ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 111 (1997) 205–213

Journal of PHOTOCHEMISTRY AND PHOTOBIOLOGY A: CHEMISTRY

In vitro photochemical damage to DNA, RNA and their bases by an inorganic sunscreen agent on exposure to UVA and UVB radiation

Hisao Hidaka [a,*], Satoshi Horikoshi [a], Nick Serpone [b,*], John Knowland [c]

[a] *Department of Chemistry, Meisei University, Hino, Tokyo, Japan*

[b] *Department of Chemistry and Biochemistry, Concordia University, Montreal, Quebec, H3G 1M8, Canada*

[c] *Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, UK*

Accepted 24 July 1997

Abstract

The fate of DNA, RNA and their pyrimidine and purine bases was examined on exposure to UVA and UVB radiation in the presence of a physical sunscreen agent (TiO_2 anatase/rutile particles) to assess the potential damage that such an agent may cause on contact with such substrates. DNA and RNA were partially decomposed and the bases were converted to carbon dioxide (nitrogen atoms to ammonia and nitrate ions) in a Pyrex reactor under conditions simulating UVA and UVB sunlight. The physical and chemical damage inflicted on DNA and RNA was also confirmed by scanning electron microscopy and gel permeation chromatography. © 1997 Elsevier Science S.A.

Keywords: DNA; In vitro photochemical damage; RNA; TiO_2; UVA; UVB

1. Introduction

Sunlight radiation reaching the terrestrial surface comprises UVA, UVB, visible and IR radiation. UVB (290–320 nm) radiation has long been recognized as the principal component of sunlight causing erythema of the skin and has been implicated in structural and cellular skin damage (elastosis, actinic keratosis, telangiectasis and skin cancers) [1]. To protect human skin from such damage, a variety of sunscreen lotions have been formulated and commercialized. These topical sunscreens provide a protective layer of exogenous UV chromophores on the skin surface to absorb or block UV radiation before it can penetrate into the epidermis and affect endogenous UV chromophores such as deoxyribonucleic acid (DNA). Therefore, sunscreens reduce significantly endogenous photochemistry and subsequent photobiology [2]. Presently, the most widely used sunscreen agent in the world is octylmethoxycinnamate (OMC) [2]; earlier, many commercial formulations contained *p*-aminobenzoic acid (PABA). Both have been used as chemical absorbers (chemical filters) to block UVB radiation. The latter was discontinued when the potential adverse effects of irradiated PABA were recognized [3]. Another popular sunscreen agent being examined is octyldimethyl-PABA (padimate-O) which appears to be harmless in the dark, but becomes mutagenic on exposure to sunlight, attacking DNA directly [4–6]. Indeed, several sunscreen-active ingredients, with PABA being the most efficient, are very good triplet sensitizers converting harmless triplet oxygen ($^3O_2$) into singlet oxygen ($^1O_2$), a well-known cytotoxic species [7]. Some irradiated chemical filters either increase the rate of formation of potentially carcinogenic DNA photoproducts, such as cyclobutane-type pyrimidine dimers [8], or undergo photochemical changes that result in a loss of UVB blocking ability [9].

The UVA sunlight component (320–400 nm) has been thought to be relatively harmless. It is now acknowledged, however, that it contributes significantly to actinic premature skin aging, dryness and exfoliation, dermatological photosensitivity and skin cancer [10,11]. Moreover, utilization of sunscreen agents that predominantly block UVB radiation may, in fact, accentuate skin damage [12,13]. A UV protecting agent that is opaque to both UVA and UVB radiation, biologically inert, chemically inert, cosmetically acceptable, compatible with conventional components of sunscreen formulations, stable under the conditions of use (a criterion not met by most organic sunscreen agents [14–18]) and resistant to water and perspiration is therefore desirable. Titanium dioxide (titania, TiO_2) is a suitable material [1,20–22], and was stated in 1978 by an OTC Advisory Review Panel on sunburn prevention and treatment drug products [23], "to

* Corresponding authors. E-mail: serpone@vax2.concordia.ca; e-mail: hidaka@chem.meisei-u.ac.jp

PII S1010-6030(97)00229-3

be an effective opaque chemical for use as a *physical sunscreen* because it reflects and scatters both UV (290–400 nm) and visible (400–700 nm) radiation, rather than absorbing the sun's rays, thereby providing a barrier for sun-sensitive individuals". Several commercial sunscreen lotions claim to contain no chemical filters. The Panel also concluded that titania was both safe and effective for sunscreen use.

Photoexcited titania particles are cytotoxic (in vitro) to HeLa cells and suppress their growth when implanted in nude mice [24]; T-24 human cancer cells can be destroyed in vitro and in vivo by illuminated titania [25]. Of interest was the finding that TiO_2 particles were observed on the surface of the cells and inside the cells. Confirming the work of Fujishima and coworkers [25,26], Knowland [27] found, from in vitro studies on human cells in culture using TiO_2 irradiated with simulated sunlight, that DNA is damaged. Wamer [28] also reports that UVA-excited TiO_2 is photocytotoxic to skin fibroblasts and is accompanied by photo-oxidative damage to cellular ribonucleic acid (RNA). Such damage to endogenous chromophores probably occurs by a process implicating radical species.

To the extent that such radicals are produced when TiO_2 particles are illuminated with UVA and/or UVB light and that TiO_2 particles have been seen in the interior and exterior of cell walls [26], the question about the "safe" utilization of this material in sunscreen lotions begs exploration, despite the suggestion that the photocatalytic activity can be controlled by coating the particles with an inorganic hydroxide, e.g. amphoteric $Al(OH)_3$, in the Kemira process [29], and that these in vitro findings have not been confirmed in studies using in vivo animals or clinical models [30].

For the past several years, we have been interested in the fate of nitrogen in amino acids and other N-containing products (surfactants, phenols) [31]. As part of these systematic studies, we report the effect(s) of a commercial titanium dioxide material (Degussa P25 TiO_2; 80% anatase, 20% rutile; Brunauer–Emmett–Teller (BET) surface area, approximately 55 $m^2 g^{-1}$) when in contact with DNA, RNA and their pyrimidine and purine bases on irradiation at wavelengths below 380 nm. The average size of the TiO_2 particles is about 20–40 nm, which falls within the recommended 20–50 nm optimum range for sunscreen formulation [22].

