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DNA archiving on FTA^R paper: photosensitizer-initiated attacks as models of aging

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Abstract

Long-term aging of dry DNA is thought to be due to the attack of diverse cascades of reactive species with probably no one single initiator of the cascades explaining all circumstances. Photosensitizer-initiated reactions from hematoporphyrin and riboflavin were used to generate two model systems of reactive species around dry DNA in order to understand such systems and how to block them. Damage was assessed using plasmid DNA as a substrate with an in-situ microgel electrophoretic technique. The DNA damage profiles from the photosensitizer-initiated reactions on dry media are markedly different from those in aqueous systems, so water tension is an important factor in these pathways. Photodynamic hematoporphyrin was oxygen-dependent but not that of riboflavin. This indicates that indirect type II pathways, probably via singlet oxygen were more important for hematoporphyrin than for riboflavin. The application of liquid paraffin wax, in an attempt to reduce oxygen exposure in hematoporphyrin-initiated attacks, did not retard photodynamic damage, but a nitrogen atmosphere did. In both the absence and presence of oxygen, the DNA protection offered by tris-urate (the anti-free radical component of the FTA^R matrix) and tris-caffeine (to a certain extent) indicated that most DNA attacks were via electrophilic species. Overall, protection of dry archived DNA from spontaneously reactive species such as free radicals appears to be a real issue and, as expected, the predominant species in air appears to involve oxygen but not exclusively or necessarily so and in at least one, probably atypical circumstance, oxygen was protective. This has implications for long-term storage of DNA and for an understanding of the pathways of reactive species attack on DNA.

Keywords: DNA archiving; Riboflavin; Hematoporphyrin; Singlet oxygen; Tris-urate

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1. Introduction

Over time, DNA is susceptible to many forms of environmental damage and destruction [1,2]. These include hydrolytic cleavage of phosphodiester bonds and base linkages, oxidative damage to base and sugar moieties, and pyrimidine dimerization (greatly accelerated by ultraviolet irradiation). Dry storage of DNA eliminates a number of degradative processes which are water-dependent [3,4]. In the long-term preservation of dry DNA, the aerobic atmosphere (from reactive or reduced forms of oxygen [1,5]) and the presence of reactive species can produce oxidative damage akin to molecular aging. But the spontaneous rates of many of these processes can be extremely slow in relation to biological lifetimes [6]. An accelerated aging model was thus required.

A model system was developed using plasmids as targets and photosensitizer reactions of riboflavin and hematoporphyrin to produce a variety of reactive-species attacks on DNA as a mimic of aging processes. Such photodynamically driven reactions can be readily induced within solid media like paper containing DNA. The two major pathways (Type I and Type II pathways) by which each of the above photosensitizers can react with DNA can be differentiated by their dependence on oxygen. The imposition of anaerobic conditions (e.g., a nitrogen atmosphere) should disfavour Type II attacks such as those singlet oxygen-mediated with respect to Type I attacks. Under aqueous environments, riboflavin has been reported to attack mainly via the Type I mechanism [7,8] and hematoporphyrin [9,10] reacts mainly via the Type II mechanism.

The observations that photodynamic attack is electrophilic [11–13] suggest that the greater the electron donor ability of the molecule, the better the chances that the free radical will react with a specific molecule. Among the four DNA bases, guanine has the lowest ionization potential and is predictably, the most susceptible to photodynamic and singlet oxygen oxidation [14,15]. This implies therefore, that inhibition of electrophilic attack would require a structural family member with a greater electron-donor ability than guanine.

This study investigated the potential of tris-urate (the anti-free radical component of the FTA^{R} paper) to assume this role as urate has been found to be a very effective singlet oxygen scavenger compared to deoxynucleosides [16], and probably has a lower ionization potential than guanine [17].

2. Materials and methods

2.1. Microgel electrophoresis

A microgel electrophoresis technique [18] using a 1-mm-thick gel and 2-mm diameter DNA-containing paper discs under neutral conditions was used to assay the damage. Strand breaks and the incision of repair enzymes were readily quantified with plasmids, as nicking of the sugar-phosphate backbone converts supercoiled DNA to the relaxed (open circular) forms.

