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# Novel reagents for chemical cleavage at abasic sites and UV photoproducts in DNA

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## ABSTRACT

**Hot piperidine is often used to cleave abasic and UV-irradiated DNA at the sites of damage. It can inflict non-specific damage on DNA, probably because it is a strong base and creates significant concentrations of hydroxyl ions which can attack purines and pyrimidines. We show that several other amines can cleave abasic DNA at or near neutral pH without non-specific damage. One diamine, *N,N'*-dimethylethylenediamine, efficiently cleaves abasic DNA at pH 7.4 by either β- or β,δ-elimination, depending on temperature. Using end-labelled oligonucleotides we show that cleavage depends mainly on elimination reactions, but that 4',5'-cyclization is also significant. This reagent also cleaves at photoproducts induced by UVC and UVB, producing the same overall pattern as piperidine, but with no non-specific damage. It should prove valuable in locating low levels of photoproducts in DNA, such as those induced by natural sunlight.**

## INTRODUCTION

Chemical detection of photoproducts in UV-irradiated DNA relies almost entirely on treatment with 1 M piperidine at 90°C, which first displaces photoproducts and then cleaves the DNA at the abasic sites created. A potential problem with this procedure is that, as several investigators have noted, it can sometimes cause significant background cleavage in unirradiated DNA (1,2). This is of little consequence if the amount of photo-induced damage is large, but can constitute a problem if it is small, and it would be helpful to find conditions which cleave DNA at photoproducts without inflicting non-specific damage. Background cleavage of DNA by piperidine probably results from the fact that it is a very strong base, producing significant concentrations of hydroxyl ions which can open or displace some bases in DNA (3,4), leaving abasic sites which are cleaved by the free amine (5). Reagents which can first displace photoproducts and then cleave at abasic sites at neutral pH should avoid this problem. Some small proteins, peptides, amino acids and amines can cleave abasic sites at neutral pH (6–8), mainly by elimination reactions (Fig. 1), but it is not known whether they can displace photoproducts. Here we first test several amines for ability to catalyse β- or β,δ-elimination of abasic DNA at or near neutral pH, assessing non-specific cleavage by assaying for relaxation of a supercoiled plasmid and specific cleavage using a defined oligonucleotide. Next, we test amines that give clean elimination without background cleavage for their ability to cleave at photoproducts in UV-irradiated DNA.

## MATERIALS AND METHODS

### Amines

Piperidine, morpholine, piperazine, 1,2-ethylenediamine and *N,N'*-dimethylethylenediamine were obtained from Aldrich; putrescine from Sigma. Fresh solutions were used for each experiment. Piperidine and morpholine were removed from samples for gel analysis using Sephadex G-50 spin columns (9) followed by precipitation.

### Plasmid preparation

Supercoiled pBluescript-II SK$^+$ DNA (Stratagene) was prepared on CsCl/ethidium bromide density gradients (9).

### Labelling and enzyme digestions of oligonucleotides and DNA

Gel-purified oligonucleotide (0.5 μg) (5'-ATTACTGTCGCCA-TCGTTA-3') was 5'-end-labelled in 20 μl using 40 μCi [γ-$^{32}$P]ATP and 10 U T4 polynucleotide kinase. Alternatively, 0.05 μg was 3'-end-labelled in 50 μl using 40 μCi [γ-$^{32}$P]ddATP and 20 U terminal deoxynucleotidyl transferase (Promega). Labelled oligonucleotides were purified on Sephadex G-50 spin columns (9). M13 –40 primer (9 ng) was 5'-end-labelled and purified as above, annealed to 3 μg single-stranded M13 mp18 DNA and extended using Sequenase version 2.0 (US Biochemicals) as recommended by the manufacturer. The product was digested with 5 U *Ava*II to give a 426 bp fragment. The enzyme was inactivated at 65°C and the DNA recovered by precipitation.

### Depurination of DNA

Plasmid DNA (10–50 μg) in 20 μl 25 mM sodium acetate, pH 4.8, was heated at 70°C for 12 min and precipitated with 3 vol. ethanol. This introduces ~3 apurinic sites/molecule (10). End-labelled oligonucleotide (50 μl) in water was added to 130 μl 98% formic acid and incubated at 37°C for 18 min. The reaction was terminated by freezing and repeated lyophilization. This introduces ~1 apurinic site/molecule (11).

