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The biological consequences of oxidized DNA bases

S.S. Wallace

Department of Microbiology and Immunology, New York Medical College, Valhalla, New York 10595, USA.

Agents that damage DNA by means of free radical intermediates produce a spectrum of DNA damages. Thus it is difficult, if not impossible, to determine which particular lesion(s) is responsible for a particular endpoint(s), be it lethality, mutagenesis or carcinogenesis. In order to circumvent this problem, we have chosen to assess the biological consequences of unique modified DNA bases.

Thymine glycol and urea residues as models for oxidative DNA base damage

Thymine glycol and urea residues are good models for free radical-induced DNA damage for a number of reasons. First, both are found as stable radiolysis products in DNA X-irradiated *in vitro* (Teoule *et al.*, 1977) and *in vivo* (Breimer & Lindahl, 1985; Leadon & Hanawalt, 1983). In addition they appear to be formed as a consequence of oxidative stress (Cathcart *et al.*, 1984). Thymine glycol is a good model for minor DNA base modifications since the saturation of the 5,6 double bond alters the base stacking properties of thymine, but the base pairing properties presumably are retained. The urea residue is an example of a fragmented product of the breakdown of hydroperoxides and is a non-instructive DNA lesion.

Both thymine glycol and urea residues can be relatively easily quantitated in DNA. Thymine glycols can be measured by the acetol fragment assay (Hariharan, 1980) as well as by various chromatographic procedures (Teoule *et al.*, 1977; Frenkel *et al.*, 1981; Cathcart *et al.*, 1984; Breimer & Lindahl, 1985*a*). They can also be quantitated by enzyme and antibody assays (Wallace, 1983; West *et al.*, 1982; Leadon & Hanawalt, 1983; Rajagopalan *et al.*, 1984). Urea residues can be quantitated by their susceptibility to various enzymes (Breimer & Lindahl, 1980; Katcher and Wallace, 1983; Kow & Wallace, 1985).

The most important reason for choosing these two modified bases to study with respect to biological consequences is that they can be selectively produced in DNA. Figure 1 shows the production of thymine glycol by osmium tetroxide oxidation of DNA thymine and its subsequent conversion to urea by alkali hydrolysis. Osmium tetroxide has been shown to selectively oxidize DNA thymine (Beer *et al.*, 1966; Frenkel *et al.*, 1981). We have shown that alkali hydrolysis of these oxidized products quantitatively converts them to urea residues (Kow & Wallace, 1985; Ide *et al.*, 1985).

As a point of departure for most of our studies, we have compared thymine glycols and urea residues to apurinic sites. Apurinic sites are models for alkali-labile oxidative DNA damage. They can be detected by both physical and enzymatic methods, and can be selectively produced in DNA by heat/acid treatment (Lindahl & Andersson, 1972).

The questions we wish to ask about these model DNA base lesions are the following: First, can they be detected and quantitated in the background of other DNA damages? Secondly, do these lesions have biological consequences, that is, are they lethal, mutagenic? Thirdly, are they capable of being recognized by putative cellular repair enzymes?

Available methods to quantitate DNA base damages

Various chromatographic procedures have been employed over the years to detect damaged DNA bases in hydrolyzed irradiated DNA. Recently high pressure liquid chromatographic and gas chromatographic mass spectrometry (Dizdaroglu, 1985) have allowed both better resolution and higher sensitivity. One advantage of these procedures is that a wide variety of modified DNA bases can be detected. However, chemical methods suffer from a relative lack of sensitivity and the fact that the modified DNA base must be stable to the hydrolysis procedures used to release it for chemical analysis. More recently enzymes have been used to release nucleosides, nucleotides (Dizdaroglu *et al.*, 1978) or the damaged DNA base (Breimer & Lindahl, 1985*a*) itself. This modification offers the advantage of eliminating the harsh hydrolysis procedures. However in the case of the DNA glycosylases, the base in question must be susceptible to the enzyme used to release it.

For thymine glycol and other thymine ring saturation products, the acetol fragment assay developed by Hariharan and Cerutti (1972) has been extensively used both *in vitro* and *in vivo*. This assay is simple to use and is reproducible. However it requires high specific radioactivity in DNA thymine for sensitivity and measures a spectrum of thymine ring saturation products.

Enzymatic methods are also routinely used (Wallace *et al.*, 1981; Paterson *et al.*, 1981) to quantitate thymine glycol,

