DNA Damage Photoinduced by Cosmetic Pigments and Sunscreen Agents under Solar Exposure and Artificial UV Illumination

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Edited by T. Itoh, Kitasato Univ., and accepted February 3, 2006 (received for review November 18, 2005)

Abstract: Nanostructured ZnO particles present in skin-care cosmetics and UVB/UVA sunscreen products generate strong oxidizing species (free radicals) when illuminated with UV radiation that can damages human skin and the horny layer. Damage to DNA by ZnO and other pigmentary ingredients in sunscreen formulations under artificial and solar UV exposure has been examined by Agarose gel electrophoresis using *pUC 18* DNA plasmids (2686 base-pairs). Initial photoinduced oxidative damage done to DNA plasmids have been probed by nicking assays under *in vitro* conditions for ZnO. The effects of nanosize ZnO and CeO₂ particles, and the newly developed CaO-doped and SiO₂-coated CeO₂ pigment are compared when subjected to artificial (75-W Hg-lamp) and solar UV radiation. Supercoiled DNA plasmids undergo one nick to produce the relaxed form, followed by a second nick yielding the linear form of the plasmids. The DNA constituents deoxyadenosine-5'-monophosphate (dAMP), guanosine-5'-monophosphate (GMP) and cytidine-5'-monophosphate (CMP) have been examined to assess the photooxidative damage done to these nucleotides under photocatalytic conditions using the cosmetic/sunscreen ZnO pigment.

Adsorption of the nucleotide through the phosphate on the positively charged ZnO surface, followed by attack of the ribose/phosphate backbone by photogenerated •OH (and/or •OOH) radicals on the ZnO surface lead to the degradation of the dAMP's ribose moiety and subsequently to decomposition of the adenine base residue. About 90% mineralization of the ribose/phosphate backbone occurred as evidenced by formation of H₂PO₄⁻ ions after only 30 min of UV irradiation. The nitrogen atoms of the adenine base were converted to NO₃⁻ and NH₄⁺ ions. About 45% of the organic carbons constituting the dAMP ribose backbone was mineralized to CO₂ within 8 h of UV irradiation occurring through formation of carboxylic acid intermediates (succinic, acetic and formic), with 85% of the remaining nucleobase ultimately mineralized after 48 h of UV irradiation. Similar occurrences were seen for the GMP and CMP

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nucleotides.

Key words: gel electrophoresis, DNA damage, sunscreen agent, cosmetic pigment, photooxidation, DNA plasmid

1 Introduction

Solar radiation induces acute and chronic reactions in human and animal skin, so much so that chronic repeated exposures to solar UVB/UVA radiation are the primary cause of benign and malignant skin tumours, including malignant melanoma (1). The solar UVA radiation (320-400 nm) reaching the surface of the Earth is about 20-fold greater than solar UVB (290-320 nm) radiation (2). The longer wavelength UVA radiation penetrates the human skin to a greater (fivefold) depth than does UVB (3-5), reaching the region below the dermis that contains fibroblasts, dermal dendrocytes (dendritic cells with immune function), mast cells, macrophages, and lymphocytes (6). However, contrary to UVB radiation, UVA radiation is not readily absorbed by DNA. Rather, UVA is absorbed by endogenous photosensitizers generating reactive oxygen species such as superoxide radical anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and/or $\cdot OH$ radicals (7, 8) that can seriously damage DNA, membranes and other cellular components (2). Specific absorbers such as flavins, dehydrogenase co-enzymes of nicotinamide-adenine dinucleotide (NAD), flavin/adenine-dinucleotide (FAD), and flavin-mononucleotide (FMN) are present in living human cells. Accordingly, exposure to UVA light causes oxidative stress to these cells and radiation-induced immunosuppression (1, 8-10). For instance, oxidative stress causes such damage as formation of

thymine glycol by peroxidation of thymine and hydrolysis of pyrimidine bases (11-14). As well, the adenine and guanine (purine) bases are also attacked by the reactive oxygen species, with cleavage of the imidazole ring in the guanine base.