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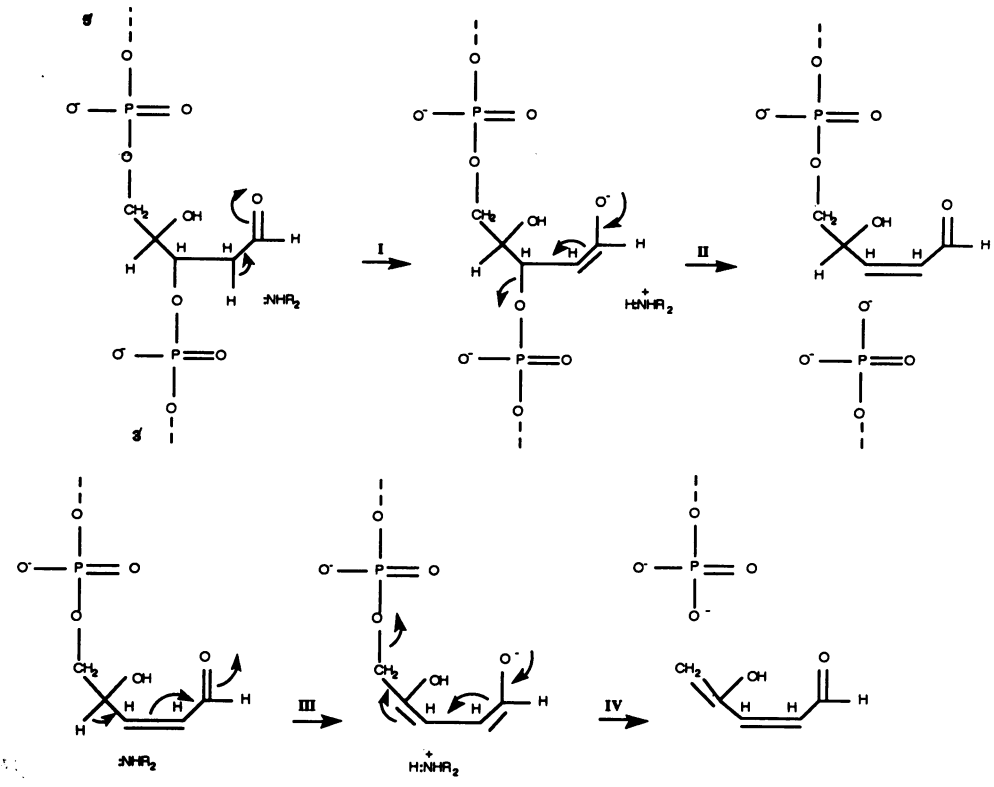

**Figure 1.** Elimination reactions at abasic sites catalysed by amines. At an abasic site in a DNA chain, the open chain aldehyde form of the deoxyribose is a minor but reactive species (27). In β-elimination, a free amine deprotonates C2 of the abasic deoxyribose (step I) and subsequent electron shifts cleave the 3′-phosphodiester bond (step II). The sugar, now an unsaturated aldehyde, remains attached to the 3′-end of the 'upstream' fragment that is released and the phosphate now forms the 5′-end of the 'downstream' fragment. In δ-elimination, which can occur only if β-elimination has occurred first, the amine deprotonates C4 of the sugar (step III) and subsequent electron shifts cleave the 5′-phosphodiester bond (step IV), detaching the sugar completely and leaving a phosphate group on the 3′-end of the upstream fragment, as well as on the 5′-end of the downstream fragment.

### UV irradiations

UVC irradiations used a 254 nm source at a fluence of 1 J/m$^2$/s, UVB irradiations two unfiltered Westinghouse FS20 sunlamp tubes 10 cm from the sample.

### Maxam–Gilbert sequencing reactions and sequencing gels

These were conducted as described for oligonucleotides (12) and longer DNA fragments (13). Sequencing gels contained 20 or 6% w/v acrylamide/bisacrylamide (19:1), 50% w/v urea and 1 × TBE buffer, pH 8.3. They were pre-run for at least 40 min. The 20% gels were run for 14 h at 750 V (bromophenol blue migrates 32 cm), 6% gels for 2 h at 1500 V (bromophenol blue migrates 40 cm).

## RESULTS

In elimination at abasic sites, amines accept protons (Fig. 1) and so must be uncharged. At or near neutral pH they remain uncharged only if their p$K_a$ values are relatively low. We first tested morpholine because it has, like piperidine, one secondary amino group, but a much lower p$K_a$ (Table 1). Figure 2A shows that 1 M piperidine at 90°C causes considerable degradation of a supercoiled plasmid. The closed circular plasmid rapidly disappears (lanes 1–5) and very little is left by 30 min, which is consistent with the non-specific effects noticed by others (1,2). Morpholine (3 M) at pH 8.0 has much less effect (lanes 6–8), although there is some relaxation at 37°C and a little more at 65°C. This is probably due to a little non-specific base displacement caused by the high temperature and alkaline pH followed by cleavage catalysed by free morpholine, which cuts abasic sites very easily (lanes 9–11). Thus morpholine cleaves abasic sites near neutral pH, but may cause some background degradation.

