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DNA repair mechanisms affecting cytotoxicity by streptozotocin in *E. coli*

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Summary

Mechanisms underlying cytotoxicity by the monofunctional nitrosourea streptozotocin (STZ) were evaluated in DNA repair-deficient *E. coli* mutants. Strains not proficient in recombinational repair which lack either RecA protein or RecBC gene products were highly sensitive to STZ. In contrast, cells that constitutively synthesize RecA protein and cannot initiate SOS repair mechanisms because of uncleavable LexA repressor (*recA098 lexA3*) were resistant to this drug compared to a *lexA3* strain. Further, *E. coli* cells lacking both 3-methyladenine DNA glycosylases I (*tag*) and II (*alkA*) also were highly sensitive to STZ. DNA synthesis was most inhibited by STZ in *recA* and *alkA tag E. coli* mutants, but was suppressed less markedly in wild-type and *recBC* cells. DNA degradation was most extensive in *recA E. coli* after STZ treatment, while comparable in *recBC*, *alkA tag*, and wild-type cells. Although increased single-stranded DNA breaks were present after STZ treatment in *recA* and *recBC* mutants compared to the wild type, no significant increase in DNA single-stranded breaks was noted in *alkA tag E. coli*. Further, DNA breaks in *recBC* cells were repaired, while those present in *recA* cells were not. These findings establish the critical importance of both recombinational repair and 3-methyladenine DNA glycosylase in ameliorating cytotoxic effects and DNA damage caused by STZ in *E. coli*.

Streptozotocin (STZ) is a monofunctional nitrosourea employed in the therapy of patients with islet cell tumors (Byrne and Schein, 1981; Moertel and Hanley, 1979). This agent causes diabetes in animals and is also a potent mutagen and carcinogen (Rakieten et al., 1963, 1971; Like and Rossini, 1976; Chick et al., 1977; Fram et al.,

1986). Mechanisms underlying cytotoxicity by this agent are poorly understood.

Our prior work has demonstrated that when *Escherichia coli* cells are treated with STZ 2 independently regulated sets of genes are induced: the SOS regulon and the adaptive response to alkylation damage (Fram et al., 1986). 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.

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This cleavage appears to be autocatalytic and results in destruction of the repressor function of LexA protein and induction of the chromosomal genes it controls (Walker, 1984). Among these SOS genes are those required for DNA damage repair.

RecA protein also participates in recombinational repair (Walker, 1984). The latter repair mechanism permits gap filling in daughter DNA strands opposite lesions in the parental DNA strand, thus allowing the formation of intact daughter DNA strands after DNA synthesis. RecA protein serves as a DNA-dependent ATPase that enhances homologous pairing of DNA molecules and strand transfer during recombination. Another enzyme activity specified by *recBCD* also contributes to recombinational repair. This multifunctional protein is an ATP-dependent exonuclease, has unwinding activity for single-stranded DNA as well as a DNA-dependent ATPase activity (Walker, 1984; Weinstock, 1987). RecBCD gene products may contribute early in recombinational repair by unwinding and incising DNA to provide a substrate for *recA*-mediated strand transfer. The RecBCD gene products may also participate in this repair process by aiding in the resolution of recombinants (Walker, 1984; Weinstock, 1987).

The adaptive response in *E. coli* is induced by methylation damage to DNA and by some ethylating agents (Volkert, 1988; Lindahl et al., 1988). Four genes arranged in 3 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 1 of 2 methyl acceptors 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 (Volkert, 1988; Lindahl, 1988). Repair of *O*⁶-methylguanine is critical since this lesion is highly mutagenic.

The regulatory function of Ada protein is activated when its methylphosphotriester acceptor site is occupied. This form of Ada protein then binds to a sequence adjacent to the promoters of the genes it controls and serves as a transcrip-

tional activator, thus inducing the adaptive response genes. Among the genes induced by Ada protein is *alkA* (Volkert, 1988; Lindahl, 1988). This gene specifies 3-methyladenine DNA glycosylase II, an enzyme that participates in the excision of a highly cytotoxic DNA lesion (3-methyladenine) as well as 3-methylguanine, 7-methylguanine, *O*²-methylthymine, and *O*²-methylcytosine (Volkert, 1988; Lindahl, 1988). 3-Methyladenine DNA glycosylase I is constitutively expressed and is not regulated by Ada protein. This enzyme is more restrictive and solely participates in the excision of 3-methyladenine from DNA; the *tag* gene codes for this repair protein (Volkert, 1988; Lindahl, 1988).

