

Purification and Characterization of an Inducible *Escherichia coli* DNA Polymerase Capable of Insertion and Bypass at Abasic Lesions in DNA*

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We have investigated the ability of DNA polymerases from SOS-induced and uninduced *Escherichia coli* to incorporate nucleotides at a well-defined abasic (apurinic/apyrimidinic) DNA template site and to extend these chains from this unpaired 3' terminus. A DNA polymerase activity has been purified from *E. coli*, deleted for DNA polymerase I, that appears to be induced 7-fold in cells following treatment with nalidixic acid. Induction of this polymerase (designated DNA polymerase X) appears to be part of the SOS response of *E. coli* since it cannot be induced in strains containing a noncleavable form of the LexA repressor (Ind⁻). The enzyme is able to incorporate nucleotides efficiently opposite the abasic template lesion and to continue DNA synthesis. Although we observe an approximate 2-fold induction of DNA polymerase III in cells treated with nalidixic acid, several lines of evidence argue that DNA polymerase X is unrelated to DNA polymerase III (pol III). In contrast to pol X, pol III shows almost no detectable ability to incorporate at or extend beyond the abasic site; incorporation efficiency at the abasic lesion is at least 100-fold larger for pol X compared to pol III holoenzyme, pol III core, or pol III* (the polymerase III holoenzyme subassembly lacking the β subunit). Pol X does not cross-react with polyclonal antibody directed against pol III holoenzyme complex or with monoclonal antibody prepared to the α subunit of pol III. Despite these structural and biochemical differences, pol X appears to interact specifically with the β subunit of the pol III holoenzyme in the presence of single-stranded binding protein. Pol X has a molecular mass of 84 kDa. Our results indicate that this novel activity is likely to be identical to DNA polymerase II of *E. coli*.

Severe DNA damage caused by ultraviolet and ionizing radiation or a variety of chemical mutagens induces either directly or through inhibition of DNA replication a stress response known as the "SOS response" (1-3). At least 15 genes are currently known to be involved in the pleiotropic SOS response (3) including genes involved in DNA repair. These genes, normally repressed by the Lex A repressor, are

turned on following proteolytic inactivation of the Lex A protein mediated by an activated form of Rec A protein (3).

An important property of the SOS repair pathway is that it is accompanied by a significant elevation in mutation frequencies, which increase from several hundred- to several thousand-fold (2). Pyrimidine dimers, caused by UV radiation, and abasic (apurinic and apyrimidinic) lesions are common forms of DNA damage that can act as effective, although not absolute blocks, to DNA synthesis by polymerases *in vitro* (4-8). Insertion of nucleotides opposite unrepaired noncoding DNA lesions and subsequent synthesis past these lesions would seem to be necessary for survival of a cell that had suffered significant DNA damage. The increased mutagenesis accompanying lesion bypass appears to require the presence of the proteins UmuC and UmuD (9) and pol III¹ (10-12). It is also possible that DNA polymerase I, perhaps in a modified form, plays a role in the induction of errors (13). At present, the biochemical basis of SOS-induced mutagenesis remains a mystery.

In this paper, we utilize a polyacrylamide gel assay (8, 14) measuring nucleotide insertion and bypass at a defined abasic lesion to compare DNA polymerases purified from uninduced and SOS-induced *Escherichia coli*.² Using cells deleted for the pol A (15) gene (DNA polymerase I), induced for the SOS response, we have purified a polymerase activity, distinct from pol III that is able to insert opposite an abasic site and to extend the chain much more efficiently than pol III. Evidence is presented which suggests that this polymerase activity is partially under control of the Lex A repressor and is likely to be identical to DNA polymerase II of *E. coli* (16, 17).

EXPERIMENTAL PROCEDURES

Bacterial Strains—The *E. coli* strains used were CM4722 [F⁺, Δ (gal-bio), *thi-1*, *relA*, *spoT*], CJ261 [as CM4722, also Δ pol A, Km^r], CJ278 [as CM4722, also Δ pol A, Km^r], and CJ229 [CM4722 Δ pol A, Km^r/pCJ102 (F'5'ExoCm^r)] supplied by Dr. C. Joyce, Yale University (15), AB2474 (F⁻, *thr-1*, *leu-6*, *proA2*, *his-4*, *thi-1*, *argE3*, *lacY1*, *galK2*, *ara-14*, *xy1-5*, *mtl-1*, *tsx-33*, *strA31*, *sup-37*, *lex-1*, *uvrA6*, λ ⁻) supplied by Dr. B. Bridges, University of Sussex (18), and HMS-

¹ The abbreviations used are: pol III, DNA polymerase III; pol III*, the polymerase III holoenzyme subassembly lacking the β subunit; pol I, DNA polymerase I; pol II, DNA polymerase II; SSB, single-stranded binding protein from *E. coli*; ssM13 DNA, bacteriophage single stranded M13 DNA.

² A preliminary report of this work was presented at the ASM Conference On DNA Replication And Mutagenesis, November 8-12, 1987, Marco Island, FL.

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83 pol A1, pol B100, thy, lys, loc zam, was supplied by Dr. Robb Moses, Baylor College of Medicine.

Nucleotides—All nonradioactive nucleotides were purchased from Pharmacia P-L Biochemicals. [γ - 32 P]ATP (4000 Ci/mmol) and [methyl]- 3 H]TTP (20 Ci/mmol) were from ICN Radiochemicals.

