Role for Deoxyribonucleic Acid Ligase in Deoxyribonucleic Acid Polymerase I-Dependent Repair Synthesis in Toluene-Treated Escherichia coli

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In a toluene-treated mutant of Escherichia coli K-12 having a temperature-sensitive, conditionally lethal mutation in the structural gene for deoxyribonucleic acid (DNA) ligase, an extensive DNA repair synthesis occurred in X-irradiated cells at the nonpermissive temperature, 42°C. At the permissive temperature, 30°C, nearly normal semiconservative synthesis and limited repair synthesis were observed when DNA ligase was activated by the addition of nicotinamide adenine dinucleotide.

In toluene-treated Bacillus subtilis strains possessing normal deoxyribonucleic acid (DNA) ligase activity, nicotinamide adenine dinucleotide (NAD) reduces and nicotinamide mononucleotide (NMN) stimulates a DNA polymerase I-dependent DNA repair synthesis after X-ray exposure (1, 2). Since NAD is the cofactor for DNA ligase in bacteria (7, 13) and NMN is an inhibitor of DNA ligase activity (8), the tentative conclusion was drawn that DNA ligase is involved in the limiting of DNA polymerase I-dependent repair synthesis.

For more direct evidence of the role of DNA ligase in controlling the extent of nucleotide insertion, we constructed a double mutant of Escherichia coli K-12 with a temperature-sensitive, conditionally lethal mutation in the structural gene for DNA ligase (4, 6, 11) and a mutation in the endA gene.

At 30°C the double mutant exhibits X-ray-induced repair synthesis similar to that shown in toluene-treated wild-type Bacillus subtilis (1, 2). X-ray-induced repair synthesis is markedly reduced when NAD is present in an assay mixture containing toluene-treated cells from which endogenous NAD has been partially removed by washing (10). At the nonpermissive temperature of 42°C, NAD no longer reduces X-ray-induced repair synthesis.

The E. coli strain used in this study, OR129 lig-7 (ts) endA1 thi nal' strA', was constructed by conjugal transfer of the lig-7 mutation into strain AB3063 endA1 thi strA' (obtained from R. P. Boyce). First a spontaneous mutant of E. coli N2668 F- lig-7 (ts) (4) resistant to 40 μg of nalidixic acid per ml was isolated and made F+ by mating it with an F+ E. coli strain (3). Ultraviolet light mutagenesis, followed by penicillin selection, resulted in a mutant that was F+lig-7 (ts) nal'leu. The nalA gene at 42.3 min on the E. coli chromosome and the lig-7 gene at 45.5 min (12) are close enough on the chromosome to allow nalidixic acid resistance to be used to select for recombinants with defective ligase. One recombinant (OR129) of several that failed to grow at 42°C was selected for further study.

E. coli OR129 was grown at 30°C in L broth or in a minimal salts medium (MMA) containing 4.5 g of KH₂PO₄, 10.5 g of K₂HPO₄, 1.0 g of (NH₄)₂SO₄ and 0.5 g of sodium citrate·2H₂O per liter supplemented with 0.4% glucose, 0.2% vitamin-free Casamino Acids (Difco), 0.01 M MgSO₄, and 10 μg of thiamine per ml. DNA was bulk labeled by growing cells in MMA in the presence of 50 μg of 2'-deoxyadenosine and 0.1 μCi of [³²P]thymidine (56 mCi/mmol; Schwarz/Mann) per ml. Toluene treatment, X-irradiation, assay conditions for measuring DNA synthesis, and isopycnic analysis were as previously described (1).

X-ray exposure of toluene-treated B. subtilis cells causes a two- to tenfold increase of DNA polymerase I-dependent [³H]thymidine triphosphate incorporation into DNA (1, 2). When toluene-treated E. coli OR129 was incubated at 30°C, there was an increase in the amount of DNA synthesized after an X-ray dose of 20 krad (Fig. 1). At the nonpermissive temperature of 42°C, there was a marked additional stimulation of DNA synthesis in the irradiated cells but little change in [³H]thymidine triphosphate incorporation in the controls.