The lower wavelength UVB radiation passes through the horny layer (*stratum corneum*) of the skin to reach the *basement membrane* below the *stratum basale*, and although it does not reach the dermis it nevertheless UV-illuminates the *Langerhans cells*, the *keratinocytes* and *melanocytes* present in the *stratum spinosum* and *stratum basale* (6). Compared to UVA, the solar UVB radiation is highly mutagenic and carcinogenic as evidenced in animal experiments. Epidemiological studies have also inferred that solar UV radiation is responsible for skin tumour development via gene mutations and immuno-suppression (1). Consequently, the UVB light that passes through the ozone layer in the stratosphere (15) and absorbed by the DNA in the various cells that constitute the epidermis causes the photo-production of DNA anomalous bases and derivatives in the configurational states of the DNA double helical chains. For instance, the cytosine and thymine bases in the DNA are either hydroxylated or deaminolyzed to generate unusual bases (16-19). In some cases, a pyrimidine dimer is formed (20).

White metal-oxide pigments such as TiO_2 , Al_2O_3 , ZnO and others are commonly employed in cosmetic and sunscreen products. The pigments typically consist

of ultrafine particles with sizes smaller than 100 nm diameter. They screen ultraviolet light, are transparent and find acceptability as cosmetic and sunscreen materials. Sunscreen formulations are generally characterized by the sun protection factor (SPF) index in evaluating their performance at screening UV radiation (21-23). (Note that the SPF stated for a sun protection product refers mostly to the UVB sun protection factor (24, 25)). The TiO₂ rutile pigment presents better SPF characteristics than ZnO. This notwithstanding, such metal-oxide pigments play both a beneficial and a deleterious role. Although they screen UVA/UVB radiation efficiently, they can also generate harmful reactive oxygen species such as O₂⁻, HOO[•] and [•]OH, radicals (and even singlet oxygen, ¹O₂, in the case of TiO₂ (26)) when subjected to UVA/UVB radiation. These pigments are also well-known semiconductor photocatalysts having bandgap energies of 3.2 eV (absorption edge, 387 nm) for TiO₂ anatase and ZnO, and 3.0 eV (414 nm) for rutile TiO₂. They have been investigated extensively in Advanced Oxidation Processes for environmental remediation. The bandgap energy of CeO₂ particles is 3.1 eV (absorption edge, 400 nm). The ZnO pigment has lately been widely employed as the physical filter of choice in some sunscreen lotions over the *micronized* TiO₂ (27).

DNA plasmids have been adopted as indicators of the photoactivity of metal-oxide specimens for examining *in vivo* damage to skin, injury to the *stratum corneum* and in some cases to DNA failure when exposed to solar UVB/UVA radiation. In this regard, in earlier studies we examined the photochemical damage done

to DNA in the presence of TiO₂ particles under exposure to UVA and UVB radiation (28-31). Possible effects that CeO₂ (ceria) particulates might have on the initial photoinduced interaction with the DNA double helix and information on the photooxidation of DNA constituents such as deoxyadenosine-5'-monophosphate (dAMP), guanosine-5'-monophosphate (GMP), and cytidine-5'-monophosphate (CMP) were considered following recent articles by Yabe and Sato (32-36) on the synthesis and potential applications of various doped and coated CeO₂ specimens proposed as potential cosmetic and sunscreen active agents. In the present report, we examine the DNA damage caused by the ZnO pigment in commercial sunscreen formulations (available in Japan) under *in vitro* conditions by UVB/UVA illumination. Changes to the forms of DNA plasmids from the Supercoiled (S), to the Relaxed (R), to the Linear (L) forms were investigated by Agarose gel electrophoresis for the newly developed CaO-doped and CaO-doped-SiO₂-coated CeO₂ pigments. These changes are compared to those effected by naked TiO₂, ZnO and CeO₂ used as standards. In addition, the photooxidative damage induced by the ZnO pigment specimens extracted from the sunscreen lotions on the DNA plasmids and the constituent nucleotides dAMP, GMP, and CMP has been examined by monitoring the fate of the phosphate groups, the fate of the nitrogen atoms (formation of NH₄⁺ and/or NO₃⁻ ions) and the fate of carbon atoms (evolution of CO₂) of the ribose and the pyrimidine and purine nucleobase residues.

