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The yeast 8-oxoguanine DNA glycosylase (Ogg1) contains a DNA deoxyribophosphodiesterase (dRpase) activity

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ABSTRACT

The yeast *OGG1* gene was recently cloned and shown to encode a protein that possesses N-glycosylase/AP lyase activities for the repair of oxidatively damaged DNA at sites of 7,8-dihydro-8-oxoguanine (8-oxoguanine). Similar activities have been identified for *Escherichia coli* formamidopyrimidine-DNA glycosylase (Fpg) and *Drosophila* ribosomal protein S3. Both Fpg and S3 also contain a deoxyribophosphodiesterase (dRpase) activity that removes 2-deoxyribose-5-phosphate at an incised 5′ apurinic/apyrimidinic (AP) sites via a β-elimination reaction. *Drosophila* S3 also has an additional activity that removes *trans*-4-hydroxy-2-pentenal-5-phosphate at a 3′ incised AP site by a Mg^{2+}-dependent hydrolytic mechanism. In view of the substrate similarities between Ogg1, Fpg and S3 at the level of base excision repair, we examined whether Ogg1 also contains dRpase activities. A glutathione S-transferase fusion protein of Ogg1 was purified and subsequently found to efficiently remove sugar–phosphate residues at incised 5′ AP sites. Activity was also detected for the Mg^{2+}-dependent removal of *trans*-4-hydroxy-2-pentenal-5-phosphate at 3′ incised AP sites and from intact AP sites. Previous studies have shown that DNA repair proteins that possess AP lyase activity leave an inefficient DNA terminus for subsequent DNA synthesis steps associated with base excision repair. However, the results presented here suggest that in the presence of $MgCl_2$, Ogg1 can efficiently process 8-oxoguanine so as to leave a one nucleotide gap that can be readily filled in by a DNA polymerase, and importantly, does not therefore require additional enzymes to process *trans*-4-hydroxy-2-pentenal-5-phosphate left at a 3′ terminus created by a β-elimination catalyst.

INTRODUCTION

Reactive oxygen species (ROS) can be generated by normal physiological processes such as the reduction of molecular oxygen to water, or by external sources such as ionizing radiation. Some of these ROS are highly active on DNA, producing, among many diverse lesions, an oxidatively damaged form of guanine, namely 7,8-dihydro-8-oxoguanine (8-oxoguanine). Left unrepaired, 8-oxoguanine will mispair with adenine (1–3) during DNA replication, leading to GC→TA transversion mutations (1,3–5) that may have a role in both carcinogenesis and aging (6,7).

The first enzyme that was found to efficiently remove 8-oxoguanine from DNA was discovered in *Escherichia coli* as the product of the *mutM* locus (8) that encodes formamidopyrimidine-DNA glycosylase (Fpg). This enzyme possesses N-glycosylase activity for the removal of 8-oxoguanine (9) forming an apurinic/apyrimidinic (AP) site in its place. The enzyme further processes the AP site by a β-,δ-elimination reaction at the abasic lesion (10), leading to the removal of the sugar and the creation of DNA termini containing 3′ and 5′ phosphoryl groups.

Notably, Fpg also possesses an activity that is able to remove a terminal 5′ deoxyribose phosphate that contains a pre-existing nick 5′ and adjacent to the AP site (11). Since nick translation by the 5′→3′ exonuclease activity of *E.coli* DNA polymerase I is inhibited by a terminal deoxyribose-phosphate (12), the action of Fpg at a 5′ incised AP site may have a biological role in *E.coli*.

In eukaryotic organisms, we recently reported that the *Drosophila* ribosomal protein S3 possesses similar activities to *E.coli* Fpg with regard to analogous N-glycosylase specificities and the processing of sugar–phosphate groups at AP sites by a β-,δ-elimination reaction (13). We further reported that, like Fpg, S3 was also able to remove 2-deoxyribose-5-phosphate at a 5′ incised AP site via a β-elimination reaction (14). Unlike Fpg, S3 was also found to be able to efficiently remove *trans*-4-hydroxy-2-pentenal-5-phosphate at a 3′ incised site via a Mg^{2+}-dependent hydrolytic mechanism.

In yeast, the OGG1 gene has been reported to encode N-glycosylase/AP lyase activities similar to Fpg and S3 for the removal of 8-oxoguanine (15,16). Here, we have examined the yeast Ogg1 protein to see if it also contains dRpase activities, and report that the activities possessed by this yeast protein are very similar to those described for *Drosophila* S3 (13,14).

