

Lesion Bypass Activity of DNA Polymerase A from the Extremely Radioresistant Organism *Deinococcus radiodurans**

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The bacterium *Deinococcus radiodurans* survives extremely high exposure to ionizing radiation and extended periods of desiccation. Radiation at the survival doses is known to cause numerous DNA damage, such as hundreds of double strand breaks and single strand breaks, as well as damage of the nucleobases. The mechanisms of *D. radiodurans* to survive the depicted threats are still only beginning to be understood. DNA polymerase A (PolA) has been shown to be crucially involved in irradiation resistance mechanisms of *D. radiodurans*. We expressed and characterized the DNA polymerase domain of PolA for the first time *in vitro*. The obtained enzyme is able to efficiently catalyze DNA-dependent DNA synthesis requiring Mg(II) as divalent metal ion. Additionally, strand displacement synthesis of the DNA polymerase, which is required in several repair processes, could be detected. We further found that DNA polymerase function of PolA is modulated by the presence of Mn(II). Whereas proceeding DNA synthesis of PolA was blocked by certain DNA damage that occurs through radiation of DNA, bypass was facilitated by Mn(II). Our results suggest an enzyme modulator function of Mn(II). These observations parallel reports that *D. radiodurans* accumulates intracellular Mn(II) in cases of irradiation and that the level of irradiation protection correlates with Mn(II) concentrations.

The bacterium *Deinococcus radiodurans* is an organism that survives extremely high exposure to ionizing radiation and extended periods of desiccation (1–5). *D. radiodurans* is able to grow without mutation formation at radiation doses more than 250 times higher than that used to kill *Escherichia coli* or are lethal to humans. Irradiation at these doses cause numerous DNA damage, such as hundreds of double strand breaks and single strand breaks as well as other DNA damage (1–5). DNA double strand breaks in these numbers result in breakage of the *D. radiodurans* genome into hundreds of short DNA fragments. However, the genome is repaired accurately before initiation of the next cycle of cell division. Albeit *D. radiodurans* was discovered 50 years ago (6), the underlying mechanisms of its strategies to survive the depicted threats are still only sparsely understood.

One of the striking features of *D. radiodurans* is its intracellular accumulation of Mn(II) in cases of irradiation (7). Interestingly, the level of irradiation protection decreased with lower Mn(II) concentrations. However, the level of DNA double strand breaks seem to be unaffected by the Mn(II) concentration. Thus other mechanisms rather than direct double strand protection from irradiation damage have been suggested to be at work (7–10).

DNA polymerases catalyze the entire DNA synthesis in DNA repair, recombination, and replication, and thus are suggested to be involved in genome repair and rearrangement of *D. radiodurans* (4, 5). Sequence analysis of the *D. radiodurans* genome indicates the presence of three DNA polymerases (11, 12). PolX, a DNA polymerase of the X family, has recently been purified and thoroughly characterized (13, 14). It was found that PolX is stimulated by Mn(II) and plays an important role in double strand repair in *D. radiodurans*. Besides its capability to catalyze DNA synthesis PolX harbors a structure-modulated 3' → 5'-exonuclease activity. The PolA² gene was identified and it has been shown that PolA is necessary for DNA damage resistance of *D. radiodurans* (15, 16). Interestingly, PolA has a 35% sequence identity (49% similarity) with *E. coli* DNA polymerase I, which might indicate similar functions of both DNA polymerases. Most recently, it was found that *D. radiodurans* PolA is crucial for the reassembly of DNA fragments into intact circular chromosomes by a process termed extended synthesis-dependent strand annealing involving significant synthesis of new DNA (17).

Due to the vitally important role of PolA in irradiation resistance of *D. radiodurans* we became interested in this enzyme. For the first time PolA was expressed, purified, and thoroughly characterized. We found that PolA is active in the presence of Mg(II). However, when investigating the DNA polymerases response to DNA damage, it was found that certain DNA lesions that occur through irradiation of DNA block the enzyme. We further found that the enzyme tolerates the presence of Mn(II). Moreover, in the presence of Mn(II) the enzyme is able to catalyze DNA bypass synthesis of the lesions that block the enzyme in the absence of Mn(II). Our results suggest an enzyme modulator function of Mn(II), a role of Mn(II) that was so far not anticipated in the mechanisms that provide *D. radiodurans* with its high irradiation resistance.

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² The abbreviations used are: PolA, DNA polymerase A; nt, nucleotide(s); ESDSA, extended synthesis-dependent strand annealing.

EXPERIMENTAL PROCEDURES

Strain—Dried *D. radiodurans* R1 type strain was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (ATCC number 13939) and cultured as described by the supplier.

Cloning, Protein Expression, and Purification—Genomic DNA was isolated and the DNA polymerase domain of *D. radiodurans* DNA polymerase A (PolA*) amplified by using Phusion DNA polymerase (New England Biolabs) with the upstream primer (containing His₆ tag): 5'-CGCATCATGCC-ATGGGCAGCAGCCATCATCATCATCACAGCAGCGAACGCGAACACGCGCAGACCCCGAGGAG-3' and downstream primer, 5'-CGGGAATTCATATGTCACTT-CGTGTCAAACCAGTTCGGCCCCGTCCC-3' (amino acid 337–956). PolA* was obtained by cloning the gene into the NcoI and NdeI sites of pET15b (Novagene). Constructs were transformed into *E. coli* BL21 Rosetta pLysS cells (Stratagene) and expressed with 1 mM isopropyl 1-thio- β -D-galactopyranoside at $A_{600} = 0.5$ for 3 h. Harvested cells were lysed with 2 mM lysozyme (Roth) and His-tagged PolA* was purified by using nickel-nitrilotriacetic acid-agarose (Qiagen). The eluted PolA* fraction was concentrated via Amicon (molecular mass cut off 50 kDa) and protein concentration was detected with SDS-PAGE by comparison with bovine serum albumin.

