The use of thioglycollate to demonstrate DNA AP (apurinic/apyrimidinic-site) lyase activities

Biological consequences of thiol addition to the 5' product of a β -elimination reaction at an AP site in DNA

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Thioglycollate reacts with the 5' product of AP lyase activity on apurinic/apyrimidinic (AP) sites in DNA. The 3'-terminal thioglycollate-unsaturated sugar 5-phosphate adduct can be released by the use of *Escherichia coli* endonuclease IV or endonuclease VI, and identified by DEAE-Sephadex chromatography. In contrast, the mammalian AP endonuclease is unable to excise a 3'-terminal thiol-unsaturated sugar adduct; this lesion, which must sometimes occur *in vivo*, might be irreparable and have pathological consequences.

INTRODUCTION

AP lyases are enzymes that cleave the C(3')–O–P bond 3' to an AP (apurinic/apyrimidinic) site in DNA by catalysing a β -elimination reaction (Bailly & Verly, 1989a). The problem raised here, to qualify an enzyme as an AP lyase, is to distinguish such a cleavage from the hydrolysis of the same bond.

In a previous investigation (Bricteux-Grégoire & Verly, 1989), the following strategy was used. The C(3')–O–P bond 5' to the AP site was first hydrolysed with AP endonuclease from rat liver chromatin; the reaction product was then submitted to the action of the enzyme nicking the C(3')–O–P bond 3' to the AP site in the presence of 50 mM-thioglycollate. The unsaturated sugar 5-phosphate released by the AP lyase reacted with thioglycollate to give an adduct that was well separated from the sugar phosphates by DEAE-Sephadex chromatography. In contrast, the added deoxyribose 5-phosphate marker did not react with thioglycollate and was eluted in the region of the sugar phosphates.

This method has a drawback: after nicking with the AP endonuclease, the 5'-terminal base-free deoxyribose 5-phosphate is labile; it is spontaneously released by β -elimination with a half-life of 2 h at 37 °C (Bailly & Verly, 1989b). Of course, one can use a high concentration of AP lyase and a short incubation time so that the background of spontaneous β -elimination is reduced to a small percentage of the β -elimination catalysed by the AP lyase. But AP lyases are sluggish enzymes when compared with AP endonucleases.

In this work, we reversed the nicking order: the DNA containing the AP site was first incubated with AP lyase in the presence of thioglycollate, then submitted to the action of an AP endonuclease. This order is more like what must happen *in vivo*. The problem was to find an enzyme able to hydrolyse the C(3')–O–P bond 5' to a 3'-terminal thioglycollate-unsaturated sugar adduct. We show here that *Escherichia coli* endonuclease VI (exonuclease III) and endonuclease IV have such a capability.

Rat liver AP endonuclease is unable to hydrolyse the C(3')–O–P bond 5' to a 3'-terminal unsaturated sugar produced by a β -elimination reaction (Bailly & Verly, 1988a). We show here that this inability is not relieved when thioglycollate is added to the

β -elimination product. This observation probably has biological implications, as will be commented on in the Conclusions and final remarks.

MATERIALS AND METHODS

The enzymes

One unit of AP endonuclease activity is the amount of enzyme needed to produce 1 pmol of breaks near AP sites per min under the conditions described by Bricteux-Grégoire *et al.* (1986); the same unit is used for AP lyases.

E. coli [pyrimidine hydrate]DNA glycosylase (PHDG) (previously wrongly named *E. coli* endonuclease III; see Bailly & Verly, 1989a) was prepared from *E. coli* BW 531 transformed by a plasmid containing the gene encoding the glycosylase (kindly given by Dr. B. Weiss). The enzyme was purified by the method of Weiss & Duker (1986) and kept in 25 mM-potassium phosphate buffer, pH 7.0, containing 5 mM-EDTA, 5 mM-2-mercaptoethanol and 50 % (v/v) glycerol, at –70 °C. Its AP lyase activity, measured in the absence of Mg²⁺, was about 50 units/ml. After addition of Mg²⁺, the preparation showed a 5' nicking activity that was probably due to contamination with endonuclease VI (see the Results and discussion section).

