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Enhancement of Deoxyribonucleic Acid Polymerase I-Directed Repair Synthesis in Toluene-Treated *Escherichia coli* After Growth in the Presence of Low Levels of *N*-Methyl-*N'*-Nitro-*N*-Nitrosoguanidine

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Deoxyribonucleic acid polymerase I-directed repair synthesis can be selectively measured in toluene-treated *Escherichia coli* cells exposed to alkylating chemicals such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Prior growth of the cells in the presence of a low dose of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine results in an enhanced deoxyribonucleic acid polymerase I-directed repair synthesis and an increase in single-strand breaks.

Many microorganisms containing DNA modified by mutagens and carcinogens such as alkylating agents exhibit the capacity to repair some or all of the damage. In *Escherichia coli*, the repair of this damage reflects expression of so-called "error-prone" and/or "error-free" systems. These repair pathways may exist as constitutive systems in the cell, as in the excision repair of UV-light-induced pyrimidine dimers, or they may be inducible as in error-prone (10) or error-free (8) repair systems.

A series of recent reports (6, 8, 9) has described an inducible error-correcting pathway in *E. coli* which is enhanced if the cells are first "adapted" to nontoxic levels of the alkylating agent *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG). Such cells are less likely to be mutated because they are better able to cope with larger amounts of alkylation damage than nonadapted cells possessing only the constitutive levels of repair systems.

Repair of DNA modified by alkylating agents is thought to occur through a number of cellular systems, one of which is the base excision-repair pathway. This system involves removal of alkylated bases by appropriate *N*-glycosylases followed by an endonuclease-directed nicking at the apurinic or apyrimidinic site (7). Excision of the damaged region could result in the production of a 3'-OH primer end on the affected strand (7).

N-Glycosylases and apurinic-site endonucleases have been identified in cells not exposed to alkylating agents. However, the production of greater levels of these enzymes or the induction of new *N*-glycosylases could account for the reduction in mutation induction observed in cells pretreated with low levels of the chemicals.

E. coli made permeable to nucleotide triphosphates by toluene treatment has been used to assay DNA polymerase I-directed repair synthesis after exposure of the toluene-treated cells to ionizing radiation (2) or alkylating agents including NTG (3). This DNA polymerase I-directed repair synthesis reflects in part the availability of 3'-OH primer ends in DNA and represents the repair synthesis component of the excision-repair pathway. In this system the absence of ATP in the assay mixture precludes DNA polymerase II- and III-directed synthesis (2). The addition of nicotinamide mononucleotide to the assay mixture prevents ligation, thereby allowing excessive nick translation and increase in the length of the repair patch (1).

The study described here was undertaken to determine whether pretreatment of growing cells with low doses of NTG before toluene treatment would increase the repair synthesis activity of the toluene-treated cells exposed to higher doses of NTG.

We found that pretreatment of cells in logarithmic growth with low levels of NTG (1 to 10 μM) enhanced polymerase I-directed repair synthesis and increased the yield of strand breaks (as measured in alkaline sucrose gradients) when the cells were subsequently tested against higher levels of MTG (2 to 10 mM) after permeablization.

The procedures for growth, toluene treatment, assay for DNA polymerase I-directed repair synthesis, and alkaline sucrose sedimentation analysis were as previously described (2). Pretreatment of the cells was accomplished by exposing portions of a log-phase culture of *E. coli* to low

levels of NTG for 30 min at 37°C. The cells were then harvested and processed as previously described (2) for the preparation of permeable cells. Challenge doses of NTG were added to the assay mixture used for assessing DNA polymerase I-directed repair synthesis by the toluene-treated cells (2, 3).

The bacterial strains used in this study were derivatives of *E. coli* K-12. Strain AB3063 F^- λ^-_{ind} *endA1 thi str* is a derivative of strain 1100 of Dürwald and Hoffmann-Berling (4). The strains used to test for the involvement of the *recA* gene product in the enhancement process were AB2463, which carries the *recA13* mutation, and its parent, AB1157, which is $recA^+$ (5). Both strains were originally obtained from Richard Boyce (University of Florida, Gainesville). Strain AB2463 *recA13* was checked for sensitivity to UV light.

DNA polymerase I activity in toluene-treated cells is increased by the addition of NTG at concentrations of 1 to 5 mM (3). In the work reported here, 2 mM NTG was used as the test dose to toluene-treated cells because concentrations above 2 mM NTG have been shown to inhibit DNA polymerase I-directed repair synthesis in X-irradiated, permeable cells (G. R. Hellermann and D. Billen, Chem. Biol. Interact., in press).

Prior growth of *E. coli* strain AB3063 in the presence of 0.1 to 10.0 μM NTG resulted in an elevated level of repair synthesis when the cells were subsequently treated with toluene and exposed to 2 mM NTG (Fig. 1B). Prior growth with levels of NTG greater than 1 μM resulted in an increase in "background" repair synthesis as measured by dTMP incorporation into an acid-insoluble material in the toluene-treated cells (Fig. 1A). This value was subtracted from the synthesis measured in the presence of 2 mM NTG to arrive at the net dTMP incorporated into DNA (Fig. 1C). These data show that growing cells responded to NTG levels as low as 0.1 μM, since enhancement of DNA polymerase I-directed repair synthesis was observed in such cells subsequently tested for repair activity (Fig. 1C).