Deoxyadenosine monophosphate (dAMP)

Guanosine monophosphate (GMP)

Cytidine monophosphate (CMP)

2 Experimental Section

2.1 Materials

Nanostructured TiO₂ (Degussa P-25) and ZnO (Wako) powdered specimens were used as reference samples. Since commercial sunscreens commonly include several additives (e.g. squalene, higher alcohols, fatty acid esters, glycerine, perfume and others), 5 g of the milky lotions containing the sunscreen active agents were treated with mixed acetone/methanol solvent (200 mL) followed by sonication and filtration yielding a white powder that was subsequently used for further measurements. X-ray diffraction analysis of the crystalline structure of all the extracted sunscreen active agents confirmed the metal-oxide to be ZnO. The following four newly CeO₂ specimens developed by Yabe and Sato (32) were examined in powdered form: (a) naked CeO₂, (b) CaO-doped (20 mol %) CeO₂, (c) CaO-doped (20 mol %) CeO₂ calcined at 700°C, and (d) CaO-doped (20 mol %)-amorphous-SiO₂-coated (10 wt %) CeO₂.

The ZnO pigments were extracted from four Japanese commercially available sunscreen lotions: (1) UV-Shield Sensitive J {Oomi Kyoudaisha Mentam; SPF 32; PA ++}, (2) Anessa Neosunscreen EX {Shiseido; SPF 50; PA +++}, (3) Alli EX Cut Sunscreen R {Kanebo; SPF 50; PA +++}, and (4) Super UV Screen N Waterproof {Kose; SPF 50; PA +++}. The DNA plasmids were obtained from the *pUC 18* DNA ($A_{260}/A_{280} = 1.76$) that contained 2686 base-pairs; they were supplied by Nippon Gene Ltd. And were used as received without further treatment.

2.2 Analytical Procedures

The concentration of phenol which was employed as a standard sample in the time profile of photocatalytic degradation activity in the filtrate was determined from its UV spectra using a JASCO V-570 spectrophotometer. The extent of mineralization of the substrates to CO₂ was obtained with a Shimadzu GC-8A gas chromatograph equipped with a TCD detector and a Porapak Q column; helium was the carrier gas. Formation of H₂PO₄⁻, NH₄⁺ and NO₃⁻ ions was assayed with a JASCO HPLC chromatograph, equipped with a CD-5 conductivity detector using either a Y-521 cationic column or an I-524 anionic column. Formation of carboxylic acid intermediates such as formic acid, acetic acid and succinic acid during the photooxidation of the

various specimens was followed with a JASCO liquid chromatograph (HPLC) containing bromothymol blue (BTB); the column was a KC-811 and the UV detector was set at 445 nm.

2.3 Gel Electrophoresis Assessment for UV-illuminated DNA *in vitro*

Gel electrophoretic experiments were carried out on a Funakoshi, model No. 1316R instrument (Tokyo, Japan) equipped with an ESP 250 power supply. A Kodak digital Science EDAS-290 analyzer system (Invistrogen Co. Ltd.) was used together with a Funakoshi (NTFM-20) 20-Watt UV-trans-illuminator.

The Agarose gel (1.75%, Agarose S, Nippon Gene Ltd.) was added to 2 M *tris*-acetate, 0.05-M EDTA buffered solution (pH 8.0; 50 mL) and subsequently dissolved using microwave-generated heat. The resulting Agarose solution was then poured into a UVT gel tray (13 cm × 16 cm) to prepare a 3.5-mm gel thin film. The above buffered solution was added to the gel electrophoresis tank (10mm deep), and before solidification of the thin layer gel a twenty-holes comb was appropriately located in the tray to prepare the thin film (30 min). The white ZnO specimens (10 mg) extracted from the sunscreen lotions were placed in a 10-mL cylindrical vessel containing deionized water (9 mL). Subsequently, a 1-μL specimen of *pUC 18* DNA was added to 20 μL of the ZnO dispersion in Petri dish. Irradiation of the dispersions was carried out with a 75 W Hg lamp (Toshiba SHL-100UVQ-2) emitting an irradiance of *ca.* $3.4 \pm 0.3 \text{ mW cm}^{-2}$ in the wavelength range 310 to 400 nm (maximal emission at $\lambda = 360\text{nm}$). Each experiment at all wavelengths without interference filter were done. After illumination, a 1-μL sample was collected in a microtube and stored in a refrigerator for later use. Subsequently, a 1-μL solution of an ethidium bromide dye solution was added to the stored illuminated samples, followed by addition of 8 μL of deionized water and further agitation to insure good mixing. The samples were then injected into each well of the Agarose gel tank. Electrophoresis was performed under an applied voltage of 100 V for 2 h. The gel samples were irradiated with the UV trans-illuminator, and the emission from the dye-strained DNA was recorded with a digital camera. The relative quantity of the three configurations (S, R, and L) of the DNA plasmids present in the gel samples was determined using a EDSA-290 densitometer.