E. coli endonuclease IV was prepared by the method of Ljungquist (1977) from *E. coli* B41 cells induced by paraquat (Chan & Weiss, 1987). The enzyme preparation obtained after the DNA-cellulose step (which was shown to be nearly pure by SDS/PAGE) was kept in 10 mM-Tris/HCl buffer, pH 8.0, containing 0.5 M-NaCl, 0.5 mM-dithiothreitol and 50 % (v/v) glycerol at –70 °C. It contained 9 μ g of protein and 50 units per ml.

E. coli endonuclease VI (Gossard & Verly, 1978) (exonuclease III) was from BRL or Boehringer Pharma. To ensure that it did not contain any AP lyase activity (which would then probably be due to PHDG), it was purified by chromatography on a phosphocellulose column (1 cm $\times$ 7 cm) equilibrated in 40 mM-sodium phosphate buffer, pH 6.5, containing 0.1 mM-EDTA and 0.1 mM-2-mercaptoethanol, and eluted with a 200 ml 0–0.6 M-NaCl linear gradient in the same buffer (Verly & Rassart, 1975); under these conditions, the PHDG is not eluted from the column

Abbreviations used: AP, apurinic/apyrimidinic; AP lyase, DNA apurinic/apyrimidinic-site lyase; d(-), symbol for base-free deoxyribose (AP site); PHDG, [pyrimidine hydrate]DNA glycosylase.

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(Katcher & Wallace, 1983). After dialysis and concentration by ultrafiltration, the endonuclease VI preparation was kept in 20 mM-Hepes/KOH buffer, pH 8.0, containing 0.4 mM-EDTA, 50 mM-KCl, 0.015 % BSA, 7 mM-2-mercaptoethanol and 25 % (v/v) glycerol at -20°C . In the absence of Mg^{2+} it was completely devoid of activity on depurinated DNA. With 10 mM- Mg^{2+} its activity was 22000 units/ml.

Mammalian AP endonuclease was extracted from rat liver chromatin (César & Verly, 1983) and purified by successive chromatography on HA-Ultrogel, Bio-Gel P-150 and single-stranded DNA-Ultrogel. The enzyme was kept in 25 mM-Hepes/KOH buffer, pH 7.8, containing 25 mM-NaCl, 0.01 % BSA, 0.005 % Triton X-100 and 50 % (v/v) glycerol. Its activity was 19000 units/ml.

DNA with ^{32}P - and ^3H -labelled AP sites

DNA labelled with ^3H in the 1' and 2'-positions of the base-free deoxyribose residues and with ^{32}P 5' to these residues was prepared as described by Bricteux-Grégoire & Verly (1989). The AP sites/T ratio was 1:250 or 1:750. This partially depurinated doubly labelled DNA is represented by $[\text{d}(-), 5'-^{32}\text{P}, 1', 2'-^3\text{H}]$ -DNA.

Nicking 3' to the AP sites with *E. coli* PHDG

The incubation mixture (450 μl) contained 20 μg of $[\text{d}(-), 5'-^{32}\text{P}, 1', 2'-^3\text{H}]$ -DNA (264000 c.p.m. of ^3H ; 800000 c.p.m. of ^{32}P) and 10 units of PHDG. Before addition of this enzyme, 1 M-KCl and 2 M-thioglycollate were added to give 50 mM-KCl and the indicated thiol concentration in the incubation mixture. It was left at 37°C for 60 min.

Inactivation and extraction of the enzymes

After addition of 100 μg of BSA, enzyme activities were extracted twice with an equal volume of phenol/chloroform (1:1, v/v).

Removal of small molecules by several dilutions-ultrafiltrations

The DNA substrate in the upper aqueous phase was concentrated by centrifugation on an Ultrafree-MC unit (10000 NMWL Millipore); the concentrate was twice diluted with 100 μl of 50 mM-Hepes/KOH buffer, pH 8.0, containing 1 mM-EDTA and reconcentrated, before finally being taken up in 100 μl of this Hepes/EDTA buffer.

Treatment with *E. coli* endonuclease IV, *E. coli* endonuclease VI or mammalian AP endonuclease

A 25 μl portion of the solution of $[\text{d}(-), 5'-^{32}\text{P}, 1', 2'-^3\text{H}]$ -DNA nicked by PHDG was used in all cases. The addition of 5 μl of *E. coli* endonuclease IV (0.25 unit) solution was followed by a 60 min incubation at 37°C . The addition of 10-fold concentrated buffer to reach 30 mM-Tris/HCl buffer, pH 7.5, 16 mM- MgCl_2 and 16 mM-NaCl final concentrations and 1 μl of *E. coli* endonuclease VI (22 units) solution was followed by a 30 min incubation at 37°C ; the reaction was stopped by the addition of 2 μl of 0.5 M-EDTA. The addition of 2 μl of 200 mM- MgCl_2 and 2 μl of rat liver chromatin AP endonuclease (38 units) solution was followed by a 30 min incubation at 37°C ; the reaction was stopped by addition of EDTA as above.

