Bipyrimidine Photoproducts Rather than Oxidative Lesions Are the Main Type of DNA Damage Involved in the Genotoxic Effect of Solar UVA Radiation†

Thierry Douki,‡ Anne Reynaud-Angelin,§ Jean Cadet,‡ and Evelyne Sage*,§

Laboratoire "Lésions des Acides Nucléiques", Service de Chimie Inorganique et Biologique & CNRS FRE 2600, CEA/DSM/Département de Recherche Fondamentale sur la Matière Condensée, CEA Grenoble, F-38054 Grenoble Cedex 9, France, and CNRS UMR 2027, Institut Curie, Centre Universitaire, F-91405 Orsay, France

Received April 14, 2003; Revised Manuscript Received June 3, 2003

ABSTRACT: Exposure to solar UV radiation gives rise to mutations that may lead to skin cancer. UVA (320−340 nm) constitutes the large majority of solar UV radiation but is less effective than UVB (290−320 nm) at damaging DNA. Although UVA has been implicated in photocarcinogenesis, its contribution to sunlight mutagenesis has not been elucidated, and DNA damage produced by UVA remains poorly characterized. We employed HPLC−MS/MS and alkaline agarose gel electrophoresis in conjunction with the use of specific DNA repair proteins to determine the distribution of the various classes and types of DNA lesions, including bipyrimidine photoproducts, in Chinese hamster ovary cells exposed to pure UVA radiation, as well as UVB and simulated sunlight ($\lambda > 295$ nm) for comparison. At UVA doses compatible with human exposure, oxidative DNA lesions are not the major type of damage induced by UVA. Indeed, single-strand breaks, oxidized pyrimidines, oxidized purines (essentially 8-oxo-7,8-dihydroguanine), and cyclobutane pyrimidine dimers (CPDs) are formed in a 1:1:3:10 ratio. In addition, we demonstrate that, in contrast to UVB and sunlight, UVA generates CPDs with a large predominance of TT CPDs, which strongly suggests that they are formed *via* a photosensitized triplet energy transfer. Moreover, UVA induces neither (6−4) photoproducts nor their Dewar isomers via direct absorption. We also show that UVA photons contained in sunlight, rather than UVB, are implicated in the photoisomerization of (6−4) photoproducts, a quickly repaired damage, into poorly repaired and highly mutagenic Dewar photoproducts. Altogether, our data shed new light on the deleterious effect of UVA.

Exposure to solar ultraviolet radiation is a major risk factor in the occurrence of skin cancer. UVC (190−280 nm) and short-wavelength UVB (280−295 nm) are totally blocked by the atmosphere and thus are not relevant for human health. The long-wavelength UVB (295−320 nm), the most energetic terrestrial wavelengths, represents only a few percent of the solar UV spectrum at the surface of Earth. UVB radiation is, however, most efficient at producing DNA damage, essentially bipyrimidine photoproducts, which leads to the mutagenic events at the origin of tumors (*1*, *2*). In contrast, lower-energy UVA photons (320−400 nm) constitute the large majority of terrestrial UV radiation but are less cytotoxic than UVB light. However, the recent widespread use of efficient UVB-blocking sunscreens is accompanied by prolonged periods of sunbathing, and thus leads to a large increase in the level of human exposure to UVA. This last trend is also emphasized by the popular use of high-intensity UVA-tanning equipments. The deleterious effect of UVA radiation has, as a consequence, emerged as a major concern in public health (*3*). Indeed, UVA is mutagenic at physiological radiation fluences (*4*−*6*). At present, the premutagenic DNA lesions induced by UVA have not been identified.

The nature of the DNA damage induced by UV radiation strongly depends on the wavelength of the incident photons that hit the cell (*7*). In the UVB range, direct light absorption by DNA results mainly in dimerization reactions between adjacent pyrimidine bases. Several types of lesions are produced, including cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6−4) pyrimidone photoproducts [(6−4) photoproducts].[1] The latter lesions may be converted into their Dewar valence isomers (Dewars) upon further exposure to near-UV radiation. UVA radiation is extremely weakly absorbed by DNA, but rather excites endogenous chromophores, leading to DNA damage. This occurs through the production of reactive singlet oxygen that specifically reacts with guanine within DNA (*8*, *9*). An additional but less likely pathway involves a one-electron oxidation of guanine by a photosensitizer in the excited state. Guanine is the major target of such photoreactions because it exhibits the lowest

† This work was supported by Institut Curie (genotoxicology program), Centre National de la Recherche Scientifique, and Commissariat à l'Énergie Atomique.