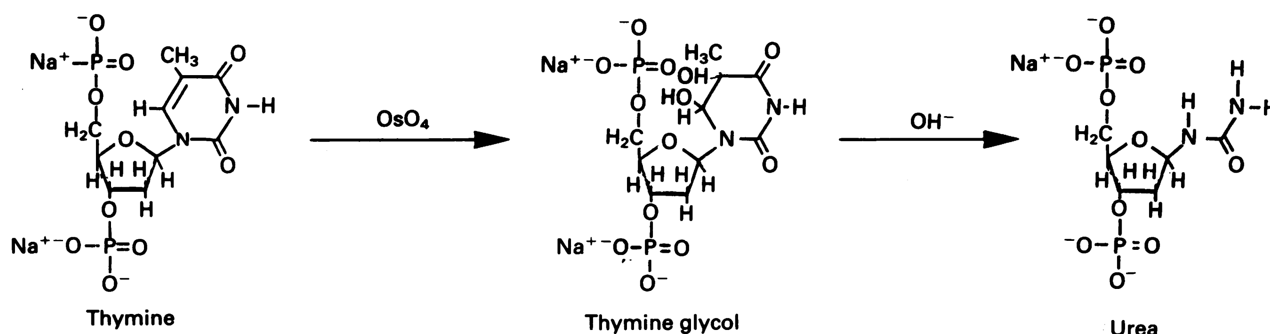


Figure 1 Diagrammatic representation of osmium tetroxide oxidation of thymine to thymine glycol and its subsequent alkali hydrolysis to urea. Reaction conditions have been described (Kow & Wallace, 1985; Ide *et al.*, 1985).

urea residues and other damaged DNA bases. These methods can be made very sensitive especially with supercoiled DNA substrates (femtomoles). They are also very easy to use for *in vitro* studies. One of the shortcomings of using enzymatic methods to quantitate DNA damages is that the enzymes themsevles require purification. Further, the reagent enzymes currently in use, *Escherichia coli* endonuclease III and *Micrococcus luteus* γ-endonuclease, recognize a spectrum of pyrimidine radiolysis products (for review see Lindahl, 1982). Thus unique lesions cannot be quantified. Also with supercoiled substrates, the upper level of measurement is limited by the Poisson distribution. The most important shortcoming in the use of enzymatic procedures to quantitate DNA base damages is that the enzyme-induced nicks must be able to be detected above a background of strand breaks. This is a formidable problem when one is dealing with DNA damaged by agents that produce free radicals such as ionizing radiation and hydrogen peroxide.

In recent years immunochemical methods have received wide-spread use for the detection and quantitation of DNA base damages produced by chemical carcinogens (Strickland & Boyle, 1984; Poirier, 1984). Antibodies to modified DNA bases have also been useful in detecting UV-induced pyrimidine dimers (Van Vunakis, 1980) as well as a number of radiolysis products (Lewis *et al.*, 1978; West *et al.*, 1982*a*, *b*; Leadon & Hanawalt, 1983; Rajagopalan *et al.*, 1984; Fuciarelli *et al.*, 1985). Immunochemical procedures offer a number of advantages. They can be made extremely sensitive, to the femtomole level, and highly specific with respect to the lesion in question. Also the assays are simple and reproducible. Perhaps the most important advantage is, that with a specific antibody, the assay is insensitive to the presence of strand breaks and to a large extent other modified DNA bases. Sensitivity of the immunoassay is limited by the affinity of the antibody and the amount of DNA that can be used in the assay. Also the hapten must be stable to the immunization procedures.

Figure 2 shows the detection of thymine glycol in osmium tetroxide oxidized φX-174 duplex DNA as measued by both the antibody assay (ELISA) and susceptibility to *E. coli* endonuclease III. The production of thymine glycol is linear with respect to osmium tetroxide concentration. Urea residues produced in this same DNA by alkali hydrolysis become susceptible to *E. coli* exonuclease III (Figure 2) and as testimony to the specificity of the anti-thymine glycol antibody, reactivity is now eliminated. These data demonstrate the sensitivity and specificity of both the antibody and enzyme assays.

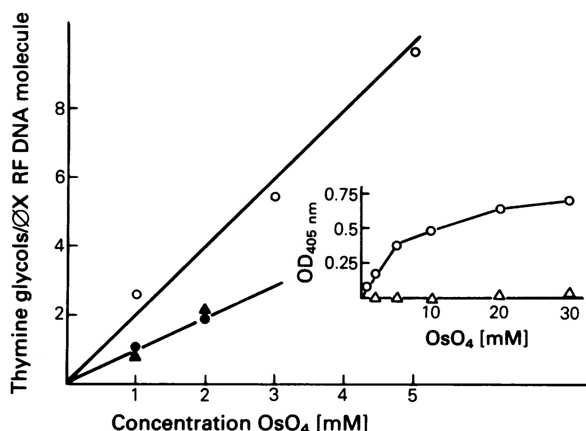


Figure 2 Quantitation of thymine glycols (●, ○) and urea residues (▲, △) in φX-174 duplex DNA by the production of enzyme-sensitive sites (●, ▲) and reactivity with anti-thymine glycol antibody (○, △). The number of *E. coli* endonuclease III-sensitive sites in thymine glycol-containing DNA (●) was determined according to the procedure of Katcher and Wallace (1983). The number of *E. coli* exonuclease III-sensitive sites in urea-containing DNA (▲) was determined according to the procedure of Kow and Wallace (1985). Reactivity of anti-thymine glycol antibody with thymine glycol-containing DNA (○) or lack thereof with urea-containing DNA (△, insert) was determined by ELISA (Rajagopalan *et al.*, 1984). The number of antibody-reactive sites was standardized to the acetol fragment assay.