DEAE-Sephadex chromatography

The samples were diluted with 10 ml of 0.1 M-sodium borate buffer, pH 8.3, supplemented with 5 μl of 1 M-deoxyribose 5-phosphate. The DEAE-Sephadex A-25 (Pharmacia) column (0.9 cm $\times$ 30 cm) was equilibrated with the dilution buffer. The elution was carried out with 200 ml of a 0–0.25 M-NaCl linear gradient in the same buffer at a rate of 25 ml/h. Fractions (3 ml)

were collected; samples were taken to measure the ^3H and ^{32}P radioactivities, and, after a diphenylamine reaction, to assay the deoxyribose 5-phosphate.

RESULTS AND DISCUSSION

E. coli endonuclease IV is inhibited by thioglycollate

When $[\text{d}(-), 5'-^{32}\text{P}, 1', 2'-^3\text{H}]$ -DNA was treated with *E. coli* PHDG, then with *E. coli* endonuclease IV, DEAE-Sephadex chromatography indicated the release of a sugar phosphate that emerged one fraction after the deoxyribose 5-phosphate marker (Fig. 1a). It is known that PHDG is an AP lyase that breaks the $\text{C}(3')\text{--O--P}$ bond 3' to an AP site by catalysing a β -elimination reaction (Bailly & Verly, 1987). *E. coli* endonuclease IV is thus capable of releasing a 3'-terminal base-free unsaturated sugar phosphate, confirming a previous observation (Bailly & Verly, 1989b).

When the incubation with PHDG was carried out in the presence of 50 mM-thioglycollate, *E. coli* endonuclease IV released very little unsaturated sugar phosphate, free or combined with thioglycollate (Fig. 1b). Two explanations seem possible: either endonuclease IV is unable to excise the 3'-terminal sugar phosphate after addition of thioglycollate, or thioglycollate inhibits endonuclease IV. To choose between these possibilities, $[\text{d}(-), 5'-^{32}\text{P}, 1', 2'-^3\text{H}]$ -DNA was incubated with PHDG in the presence of 200 mM-thioglycollate and the thioglycollate removed by successive dilutions-ultrafiltrations before endonuclease IV was added; Fig. 1(c) shows that a thioglycollate-unsaturated sugar phosphate adduct was released together with some unsaturated sugar phosphate in the ratio 1.8:1. The removal of thioglycollate before addition of endonuclease IV makes it clear that the adduct formed before the 5' cleavage. Thus *E. coli* endonuclease IV is able to release the 3'-terminal thioglycollate-unsaturated sugar phosphate adduct; the enzyme is inhibited by thioglycollate, as was confirmed directly with a depurinated DNA as substrate.

E. coli endonuclease VI is not inhibited by thioglycollate: the reaction with thioglycollate of the 3'-terminal base-free unsaturated sugar is incomplete

When $[\text{d}(-), 5'-^{32}\text{P}, 1', 2'-^3\text{H}]$ -DNA was preincubated with PHDG, *E. coli* endonuclease VI, in the presence of Mg^{2+} , released a sugar phosphate (Fig. 2a). This is confirmation that endonuclease VI is able to release a 3'-terminal base-free unsaturated sugar produced at an AP site by a β -elimination reaction (see also Bailly & Verly, 1988a).

When 50 mM-thioglycollate was present from the beginning of the PHDG preincubation, endonuclease VI, in the presence of Mg^{2+} , released a mixture of thioglycollate-unsaturated sugar phosphate adduct and unsaturated sugar phosphate in the ratio 0.6:1 (Fig. 2b). This ratio increased to 6:1 when the thiol concentration was raised to 200 mM.

E. coli endonuclease VI, in contrast with endonuclease IV, is thus not inhibited by thioglycollate; on the other hand, like endonuclease IV, it is capable of removing a 3'-terminal base-free thioglycollate-unsaturated sugar phosphate adduct. The advantage of endonuclease VI is thus that it is not necessary to remove the thioglycollate after the AP lyase treatment before the addition of the AP endonuclease.