3 Results and Discussion

Sunscreen active agents are so-called either physical or chemical filters. The former typically comprise white ZnO and micronized TiO₂, whereas the latter (organic) filter comprises such substances as Padimate-O, Parsol 1789, octylmethoxycinnamate, and others (37). Metal oxides such as CeO₂ and ZrO₂ particulates with sizes smaller than *ca.* 100 nm are also being examined for incorporation in cosmetics and sunscreen commercial products (38-49). However, naked ceria particles are pale yellow and highly oxidizing, but less than TiO₂ and ZnO (48), making them nonetheless unsuitable for use in cosmetics and as sunscreen active agents (46). Accordingly, the CeO₂ particles have in some cases been modified by coating with boron nitride (BN), which effectively reduce both thermal and photocatalytic activity toward the oxidation of castor oil. The BN-coated CeO₂ specimens displayed higher transparency and greater effectiveness at UV shielding than either TiO₂ or ZnO particles (41, 44, 46). A variety of metal-ion doped ceria particulate systems have also been reported. They displayed small particle size (2-4 nm), increased absorption characteristics in the UV region and greater transparency in the visible region relative to an equivalent quantity of micronized TiO₂ (32-34, 40, 45). Calcium oxide doping of CeO₂ (20 mol %) imparts greater photostability relative to naked ceria particles, and coating with a layer of amorphous SiO₂ enhances particle integrity (48). It was therefore appropriate to examine the behaviour of these particulate specimens toward DNA.

The double helical DNA structure undergoes damage under UV illumination with the white pigments (*e.g.*, TiO₂) used as active physical agents in cosmetic and in sunscreen formulations (37). The initial DNA plasmids typically consist of the supercoiled form (S), which upon UV exposure to UV radiation showed a pattern of two bands in the nicking assay (**Fig. 1(a)**): a lower band corresponds to the supercoiled (S) plasmids and the upper band belongs to the relaxed (R) form of the plasmids. To assess the behaviour of the various CeO₂ specimens toward DNA, two other white pigments TiO₂ (P-25) and ZnO (Wako) were also examined as reference and were subjected to otherwise identical conditions as the ceria specimens.

In the presence of TiO₂, the supercoiled DNA configuration decreased significantly within *ca.* 30 min of

exposure to UV illumination, the band becoming barely visible after 30 min (**Fig. 1(b)**). Concomitantly, the band intensity of the relaxed form increased rapidly after only 15 min of irradiation. Further UV illumination of the TiO₂-DNA specimen caused another band to appear after *ca.* 30 min sandwiched between the S and R bands: this band corresponds to the linear (L) form of the DNA plasmids (28, 50). In the presence of the ZnO pigment, the S band intensity decreased relatively early after only 15 min of UV irradiation (**Fig. 1(c)**); at the same time the L band appeared intensified. Thereon, the R band decreased in intensity, whereas the L band became more pronounced. Beyond 30 min of irradiation, the supercoiled structure of the DNA plasmids was no longer observable. Compared to the behaviour of the TiO₂-DNA sample, it is evident that the ZnO pigment imparted a faster and greater damage to the DNA plasmids.