MATERIALS AND METHODS

Cloning and expression of yeast OGG1

The yeast OGG1 gene was cloned by using PCR, in a similar fashion as previously used by us for the cloning and expression

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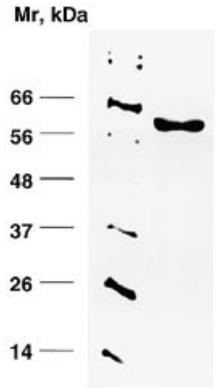

Figure 1. SDS-polyacrylamide gel electrophoresis of GST-Ogg1. The yeast fusion construct GST-Ogg1 was overexpressed in RPC501, and purified using a glutathione-agarose (Sigma) affinity column. Only a single band of purified GST-Ogg1 was observed by Coomassie staining. Molecular weight standards (New England Biolabs) are to the left.

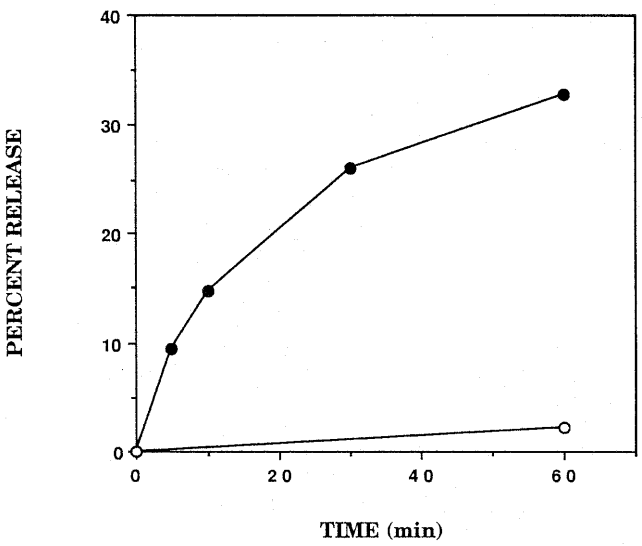

Figure 2. Time course for the release of *trans*-4-hydroxy-2-pentenal-5-phosphate from a M13mp18 double-stranded DNA substrate containing 3′ incised AP sites in the presence of 5 mM $MgCl_2$. Reactions incorporated M13mp18 DNA containing 3 nM ^{33}P-labelled 2-deoxyribose-5-phosphate end-groups. The release of 4-hydroxy-2-pentenal-5-phosphate was determined by precipitation with trichloroacetic acid in the presence of Norit charcoal. Reactions contained no enzyme (○) or 150 ng of GST-Ogg1 (●).

of the *E.coli* Fpg and rat MPG genes (13). We used genomic DNA from S*accaromyces cerevisiae* as a template for the PCR reaction. Oligonucleotides used in the PCR reaction corresponded to the 5′ end (CGCGGATCCATGTCTTATAAATTCGGC) and 3′ end (CCGGAATTCCTAATCTATTTTTGCTTC). The 5′ primer included a *Bam*HI site, while the 3′ primer included an *Eco*RI site for cloning into the pGEX-4T-1 vector (Pharmacia) for protein production (ATG and CTA stop codons are underlined in the oligonucleotides). The PCR product was purified using the High Pure PCR Product purification kit (Boehringer Mannheim), digested with *Bam*HI and *Eco*RI and ligated into pGEX-4T-1. Colonies were isolated containing the inserted fragment and sequenced in their entirety to confirm the correct yeast OGG1 sequence and that the correct reading frame was used. This construct was used for expression of Ogg1 protein as a GST fusion protein (GST-Ogg1).

GST-Ogg1 was overexpressed in *E.coli* RPC501 (17), which is deficient for the AP endonuclease activity of exonuclease III (*xth*) and endonuclease IV (*nfo*). The conditions for overexpression and purification of GST-Ogg1 were identical to those previously reported for *Drosophila* S3 (13).