It is noteworthy that the reactivity of the 3'-terminal base-free unsaturated sugar produced at an AP site by a β -elimination reaction is lower than that of the free unsaturated sugar 5-phosphate. With the latter, 50 mM-thioglycollate was sufficient to cause complete addition (Bricteux-Grégoire & Verly, 1989), whereas the reaction is only partial when the sugar is still

Fig. 1. *E. coli* endonuclease IV is able to release a 3'-terminal unsaturated sugar phosphate or a 3'-terminal thiol-unsaturated sugar phosphate adduct

(a) *E. coli* endonuclease IV releases an unsaturated sugar phosphate (fraction 19) from [d(-),5'- ^{32}P ,1',2'- ^3H]DNA preincubated with PHDG; (b) little release occurs when the two successive incubations are carried out in the presence of 50 mM-thioglycollate; (c) when 200 mM-thioglycollate is present only during the preincubation with PHDG, *E. coli* endonuclease IV releases a mixture of thioglycollate-unsaturated sugar phosphate adduct (fraction 34) and unsaturated sugar phosphate. The peak of the deoxyribose 5-phosphate marker (—) is at fraction 18; *, ^{32}P ; $\triangle$, ^3H . In (a) and (b), the ^3H peak centred on fraction 9 comes from the labelled 2'-H of the substrate lost during the β -elimination catalysed by PHDG (see Bailly & Verly, 1987).

Fig. 2. *E. coli* endonuclease VI is able to release a 3'-terminal unsaturated sugar phosphate or a 3'-terminal thiol-unsaturated sugar phosphate adduct

(a) *E. coli* endonuclease VI releases an unsaturated sugar phosphate from [d(-),5'- ^{32}P ,1',2'- ^3H]DNA preincubated with PHDG; (b) it releases a mixture of thioglycollate-unsaturated sugar phosphate adduct and unsaturated sugar phosphate when the two successive incubations are carried out in the presence of 50 mM-thioglycollate. See Fig. 1 legend for explanation of symbols.

attached to the 3'-end of the polynucleotide chain (see also Bailly & Verly, 1988b); in this case, even 200 mM-thioglycollate is insufficient to cause a complete reaction.

The PHDG preparation is contaminated with a Mg^{2+} -requiring AP endonuclease

No sugar phosphate was released into the solution when

[d(-),5'- ^{32}P ,1',2'- ^3H]DNA was only incubated with PHDG (Fig. 3a). However, when Mg^{2+} was added after this preincubation, the 3'-terminal base-free unsaturated sugar phosphate was released (Fig. 3b). One must conclude that the PHDG preparation was contaminated with a Mg^{2+} -requiring AP endonuclease, very probably endonuclease VI. This is not very troublesome when the protocol is to add endonuclease VI after the AP lyase

Fig. 3. The PHDG preparation is contaminated with an Mg^{2+} -requiring AP endonuclease

[d(-),5'- ^{32}P ,1',2'- 3H]DNA is incubated for 60 min with PHDG; Mg^{2+} is then added (b) or not (a), and the incubation continued for another 60 min. See Fig. 1 legend for explanation of symbols.

preincubation; the only drawback is not having a clean control (in which there is no production of free sugar phosphate) when endonuclease VI is not added. But the situation is totally different when the aim is to test another Mg^{2+} -requiring AP endonuclease on the preincubation product. It was decided to inactivate all enzymes after the PHDG preincubation.

Heating the solution in the hope of inactivating endonuclease VI was tried first; the test of this inactivation was the absence of sugar phosphate release during subsequent incubation with Mg^{2+} . Heating for 15 min at 80 °C was found to be necessary. However, when *E. coli* endonuclease IV was added after this heat treatment, very little sugar phosphate was formed. The probable explanation is that 15 min at 80 °C was sufficient to cause an important δ -elimination so that little 3'-terminal base-free unsaturated sugar phosphate was left for the endonuclease IV to release.

Another method was therefore tried: after the PHDG preincubation, the enzymes were denatured and extracted twice with phenol/chloroform and the solution subjected to several dilutions-ultrafiltrations. When Mg^{2+} was added, no sugar phosphate was released; nevertheless, endonuclease IV still released sugar phosphate. The conclusions are that the phenol/chloroform extraction eliminated the parasitic Mg^{2+} -requiring AP endonuclease but the treatment did not influence the activity of the AP endonuclease used in the second part of the experiment.