2. Experimental details

The pyrimidine (cytosine, thymine and uracil) and purine (adenine and guanine) bases, together with DNA (from herring sperm; average molecular weight, around 10^6–10^7 g mol^{-1}; $A_{260}/A_{280} \sim 1.43$) and RNA (from yeast; average molecular weight, approximately 25 000 g mol^{-1}) examined for their photo-oxidative degradation, were obtained from Tokyo Kasei Co. Ltd. The titanium dioxide (P25 TiO_2) was supplied by Degussa AG (Germany). Calf thymus DNA (Pharmacia Biotech; $A_{260}/A_{280} = 1.456$) was used to examine the effect of irradiated titania on DNA strands by scanning electron microscopy (SEM). All solutions were prepared using deionized distilled water (pH 6.33).

Oxygen-saturated aqueous solutions of the pyrimidine and purine bases (0.1 mM; 50 ml; pH 6–7) containing 100 mg TiO_2 were illuminated in a Pyrex reactor at wavelengths longer than 290 nm with a 100 W Hg lamp (see Fig. 1; Toshiba SHLS-1002A). NH_4^+ ions were analysed with a JASCO ion chromatograph, equipped with a CD-5 conductivity detector, using a Y-521A cationic column and a 4 mM solution of HNO_3 as the eluent; NO_3^- ions were also assayed by ion chromatography with an I-524 anionic column; the eluent was a mixture of phthalic acid (2.5 mM) and tris(hydroxymethyl)aminomethane (2.3 mM) with the pH adjusted to pH 4. Nitrate ions from the oxidative damage to DNA were determined by a Shodex I-524A anionic column with 10 mM phthalic acid as eluent. The temporal evolution of carbon dioxide was assayed with an Ohkura gas chromatograph (model 802) fitted with a Porapak Q column and thermal conductivity detector; He gas was used as the eluent. The peroxide value (POV) of the photodegraded aqueous mixtures (0.1 ml aliquots; 50 ml solutions; 100 mg TiO_2) was measured in an iso-octane–acetic acid solvent mixture (ratio, 1 : 4) using the KI procedure [32].

Gel permeation chromatograms following the photolysis (Hg lamp delivering 1.454 mW cm^{-2} at 360 nm; irradiation period, 0–12 h) of 25 mg of DNA or RNA in 50 ml of H_2O in the presence of 100 mg of titania particles were obtained using a JASCO high performance liquid chromatography (HPLC) instrument with an RI-930 refractive index detector and an Asahipak GF-510 HQ (Shodex®) column with water as the eluent. The photocatalysed degradation of DNA or RNA solution (pH 3.38 and pH 3.42 respectively) was performed using a 75 W Hg lamp (Toshiba SHL-100UVQ-2) delivering 1.247 mW cm^{-2} at 310–400 nm; control experiments were also performed in the absence of TiO_2. Assays of carbon dioxide and POV were carried out as described above. Analyses of nitrate and phosphate ions were carried out by ion chromatography as described above with 10 mM phthalic acid solution.

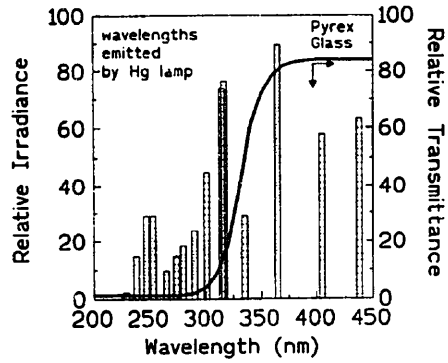


Fig. 1. Relative irradiance of the Hg lamp used in photolysis and relative transmittance of the Pyrex reactor. Note that only UVA (320–400 nm) and UVB (290–320 nm) are used in this work.

The physical/chemical damage to DNA by irradiated TiO_2 nano-particles was probed by SEM or transmission electron microscopy (TEM); 5 mg of calf thymus DNA (sodium salt) was placed in a dispersion containing 20 mg of TiO_2 and 15 ml of H_2O in a 75 ml vial and irradiated for 0, 1 and 3 h without agitation. Subsequently, the solutions were filtered through a 0.22 μm membrane filter. The residual solid TiO_2/DNA was examined by SEM using a Hitachi S-570 electron microscope (30 Å resolution) or by TEM with a Hitachi H-800 electron microscope (1.4 Å resolution).

3. Results and discussion

Titanium dioxide normally exists in two crystalline forms: anatase and rutile. In the 400–700 nm region, titania particles reflect and scatter light, resulting in the expected $1/\lambda^4$ dependence [33], thereby making TiO_2 an excellent physical screen against certain photodermatoses (e.g. porphyria) that occur at visible wavelengths [34,35]. However, nano- and micro-crystallites of titania absorb significantly and, to a much lesser extent, scatter UVA and UVB radiation [24,25], i.e. they absorb at wavelengths below 385 nm (absorption threshold, 3.20 eV; bandgap energy) for anatase and below approximately 405 nm (3.02 eV) for rutile. The anatase polymorph has been shown repeatedly to be a very photoactive UV semiconductor, and has been examined extensively as a photocatalyst for the mineralization of a variety of environmental organic pollutants [36,37]. The rutile TiO_2 polymorph is also a good photocatalyst [38].