To analyse the cleavage mechanism we incubated a 5′-labelled oligonucleotide with morpholine and compared the patterns produced with those found using the 'strong' and 'mild' alkali treatments used by Bailly *et al.* (14) to distinguish β- from β,δ-elimination at an abasic site (Fig. 2B). Like piperidine, 'strong' alkali (0.2 M NaOH, 65°C, 30 min) catalyses complete β,δ-elimination, generating the same bands on a sequencing gel as piperidine. 'Mild' alkali (0.2 M NaOH, 37°C, 15 min) produces, in addition to the δ-elimination product, two bands with lower mobility, which are due to β-elimination. The faster one represents the 5′ (i.e. upstream)-fragment resulting from β-elimination at an abasic deoxyribose and it carries an α,β-unsaturated aldehyde on its 3′-terminus. The slower one is due to reaction between the aldehyde and Tris in the gel buffer to form an imine (15). The single δ band and the two β bands act as very convenient reference points for deducing the effects of other reagents. Figure 2B shows that buffered morpholine causes reasonably clean β,δ-elimination at apurinic sites in DNA, the yields being higher at 65°C than at 37°C.

**Table 1.** Ionic species present in relation to pH and $pK_a$ values

| Compound | Amino groups[a] | | | pH at 25°C | Percentage in each form | | | |
|---|---|---|---|---|---|---|---|---|
| | $pK_{a1}$ | $pK_{a2}$ | $pK_{a3}$ | | Unprotonated | Monoprotonated | Diprotonated | Triprotonated |
| Piperidine | 11.12 | – | – | Unbuffered | 96 | 4 | – | – |
| | | | | 8.0 | 0 | 100 | – | – |
| | | | | 7.0 | 0 | 100 | – | – |
| Morpholine | 8.49 | – | – | 8.0 | 25 | 75 | – | – |
| | | | | 7.0 | 3 | 97 | – | – |
| 1,4-Diaminobutane (putrescine) | 9.2 | 10.65 | – | 7.4 | 0 | 2 | 98 | – |
| Lysine | 8.95 $\alpha$-$NH_2$ | 10.5 $\varepsilon$-$NH_2$ | – | 7.4 | 0 | 3 | 97 | – |
| Lys–Trp–Lys | 7.5 $\alpha$-$NH_2$ | 10.05 $\varepsilon$-$NH_2$ | 11.01 $\varepsilon$-$NH_2$ | 7.4 | 0 | 0 | 45 | 55 |
| Piperazine | 5.55 | 9.81 | – | 7.4 | 1 | 98 | 1 | – |
| 1,2-ethylenediamine | 6.85 | 9.93 | – | 7.4 | 0 | 78 | 22 | – |
| *N,N'*-dimethylethylenediamine | 7.43 | 10.23 | – | 7.4 | 0 | 48 | 52 | – |

[a]$pK_a$ values for Lys–Trp–Lys are based on figures given in references 28 and 29. All others are taken from reference 30.

Abasic sites can also be cleaved near neutral pH by much lower concentrations of compounds with two or more amino groups, such as spermine, spermidine (8), 1,4-diaminobutane (putrescine), lysine (6) and the tripeptide Lys–Trp–Lys (10). Their efficiency is probably due to strong binding to DNA promoted by their positively charged amino groups (16), resulting in a high concentration of unprotonated amine near the abasic sites. Results using Lys–Trp–Lys support this view. At pH 7.4 about half of it is in the active (diprotonated) form, with an uncharged N-terminal amino group, two protonated ε-amino groups and a discharged carboxyl group, giving it a net positive charge (Table 1). Clear evidence that the positive charge is indeed important comes from the finding that $Na^+$ ions inhibit cleavage by Lys–Trp–Lys, due to competition for binding to DNA (10,15).

Thus we expect diamines to constitute useful catalysts for elimination at abasic sites. The $pK_a$ of one amino group should be relatively high, so that it is positively charged at neutral pH and promotes binding to DNA. The $pK_a$ of the other should be lower. We examine three in detail: piperazine, 1,2-ethylenediamine and *N,N'*-dimethylethylenediamine. In each case, protonation of one amino group reduces the availability of the lone pair of electrons on the other, which becomes more difficult to protonate. Consequently, each of these symmetrical compounds has two very different $pK_a$ values. As shown in Table 1, the higher value is very similar in all three compounds, while the lower one steadily increases.

Piperazine at pH 7.4 inflicts only a little non-specific damage on untreated DNA at temperatures up to 90°C, but does cleave abasic DNA (Fig. 3A). The cleavage pattern (Fig. 3B) shows that at 37°C it catalyses β-elimination, but little if any β,δ-elimination, that at 65°C it achieves both β- and β,δ-elimination and that at 90°C β,δ-elimination predominates. However, we always find a significant background of β-elimination, as well as some other products which we cannot identify reliably. Piperazine at neutral pH does not meet our criteria, probably because its $pK_{a1}$ is too low for it to complete clean elimination reactions. Other workers have found that even unbuffered piperazine at 90°C is less efficient than piperidine at cleaving apurinic DNA (17) and a further problem is that samples occasionally fail to migrate from wells during electrophoresis (Fig. 3A, lanes 4 and 9).