M13 double stranded DNA containing labelled AP sites

An M13 DNA substrate containing ^{33}P-labelled AP sites was prepared essentially as described previously (8,11). [α-^{33}P]dUTP was prepared by deamination of [α-^{33}P]dCTP (Dupont, 2000 Ci/mmol) as described previously (19) and was incorporated into DNA in a reaction (100 µl), containing 1 µg single stranded M13mp18 DNA, 30 ng 24mer primer, 2.5 nmol of dATP, dCTP, dGTP, 0.25 nmol dTTP, 0.15 nmol dUTP, 15 µCi [α-^{33}P] dUTP, 50 mM Tris–HCl pH 7.8, 10 mM $MgCl_2$, 1 mM DTT and 3 U of large fragment (Klenow) of DNA polymerase I. Following incubation at 37°C for 2 h, the reaction was supplemented with an additional 2.5 nmol of dATP, dTTP, dGTP, dCTP and 3 U of large fragment (Klenow) of DNA polymerase I and incubation was continued for 1 h. The reaction was stopped by the addition of 4 µl 0.5 M Na_2EDTA, followed by incubation at 65°C for 5 min. The DNA was purified from unincorporated nucleotides on a 1 ml Sephadex G-50 column, precipitated with ethanol, then lyophilized. The [^{33}P]dUMP-containing M13 DNA was treated with uracil-DNA glycosylase and subsequently treated with either *E.coli* endonuclease IV to create a substrate containing 5′ incised AP sites or with *E.coli* endonuclease III to create a substrate containing 3′ incised AP sites as described previously (20).

Enzyme assays

DNA dRpase activity was assayed in a reaction measuring either the release of sugar–phosphate from a double stranded M13mp18 DNA substrate containing intact AP sites, 5′ incised AP sites or 3′ incised AP sites. A typical reaction (100 µl) contained 220 fmol double stranded M13mp18 DNA substrate containing intact or incised AP sites, 150 ng GST-Ogg1 enzyme, 50 mM HEPES-KOH pH 7.4, 50 mM KCl, 1 mM DTT, 2 mM Na_2EDTA. Some reactions were supplemented with 5 mM $MgCl_2$. Release of sugar–phosphate products was determined either by precipitation with trichloroacetic acid in the presence of Norit charcoal or by HPLC anion exchange chromatography, as described previously (18,20).

Sugar–phosphate product analysis

M13mp18 double-stranded DNA substrate containing 5′ incised AP sites (220 fmol; 55 000 c.p.m.) was incubated with either 100 ng GST-Ogg1 enzyme or 2 mM spermidine in the absence of enzyme in a 50 µl reaction containing 100 mM sodium thioglycolate, 30 mM HEPES-KOH pH 7.4, 50 mM KCl, 1 µg/ml BSA, 1 mM DTT, 0.05% Triton X-100, 0.1 mM Na_2EDTA for 30 min at 37°C, The reaction mixture was then injected onto a Brownlee MPLC AX cartridge column (4.6 mm × 3 cm), which was eluted with 6 ml of 25 mM KH_2PO_4 pH 4.5, followed by a 1 ml linear gradient to 250 mM KH_2PO_4 pH 4.5, followed by 15 ml of 250 mM KH_2PO_4, pH 4.5, at a flow rate of 1 ml/min. Fractions (0.5 ml) were collected and radioactivity contained in each fraction was determined by liquid scintillation counting.

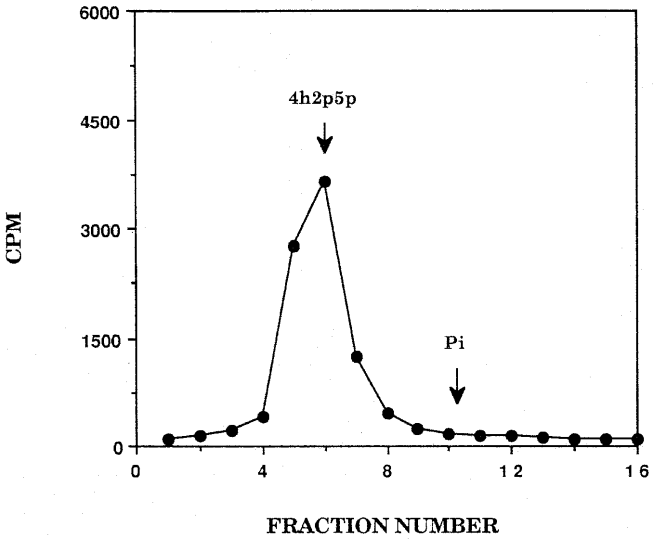

Figure 3. Enzymatic release of *trans*-4-hydroxy-2-pentenal-5-phosphate from an M13mp18 double-stranded DNA substrate containing 3′ incised AP sites. The reaction products were resolved on a MPLC AX HPLC column (14). The sugar–phosphate product *trans*-4-hydroxy-2-pentenal-5-phosphate (4h2p5p) released by GST-Ogg1 elutes between fractions 6 and 7 (3.5 min) under these conditions; inorganic phosphate (Pi) elutes between fractions 10 and 11 (5.5 min), as determined by authentic standards (14,24).