Typically, the absorption of radiation of suitable energy (see above) by TiO_2 nano- and micro-crystallites leads to a low-energy, non-vertical, indirect electronic transition from the valence band to the conduction band (indirect bandgap of anatase, 3.20 eV) and to direct, vertical transitions at higher energies (wavelengths of less than 380 nm) [39].

$$\{Ti^{IV}-O^{2-}-Ti^{IV}=\}-OH+h\nu \rightarrow \{Ti^{III}-O^{\bullet-}-Ti^{IV}=\}-OH \quad (1a)$$

that is

$$TiO_2+h\nu \rightarrow (e^-/h^+) \rightarrow e_{cb}^- + h_{vb}^+ \quad (1b)$$

$$e_{cb}^- \rightarrow e_{tr}^- \{\text{as } Ti^{III}\} \quad (1c)$$

$$h_{vb}^+ \rightarrow h_{tr}^+ \{\text{as } {}^{\bullet}OH_{surf}\} \quad (1d)$$

These transitions form bound excitons (bound electron–hole pairs, Eq. (1b)) which, subsequent to their separation and migration (Eq. (1b)) towards the crystallite surface, while spanning various lattice and surface defects, ultimately yield surface-trapped electrons (e_{tr}^-) and holes (h_{tr}^+) in a very short time (less than 10^{-11} s [40]), which are then poised to initiate photoreductions and photo-oxidations. The trapped holes have been identified with surface-bound $^{\bullet}OH$ radicals which can combine to yield H_2O_2 [41,42].

$$\{Ti^{III}-O^{\bullet-}-Ti^{IV}=\}-OH \leftrightarrow \{Ti^{III}-O^{2-}-Ti^{IV}=\}-{}^{\bullet}OH \quad (2)$$

The $^{\bullet}OH$ radical causes cellular damage in vivo [43] and, although it does not diffuse from the surface of TiO_2 particles, its dimeric product H_2O_2 can diffuse for considerable distances and produce serious damage [44].

To assess how DNA and RNA may be affected in a dispersion of aqueous titania (TiO_2) illuminated by UVA and UVB radiation, we examine first how the corresponding pyrimidine and purine bases (Table 1) in DNA and RNA are affected under otherwise identical conditions.

3.1. Pyrimidine and purine bases

The temporal evolutions of NH_4^+ and NO_3^- ions from the conversion of nitrogen atoms in uracil (Ura), thymine (Thy) and cytosine (Cyt) are illustrated in Fig. 2(a)–(c) respectively. The formation of NH_4^+ from Ura is prompt (approximately 0.009 min^{-1}), reaches a maximum value and subsequently decreases at longer irradiation periods; possibly NH_4^+ ions are oxidized at these longer times. A similar behaviour is displayed by Cyt (Fig. 2(c)). Nitrate ion formation (0.0015 min^{-1}) is slow initially, but evolves rapidly after approximately 5 h of illumination in greater yields than NH_4^+ ions (18% versus approximately 5%: $NO_3^-/NH_4^+ \approx 4$ after 30 h of irradiation).

Ammonium ions evolve slowly initially on irradiation of Thy (0.0003 min^{-1}), whereas nitrate ions are detectable only after a 5 h induction period (Fig. 2(b)); the yields are 31% for NO_3^- and 6.5% for NH_4^+ (NO_3^-/NH_4^+ ratio approximately 5 : 1 after 30 h). By contrast, NH_4^+ ion formation from the illumination of a Cyt/TiO_2 dispersion is faster (0.007 min^{-1}), reaching a maximum value after approximately 8 h; subsequently, NH_4^+ may be oxidized on further irradiation to 30 h (Fig. 2(c)). Evolution of NO_3^- is slower (0.0025 min^{-1}); at the longest irradiation time, the NO_3^-/NH_4^+ ratio is approximately unity.

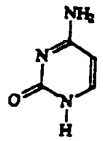
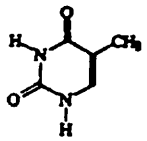
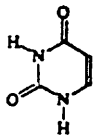
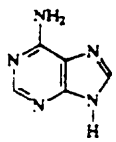
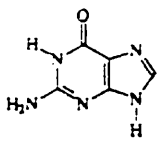
The conversion of the amino group and ring nitrogens in adenine, produced by the TiO_2-photocatalysed process, is depicted in Fig. 3(a); NH_4^+ ions evolve rapidly (0.0059 min^{-1}) and, after a time lag of approximately 2 h, NO_3^- ions begin to form (0.00097 min^{-1}). Yields of both ionic species are approximately 18%–20%.

The photocatalysed conversion of guanine produces nitrate ions (0.00030 min^{-1}) in greater yields than ammonium ions (0.0026 min^{-1}) after a 30 h illumination period (NO_3^-/NH_4^+ ratio approximately 1.7); however, in the first 8 h of illumination, a slightly greater quantity of NH_4^+ is formed (see Fig. 3(b)).

3.2. DNA and RNA

Fig. 4(a) illustrates the temporal behaviour during the formation and decay of peroxides on illumination of aqueous solutions of DNA and RNA in the presence of titania parti-

Table 1
Structures and rates of formation of NH_4^+ and NO_3^- ions in oxygen-saturated aqueous TiO_2 dispersions for some pyrimidine and purine bases

Compound	Structure	k (10^{-3} min^{-1})		Percentage yield[a]	
		NH_4^+	NO_3^-	NH_4^+	NO_3^-
Pyrimidine bases					
Cytosine		7.1 ± 1.3	2.5 ± 0.2	~5	~6
Thymine		0.3 ± 0.2	1.4 ± 0.4	6.5	31
Uracil		9 ± 4	1.5 ± 0.3	~5	18
Purine bases					
Adenine		5.9 ± 0.6	0.97 ± 0.13	20	18
Guanine		2.6 ± 0.4	0.30 ± 0.11	13	22

[a] After 30 h of irradiation.

cles. These peroxides are probably formed by reaction of the $^{\bullet}OH$ radicals with the nucleic acids, with subsequent reaction with molecular oxygen O_2 as depicted in Eq. (3) for DNA

$$\begin{aligned} &\text{DNA} + {}^{\bullet}\text{OH} \rightarrow \text{HO-DNA}^{\bullet} \xrightarrow{O_2} \text{HO-DNA} \\ &\quad \xrightarrow[H_2O]{} -\text{OO}^{\bullet} \rightarrow \text{HO-DNA-OOH} \end{aligned} \qquad (3)$$

The behaviour shown in Fig. 4(a) suggests that a variety of peroxide species are formed; we were unable to identify them.