2.2. Plasmid preparation and irradiation conditions

The plasmid pUC19 (2686 bp) was propagated in *Escherichia coli* TOP F′ and the DNA isolated using alkali lysis followed by RNase treatment.

Tris (160 mM)-urate (40 mM), the main trap for reactive species in the FTA matrix was loaded onto cotton paper (density 0.0187 g cm^{-2}, thickness 0.45 mm) at 1 ml per 20 cm^2 paper and the papers were allowed to dry thoroughly overnight at room temperature. The tris-urate paper was used in conjunction with the same cotton paper without the reagent mixture designated as plain paper in this experiment. Tris (160 mM)-caffeine (40 mM) was loaded onto similar cotton paper in the same way.

Riboflavin and hematoporphyrin were, respectively, loaded onto cotton paper at 1 ml per 40 cm^2 and the papers were air dried. Discs of 2-mm diameter were punched out from the photosensitizer-loaded papers. Approximately 3–5 μg plasmid DNA was then loaded onto each disc and air dried before illumination. The exposure of plasmid DNA on photosensitizer-bearing discs to irradiation was performed on glass slides in contact with melting ice. The light source was an 8-W white fluorescent lamp (Minilite ML-8 series) at a distance of 6 cm from the discs. The intensity of the lamp output at this distance was determined to be 3.02 kW m^{-2} by potassium ferrioxalate actinometry of Murov [19]. Irradiation times for riboflavin and hematoporphyrin were, respectively, standardized for 30 and 60 min.

2.3. Repair enzymes

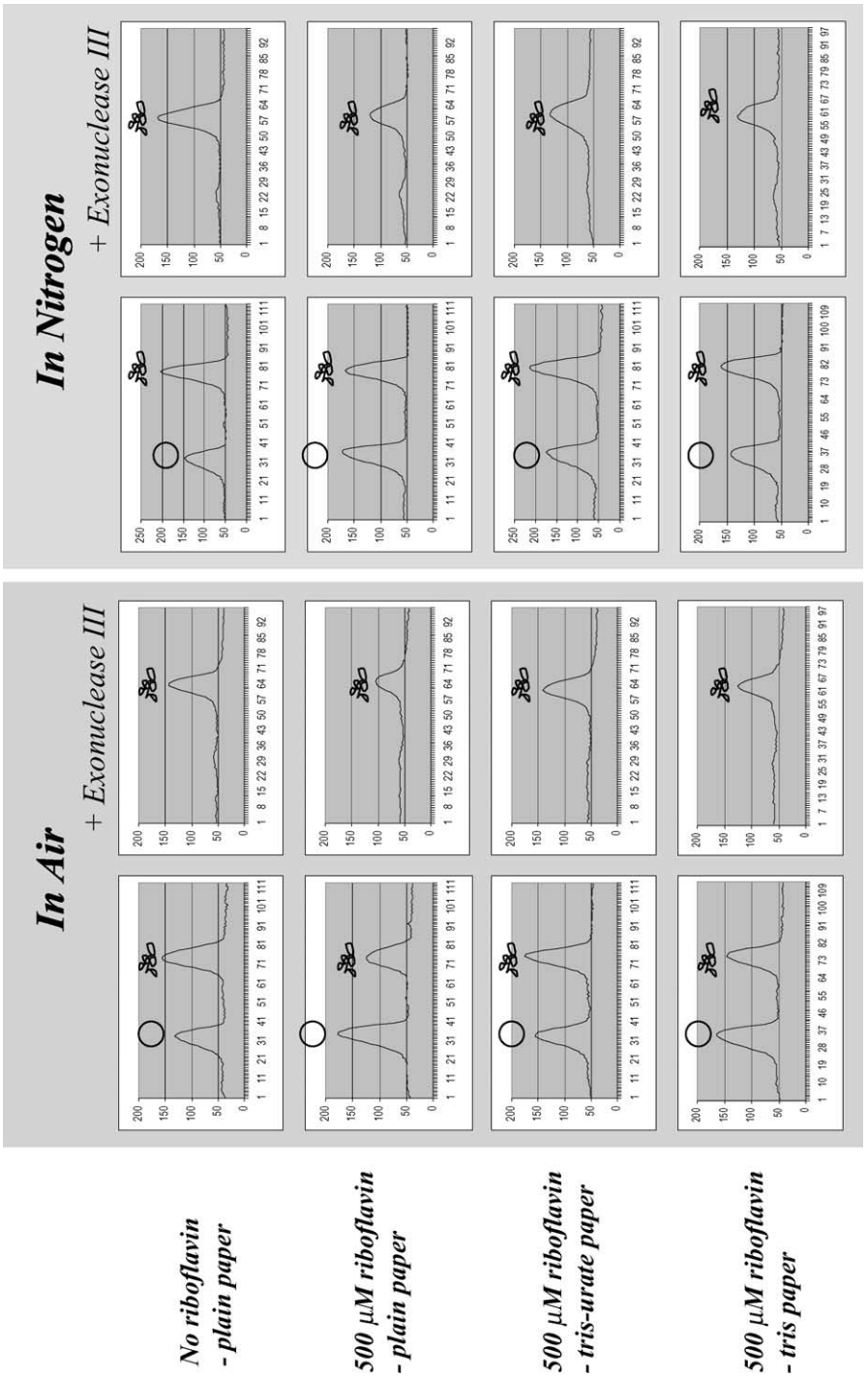
Fpg protein (1 U/μl, from Trevigen) has been demonstrated to recognize 8-hydroxyguanine, formamidopyrimidines and sites of base loss (AP sites). Exonuclease III (200 U/μl, from Epicentre Technologies) recognizes AP sites only.