Pretreatment with 100 μM NTG resulted in a high level of background repair synthesis in the toluene-treated preparation (Fig. 1A). In this case addition of 2 mM NTG to the toluene-treated cells resulted in little or no additional DNA synthesis (Fig. 1C). We interpret this finding as an indication of saturation of the DNA-polymerase I-directed repair system by the in vivo pretreatment.

The length of pretreatment with the mutagen also affected the enhancement response. Maximum enhancement of repair synthesis was observed in cells pretreated with 10 μM NTG for 30 to 40 min (Fig. 2). However, additional growth in the presence of the agent resulted in a reduction in the enhancement phenomenon. The reason for the decrease in unknown. Increasing toxicity with prolonged exposure is one possibility. Background repair synthesis as observed in the toluene-treated cells did increase with in-

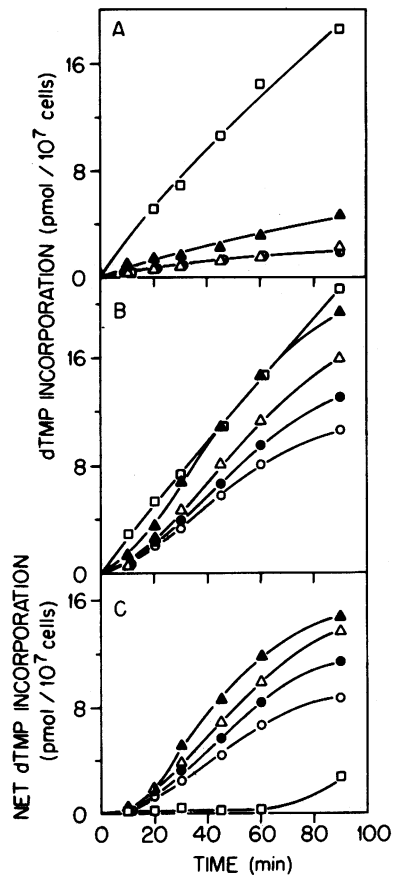

FIG. 1. *Kinetics of dTMP incorporation in E. coli AB3063 challenged with NTG after pretreatment with various concentrations of NTG. Cells in logarithmic growth were treated with NTG for 30 min at 37°C, washed, permeablized with 1% toluene for 5 min, frozen in liquid nitrogen, and stored at −65°C. For assay of polymerase I activity, thawed, toluene-treated cells were diluted into standard assay mixtures which lacked ATP, contained nicotinamide mononucleotide (2), and were without (A) or with (B) 2 mM NTG. (C) Net incorporation of dTMP (incorporation in the presence of NTG minus incorporation in the absence of NTG). At the indicated times of incubation at 37°C, samples were withdrawn and the amount of radioactivity in acid-insoluble material was determined. The cells had been pretreated as follows:* ○, *no pretreatment;* ●, *0.1 μM NTG;* △, *1.0 μM NTG;* ▲, *10 μM NTG; and* □, *100 μM NTG.*

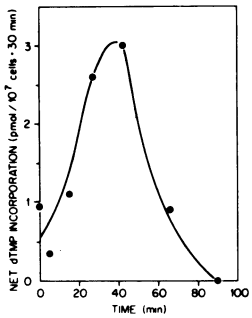

FIG. 2. *Effect of length of time of pretreatment with NTG on dTMP incorporation in E. coli AB3063 challenged with NTG. Cells in logarithmic growth were pretreated with NTG at a final concentration of 10 μM for the indicated lengths of time before being permeablized by toluene treatment as described in the legend to Fig. 1. Polymerase I activity in the permeablized cells in response to challenge with 2 mM NTG was assayed at 37°C as described in the legend to Fig. 1. Net dTMP incorporation in 30 min is plotted against length of time of NTG pretreatment.*

creasing length of pretreatment up to 40 min, but then decreased (data not shown).

DNA polymerase I requires a 3'-OH terminus for the initiation of polymerization. The addition of certain alkylating agents to toluene-treated bacteria results in the formation of such sites. In previous studies with alkylating agents we observed a correlation between the level of DNA polymerase I-directed repair synthesis and the number of single-strand breaks as measured in an alkaline sucrose gradient (2, 3). A small number of these sites may be produced by spontaneous chemical breakdown of the alkylated DNA, but the majority probably result from enzymatic action at the site of damage on the DNA (7). Analysis of DNA sedimented in alkaline sucrose gradients gives the number of all spontaneous and enzymatic breaks plus those breaks that arise as a result of the alkaline lability of the phosphodiester bond at the apurinic or apyrimidinic sites. Since the assay system used here precludes break rejoining because of the presence of nicotinamide mononucleotide (2), spontaneous and enzymatically produced nicks will not be rejoined during incubation of the toluene-treated cells in the assay mixture. Table 1 shows the number of single-strand breaks measured on alkaline sucrose gradients in the DNA of cells that have been grown in the presence of 10 μM NTG for 30 min prior to toluene treatment compared with the number in cells not so pretreated. Toluene-treated cells exposed to 2 mM NTG for 30 min show an increased number of single-strand breaks compared with controls (no NTG added to the assay mixture). However, if the cells had been grown in the presence of 10 μM NTG before permeablization, a substantial increase in the number of single-strand breaks was observed when these cells were challenged with 2 mM NTG. The pretreatment resulted in a small increase in the number of background single-strand breaks in the toluene-treated cells. This was subtracted from the number of single-strand breaks measured in the presence of 2 mM NTG to arrive at the values for yield of single-strand breaks shown in Table 1.