In Fig. 4(b), the release of phosphate, as $H_2PO_4^-$, from the sugar/phosphate backbone of both DNA and RNA is shown in the first 2 h of irradiation ($k_i \sim (1.7\text{–}1.8) \times 10^{-2}$ min^{-1}), indicating that the backbone undergoes degradation by the UV-illuminated sunscreen agent TiO_2. The take-up (or loss; $k_d \sim 1.3 \times 10^{-2}$ min^{-1} for DNA and 1.9×10^{-2} min^{-1} for RNA; Table 2) of phosphate after 2 h is probably due to the recombination or reattachment of phosphate to remnants of the backbone or to intermediates from the decomposition of the pyrimidine and/or purine bases.

Evidence that the $^{\bullet}OH$ radicals formed in Eq. (1d) and Eq. (2) also attack the pyrimidine and/or purine bases of DNA and RNA is illustrated in Fig. 5(a), in which we summarize the temporal growth of NH_4^+ and NO_3^- ions from the conversion of the nitrogen atoms contained in these bases (see Scheme 1). The NO_3^- ions appear only after 3 h of irradiation, whereas NH_4^+ ions emerge promptly. The rates of formation of these ions are comparable with those from the photodegradation of the bases (see Table 1), but about ten times slower than the release of phosphate from the backbone. Carbon dioxide is also formed from the partial photomineralization of the carbonaceous fragments of DNA and RNA, but at a rate about four orders of magnitude slower ($k_{CO_2} \sim 10^{-6}$ min^{-1}) than the conversion of nitrogen. We take this as indicating the formation of a variety of intermediates along the path to complete mineralization.

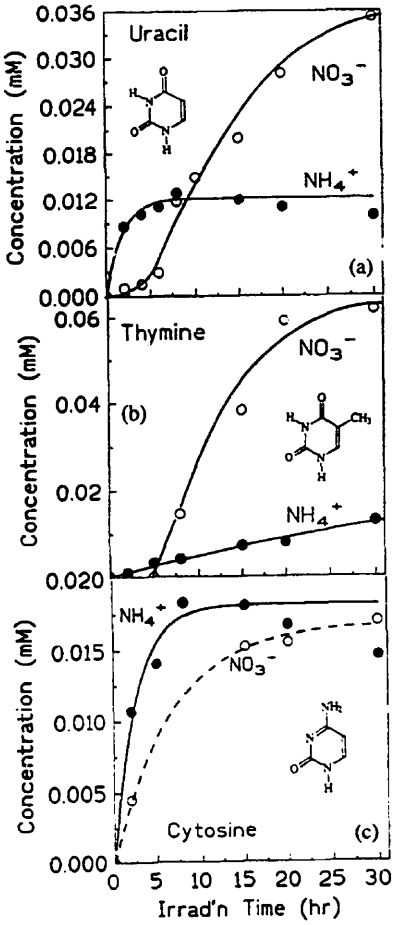


Fig. 2. Graphs illustrating the temporal evolution of NH_4^+ and NO_3^- ions in the photocatalysed transformation of the pyrimidine bases: (a) uracil; (b) thymine; (c) cytosine.

A control experiment carried out under otherwise identical conditions in which DNA and RNA were exposed to UVA and UVB radiation, but in the absence of TiO_2 particles, reveals that CO_2 begins to evolve after 1 h for DNA and after 5 h for RNA. The yield of carbon dioxide after 8 h of irradiation is 0.05 mmol l^{-1} for DNA and approximately 0.016 mmol l^{-1} for RNA; these yields can be compared with the yields of CO_2 evolved in the DNA/TiO_2 and RNA/TiO_2 systems: 25 mmol l^{-1} and approximately 13 mmol l^{-1} respectively (see Fig. 5(b)). The increase in the decomposition of DNA and RNA is nearly 500–800 times in the presence of the sunscreen agent titania (TiO_2).

Added confirmation of the photodegradative changes occurring in DNA and RNA substrates on illumination (Hg lamp; 360 nm; 1.454 mW cm^{-2}) in the presence of TiO_2 particles is depicted in Fig. 6, which shows the temporal evolution of gel permeation chromatograms. Initially, in the dark, a broad peak at approximately 15–20 min is seen for

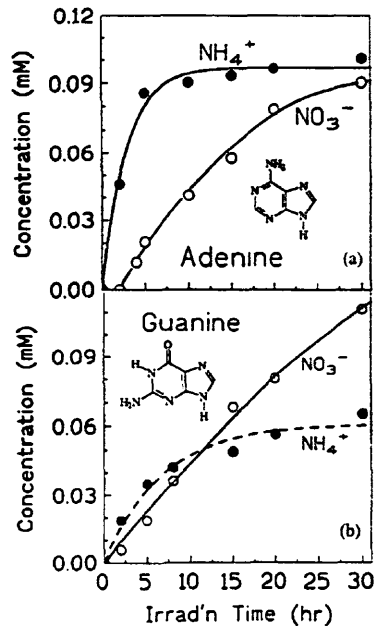


Fig. 3. Temporal evolution of NH_4^+ and NO_3^- during the photoconversion of the two purine bases: (a) adenine; (b) guanine.