Our prior work demonstrated that *recA*, *ada*, and *alkA* *E. coli* mutants are more sensitive than wild-type cells to STZ (Fram et al., 1986). These findings suggest that RecA protein enhances survival either by derepressing SOS genes or by participating in recombinational repair or both. Our previous results also demonstrated the participation of the adaptive response in ameliorating survival in cells after STZ treatment. In this study, we wished to clarify the involvement of recombinational repair and of 3-methyladenine DNA glycosylases in affecting cellular survival after exposure to STZ. An additional goal was to analyze the effects of these repair mechanisms on macromolecular synthesis and on DNA damage in cells treated with this agent. Lastly we evaluated the effect of this agent on survival of *polA* *E. coli* mutants deficient in DNA polymerase I. Polymerase I catalyzes DNA repair synthesis in *E. coli* on both nicked and gapped DNA template-primers; we reasoned that such a process was likely important in repairing damage by an agent that causes DNA-strand scission (McMacken et al., 1987).

Methods

Reagents

Streptozotocin was obtained from Sigma (St. Louis, MO). A 10⁻³ M stock solution was prepared in double-distilled water and frozen at -20°C. Methyl ³H-dThd (77.9 Ci/mmmole), 5-³H-UdR (26.7 Ci/mmmole), 1-(3,4,5-³H(N))leucine (176.5 Ci/mmmole) were obtained from New England Nuclear (Boston, MA). 2,5-Diphenyloxazole

TABLE 1
BACTERIAL STRAINS

Strain	Synonym	Genotype
AB1157	wild type	<i>thr-1 leuB6 thi-1 argE3</i> <i>hisG4 proA2 lacY1</i> <i>galK2 mtl-1 xyl-1 ara-14</i> <i>rpsL31 tsx-33</i> <i>glnV44 rfbD1 kdgK51</i>
AB2463	<i>recA</i>	As AB1157 but <i>recA13</i>
MS23	<i>alkA</i>	As AB1157 but <i>alkA his⁺</i>
JC5519	<i>recBC</i>	As AB1157 but <i>recB21 recC22</i>
AB2497	wild type	As AB1157 but <i>thyA12 deoB6</i>
AB2487	<i>recA</i>	As AB2497 but <i>recA13</i>
GM3843	<i>recBC</i>	As JC5519 but <i>thy⁻</i>
BK2118	<i>alkA tag</i>	<i>alkA tag</i>
GM3840	<i>alkA tag</i>	<i>alkA tag tsx^R srl-300::Tn10 thy⁻</i>
DM2251	wild type	(<i>lac-pro</i>) _{XIII} <i>ntl-1 rpsL31 thi-1</i>
DE192	<i>lexA51</i>	As DM2251 but <i>lexA51 sulA211</i>
DM2573	<i>recA430</i>	
	<i>lexA51</i>	As DE192 but <i>recA430</i>
DE464	<i>recA730</i>	
	<i>lexA51</i>	As DE192 but <i>recA730</i>
DE407	<i>lexA3</i>	As DM2251 but <i>lexA3</i>
DM2210	<i>recA098</i>	As AB1157 but <i>lexA3</i>
	<i>lexA3</i>	<i>recA098 sulA211</i>
CM4722	wild type	
CJ278	<i>polA</i>	As CM4722 but <i>polA* Km^R</i>

* Contains secondary mutation(s) which allow growth on rich medium. Individual gene loci are italicized.

(PPO) and *p*-bis[2-(5-phenyloxazolyl)]-benzene (POPOP) were obtained from New England Nuclear.