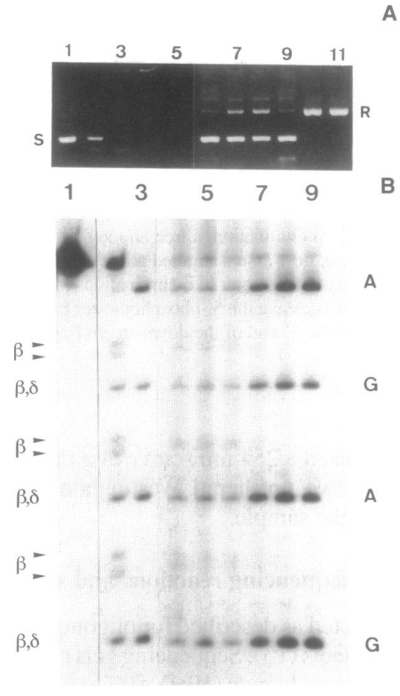


**Figure 2.** Effects of unbuffered piperidine and of morpholine at pH 8.0 on intact and apurinic DNA. (**A**) Assay for non-specific strand breakage. Supercoiled $SK^+$ DNA was incubated with 1 M piperidine or with 3 M morpholine acetate, pH 8.0. Lane 1, normal DNA with no treatment. Lanes 2–5, normal DNA after incubation with piperidine at 90°C for 1, 5, 10 and 30 min. Lanes 6–8, normal DNA after no treatment (lane 6) or after incubation with 3 M morpholine acetate, pH 8.0, for 60 min at 37 or 65°C. Lanes 9–11, as lanes 6–8 but with apurinic DNA. The positions of supercoiled (S) and relaxed (R) plasmids are marked. (**B**) Cleavage mechanisms at apurinic sites catalysed by alkali or by morpholine at pH 8.0. The 5'-labelled apurinic oligonucleotide was run on a 20% sequencing gel after no treatment (lane 1), after treatment with 0.2 M NaOH at 37°C for 15 min (lane 2) or at 65°C for 30 min (lane 3) and after incubation with 3 M morpholine acetate, pH 8.0, for 30, 60 and 120 min at 37°C (lanes 4–6) or 65°C (lanes 7–9). The oligonucleotide was cleaved by either β- or β,δ-elimination at apurinic sites.

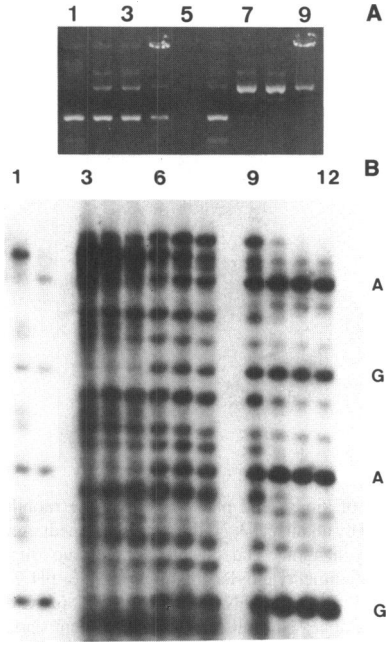


**Figure 3.** Effects of piperazine at pH 7.4 on intact and apurinic DNA. (**A**) Assay for non-specific strand breakage. Supercoiled SK+ DNA was incubated with 100 mM piperazine acetate, pH 7.4, either before or after depurination. Lanes 1–4, normal DNA with no further treatment (lane 1) or after incubation with piperazine acetate for 1 h at 37, 65 and 90°C. Lane 5, empty. Lanes 6–9, as lanes 1–4 but with apurinic DNA. (**B**) Cleavage at apurinic sites catalysed by piperazine at pH 7.4. The 5′-labelled apurinic oligonucleotide was run on a 20% sequencing gel after treatment with 0.2 M NaOH at 37°C for 15 min (lane 1) or 65°C for 30 min (lane 2) and after incubation with 100 mM piperazine acetate, pH 7.4, for 1, 2 or 3 h at 37 (lanes 3–5) and 65°C (lanes 6–8) and at 90°C for 0.5, 1, 2 or 3 h (lanes 9–12).