PM2 bacteriophage as a model for assessing the production and biological consequences of DNA damage produced by ionizing radiation

Based on the classic studies of Taylor and Ginoza with φX-174 (1967) and David Freifelder with the T-even phages and bacteriophage λ (1968), we (Moran & Wallace, 1985) X-irradiated PM2 bacteriophage under conditions that minimized damage to the protein coat of the phage. The number of lethal hits per radiation dose given to the phage was assessed by the production of plague-forming units. The DNA was then extracted from the irradiated phage and various classes of damage were measured. These included single strand breaks, double strand breaks, alkali labile lesions and thymine ring saturation products. When the phage were irradiated under oxic conditions, for each lethal X-ray hit there were 2.09 single strand breaks, 1.06 alkali labile lesions, 0.4 thymine ring saturation products, and 0.11 double strand breaks in the PM2 genome. These data gave the rate of production of these four classes of damages related to the number of lethal hits but they did not give the contribution of each of these classes to the X-ray induced lethality of the PM2 bacteriophage. In order to ascertain this information, we used a PM2 transfection system. Here each unique type of lesion was separately introduced into the DNA and the inactivation efficiency determined by using transfection as an endpoint. As a model for thymine ring saturation products, thymine glycol was produced in PM2 DNA *in vitro* by osmium tetroxide oxidation. As a model for alkali labile sites, apurinic sites were produced by heat/acid treating the DNA. The results of these data showed that the inactivation efficiency of thymine glycol and apurinic sites were essentially equal, that is, it took about 7 or 8 lesions to produce a lethal event in PM2 transfecting DNA.

Thus when one takes into account the inactivation efficiency of thymine glycol, as a model for thymine ring saturation products, and apurinic sites, as models for alkali labile lesions, together with the inactivation efficiencies previously determined (Van der Schans *et al.*, 1973) for single and double strand breaks, the estimated contribution to lethality of each of these four classes of damages can be calculated. These results are diagrammatically depicted in Figure 3. It can be seen that thus far we have accounted for some 34% of the inactivating events produced in PM2 bacteriophage by X-irradiation under oxic conditions. Most

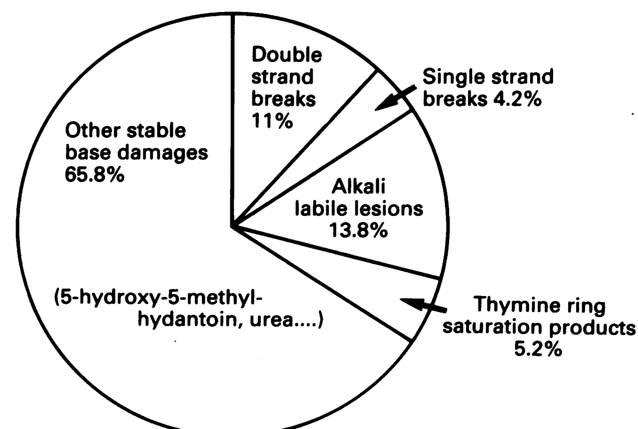


Figure 3 Contribution of various classes of DNA damage to the X-ray inactivation of PM2 bacteriophage (O_2).

of the remaining inactivating events are caused by as yet unidentified stable base damages.

Base damages as replicative blocks to DNA synthesis *in vitro*

It was initially surprising to us that a non-instructive lesion like an apurinic site had the same inactivation efficiency as a relatively minor base modification as exemplified by thymine glycol. However, it must be remembered that in repair proficient cells such as the ones used here, the efficiency of inactivation of a particular lesion includes both the ability of the lesion in question to constitute a replicative block or some other inactivating event as well as the efficiency of repair of that lesion. Thus it is possible that the apurinic site could constitute a major replicative block to the synthesizing apparatus, however, because of the number of apurinic endonucleases present in the cell, this inactivation efficiency might be low. In contrast, it might be that thymine glycols are minor blocks to DNA replication, however, they might persist in greater numbers because they are repaired less efficiently. In order to address the first part of this question, we (Ide *et al.*, 1985) asked whether thymine glycols and urea residues, like apurinic sites (Sagher & Strauss, 1983; Schaaper *et al.*, 1983), were replicative blocks to DNA polymerases *in vitro*.

In order to do this, single stranded DNA was isolated from bacteriophage M13, oxidized with osmium tetroxide to produce thymine glycols, and annealed to a primer. We then asked whether this primer-template containing thymine glycols was efficient as a substrate for DNA polymerase I of *E. coli*. We found that the presence of thymine glycols in the template strand significantly reduced the incorporation of triphosphates into DNA and that the inhibition was dependent upon the number of thymine glycols present in the template strand. Further, when the thymine glycols were alkali hydrolyzed to urea residues, the same results were obtained. Thus both thymine glycols and urea residues were efficient inhibitors of DNA polymerase I *in vitro*.