Bacterial strains

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

Cytotoxicity

Cells were grown in brain-heart infusion to mid-exponential phase, centrifuged at 12,000 rpm for 3 min and resuspended in 10 ml phosphate buffer. 1 ml of cells in Eppendorf tubes was exposed to either no drug or 10^{-4} – 10^{-6} M STZ for 30 min at 37°C, washed twice, and plated on brain-heart nutrient agar. Colonies were counted after incubation overnight.

DNA, RNA and protein synthesis

Cells were grown overnight in K medium (100 ml minimal salts, 10 ml casamino acids (20%), 1 ml 20% glucose, and 0.2 ml vitamin B1) supplemented with 2 µg/ml dThd (except for *alkA tag* and *recBC* *E. coli* mutants which required 20 µg/ml dThd). After a density of 10^8 cells/ml was reached, cells were exposed to either no drug or 10^{-6} – 10^{-4} M STZ. 1 µCi/ml ³H-dThd was added. 100 µl of cells was removed at various intervals, added to 3-MM cellulose filter disks (Whatman, Maidstone, U.K.) and allowed to settle for 30 s. The disk was placed in ice-cold, 10% TCA (trichloroacetic acid). After 30 min, disks were washed 3 times in 5% TCA at room temperature, washed once with 95% ethanol and once with acetone. Disks were dried and radioactivity measured after the addition of 5 ml scintillation fluid (4.0 g PPO, 50 mg POPOP/1 toluene).

The same approach was used in measuring RNA and protein synthesis except as noted below. To analyze RNA synthesis, 5 µCi UdR/ml was added with 10 µg carrier UdR/ml. In the case of protein synthesis 1 µCi/ml ³H-leucine and 10 µg carrier leucine/ml was employed.

DNA degradation

The procedure for analyzing DNA degradation is published (Clark et al., 1966). Cultures were prepared by inoculating cells into 10 ml K medium supplemented with 100 µl dThd (2 mg/ml), 1 ml deoxyadenosine (200 µg/ml) and 2 µCi/ml ³H-dThd (10 Ci/mM; New England Nuclear). Cells were grown to a density of 10^8 /ml. 4 ml of cells was added to each of 2, 15-ml conical tubes, spun for 5 min at 5000 rpm, and resuspended in 3.6 ml K medium with 400 µl dThd (2 mg/ml). Cells were centrifuged and resuspended in the same medium or, in the case of drug-treated cells, resuspended in 3.2 ml K medium, 400 µl dThd, and 400 µl of 10^{-3} M STZ. Cells were incubated for 30 min at 37°C and then washed twice and resuspended in 3.6 ml K medium and 400 µl dThd. Cells were incubated at 37°C with aeration in the dark for 0–200 h. After each incubation period, 500 µl of cells and 20 µl of salmon sperm DNA (2 mg/ml) were added to a screw-top Eppendorf, vortexed, and then 500 µl of 10% TCA was added. The solution was mixed and placed on ice for 30

min, and spun in a microfuge for 10 min. 100 μ l of each supernatant was added to a scintillation vial. Pellets were heated at 95°C for 30 min, vortexed, and allowed to precipitate. 100 μ l of the solution was added to a plastic vial. Radioactivity was then measured on a scintillation counter and the % DNA degradation calculated by dividing the counts per minute obtained from the initial supernatant after TCA precipitation divided by the numbers of counts obtained after the pellets were heated. This value was then multiplied by 100 to give the % DNA degraded.

Measurement of DNA single-strand breaks

An overnight culture of cells was prepared by inoculating 1 ml K medium with 5 μ l dThd (mg/ml) and incubating overnight at 37°C. 50 μ l of cells was added to 10 ml K medium and 5 μ l dThd (mg/ml) and 10 μ l 3 H-dThd. Cells were grown overnight to a density of 2–4 $\times 10^8$ /ml. The culture is split and diluted to a density of 1–2 $\times 10^8$ cells/ml. Cells are exposed to either no drug or STZ, incubated for 30 min at 37°C, washed twice with phosphate buffer, and resuspended in 1 ml of 10% sucrose, 0.05 M Tris, pH 8.0, 0.2 ml fresh lysozyme (5.0 mg/ml in 0.25 M Tris, pH 8.0) was added as well as 0.4 ml, 0.25 M EDTA, pH 8.0. The solution was placed on ice for 5 min and occasionally swirled. 0.4 ml of 2% sarkosyl in 0.01 M EDTA, pH 8.0, was added. The solution was allowed to clear on ice and vortexed for 15 s.

