Gene Expression Caused by Alkylating Agents and cis-Diaminedichloroplatinum(II) in *Escherichia coli*¹

Robert J. Fram,² Jay Crockett, and Michael R. Volkert

Departments of Medicine and Pharmacology [R. J. F.] and Molecular Genetics and Microbiology [J. C., M. R. V.], University of Massachusetts Medical School, Worcester, Massachusetts 01655

**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 MuΔl (Ap* 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-diaminedichloroplatinum(II) (cis-DDP). The results demonstrate heterogeneous effects by these agents on gene expression. While 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea induces aidC, other nitrosoureas, methotrexate, 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 β¹-methylguanine, β¹-methylthymine, and methyaphosphotriesters by transferring the methyl group from the lesion to 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 O⁶-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 requirements. Further, both mutagenesis and cytotoxicity by cis-DDP are critically requirements. Therefore, it is not surprising that both mutagenesis and cytotoxicity by cis-DDP are critically requirements. Therefore, it is not surprising that both...
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 10⁸ 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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
</tr>
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<tbody>
<tr>
<td>MV1161*</td>
<td>Wild type</td>
</tr>
<tr>
<td>MV1571</td>
<td>alkA5::Mu-dl (Ap'/ lac)</td>
</tr>
<tr>
<td>MV1563</td>
<td>aidD2::Mu-dl (Ap'/ lac)</td>
</tr>
<tr>
<td>MV1601</td>
<td>aidC::Mu-dl (Ap'/ lac)</td>
</tr>
<tr>
<td>MV1608</td>
<td>aidC::Mu-dl (Ap'/ lac)</td>
</tr>
<tr>
<td>DM4000P</td>
<td>sulA::Mu-dl (Ap'/ lac):Tn9</td>
</tr>
</tbody>
</table>

* All strains are derivatives of MV1161 and contain the following additional markers: argE3 his-4 lew-6 pro42 ara-14 galK2 lacY1 met-11-1 xyl-5 thi-1 rpsL31 supE44 tss-33 fts-550 (14).

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

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
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 O₆-alkylguanine-DNA-alkyltransferase prevents formation of DNA interstrand cross-links by removing adducts formed at the O₆ 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 N₇ 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 cytotoxicity 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 N7 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 N7 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.

ACKNOWLEDGMENTS

The authors acknowledge the excellent assistance of Dianne Sullivan and Karen Cawrse in manuscript preparation as well as helpful suggestions by Dr. Martin Marinos.

REFERENCES


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