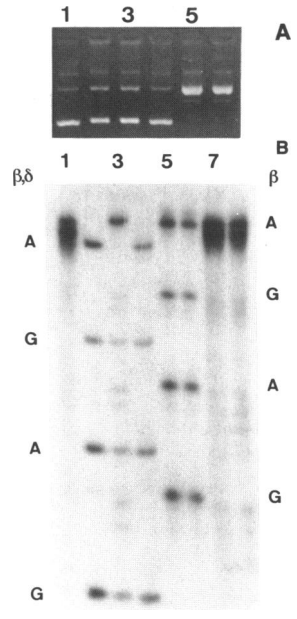


**Figure 4.** Effects of 1,2-ethylenediamine and putrescine on intact and apurinic DNA. (**A**) Assay for non-specific strand breakage. Supercoiled SK+ DNA was incubated with 20 mM 1,2-ethylenediamine acetate, pH 7.4, either before or after depurination. Lanes 1–3, normal DNA with no further treatment (lane 1) or after incubation with 1,2-ethylenediamine for 1 or 2 h at 37°C. Lanes 4–6, as lanes 1–3 but with apurinic DNA. (**B**) Cleavage at apurinic sites catalysed by 1,2-ethylenediamine and putrescine at pH 7.4. The 5′-labelled apurinic oligonucleotide was run on a 20% sequencing gel after no treatment (lane 1), treatment with 1 M piperidine for 30 min at 90°C (lane 2), 0.2 M NaOH at 37°C for 15 min (lane 3) or at 65°C for 30 min (lane 4) or 20 mM 1,2-ethylenediamine acetate, pH 7.4, at 37°C for 1 or 2 h (lanes 5 and 6), 20 mM putrescine acetate, pH 7.4, at 37°C for 1 or 2 h (lanes 7 and 8). The bands due to β- or β,δ-elimination at apurinic sites are marked.

1,2-Ethylenediamine, with a $pK_{a1}$ of 6.85, has virtually no effect on untreated DNA, but can cleave apurinic DNA at 37°C at pH 7.4 (Fig. 4A). The cleavage pattern (Fig. 4B) is clean and shows clearly that these conditions catalyse β- but not δ-elimination. Incubation at 65°C did not significantly affect the results (not shown), which strongly suggests that this reagent does not catalyse δ-elimination. The cleavage bands have slightly lower mobilities than those found with β-elimination catalysed by mild alkali. Bailly and Verly found a similar effect with Lys–Trp–Lys (8), due to reaction between the aldehyde group in the cleavage product and the N-terminal amino group in the tripeptide to form a Schiff base (15). A similar reaction may also occur with 1,2-ethylenediamine. Putrescine, with a much higher $pK_{a1}$, also cleaves apurinic DNA by β-elimination, but it is much less efficient, as very little exists in the monoprotonated, active form at pH 7.4.

*N,N′*-dimethylethylenediamine has a significantly higher $pK_{a1}$ than 1,2-ethylenediamine, which should increase its efficiency as an elimination catalyst. Figure 5A shows that it has no non-specific effects on DNA and Figure 5B shows that its effects on abasic DNA depend upon temperature. The products formed at 37°C appear within 15 min and run just between the β-elimination doublet bands found with mild alkali. After 30–60 min at 90°C the main products coincide precisely with the δ-elimination bands found with strong alkali, and very few others are found. At 65°C both sets of products are found. It appears that this reagent catalyses β-elimination at 37°C, β,δ-elimination at 90°C and both at 65°C, when some minor products, discussed below, also appear.

After β-elimination at an abasic site, the 3′-phosphate on the upstream fragment still has the remnants of the deoxyribose attached to it and bears one negative charge (Fig. 1). After β,δ-elimination, the deoxyribose is completely eliminated and the 3′-phosphate group bears two negative charges. These differences explain the increased mobilities of the 5′-labelled fragments resulting from β,δ-elimination (Fig. 5B). In both cases, however, the downstream fragments start with 5′-phosphate groups. Consequently, if cleavage proceeds exclusively by elimination reactions, an abasic oligonucleotide labelled at its 3′-end should give the same, simple pattern of fragments whether it is cleaved by β- or β,δ-elimination. In practice, however, the 3′-labelled oligonucleotide gave a rather complex pattern of products (Fig. 6). Both *N,N′*-dimethylethylenediamine and hot alkali gave, in addition to strong bands attributable to β,δ-elimination, a variety of weaker bands, indicating some alternative cleavage mechanism. The most likely alternatives are 3′,4′- and 4′,5′-cyclization. These mechanisms are often discounted, although results reported by Lindahl and Andersson (6) suggest that they do occur to a significant extent. 3′,4′-Cyclization produces downstream fragments with 5′-hydroxyl groups (Fig. 7). If the minor bands found in Figure 6 (lanes 1 and 2) represent such fragments,