Primer-Template—Synthesis of the 28-nucleotide abasic template has been previously described (19). 5'-End labeling of the 15-base primer and annealing to the 28-base template has also been described (8). Annealing of the 32 P-labeled 23-base primer to ssM13 has been described (14).

Enzymes—*E. coli* DNA polymerase III (holoenzyme, core, and pol III*) was a gift of Dr. A. Kornberg, Stanford University and Dr. C. McHenry, University of Colorado. *E. coli* DNA polymerase III* was purified from strain CJ229 as described below.

Other Materials—Antibody prepared against DNA polymerase I was a gift of Dr. S. Linn, University of California, Berkeley. Antibody prepared to DNA polymerase III (holoenzyme and α subunit) was a gift of Dr. C. McHenry, University of Colorado. Rec A protein was purified as described (20). The β subunit of DNA polymerase III holoenzyme and single-stranded DNA-binding protein (SSB) were gifts of Dr. A. Kornberg, Stanford University. Whatman phosphocellulose 11 and Pharmacia LKB Biotechnology Inc. Sephacryl S-200 were used for column chromatography. Sodium dodecyl sulfate (SDS) for the activity gel was purchased from Bio-Rad lot M2697. Behring Diagnostics anti-rabbit IgG peroxidase conjugate and Sigma 4-chloronaphthol were used in Western immunoblot analysis.

DNA Polymerase Primer Extension Reactions—Primer extension reactions for the gel electrophoresis assay were started by adding 3 μ l of annealed primer-abasic site template to 7 μ l of enzyme/nucleotide mix. Final concentrations were 20 mM Tris-HCl, pH 7.5, 7.3 mM MgCl₂, 2.5 mM dithiothreitol, 2.5 mg/ml bovine serum albumin, 3 mM spermidine, 50 μ M each of dCTP and dTTP, 800 μ M dATP, β subunit (1 μ g/ml) and SSB protein (250 μ g/ml) were used with SSM13 template as described (21). The polymerization reactions were allowed to proceed at 37 °C for 5 min and terminated by the addition of 20 μ l of 20 mM EDTA in 95% formamide. Samples of this mixture were then subjected to gel electrophoresis as described below.

Gel Electrophoresis and Autoradiography—Samples of the polymerase reaction mixtures (5 μ l) were heat denatured at 100 °C for 5 min, cooled on ice, and loaded onto a 8% (ssM13 template) or 16% (28-base template) polyacrylamide gel containing 8 M urea. Electrophoresis was performed at 2000 V for 2–4 h to resolve extended primers. Autoradiograms of gels were made by overlaying medical x-ray film (Kodak GPB-1) with an intensifying screen and exposing overnight.

Purification of *E. coli* DNA Polymerases—*E. coli* pol III* was prepared through Fraction III as described by McHenry (22). The column fractions were immediately assayed for polymerase activity and peak fractions were stored at –70 °C.

E. coli—DNA polymerase X (enzyme that inserts nucleotides at abasic lesions) was purified through Fraction II as described by McHenry (22). Fraction II was dialyzed overnight against 50 mM Tris-HCl, pH 7.5, 30% glycerol, 1 mM EDTA, 5 mM dithiothreitol (Buffer PC) + 25 mM NaCl and applied to a phosphocellulose column (P11) equilibrated with the same buffer. The column was first washed with two-column volumes of equilibration buffer followed by elution with an eight-column volume gradient (from Buffer PC + 50 mM NaCl \rightarrow Buffer PC + 400 mM NaCl). Column fractions were assayed for DNA polymerase activity, and active fractions (270 mM NaCl) pooled resulting in Fraction IIIB. Fraction IIIB was dialyzed 24 h against Buffer PC + 25 mM NaCl then concentrated 10-fold on a phosphocellulose column by eluting with Buffer PC + 500 mM NaCl. All enzyme activity was applied to a Sephacryl S-200 column and eluted with Buffer PC + 40 mM NaCl. The enzyme was assayed and pooled fractions were again concentrated on a phosphocellulose column (Fraction IV).

DNA Polymerase Activity on Gapped DNA—DNA polymerase activity was monitored throughout the purification by measuring the incorporation of [methyl- 3 H]TTP into activated salmon sperm DNA as described (23) except that reactions were performed for 15 min at 37 °C and were terminated by the addition of 0.2 M sodium pyrophosphate in 15% trichloroacetic acid. One unit of enzyme catalyzes the incorporation of 1 pmol of [3 H]TTP into acid-insoluble material in 1 min at 37 °C.

Activity Gel—The *in situ* SDS-polyacrylamide DNA polymerase "activity" gels were run as described (13). Gels were dried and autoradiography performed with Kodak GPB-1 film.

Induction of Rec A Protein by Nalidixic Acid—Rec A protein was

induced by treatment of logarithmically growing cells with nalidixic acid. Cells were grown in LB media to mid-log phase at which time nalidixic acid (40 μ g/ml) was added and incubation continued for 2 h at 37 °C. Cells were immediately pelleted and extracts were prepared according to McHenry (22).

Western Immunoblot Analysis of Proteins—Polyacrylamide gels for protein transfer blots were prepared as described (24). Protein bands were transferred electrophoretically to nitrocellulose filter paper in a Bio-Rad transblot apparatus, incubated with rabbit anti-Rec A protein antibody and subsequently incubated with excess goat anti-rabbit IgG peroxidase conjugate and stained with 4-chloronaphthol.