The gel electrophoresis patterns with respect to naked CeO₂-DNA system are illustrated in **Fig. 1(d)**. Contrary to the TiO₂ and ZnO pigments, the number of

Fig. 1 Pictures of Gel Electrophoresis Samples Showing DNA Damage as a Result of UV Irradiation (Hg Lamp; Light Irradiance, $3.4 \pm 0.3 \text{ mW cm}^{-2}$ at 360 nm): (a) pigment-free DNA, (b) DNA plasmids in the presence of TiO₂ particles (Degussa P-25), (c) DNA plasmids in the presence of ZnO, and (d) DNA in the presence of naked CeO₂ particles.

supercoiled DNA plasmids (S band) in the CeO₂-DNA system gradually decreased with increasing illumination time, whereas the number of relaxed plasmids increased. No L band was evident even after 180 min of UV irradiation. Clearly, the naked CeO₂ particles caused far less damage to the DNA plasmids upon UV illumination than either of the more photoactive TiO₂ and ZnO reference pigments.

The temporal changes in the relative intensity of supercoiled, relaxed and linear bands in the gel electrophoresis patterns for damage to the DNA plasmids caused by the ZnO pigment, which was extracted from

Fig. 2 Relative Intensity of the Bands Corresponding to the Supercoiled (●), Relaxed (Δ) and Linear (■) Forms of the DNA Plasmids as a Function of Irradiation Time of the white ZnO Pigments: (a) pristine ZnO (Wako); and ZnO from commercial sunscreen lotions (b) Anessa Neosunscreen EX, (c) UV-Shield Sensitive J, (d) Alli EX Cut Sunscreen R, and (e) Super UV Screen N Waterproof.

the commercial sunscreen lotions, are shown in **Fig. 2(a)-2(e)**. The corresponding results for a pristine sample of ZnO (Wako) are displayed in **Fig. 2(a)**.

All the ZnO specimens extracted from sunscreen lotions caused considerable damage to the DNA plasmids which increased in the order: {Wako ZnO} ≈ {Super UV Screen N Waterproof (SPF 50, PA +++)} < {Alli EX Cut Sunscreen R (SPF 50, PA +++)} ≈ {Anessa Neosunscreen EX (SPF 50, PA +++)} < {UV-Shield Sensitive J (SPF 32, PA ++)}. Clearly, the most photoactive sunscreen active ZnO pigment for DNA damage amongst the commercial sunscreens was from the UV-Shield Sensitive J lotion, whereas the least photoactive ZnO specimen was from the Super UV Screen N Waterproof sunscreen lotion. These observations accord with the SPF index and the PA index used for commercial inorganic/nanosized UV shielding metal-oxide pigments. Interestingly, the pristine ZnO specimen (Wako) displayed lower photoactivity toward nicking DNA plasmids than the commercial pigments.

The CeO₂ specimen has most of the prerequisite properties in applications as a skin-protective sunscreen agent since it is transparent to visible light, it has excellent characteristics for adsorption of UV light, the particle size is below 100 nm, and displays natural skin transparency (38, 39, 42, 47, 48). These fine characteristics notwithstanding, however, CeO₂ particles also display unusually high oxidation catalytic activity toward oxidation of many organic substances (31, 43-45). Consequently, CeO₂ particles have seldom been used until now. Accordingly, CeO₂ particulates were modified by doping with various metal ions, in particular with Ca²⁺ (CaO after calcinations), and coated with an amorphous layer of SiO₂. The photoactivity of these modified ceria specimens were examined to assess the oxidative activity toward DNA plasmids and oxidation of phenol (compared with the photooxidative ability of P-25 TiO₂ - see below), and their suitability as possible sunscreen and cosmetic pigments.

The effects of surface-modified CeO₂ powder specimens toward injury to DNA plasmids subjected to artificial UV radiation (Hg light source) are reported in **Fig. 3(a)- 3(d)**, where in the temporal damage done to the supercoiled (S) DNA plasmids and the evolution of the relaxed (R) and linear (L) configurations of DNA for (a) naked CeO₂ alone, (b) CaO-doped (20 mol %) CeO₂, (c) CaO-doped (20 mol %) CeO₂ calcined at 700 °C and (d) CaO-doped (20 mol %)-amorphous-SiO₂-

coated (10 wt %) CeO_2 is compared. The coating with a SiO_2 layer should prevent conversion of the supercoiled configuration to relaxed and/or linear forms. Results of exposing the same CeO_2 specimens/DNA plasmid systems to solar UV radiation are illustrated in **Fig. 3(e)-3(h)**.

