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Release of 5′-terminal deoxyribose-phosphate residues from incised abasic sites in DNA by the *Escherichia coli* RecJ protein

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ABSTRACT

Excision of deoxyribose-phosphate residues from enzymatically incised abasic sites in double-stranded DNA is required prior to gap-filling and ligation during DNA base excision-repair, and a candidate deoxyribophosphodiesterase (dRpase) activity has been identified in *E.coli*. This activity is shown here to be a function of the *E.coli* RecJ protein, previously described as a 5′→3′ single-strand specific DNA exonuclease involved in a recombination pathway and in mismatch repair. Highly purified preparations of dRpase contained 5′→3′ exonuclease activity for single-stranded DNA, and homogeneous RecJ protein purified from an overproducer strain had both 5′→3′ exonuclease and dRpase activity. Moreover, *E.coli* recJ strains were deficient in dRpase activity. The hydrolytic dRpase function of the RecJ protein requires Mg^{2+}; in contrast, the activity of *E.coli* Fpg protein, that promotes the liberation of 5′→3′ Rp residues from DNA by β-elimination, is suppressed by Mg^{2+}. Several other *E.coli* nucleases, including exonucleases I, III, V, and VII, endonucleases I, III and IV and the 5′→3′ exonuclease function of DNA polymerase I, are unable to act as a dRpase. Nevertheless, *E.coli* fpg recJ double mutants retain capacity to repair abasic sites in DNA, indicating the presence of a back-up excision function.

INTRODUCTION

Spontaneous hydrolytic or oxidative loss of bases from DNA, and enzymatic removal of altered residues by cleavage of base–sugar bonds, leads to the formation of non-coding apurinic/apyrimidinic (AP) sites (1,2). These abasic sites in DNA are subject to rapid incision and repair by the base excision-repair pathway in both prokaryotic and eukaryotic cells (3). Their correction by the sequential action of several enzymes usually results in the excision of a sugar–phosphate residue, insertion of a single nucleotide by a DNA polymerase, and sealing of the nick by a DNA ligase. Thus, in most base excision-repair events only the damaged nucleotide is excised from the DNA and replaced by a single normal nucleotide (4).

To initiate the repair process, an AP endonuclease catalyses the hydrolysis of a phosphodiester bond linking the sugar–phosphate residue with a normal nucleotide. The abundant *E.coli* AP endonucleases, exonuclease III and endonuclease IV, incise the phosphodiester bond on the 5′ site of the AP site, thus generating a strand break with a 5′-terminal 2-deoxyribose-5-phosphate residue (dRp) (5). The mechanism of the subsequent excision of the dRp moiety has remained the least understood event in the base excision-repair process. A search for enzymes that can remove 5′-terminal sugar phosphate residues in free form from 5′ incised AP sites has revealed two candidate activities. One of these is ascribed to a β-elimination reaction promoted by the Fpg protein, a DNA glycosylase with associated AP lyase activities (6,7). Hydrolytic excision of dRp is catalysed by a different protein, that has been named DNA deoxyribophosphodiesterase or dRpase (8,9).

In this paper, we present evidence that the Mg^{2+}-dependent dRpase activity described previously (8) is associated with the 5′→3′ single strand-specific DNA exonuclease encoded by the *E.coli* recJ gene (10)

MATERIALS AND METHODS

DNA substrates

Oligonucleotides were prepared on a commercial DNA synthesizer. 5′-^{32}P-labelling of oligonucleotides with polynucleotide kinase and annealing of oligonucleotides to form double-stranded DNA substrates were performed as described

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previously (4). Substrates with a 5'-[^{32}P]dRp residue (Fig. 1A and 1B) were made as follows: an oligonucleotide was synthesized with a uracil residue in the 5'-terminal position, phosphorylated with polynucleotide kinase using [γ-^{32}P]ATP as cofactor and annealed with a complementary strand as required. The DNA was then treated with a high concentration of uracil–DNA glycosylase (2 units per μg DNA) in 70 mM Hepes·KOH (pH 7.8), 1 mM EDTA, 1 mM dithiothreitol for 1 h at 37°C. Uracil–DNA glycosylase can remove free uracil from a 5'-dUMP residue in DNA at a slow rate (11). The reaction mixture was treated with phenol/chloroform, and the DNA substrate purified by gel filtration on a Sephadex G-50 (Pharmacia) column (10×0.1 cm) equilibrated with 10 mM Hepes·KOH (pH 7.8), 1 mM EDTA. More than 80% of the ^{32}P-labelled 5'-residues were present as dRp as estimated by their alkali sensitivity (released as Norit charcoal/acid-soluble material on incubation with 0.5 M NaOH for 15 min at 37°C).