Protein Assays—Protein concentrations were measured according to the Bradford assay using materials and protocol supplied in the Bio-Rad Protein Assay Kit. Staining of protein gels by Coomassie Brilliant Blue R(Sigma) has been described (25).

RESULTS

Our initial objective was to determine if there is a significant increase in the activity of nucleotide incorporation and bypass at noncoding abasic lesions in DNA polymerase fractions prepared from SOS-induced cells. To investigate incorporation opposite a noncoding lesion, we synthesized DNA oligonucleotide templates containing a reduced abasic site (19) (Fig. 1). Primer molecules labeled with 32 P at their 5'-ends were annealed to the oligonucleotide templates containing the abasic site. After reaction with different polymerase fractions, primer molecules extended to different extents were resolved by polyacrylamide gel electrophoresis and visualized by autoradiography (Fig. 2).

DNA synthesis rates can be determined by measuring the fraction of primer molecules elongated by the addition of one or more nucleotides/unit reaction time, as previously described (8, 14). Incorporation of three complementary nucleotides (dTTP, dCMP, dTMP) is permitted to occur before the enzyme reaches the abasic site on the template strand. This type of configuration was chosen so that polymerase activity at normal template sites can be determined separately from incorporation and extension at the noncoding abasic lesion (8). DNA polymerases were purified from strain CJ229 in order to eliminate trace amounts of DNA polymerase I which copurify with pol III* (26). CJ229 contains a chromosomal deletion of the pol I gene and carries the portion of the pol I gene encoding the 5'-3' exonuclease on an F' episome (15).

Ability of DNA Polymerases from SOS-induced and Uninduced Cells to Incorporate and Extend at Abasic Lesions—DNA polymerases purified from uninduced and SOS-induced cells were examined for their ability to incorporate dAMP opposite the abasic site (*X band*, Fig. 2) and to extend beyond the lesion (*T bands*, Fig. 2). DNA polymerase III* purified from both uninduced and induced cells shows virtually no insertion or extension activity at the abasic site (Fig. 2, lanes 4 and 5). Pol III* is obtained as a peak of activity from a phosphocellulose column eluted with a linear salt gradient in



FIG. 1. Primer-template configurations used to evaluate the ability of *E. coli* DNA polymerases to synthesize opposite abasic lesions (X). The 5'- 32 P end-labeled primer is 15 nucleotides long; the unlabeled template strand is 28 (a) or 26 (b) nucleotides long, of which 15 residues at the 3'-end are complementary to the primer.

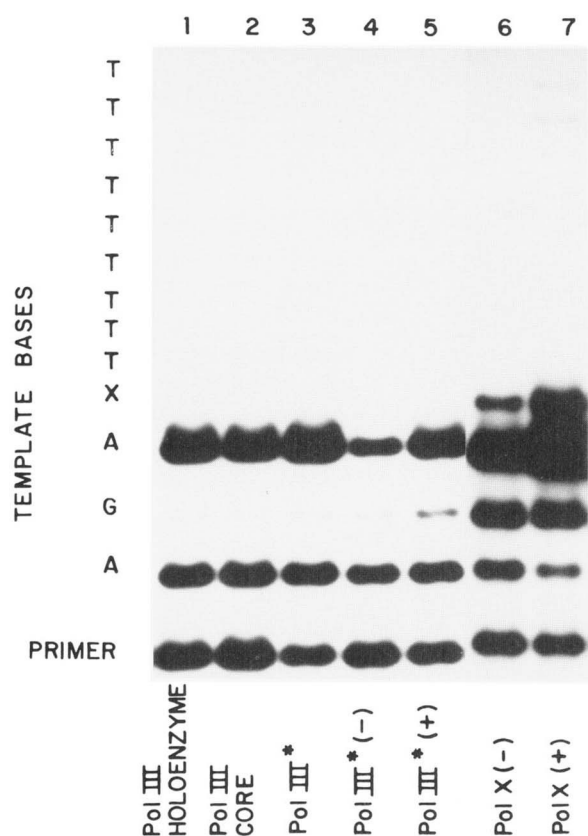


FIG. 2. Gel autoradiogram of primer extension assay for *E. coli* DNA polymerases on a template containing an abasic site (X). DNA polymerase reactions were performed as described under "Experimental Procedures" using 3 units of DNA polymerase purified from strain HMS83 (lanes 1–3) and strain CJ229 (lanes 4–7). In lanes 4–7 the \pm indicates whether the enzymes were purified from uninduced (–) or induced (+) cells.

the range of 74–150 mM NaCl (Fig. 3); this fraction, designated as Fraction IIIA, represents the pol III holoenzyme minus the β subunit required for processivity (21).

Highly purified fractions of pol III holoenzyme (lane 1), pol III core (complex consisting of protein subunits α , ϵ , and θ ; lane 2) and pol III* (lane 3), obtained from the laboratories of Dr. A. Kornberg and Dr. C. McHenry, showed almost no incorporation activity at the abasic site, which is similar to what was found for partially purified pol III* from SOS-induced and uninduced cells. Note that the presence of an intense band immediately prior to the abasic site (A band, Fig. 2) indicates that incorporation of dAMP at the abasic site is highly inefficient. The presence of a relatively intense band at the abasic site (X band, Fig. 2) shows that incorporation is occurring opposite the lesion but that the enzyme has difficulty synthesizing beyond the lesion.