Hydrolysis of the C(3')-O-P bond 5' to an AP site is followed by a spontaneous β -elimination of the 5'-terminal sugar phosphate

Bailly & Verly (1989b), using labelled oligonucleotides and gel electrophoresis and other techniques, have shown the lability of a 5'-terminal AP site that is lost with a half-life of 2 h at 37 °C by a process of β -elimination. We fully confirm this observation.

An unsaturated sugar phosphate was released when [d(-),5'- ^{32}P ,1',2'- 3H]DNA was incubated with *E. coli* endonuclease IV alone. In a similar way, a progressive release of sugar phosphate was observed when the same substrate was incubated with *E. coli* endonuclease VI in the presence of Mg^{2+} (Fig. 4a, 15 min; Fig. 4b, 60 min); the rate of release did not change if, after a 10 min incubation with enzyme sufficient to nick 5' to all AP sites, the Mg^{2+} was neutralized with EDTA.

The lability of the 5'-terminal AP site must be taken into account when the problem is to ascertain, by the chromatographic

method described in this paper, that the nicking 3' to an AP site is due to an AP lyase; one must be sure that the 3' nicking is complete before adding *E. coli* endonuclease VI (+ Mg^{2+}) or *E. coli* endonuclease IV.

Rat liver chromatin AP endonuclease does not release the 3'-terminal sugar phosphate produced by a β -elimination reaction at an AP site; addition of thioglycollate does not relieve this inability

[d(-),5'- ^{32}P ,1',2'- 3H]DNA was incubated with PHDG in the absence or presence of 200 mM-thioglycollate; the solution was extracted twice with phenol/chloroform, subjected to successive dilutions-ultrafiltrations and finally taken up in Hepes/EDTA buffer (see the Materials and methods section).

Portions of these solutions were incubated at 37 °C for 60 min with *E. coli* endonuclease IV (0.25 unit), and then analysed on DEAE-cellulose. In the absence of thioglycollate (Fig. 5a), only one peak containing both 3H and ^{32}P appeared, the maximum of which is just one fraction after the maximum of the deoxyribose 5-phosphate marker. When thioglycollate was present during the PHDG preincubation (Fig. 5b), a second peak appeared (around fraction 31), which corresponds to the addition product of thioglycollate to the unsaturated sugar created by β -elimination, and subsequently released by 5' nicking.

To other portions of the DNA that had been treated with PHDG, in the absence or presence of 200 mM-thioglycollate, were added *E. coli* endonuclease VI (22 units) and Mg^{2+} , and the mixture was incubated for 30 min at 37 °C. The reaction was stopped by the addition of EDTA and the solution submitted to DEAE-Sephadex chromatography. The results (not shown) are quite similar to those obtained with endonuclease IV. When no endonuclease VI was added, Mg^{2+} alone was unable to liberate any radioactive product visible on DEAE-Sephadex chromatography.

All these results are in agreement with what was reported in the previous sections.

To still other portions of the substrate nicked with PHDG, in the absence or in the presence of 200 mM-thioglycollate, were added Mg^{2+} and rat liver chromatin AP endonuclease (38 units). After an incubation of 30 min at 37 °C and addition of EDTA, the solution was submitted to DEAE-Sephadex chromatography.

Fig. 4. Nicking 5' to an AP site by an AP endonuclease is followed by the spontaneous loss of the 5'-terminal base-free sugar 5-phosphate

In the presence of Mg^{2+} , $[d(-), 5'-^{32}P, 1', 2'-^3H]DNA$ is incubated with *E. coli* endonuclease VI (amount sufficient to nick 5' to all AP sites in less than 10 min) for 15 min (a) or 60 min (b). See Fig. 1 legend for explanation of symbols.

Fig. 5. The mammalian AP endonuclease is incapable of releasing a 3'-terminal unsaturated sugar phosphate or a 3'-terminal thiol-unsaturated sugar phosphate adduct

$[d(-), 5'-^{32}P, 1', 2'-^3H]DNA$ is preincubated with PHDG in the absence (a and c) or in the presence (b and d) of 200 mM-thioglycollate; the enzyme is denatured and extracted and the solution ultradialyzed. To portions of this solution are added either *E. coli* endonuclease IV (a and b) or Mg^{2+} and rat liver chromatin AP endonuclease (c and d). See Fig. 1 legend for explanation of symbols.