In order to determine whether this inhibition was at the site of the thymine glycol or urea residue in the template strand, high resolution sequencing gels were used to analyze the newly synthesized strand. The procedure we used, which was pioneered by Strauss for UV-induced damage (Moore & Strauss, 1979), is shown in Figure 4. The sequence of the template strand is first determined by the stop sites in the newly synthesized strand using reaction mixes containing one nucleotide with a dideoxy 3′ terminus (Sanger *et al.*, 1977). These are depicted for the four bases in lanes 1, 2, 3, 4. In order to determine potential stop sites opposite the lesion, the damaged, primed template strand is incubated with the four normal nucleoside triphosphates and any stop sites in the newly synthesized strand are observed on the gel. If stops occur opposite T in the template strand, which in this case would be the putative thymine glycol, then a band would show up adjacent to the A band determined by the dideoxy sequencing as is depicted in lane 5. If no stops occur, then the newly synthesized DNA would be highly polymerized and would show up in a high molecular weight control band as depicted in lane 6.

When the M13 DNA template strand contained thymine glycols, stops were observed opposite the putative thymine glycol. Thus DNA polymerase I was capable of inserting a base, A, opposite the thymine glycol but polymerization could continue no further. Similar results were obtained with T4 DNA polymerase. When the thymine glycol residues in the template strand were converted to urea residues, stops occurred at one base prior to the putative urea residue indicating that either polymerization could extend no further or that the proof reading 3→ 5 exonuclease of DNA polymerase I removed the non pairing base after it was inserted opposite the non-instructive lesion. The results obtained with urea residues are similar to results that had

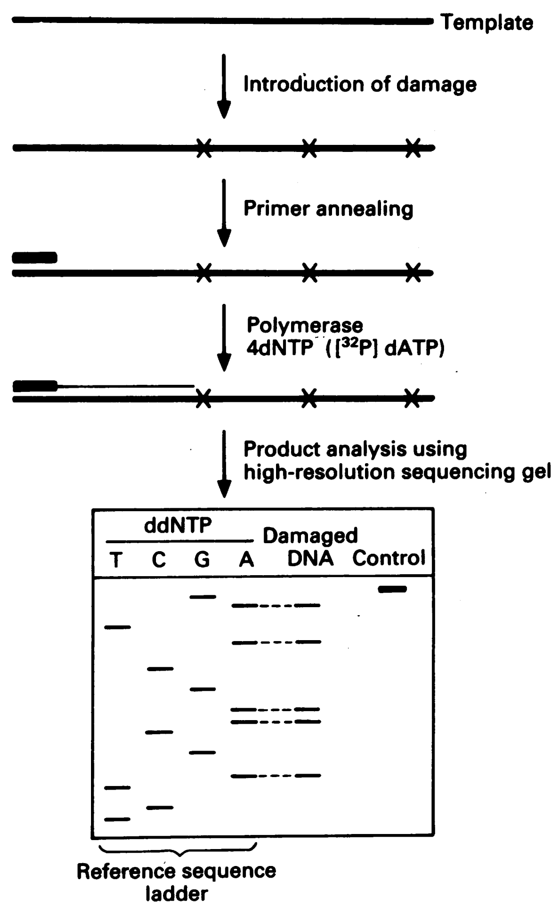


Figure 4 Detection of termination sites opposite DNA base damages on high resolution sequencing gels.

been obtained previously with the non-instructive apurinic site (Strauss *et al.*, 1982; Sagher & Strauss, 1983, 1985). Thus both thymine glycols and urea residues appear to be replicative blocks to DNA polymerases *in vitro* suggesting that they would be lethal lesions *in vivo*, as we had already observed for thymine glycols. Further, the data predict that thymine glycols would not be mutagenic lesions since A appears to be incorporated opposite this putative damage *in vitro* (Clark & Beardsley, 1986). In fact, in a collaborative study we (Hayes, Huang, Wallace and LeClerc, unpublished observations) have shown, using an M13 *lacZ* forward mutation system, that thymine glycols are not mutagenic. Sequencing analysis revealed that most of the mutants produced by osmium tetroxide oxidation were the result of C→ T transitions implicating oxidized cytosine residues as the mutagenic lesion produced by this oxidizing agent.

Repair of oxidative DNA base damage in *Escherichia coli*

Table I summarizes the four enzymes that have been isolated from *E. coli* that recognize oxidized DNA bases. These enzymes act via an excision repair mechanism where the damaged base is either recognized by an endonucleolytic or glycosylic reaction. If the reaction is glycosylic, the damaged base is removed as a free base and the resulting abasic site is recognized by a cellular apurinic endonuclease which incises adjacent to this site. The incising event is followed by exonuclease action which removes the damaged abasic residue from the DNA, then by polymerization and ligation. If the reaction is endonucleolytic, the glycosylic step is omitted but the remainder of the series of reactions is the same.