Addition of β subunit and SSB protein to pol III* prepared from both uninduced and SOS-induced cells resulted in processive DNA synthesis by both fractions. However, this increased processivity did not result in a measurable increase of incorporation at or extension beyond the abasic site. The integrity of pol III* purified from both uninduced and SOS-induced cells was verified by identifying individual subunit components of the pol III* complex by Western immunoblot analysis using the polyclonal antibody directed against the pol III holoenzyme and the monoclonal antibody prepared to the pol III α subunit (data not shown).

A second peak of polymerase activity (Fraction IIIB) elutes from the phosphocellulose column between 250–350 mM NaCl

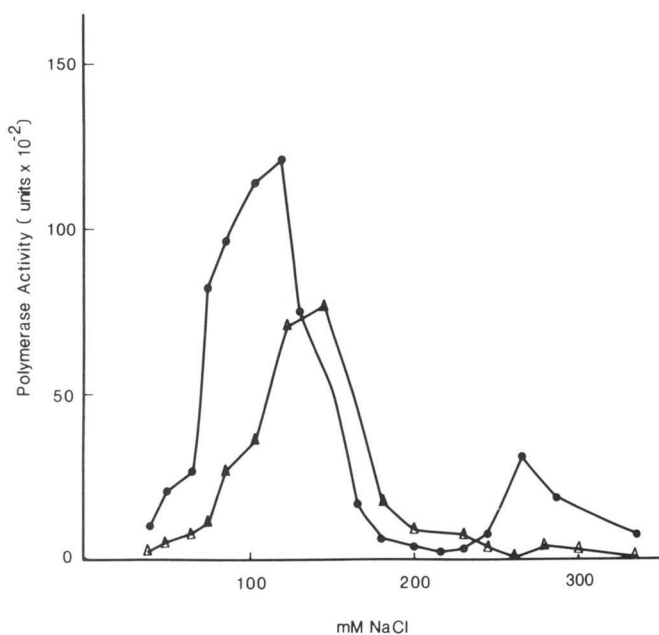


FIG. 3. Gradient elution of DNA polymerase activities on phosphocellulose from SOS-induced and uninduced cells. Extracts prepared from strain CJ229 induced for SOS (solid circles) and from strain CJ229 not induced for SOS (open triangles) were applied to a P11 phosphocellulose column and eluted with a linear NaCl gradient. Purification procedures and DNA polymerase activity measurements are described under "Experimental Procedures."

and is clearly evident in SOS-induced cells (Fig. 3). The total number of polymerase units in Fraction IIIB is about 7-fold greater in preparations from SOS-induced compared to uninduced cells. In contrast to pol III*, pol X (Fraction IIIB) has the remarkable property that it can incorporate dAMP at the abasic site and continue synthesis beyond the lesion (Fig. 2, lanes 6 and 7). Based on prolonged exposure of the pol III* autoradiograms, we estimate that the rate of dAMP incorporation at the abasic site is at least a 100-fold greater for pol X (Fraction IIIB) compared to pol III* (Fraction IIIA).

The template configuration shown in Fig. 1b was used to investigate the possibility that the A·X bands (Fig. 2) might result from incorporation of dAMP opposite the template T site adjacent to the abasic site X (Fig. 1a) rather than incorporation opposite template X. A mechanism has been proposed to explain certain mutagenic hot spots in terms of template dislocation instead of nucleotide misincorporation (27). The gel assay was repeated using the template 3'... AXGC8 (Fig. 1b) and dATP and dTTP as substrates for primer elongation. The bands corresponding to insertion at the abasic site were found to be equally intense in the presence or absence of dCTP suggesting that dAMP was incorporated opposite template X and not dCTP opposite template G (data not shown). However, addition of dCTP to the reaction allowed the addition of a single dCMP residue beyond the abasic site. Further extension was observed in the presence of both dCTP and dGTP (data not shown). Therefore, we conclude that template slippage cannot account for insertion and extension at the abasic site.

Induction of Rec A Protein by Nalidixic Acid in Pol I Deletion Strains—Five different *E. coli* strains were examined for their ability to respond to an SOS-inducing treatment by observing the level of Rec A protein produced (Fig. 4). Rec A protein induction was examined in each strain following treatment with nalidixic acid by Western immunoblot analysis using a polyclonal anti-Rec A antibody. Equal amounts of protein