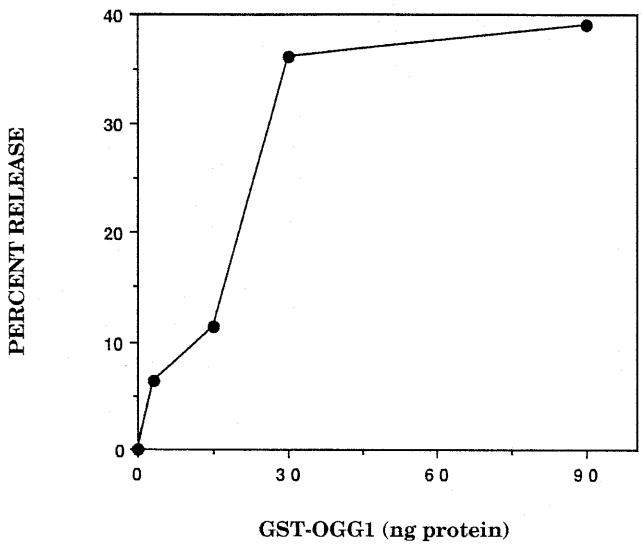

Figure 4. Enzymatic release of *trans*-4-hydroxy-2-pentenal-5-phosphate from a M13mp18 double-stranded DNA substrate containing labelled AP sites following treatment with GST-Ogg1 in the presence of 5 mM $MgCl_2$. Reactions incorporated M13mp18 DNA containing 0.4 nM ^{33}P-labelled AP sites. The release of sugar–phosphate was determined by precipitation with trichloroacetic acid in the presence of Norit charcoal.

RESULTS

Ogg1 removes sugar–phosphate products from a DNA substrate containing 3′-incised AP sites

To test the ability of GST-Ogg1 to remove sugar–phosphate products at a 3′ incised AP site, a double stranded M13mp18 DNA substrate containing 3′ *trans*-4-hydroxy-2-pentenal-5-phosphate end-groups was utilized. M13mp18 DNA containing [^{33}P]dUMP was treated with uracil-DNA glycosylase followed by treatment with *E.coli* endonuclease III, a known β-elimination catalyst (21). GST-Ogg1 was found to remove the product *trans*-4-hydroxy-2-pentenal-5-phosphate from the substrate in a Mg^{2+}-dependent fashion. Since trace bacterial contamination by class II, 5′-acting AP endonucleases could have been a reason for our result, GST-Ogg1 was subsequently generated in *E.coli* RPC501, which is deficient for the AP endonuclease activity associated with exonuclease III and endonuclease IV. The purification of GST-Ogg1 from this strain resulted in a homogeneous preparation of fusion construct as judged by SDS-PAGE and Coomassie staining (Fig. 1). As seen in the time course in Figure 2, GST-Ogg1 was once again found to remove the product *trans*-4-hydroxy-2-pentenal-5-phosphate. The identity of the product was subsequently confirmed by anion exchange HPLC, in which a single peak was observed (Fig. 3). As seen in Figure 3, no inorganic phosphate was released from the substrate following treatment with the GST-Ogg1, indicating that the preparation does not contain a phosphatase activity.

Ogg1 removes sugar–phosphate products from a DNA substrate containing intact AP sites

Yeast Ogg1 has previously been determined to catalyze a β-elimination reaction at an abasic site in DNA (15,16). We therefore eliminated the pre-incision step with *E.coli* endonuclease III, and tested the ability of GST-Ogg1 to directly catalyze the excision of the 3′ sugar–phosphate. Double stranded M13mp18 DNA containing [^{33}P]dUMP that had been treated only with uracil-DNA glycosylase was employed as a substrate containing unincised AP sites. As seen in Figure 4, GST-Ogg1 was able to catalyze the removal of *trans*-4-hydroxy-2-pentenal-5-phosphate from the substrate in a protein dependent manner. No release of sugar–phosphate was evident in the absence of $MgCl_2$; release of *trans*-4-hydroxy-2-pentenal-5-phosphate was confirmed by anion exchange HPLC (data not shown).