Primer Extension Assays—A 33-nt template 5'-d(AAATCAACCTATCCTCCTTCAGGACCAACGTAC)-3' was primed with a 5'-³²P-end-labeled 20-nt 5'-d(CGTTGGTCTGAAGGAGGAT)-3'. The reaction mixture (30 μ l) contained 50 mM Tris (pH 8.8), 1 mM dithiothreitol, 0.05% Triton X-100, 10 mM MgCl₂, 10 μ M dNTPs, 150 nM primer-template, and 0 or 1 mM MnCl₂. Reactions were initiated by adding PolA* and incubated at 30 °C for 10 min. All DNA polymerase concentrations are given in the figure legends. The reactions were terminated with 3 volumes of 80% formamide, 20 mM EDTA, and 0.1% bromphenol blue. Products were analyzed by electrophoresis in a 12% denaturing polyacrylamide gel. Oligonucleotides for investigation of lesion bypass were obtained from Purimex (Grebenstein, Germany). Sequences were: abasic site template, 5'-d(AAATCAaCCTATCCTCCTTCAGGACCAACGTAC)-3', 23-nt oligonucleotide for standing start conditions, 5'-d(CGTTGGTCTGAAGGAGGATAGG)-3'; oxoA/oxoG template, 5'-d(AAATQAACCTATCCTCCTTCAGGACCAACGTAC)-3', and 25-nt oligonucleotide for standing start experiments, 5'-d(CGTTGTCTCCTGAAGGAGGATAGGT)-3'.

DNA Polymerase Thermostability and Metal Ion Dependence—For testing the thermostability of PolA* primer extension assays were performed as described above. Samples were incubated 10 min with 1 nM PolA* at increasing reaction temperatures ranging from 30 to 60 °C. To test the metal ion dependence of PolA* primer extension was done as described above using the same buffer without divalent metal ion. Metal ions (MgCl₂, MnCl₂, or a mixture of MgCl₂ and MnCl₂, respectively) were added to the reaction mixture in concentrations ranging from 10 μ M to 20 mM. Reactions were started with 0.5 nM PolA* and incubated for 10 min at 30 °C. Reactions were terminated with 3 volumes of 80% formamide, 20 mM

EDTA, and 0.1% bromphenol blue and analyzed by 12% denaturing PAGE. Data were quantified by phosphorimager analysis (Molecular Imager FX, Bio-Rad). Activity was measured by quantifying the intensity of each band. From this quantification the amount of incorporated nucleotides was calculated. The total amount of incorporated nucleotides for each reaction equals the sum of incorporated nucleotide of each band. All reactions were done in triplicates.

Steady State Kinetics—DNA substrates were prepared by hybridizing the 5'-³²P-end-labeled 20-nt primer to a 33-nt template. Reactions (30 μ l) comprised the same buffer as described above and contained 150 nM primer-template complex. Reactions were incubated (with unmodified templates, 1 nM PolA*; with templates containing lesions, 10 nM PolA*) at 30 °C for 10 min and were conducted in the presence of one respective dNTP. At least eight different concentrations were chosen to result in product conversion between 1 and 20% to ensure single completed hit conditions as reported (24). All reactions were done in triplicate and terminated with 3 volumes of 80% formamide, 20 mM EDTA, and 0.1% bromphenol blue and analyzed by 12% denaturing PAGE. Data were quantified by phosphorimager analysis (Molecular Imager FX). The kinetic constants were derived from linear Eadie-Hofstee plots (product formation/dNTP concentration *versus* dNTP concentration) with linear regression using Microsoft Excel as described (24).

Strand Displacement Assay—A 90-nt template, 5'-d(CCGT-CAGCTGTGCCGTCGCGCAGCACGCGCCGCCGTGGAC-AGAGGACTGCAGAAAATCAACCTATCCTCCTTCAGGACCAACGTACAGAG)-3', was primed with a 5'-³²P-end-labeled 16-nt oligonucleotide 5'-d(CTCTGTACGTTGGTCC)-3' as primer and an additional 24-nt oligonucleotide, 5'-d(GGATAGGTTGATTTTCTGCAGTCC)-3' (binding region in the 90-nt template is underlined) to test strand displacement. The reaction mixture (30 μ l) contained 50 mM Tris (pH 8.8), 1 mM dithiothreitol, 0.05% Triton X-100, 10 mM MgCl₂, 10 μ M dNTPs, 150 nM primer-template and MnCl₂, 0 or 1 mM. Reactions were initiated by adding polA* and incubated at 30 °C for 10 min. The reactions were determined with 3 volumes of 80% formamide, 20 mM EDTA, and 0.1% bromphenol blue. Products were analyzed by electrophoresis in a 12% denaturing polyacrylamide gel.

RESULTS

Cloning and Expression—From sequence analysis and comparison with *E. coli* DNA polymerase I it was suggested that the gene of *D. radiodurans* PolA (gene ID DR1707) harbors both a 5' \rightarrow 3' exonuclease and a DNA polymerase domain (11, 12). To clone and express the DNA polymerase domain of *D. radiodurans* PolA (amino acid 337–956) we used genomic DNA that was derived from the *D. radiodurans* R1 type strain. The restricted *polA* gene (*polA**) was PCR amplified and cloned into an *E. coli* expression vector containing an N-terminal His₆ tag. Protein expression was performed in BL21 Rosetta cells. Purification led to a clean protein fraction containing the anticipated DNA polymerase domain henceforth termed PolA* (Fig. 1A).