Double-stranded bacteriophage M13 DNA containing several strand interruptions with 5'-^{32}P-labelled dRp residues was made essentially as described by Graves *et al.* (6). Briefly, oligonucleotide-primed DNA synthesis on a single-stranded M13 DNA template was carried out using the Klenow fragment of *E.coli* DNA polymerase I in the presence of the four common deoxynucleoside triphosphates and [α-^{32}P]dUTP. The product was treated with *E.coli* uracil–DNA glycosylase and endonuclease IV to generate incised abasic sites, and purified by phenol/chloroform extraction and gel filtration.

Reagent enzymes

The following enzymes were purified from *E.coli* overproducer strains as described: RecJ protein (10), Fpg protein (12), and endonuclease IV (13). Uracil–DNA glycosylase was purified as described previously (14). Exonuclease I was obtained from United States Biochemicals, and exonuclease VII from Bethesda Research laboratories.

Purification of dRpase

This enzyme was purified approximately 5000-fold from 80 g *E.coli* cells, essentially by the method described previously (8). The starting material was *E.coli* K12 NH5033, a strain deficient in exonuclease I, Rec BCD nuclease, and endonuclease I (15). The yields from individual purification steps were similar to those obtained previously with an *E.coli* strain deficient in exonuclease III and DNA polymerase I (8). After the final Mono Q step of the published procedure (8), the partially purified enzyme preparation was passed through a Mono S column (in 50 mM Hepes·KOH (pH 7.8), 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol), to which the activity did not adsorb, and then rechromatographed on a Mono Q column retaining only the peak fraction of activity. These latter two steps resulted in an additional 2-fold purification by Mono S chromatography and a further 6-fold purification by Mono Q chromatography. However, several protein bands could still be detected by silver staining after SDS-polyacrylamide gel electrophoresis, so the enzyme was only partially purified; specifically, 3–4 different protein bands were present in the relevant 50–60 kDa region.

Fractionated cell extracts from different bacterial strains were made from cells lysed by lysozyme/EDTA treatment (16). After clarification of the lysates, ammonium sulfate was added to 0.35 g·ml^{-1}. Each protein precipitate was dissolved in 50 mM Hepes·KOH (pH 7.8), 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol, dialysed against the same buffer, and applied to a DEAE–Sepharose column. Proteins were eluted from the column with a linear NaCl gradient from 0 to 0.5 M, and fractions assayed for dRpase and 5'→3' exonuclease activity in 50μl reaction mixtures at 37°C for 10 min as detailed below.

Enzyme assays

Enzymatic release of 5'-dRp residues was measured using 0.2–2 pmol DNA substrates with ^{32}P-labelled 5'-dRp residues. The reaction mixture (250 μl) also contained 100 mM Hepes·KOH (pH 7.8), 5 mM $MgCl_2$, 2 mM Na_2HPO_4, 1 mM dithiothreitol, and a limiting amount of enzyme, and was incubated at 37°C. Fifty μl aliquots were removed at various times, and carrier DNA (50 μl 0.5% calf thymus DNA) and 50 μl cold 15% trichloracetic acid (TCA) added. After 5 min on ice, 50 μl 5% Norit charcoal in 5% TCA was added to adsorb nucleotides. ^{32}P-labelled Norit charcoal/acid soluble material in the supernatants was estimated by liquid scintillation counting. Where indicated, 5 mM $MgCl_2$ in the reaction mixture was replaced with 1 mM EDTA. All kinetic experiments were repeated 3–5 times with consistent results.

5'→3' exonuclease assays were carried out in reaction mixtures (250 μl) containing 0.2–2 pmol single- or double-stranded DNA substrate with a 5'-^{32}P-labelled nucleotide residue (Fig. 1C and Fig. 1D) in 100 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 2 mM Na_2HPO_4, 1 mM dithiothreitol, with a limiting amount of enzyme. After incubation at 37°C for various times, 50μl samples were moved, chilled on ice, and DNA precipitated with TCA in the presence of carrier. The radioactivity in the supernatant was determined by liquid scintillation counting.