No peak of radioactivity appeared in absence of thioglycollate (Fig. 5c), showing that the mammalian enzyme is unable to excise a 3' unsaturated base-free sugar produced at an AP site by β -elimination; this confirms what has been previously published (Bailey & Verly, 1988a). But the result was the same (Fig. 5d) when thioglycollate was present during the preincubation with PHDG; this indicates that the enzyme is also unable to excise a 3'-terminal thioglycollate-unsaturated sugar phosphate adduct.

CONCLUSIONS AND FINAL REMARKS

Two aspects of thiol addition to the 5' product of β -elimination at an AP site in DNA are dealt with here, namely methodological and biological.

(1) To name an enzyme AP lyase, one must demonstrate that the nicking of the $C(3')-O-P$ bond 3' to the AP site results from a β -elimination and not from a hydrolysis. It can be done by

subsequently hydrolysing the C(3')-O-P bond 5' to the AP site with *E. coli* endonuclease IV or *E. coli* endonuclease VI. However, on DEAE-Sephadex chromatography, the released unsaturated sugar phosphate hardly separates from the deoxyribose 5-phosphate that would have been produced if the 3' cleavage had occurred by hydrolysis. A way out of this difficulty is to add thioglycollate during the AP lyase incubation; the subsequent treatment with *E. coli* AP endonuclease releases a thioglycollate-unsaturated sugar phosphate adduct that separates well from the deoxyribose 5-phosphate on DEAE-Sephadex chromatography.

However, several problems must be faced. One is that thioglycollate inhibits *E. coli* endonuclease IV so that the thiol must be removed before addition of the 5' nicking enzyme. This is not without advantages: thioglycollate addition cannot take place during the *E. coli* endonuclease IV treatment and the appearance of thioglycollate-unsaturated sugar phosphate adduct on the DEAE-Sephadex chromatogram shows that the first enzyme indeed catalysed a β -elimination and can truly be called an AP lyase.

Thioglycollate does not inhibit *E. coli* endonuclease VI so it does not have to be removed after the first incubation with the 3'-nicking enzyme. But one must be sure that the 3' cleavage is complete before *E. coli* endonuclease VI is added. Indeed, when the 5' nicking by the AP endonuclease occurs without previous 3' nicking, it is followed by a spontaneous β -elimination and the unsaturated sugar phosphate released reacts quantitatively with thioglycollate; the meaning of the adduct seen on the chromatogram can thus be ambiguous.

Another problem is that the 5'-terminal unsaturated sugar produced by β -elimination at an AP site does not react quantitatively with thioglycollate even when its concentration is 200 mM. Thus *E. coli* endonuclease IV or *E. coli* endonuclease VI always releases a mixture of thioglycollate-unsaturated sugar phosphate adduct and unsaturated sugar phosphate.

(2) Since, *in vivo*, AP lyases or other β -elimination catalysts work in a thiol-containing medium, some of the 5'-cleavage products must form thiol adducts. This addition is not a dead end for the repair of AP sites in *E. coli* since, in these bacteria, two enzymes, endonuclease IV and endonuclease VI, are capable of removing the 3'-terminal adduct.

In eukaryotic cells, a minor route of AP-site repair involving a first step of 3' nicking by β -elimination catalysed by the chromatin histones and polyamines has been described. Since no

enzyme able to remove the resulting 3'-terminal unsaturated sugar is known to be present in the nucleus, the repair probably proceeds via a δ -elimination, also catalysed by histones and polyamines (Bailly *et al.*, 1989). However, addition of a thiol to the unsaturated sugar prevents the δ -elimination (Bailly & Verly, 1988b). Since the mammalian AP endonuclease can no more remove a 5'-terminal thiol-unsaturated sugar adduct than it can the simpler 5'-terminal unsaturated sugar, and because no other enzyme has yet been shown to achieve such a feat, it is possible that the modified lesion is no longer reparable. Although it seldom occurs, this lesion might be of great importance, playing a role in the genesis of cancers.

One is brought back to an ever-recurring question on the action of small doses of DNA-damaging agents: is it proportional to the dose or is there a threshold below which no harm occurs? It is obvious that the answer is different depending on whether the lesion is reparable or not. For irreparable damage, as the 5'-terminal thiol-unsaturated sugar adduct seems to be, there must be a direct relationship between dose and effect.

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