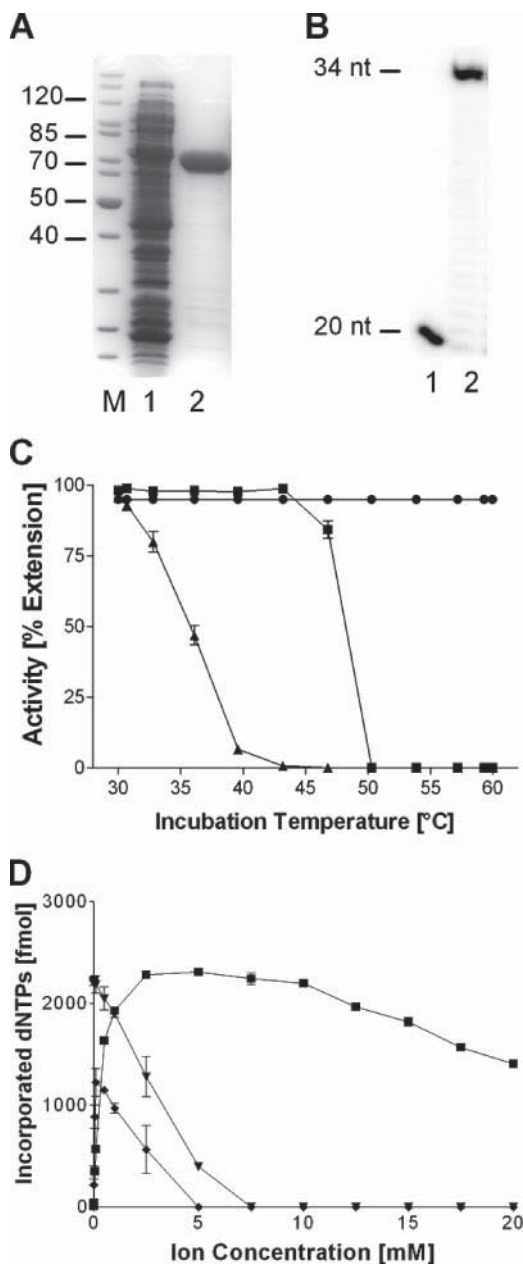


FIGURE 1. Expression and activity of PolA*. A, SDS-PAGE analysis of PolA* expression in *E. coli* BL21 Rosetta (lane 1) and the elution fraction by nickel-nitrilotriacetic acid chromatography (lane 2). B, phosphorimage of primer extension assay without polA* (lane 1) and with 10 nM PolA* (lane 2) after 10 min incubation at 30 °C. C, graphical representation of the polymerase activity at different incubation temperatures for Klenoq (triangles), *D. radiodurans* PolA* (squares), and Klenow fragment of *E. coli* DNA polymerase I (circles) ($n = 3$). D, graphical representation of the polymerase activity dependence on metal ion concentration of Mg(II) (squares), Mn(II) (diamonds), or a combination of 10 mM Mg(II) and various Mn(II) concentrations (triangles) in the reaction buffer. The presented data derives from averages of at least three independently repeated experiments.

PolA Is a DNA-dependent DNA Polymerase*—To access whether the cloned protein has indeed DNA polymerase activity we employed primer extension assays comprising a 5'-³²P-labeled oligonucleotide as primer annealed to a 33-nt template. These assay formats have been used numerous times in investigation of DNA polymerase properties such as selectivity and substrate spectra (18, 19). The reactions were analyzed by dena-

turing PAGE and phosphorimaging. We found that PolA* catalyzes proceeding DNA synthesis employing a DNA template in the presence of dNTPs (Fig. 1B). Like all known DNA polymerases, PolA* activity is dependent on the presence of divalent metal ions (20, 21). Thus, in the absence of Mg(II) no activity was observed (data not shown). Next, we investigated the nucleic acid substrate spectra of PolA*. We found that all combinations such as a DNA-template with rNTPs or an RNA-template with either dNTPs or rNTPs failed to result in any significant nucleotide incorporation (data not shown).

Due to its high sequence homology to *Thermus aquaticus* DNA polymerase and the tight relations of the *Deinococcae* to the *Thermus* family (1–5), thermostability of PolA* activity was investigated next. To compare PolA* with the large fragment of *T. aquaticus* DNA polymerase (Klenoq) and the Klenow fragment of *E. coli* DNA polymerase I (22, 23), we comparatively investigated the thermostability of these enzymes. The respective enzymes were incubated for 15 min at various temperatures. Subsequently their activity was measured in a primer extension assay. It was found that PolA* remains active until about 50 °C, whereas the Klenow fragment of *E. coli* DNA polymerase I was found to be inactivated already at 40 °C. However, *D. radiodurans* PolA* is not a thermophilic enzyme because its activity significantly drops at 50 °C (Fig. 1C).

As mentioned, an increased Mn(II) concentration is an important factor in the radioresistance of *D. radiodurans* (7, 8). Thus, we next investigated the response of PolA* to Mn(II). Reactions were conducted with increasing Mn(II) concentrations (0–10 mM) and keeping Mg(II) constant at 10 mM. We find that up to 1 mM Mn(II) the polymerase activity of PolA* is retained (Fig. 1D). At higher Mn(II) concentrations the activity is decreased and over 7.5 mM totally lost. On the other hand, Mn(II) has no significant effect on the nucleic acid substrate spectrum, no broader tolerance to RNA templates or rNTP incorporation was observed (data not shown). Taken together, these results show that PolA* is a DNA-dependent DNA polymerase.

PolA Selectivity*—To examine the fidelity of PolA* for Watson-Crick base pair formation we performed primer extension studies. First we investigated single nucleotide incorporation opposite the canonical, as well as opposite the non-canonical, nucleotide in the template strand. A 20-nt primer-33-nt template duplex containing one of the four nucleobases at the position directly after the 3' terminus was employed for these investigations (Fig. 2). Incorporation was monitored by use of radiolabeled primer in the presence of one of the four dNTPs. We found that the primer was extended efficiently only in cases when nucleobase pairs according to the Watson-Crick rule were formed. To quantify the observed effects we investigated the efficiencies of nucleotide incorporation and mismatch formation under steady-state and single completed hit conditions as described (24). The data indicates that the selectivity within the investigated sequence context is somewhat sequence dependent and varies between 1:10,000 and 1:100 (Table 1).