Exonuclease I and exonuclease VII activities (17) were assayed using uniformly ^{14}C-labelled single-stranded *E.coli* DNA as substrate. Exonuclease I was measured in the standard reaction buffer and exonuclease VII in 70 mM Hepes·KOH (pH 7.8), 2 mM EDTA and 1 mM dithiothreitol.

Bacterial strains

E.coli K12 strains were AB1157 (F$^-$ *thr-1 leu-6 proA2 his-4 thi-1 argE3 lacY1 galK2 ara-14 xyl-15 mtl-1 tsx-33 rpsL31 supE44)* , BH20 (as AB1157 but *fpg-1 :: Kan*^{r}) (18), BW9109 (as AB1157 but Δ*xth*), RPC501 (as AB1157 but *nfo-1 :: Kan*^{r} Δ*xth*) (19), KLC381 (Hfr Δ[*gal–bio*] Δ[*gua–xse*]) (20), CJ236 (*dut-1 ung-1 thi-1 relA1*/pCJ105) and BD10 (*thyA36 deoC ung-1*) (21). BS100, BS101, BS102 and BS105 were *fpg-1::Kan*^{r} derivatives of NH5033 (F$^-$ *endA1 recB21 sbcB15 sbcC gal-44 thi-1 lac-61 deo-27*) (15), N2446 (as AB1157 but *recJ284::Tn10*, obtained from R.G.Lloyd), JC13030 (as AB1157 but *recJ77*, also from R.G.Lloyd) and AB1157 respectively, and were constructed by generalised P1 transduction as described previously (22) using a thermoinduced P1 *cml clr* 100 lysate of BH20 (*fpg-1::Kan*^{r}). BS104 was a *recJ284::Tn10* transductant of BS100. Kanamycin resistant transductants were selected on L agar (23) containing 50 μg/ml kanamycin.

Assessment of MMS sensitivity

Agar plates containing a gradient of methylmethanesulfonate (MMS, Aldrich Co.) were prepared by a method modified from that of Cunningham *et al.* (19). M9 minimal agar was supplemented with 0.2% Difco casamino acids and 1 μg ml^{-1} vitamin B1. Agar (50ml) containing 18mM MMS was poured into a 10 cm square Petri dish which was tilted to form a triangular wedge. The solidified wedge was overlaid with 50 ml agar (without added mutagen). *E.coli* strains were grown in

supplemented liquid M9 minimal medium to A_{540} 0.4 to 0.5. Cultures (10 μl) were spotted and then spread with a glass rod across the gradient plates and incubated for 40h at 30°C.

Phage λ with uracil-containing DNA

A 20 ml λ_{gv} liquid lysate of strain CJ236 (*dut ung*) was prepared essentially as described previously (23). Cells from 1 ml of overnight culture were initially infected with approximately 5×10^6 λ_{gv} pfu. The λ lysate was titered on various strains; triplicate 100 μl samples of a serial dilution of the lysate and 0.3 ml exponential culture were added to 3 ml molten top L-agar containing 10 mM $MgSO_4$, poured on to a L-agar plate containing 10 mM $MgSO_4$ and incubated overnight at 37°C. The titre of the lysate on strain BD10 (*ung*) was 3.9×10^{10} pfu/ml.

RESULTS

Excision of 5′-terminal deoxyribose-phosphate residues from DNA strand interruptions by various *E.coli* enzymes

DNA containing small numbers of abasic sites is incised by AP endonucleases resulting in single-strand breaks with 5′-dRp moieties. In agreement with previous results (8), such dRp residues were liberated in free form from a double stranded DNA substrate (Fig.1A) by hydrolysis on incubation with purified fractions of dRpase, and the reaction was Mg^{2+}-dependent (Fig. 2). Another *E.coli* enzyme, the Fpg protein, promoted the release of free sugar−phosphate residues from this substrate by a β-elimination process, in confirmation of the results of Graves *et al.* (6). The latter reaction was strongly suppressed by the presence of Mg^{2+} (Fig. 2). A report that exonuclease I has an associated dRpase activity (24) was not reproduced in our experiments; no detectable release of 5′-dRp residues by a large amount of enzyme (4 units per standard reaction mixture) was observed (Fig. 2), whereas uniformly ^{14}C-labelled single-stranded DNA was efficiently digested to more than 80% acid-soluble products (data not shown). At very high exonuclease I concentrations (40 units per standard reaction mixture), 10−15% of the radioactive material in a 5′-^{32}P-labelled double-stranded DNA substrate (Fig. 1A) was converted to an acid-soluble form, but comparable activity was seen with a control double-stranded DNA substrate without a 5′-dRp residue (Fig. 1D). Exonuclease VII, which degrades single-stranded DNA from both the 5′ and 3′ ends in the absence of Mg^{2+}(25) was also unable to release 5′-^{32}P-labelled dRp residues (less than 5% release) from preincised AP sites in a M13 DNA substrate, even at the high enzyme concentration of 2 units per reaction mixture (data not shown).