Cerium oxide particles alone show little photoactivity toward damage to the DNA plasmids as evidenced by the relative stability of the supercoiled form and the slow evolution of the relaxed DNA plasmids under both artificial and solar UV exposure (**Fig. 3(a)** and **3(e)**). By comparison, although barely visible, the CaO-doped CeO_2 specimen caused some of the supercoiled plasmids to evolve also into the linear form at the longer irradiation times (beyond 60 min; **Fig. 3(b)** and **3(f)**) through consecutive nicks of the DNA structure. The calcined CaO-doped CeO_2 specimen shows some linear plasmids (the L band) as well, subsequent to solar UV exposure (**Fig. 3(g)**), but not to artificial UV radiation

(**Fig. 3(c)**). By contrast, no linear plasmids are evidently formed when the DNA plasmids are subjected to artificial and solar UV radiation in the presence of the CaO-doped-amorphous- SiO_2 -coated ceria specimen (**Fig. 3(d)** and **3(h)**).

Both TiO_2 and ZnO fine particles used in cosmetic and sunscreen products exhibit relatively high photoactivity toward degradation of cosmetic constituents, damage to DNA (28), and promotion of skin senescence (photoaging) by photogenerated reactive oxygen species (51, 52), such as $\cdot\text{OH}$ and $\cdot\text{OOH}$ radicals, superoxide radical anions ($\text{O}_2^{\cdot-}$) and singlet oxygen ($^1\text{O}_2$), and H_2O_2 . Incomplete photoprotection against the solar UVA radiation also causes skin senescence (53). Another troubling issue with these cosmetic pigments is the whitening phenomenon on human skin. Coating of CeO_2 particles with amorphous silica diminishes UV intrusion into the skin as they are stronger UV absorbers and thus better UV blockers. Moreover, their

Fig. 3 Left Hand Panels Display the Nicking Results from Gel Electrophoresis Measurements Showing the Photoinduced Damage Caused to DNA Plasmids under UV-illumination with a Hg Lamp Emitting a Light Irradiance of $3.8 \pm 0.4 \text{ mW cm}^{-2}$ at 360 nm for (a) naked CeO_2 alone, (b) CaO-doped (20 mol%) CeO_2 , (c) CaO-doped (20 mol%) CeO_2 calcined at 700°C and (d) CaO-doped (20 mol%)-amorphous- SiO_2 -coated (10 wt %) CeO_2 . The right hand panels illustrate the nicks photoinduced on the DNA plasmids under solar exposure (light irradiance, $3.7 \pm 0.2 \text{ mW cm}^{-2}$ at 360 nm) for (e) naked CeO_2 alone, (f) CaO-doped (20 mol%) CeO_2 , (g) CaO-doped (20 mol %) CeO_2 calcined at 700°C , and (h) CaO-doped (20 mol%)-amorphous- SiO_2 -coated (10 wt %) CeO_2 . In all the panels, the lowest band corresponds to the S form of DNA, the upper band is that of the R form, and the middle band (where visible) corresponds to the L form of the DNA plasmids.

photoactivity is also diminished. Doping CeO_2 particles with metal ions also depresses the photoactivity of ceria (32, 44-46).

The temporal relative stability of the supercoiled DNA plasmid structure and the evolution of the relaxed structure under artificial UV light are illustrated in **Fig. 4**. Formation of relaxed plasmids increased during the 3 h of UV irradiation, indicating that some of the supercoiled plasmids were nicked to evolve into the relaxed form. Since the hydrogen bonds composing the double helical supercoiled configuration are *ca.* 25-fold weaker

than the common covalent bond, the supercoiled helix easily unwinds to the relaxed configuration when nicked by reactive oxygen species produced under irradiation. By comparison relative to the DNA plasmids are subjected to the UV radiation (**Fig. 4(a)**), the presence of the other four modified ceria specimens showed a slightly greater number of relaxed plasmids after 3 h of irradiation (**Fig. 4(b) to 4(e)**).