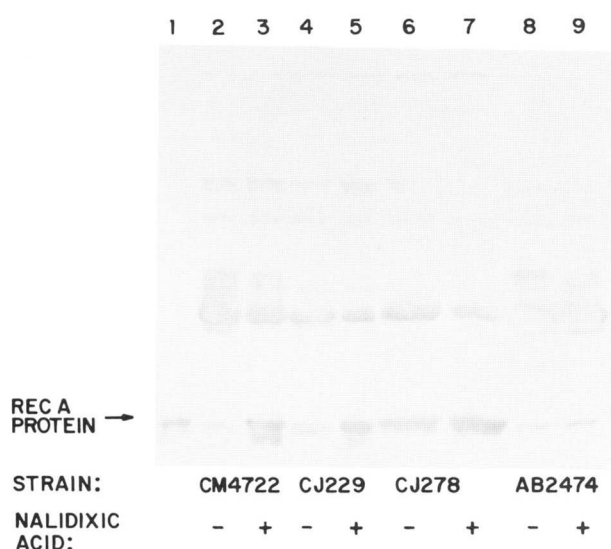


FIG. 4. Immunoblot analysis of Rec A protein induction by nalidixate treatment of pol A and lex A mutant strains. Lane 1, purified Rec A protein. Lanes 2–9, crude extracts of CM4722 (pol I⁺), CJ229 (Δ pol I, F' 5'–3' exo), CJ278 (Δ pol I), and lex A (Ind[–]) AB2474 (pol I⁺) (75 μ g of protein in each lane) prepared from cells grown with (+) and without (–) the addition of nalidixic acid. Gel electrophoresis and Western transfer were performed as described under "Experimental Procedures" using antibody to Rec A protein.

from crude extracts were electrophoresed to allow visual estimates of Rec A protein induction (Fig. 4, lanes 2–9).

The parent strain CM4722 shows at least a 5-fold increase in Rec A protein after treatment with nalidixic acid (lanes 2 and 3). Strain CJ229 (Δ pol I, F' 5'–3' exonuclease; lanes 4 and 5) closely resembles the parental strain. However, strain CJ278 (Δ pol I), containing a deletion of the entire pol I gene shows no apparent change in Rec A protein levels after nalidixic acid treatment (lanes 6 and 7).

Note that the basal level of Rec A protein (lane 6) appears significantly elevated compared to both the parental strain (lane 2) and to strain CJ229 (lane 4), suggesting partial induction of Rec A protein in strain CJ278. We did not include strain CJ261 (Δ pol I) among the strains tested for induction of Rec A protein (Fig. 4) because it grows poorly in rich medium; however, we have verified that CJ261 resembles CJ278 in the partial induction of Rec A protein when grown in minimal medium (data not shown). The data showing that Rec A protein appears partially induced in the absence of nalidixic acid when the 5'–3' exonuclease is absent suggest a possible role for the polymerase associated 5'–3' exonuclease in lowering the basal level of Rec A protein. Strain AB2474 (lex A Ind[–]) contains the noncleavable lex-1 gene product that prevents SOS induction (18). As expected, AB2474 shows no induction of Rec A protein (Fig. 4, lanes 8 and 9).

Pol X Appears to be Under Lex A Control—A comparison of SOS-induced and uninduced levels of pol III* (Fraction IIIA) and pol X (Fraction IIIB) shows that both enzymes appear to respond to treatment by nalidixic acid, pol III* by a factor of about two and pol X (Fraction IIIB) by a factor of about seven. The 2-fold induction observed for pol III* is consistent with the recent finding that a possible consensus Lex A operator sequence is located slightly upstream from the *dnaE* gene promoter (28). To determine whether pol X (Fraction IIIB) was also controlled by the Lex A repressor, and not just responding to the presence of nalidixic acid, we carried out an enzyme purification through the phosphocellulose chromatography step using as the source of the activity nalidixate-treated strain AB2474, which is noninducible for Rec

TABLE I

A comparison of DNA polymerase activity of pol X purified under both SOS-induced and uninduced conditions

Pol X was purified through Fraction IIIB from cells grown with or without an SOS-inducing treatment (nalidixic acid) and assayed for polymerase activity as described under "Experimental Procedures." Activity is reported as units/g of wet cell mass. The numbers represent the mean \pm S.E. Numbers in parentheses refer to the number of trials.

Strain	DNA polymerase activity	
	–Nalidixic acid	+Nalidixic acid
	units/g	
CJ229	15.5 \pm 8.6 (3)	129.2 \pm 46.0 (4)
Δ pol A/F' 5'–3' exo		
CJ278	17.2 (1)	95.4 (1)
Δ pol A		
AB2474	14.5 (1)	23.7 \pm 6.4 (2)
lex A (Ind [–])		

A protein and other SOS functions.

A comparison of normalized pol X (Fraction IIIB) polymerase units³ from strains of *E. coli* used in this experiment are given in Table I. Measurements on several independent preparations of CJ229 (Δ pol I, F' 5'–3' exonuclease) show an approximate 7-fold increase in the level of pol X (Fraction IIIB) in induced cells. Induced and uninduced levels of pol X (Fraction IIIB) obtained from CJ278 (Δ pol I) do not differ significantly from CJ229. Thus, induction of pol X (Fraction IIIB) cannot be attributed to the presence or absence of the 5'–3' exonuclease fragment of pol I.

When grown in the presence of nalidixic acid, the uninducible lex A (Ind[–]) strain AB2474 contains a level of pol X (Fraction IIIB) that appears comparable to values obtained from uninduced cells. Thus, induction of pol X (Fraction IIIB) by nalidixate treatment likely results from expression of the SOS regulon in treated cells rather than a specific response to the presence of nalidixic acid. Note that elevated levels of Rec A protein are present in the extract of CJ278 (Δ pol I) in the absence of nalidixic acid (Fig. 4, lane 6), yet pol X (Fraction IIIB) activity remains at low, uninduced levels (Table I). It is likely, therefore, that induction of pol X activity requires full induction of the SOS response and does not increase under conditions of suboptimal induction such as during the growth of the pol A deletion strain.