* To whom correspondence should be addressed: CNRS UMR 2027, Institut Curie, Bât. 110, Centre Universitaire, 91405 Orsay Cedex, France. Telephone: 33 (0)1 69 86 71 87. Fax: 33 (0)1 69 86 94 29. E-mail: Evelyne.Sage@curie.u-psud.fr.

‡ CEA Grenoble.

§ Institut Curie.

[1] Abbreviations: (6−4) photoproducts, pyrimidine (6−4) pyrimidone photoproducts; 8-oxoGua, 8-oxo-7,8-dihydroguanine; CHO, Chinese hamster ovary; CPDs, cyclobutane pyrimidine dimers; Nth, endonuclease III; Fpg, formamidopyrimidine DNA *N*-glycosylase; HPLC−MS/MS, high-performance liquid chromatography coupled to tandem mass spectrometry; SSL, simulated sunlight; T4 endoV, endonuclease V of bacteriophage T4.

10.1021/bi034593c CCC: $25.00
Published on Web 07/12/2003

ionization potential and thus behaves as a sink for the positive charges that migrate through the DNA double helix (*10*−*12*). Therefore, guanine lesions, and particularly the widely studied 8-oxo-7,8-dihydroguanine (8-oxoGua), are expected to be the most frequent damage resulting from the photooxidation of DNA. UVA may also promote the formation of hydroxyl radicals *via* the photosensitized production of superoxide anions. Because of their high reactivity and low specificity, hydroxyl radicals likely induce a wide range of DNA damage.

However, the effects of solar light cannot be simply rationalized in terms of UVB-induced pyrimidine dimerization and UVA-triggered oxidative DNA damage. Indeed, UVA has been reported to give rise to CPDs (*13*−*15*), but much less efficiently than UVB radiation does. The mechanism of CPD formation by UVA has not been elucidated. There are also indications that, in UVA-irradiated cells, CPDs can be more frequently produced than 8-oxoGua (*14*, *16*), in agreement with the extremely low frequency of GC to TA transversions, the mutagenic hallmark of 8-oxoGua, in UVA-induced mutation spectra in mammalian cells (*5*, *6*, *17*).

In the work presented here, we employed different techniques to more extensively describe the distribution of DNA damage within Chinese hamster ovary (CHO) cells exposed to pure UVA radiation, in comparison with UVB (295−320 nm) and broad spectrum simulated sunlight (SSL, $\lambda > 295$ nm). Electrophoretic analysis in combination with the use of specific DNA repair enzymes showed that strand breaks, oxidized pyrimidines, and purines were less frequently formed than CPDs upon UVA irradiation. HPLC−tandem mass spectrometry (HPLC−MS/MS) quantification (*18*, *19*) of CPDs and (6−4) and Dewar photoproducts at the four possible bipyrimidine sites shed light on their mechanism of formation upon exposure to UVA and SSL. Altogether, our data establish that pyrimidine dimerization products rather than oxidative lesions are predominantly involved in UVA-induced DNA damage in cultured mammalian cells, as in the case of UVB. These observations bring new insight into the role of UVA in solar mutagenesis.