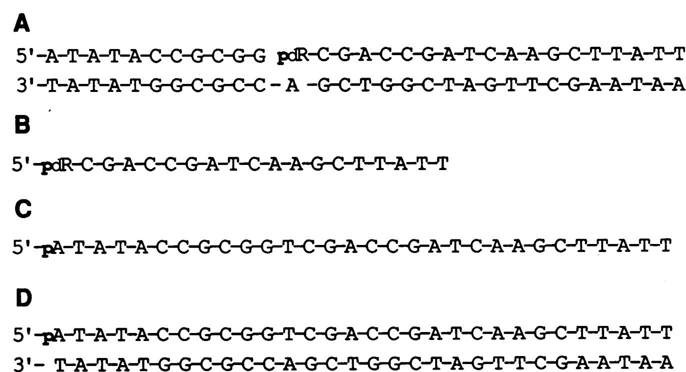

Figure 1. Sequences of double-stranded and single-stranded DNA substrates. Abasic deoxyribose-phosphate residues are shown as pdR, and ^{32}P-labelled phosphate moieties are shown in bold text.

dRpase preparations contain a 5′→3′ exonuclease activity specific for single-stranded DNA

The action of dRpase at incised AP sites in double-stranded DNA was found to be restricted to removal of the 5′-dRp residue, and no exonuclease activity was detected (8). However, the unpaired sugar−phosphate residue might resemble a short 5′-single-stranded tail, and therefore the ability of purified dRpase preparations to act on a single-stranded DNA substrate was investigated. Efficient removal of the 5′-^{32}P-label from single-stranded oligonucleotides with either a 5′-nucleotide or a 5′-dRp residue (Fig. 1B and 1C) was observed, with no clear difference (less than two-fold) between the two substrates (Fig. 3A and 3B). The removal of 5′-dRp from a single stranded substrate was 8−10 times more rapid than from the double-stranded DNA substrate (Fig. 3A). A small amount of acid soluble material was released from a double-stranded DNA substrate containing a 5′-^{32}P-labelled nucleotide residue and may have resulted from breathing and degradation of the A−T rich 5′-region of this substrate (Fig. 1D and 3B). The single strand exonuclease activity was dependent on the presence of Mg^{2+} in the reaction mixture (Fig. 3B), and therefore could not be ascribed to exonuclease VII. Furthermore, experiments with 5′-*vs* 3′-end-labelled substrates, using substrate C (Fig. 1) either 5′-^{32}P-labelled with polynucleotide kinase or 3′-^3H-labelled after addition of a radioactive nucleotide residue with terminal transferase, established that degradation of the single-stranded substrate was in the 5′→3′ direction (data not shown). The exonuclease activity showed a broad pH optimum in the range pH 6.8−8.8, and both this activity and the dRpase function are associated with a protein of 50−60 kDa as estimated by gel filtration (8). A Mg^{2+}-dependent exonuclease of 62 kDa that degrades single-

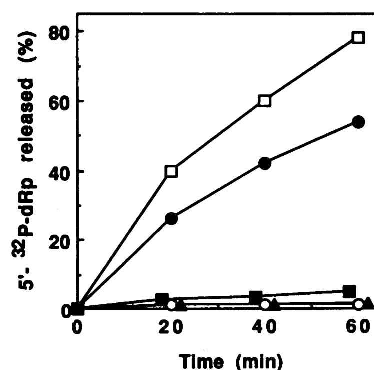

Figure 2. Enzymatic release of a 5′-dRp from a double-stranded DNA substrate in the presence or absence of Mg^{2+}. *E.coli* dRpase (1 μg protein; circles), Fpg protein (1 μg protein; squares) or exonuclease I (4 units; triangles) were assayed in 250 μl reaction mixtures containing 1.25 pmol double-stranded DNA substrate A (see Fig.1). The reaction mixtures were incubated at 37°C and 50 μl aliquots removed at the times indicated. After precipitation of DNA with 5% TCA and removal of nucleotides with Norit charcoal, the radioactivity in the supernatant was quantitated. Background release of ^{32}P-labelled dRp by non-enzymatic β-elimination was ~4% in 1h and has been subtracted. Filled symbols show results in the presence of 5 mM $MgCl_2$, and open symbols in the presence of 1mM EDTA and the absence of $MgCl_2$.