Because it is known that Mn(II) may affect properties of DNA polymerases (25–28), we next investigated the impact of Mn(II) at 1 mM in the presence of 10 mM Mg(II) on the selectivity of PolA* (Fig. 2C, Table 1). In general, our results indicate that

PolA* selectivity is decreased because nucleotide misinsertion efficiency is increased compared with the results obtained in the absence of Mn(II), whereas incorporation efficiency of canonical substrates are affected only to a minor extent.

DNA Strand Displacement—Recent investigations suggest the involvement of PolA in extended synthesis-dependent strand annealing (17). This process involves the ability of the enzyme to displace a DNA strand annealed to the template strand to proceed with DNA synthesis. To access the ability of *D. radiodurans* PolA* to catalyze strand displacement we set up an assay comprising a radioactively labeled 16-nt primer that is annealed to the complementary site of the 90-nt template (see “Experimental Procedures”). Leaving a short 7-nt gap a second 24-nt oligonucleotide anneals to the complementary template site. This construct was incubated in the presence of all four dNTPs and PolA* in increasing concentrations. If PolA* is

devoid of strand displacement activity primer extension should abort after incorporation of seven nucleotides. On the other side, strand displacement activity should allow the enzyme to synthesize longer reaction products. We found that at low enzyme concentrations the enzyme significantly pauses after incorporating seven nucleotides (Fig. 3, lanes 2). However, when increasing the enzyme concentration nearly to equality to the primer-template complex concentration or prolonging the incubation time strand displacement activity was clearly detectable (Fig. 3, A and B). Similar results were obtained in the presence of Mn(II) indicating no significant effect of this metal ion on strand displacement activity.

Lesion Bypass Synthesis—Radiation of DNA causes several DNA lesions, such as damage to the nucleobases and sugar backbone (29, 30). These lesions have been found to impose severe impediments to the replication machinery if not repaired efficiently (31–33). To further characterize PolA* the sensitivity of the enzyme to DNA lesions was investigated. For these experiments we used templates containing site-specifically introduced frequently occurring DNA lesions like an abasic site, 8-oxo-adenine (oxoA) and -guanine (oxoG), respectively (29, 30). The investigations were performed under “running start” conditions where several undamaged nucleotides in the template have to be replicated before the DNA polymerase encounters the respective damaged site. First we investigated bypass synthesis of an abasic site analogue. In the absence of Mn(II) and in the exclusive presence of Mg(II), PolA* arrests prior to DNA damage (Fig. 4A). Only at increased enzyme concentrations was insertion of a nucleotide opposite the abasic site analogue observed without any significant further extension despite the presence of all four dNTPs. Single nucleotide incorporation studies indicate that dA is predominantly incorporated opposite the lesion (Fig. 5A and Table 2).

Because it is known for other DNA polymerases that Mn(II) has significant effects on DNA polymerase catalysis the same reactions were conducted in the presence of Mn(II). We found that even at the lowest enzyme concentration used nucleotide insertion opposite the lesion is observed indicating that PolA* is

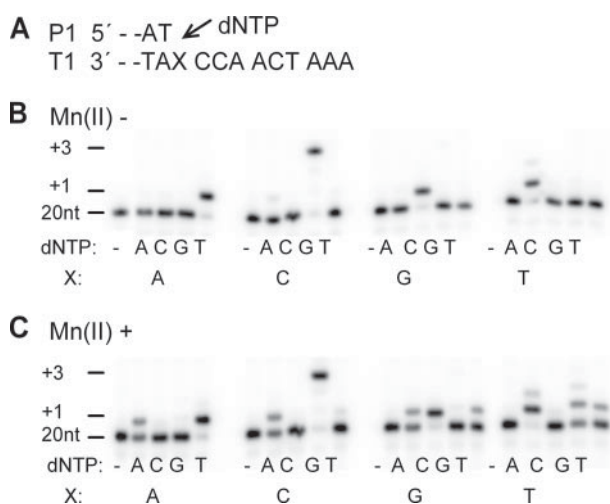


FIGURE 2. Nucleotide incorporation by *D. radiodurans* PolA*. A, partial sequence of employed primer-template complex used for single-nucleotide incorporation studies. B, single-nucleotide incorporation in all possible combinations of template and incorporated nucleotide without Mn(II). For all reactions equal concentrations of buffer, primer-template complex, dNTPs, and 1 nM DNA polymerase were employed. C, as described B but in the presence of 1 mM Mn(II).

TABLE 1
Kinetic parameter of match and mismatch incorporation

The presented data derives from averages of at least three independently repeated experiments.