Table I *Escherichia coli* enzymes that recognize oxidized DNA bases[a]

Enzyme	*Base(s) recognized*	*DNA substrates*
endonuclease III	thymine glycol urea 5-hydroxy-5-methylhydantoin methyltartronyurea dihydrothymine (duplex DNA)	X-irradiated DNA heavily UV-irradiated DNA DNA treated with: osmium tetroxide osimum tetroxide followed by alkali hydrolysis potassium permanganate hydrogen peroxide sodium bisulfite-low concentration (apurinic and apyrimidinic DNA)
exonuclease III	urea (duplex DNA)	DNA treated with: osmium tetroxide followed by alkali hydrolysis hydrogen peroxide (apurinic and apyrimidinic DNA)
formamidopyrimidine-DNA glycosylase	MeFAPyr FAPyr (duplex DNA)	alkylation followed by alkali hydrolysis X-irradiated DNA
uracil-DNA glycosylase	uracil (single stranded or duplex DNA)	DNA treated with: sodium bisulfite-high concentration nitrous acid Any DNA containing uracil

[a]For reviews and references therein see Breimer & Lindahl, 1985*b*; Lindahl, 1982; Linn, 1982; Teebor & Frenkel, 1983; Wallace, 1983.

The formamidopyrimidine DNA glycosylase (Chetsanga & Lindahl, 1979; Breimer, 1984) is an enzyme that has been shown to recognize two fragmented purine products in duplex DNA. The enzyme has no associated endonucleolytic activity. Uracil DNA glycosylase (Lindahl *et al.*, 1977) recognizes the oxidative deamination product of cytosine, uracil (DaRoza *et al.*, 1977; Duncan & Weiss, 1982). This enzyme acts on single or duplex DNA and releases the free base. The enzyme has no associated endonucleolytic activity.

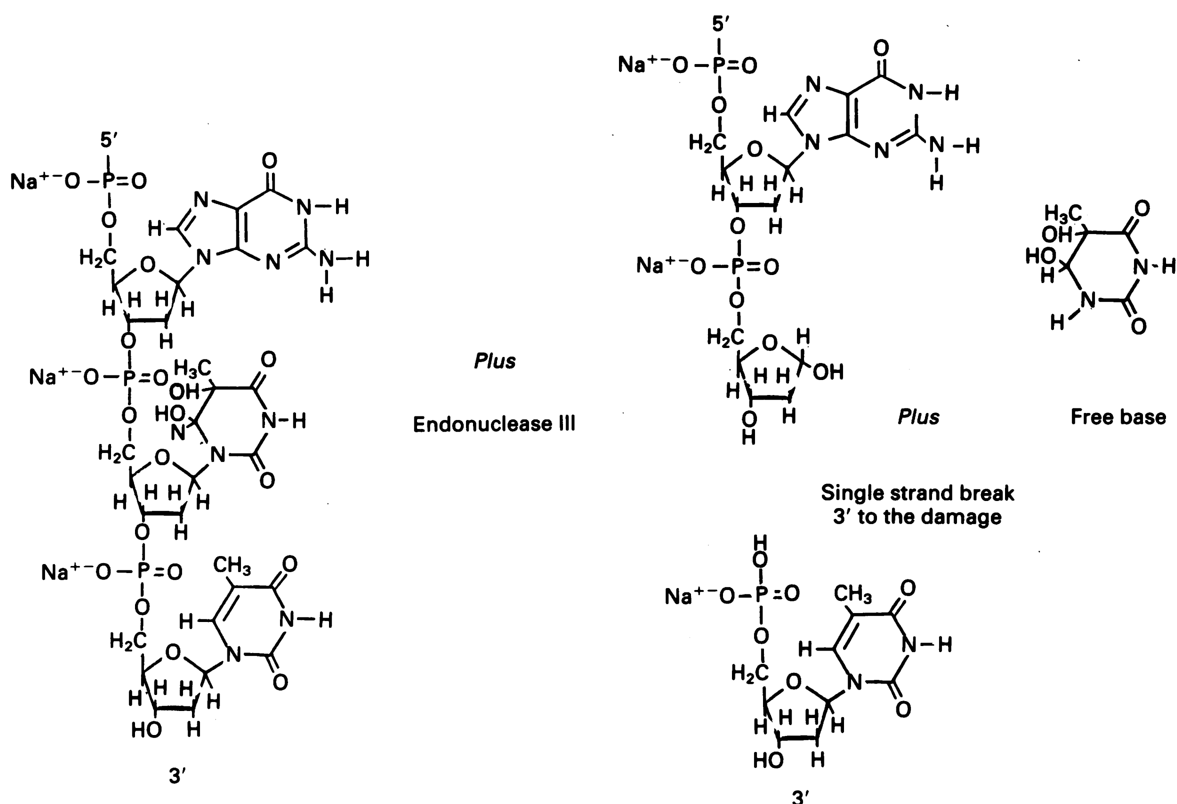
Endonuclease III was identified in our laboratory as an activity that incised X-irradiated DNA (Strniste & Wallace, 1975). It was subsequently purified using a heavily UV-irradiated DNA substrate by Radman (1976), and its purification, characterization and substrate specificity was determined in a number of laboratories including our own (Armel *et al.*, 1977; Gates & Linn, 1977; Demple & Linn, 1980; Katcher & Wallace, 1983; Breimer & Lindahl, 1984). The enzyme acts exclusively on duplex DNA and releases, in a glycosylic reaction, thymine glycol and urea residues, as well as other breakdown products of pyrimidines. In addition, it contains an associated class I apurinic activity that nicks on the 3′ side of the resulting apyrimidinic site leaving a poor substrate for DNA polymerase I (Warner *et al.*, 1980; Katcher & Wallace, 1983). The action of this enzyme on a DNA substrate containing thymine glycol is depicted in Figure 5. Whether an enzyme has endonucleolytic or glycosylic activity can be assessed by determining whether or not the damaged base is released as a free base or as a nucleoside or nucleotide. The specific damaged bases thus far shown to be released by endonuclease III are listed in Table I.

