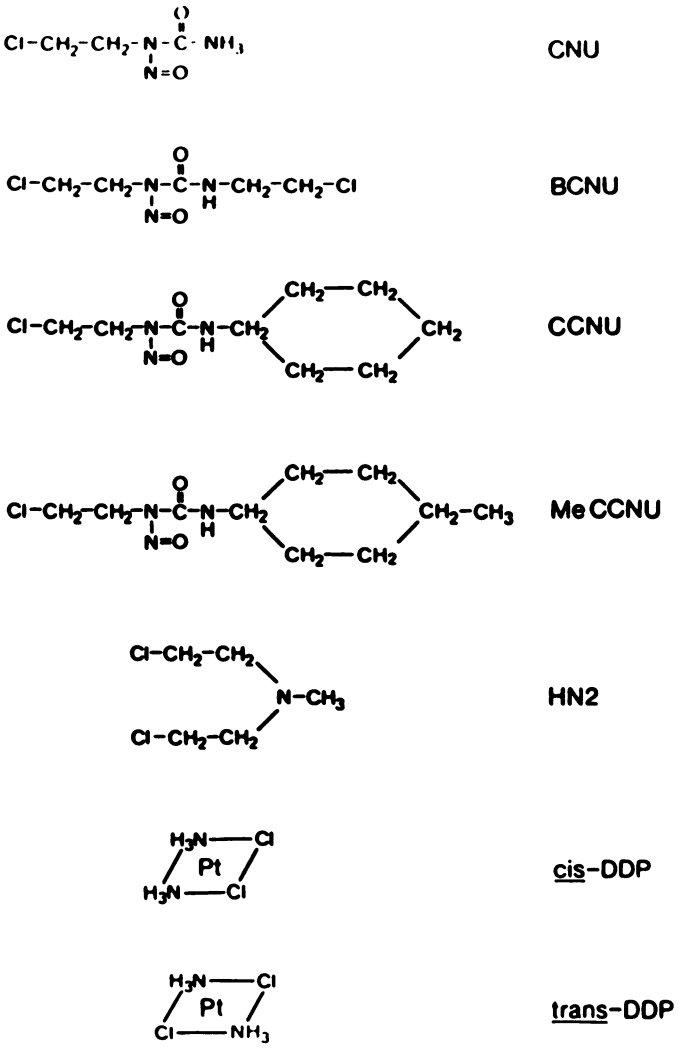


Fig. 1. Structures of CNU, BCNU, CCNU, MeCCNU, *cis*-DDP, and *trans*-DDP.

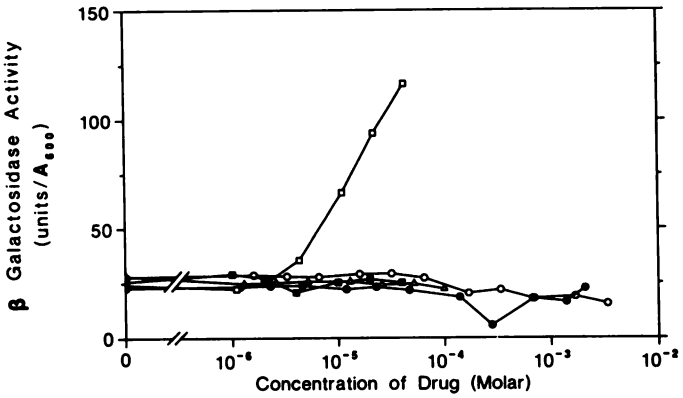


Fig. 2. Expression of *alkA51*::Mu-dl (Ap$^r$ *lac*). An overnight culture was diluted 1:50 and cells were grown without shaking to a density of $10^8$ cells/ml. Cells were exposed to drug for 3 h at 30°C. β-Galactosidase activity was measured as described in the text. Symbols represent exposure to CNU (○), BCNU (●), CCNU (□), MeCCNU (■), HN2 (△).

The SOS response, as indicated by the *sulA-lac* fusion is induced strongly by at least two agents, CNU and HN2 (see Fig. 4). A weak but reproducible induction is also seen upon BCNU and CCNU treatment, while MeCCNU does not appear to cause induction of the SOS response.