DNA	Mn(II)	dATP				dGTP			
		K_M	k_{cat}	Efficiency	Selectivity	K_M	k_{cat}	Efficiency	Selectivity
		μM	$\times 10^{-3} min^{-1}$	$\times 10^{-3}$		μM	$\times 10^{-3} min^{-1}$	$\times 10^{-3}$	
A	–	60.9 ± 8.4	23 ± 4	0.4	0.0002	6.14 ± 0.3	24 ± 3	3.9	0.0022
	+	15.6 ± 4	61 ± 8	3.9	0.0036	13.1 ± 1.3	29 ± 1	2.2	0.0021
G	–	101 ± 6.6	74 ± 6	0.7	0.0006	120 ± 14.5	21.3 ± 2	0.18	0.0001
	+	0.61 ± 0.08	58 ± 7	96	0.07	46.9 ± 3.7	32.5 ± 2	0.74	0.0005
C	–	58.9 ± 15	65 ± 10	1.1	0.0005	0.01 ± 0.005	31.7 ± 4	2317	1
	+	0.60 ± 0.06	47 ± 4	73	0.025	0.01 ± 0.002	29.3 ± 1	2047	1
T	–	0.04 ± 0.01	44 ± 6	1167	1	7.92 ± 0.5	22.5 ± 1	2.8	0.0024
	+	0.04 ± 0.01	46 ± 10	1149	1	1.58 ± 0.3	19 ± 1	12	0.01
		dCTP				dTTP			
A	–	50.4 ± 11	32 ± 7	0.6	0.0003	0.02 ± 0.001	27 ± 1	1805	1
	+	13.5 ± 1.4	23 ± 1	1.7	0.0016	0.04 ± 0.01	42 ± 3	1077	1
G	–	0.03 ± 0.01	41 ± 8	1265	1	1.06 ± 0.15	8 ± 1	7.8	0.0062
	+	0.03 ± 0.01	39 ± 7	1398	1	1.04 ± 0.07	51 ± 2	49	0.035
C	–	2700 ± 471	43 ± 6	0.02	0.00	165 ± 9.8	40 ± 2	0.3	0.0001
	+	90.1 ± 11	19 ± 1	0.2	0.0001	0.01 ± 0.003	9 ± 1	114	0.056
T	–	12.3 ± 2	33 ± 4	2.7	0.0023	6.38 ± 1	14 ± 1	2.2	0.002
	+	1.09 ± 0.1	24 ± 2	22	0.019	1.69 ± 0.3	59 ± 2	35	0.031

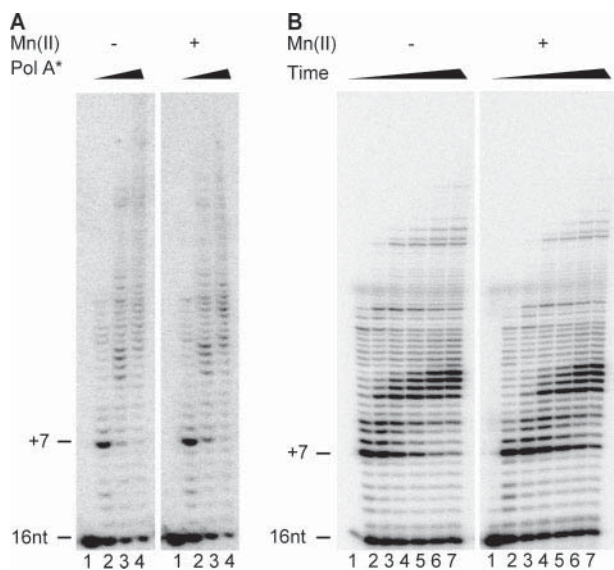


FIGURE 3. Strand displacement experiment catalyzed by *D. radiodurans* PolA*. *A*, increasing concentrations of PolA* (lanes 1, 0 nM; lanes 2, 1 nM; lanes 3, 10 nM; lanes 4, 100 nM) were incubated 10 min at 30 °C without Mn(II) or with 1 mM Mn(II) as indicated. *B*, time dependence of strand displacement with 1 nM PolA* (lane 1, 0 min; lane 2, 5 min; lane 3, 10 min; lane 4, 20 min; lane 5, 30 min; lane 6, 45 min; lane 7, 60 min). For all reactions equal concentrations of buffer, primer-template complex, and dNTPs were employed.

more efficient in processing lesions in the presence of Mn(II). However, further extension is significantly hampered. Single-nucleotide incorporation studies confirm that the efficiency of nucleotide insertion is increased in the presence of Mn(II) in general. Predominantly incorporated opposite to the abasic site is dA and with a 5-fold lower efficiency dG (Table 2).

Next we investigated the ability of PolA* to bypass oxoA and oxoG lesions (Fig. 4, *B* and *C*). In the absence of Mn(II) nucleotide insertion significantly pauses prior to the lesions at low enzyme concentration and opposite to the lesion when incubated at higher enzyme concentrations. Single nucleotide incorporation experiments indicate that opposite to the oxoA-lesion mainly dT and opposite to the oxoG-lesion mainly dC are incorporated. However, in the presence of Mn(II) at higher enzyme concentrations even synthesis past the lesions is observed (Fig. 5, *B* and *C*). Here again addition of Mn(II) increased lesion bypass synthesis significantly. Taken together, the ability of PolA* to deal with DNA template lesions as they occur from radiation of DNA is significantly increased in the presence of Mn(II).

DISCUSSION

PolA has been shown in the past to be an essential enzyme for the extreme radioresistance of *D. radiodurans*. For instance it was found that a *D. radiodurans* strain that has mutations in the *polA* gene loses the ability to polymerize DNA accompanied by a loss of radioresistance (15, 16). Transformation of an intact gene from wild-type *D. radiodurans* to the mutant strain restored both DNA polymerase activity and DNA damage resistance. More recently it was suggested that PolA was involved in a mechanism of *D. radiodurans* to reassemble hundreds of DNA fragments into intact circular chromosomes (17). In a PolA-dependent process termed extended synthesis-de-