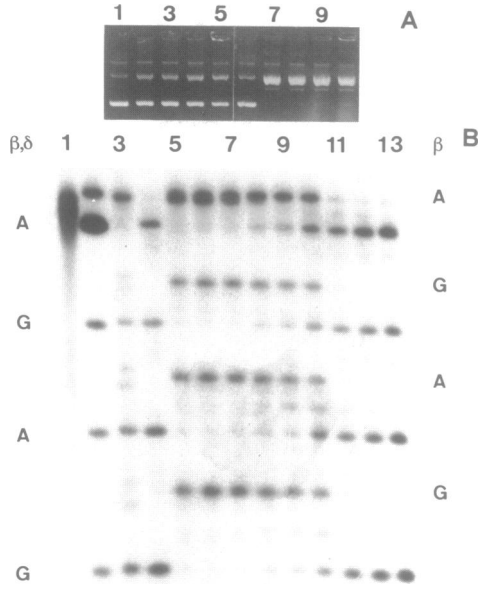


**Figure 5.** Effects of *N,N′*-dimethylethylenediamine on intact and apurinic DNA. (**A**) Assay for non-specific strand breakage. Supercoiled SK+ DNA was incubated with 100 mM *N,N′*-dimethylethylenediamine acetate, pH 7.4, either before or after depurination. Lanes 1–5, normal DNA with no further treatment (lane 1) or after incubation with *N,N′*-dimethylethylenediamine acetate at 37°C for 30 or 60 min (lanes 2 and 3) or at 90°C for 30 or 60 min (lanes 4 and 5). Lanes 6–10, as lanes 1–5 but with apurinic DNA. (**B**) The 5′-labelled apurinic oligonucleotide was run on a 20% sequencing gel after no treatment (lane 1), treatment with 1 M piperidine for 30 min at 90°C (lane 2), 0.2 M NaOH for 30 min at 37 (lane 3) or 65°C (lane 4) or 100 mM *N,N′*-dimethylethylenediamine acetate, pH 7.4, for 15, 30 and 60 min at 37°C (lanes 5–7), 65°C (lanes 8–10) or 90°C (lanes 11–13). The bands due to β- or β,δ-elimination at apurinic sites are marked.

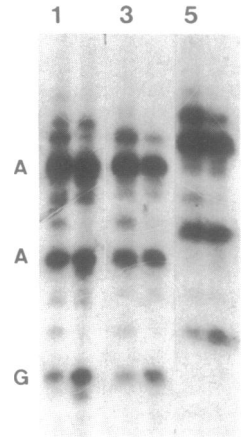


**Figure 6.** Effects of kinase and phosphatase on the mobility of 3′-labelled fragments released by alkali and *N,N′*-dimethylethylenediamine which indicate significant 4′,5′-cyclization. The 3′-labelled apurinic oligonucleotide was cleaved using *N,N′*-dimethylethylenediamine acetate, pH 7.4, for 30 min at 90°C (lanes 1, 3 and 5) or 0.2 M NaOH for 30 min at 65°C (lanes 2, 4 and 6). The products were analysed after no further treatment (lanes 1 and 2), after treatment with T4 polynucleotide kinase and 1 mM unlabelled ATP (lanes 3 and 4) and after treatment with calf intestinal phosphatase for 1 h at 37°C (lanes 5 and 6). Only dephosphorylation altered the mobilities of the bands, indicating that there is no 3′,4′-cyclization, but that there is 4′,5′-cyclization (see text).

phosphorylation should convert them to the main fragments released by elimination reactions and they should disappear. However, the banding pattern was not affected by treating the fragments with a kinase (lanes 3 and 4), which strongly suggests that neither hot alkali nor *N,N′*-dimethylethylenediamine catalyse significant 3′,4′-cyclization. 4′,5′-Cyclization produces downstream fragments with cyclic deoxyribose phosphates on their 5′-termini. Such fragments, like those produced by β-elimination, should be susceptible to phosphatase and in practice the major and minor bands all moved to positions of lower mobility after treatment with alkaline phosphatase (lanes 5 and 6). This confirms the presence of terminal phosphate groups on these fragments and shows that β,δ-elimination is accompanied by a small amount of 4′,5′-cyclization. After β,δ-elimination, upstream fragments terminate in 3′-phosphates, while after 4′,5′-cyclization they terminate in 3′-hydroxyls and consequently have lower mobility. Thus we can attribute the minor bands released from the 5′-labelled apurinic oligonucleotide which run between the β- and β,δ-elimination bands in Figure 5B (lanes 5–13) to 4′,5′-cyclization. These bands are clearly visible at 65°C (lanes 8–10), but are very weak with either short incubations at 37°C (lane 5) or longer incubations at 90°C (lane 13), showing that 4′,5′-cyclization at abasic sites can be minimized by careful choice of conditions. The above results show that *N,N′*-dimethylethylenediamine at neutral pH catalyses efficient cleavage at abasic sites, principally by elimination reactions, the pattern (β or β,δ) depending on temperature. In order to see whether it can also cleave at photoproducts, we tested its effects on labelled oligonucleotides after UV irradiation. Figure 8 shows that after incubating for 30 min at 90°C it successfully detects photoproducts induced by UVC (lanes 3–5), 37°C being inadequate (lane 6). As expected from the results shown in Figure 6, the banding pattern was more complicated when the oligonucleotide was labelled at its 3′-end than when it was 5′-end-labelled, but the essential point is that in both cases the pattern produced by the diamine at neutral pH was very similar to that produced by piperidine at high pH. It is, however, striking that the bands found with both *N,N′*-dimethylethylenediamine and piperidine in lanes 3 and 4 do not coincide with those found with Maxam–Gilbert treatment in lanes 1 and 2. This anomalous mobility, which is especially pronounced in short oligonucleotides such as the one used here, has been noted and discussed before (18). It has been attributed to the presence of pyrimidine (6–4) pyrimidone photoproducts at the 3′-ends of 5′-end-labelled fragments. On this basis we can assign the bands found in lanes 3 and 4 to photoproducts at particular bases and it would appear from these findings that some photoproducts are formed at purines as well as at pyrimidines.