The UmuC gene product is not required for induction of the SOS response but is required for induced mutagenesis (3). Pol X is present in fully induced amounts in a *umuC*[–] strain containing the Rec A730 allele, which constitutively expresses the SOS response (3) (data not shown). This polymerase fraction is also present in strain CJ278 *umuC*[–] (Δ pol I) when grown in the presence of nalidixic acid (data not shown). Thus, if pol X plays a role in error prone repair, its presence at induced levels may be necessary but certainly not sufficient to catalyze bypass at noncoding lesions.

Purification of Pol X to a Single 84-kDa Protein—Pol X (Fraction IIIB) eluting between 250–350 mM NaCl from a phosphocellulose column (Fig. 3) was further purified by gel filtration using a Sephacryl S-200 gel filtration column. DNA polymerase fractions at various stages of purification were separated on a denaturing polyacrylamide gel (Fig. 5). The concentrated polymerase peak (Fraction IV) was found to

³ Enzyme activity was normalized using wet cell weight because of potential differences in protein concentrations comparing SOS-induced to uninduced conditions. However, the same relative values of specific activities for induced versus uninduced polymerase (Table I) were obtained when enzyme activity was normalized to milligrams of protein in Fraction I.

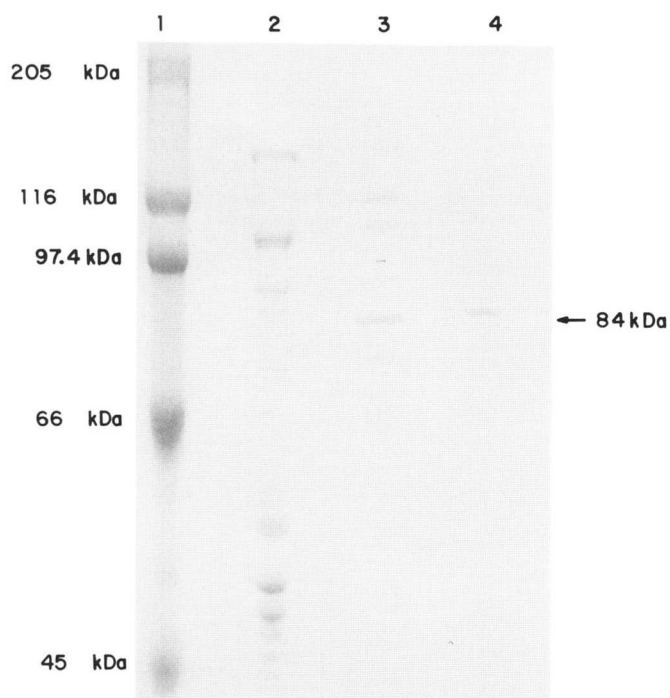


FIG. 5. Purified pol X from induced strain CJ229 contains a major 84-kDa polypeptide. Purification procedures and Coomassie staining of the 11% SDS-polyacrylamide gel are described under "Experimental Procedures." Lane 1, Sigma SDS molecular weight markers. Lane 2, Fraction II. Lane 3, Fraction IIIB. Lane 4, Fraction IV.

contain a single major protein band with a molecular mass of about 84 kDa (Fig. 5, lane 4). The specific activities of pol X Fractions IIIB and IV are 7,000 units/mg and roughly 70,000 units/mg, respectively. Side fractions of polymerase Fraction IV showed a minor band of about 42 kDa (data not shown).

In order to determine whether the 84-kDa protein in Fraction IV preparations contained the polymerase activity, Fraction IV was run on a denaturing polyacrylamide gel and then "renatured" *in situ* (13) to measure polymerase activity on the gel (Fig. 6, lane 1). The activity gel revealed two active polymerase bands. A major band of activity migrates at a molecular mass of about 84 kDa in accordance with the location of the major protein band (Fig. 5, lane 4). A second minor band of activity migrates at a molecular mass of 102 kDa and was not detected in the Coomassie-stained protein gel.

Evidence That Pol X Is Identical to DNA Polymerase II—At least one report indicates that the molecular mass of DNA polymerase II of *E. coli* is 86 kDa (29), a value similar to that of pol X (Fraction IV). In order to determine the relationship, if any, between these activities, we purified DNA polymerase II as described in Ref. 30 and analyzed it on the activity gel as described for pol X (Fraction IV). The major activity band observed for pol II is 84 kDa suggesting that pol X (Fraction IV) and pol II may be the same enzyme(s) (Fig. 6, lane 2). Two different molecular mass values for pol II have been reported in the literature, 86 (29) and 120 kDa (30). Purified DNA polymerase II has similar characteristics as pol X Fractions IIIB and IV (Fig. 2) with respect to nucleotide insertion at the abasic site and extension beyond the lesion (data not shown). At the present time we have not been successful in separating the 102-kDa component from the 84-kDa activity in enzymatically measurable amounts for further study. Fraction IIIB (Fig. 6, lane 3) purified from strain HMS83 (pol A⁻ pol B⁻) contains only the minor 102-kDa band and no detect-