The photooxidative activity of various sunscreen ingredients on the degradation of phenol (0.1 mM) was examined. Results are reported in **Fig. 5**. Phenol decomposed in less than 40 min with TiO_2 particles (P-25) through first order kinetics. Compared to this very active titania specimen, the four ceria specimens were relatively photoinactive in degrading phenol (naked CeO_2 alone was *ca.* 50 times less active than TiO_2). Amongst the four CeO_2 systems, the CaO-doped-amorphous- SiO_2 -coated CeO_2 specimen was the least photoactive in degrading phenol for the 1 h of UV irradiation. The quantity of phenol that degraded in the presence of naked CeO_2 amounted to about a 10% drop in the initial concentration after 60 min of UV irradiation.

We next examined the fate of the DNA linear plasmids further under UV irradiation (Hg light source; 75 Watts). These linear plasmids were produced from *pUC 18* DNA using the Hind III restriction enzyme and then were examined by gel electrophoresis in the absence of

Fig. 4 Temporal Relative Intensity of Supercoiled (●) and Relaxed (Δ) Configurations Subsequent to UV Irradiation of DNA Plasmids with the 75-Watt Hg Light Source in the Presence of (a) pigment-free system, (b) naked CeO_2 alone, (c) CaO-doped (20 mol %) CeO_2 , (d) CaO (20 mol %)-doped CeO_2 calcined at 700°C, and (e) CaO (20 mol %)-doped-amorphous- SiO_2 -coated (10 wt %) CeO_2 .

Fig. 5 Temporal Course of the Degradation of Phenol (0.1 mM) under UV Irradiation in the Presence of TiO_2 (P-25) particulates (●) and CeO_2 Specimens: naked CeO_2 (○); CaO-doped (20 mol %) CeO_2 (■); CaO-doped (20 mol %) CeO_2 calcined at 700°C (Δ); and CaO-doped (20 mol %)-amorphous- SiO_2 -coated (10 wt %) CeO_2 (◆).

ZnO (**Fig. 6(a)**) and presence of the ZnO pigment (**Fig. 6(b)**). The linear DNA plasmids were very stable to UV irradiation for up to 6 h when no pigmentary ZnO was present. However, in the presence of ZnO followed by UV irradiation the linear plasmids were totally degraded after 3 h (the L band was no longer visible after this time - **Fig. 6(b)**). {Note that the first assay in **Fig. 6(b)** is that of the supercoiled DNA plasmids and is shown for comparison only}. Clearly, the above evidence suggests that the double helical configuration of the linear plasmid was not damaged by UV radiation alone. However, in the presence of the ZnO pigment known to produce $\bullet\text{OH}$ radicals (and other reactive oxygen species - see above) when subjected to UV irradiation, these radicals induced considerable damage to the plasmids through oxidation of a number of the 2686 base pairs.

To examine what the fate of some of the constituents of the DNA plasmids may be in the presence of such a photoactive sunscreen pigment as ZnO when subjected to UV irradiation for long periods, we next examined the photooxidative degradation of three dAMP, GMP and CMP nucleotides, under photocatalytic conditions otherwise typically used in Advanced Oxidation Processes. The temporal course of the photodegradation of the dAMP nucleotide monitored through the evolution of mineralized products is illustrated in **Fig. 7**.

Exposure of dAMP to UV irradiation in the presence

Fig. 6 Pictures of Gel Electrophoresis Patterns on the Photodamage Caused to the Linear Structure of the DNA Plasmids Prepared from *pUC 18* DNA using the Hind III Restriction Enzyme for (a) a ZnO-free system and (b) in the Presence of the ZnO Pigment.

of ZnO for 36 h (1.5 d) led to the photomineralization of this nucleotide, as evidenced by formation of dihydrogenphosphate anions, ammonium ions and nitrate ions. During the initial stage, the H_2PO_4^- ion is rapidly formed in less than 0.5 h (*ca.* 0.045 mM) and then just rapidly (in less than 1 h) about half of the initial quantity of H_2PO_4^- adsorbed on the positively charged ZnO particle surface. Continued irradiation gradually increases the quantity of the H_2PO_4^- ions to *ca.* 0.026 mM after 36 h. Concomitantly, NH_4^+ cations and NO_3^- anions also formed rapidly initially and then more slowly tending to *ca.* 0.040 mM for nitrate ions and *ca.* 0.055 mM for ammonium ions. On continued irradiation, the latter were further oxidized to NO_3^- reaching 0.1 mM after 36 h through prior formation of a hydrox-

Fig. 7 Photocatalytic Mineralization of the dAMP Nucleotide in the Presence of ZnO Yielding H_2PO_4^- Ions from the Breakup of the Ribose-Phosphate Backbone of the Nucleotide, and NH_4^+ and NO_3^- Ions from the Degradation of the Adenine Base Residue.