In contrast to results obtained with nitrosoureas, *cis*-DDP

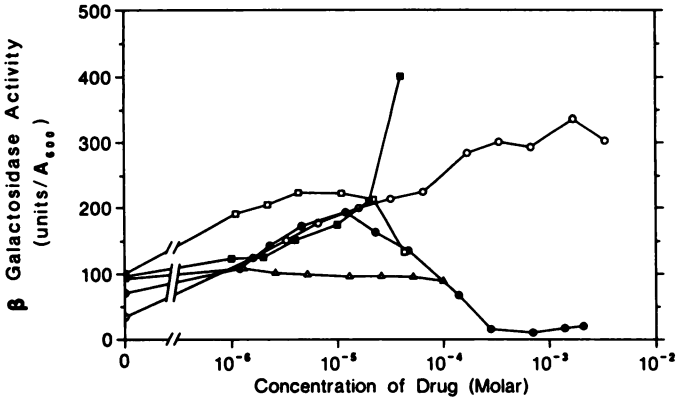


Fig. 3. Expression of *aidC*::Mu-dl (Ap$^r$ *lac*). Cells were exposed to drug for 3 h. Symbols are the same as in Fig. 2.

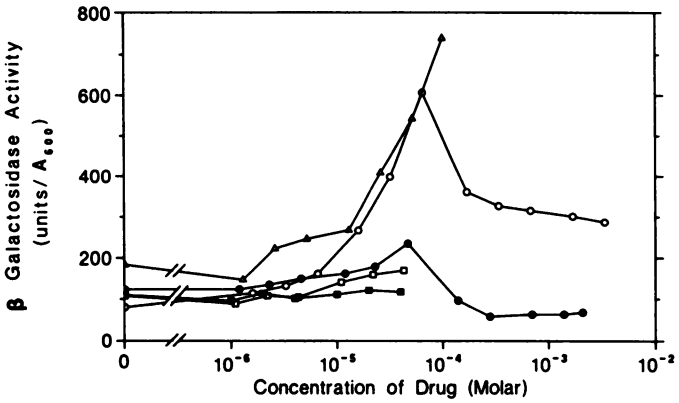


Fig. 4. Expression of *sulA*::Mu-dl (Ap$^r$ *lac*)*Xcam*. Cells were exposed to drug for 3 h at 30°C. Symbols are the same as in Fig. 2.

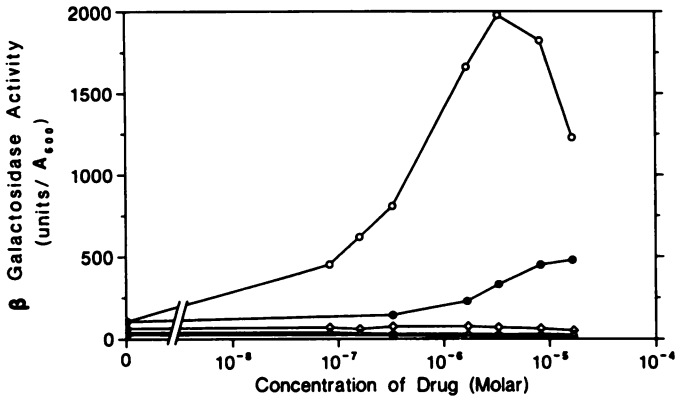


Fig. 5. Gene expression in *E. coli* fusion mutants by *cis*-DDP and *trans*-DDP. Cells were exposed to drug for 3 h at 30°C. *Open symbols*, cells exposed to *cis*-DDP; *closed symbols*, cells treated with *trans*-DDP. The *E. coli* fusion mutants evaluated were *sulA*::Mu-dl (Ap$^r$ *lac*)*Xcam* (○, ●); *aidD*::Mu-dl (Ap$^r$ *lac*) (△); *alkA*::Mu-dl (Ap$^r$ *lac*) (□); and *aidC*::Mu-dl (Ap$^r$ *lac*) (◇).

and *trans*-DDP only induced the *sulA* gene (see Fig. 5). Neither agent induced *aidD*, *alkA*, *aidB*, or *aidC* (Fig. 5).[4]

## DISCUSSION

Chloroethylnitrosoureas are complex alkylating agents that cause interstrand cross-links in DNA (16). This lesion is thought to underlie cytotoxic effects by these agents (17). *In vitro* experiments demonstrate that $O^6$-alkylguanine-DNA-alkyltransferase prevents formation of DNA interstrand cross-links by removing adducts formed at the $O^6$ position of guanine (18–19). These drugs also cause a variety of other DNA lesions including monofunctional base adducts, phosphate esters, and DNA intrastrand cross-links (19). A role for excision repair in ameliorating cytotoxicity by these agents is implicated by enhanced cytotoxicity of these compounds in *E. coli* mutants deficient in *uvr* endonuclease (6–7). HN2, a nitrogen mustard, also cross-links DNA and this lesion appears to underlie its cytotoxic effects (16).