MATERIALS AND METHODS

Cell Irradiation. Nucleotide excision repair-deficient (ERCC1) UVL9 CHO cells were grown in αMEM supplemented with 10% fetal bovine serum. Twenty-four hours prior to irradiation, 2.5×10^6 cells were plated in 60 mm Petri dishes. Cells were irradiated on ice in PBS buffer. Irradiation conditions have been extensively described elsewhere (*15*, *16*). Briefly, cells were exposed to filtered broad band UVB radiation (295−320 nm) produced by a series of standard 15 W fluorescent tubes (Vilber-Lourmat, Torcy, France) and to simulated sunlight ($\lambda > 295$ nm) produced by a xenon arc lamp (XBO, OSRAM, Molsheim, France). Polychromatic UVA radiation was obtained from a SUPERSUN 5000 lamp (Mutzhas, Münich, Germany) which emits virtually exclusively in the UVA1 range (340−440 nm, Figure 1). The spectral outputs of the lamps and the properties of the cutoff filters (Schott, Mainz, Germany) employed in the case of UVB and SSL to eliminate contaminating UVC and short UVB wavelengths have been rigorously characterized (*20*). Cells received either 750−4500 kJ/m^2 of UVA radiation at a fluence rate of 1 kJ m^{-2} s^{-1}, 0.5−2 kJ/m^2 of UVB at a fluence rate of 8.5 J m^{-2} s^{-1}, or 2500−7500 kJ/m^2 of SSL at a fluence rate of 1600 kJ m^{-2} s^{-1}.

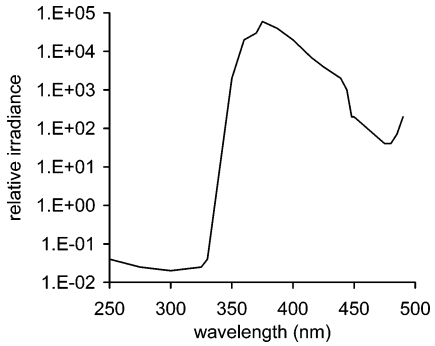

FIGURE 1: Emission spectrum of the UVA lamp that was used. Note the log scale for the intensity.

Identification of DNA Photolesions by Repair Enzymes and Alkaline Gel Electrophoresis. After irradiation, cells were harvested and DNA was immediately extracted as previously reported (*16*). Aliquots (2 ng) of DNA from irradiated or nonirradiated cells were incubated for 45 min at 37 °C, in a reaction buffer (12 μL) that contained 250 ng of either *Escherichia coli* Nth, Fpg protein, or T4 endoV (obtained from S. Boiteux, CEA, Fontenay-aux Roses, France, and J. Brouwer, Leiden University, Leiden, The Netherlands). Prior to being loaded on a 0.6% alkaline agarose gel, samples were denatured for 20 min at room temperature by adding 10 μL of 100 mM NaOH and 4 mM EDTA and 10 μL of denaturing gel loading buffer (50% glycerol, 1 N NaOH, and 0.2% bromocresol green). Electrophoresis was carried out overnight at 1.3 V/cm at room temperature. Electrophoretic profiles were analyzed with the Biocom image analyzer and Lecphor software (Les Ulis, France). Then, the number of strand breaks per Mbp was assessed according to the method of Sutherland *et al*. (*21*).

HPLC−MS/MS Quantification of Bipyrimidine Photoproducts. Following irradiation, cells were harvested and DNA was extracted using a chaotropic method (*22*). It was then first digested with P1 nuclease and phosphodiesterase II, and further incubated with alkaline phosphatase and phosphodiesterase I as previously described (*18*). The reaction mixture contained normal nucleosides and the bipyrimidine photoproducts under the form of modified dinucleoside monophosphates. These reaction products were separated on an Uptisphere ODB (Montluçon, France) octadecylsilyl silica gel column [3 μm particle size, 150 mm × 2 mm (inside diameter)]. The column oven was set at 28 °C, and the flow rate was 200 μL/min. The mobile phase was a gradient of acetonitrile [maximum proportion of 20% (v/v)] in 2 mM triethylammonium acetate. Methanol was added at a flow rate of 100 μL/min at the outlet of the column. Normal nucleosides were quantified by a HPLC UV detector set at 280 nm. The level of each type of modified dinucleoside monophosphates was determined by on-line electrospray ionization tandem mass spectrometry as previously described (*18*, *19*). For this purpose, deprotonated ions of each of the modified dinucleoside monophosphates were isolated and fragmented. For each of those, the intensity of a resulting specific daughter ion was monitored. The amount of each type of bipyrimidine photoproduct was inferred from

an external calibration obtained by injecting known amounts of the authentic compound. The yield of formation of a given photoproduct was calculated from the linear regression of the dose−response curve.