DNA single-strand breaks were analyzed on alkaline sucrose gradients. 4.8-ml gradients were prepared by mixing 2.4 ml 5% sucrose with 2.4 ml 20% sucrose in 0.3 N NaOH, 0.7 M NaCl, and 1 mM EDTA. 50 μ l 1 N NaOH was layered on the top of the gradient with 0.1 ml of the DNA sample. The gradients were spun at 30,000 rpm for 90 min in a Ti55 swinging bucket rotor in an ultracentrifuge. Fractions were collected from the bottom of the tube using a peristaltic pump (Rainin Instruments, Woburn, MA). Seven drops were placed on a 3-MM cellulose filter disk (Whatman). Disks were washed once in 10% TCA for 30 min, washed once in 95% ethanol, placed in acetone for 10 min, and air-dried. Radioactivity was measured after the addition of 5 ml scintillation fluid as previously described.

In order to analyze repair of single-stranded DNA breaks, cells were spun, after drug exposure, washed twice in minimal salts and incubated on a shaking water bath at 37°C for 90 min. DNA was extracted and analyzed on alkaline sucrose gradients.

Results

The effect of STZ on survival in *E. coli* *recA*, *recBC* and *alkA tag* mutants is shown in Fig. 1. The results demonstrate that *alkA tag* and *recA* *E. coli* mutants are highly sensitive to STZ, while *recBC* cells are moderately sensitive compared to wild-type cells. Cells lacking the *alkA* gene were more sensitive to STZ than the *E. coli tag* mutant, while the *alkA tag E. coli* mutant was more sensitive to STZ than the *alkA* mutant (data not shown).

In order to further clarify the role of RecA protein with regard to survival in STZ-treated cells, cytotoxicity was analyzed in *lexA51* and *lexA3* bacteria. Since the expression of the *recA* gene is itself SOS-dependent, the gene is not expressed in *lexA3* bacteria but is fully expressed in *lexA51*. Fig. 2 shows that *lexA51* cells have a higher survival than *lexA3* as anticipated. Further, survival of *lexA51* is superior to that of wild-type cells as would occur if levels of *recA* protein were

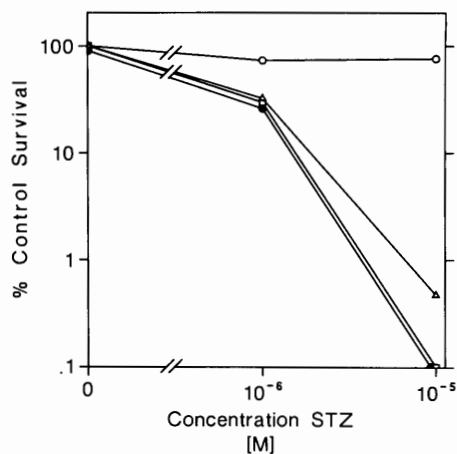


Fig. 1. Cytotoxicity by STZ in *E. coli* mutants. Cells were treated with drug for 30 min at 37°C, washed twice and plated. Colonies were counted after overnight incubation. Symbols represent: wild type, AB2497 (○) *recBC*, GM3843 (△); *recA*, AB2487 (■); *alkA tag*, GM3840 (□). See Methods section for details.