To examine the effects of UVB we used a longer, double-stranded fragment, partly because the damage inflicted by UVB is likely to be less severe, but also because this fragment is more similar to normal DNA. Figure 9 shows that the banding pattern found with *N,N′*-dimethylethylenediamine is virtually identical to that found with piperidine. Thus, it can be used to detect the range of photoproducts which can be detected with piperidine, but without the background degradation. Here the fragments are much longer than those in Figure 8 and the nature of the 3′-terminus has much less effect on electrophoretic mobility (19). Consequently, we can identify photoproducts by reference to Maxam–Gilbert fragments (not shown). The majority of the

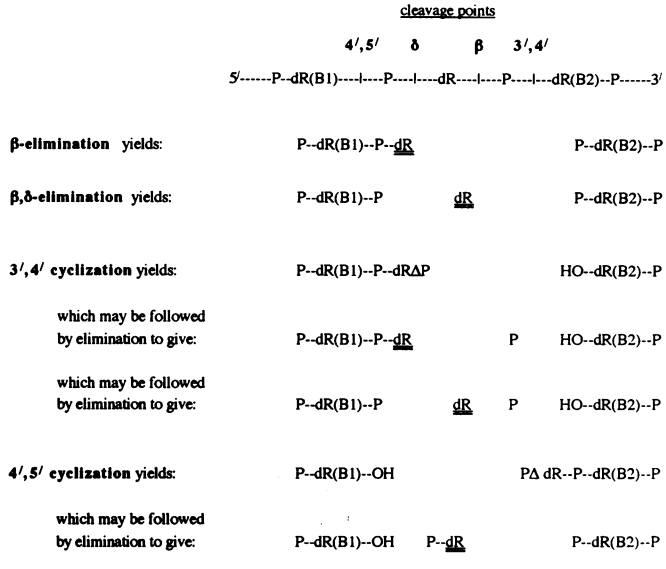


**Figure 7.** Fragments released by elimination or cyclization at an abasic site. The scheme shows how the cleavage mechanism determines the 5′- and 3′-termini of the fragments released. B1 and B2 represent normal bases, dR an abasic site, dR an unsaturated aldehyde. P represents free phosphate, Δ indicates a cyclic phosphate.

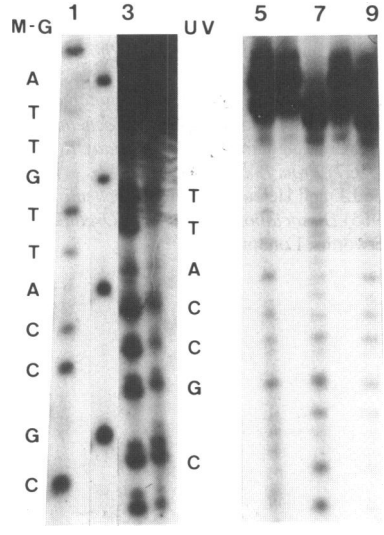


**Figure 8.** Fragments released by *N,N′*-dimethylethylenediamine from a UVC-irradiated oligonucleotide. The oligonucleotide was labelled at either its 5′- or its 3′-end, irradiated with UVC for 35 min and then incubated with *N,N′*-dimethylethylenediamine, pH 7.4, piperidine or NaOH. Lanes 1 and 2 show the reference ladders found with Maxam–Gilbert cleavage of the 5′-labelled, unirradiated oligonucleotide at C and T (lane 1) and at G and A (lane 2). Lanes 3 and 4 compare the effects of 100 mM *N,N′*-dimethylethylenediamine, pH 7.4, at 90°C for 30 min (lane 3) with those of 1 M piperidine at 90°C for 30 min (lane 4). Lanes 5–9 show the patterns obtained with the 3′-labelled oligonucleotide and *N,N′*-dimethylethylene diamine, pH 7.4, at 90 and 37°C (lanes 5 and 6), 0.2 M NaOH at 65°C for 30 min or at 37°C for 15 min (lanes 7 and 8) and 1 M piperidine at 90°C (lane 9).

photoproducts detected in this experiment are at dipyrimidines and are probably (6–4) photoproducts or their Dewar isomers, although we cannot exclude the possibility that there are some other lesions as well.