Wild type *E. coli* cells contain about 300–400 molecules of endonuclease III (Breimer & Lindahl, 1984) which accounts for all the known activity on thymine ring saturation products. Recently mutants defective in this activity, *nth*, have been isolated (Cunningham & Weiss, 1985). Surprisingly these mutants are not sensitive to X-rays, hydrogen peroxide or a number of other DNA damaging agents. Since the *in vitro* properties of this enzyme have been well delineated, and it is known to act on X-irradiated and oxidized DNA substrates, it was of interest to know if the enzyme was actually capable of removing damaged DNA bases *in vivo*. In order to ascertain this, we (Laspia, Petrullo & Wallace, submitted) treated the duplex form of φX-174 DNA with osmium tetroxide to generate thymine glycols. The DNA was then transfected into either wild type hosts or hosts lacking endonuclease III. As we observed with PM2 phage DNA and its host *Alteromonas espejiana* (Moran & Wallace, 1985), thymine glycols are lethal lesions in φX-174 DNA when transfected into an *E. coli* host. It took about 12 thymine glycols to produce a single inactivating event in duplex φX transfecting DNA. However, when this same DNA was transfected into *nth* mutants lacking endonuclease III, the transfecting DNA was inactivated at a two to three fold greater rate. Thus endonuclease III appears to recognize and remove thymine glycols *in vivo* functioning to repair these lesions in the wild type host.

Exonuclease III is an enzyme having multiple activities (Weiss, 1981) that was isolated a number of years ago as a by-product of the purification of DNA polymerase I (Richardson & Kornberg, 1964; Richardson *et al.*, 1964). Exonuclease III also requires duplex DNA and its primary endonucleolytic activity is a class II apurinic activity that nicks on the 5′ side of the AP site leaving a good substrate for DNA polymerase I (Warner *et al.*, 1980).

During the course of our studies with oxidized DNA substrates we (Kow & Wallace, 1985) made the observation that exonuclease III incised an osmium tetroxide oxidized duplex DNA substrate that had been subjected to alkali hydrolysis. Thus it appeared that exonuclease III was capable of recognizing urea residues and perhaps other fragmented pyrimidine products in an *in vitro* reaction. We showed that this activity was endonucleolytic not glycosylic thus resembling its activity on apurinic sites. The activities of endonuclease III and exonuclease III on a DNA substrate containing urea residues in depicted in Figure 6.

Wild type *E. coli* cells contain about 3,500 exonuclease III molecules per cell which accounts for between 85 and 90% of the cellular AP endonuclease activity (Ljungquist *et al.*, 1976; Yajko & Weiss, 1975). However mutants defective in exonuclease III, *xth*, are only slightly sensitive, if at all, to agents such as alkylating agents that introduce AP sites into DNA (Ljungquist *et al.*, 1976; Yajko & Weiss, 1975). Wild type cells contain about 98% of the cellular $3' \rightarrow 5'$ exonuclease activity (Milcarek & Weiss, 1982) yet *xth* mutants are not sensitive to agents, such as X-rays, that introduce frayed single stranded regions into DNA that would be susceptible to clean up by this activity (Yajko & Weiss,



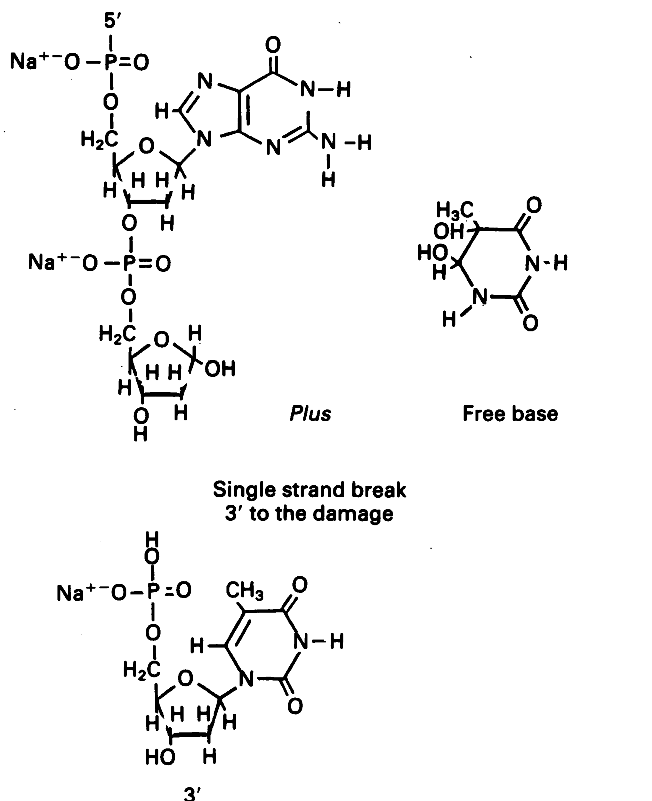


Figure 5 Enzymatic release of thymine glycol from one strand of a duplex DNA molecule by *E. coli* endonuclease III.