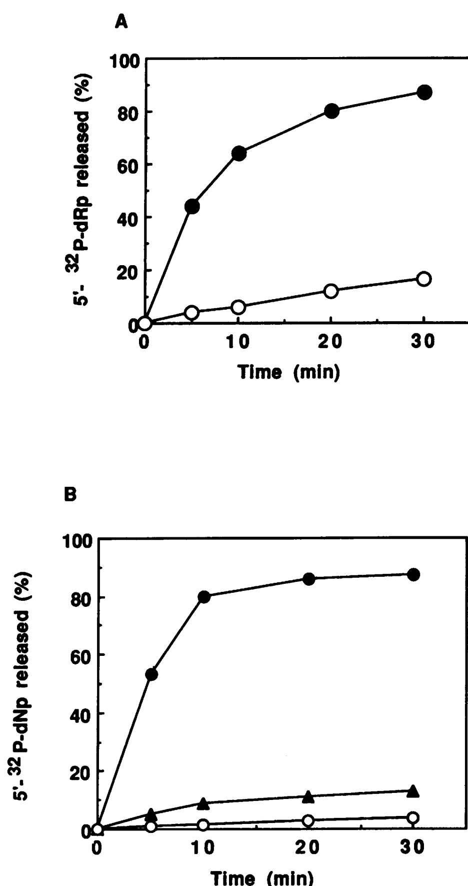

Figure 3. Excision of a 5′-dRp or a 5′-nucleotide residue from a single-stranded or double-stranded DNA substrate by dRpase. **A.** Reaction mixtures (250 μl) contained 1.25 pmol double-stranded substrate with a 5′-dRp (Fig.1A) (open circles) or single-stranded substrate with 5′-dRp (Fig. 1B) (closed circles). As in Fig.2, background release of ^{32}P-dRp has been subtracted. **B.** Reaction mixtures (250 μl) contained 1.25 pmol double-stranded substrate (Fig.1D) (closed triangles) or single-stranded substrate (Fig. 1C) with (closed circles) or without (open circles) magnesium . Incubations were at 37°C, and 50 μl aliquots were removed at the times indicated and analysed by precipitation with TCA and Norit charcoal (for reactions in A) or with TCA alone (for reactions in B).

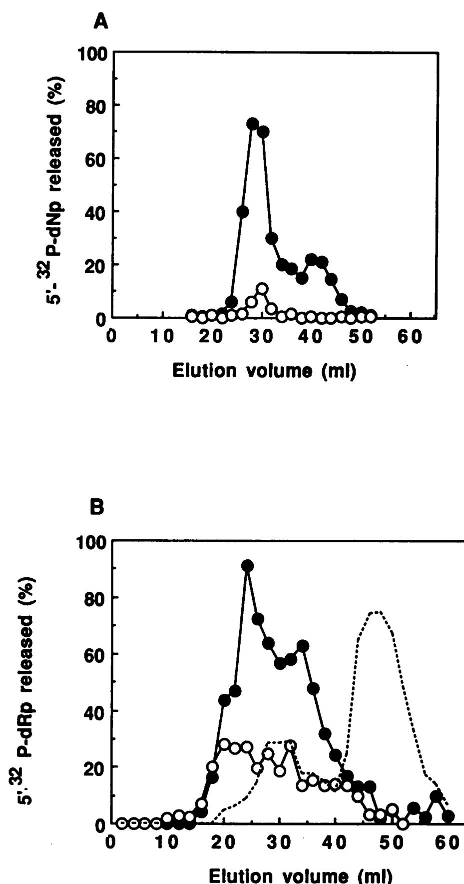

Figure 4. Co-chromatography of 5′→3′ single-strand exonuclease and double-strand dRpase activities in fractionated extracts of *E.coli*. Crude cell extracts were subjected to ammonium sulphate fractionation and DEAE–Sepharose chromatography as described in Materials and Methods. Column fractions (5 μl aliquots) were assayed for; (**A**) 5′→3′ single-strand exonuclease activity (substrate C, Fig. 1) and (**B**) dRpase activity (substrate A, Fig. 1) as described in Materials and Methods. Each 50 μl reaction mixture contained 0.2 pmol oligonucleotide substrate. Filled symbols represent assays of BH20 (*fpg*) fractionated extracts, and open symbols assays of BS101 (*fpg recJ*) fractionated extracts. The dashed line shows protein concentrations with the peak fraction (number 48) containing 1.4 mg·ml^{-1} protein.