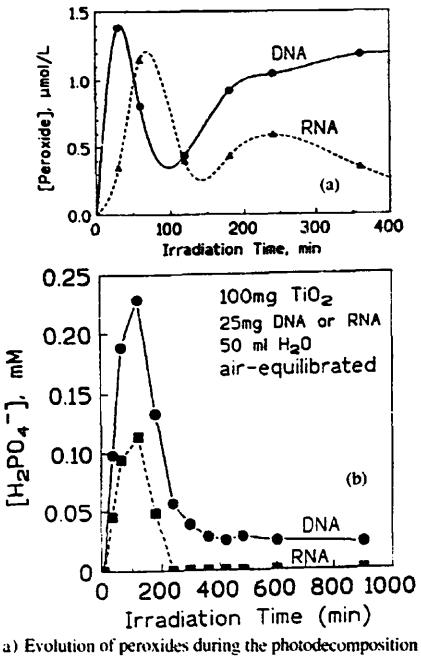


Fig. 4. (a) Evolution of peroxides during the photodecomposition of DNA and RNA in the presence of titania nano-particles. (b) Temporal evolution of phosphate, as $H_2PO_4^-$ ions.

both DNA and RNA with two smaller peaks at longer retention times. As irradiation is continued to 12 h, the chromatograms shift to longer retention times, indicating the breakup

Table 2
Summary of kinetic data for the photo-oxidation of DNA and RNA[a]

Species	Parameter	DNA	RNA
$H_2PO_4^-$	k_i (10^{-2} min^{-1})	1.83±0.44	1.69±0.57
	k_a (10^{-2} min^{-1})	1.27±0.12	1.85±0.35
CO_2	k_i (10^{-6} min^{-1})	9.9	5.3
	Rate$_i$ (10^{-5} M min^{-1})	5.11±0.06	2.76±0.06
NH_4^+	k_i (10^{-3} min^{-1})	3.6±0.3	5.3±0.5
NO_3^-	k_i (10^{-3} min^{-1})	2.3±0.3	2.4±0.2

[a]100 mg TiO_2 Degussa P25 in 50 ml of air-equilibrated aqueous dispersion; 25 mg DNA or RNA; ambient temperature.

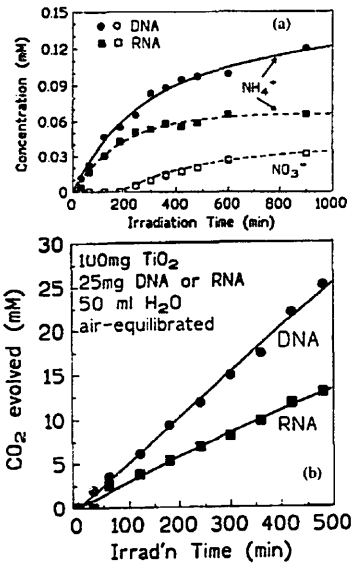


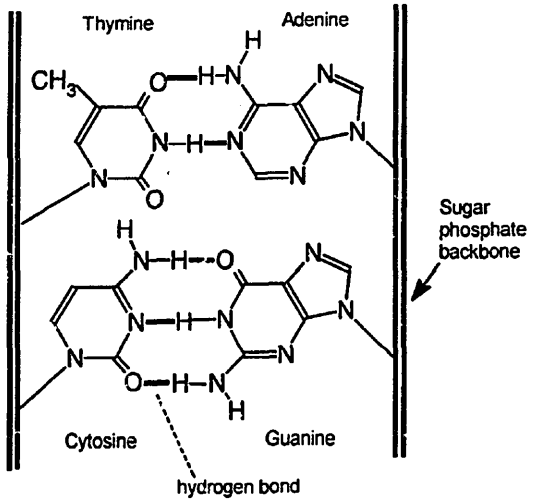
Fig. 5. Temporal evolution of NH_4^+ and NO_3^- ions (a) and carbon dioxide (b) during the photodecomposition of DNA and RNA in the presence of titania nano-particles.

of the DNA and RNA substrates and the formation of species of smaller size that elute at later times.

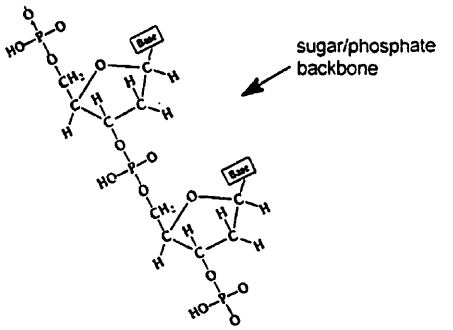
3.3. Electron microscopy on the DNA/TiO_2 system

Fig. 7 shows a transmission electron micrograph at 600 000× magnification of TiO_2 particles embedded in a DNA gel (sodium salt of calf thymus DNA) after 1 h of irradiation with UVA and UVB light; the average P25 titania particle size under these conditions was approximately 26 nm.

Physical and chemical damage of DNA by irradiated titania particles is best illustrated in the scanning electron micrographs at a 2500× magnification; this is shown in Fig. 8(a)–(b) for 0, 1 and 3 h of illumination respectively. Before irradiation, the DNA-protein strands appear normal. However, after 1 h of irradiation, there is clear evidence of surface decomposition of the strands, which is further accentuated following 3 h of illumination (Fig. 8(c)).



The four bases of DNA showing the H-bonding between base pairs



Scheme 1. Bases in DNA; note the H bond (bold line) that connects thymine and adenine and cytosine and guanine. The nature of the sugar/phosphate backbone is also shown.

3.4. Mechanistic implications

In addition to the surface-trapped electrons and trapped holes, H atoms and $HO_2^\bullet$ radicals can also be formed under acidic conditions via

$$e_{cb}^- + H^+ \rightarrow H_{surf} \quad (4)$$

$$O_2 + e_{cb}^- \rightarrow O_2^{\bullet -} \quad (5a)$$

$$O_2^{\bullet -} + H^+ \rightarrow HO_2^\bullet \quad (5b)$$

These radicals, together with e_{tr}^- and the $^\bullet$OH radicals, can lead to substantial chemistry in the pyrimidine and purine bases and DNA and RNA nucleotides. Extensive literature exists on the reaction of these various radical species with the bases and the nucleotides [45].