1975). The principal phenotype associated with *xth* mutants is their extreme hypersensitivity to hydrogen peroxide (Demple *et al.*, 1983). *Xth* mutants are also sensitive to near UV light (Sammartano & Tuveson, 1983) which appears to inactivate cells via a free radical mechanism.

Perhaps the hypersensitivity of *xth* mutants to hydrogen peroxide is partially associated with their inability to remove urea or fragmented pyrimidine residues from oxidized DNA. In fact, we (Kow & Wallace, 1985) showed *in vitro*, that exonuclease III is capable of recognizing stable residues generated in DNA by hydrogen peroxide. It was therefore of interest to examine whether wild type cells containing exonuclease III were capable of removing urea residues from DNA treated *in vitro*. In order to do this, we (Laspia, Petrullo & Wallace, submitted) oxidized duplex φX-174 DNA with osmium tetroxide and subsequently alkali hydrolyzed it to produce urea residues. This DNA was then used to transfect either wild type of *xth* mutants lacking exonuclease III. We found that urea residues had the same inactivation efficiency as thymine glycols in wild type cells, and DNA containing urea residues was inactivated at the same rate in xth mutants lacking exonuclease III. Thus urea residues are also lethal lesions *in vivo* having approximately the same inactivation efficiency as thymine glycols and apurinic sites but other enzymes such as endonuclease IV may be able to substitute for exonuclease III *in vivo*.

Summary and future perspectives

The biological consequences of apurinic sites, thymine glycols and urea residues are summarized in Table II. All three are replicative blocks to *in vitro* DNA synthesis, can be recognized by a variety of repair enzymes and are lethal lesions in viral transfecting DNA. Apurinic sites are mutagenic lesions *in vivo* while thymine glycols are not. Preliminary results (Petrullo & Wallace) suggest that urea residues are mutagenic in single stranded f1 DNA using a forward mutation assay system.

From these data, one can begin to generalize about the biological consequences of unique DNA base damages. It appears that non instructive lesions, whether they be derived from purines (apurinic sites) or pyrimidines (urea residues), are capable of being both lethal and mutagenic. Even more interestingly, a product such as thymine glycol, that is only a minor base modification of thymine, is capable of blocking DNA synthesis *in vitro* and being lethal *in vivo*. Thus it would be of interest to investigate the consequences of other stable DNA products that contain minor modifications that might have the potential to be mutagenic and/or carcinogenic.

With the exception of the cytosine deamination product, uracil, a mutagenic lesion, very little is known about the consequences of free radical induced damage to cytosine or to purines. The reason that we were able to address these questions with DNA containing thymine glycols or urea residues was that we could quantitate these damages, and more importantly, we could selectively produce them. Even in this case, it turned out that the mutagenic lesion produced by osmium tetroxide was a minor cytosine oxidation product. Thus a major stumbling block is the ability to introduce unique DNA lesions. Since chemical modification is notorious for introducing a spectrum of DNA damage, we (Ide, Melamede & Wallace, submitted) are currently attempting to engineer stable radiolysis products into DNA. Our approach is to chemically synthesize the desired modified nucleoside triphosphate and to use it as a substrate for DNA polymerase *in vitro*. Certain stable O-alkylated thymidine triphosphates have been shown to be substrates for DNA polymerase I (Singer *et al.*, 1983). Using DNA polymerase I from *Escherichia coli*, we have been able to incorporate dihydrothymidine triphosphate into DNA. By using different DNA templates unique substrates can be produced for both enzyme and transfection assays. These studies should enable us to assess the potential recognition of dihydrothymine by repair enzymes as well as its lethal and mutagenic potential.