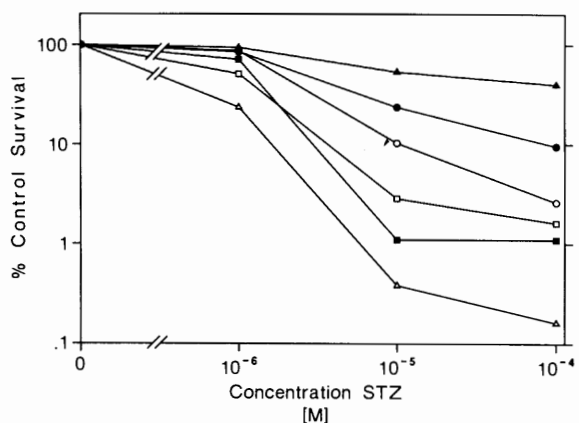


Fig. 2. Cytotoxicity by STZ in *E. coli* strains with a mutation in *recA*. Cells were exposed to drug for 30 min at 37°C, washed and plated. Symbols represent: *recA98 lexA3*, DM2210 (▲), *lexA51*, DE192 (●); wild type, DM2251 (○); *recA430 lexA51*, DM2573 (□); *recA730 lexA51*, DE464 (■) and *lexA3*, DE407 (△).

consistently elevated in the former strain and if RecA protein directly contributes to repairing STZ-induced DNA damage.

A direct role for RecA protein separate from its regulatory function in SOS repair is confirmed by the enhanced survival of *RecA98 lexA3* cells compared to *lexA3* cells. The former strain con-

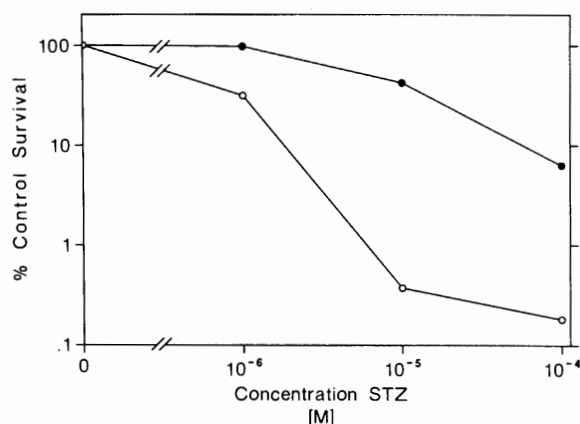


Fig. 3. Cytotoxicity by STZ in *polA E. coli*. Cells were exposed to drug for 30 min at 37°C, washed and plated. Symbols represent wild type, CM4722 (●) and *polA E. coli* mutant cells, CJ278 (○). See Methods for details.

stitutively synthesizes RecA protein and cannot induce the SOS response since the *lexA3* gene specifies an uncleavable LexA repressor. Lastly, the protease function of RecA protein, a function involved in derepressing SOS repair genes, does not affect results since survival is similar in a strain with high RecA protease activity (*recA730*) and a strain with low protease activity (*recA430*).

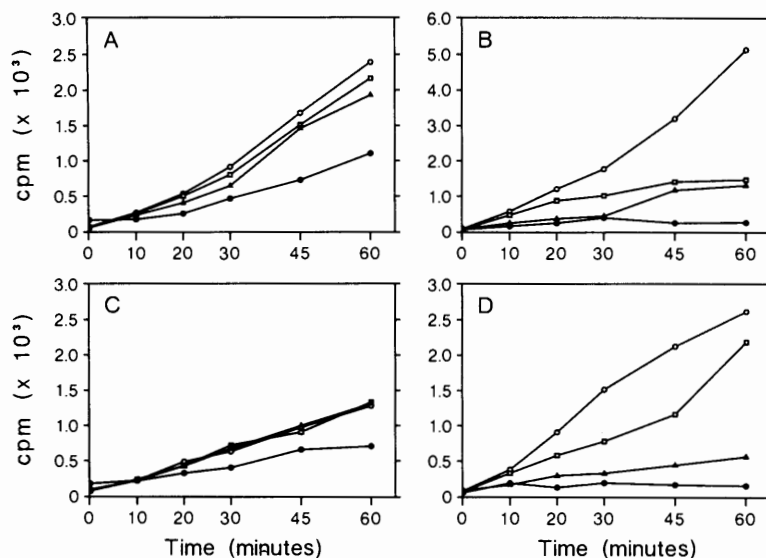


Fig. 4. Inhibition of DNA synthesis by STZ in *E. coli*. Cells were exposed to 1 μ Ci/ml tritiated thymidine and either no drug or 10⁻⁶–10⁻⁴ M STZ for various intervals. Acid-precipitable radioactivity was analyzed as described in the text. Results with wild type (AB2497) are shown in A, *recA* (AB2487) in B, *recBC* (GM3843) in C, and *alkA tag* (GM3840) in D. Symbols represent: no drug (○), 10⁻⁶ M STZ (□), 10⁻⁵ M STZ (▲), and 10⁻⁴ M STZ (●).