In neutral solutions, the pyrimidines Thy, Ura and Cyt are efficient scavengers of $^\bullet$OH radicals and have equal reactivity

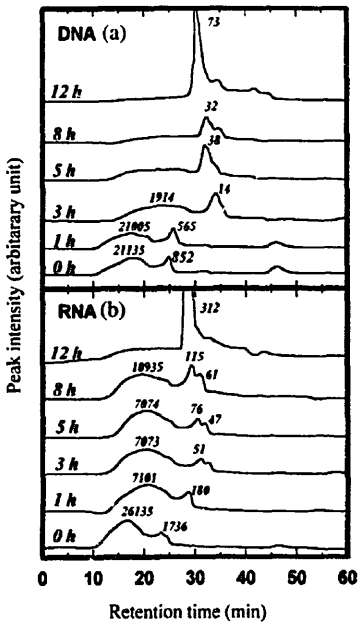
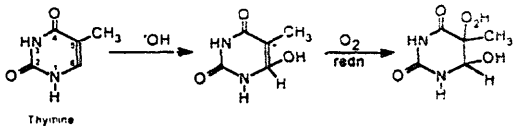


Fig. 6. Gel permeation chromatograms as a function of irradiation time depicting the molecular weight distribution during the photodecomposition of DNA (a) and RNA (b) in the presence of titania nano-particles.

Fig. 7. Transmission electron micrograph showing TiO_2 (dark spots; size of particles, approximately 27 nm) in a DNA gel after 1 h of illumination with UVA and UVB radiation; magnification: 600 000×.

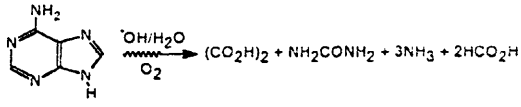
($k \sim (4–6) \times 10^9\ M^{-1}\ s^{-1}$) towards $^{\bullet}OH$, although calculations indicate that the expected reactivity should follow the trend Thy > Ura > Cyt. Attack of DNA by $^{\bullet}OH$ is slower by about an order of magnitude ($k \sim 3 \times 10^8\ M^{-1}\ s^{-1}$ [45]). H atom reaction with the nucleic acid components seems to be about an order of magnitude slower ($k \sim (1–5) \times 10^8\ M^{-1}\ s^{-1}$) than $^{\bullet}OH$ radical attack and follows the expected trend Thy > Ura > Cyt, whereas electron attachment to these pyrimidine bases is ten times faster ($k \sim (1–2) \times 10^{10}\ M^{-1}\ s^{-1}$); however, with DNA, electron attachment is slower (approximately $10^8\ M^{-1}\ s^{-1}$) owing to the negatively charged phosphate group. The $^{\bullet}OH$ radical appears to attack preferentially the 5,6-ethylenic bond of the pyrimidine bases. Indeed, according to estimates of valence indices on pyrimidines, the most suitable position for radical attack is at C(6) for Thy (Eq. (6))



(6)

C(5) for Ura and C(5) or C(6) for Cyt. By contrast, electrical charge indices suggest that electrophilic attack should occur at C(5), whereas nucleophilic attack should take place at C(6) (see references in Ref. [45]). Because of the scavenging role of molecular oxygen for e^- (Eq. (5a)), much of the photodegradation of the pyrimidine bases examined here will occur by $^{\bullet}OH$ radical attack under saturated O_2 conditions (see Section 2). Teoule and Cadet [46] identified no less than ten hydroperoxides from the radiolysis of aqueous air-equilibrated thymine solutions, together with ring opening and the formation of urea (NH_2CONH_2), formylurea ($HOC-NHCONH_2$), acetylurea ($CH_3CONHCONH_2$) and formylpyruvylurea ($CH_3COCONHCONHCHO$), all of which can, in principle, subsequently be converted to ammonia. Nitrate ion formation may probably occur [47] via a pathway involving initial $^{\bullet}OH$ radical attack on one of the nitrogens to give a hydroxylamine species, followed by further oxidation.

Some early experiments by Scholes and Weiss [48] showed that radiation-induced oxidation of purine bases in solution led to deamination, ring fission and, ultimately, production of low-molecular-weight species, such as urea and oxalic acid; reaction (7) shows the case for adenine



(7)

Under the conditions used in this work, further oxidation of the carboxylic acids yields carbon dioxide. Curiously, in contrast with our findings of substantial quantities of NO_3^- ions, the radiation-induced degradation of adenine does not appear to form these ions. Their formation must therefore be sought from the possible attack of $^{\bullet}OH$ radicals on either the amino group or alternatively on one (or more) of the nitrogens in the adenine structure to yield a hydroxylamine species.

In the case of the nucleotides DNA and RNA, $^{\bullet}OH$ radical attack must also implicate to some, perhaps minor, extent the sugar/phosphate backbone, as the release of the phosphate group is evident in Fig. 4(b). The major reaction of $^{\bullet}OH$ radicals, however, will be with the pyrimidine and purine bases to give ammonia, nitrate ions and carbon dioxide (Fig. 5) in a manner that parallels our observations with the free bases.