The irradiated discs were each incubated with the respective repair enzymes (Fpg protein 1 μl per disc), Exonuclease III, 0.2 μl per disc) in a 10-μl digest mix of the appropriate buffer at 37 °C for 2 h.

3. Results and discussion

From studies in free solution, the damage induced by many photosensitizers is dominated by base modifications sensitive to the repair endonuclease Fpg protein (formamidopyrimidine-DNA glycosylase) [20], and most of these base modifications are oxidised guanines like 8-hydroxyguanine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine [9,21]. Low DNA hydration levels as with dry stored DNA on paper was found in this study to be unfavorable for formation of oxidised guanines, but was a lesser hindrance to the formation of DNA strand breaks and AP sites.

The reduction in DNA modifications under anaerobic conditions was not appreciable from photodynamic riboflavin (Fig. 1), which supports the earlier indications that the main operating mode of photosensitizing riboflavin is by a direct Type I reaction. Interestingly, in an oxygen-deficient atmosphere, there was no impedance to depurination, implying that damage could be mitigated by oxygen (air). On the other hand, the


In Air
+ Exonuclease III
In Nitrogen
+ Exonuclease III
No riboflavin
- plain paper
500 μM riboflavin
- plain paper
500 μM riboflavin
- tris-urate paper
500 μM riboflavin
- tris paper