Although it is reasonable to suppose that the enhanced number of single-strand breaks is the cause of the increased DNA polymerase I-directed synthesis observed, another possibility is that the pretreatment resulted in a more extensive DNA chain elongation in the permeable cells. We tested this possibility by exposing toluene-treated preparations of cells grown in the presence of 10 μM NTG to 5, 10, and 25 krad of X rays. X rays have been shown to stimulate DNA polymerase I-directed repair synthesis, and we reasoned that if the chemical pretreatment causes additional chain elongation, then

TABLE 1. *Yield of single-strand breaks in DNA of E. coli AB3063 cells with or without NTG pretreatment and NTG challenge*

Pretreatment[a]	Challenge	M_n	Single-strand breaks/ 10^9 daltons	Yield of single-strand breaks
None	No incubation	103×10^6	9.7	
None	+ Incubation, no addition	65×10^6	15	
None	+ Incubation + 2 mM NTG	9×10^6	111	96
10 μM NTG	No incubation	88×10^6	11	
10 μM NTG	+ Incubation, no addition	39×10^6	26	
10 μM NTG	+ Incubation + 2 mM NTG	5.7×10^6	175	149

[a] Cells in logarithmic growth (bulk-labeled in the DNA with [^{14}C]thymidine) were treated where indicated with NTG at a final concentration of 10 μM 30 min before being permeablized with toluene as described in the legend to Fig. 1. Thawed, permeablized cells were incubated for 30 min at 37°C in the standard assay mixture (2) with or without 2 mM NTG. After incubation the cells were washed and lysed with lysozyme (500 μg/ml), and a portion of the lysate was layered onto a 5 to 20% alkaline sucrose gradient, where it was allowed to stand for 45 min before being centrifuged for 1 to 4 h at 28,000 rpm and 20°C. The profiles of radioactivity were used, with the help of a computer program, to generate the number average molecular weights (M_n) given in the table. The number of single-strand breaks in 10^9 daltons of DNA is taken to be $(1/M_n) \times 10^9$. The yield of single-strand breaks is the difference between the number of single-strand breaks per 10^9 daltons in the DNA-permeable cells incubated without NTG and the number in the DNA of permeable cells incubated with NTG.

we should observe a higher level of repair synthesis in pretreated cells after X irradiation. At all three doses of X rays there was no difference between the pretreated and non-pretreated cells (data not shown).

We conclude from this experiment that: (i) prior growth in the presence of low levels of NTG does not lead to enhanced DNA polymerase I-directed repair synthesis brought about by X-ray exposure of toluene-treated cells; and (ii) the enhanced repair synthesis at high levels of NTG seen in toluene-treated cells prepared from cultures grown in the presence of low levels of NTG is not due to additional chain elongation, but is more likely due to an increase in the number of 3'-OH termini.

Jeggo et al. have referred to the reduced mutagenesis in *E. coli* which occurs after growth at low levels of NTG as an adaptive response (6). These investigators observed that *polA* strains exhibit the adaptive response as far as mutation is concerned, but this was less so for lethality (6). This led to the suggestion by Jeggo et al. that, for the *polA* strain, "lethality and mutation are the end result of a different sequence of events" (6). Our findings support their suggestion of DNA polymerase I involvement in the adaptive response and further suggest that the enhanced levels of repair synthesis and single-strand breaks observed in our study are more likely to be related to events involved in killing than in mutagenesis.

Unlike the induction of the error-prone pathway, this adaptive system can be expressed in the absence of the *recA* gene product (6). We tested the involvement of the *recA* gene product in the enhancement of DNA polymerase I-directed repair synthesis observed in our permeable cells. Strain AB2463 *recA13* was as effective in expressing enhancement by growth in the presence of low doses of NTG as was its parent strain, AB1157, which is *recA*$^+$ (data not shown). We suggest, therefore, that the enhancement of DNA polymerase I primer site production by pretreatment with NTG followed by NTG challenge is not a *recA*-dependent function. Jeggo et al. (6) also found that *recA13* did not influence the expression of the adaptive response in their study; however *recA12* failed to show the adaptive response. Therefore, our finding with *recA13* is not sufficient to totally exclude the involvement of other *recA* alleles in the DNA polymerase I response reported here.

The pretreatment with low levels of NTG may result in the induction of specific *N*-glycosylases and endonucleases and/or increased activity of these enzymes. We are in the process of assessing these possibilities.

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