GST-Ogg1 removes a 5′ terminal deoxyribose-phosphate from a DNA substrate containing incised AP sites

To test the ability of GST-Ogg1 to remove sugar–phosphate products at a 5′ incised AP site, a double stranded M13mp18 DNA substrate containing 5′ 2-deoxyribose-5-phosphate end-groups was utilized. M13mp18 DNA containing [^{33}P]dUMP was treated with uracil-DNA glycosylase followed by treatment with *E.coli* endonuclease IV (22). As can be seen in the time course presented in Figure 5, GST-Ogg1 was able to efficiently remove the 5′ sugar–phosphate.

Mechanism of release of the sugar–phosphate product from a DNA substrate containing 5′ incised AP sites

The release of sugar–phosphate from a DNA substrate containing 5′ incised AP sites by the action of GST-Ogg1 was not dependent on the addition of $MgCl_2$ and suggested that this reaction may proceed via β-elimination. The *E.coli* Fpg protein (11) and the *Drosophila* S3 protein (13) have been shown to remove 5′ terminal deoxyribose phosphate groups by such a mechanism. To determine if the release of sugar–phosphate occurred via β-elimination, GST-Ogg1 was

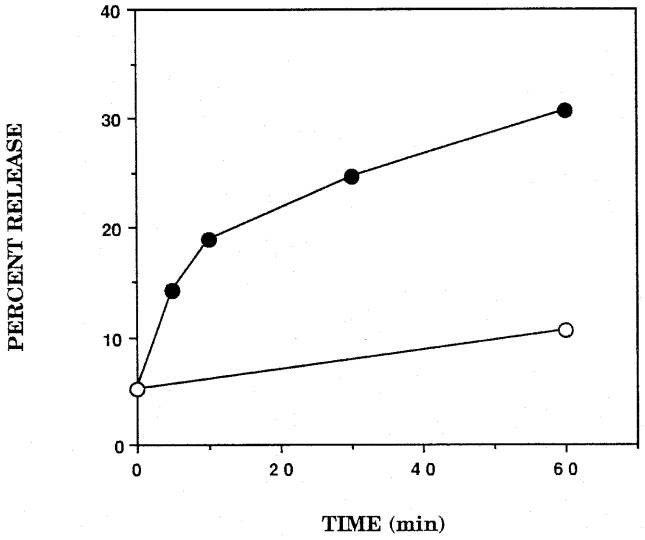

Figure 5. Time course for the release of sugar–phosphate from a M13mp18 double-stranded DNA substrate containing 5′ incised AP sites. Reactions incorporated M13mp18 DNA containing 3 nm ^{33}P-labelled 2-deoxyribose-5-phosphate end-groups. The release of sugar–phosphate was determined by precipitation with trichloroacetic acid in the presence of Norit charcoal. Reactions contained no enzyme (○) or 150 ng of Ogg1 (●).

incubated with the M13 DNA substrate containing 5′ incised AP sites in the presence of sodium thioglycolate. It has been shown previously that this reagent will react with an unsaturated sugar–phosphate product, resulting in the formation of products with altered mobilities on anion exchange chromatography (11). When the M13 DNA substrate containing 5′ incised AP sites was incubated with sodium thioglycolate and the β-elimination catalyst spermidine (2 mM), the unsaturated sugar–phosphate product released by spermidine treatment formed products that reacted with sodium thioglycolate, as is seen in Figure 6A. When the DNA substrate was incubated with sodium thioglycolate and GST-Ogg1, the released products co-migrated with the products produced by spermidine treatment, as is seen in Figure 6B. These results strongly suggest that the mechanism of release of the 5′ terminal deoxyribose-phosphate by GST-Ogg1 is via a β-elimination reaction.

Kinetics of dRpase activities

The kinetics for the removal of sugar–phosphate from DNA substrates containing both 5′ and 3′ incised AP sites was determined. As seen in Figure 7, the apparent K_m for the release of *trans*-4-hydroxy-2-pentenal-5-phosphate from a M13mp18 DNA substrate containing 3′ incised AP sites was determined by Lineweaver–Burk analysis to have a value of 6 nM; the value of V_{max} is 0.013 pmol/min. For the removal of 2-deoxyribose-5-phosphate from a M13mp18 DNA substrate containing 5′ incised AP sites, a K_m value of 70 nM was determined from Lineweaver–Burk analysis, as shown in Figure 8; the value of V_{max} is 0.06 pmol/min.