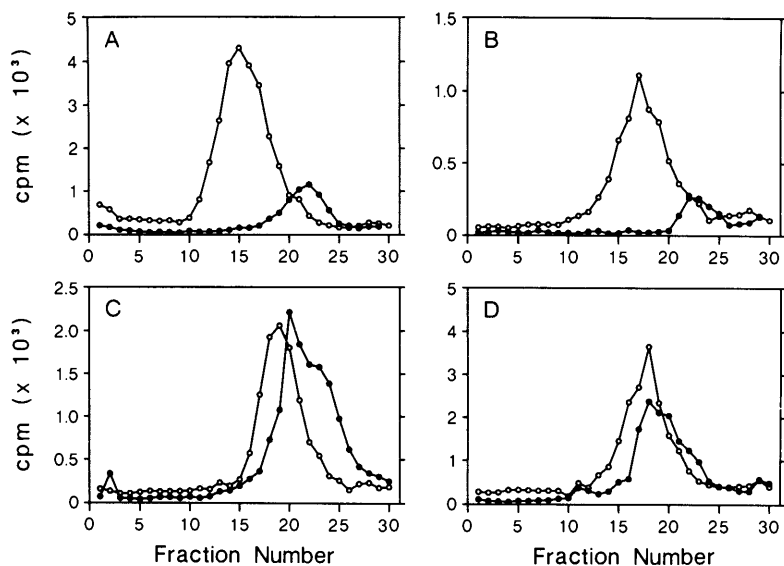


Fig. 5. Induction and repair of DNA single-strand breaks in *recA* and *recBC* *E. coli*. Cells were exposed to 10^{-4} M STZ (●) or no drug (○) for 30 min at 37°C, washed twice and either incubated an additional 90 min to assess repair of single-stranded DNA breaks or DNA-extracted and analyzed for breaks on alkaline sucrose gradients. Results with *recA* *E. coli* (AB2437) immediately after drug removal are shown in A. The effect of a 90-min recovery period after drug removal in this strain is shown in B. In panel C, the induction of single-stranded DNA breaks is analyzed in *recBC* cells (GM3843) and the effect of a further 90-min incubation at 37°C is shown in panel D. See text for details.

The effect of STZ on cytotoxicity in *polA* *E. coli* mutants which lack DNA polymerase I was analyzed because of the participation of this enzyme in catalyzing repair synthesis on nicked and gapped DNA templates. Fig. 3 demonstrates that *polA* *E. coli* are more sensitive to STZ than the wild type.

Because an important mechanism underlying cytotoxicity by STZ is likely mediated by effects on the synthesis of macromolecules, this issue was addressed by measuring DNA, RNA, and protein synthesis in the presence of drug. Fig. 4 demonstrates that STZ inhibits DNA synthesis and that the extent of inhibition of DNA synthesis is most