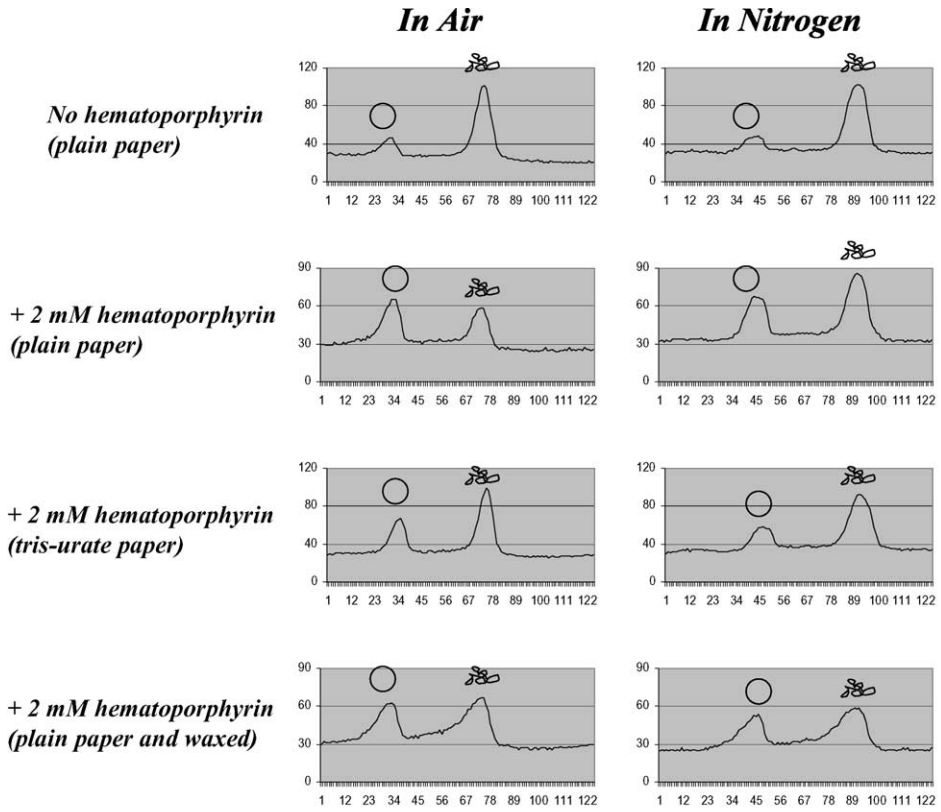


Fig. 2. Effect of photodynamic hematoporphyrin at very low oxygen pressure (paraffin wax encasing) and comparison to paper discs without wax encasing. The waxed paper discs were rinsed in xylene before being electrophoresed. Irradiation in air showed that strand breakage was not retarded by the wax encasing, and irradiation in nitrogen showed little or no reduction in damage. The uncased plain and tris-urate paper discs showed significant reduction in strand breakage from irradiation in nitrogen. Raw data expressed arbitrarily as uncorrected units of fluorescence intensity (y-axis) with distance (x-axis). [○ = relaxed, circular plasmid; ☙ = supercoiled form].

obvious reductions in strand breaks (Fig. 2) and AP (apurinic/apyrimidinic) sites (not shown) from photodynamic hematoporphyrin in nitrogen atmosphere are indications of extensive Type II mechanisms.

Hematoporphyrin-bearing discs containing plasmids were pre-coated with paraffin wax by dipping in molten wax before irradiation in air or in nitrogen. Controls on plain and tris-

Fig. 1. Effects of photodynamic riboflavin in air and in nitrogen (oxygen-deficient) atmosphere. Representative plasmid scan profiles on plain paper without riboflavin (top row), on plain paper with 500 μM riboflavin (second row), on tris-urate paper with 500 μM riboflavin (bottom row). Irradiation was in air (left panel) and in nitrogen (right panel). DNA content expressed arbitrarily as units of fluorescence intensity (y-axis) with distance (x-axis) [○ = relaxed, circular plasmid; ☙ = supercoiled form].