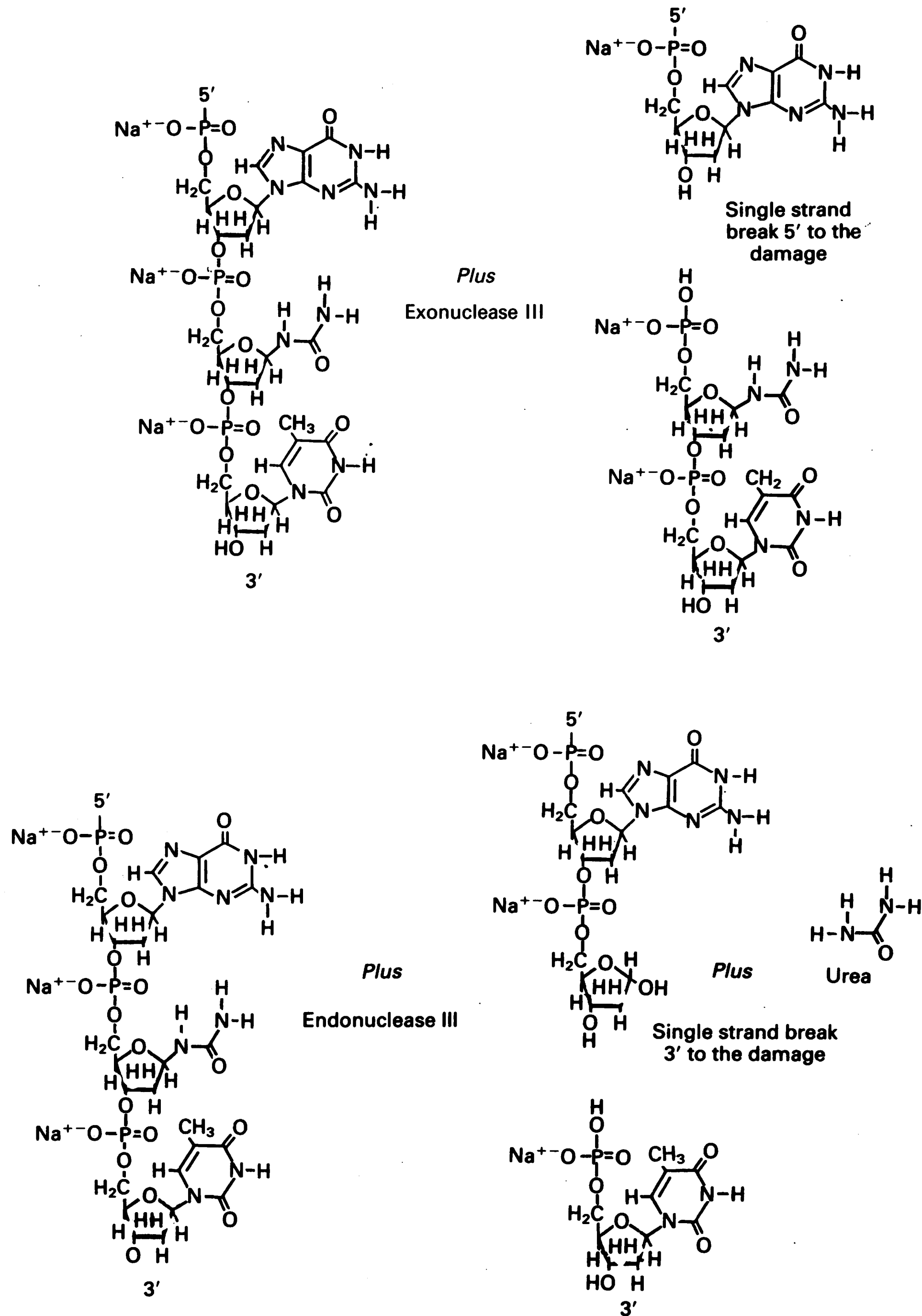


Figure 6 Enzymatic action of *E. coli* endonuclease III or exonuclease III on one strand of a duplex DNA molecule containing urea residues.

Table II Biological properties of unique DNA lesions

Lesion	*In vitro replicative blocks*	*Recognized by repair enzymes*[d]	*Lethal lesion*	*Mutagenic lesion*
apurinic site	Yes:[a] *E. coli* pol I T4 DNA pol AMV pol DNA polα	Yes: multiple species found ubiquitously	Yes:[g] duplex PM2 and ϕX-174 DNA single stranded ϕX-174 DNA	Yes:[j] single stranded ϕX-174 and M13 DNA
thymine glycol	Yes:[b] *E. coli* pol I T4 DNA pol	Yes: *E. coli* endo III *M. luteus* γ-endo[e] enzymes from Drosophila and mammalian cells	Yes:[h] duplex PM2 and ϕX-174 DNA single stranded ϕX-174 DNA	No:[k] single stranded M13 and fl DNA
urea residue	Yes:[c] *E. coli* pol I T4 DNA pol	Yes: *E. coli* exo III *S.* cerevisiae endo E[f] *E. coli* endo III *M. luteus* γ-endo[e] mammalian urea-DNA glycosylase	Yes:[i] duplex ϕX-174 DNA single stranded ϕX-174 DNA	?

[a]Sagher & Strauss, 1983; [b]Ide *et al.*, 1985; Rouet & Essigman, 1985; Hayes & LeClerc, 1986; Clark & Beardsley, 1986; [c]Ide *et al.*, 1985; Hayes & LeClerc, 1986; [d]Breimer & Lindahl, 1985*b*; Lindahl, 1982; Linn, 1982; Teebor & Frenkel, 1983; Wallace, 1983; [e]Kow & Wallace, unpublished observations; [f]Chang & Wallace, unpublished observations; [g]Kudrna *et al.*, 1979; Schaaper & Loeb, 1981; Moran & Wallace, 1985; Laspia *et al.*, unpublished observations; [h]Hariharan *et al.*, 1977; Moran & Wallace, 1985; Laspia *et al.*, unpublished observations; [i]Laspia *et al.*, unpublished observations; [j]Schaaper & Loeb, 1981; Kunkel, 1984; [k]Hayes *et al.*, unpublished observations.

We hope to extend this approach to the study of the biological consequences of stable purine and cytosine products produced by free radical intermediates.

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