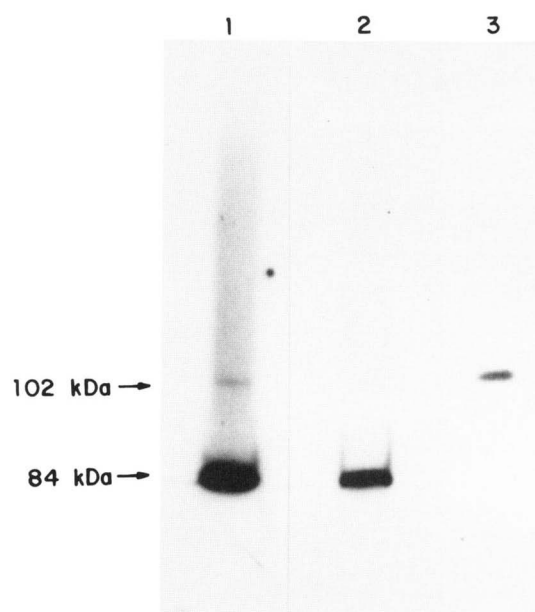


FIG. 6. Renaturation of two enzymatically active polymerase bands in a polyacrylamide gel containing purified pol X (Fraction IV). The protocol for the *in situ* activity gel for polymerases is described under "Experimental Procedures." Lane 1, pol X (from strain CJ229). Lane 2, pol II (from strain CJ229). Lane 3, Fraction IIIB (from strain HMS83). The reduced activity in the polB⁻ mutant (HMS83) necessitated a 4-fold longer exposure of lane 3 as compared to lanes 1 and 2.

able levels of the 84-kDa protein.

Additional evidence that pol X and DNA polymerase II may be the same enzyme follows from an assessment of polymerase activity using the pol II mutant HMS83 (31). We found that under both induced and uninduced conditions for HMS83, the amount of DNA polymerase eluting from the phosphocellulose column between 250–350 mM NaCl (see *e.g.* Fig. 3) was less than 1/4 the level found in uninduced CJ229 (Table I). We conclude that the pol B mutation not only reduces the basal level of pol X (Fraction IIIB) but also blocks the increased expression of this activity following SOS-inducing treatments.

Pol X Interacts with the β Subunit of the Pol III Holoenzyme Complex in the Presence of SSB Protein—A time course showing the extension of primed ssM13 DNA catalyzed by pol X (Fraction IV) and pol III* is shown in Fig. 7. The activity of pol X (lanes 1–4 and lanes 9–12) appears greater than pol III* in the presence of SSB (lanes 17 and 18), since most of the extended primers from pol X reactions (lanes 1–4) migrate on the gel more slowly than their pol III* counterparts at all reaction times.

The addition of β subunit (lanes 5–8) or SSB protein (lanes 9–12) separately to pol X has no discernible effect on activity. However, in the presence of β subunit and SSB, pol X has significantly enhanced activity (lanes 13–16) similar to pol III holoenzyme (pol III* plus β subunit) plus SSB (lanes 19 and 20). Interestingly, in the absence of the β subunit, the addition of SSB results in increased primer degradation for both pol X (lanes 9–12) and pol III* (lanes 17 and 18). We conclude that the β subunit can interact with pol X, possibly in a manner similar to pol III*, to promote a marked increase in the extent of DNA synthesis.

DISCUSSION

SOS-induced error prone repair involves replicative bypass of unrepaired noncoding lesions on DNA template strands.

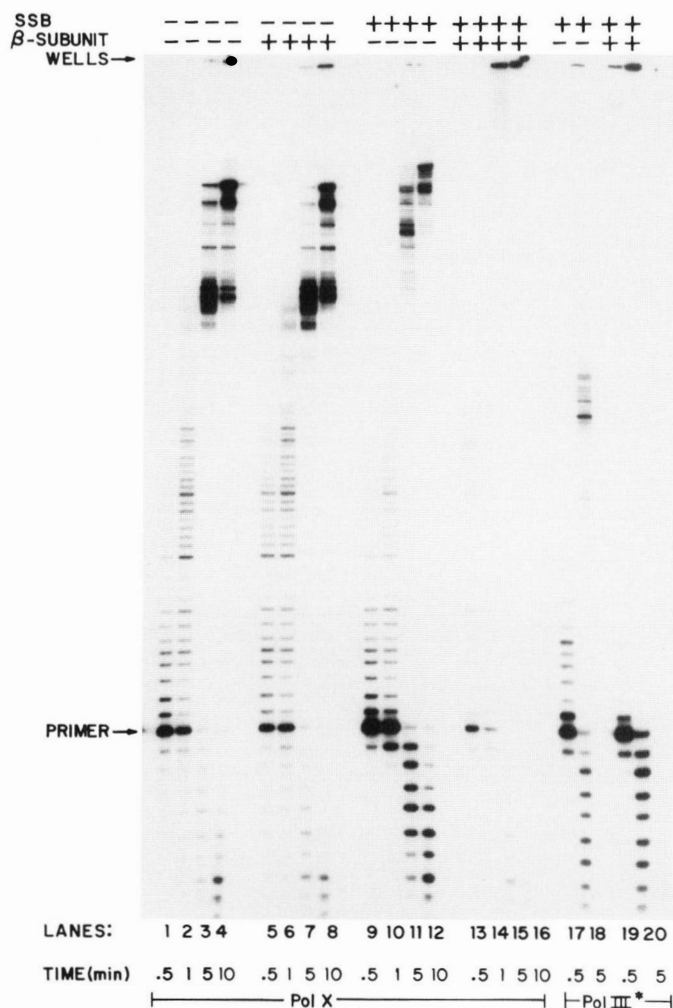


FIG. 7. Enhanced DNA synthesis by pol X in the presence of both SSB protein and β subunit on primed ssM13 template. DNA polymerase reactions were performed as described under "Experimental Procedures" using 4 units of pol X (Fraction IV) (lanes 1–16) or 4 units pol III* (lanes 17–20).