Photosensitized Formation of Cyclobutane Dimers and Photoisomerization of (6−4) Photoproducts within Isolated DNA. An argon-degassed aqueous solution (5 mL) of calf thymus DNA (1 mg/mL, pH 7) was exposed to 10−60 kJ/m^2 of UVA radiation (maximum emission of 365 nm, dose rate of 2 kJ m^{-2} min^{-1}) with magnetic stirring in the presence of benzophenone (a saturated solution) or acetophenone (10 μM), or in the absence of a sensitizer. DNA was recovered by precipitation with NaCl and cold ethanol, digested, and analyzed by HPLC−MS/MS for its content in photoproducts, as described above. A linear rate of formation of TT, TC, and CT CPDs with respect to the dose was observed. Linear regression provided the yield of lesion formation per kilojoule per square meter. To study the photoisomerization of (6−4) photoproducts by UVA, a solution (9 mL) of calf thymus DNA (1 mg/mL, pH 7) was first exposed to UVC radiation (overall dose of 2.3 kJ/m^2) to create (6−4) photoproducts. Increasing doses of UVA radiation (0, 5, 15, and 30 kJ/m^2) were then applied with magnetic stirring to aliquot fractions of 2 mL. DNA was then digested and analyzed by HPLC−MS/MS in triplicate, as described above.

RESULTS AND DISCUSSION

To gain further insight into the mechanisms underlying the genotoxic properties of UVA, we undertook a thorough determination of the distribution of various classes of DNA damage in Chinese hamster ovary (CHO) cells upon exposure to UVA radiation, in comparison with exposure to UVB and SSL. The emissions of the UVB and SSL sources were rigorously filtered using 2 mm thick WG305 and WG320 Schott cutoff filters to eliminate UVC and short-UVB photons. The UV irradiance for the filtered SSL is composed of 4.6% UVB and 95.4% UVA (*20*). The emission spectrum of the UVA lamp is constituted almost totally by radiation in the UVA1 range (340−400 nm), as seen in Figure 1. The UVA fluences corresponded to those obtained by exposure for 1−5 h to natural sunlight in Paris, at zenith, in summer (*23*).

CPDs Are Formed in Significantly Greater Yields than Oxidative Products in UVA-Irradiated Cells. Using alkaline gel electrophoresis which detects DNA strand breaks, we first determined the distribution of DNA lesions among various classes, in CHO cells exposed to either UVA, UVB, or SSL. Frank strand breaks and alkali-labile sites were directly detected. Oxidized pyrimidine and purine bases and CPDs were quantified following their conversion into single-strand breaks by specific DNA repair proteins, i.e., endonuclease III (Nth) and formamidopyrimidine *N*-glycosylase (Fpg) from *E. coli*, and endonuclease V from bacteriophage T4 (T4 endoV), respectively. The four classes of DNA lesions induced by solar UV radiation were thus revealed using a single assay. Figure 2A shows that oxidized purine bases, detected as Fpg-sensitive sites, were the most abundant oxidation products induced by UVA. It is probable that these lesions are essentially 8-oxoGua. Direct strand breaks and oxidized pyrimidine bases were also observed, but were much less frequent. In line with these results, the formation