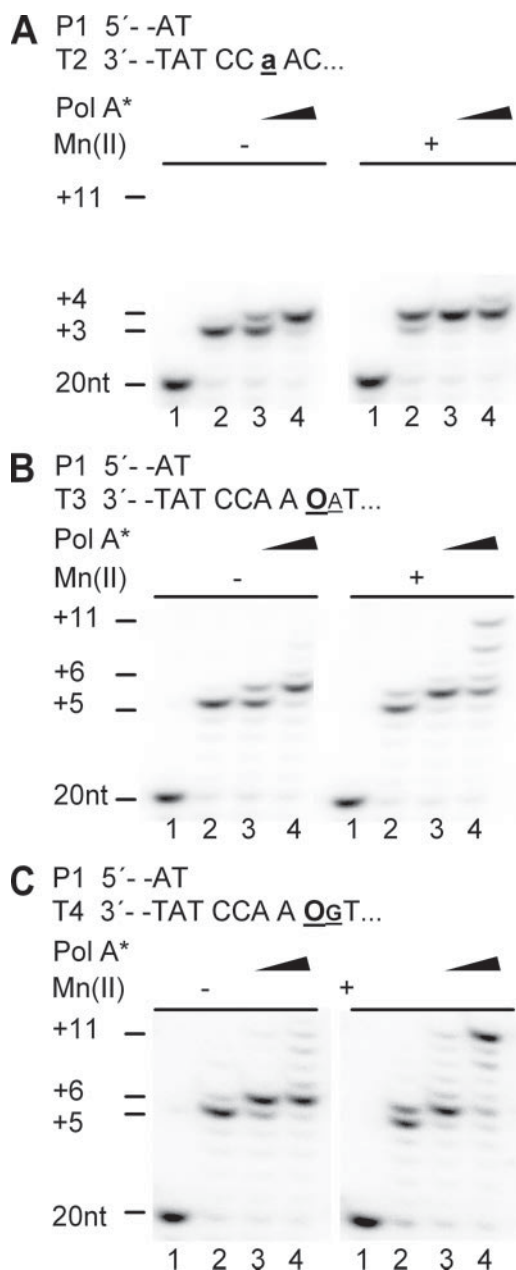


FIGURE 4. Lesion bypass synthesis. Primer extension assay using template strands containing an abasic site (*A*), oxoA (*B*), or oxoG (*C*) DNA lesion, respectively. Partial primer-template sequences are depicted on the top of each set of experiments. Reactions were conducted under running start conditions using increasing PolA* concentrations (lanes 1, 0 nM; lanes 2, 1 nM; lanes 3, 10 nM; lanes 4, 100 nM) and all four dNTPs. Reactions were performed in the absence or presence of 1 mM Mn(II) as indicated. For all reactions equal concentrations of buffer, primer-template complex, and dNTPs were employed.

pendent strand annealing (ESDSA) the enzyme contributes to DNA fragment reassembly. During the progression of ESDSA the enzyme is involved in extensive catalysis of DNA synthesis. Strikingly, an investigated inactive PolA mutant is deficient in ESDSA repair. Taken together, these findings suggest that PolA is heavily involved in DNA synthesis during repair processes that contribute to the reassembly of the scattered genome after irradiation. However, besides damaging the DNA backbone leading to DNA single and double strand breaks, irradiation also affects the integrity of the nucleobases (29, 30). Oxidation

as a consequence of attack by reactive oxygen species, such as hydroxyl radicals generated by γ -irradiation, leads to alterations of the nucleobase structure or even loss of the nucleobase. These lesions are mutagenic in other species and mostly block proceeding DNA synthesis by replicative DNA polymerases (31–33). Thus, these lesions either have to be repaired prior to DNA replication by the DNA repair machinery or being bypassed by DNA polymerases (31–33). Many species like *E. coli* comprise several specialized DNA polymerases that are competent in bypassing lesions that block replicative DNA po-

lymerases. These enzymes seem to work in tight coordination with the DNA replication machinery and have been shown to be crucial for the survival of the respective organism. The genome of *D. radiodurans* has been fully sequenced (11, 12). However, no ortholog lesion-bypass DNA polymerases such as umuC and umuD present in *E. coli* could be annotated in *D. radiodurans* by sequence analysis (11, 12). Thus, it remains to be elucidated how *D. radiodurans* DNA polymerases deal with lesions when replicating damaged DNA template strands.

We have cloned and expressed for the first time the DNA polymerase domain of *D. radiodurans* PolA termed PolA*. The protein was thoroughly characterized and it was found that the purified enzyme is indeed a DNA polymerase that does not require accessory factors to be significantly active. As it is found for all known DNA polymerases the DNA polymerase activity of PolA* is dependent on the presence of divalent metal ions such as Mg(II) (20, 21). PolA* remains active in the presence of Mn(II) until the concentration of Mn(II) reaches a certain level. Thus, PolA* significantly contrasts *D. radiodurans* PolX that has DNA synthesis activity exclusively in the presence on Mn(II) (13, 14). To address the response of PolA* to DNA damage we investigated the ability of PolA* to bypass some DNA lesions that occur through irradiation. For this purpose DNA template strands were employed that contained site-specifically introduced DNA lesions. It was found that all of the investigated lesions block proceeding DNA synthesis. However, in the presence of Mn(II) the capability of PolA* to bypass lesions was significantly increased. This feature of the enzyme is accompanied by some loss of DNA polymerase selectivity. The influence of Mn(II) ions on the properties of DNA polymerases has been extensively studied before and similar features as the discovered here have been described (25–28, 34–36). Nevertheless, it was recently reported that *D. radiodurans* accumulates very high concentrations of intracellular Mn(II) compared with other radiosensitive bacteria (7). Interestingly, resistance to radiation was found to be a concentration-dependent response to Mn(II). Because *D. radiodurans* and *E. coli* exhibit a similar degree of DNA double strand breaks after irradiation with the same dose, it is unlikely that Mn(II) directly protects DNA during irradiation itself (7). It was suggested that Mn(II)