If E. coli DNA ligase affects X-ray-induced
DNA synthesis by limiting DNA polymerase I-dependent repair synthesis, then the addition of the DNA ligase cofactor (NAD) at the non-permissive temperature will have no effect on the observed DNA synthesis since the enzyme should be inactive. This proved to be true, but only if the toluene-treated cells received a 10-min preincubation at 42°C (Fig. 2B). Without the preincubation the presence of NAD markedly decreased X-ray-induced DNA synthesis (Fig. 2A). Apparently inactivation of residual DNA ligase requires some minutes of incubation at 42°C. The once-washed, toluene-

**FIG. 1.** Effect of X-irradiation on DNA synthesis in toluene-treated E. coli OR129. Cells were grown at 30°C in L broth to a density of about $10^8$ cells/ml, harvested, toluene-treated, and concentrated 50-fold. DNA synthesis was measured in an assay mixture containing 70 mM potassium phosphate buffer (pH 7.4); 13 mM MgSO$_4$; 1.3 mM adenosine triphosphate; 33 µM each of deoxyadenosine triphosphate, deoxyctydine triphosphate, and deoxyguanosine triphosphate; 2 mM dithiothreitol; 20 µM of either deoxythymidine triphosphate or bromodeoxyuridine triphosphate; 2.5 µCi of [3H]thymidine triphosphate at a specific activity of 0.5 µCi/mmol; and about $10^8$ toluene-treated cells. dTMP, Thymidine 5'-monophosphate.

**FIG. 2.** Effect of NAD and NMN on DNA synthesis at 42°C in irradiated, toluene-treated cells. Conditions were identical to those described in the legend to Fig. 1, except that a portion of the cells was preincubated at 42°C for 10 min before adding to the assay mixture. (A) No preincubation; (B) 10-min preincubation.
Fig. 3. Isopycnic analysis of DNA from unirradiated and irradiated, toluene-treated cells. Cells were grown at 30°C in MMA containing [14C]deoxythymidine to bulk label the DNA and then toluene-treated; a portion of the toluene-treated cells were given an X-ray dose of 20 krad. Cells were then added to assay mixtures in which bromodeoxyuridine triphosphate had been substituted for thymidine triphosphate, and the mixtures were incubated at 30 or 42°C for 20 min. The final concentrations of NAD and NMN were as given in Fig. 2. Cells were preincubated at 42°C for 10 min in the assay mixture prior to the addition of [3H]thymidine triphosphate, NAD, or NMN (as indicated) before assaying at 42°C. Preparation of lysates, conditions of centrifugation, and collection of gradients were as described previously (1). LL and LH mark the position of parental and hybrid density, respectively.
treated cells apparently retain some NAD, since addition of NMN to the cells at 30°C increased X-ray-induced DNA synthesis (data not shown).

Isopycnic gradient analysis of DNA was carried out to determine the mode of DNA synthesis in the toluene-treated cells with and without irradiation. If repair-type synthesis is limited by DNA ligase as suggested by our previous studies with B. subtilis (2), then addition of NAD should effectively reduce a repair-type synthesis at the permissive temperature (30°C). However, at the nonpermissive temperature of 42°C, DNA ligase activity should be minimal and the addition of NAD should not deter extensive repair-type synthesis. The data given in Fig. 3 clearly fits this expectation. At 30°C, under conditions of minimal DNA ligase activity (addition of NMN), repair-type synthesis is enhanced and replicative synthesis is inhibited in the irradiated cells (Fig. 3B) as compared with the unirradiated cells (Fig. 3A). The activation of DNA ligase by the addition of NAD to the complete reaction mixture results in a reduction in repair synthesis while allowing the expression of a replicative synthesis mode after X-irradiation (Fig. 3C). NAD has a slight effect on DNA synthesis in unirradiated toluene-treated B. subtilis (2) and E. coli (data not shown).

At 42°C, repair synthesis was enhanced in the irradiated cells (Fig. 3E) as compared with the unirradiated cells (Fig. 3D), but the addition of NAD failed to reduce X-ray-stimulated repair synthesis (Fig. 3F). Semiconservative synthesis is not seen in the irradiated cells held at 42°C, but since semiconservative synthesis is also reduced in the unirradiated cells held at 42°C this probably reflects a response of DNA replication by these cells to this temperature.

We conclude that the NAD effect in irradiated cells results from its role as cofactor for DNA ligase.

We have evidence that the extent of DNA polymerase I activity in X-ray-induced repair synthesis in toluene-treated cells depends directly on the level of ligase activity, whereas that of DNA polymerases II and III does not (D. Billen and G. R. Hellermann, unpublished observations). From the results presented here for E. coli, and our previous observations on B. subtilis (2), we suggest that DNA ligase directly affects the extent of DNA polymerase I-dependent resynthesis during X-ray-induced repair in toluene-treated bacterial cells by termination of nucleotide reinsertion as a result of ligation.

In studies utilizing purified enzyme preparations, a similar concerted action of DNA polymerase and DNA ligase has been implicated in the final steps of DNA replication (9) and also in excision repair after irradiation of DNA with ultraviolet light (5).

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