Our experiments demonstrate significant heterogeneity among these agents in inducing gene expression. For example, CCNU, in contrast to the other nitrosoureas tested, induces *ada*. The basis for this finding is unclear. Prior studies have shown that Ada protein reacts with methylphosphotriesters, and when it becomes methylated at the cys 69 residue, it induces the adaptive response genes (21). Ethylating agents are generally less effective inducers of *ada* compared to methylating agents as shown by less marked effects on enhancing cytotoxicity and mutagenesis in *E. coli ada* mutants compared to wild type cells (22). Further, the chloroethylnitrosourea BCNU does not cause more cytotoxicity in *ada* mutants of *E. coli* than in wild type and induction of the adaptive response by prior exposure to low levels of MNNG does not alter the cytotoxic effects of this agent (19–20). The failure of CNU, BCNU, and MeCCNU to induce expression of adaptive response genes is consistent with these observations. The contrasting effects of CCNU with other nitrosoureas on expression of the *alkA* fusion mutant may suggest that either more of a particular DNA lesion is formed, such as a hydroxyethylphosphotriester, or that other phenomena, such as direct modification of the *ada* protein, occur to a greater degree than with the other nitrosoureas analyzed. As yet, no difference in the spectrum of lesions caused by CCNU, in contrast to other haloethylnitrosoureas, are known to explain the results. Our data also do not exclude the possibility that differences in the intracellular concentration of CCNU compared to the other nitrosoureas tested may contribute to the results.

The induction of *aidC* by all chloroethylnitrosoureas analyzed is consistent with the induction of this gene by a variety of agents that form adducts greater than one carbon in length.[4] The lack of expression of the *aidC* fusion mutant after treatment with HN2 may result from the failure of this agent to form adducts at extracyclic oxygens since the principal DNA adducts formed by this agent occur at the $N^7$ position of guanine (23). In this respect, HN2 resembles methylmethanesulfonate, an agent that does not preferentially form adducts at extracyclic oxygens and which does not cause *aidC* induction (24). Chloroethylnitrosoureas, on the other hand, form adducts at reactive oxygens and are effective inducers of *aidC* expression (18).

None of the agents caused expression of the *aidB* fusion mutant, another gene of the adaptive response (data not shown). Expression of this gene was previously noted after exposure to methylating agents (12). Although the expression of this gene is controlled by Ada protein in concert with the *ada-alkB* operon and *alkA*, it is generally less responsive to inducing agents. Thus, lack of *aidB* induction by agents that induce the *ada-alkB* operon and *alkA* weakly is consistent with previous observations.

The *sulA-lac* fusion was induced strongly by HN2 and CNU, less markedly by BCNU and CCNU, and not at all by MeCCNU. The induction of the *sulA* gene is consistent with the participation of SOS repair in ameliorating cytotoxic effects by these agents (19–20). The basic underlying differences in the extent of *sulA* gene expression, particularly among the chloroethylnitrosoureas is unclear. The initial event in the induction

of SOS repair is activation of RecA protein (1). This phenomenon is thought to result from the formation of single stranded regions in DNA (1). Thus, increased formation of adducts at particular sites, such as at phosphate groups or at the $N^7$ position of guanine, might cause increased signal levels and affect the extent of RecA protein activation.

*cis*-DDP also induced the *sulA* fusion mutant. The extent of expression was markedly greater than that occurring after treatment with *trans*-DDP. The latter agent is less cytotoxic and mutagenic than *cis*-DDP and is not an effective antineoplastic drug (9–10).

*cis*-DDP binds principally at the $N^7$ position of guanine (25–26). It forms monofunctional adducts as well as interstrand and intrastrand DNA cross-links (25–26). The frequency of DNA intrastrand cross-link formation between adjacent guanines is thought, on a stereochemical basis, to occur far more frequently with *cis*-DDP than *trans*-DDP. Results from experiments analyzing chain termination during *in vitro* DNA synthesis are consistent with this hypothesis (27). *cis*-DDP in contrast to *trans*-DDP also is a far more efficient inducer of DNA interstrand cross-links (28). The extent of induction of *sulA* by *cis*-DDP may result from the creation of single stranded regions in DNA resulting from strain in the double helix. The latter may result from formation of both intrastrand and interstrand cross-links in DNA by *cis*-DDP adducts.

Induction of the *sulA* fusion mutant is consistent with the critical importance of the SOS repair mechanism in affecting cytotoxicity and mutagenesis by *cis*-DDP in *E. coli* (12). Our results also clearly exclude the possibility that a failure of *cis*-DDP to induce DNA repair underlies enhanced cytotoxicity by this agent in comparison to its *trans* isomer.

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