DISCUSSION

The existence of DNA deoxyribophosphodiesterases was first discovered in *E.coli* for the removal of 5′ terminal deoxyribose-phosphate groups at incised AP sites (19). Such an activity was originally sought because it was considered at that time that

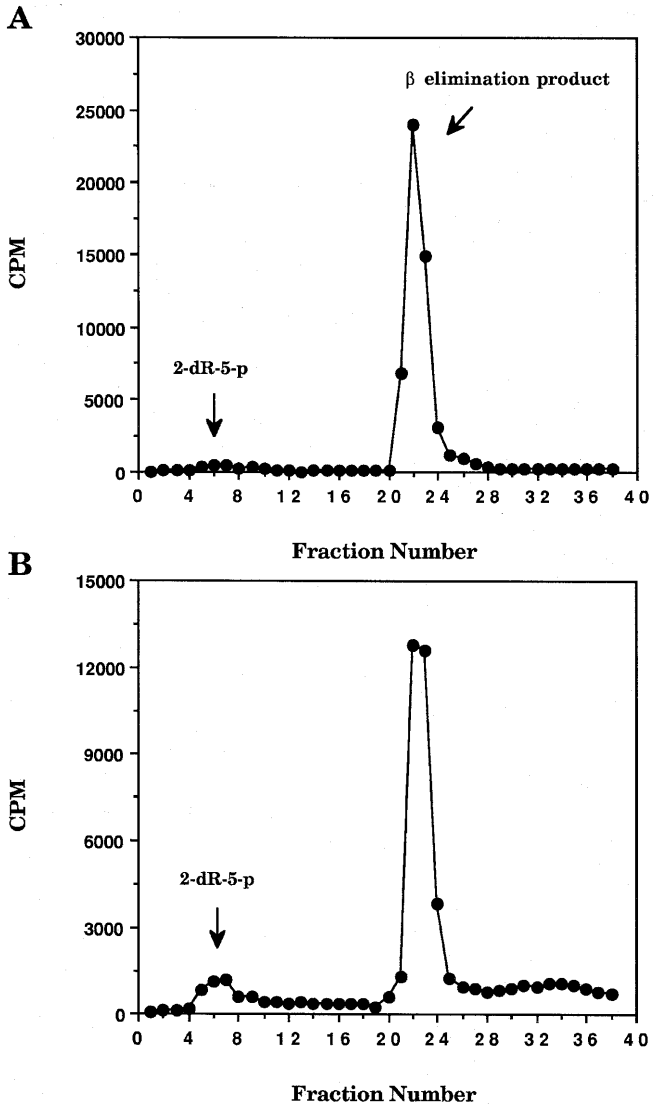

Figure 6. Anion exchange HPLC separation of sugar–phosphate products released from a M13mp18 double-stranded DNA substrate containing 5′ incised AP sites. (**A**) Products released from the substrate following treatment with 2 mM spermidine and 100 mM sodium thioglycolate. (**B**) Products released from the substrate containing 5′ incised AP sites following treatment with 100 ng GST-Ogg1 and 100 mM sodium thioglycolate. 2-dR-5-p indicates the elution position of 2-deoxyribose-5-phosphate or *trans*-4-hydroxy-2-pentenal-5-phosphate, which co-elute under these conditions (14).

β-elimination catalysts such as endonuclease III provided only modest amounts of cleavage activity at abasic sites when compared to the 5′ acting AP endonucleases. This was verified by subsequent tests for dRpase activity that might be possessed by endonuclease III (11). Conversely, another β-elimination catalyst was in fact identified in the same study, in which it was shown that Fpg efficiently catalyzed the removal of 5′ terminal deoxyribose-phosphate (11).