Fig. 8 Evolution of CO_2 Gas in the Photodegradation of dAMP with ZnO Pigment.

Fig. 9 Formation of Formic, Acetic and Succinic Acids in the Photodegradation of dAMP in Aqueous ZnO Dispersions.

Fig. 10 Formation of H_2PO_4^- , NH_4^+ and NO_3^- Ions in the Photodegradation of GMP with ZnO Pigment.

Fig. 11 Formation of H_2PO_4^- , NH_4^+ and NO_3^- Ions in the Photodegradation of CMP with ZnO Pigment.

ylamine ($-\text{HNOH}$) moiety and NO_2^- ions. The degradation of adenosine was also confirmed by monitoring the evolution of carbon dioxide by gas chromatography (**Fig. 8**). The level of mineralization of this nucleoside (includes the ribose moiety) to CO_2 gas reached ~83% after 2 d of UV irradiation through prior formation of carboxylic acid intermediate species (succinic, acetic and formic acids; **Fig. 9**).

The GMP and CMP nucleotides were also photomineralized under other conditions otherwise identical to those used for the degradation of the deoxyadenosine nucleotide. The corresponding temporal evolution of H_2PO_4^- ions, NH_4^+ and NO_3^- ions are illustrated in **Fig. 10** and **11**, respectively. In both cases, the dihydrogenphosphate anions formed fairly rapidly, followed by rapid adsorption onto the ZnO surface as evidenced by the rapid drop in concentration within a few minutes. For the guanosinemonophosphate nucleotide, a greater quantity of nitrate ions formed relative to ammonium ions. By contrast, for the CMP nucleotide, the quantity of NO_3^- ion produced was significantly lower than that of NH_4^+ ions. Such differences are likely due to structural differences between the guanine base (a purine) and the cytosine base (a pyrimidine). Note the similarities in the degradation of the adenine and guanine, both purine bases, and their contrast with the pyrimidine base cytosine.

4 Concluding Remarks

The zinc oxide pigment used in cosmetics and as a sunscreen active agent in commercial formulations causes significant damage to DNA under UV illumination. In line with earlier observations (28, 36), nicks on the supercoiled DNA plasmids were caused by $\cdot\text{OH}$ radicals photogenerated on UV-irradiated ZnO. The DNA constituent nucleotides dAMP, GMP and CMP undergo fairly rapid degradation in the presence of the ZnO pigment exposed to UV radiation producing dihydrogenphosphate, ammonium and nitrate ions. Evidently, the $\cdot\text{OH}$ radicals attack both the ribose and the deoxyadenosine, guanosine, and cytidine nucleotides within *ca.* 30 min of irradiation. The CeO_2 specimens were less photoactive than the corresponding metal oxides TiO_2 and ZnO toward phenol and DNA plasmids. Consequently, the CeO_2 specimens would be preferred as physical filters in cosmetics and sunscreen lotions over the other two metal oxides. The CaO -

doped-amorphous-SiO₂-coated CeO₂ system tended to be the least photoactive of the four ceria spheria specimens examined.

Acknowledgments

The work carried out in Tokyo was supported by the "Academic Frontier" Project for Private Universities with a matching fund subsidy from the Japanese Ministry of Education, Culture, Sports, Science and Technology (MEXT) for the period of 2003 ~ 2007, as well as from a Grant-in-Aid for Science Research (No. 17550145; MEXT: to H.H.). Studies carried out in Pavia were supported by a grant (to N.S.) from the Ministero dell' Università e della Ricerca (MIUR-Rome, Italy). Finally, we also wish to thank Prof. J. Knowland of Oxford University for several useful discussions on the matter of chemical sunscreen active agents.

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