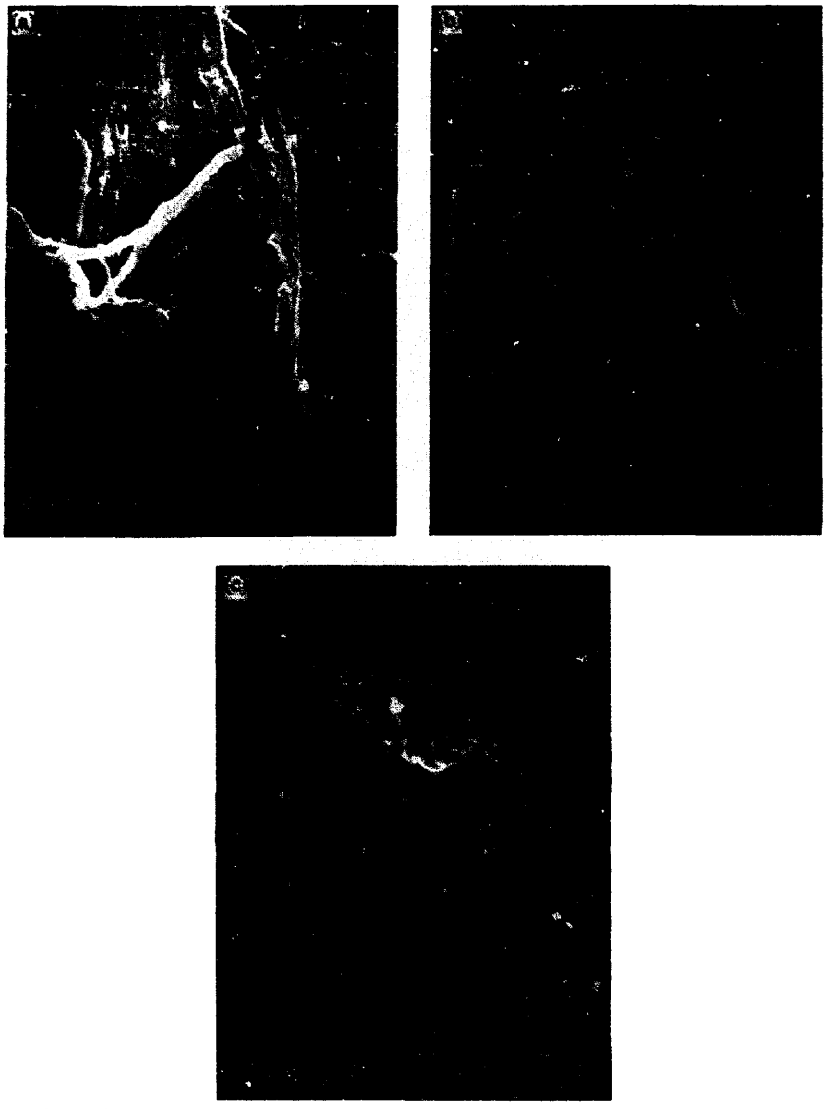


Fig. 8. Scanning electron micrographs (magnification: 2500×) depicting the physical/chemical damage inflicted on DNA-protein strands by UVA and UVB radiation in the presence of TiO_2 nano-particles: (a) in the dark; (b) after 1 h of illumination; (c) after 3 h of irradiation.

4. Conclusions

The fate of DNA, RNA and their corresponding pyrimidine and purine bases on exposure to UVA and UVB illumination (Hg lamp at radiation doses approximately 50 times smaller than 1 sun [49]) in the presence of titania nano-particles (a sunscreen agent taken to be a physical sunscreen in some quarters [23]) has been examined in vitro to probe the potential harmful effects caused by titania on these two biologically important substrates. Although these in vitro studies may not reflect actual in vivo cases, it is demonstrated that, if the sunscreen agent TiO_2 interacts with DNA or RNA and is illuminated with appropriate UV light, serious damage to these substrates can ensue. Carbon dioxide, ammonia and nitrate ions are produced following this radiation damage, together with various other (unidentified) intermediates.

Acknowledgements

The work in Tokyo was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education (No. 06640757) and that in Montreal by the Natural Sciences and Engineering Research Council of Canada.