In eukaryotic organisms, the rat DNA polymerase β was recently shown to contain a dRpase activity for the removal of 5′-terminal deoxyribose phosphate groups (23). Another eukaryotic protein shown to possess dRpase activity was the *Drosophila* ribosomal protein S3 (14). We have now demonstrated that the Ogg1 protein from *S.cerevisiae* also contains this activity. Moreover, the

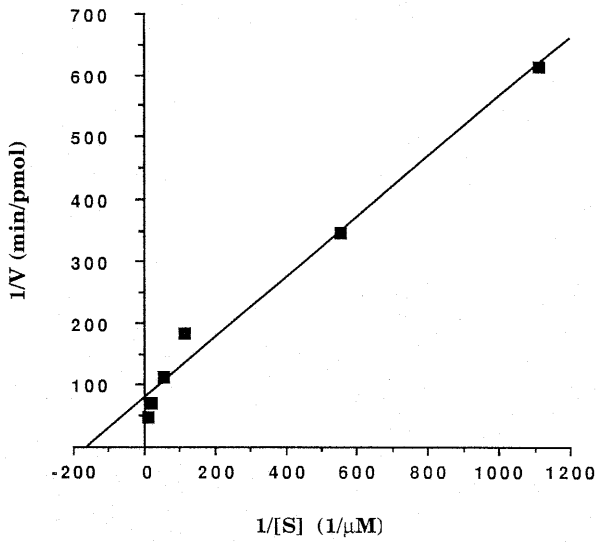

Figure 7. Lineweaver–Burk plot for the determination of K_m for the release of 4-hydroxy-2-pentenal-5-phosphate from an M13mp18 double-stranded DNA substrate containing 3′ incised AP sites. Substrate range, 0.0009–0.09 μM; K_m = 6 nM.

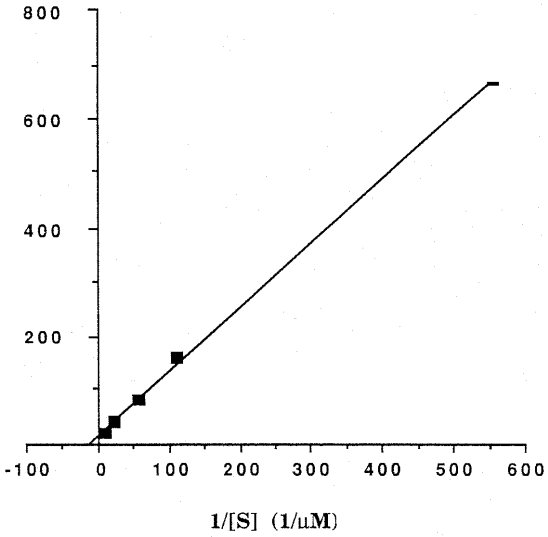

Figure 8. Lineweaver–Burk plot for the determination of K_m for the release of sugar–phosphate from an M13mp18 double-stranded DNA substrate containing 5′ incised AP sites. Substrate range, 0.0018–0.09 μM; K_m = 0.07 μM.

prokaryotic Fpg activity, and the eukaryotic proteins cited above remove the 5′-terminal deoxyribose-phosphate products by similar β-elimination reaction mechanisms.

The removal of *trans*-4-hydroxy-2-pentenal-5-phosphate product at a 3′ incised AP site is not so uniform as observed for the removal of 2-deoxyribose-5-phosphate at a 5′ incised AP site. For example, it is not anticipated that DNA polymerase β would possess this activity, and the activity is lacking for the *E.coli* Fpg protein (14). On the other hand, *Drosophila* S3 is able to remove 3′ sugar phosphates in a Mg^{2+}-dependent hydrolytic mechanism (14). As shown here, the yeast Ogg1 protein behaves similarly to the S3 protein in its ability to liberate *trans*-4-hydroxy-2-pentenal-5-phosphate at 3′ incised AP sites.

The ability to reveal dRpase activity at a 3′ terminus has generally relied upon AP DNA substrates that have been cleaved by a β-elimination catalyst. Both *Drosophila* S3 and yeast Ogg1 efficiently cleave these substrates via a Mg^{2+}-dependent hydrolytic mechanism. As shown here, however, in the presence of Mg^{2+} the pre-incision step is not necessary for Ogg1 to completely process the existing AP site, presumably leaving a one nucleotide gap and an efficient 3′ terminus for DNA polymerase binding and gap filling.

Questions have been raised in the past in regard to the significance of the AP lyase activity associated with many known N-glycosylases. In view of recent findings reported here and elsewhere (14), it may be that in the presence of certain co-factors like Mg^{2+}, these enzymes are extremely efficient in that they can process damaged DNA bases in a single concerted step that would only require the subsequent action of a DNA polymerase and DNA ligase to complete the repair process.

ACKNOWLEDGEMENTS

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