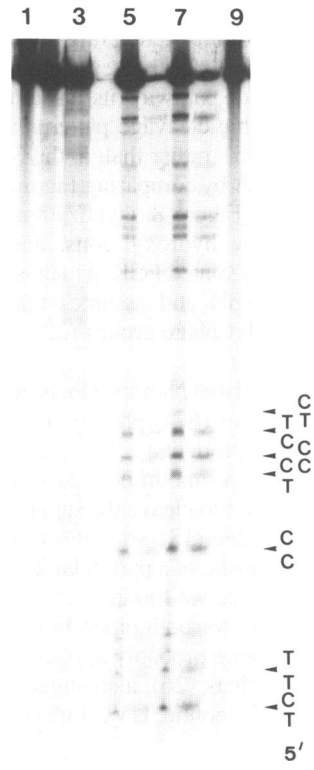


**Figure 9.** Fragments released by *N,N′*-dimethylethylenediamine from UVB-irradiated DNA. A 426 bp 5′-labelled piece of DNA was synthesized as described in Materials and Methods. Lanes 1–3 show the patterns found on a 6% sequencing gel with no further treatment (lane 1) and after incubation at 90°C for 30 min with either 100 mM *N,N′*-dimethylethylenediamine, pH 7.4 (lane 2), or 1 M piperidine (lane 3). Lane 4 is empty. Lanes 5–8 show the patterns found when the fragment was first irradiated for 60 min (lanes 5 and 6) or 120 min (lanes 7 and 8) and then incubated at 90°C for 30 min with either 100 mM *N,N′*-dimethylethylenediamine, pH 7.4 (lanes 5 and 7), or 1 M piperidine (lanes 7 and 8). Lane 9, pattern found with irradiation for 120 min but without chemical treatment.

## DISCUSSION

The main object of this study was to find reagents which can cleave DNA at both abasic sites and photoproducts at neutral pH with minimal background degradation. The most successful one we have found so far is *N,N′*-dimethylethylenediamine, which cleaves abasic sites with none of the background cleavage that piperidine can cause. Its positive amino group promotes binding to DNA, while its uncharged amino group catalyses cleavage. A 100 mM solution, buffered at pH 7.4 at room temperature with acetic acid, gives clean β-elimination after 15 min at 37°C and clean β,δ-elimination after 30 min at 90°C. At 90°C, but not at 37°C, it also cleaves at photoproducts and could be used to distinguish abasic sites from photoproducts in DNA. Presumably it first displaces damaged bases, although we have not examined the mechanism. It does not cause aggregation of DNA, which can be a problem with polyamines (20), and does not have to be removed before samples are applied to sequencing gels. It should prove useful in studies on photoproducts induced in DNA by UV irradiation, especially when the amount of damage is small, as is the case when DNA is damaged by natural sunlight acting alone or in combination with some chemicals used in sunscreens (21).

Some cleavage catalysed by *N,N'*-dimethylethylenediamine proceeds by 4',5'-cyclization, generating downstream fragments bearing cyclic deoxyribose phosphates at their 5'-ends. Although this is a relatively minor pathway, its existence means that the simplest autoradiographic cleavage patterns are obtained when DNA is labelled at the 5'- rather than at the 3'-end, as is clearly shown for apurinic DNA by comparing Figures 5B and 6 and for UV-irradiated DNA in Figure 8. Cyclization also occurs when cleavage is induced by hydroxyl ions, and this means that conventional ligation-mediated PCR, which is based on cleavage by piperidine at high pH and assumes that the downstream fragments all bear 5'-phosphate groups (22), may underestimate some lesions.

It is generally assumed that photoproducts detected by cleavage with piperidine are either (6–4)-photoproducts [i.e. pyrimidine (6–4) pyrimidone photoproducts] (22,23) or their Dewar isomers (1,24,25). However, many modified bases can be displaced by piperidine, which goes on to cleave the sugar–phosphate chain at the abasic site thus created (18), so that sensitivity to piperidine is not necessarily diagnostic of a particular kind of photoproduct. As the pattern of cleavage we found after irradiation with UVB was essentially identical for both piperidine and *N,N'*-dimethylethylenediamine, the same probably applies to the diamine. Our results, like those of others (26), also suggest that, at least in a single-stranded oligonucleotide, UVC induces purine as well as pyrimidine photoproducts.

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