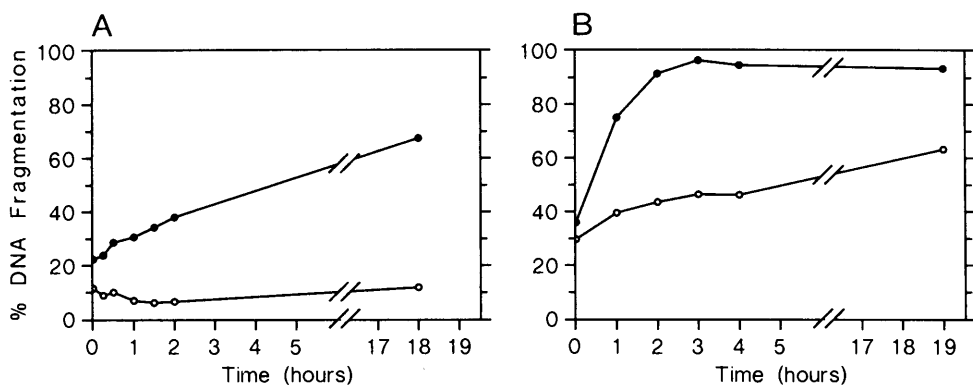


Fig. 6. DNA degradation by STZ. Cells were prelabeled overnight with tritiated thymidine ($2 \mu\text{Ci/ml}$), spun, resuspended in medium and exposed to no drug (○) or 10^{-4} M STZ (●) for 30 min at 37°C. DNA degradation was measured at various intervals after drug removal as described in the text. Results in wild type (AB1157) are shown in panel A, while panel B contains results with *recA* cells (AB2463).

marked in *recA* and *alkA tag E. coli* mutants. *recBC E. coli* resemble wild-type cells in this respect. DNA synthesis was more markedly inhibited by STZ than either RNA or protein synthesis, although synthesis of these macromolecules was also decreased in the presence of drug (data not shown).

The induction of single-stranded DNA breaks was analyzed by employing alkaline sucrose gradients after cellular DNA was labeled and cells exposed to STZ. Fig. 5 shows that the most marked increase in single-stranded DNA breaks occurs in *recA E. coli*. The reduction in total radioactivity that occurs in drug-treated *recA* cells most probably results from the enhanced DNA degradation that occurs in this strain (see below). Increased break frequency is evident, albeit to a lesser extent in *recBC E. coli*. Further, after removal of drug and incubation in media for 90 min at 37°C, there was no evidence of repair of single-stranded breaks in *recA* cells (see Fig. 5). This was not the case with *recBC* cells which were capable of repairing breaks during this recovery period. No increase in DNA-strand breaks was evident in *alkA tag E. coli* or the wild type after exposure to STZ at 10^{-4} M for 40 min at 37°C (data not shown).

DNA degradation also was assessed over longer intervals after drug exposure (see Fig. 6). Cellular DNA was prelabeled with tritiated thymidine, exposed to STZ for 30 min, and DNA degradation monitored up to 20 h after drug exposure. *recA E. coli* demonstrated marked increases in DNA degradation after drug exposure as well as significantly higher basal levels compared to wild-type cells (see Fig. 6). In contrast, enhanced DNA degradation was not evident in either *recBC* or *alkA tag E. coli* mutants (data not shown).

Discussion

Streptozotocin is a monofunctional nitrosourea that methylates DNA (Ledoux, 1986). Mechanisms that affect cytotoxicity by STZ have been studied in pancreatic islet cells. While the enhanced susceptibility of islet cells to this drug is thought to be related to the presence of a D-glucopyranose residue and increased transport, DNA single-strand breaks accompanied by cellular

metabolic derangements occurring as a result of decreased levels of NAD following the activation of poly(ADP-ribose) also may contribute (Okamoto, 1988). The precise mechanisms underlying cytotoxicity by STZ whether in normal or neoplastic B cells, however, remain unclear. In the current report an attempt was made to understand how DNA repair mechanisms affect cytotoxicity by this agent in *E. coli*. We employed *E. coli* mutants deficient in specific DNA repair pathways to clarify the involvement of various repair mechanisms and provide a conceptual framework for further studies in eukaryotic cells.