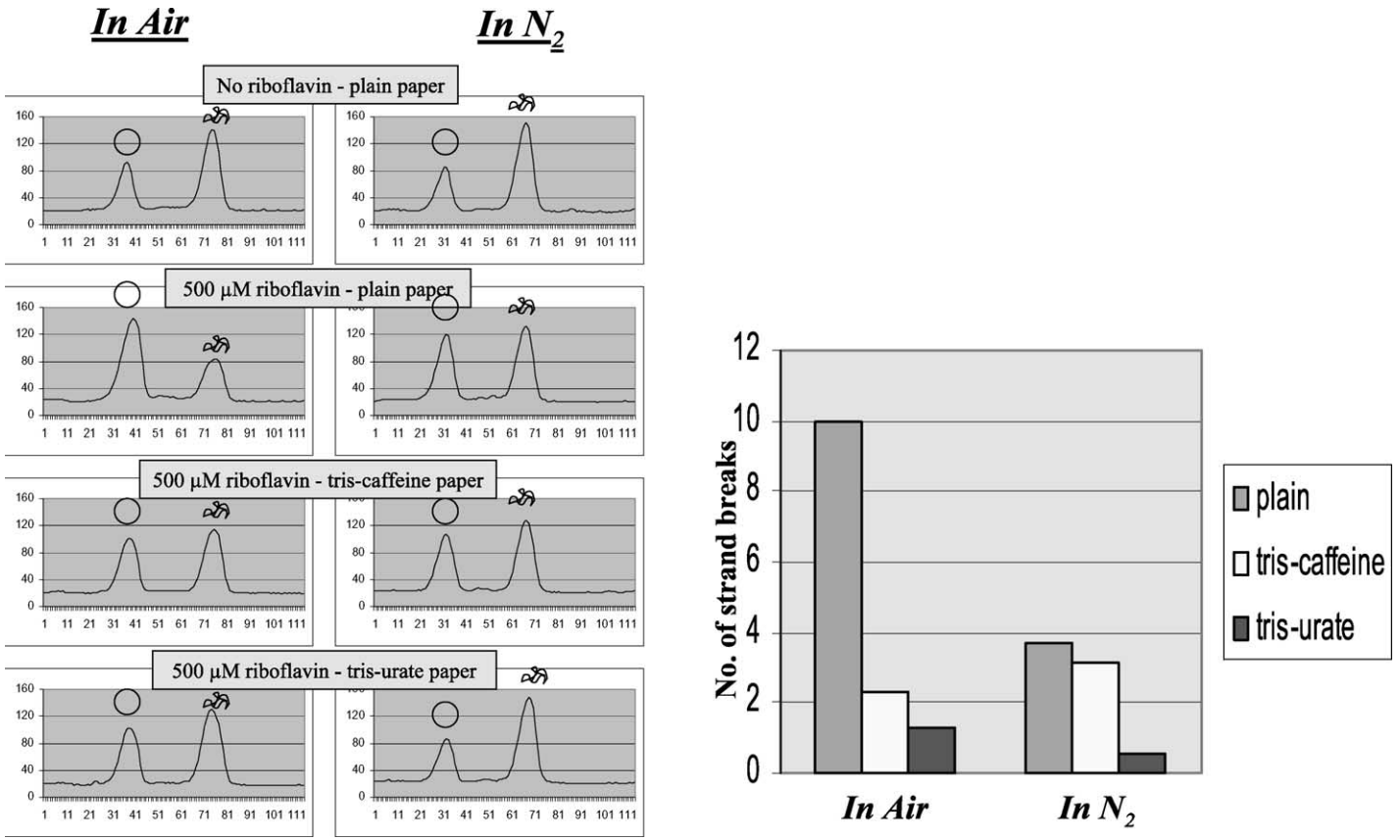


Fig. 3. Photodynamic riboflavin on plain, tris-caffeine and tris-urate papers induced in air and in nitrogen. Left panel: Representative plasmid scan profiles induced in air (left) and nitrogen (right), respectively. Raw data expressed arbitrarily as uncorrected units of fluorescence intensity (*y*-axis) with distance (*x*-axis). [○ = relaxed, circular plasmid; ∽ = supercoiled form]. Right panel: Corresponding number of strand breaks.

urate papers were performed simultaneously. The paraffin wax coated discs showed little or no reduction in strand breaks in a nitrogen atmosphere, whereas damage was significantly decreased for the unwaxed plain and tris-urate paper discs in a nitrogen atmosphere. The low oxygen exposure provided by small pockets of air trapped inside the paraffin encasing contributed the same effect to free radical damage as the relatively much higher oxygen availability of freely moving air (refer to Fig. 2).

The protective effects of tris-urate and tris-caffeine (to a certain extent) against photodynamic riboflavin damage was obvious (Figs. 1 and 3). In air irradiation, when