stranded (but not double-stranded) DNA in the 5′→3′ direction has been shown previously to be the product of the recJ gene (10).

Association of Mg^{2+}-dependent dRpase activity with RecJ protein

In order to determine whether the dRpase and RecJ activities were associated with the same protein, the occurrence of these activities in fractionated extracts of BH20 (*fpg*) and BS101(*recJ fpg*) strains was investigated. These experiments were performed with *fpg* mutants to avoid partial release of 5′-dRp residues through β-elimination by the Fpg protein. Gradient chromatography of the RecJ$^+$ cell extract on a DEAE–Sepharose column yielded a major peak, with a shoulder, of 5′→3′ exonuclease activity (Fig. 4A). This activity appeared before the bulk of the protein was eluted from the column. The exonuclease activity was absent or strongly reduced in the recJ mutant, and thus identifies it as being due to the known nuclease function of the RecJ protein (10). When the column fractions were assayed for dRpase activity, the peak fraction coincided with that of exonuclease activity, and again a shoulder of activity was observed (Fig. 4B). This activity was strongly reduced in the *recJ* strain (Fig. 4B), or by exclusion of Mg^{2+} from the reaction mixtures (data not shown). Some activity remained that was neither stimulated nor inhibited by the presence of Mg^{2+} (data not shown) and may reflect relatively non-specific β-elimination events. The data are consistent with the observed dRpase activity being due to an intrinsic function of the RecJ protein. Similar data to those shown here were also obtained using fractionated cell extracts of a second pair of isogenic strains, BS100 and BS104 (*recJ*).

Homogeneous RecJ protein has dRpase activity

RecJ protein purified from an overproducing strain was assayed in standard reaction mixtures. In addition to the previously known 5′→3′ single-stranded DNA exonuclease function, the enzyme also showed dRpase activity on a double-stranded DNA substrate containing pre-incised AP sites (Fig. 5). The activity of the apparently homogeneous, overproduced RecJ protein was similar to that of ~5,000-fold purified dRpase (Fig. 5). As observed for dRpase (Fig. 3), the RecJ protein also removed 5′-dRp from single-stranded DNA (Fig. 1B) 8–10 times more rapidly than

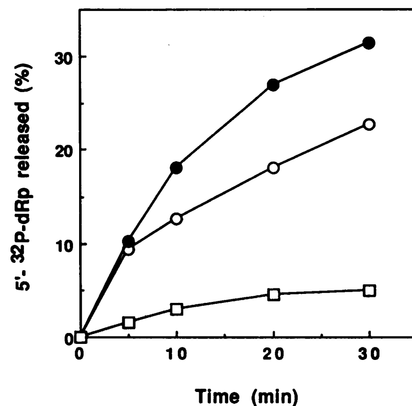

Figure 5. Excision of 5'-^{32}P-labelled dRp from a double-stranded phage M13 DNA substrate containing preincised abasic sites. The activities of purified dRpase (0.2 μg , closed circles) and overproduced RecJ protein (0.2 μg, open circles) were compared at 37°C. Open squares show non-enzymatic release of dRp residues under the assay conditions used. Each 250 μl reaction mixture contained 10 ng (15 000 cpm) double-stranded M13 DNA containing ^{32}P-labelled preincised abasic sites. Fifty μl aliquots were removed at various times from reaction mixtures and analysed after TCA/Norit charcoal precipitation as in Fig. 2. Standard deviations in three different experiments were < 10%.

from double-stranded DNA (data not shown). These results demonstrate that purified dRpase and authentic RecJ protein act in an indistinguishable manner on these DNA substrates, and confirm that the previously reported dRpase activity (8) may be ascribed to the RecJ protein.