Lesion bypass is thought to occur in two steps. The first step is insertion of a nucleotide base opposite the template lesion, perhaps by an SOS-induced or modified "mutator-like" polymerase having a reduced specificity of base selection. The second step is where the actual bypass occurs; here a nucleotide inserted opposite the lesion serves as a possibly unstable or geometrically aberrant 3'-primer terminus for continued DNA synthesis. It is believed that in addition to DNA replication proteins required for insertion opposite the lesion, the second step in bypass may require direct involvement of the proteins UmuC, UmuD (9), and activated Rec A (32–34).

In this paper, we have asked whether there are DNA polymerase activities elevated in SOS-induced *E. coli*, when compared to uninduced cells, that can insert and bypass at a well-defined abasic template lesion (Fig. 1). We purified a DNA polymerase activity (pol X, Fig. 3), which is present at about 7-fold higher levels in induced compared to uninduced cells (Fig. 3, Table I). The level of pol X appears to be under Lex A repressor control; it does not appear to be induced in a lex A (*Ind*[−]) mutant strain of *E. coli* that is uninducible for an SOS response (Table I).

The induced polymerase activity has been purified extensively. Peak fractions, eluting from a Sephacryl S-200 column, referred to as pol X (Fraction IV), migrate as a single major band on a denaturing polyacrylamide gel (Fig. 5). The molec-

ular mass of pol X is about 84 kDa and has an estimated purity greater than 90%.

The insertion and extension activity of either pol X Fractions IIIB or IV at the abasic site is markedly different from DNA polymerase III. Pol X inserts dAMP with relatively high efficiency at the abasic site and also extends, albeit at a much lower efficiency, beyond the lesion (Fig. 2). In contrast, none of the forms of the complex comprising DNA polymerase III-holoenzyme, core, pol III* (holoenzyme minus the β subunit) are capable of significant insertion or extension at the abasic lesion. We estimate that pol X has at least a hundred-fold higher dAMP insertion efficiency at the abasic site than pol III.

Several lines of evidence lead us to suggest that pol X is identical to DNA polymerase II. Polyacrylamide gel electrophoresis of pol X (Fraction IV), under denaturing conditions, reveals a major band at a molecular mass of 84 kDa (Fig. 5). A measurement of polymerase activity of pol X (Fraction IV) on a polyacrylamide gel shows a major peak of activity at 84 kDa and a significantly smaller peak at 102 kDa (Fig. 6). Molecular masses of DNA polymerase II were reported by one group to be 86 kDa (29) and by another to be 120 kDa (30). The 86-kDa mass is in approximate agreement with the 84 kDa activity band detected in our renaturation gels.

We purified pol II according to a published procedure (30) and found a peak of activity in polyacrylamide gels after renaturation which comigrated with the 84-kDa pol X activity band (Fig. 6) and was able to insert and extend at the abasic site. Fraction IIIB was also purified from the pol B mutant strain, HMS83 (31). In this mutant, the basal level of Fraction IIIB was reduced 5- to 10-fold below basal levels measured in pol B⁺ strains, grown in the absence of SOS-inducing treatments (Table I). As judged by polyacrylamide activity gels, partially purified polymerase from strain HMS83 lacked the band of activity at 84 kDa but retained the minor band at 102 kDa.

Pol X exhibits slightly higher activity on primed M13 DNA template than pol III* (Fig. 7). However, we have demonstrated that, as seen with pol III*, the addition of the β subunit and SSB protein converts pol X into a more active enzyme able to synthesize full length M13 DNA chains perhaps as efficiently as DNA polymerase III holoenzyme complex (Fig. 7).

It has been suggested that pol II is the same as pol III' (pol III core plus the τ subunit) (35). Using Western immunoblotting techniques we investigated whether pol X could cross-react with a polyclonal antibody prepared against pol III holoenzyme or a monoclonal antibody prepared against the α subunit of pol III, containing the polymerase catalytic subunit. No cross-reactivity was detected using either pol X Fractions IIIB or IV. Control experiments using pol III* (Fraction IIIA, Fig. 3) showed the expected result that Fraction IIIA had the same subunit composition as pol III* (data not shown). We conclude that while it is highly unlikely that pol X is part of the pol III holoenzyme complex, it can still interact specifically with β subunit in the presence of SSB protein to enhance DNA synthesis.

DNA polymerase II was originally isolated and purified from DNA polymerase I defective pol A mutants (36) just prior to the discovery that DNA polymerase III was the enzyme required for DNA replication (37). Since that time, the role of pol II in DNA replication or repair has not been determined. Pol X was detected by specifically seeking an enzyme that could insert and synthesize past an abasic template site and which appeared more active under SOS-induced conditions. We have presented evidence strongly suggesting

that pol X is identical to DNA polymerase II. If this supposition is correct, then the observation that this polymerase activity is induced 7-fold in *lex A*⁺ but not *lex A* (*Ind*⁻) cells may be an important clue that its role could be central to SOS-induced error prone repair in *E. coli*.

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