Our prior work demonstrates that STZ induces the adaptive response and the SOS repair mechanism in *E. coli* (Fram et al., 1988). The Ada protein specifies 2 methyltransferase activities that mediate demethylation of methylphosphotriesters as well as of *O*⁶-methylguanine and *O*⁴-methylthymine (Volkert, 1988). Further, *Ada* positively regulates the adaptive response to alkylating agents (Volkert, 1988). Induction of the adaptive response affected survival since mutants deficient in either *ada* gene function which specifies methyltransferase or *alkA* which codes for 3-methyladenine DNA glycosylase II were both highly sensitive to STZ compared to wild-type cells (Fram et al., 1986). The current study confirms these results by demonstrating a marked reduction in survival of *alkA tag E. coli* cells lacking both the constitutive as well as inducible 3-methyladenine DNA glycosylase. These findings suggest that 3-methyladenine is a critical cytotoxic lesion formed in DNA by this agent. Since 3-methyladenine DNA glycosylase II specified by *alkA* also repairs other methyl adducts in DNA, our results do not preclude the participation of other DNA adducts in mediating cytotoxicity by this agent (Volkert, 1988). Experiments analyzing macromolecular synthesis and DNA damage by STZ in cells unable to excise 3-methyladenine (*alkA tag E. coli*) demonstrate that DNA synthesis is inhibited at lower concentrations of drug than in wild-type cells and that DNA is the principal macromolecule whose synthesis is inhibited by this agent. Thus, persistent DNA adducts not excised by 3-methyladenine DNA glycosylase I and II may enhance cytotoxicity in this *E. coli* mutant by interfering with DNA synthesis.

In contrast to mutants deficient in recombinational repair, streptozotocin treatment of *alkA tag E. coli* was not associated with increased induction of DNA single-stranded breaks and *alkA tag E. coli* showed no evidence of increased DNA degradation up to 20 h after drug exposure compared to the wild type. These results suggest that failure to repair lesions recognized by this glycosylase are not associated with increased levels of DNA-strand scission. This most probably occurs because no apurinic or apyrimidinic sites are formed so that an appropriate substrate for apurinic/apyrimidinic endonuclease is not present. The absence of increased DNA fragmentation by STZ in *alkA tag* cells further emphasizes the importance of persistent adducts such as 3-methyladenine in inhibiting DNA synthesis as a mechanism underlying enhanced cytotoxicity in this strain. Wild-type cells also showed no evidence of DNA-strand scission after exposure to STZ. This finding likely occurs because DNA repair mechanisms are highly efficient in these cells.

While *recA E. coli* previously were shown to be very sensitive to treatment with STZ, the mechanism underlying enhanced cytotoxicity in these cells was unclear (Fram et al., 1986). RecA protein has multiple functions (Walker, 1984). This protein serves as a protease that cleaves LexA repressor, thus causing the expression of multiple SOS repair genes as well as *recA* itself. RecA protein also participates directly in recombinational repair, mediating pairing of homologous DNA segments and strand transfer (Weinstock, 1987). Lastly, *recA* gene product also affects mutagenic events (Ennis et al., 1985). The experiments in this report demonstrate that by facilitating recombinational repair, RecA protein enhances survival of cells treated with STZ. The involvement of recombinational repair in ameliorating effects by this drug is further emphasized by the sensitivity of cells lacking *recBC* gene products to STZ. These proteins are thought to participate in both strand transfer and the segregation of recombinants (Weinstock, 1987).

The induction of DNA single-stranded breaks was enhanced in both *recA* and *recBC E. coli* compared to the wild type. This finding is consistent with the inability of these mutants deficient

in recombinational repair to fill DNA gaps opposite adducts in parental DNA strands. Results from the DNA degradation studies show extensive DNA degradation in *recA E. coli* after treatment with STZ. In contrast, *recBC E. coli* DNA showed no evidence of enhanced degradation compared to the wild type when incubated for periods up to 20 h after drug exposure. Such a finding might be explained by the increased ability of *recBC E. coli* to repair DNA-strand scission compared to *recA* mutant *E. coli*. The biological relevance of recombinational repair to survival after exposure to STZ also is suggested by the marked sensitivity of *recA* and, to a lesser extent, *recBC E. coli* cells compared to the wild type. The presence of DNA degradation in wild-type cells after exposure to STZ may contribute to its cytotoxic effects and is consistent with results from a prior study (Lengeler, 1980).

It is possible that normal and transformed islet cells as well as some human tumors may prove more sensitive to STZ due to deficient DNA repair mechanisms. Whether 3-methyladenine DNA glycosylase and recombinational repair similarly affect survival of human tumor cells treated with STZ and underlie differences in sensitivity of human tumors to this agent are issues currently being explored.

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