Repair of AP sites in DNA *in vivo* in *fpg/recJ* mutants

The ability of the *fpg recJ* single or double mutants to repair AP sites in DNA *in vivo* was examined by two approaches, by their sensitivity to MMS and their ability to support the growth of λ_{gv} containing uracil in its DNA. Methylated bases and uracil in cellular DNA are excised by DNA glycosylases, thereby generating AP sites. In both cases, the phenotypes of the *fpg/recJ* mutants were compared to strains BW9109 (*xth*) and RPC501 (*xth nfo*) which are known to be defective in AP site repair (19). BW9109 (*xth*) lacks the major cellular AP endonuclease and RPC501 (*xth nfo*) lacks two AP endonuclease activities. Sensitivity to MMS was examined using gradients of this compound in minimal agar plates. BH20 (*fpg*), N2446 (*recJ::Tn10*), JC13030 (*recJ77*) and double *recJ fpg* mutants showed a resistance to MMS similar to that of the wild type strain, whereas BW9109 and RPC501 were clearly hypersensitive (Table 1). Lambda bacteriophage containing uracil (λ_u) was prepared by growth of λ_{gv} in strain CJ236 (*dut ung*). The *dut* strain is deficient in dUTPase and incorporates uracil residues into DNA. In the absence of cellular uracil−DNA glycosylase (*ung*), the uracil is not excised (21). The titre of the λ_u stock on strain BD10 (*ung*) was 4×10^{10} pfu/ml, but decreased to 10^6 pfu/ml on transfection of strain AB1157 (Ung$^+$) due to excision of uracil by uracil−DNA glycosylase and the generation of many toxic AP sites. This titre was decreased even further in mutants deficient in the repair of AP sites in DNA, by 60% in BW9109 (*xth*) and by 95% in RPC501 (*xth nfo*). However, survival of λ_u in the *fpg/recJ* single or double mutants was the same as that in the wild type strain, AB1157 (Table 1). Defective repair of AP sites was therefore not apparent in the mutants deficient in

Table 1. Assessment of DNA abasic site repair in various *E.coli* mutants

Bacterial Strain*	Relevant Genotype (cm)	Growth across a 10 cm gradient of 0 tp 18mM MMS (cm)	% Survival of uracil containing λ_{gv} relative to that in the wild type strain***
AB1157	wild type	7.3	100
BW9109	Δ*xth*	3.6	39
RPC501	Δ*xth nfo-1::Kan*^{r}	0.5	5
BH20**	*fpg-1::Kan*^{r}		102
N2446	*recJ284::Tn10*	7.2	140
BS101	*recJ284::Tn10 fpg-1::Kan*^{r}	7.2	136
JC13030	*recJ77*	7.7	
BS102	*recJ77 fpg-1::Kan*^{r}	7.2	

* All strains are derivatives of AB1157
**BS105, a *fpg-1::Kan*^{r} transductant of AB1157, had the same MMS resistance as the wild type in a separate experiment.
***Uracil-containing λ_{gv} stock was prepared by growth of λ_{gv} in CJ236 (*dut ung*). The survival of this stock in the wild type AB1157 was 1.5×10^6 pfu/ml.

both the Fpg and RecJ activities. Repair of abasic sites monitored by λ_u survival was also normal in an exonuclease VII deficient strain, KLC381 (Δ*xse*).

DISCUSSION

AP endonucleases such as *E.coli* exonuclease III and endonuclease IV, and the major mammalian AP endonuclease, incise DNA at the 5' side of AP sites. These endonucleases can not subsequently excise the 5' terminal dRp residue at the site of incision, so a separate function is required for this process (8). In *E.coli*, as well as in mammalian cells, the excision step usually involves removal of only the dRp residue without adjacent nucleotide residues; a one-nucleotide gap is produced and filled-in by short-patch DNA repair synthesis (4). Thus, an activity that catalyzes the release of free dRp is required for most excision events, and enzymes that release the dRp residue as part of an oligonucleotide can only account for a minor proportion. Similar results have been reported for *Xenopus laevis* oocyte extracts which apparently repair many AP sites by replacement of only the damaged residue (26, 27). In surveys of known activities using reagent enzymes or *E.coli* cell extracts, only two have been detected that are able to excise free dRp from DNA. One is a Mg^{2+}-dependent activity that catalyses the release of dRp by hydrolytic cleavage of the 5'-terminal phosphodiester bond at a pre-incised AP site (8). In this work, we present several independent lines of evidence indicating that this activity is a function of the RecJ protein. The second activity is associated with the Fpg protein (the *mutM* gene product) (6), and we have confirmed this observation. The Fpg protein catalyses the hydrolysis of base−sugar bonds at sites of purine residues that have a fragmented or oxidized imidazole ring. This protein also has an associated AP lyase activity, allowing the release of a 5'-terminal dRp residue in DNA by β-elimination.