References

[1] M.W. Brown, E. Galley, Cosmetics Toiletries 105 (1990) 69.
[2] N.A. Shaath, Photochemistry and photostability of sunscreen components, mixtures, and products, Lecture presented at the Discussion of the Photochemistry and Photobiology of Sunscreens: Public Meeting and Reopening of the Administrative Record, US Food and Drug Administration, Rockville, MD, September 19 and 20, 1996.
[3] N.D.M. Hodges, S.H. Moss, D.J.G. Davies, Photochem. Photobiol. 26 (1977) 493. P.J. Osgood, S.H. Moss, D.J.G. Davies, J. Invest. Dermatol. 79 (1982) 354. B.M. Sutherland, Photochem. Photobiol. 36 (1982) 95. B.M. Sutherland, K.P. Griffin, Photochem. Photobiol. 40 (1984) 391. F.P. Gasparro, Photodermatol. 3 (1986) 61. F.P. Gasparro, Photodermatol. 2 (1985) 151.
[4] J. Knowland, E.A. McKenzie, P.J. McHugh, N.A. Cridland, FEBS Lett. 324 (1993) 309.
[5] F.P. Gasparro, FEBS Lett. 336 (1993) 184.
[6] J. Knowland, FEBS Lett. 336 (1993) 186.
[7] J.M. Allen, C.J. Gossett, S.K. Allen, Photochemical formation of singlet molecular oxygen ($^1O_2$) in aqueous solutions of several sunscreen active ingredients, Lecture presented at the Discussion of the Photochemistry and Photobiology of Sunscreens: Public Meeting and Reopening of the Administrative Record, US Food and Drug Administration, Rockville, MD, September 19 and 20, 1996.
[8] S.Y. Wang (Ed.), Photochemistry and Photobiology of Nucleic Acids, Vol. I (Chemistry), Academic Press, New York, 1976.
[9] B.S. Martincigh, DNA photodamage induced by sunscreen absorbers, Lecture presented at the Discussion of the Photochemistry and Photobiology of Sunscreens: Public Meeting and Reopening of the Administrative Record, US Food and Drug Administration, Rockville, MD, September 19 and 20, 1996.
[10] L.H. Kligman, J. Invest. Dermatol. 84 (1985) 274.
[11] L.H. Kligman, Dermatol. Clin. 4 (1986) 517.
[12] J. Ferguson, Soap, Perf. Cosm. 63 (1990) 47.
[13] B.L. Diffey, D.M. Farr, The Lancet (1989) 429.
[14] N.M. Roscher, M.K.O. Lindemann, S.B. Kong, C.G. Cho, P. Jiang, J. Photochem. Photobiol. A: Chem. 80 (1994) 417.
[15] Comment SUP20, Docket No. 78N-0038, Dockets Management Branch, Food and Drug Administration, US Department of Health and Human Services, 1978, pp. 184–186.
[16] H. Flindt-Hansen, C.J. Nielsen, P. Thune, Photodermatol. 5 (1988) 257.
[17] J.K. Broadbent, B.S. Martincigh, M.W. Raynor, L.F. Salter, R. Moulder, P. Sjoberg, K.E. Markides, J. Chromatogr. A 732 (1996) 101.
[18] K.U. Schallreuter, J.M. Wood, D.W. Farwell, J. Moore, H.G.M. Edwards, J. Invest. Dermatol. 106 (1996) 583.
[19] N.A. Shaath, H.M. Fares, K. Klein, Cosmetics Toiletries 105 (1990) 41.
[20] B. Catlow, Seifen Oele Fette Wachse J. 119 (1993) 497.
[21] P. Alexander, Manufacturing Chemist 62 (1991) 21.
[22] Formulators fine-tune TiO_2-based screens, Manufacturing Chemist 64 (1993) 26.
[23] Federal Register, OTC Advisory Review Panel Report No. 43 FR 38206, US Food and Drug Administration, Rockville, MD, August 25, 1978.
[24] A. Saliparo, N. Serpone, unpublished results.
[25] R. Cai, Y. Kubota, T. Shuin, H. Sakai, K. Hashimoto, A. Fujishima, Cancer Res. 52 (1992) 2346.
[26] Y. Kubota, T. Shuin, C. Kawasaki, M. Hosaka, H. Kitamura, R. Cai, H. Sakai, K. Hashimoto, A. Fujishima, Br. J. Cancer 70 (1994) 1107.
[27] J. Knowland, Potential genotoxicity of inorganic sunscreens, Lecture presented at the Discussion of the Photochemistry and Photobiology of Sunscreens: Public Meeting and Reopening of the Administrative Record, US Food and Drug Administration, Rockville, MD, September 19 and 20, 1996.
[28] W. Wamer, Oxidative damage to nucleic acids photosensitized by titanium dioxide, Lecture presented at the Discussion of the Photochemistry and Photobiology of Sunscreens: Public Meeting and Reopening of the Administrative Record, US Food and Drug Administration, Rockville, MD, September 19 and 20, 1996.
[29] V.P.S. Judin, quoted in Formulators fine-tune TiO_2-based screens, Manufacturing Chemist 64 (1993) 26.
[30] G.M. Williams, Discussion of the pre-clinical and clinical study data of relevance to photoexposure and the possible photochemistry of titanium dioxide (TiO_2) and zinc oxide (ZnO), Lecture presented at the Discussion of the Photochemistry and Photobiology of Sunscreens: Public Meeting and Reopening of the Administrative Record, US Food and Drug Administration, Rockville, MD, September 19 and 20, 1996.
[31] V. Maurino, C. Minero, E. Pelizzetti, N. Serpone, in: E. Pelizzetti (Ed.), Fine Particles Science and Technology from Micro to Nanoparticles, Kluwer, Dordrecht, 1996, pp. 707–718. H. Hidaka, K. Nohara, J. Zhao, E. Pelizzetti, N. Serpone, J. Photochem. Photobiol. A: Chem. 91 (1995) 145. H. Hidaka, K. Takashima, K. Nohara, J. Zhao, E. Pelizzetti, N. Serpone, New J. Chem. 18 (1994) 541. E. Pelizzetti, C. Minero, E. Borgarello, L. Tinucci, N. Serpone, Langmuir 9 (1993) 2995. H. Hidaka, J. Zhao, K. Nohara, K. Kitamura, Y. Satoh, E. Pelizzetti, N. Serpone, in: D.F. Ollis, H. Al-Ekabi (Eds.), Photocatalytic Purification and Treatment of Water and Air, Elsevier, Amsterdam, 1993, pp. 251–260.
[32] S. Hara, Y. Kuroda, S. Nagakawa, Y. Totani, J. Jpn. Oil Chem. Soc. 43 (1994) 18.
[33] N. Serpone, J. van de Ven, unpublished results, 1995.
[34] T.M. Macleod, W. Fran-Bell, Br. J. Dermatol. 92 (1975) 149.
[35] R.M. Sayre, N. Kollias, R.L. Roberts, A. Baqer, I. Sadiq, J. Soc. Cosmet. Chem. 41 (1990) 103.
[36] D.W. Bahnemann, J. Cunningham, M.A. Fox, E. Pelizzetti, P. Pichat, N. Serpone, in: D. Crosby, G. Helz, R.G. Zepp (Eds.), Aquatic and Surface Photochemistry, Lewis Publishers, Boca Raton, FL, 1994, pp. 261–316.
[37] N. Serpone, in: The Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 18, Wiley-Interscience, New York, 1996, pp. 820–837.
[38] A. Selafani, J.-M. Herrmann, J. Phys. Chem. 100 (1996) 13 655.
[39] N. Serpone, D. Lawless, R.F. Khairutdinov, J. Phys. Chem. 99 (1995) 16 646.
[40] N. Serpone, D. Lawless, E. Pelizzetti, R.F. Khairutdinov, J. Phys. Chem. 99 (1995) 16 655.
[41] D. Lawless, N. Serpone, D. Meisel, J. Phys. Chem. 95 (1991) 5166.
[42] J.H. Arbour, J. Tromp, M.L. Hair, Can. J. Chem. 63 (1985) 204.
[43] J. Russell, J. Ness, M. Chopra, J. McMurray, W.E. Smith, J. Pharmaceut. Biomed. Anal. 12 (1994) 863.
[44] H.M. Swartz, The significance of free radicals in the pathophysiology of the skin, Lecture presented at the Discussion of the Photochemistry and Photobiology of Sunscreens: Public Meeting and Reopening of the Administrative Record, US Food and Drug Administration, Rockville, MD, September 19 and 20, 1996.
[45] G. Scholes, in: S.Y. Wang (Ed.), Photochemistry and Photobiology of Nucleic Acids, Vol. I, Chemistry, Academic Press, New York, 1976, pp. 521–577.
[46] R. Teoule, J. Cadet, Chem. Commun. (1971) 1269.
[47] K. Nohara, H. Hidaka, E. Pelizzetti, N. Serpone, J. Photochem. Photobiol. A: Chem. 102 (1997) 265.
[48] G. Scholes, J. Weiss, Biochem. J. 53 (1953) 567.
[49] H. Hidaka, H. Kubota, M. Gratzel, E. Pelizzetti, N. Serpone, J. Photochem. 35 (1986) 219.