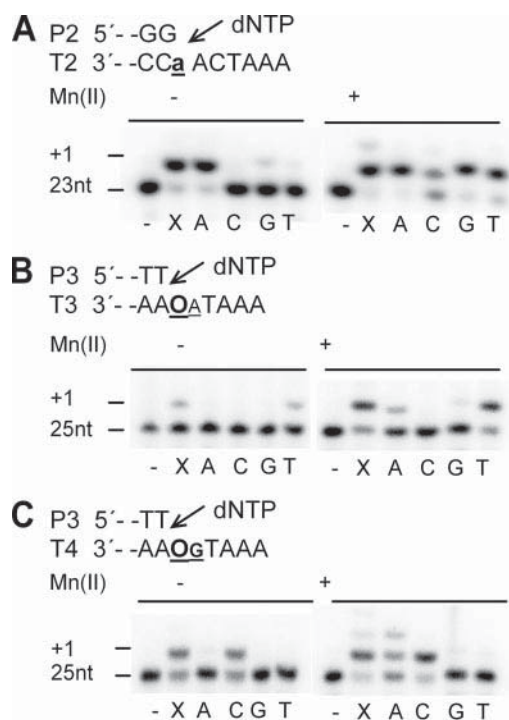


FIGURE 5. **Nucleotide incorporation opposite the damaged site.** Primer extension assay using template strands containing an abasic site (A), oxoA (B), or oxoG (C) DNA lesion, respectively. Partial primer template sequences are depicted on the top of each set of experiments. Reactions contained either all four dNTPs (X) or dATP, dCTP, dGTP, and dTTP, as indicated. Reactions were conducted in the absence or presence of 1 mM Mn(II) as indicated. For all reactions equal concentrations of buffer, primer-template complex, dNTPs, and 10 nM polymerase were employed.

TABLE 2
Single nucleotide incorporation kinetics opposite to DNA lesions (abasic site (AP), oxoA, and oxoG)

The presented data derives from averages of at least three independently repeated experiments.

DNA	Mn(II)	K_M			k_{cat}			Efficiency		
		μM	$\times 10^{-3} min^{-1}$	$\times 10^{-3}$	μM	$\times 10^{-3} min^{-1}$	$\times 10^{-3}$	μM	$\times 10^{-3} min^{-1}$	$\times 10^{-3}$
		dATP			dGTP					
AP	-	3.72 ± 0.02	3.0 ± 0	8.07	132 ± 7.5	4.6 ± 0.2	0.35			
	+	0.37 ± 0.02	9.5 ± 0.5	259	0.25 ± 0.03	1.2 ± 0.1	48			
A _{oxo}	-	63.3 ± 2.9	1.9 ± 0.1	0.3	49.3 ± 1.6	1.9 ± 0.1	0.39			
	+	1.52 ± 0	2.9 ± 0.02	19.1	4.60 ± 0.3	2.2 ± 0	4.8			
G _{oxo}	-	31.9 ± 1.7	4.8 ± 0.3	1.5	NA ^a	NA	NA			
	+	2.09 ± 0.3	5.8 ± 0.1	27.6	NA	NA	NA			
		dCTP			dTTP					
AP	-	1127 ± 91	3.4 ± 0.1	0.03	355 ± 30	6.5 ± 0.4	0.18			
	+	4.97 ± 0.8	2.3 ± 0.4	4.6	10.7 ± 1.2	6.7 ± 0.1	6.3			
A _{oxo}	-	NA	NA	NA	3.24 ± 0.1	3.3 ± 0.4	10.2			
	+	NA	NA	NA	0.44 ± 0.01	2.3 ± 0.2	52			
G _{oxo}	-	3.60 ± 0.1	0.6 ± 0.1	59	NA	NA	NA			
	+	4.90 ± 0.03	0.2 ± 0.03	241	NA	NA	NA			

^a NA, not accessible.

participates in the removal of reactive oxygen species that result from irradiation and prevent reactive oxygen species from damaging proteins (7, 8). An alternative role of Mn(II) accumulation in the irradiation survival of *D. radiodurans* was proposed to be its participation in the condensation of its genome (9, 10).

Based on our herein depicted findings it might be that Mn(II) has an additional role in the strategies of *D. radiodurans* to survive high irradiation doses. We find that Mn(II) renders *D. radiodurans* PolA* more capable in bypassing DNA template lesions that otherwise block proceeding DNA synthesis. In this sense Mn(II) rather acts as a modulator of enzyme function than being directly engaged in DNA damage prevention or repair processes. These findings are in agreement with the recent finding that *D. radiodurans* PolX, an enzyme believed to participate in double strand break repair, strongly requires Mn(II) for DNA synthesis activity (13, 14). Here as we discovered for PolA*, positive effects of Mn(II) on lesion bypass are accompanied by a drop in selectivity. Mismatches formed during DNA synthesis performed in the presence of Mn(II) might be repaired by the *D. radiodurans* DNA repair system efficiently and thus, should impose less threat to survival of the system as blockage of DNA synthesis by DNA lesions.

Our results indicate only a negligible role of Mn(II) on the strand-displacement activity of PolA*. Because the herein depicted results of PolA* are derived from a 5' → 3'-exonuclease-deficient variant one can only speculate about the impact of Mn(II) on the strand-displacement activity of the full-length PolA enzyme. It remains to be further shown whether the 5' → 3'-exonuclease activity of PolA plays an essential role on the enzymes function in strand displacement or processing of damaged templates, that DNA polymerase activity is necessary for UV and irradiation resistance is already clearly shown (16). Results obtained from studying *E. coli* (37) and *Streptococcus pneumoniae* (38) suggest an important role of the 5' → 3'-exonuclease activity in PolA for damage response. *E. coli* PolA activities are compensable during growth in minimal medium, whereas growing on rich media requires at least DNA polymerase or 5' → 3'-exonuclease activity for cell viability. Whereas for *S. pneumoniae* the 5' → 3'-exonuclease activity is not substitutable at all growing conditions, suggesting a lack of a mechanism that could replace this function of PolA.