Several *E.coli* nucleases have been shown to be unable to release a 5'-terminal dRp residue. Endonuclease III, a DNA glycosylase acting on ring-saturated and ring-fragmented pyrimidine residues, has an associated AP lyase activity that can promote β-elimination on the 3' side of an AP site, but the enzyme does not act on an AP site already incised at the 5' side

by an AP endonuclease (6,8,28). As shown here, exonuclease VII, a high-molecular weight exonuclease that can degrade single-stranded DNA from both the 3′ and 5′ ends in a Mg^{2+}-independent reaction, also is unable to excise a 5′-terminal dRp residue in free form. Moreover, *E.coli* mutant strains deficient in several other nucleases such as endonuclease I or RecBCD nuclease yielded cell extracts with unaltered dRpase activity (4). The 5′→3′ exonuclease function of *E.coli* DNA polymerase I has often been proposed as a possible candidate to catalyse the removal of dRp from incised AP sites. However, this function of Pol I is strongly suppressed by the presence of a base-free residue at a DNA 5′ terminus and, instead of excising the moiety in a nick translation reaction, displaces the strand with the 5′-dRp residue (29). The enzyme is unable to release dRp in free form, but slow excision of the residue as part of an oligonucleotide has been detected (30).

A recent report by Sandigursky and Franklin (24) indicated that *E.coli* exonuclease I might act as a 5′-dRpase. We have been unable to reproduce these experiments, and find no detectable dRpase activity associated with exonuclease I (Fig. 2). This enzyme acts exclusively as a 3′→5′ exonuclease on single-stranded DNA; the DNA is hydrolytically degraded to mononucleotides except for the 5′-terminal two residues, that remain as a dinucleotide (17). The size and fractionation properties of exonuclease I are similar to those of the RecJ protein, and may have resulted in their copurification. In this work, as well as in previous studies on base excision-repair by a one-nucleotide gap-filling event, no involvement of the *sbcB* gene product (exonuclease I) was observed (4).

Mutations in the *recJ* gene do not confer a detectably altered phenotype to wild-type *E.coli* cells, aside of reduced recombination between plasmids. However, in a *recBCD* background, a *recJ* mutation leads to a severe reduction in recombination frequency (31). It seems probable that the exonuclease activity associated with the RecJ protein can substitute for that of the RecBCD enzyme, and that RecJ may play a role in recombinational repair. Moreover, in mismatch correction involving a DNA strand break located at an unmethylated d(GATC) sequence 5′ to the mismatched residue a 5′→3′ excision function is required, and either the RecJ protein or exonuclease VII is needed for this step (32). The identification of a dRpase activity associated with the RecJ protein suggests an additional role for the RecJ protein in base excision-repair. However, no defect in the repair of AP sites was detected *in vivo* in *recJ* or *recJ fpg* mutants. For comparison, *E.coli nfo* mutants, deficient in a single AP endonuclease, are not sensitive to MMS, and a yeast mutant lacking DNA polymerase β that is likely to be involved in short-gap filling, does not have an apparent DNA repair defective phenotype (33). The proficient repair of AP sites in *recJ/fpg* mutants is not consistent with either the RecJ or Fpg protein being the only means of catalysing an obligatory dRp excision step in the base excision-repair DNA repair pathway. Moreover, the RecJ protein is present at only about 50 molecules per *E.coli* cell, a level considerably lower than that of the major AP endonuclease (exonuclease III), DNA polymerase I, and DNA ligase, while the activity of the Fpg protein is strongly suppressed at physiological Mg^{2+} concentrations. In consequence, other modes of dRp excision may be relevant as back-up reactions, such as slow non-enzymatic release of 5′-terminal dRp residues promoted non-specifically by cellular polyamines and basic proteins (34), or inefficient excision of dRp residues in oligonucleotide form by DNA polymerase I or other exonuclease functions (30). Thus, the crucial excision step that removes informationless dRp residues from damaged DNA, that occurs efficiently *in vivo* (3), may be due to the combined effect of several activities.

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