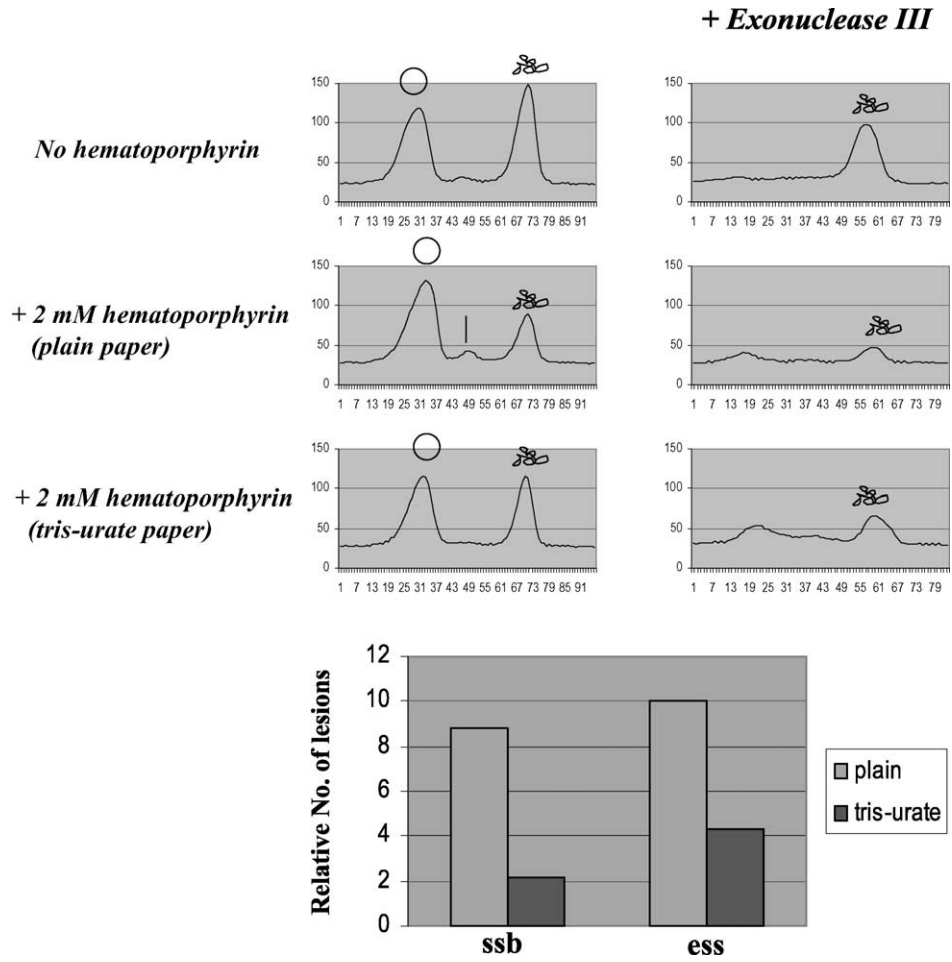


Fig. 4. Effects of photodynamic hematoporphyrin on plain and tris-urate papers. Representative plasmid scan profiles after hematoporphyrin + light, and subsequent treatment of irradiated profiles with exonuclease III (top panel); and corresponding number of single-strand breaks (ssb) and AP sites (ess) shown in bottom panel. Raw data expressed arbitrarily as uncorrected units of fluorescence intensity (*y*-axis) with distance (*x*-axis). [○ = relaxed, circular plasmid; | = linear form; ❧ = supercoiled form].

compared to tris-caffeine, the protection from tris-urate was approximately 2 times greater in terms of strand breakage, and approximately 1.2 times better in terms of depurination. In a nitrogen atmosphere, the differences were more pronounced, protection from tris-urate being six-fold greater in terms of strand breaks, and approximately 2.3 times better in terms of depurination.

Protection by tris-urate against the reactive species from photodynamic hematoporphyrin was also quite significant. There was a bigger loss of superhelicity on the plain papers with observable linear plasmid forms, but on the tris-urate papers, strand breaks were reduced by 25% with linear forms not observable (Fig. 4). The numbers of AP sites were approximately 2.3-fold higher on the plain papers compared to the tris-urate papers.

4. Conclusion

The FTA^{R} paper has been demonstrated to be an effective long-term DNA storage device from considerations of free radical attacks from aging simulators, biological assault [18] and UV exposure [18]. The results have highlighted an important issue in long-term protective strategies against DNA degradation, that is, an analogue (perhaps even a 'designer' analogue) with a clearly lower ionization potential than guanine or even urate will be protective. The use of anaerobic storage conditions will not be preventive against the direct attack of Type I reactions, and even the use of restricted oxygen access will not retard the singlet oxygen mediated reactions of Type II radicals although absolute nitrogen does. The absence of free liquid water, although inhibitive to enzymatic activity and microbial growth, is no blanket protection to free radical attacks on DNA, but it changes the relative importance of the attacking agents. Finally, as free radical reactions are often light initiated, light shielding will be an important consideration for long-term DNA preservation.

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