Taken together, the DNA polymerase domain of *D. radiodurans* PolA was expressed and characterized for the first time. It was found that the derived protein is indeed a DNA polymerase that exhibits Watson-Crick DNA polymerization selectivity. DNA lesions as they occur after radiation seem to be a significant blockage for proceeding DNA synthesis. Mn(II) increases the enzymes ability to bypass lesions, a process that has been shown to be crucial in DNA repair of other bacteria. This finding suggests a role of Mn(II) in *D. radiodurans* as modulator of enzyme function that might add to the strategies of the bacteria to survive threats such as high doses of radiation or long periods of desiccation.

REFERENCES

- Minton, K. W. (1994) *Mol. Microbiol.* **13**, 9–15
- Battista, J. R., Earl, A. M., and Park, M. J. (1999) *Trends Microbiol.* **7**,

- 362–365
- Mattimore, V., and Battista, J. R. (1996) *J. Bacteriol.* **178**, 633–637
- Cox, M. M., and Battista, J. R. (2005) *Nat. Rev. Microbiol.* **3**, 882–892
- Battista, J. R. (1997) *Annu. Rev. Microbiol.* **51**, 203–224
- Anderson, A. W., Nordon, H. C., Cain, R. F., Parish, G., and Duggan, D. (1956) *Food Technol.* **10**, 575–578
- Daly, M. J., Gaidamakova, E. K., Matrosova, V. Y., Vasilenko, A., Zhai, M., Venkateswaran, A., Hess, M., Omelchenko, M. V., Konstandarites, H. M., Makarova, K. S., Wackett, L. P., Fredrickson, J. K., and Ghosal, D. (2004) *Science* **306**, 1025–1028
- Chou, F. I., and Tan, S. T. (1990) *J. Bacteriol.* **172**, 2029–2035
- Levin-Zaidman, S., Englander, J., Shimoni, E., Sharma, A. K., Minton, K. W., and Minsky, A. (2003) *Science* **299**, 254–256
- Englander, J., Klein, E., Brumfeld, V., Sharma, A. K., Doherty, A. J., and Minsky, A. (2004) *J. Bacteriol.* **186**, 5873–5977
- Makarova, K. S., Aravind, L., Wolf, Y. I., Tatusov, R. L., Minton, K. W., Koonin, E. V., and Daly, M. J. (2001) *Microbiol. Mol. Biol. Rev.* **65**, 44–79
- White, O., Eisen, J. A., Heidelberg, J. F., Hickey, E. K., Peterson, J. D., Dodson, R. J., Haft, D. H., Gwinn, M. L., Nelson, W. C., and Richardson, D. L. (1999) *Science* **286**, 1571–1577
- Blasius, M., Shevelev, I., Jolivet, E., Sommer, S., and Hübscher, U. (2006) *Mol. Microbiol.* **60**, 165–176
- Lecointe, F., Shevelev, I. V., Bailone, A., Sommer, S., and Hübscher, U. (2004) *Mol. Microbiol.* **53**, 1721–1730
- Gutman, P. D., Fuchs, P., and Minton, K. W. (1994) *Mut. Res. DNA Repair* **314**, 87–97
- Gutman, P. D., Fuchs, P., Ouyang, L., and Minton, K. W. (1993) *J. Bacteriol.* **175**, 3581–3590
- Zahradka, K., Slade, D., Bailone, A., Sommer, S., Averbeck, D., Petranovic, M., Lindner, A. B., and Radman, M. (2006) *Nature* **443**, 569–573
- Loeb, L. A., and Kunkel, T. A. (1982) *Annu. Rev. Biochem.* **52**, 429–457
- Goodman, M. F., Creighton, S., Bloom, L. B., and Petruska, J. (1993) *Crit. Rev. Biochem. Mol. Biol.* **28**, 83–126
- Mildvan, A. S., and Loeb, L. A. (1972) *Crit. Rev. Biochem.* **6**, 219–244
- Kornberg, A., and Baker, T. (1991) *DNA Replication*, W. H. Freeman and Co., New York
- Barnes, W. M. (1992) *Gene (Amst.)* **112**, 29–35
- Setlow, P., Brutlag, D., and Kornberg, A. (1972) *J. Biol. Chem.* **247**, 224–231
- Creighton, S., Bloom, L. B., and Goodman, M. F. (1995) *Methods Enzymol.* **262**, 232–256
- Sirover, M. A., and Loeb, L. A. (1976) *Science* **194**, 1434–1436
- Sirover, M. A., Dube, D. K., and Loeb, L. A. (1979) *J. Biol. Chem.* **254**, 107–111
- Kunkel, T. A., and Loeb, L. A. (1979) *J. Biol. Chem.* **254**, 5718–5725
- Tabor, S., and Richardson, C. C. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 4076–4080
- Friedberg, E. C., Walker, G. C., and Siede, W. (1995) *DNA Repair and Mutagenesis*, American Society for Microbiology Press, Washington, D. C.
- Schärer, O. D. (2003) *Angew. Chem. Int. Ed.* **42**, 2946–2974
- Prakash, S., Johnson, R. E., and Prakash, L. (2005) *Annu. Rev. Biochem.* **74**, 317–353
- Goodman, M. F. (2002) *Annu. Rev. Biochem.* **71**, 17–50
- Hübscher, U., Maga, G., and Spadari, S. (2002) *Annu. Rev. Biochem.* **71**, 133–163
- Moore, P. D., Bose, K. K., Rabkin, S. D., and Strauss, B. S. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78**, 110–114
- Rabkin, S. D., Moore, P. D., and Strauss, B. S. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80**, 1541–1545
- Villani, G., Le Gac, N. T., Wasungu, L., Burnouf, D., Fuchs, R. P., and Boehmer, P. E. (2002) *Nucleic Acids Res.* **30**, 3323–3332
- Joyce, C. M., and Grindley, N. D. F. (1984) *J. Bacteriol.* **158**, 636–643
- Diaz, A., Lacks, S. A., and Lopez, P. (1992) *Mol. Microbiol.* **6**, 3009–3019