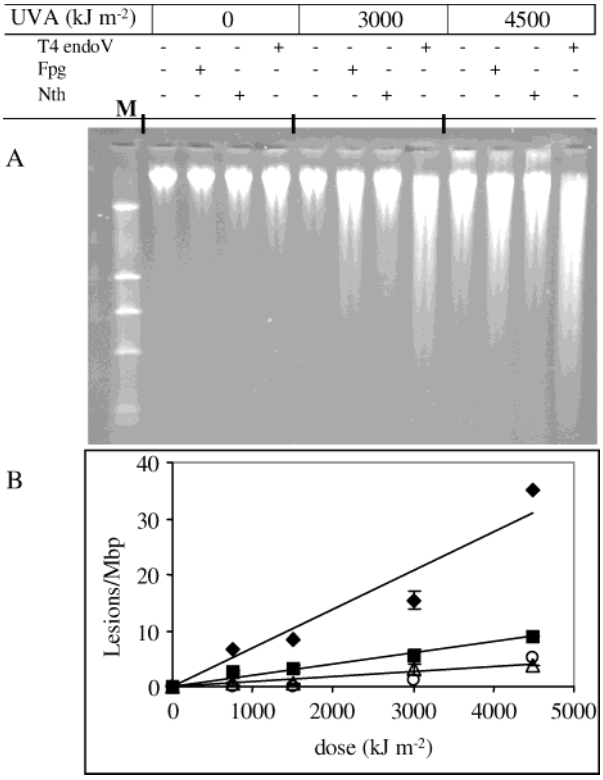

FIGURE 2: Direct and DNA repair enzyme-induced strand breaks within CHO cells exposed to UVA radiation. (A) Electrophoregram of DNA extracted from CHO cells either unirradiated or irradiated with UVA at doses of 3000 or 4500 kJ/m^2, and incubated in the absence or presence of T4 endoV, Nth, or Fpg proteins. (B) Induction of frank strand breaks with alkali-labile sites (△) or Nth-, Fpg-, and T4 endoV-sensitive sites, corresponding to oxidized pyrimidine (○), oxidized purines (■), and CPDs (◆), respectively, as a function of UVA dose. From the linear regression, the yields of the above UVA-induced photolesions were 9×10^{-4}, 8×10^{-4}, 2×10^{-3}, and 7×10^{-3} per 10^6 bases per kilojoule per square meter, respectively.

of thymine oxidation products could not be detected using the sensitive HPLC−MS/MS method, while induction of 8-oxoGua was unambiguously observed (data not shown). A low level of production of single-strand breaks and Nth-sensitive sites, relative to the level of Fpg-sensitive sites in UVA-irradiated cells, has also been observed using alkaline elution (*14*) and the comet assay (*24*). The yield of formation of single-strand breaks in our study is in excellent agreement with earlier reports on CHO and human cells irradiated with nearly monochromatic 365 nm radiation (*25*, *26*). These results provide further support for a minor contribution of hydroxyl radicals and a major role of singlet oxygen in the formation of oxidative DNA lesions by UVA (*14*, *24*).

Following irradiation with UVB or SSL, oxidized bases and DNA strand breaks were barely detected (data not shown). However, for the three types of radiation, CPDs were by far the major lesions that were formed. Particularly striking, UVA irradiation produced CPDs in a much higher yield than oxidative damage (Figure 2A,B). The latter observation confirms our previous data and a published report which indicated a higher level of production of CPDs than 8-oxoGua by UVA, using detection methods different from those used here and a different UVA source (*14*, *16*). The comparative rates of UVA-induced formation of CPDs, Fpg- and Nth-sensitive sites, and DNA strand breaks yield a ratio of approximately 10:3:1:1. Our data demonstrate that oxida-

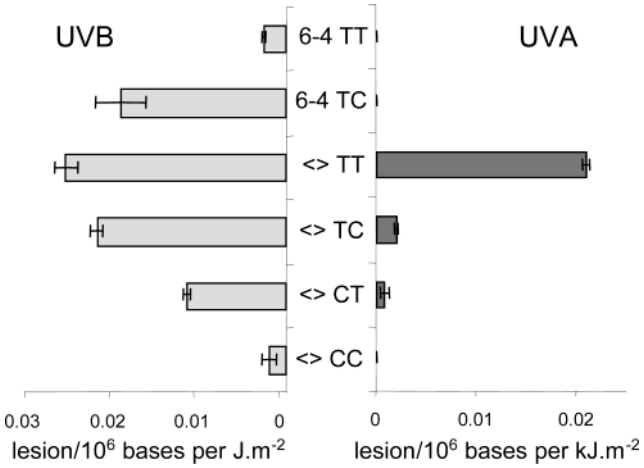

FIGURE 3: Distribution of cyclobutane dimers (< >) and (6−4) photoproducts (6−4) at the four possible bipyrimidine sites within the DNA of CHO cells upon exposure to either UVA or UVB radiation. Neither type of Dewar was detected. Results represent the yield of formation (±standard error) obtained by linear regression of the level of lesion with respect to the applied dose ($n = 12$).

tive DNA lesions, mainly constituted by 8-oxoGua, are not the major class of damage produced by UVA while, unexpectedly, bipyrimidine photoproducts as CPDs are.

UVA-Induced CPDs Are Predominantly Formed at Thymine−Thymine Sites by Photosensitization. The observation that CPDs, revealed as T4 endoV-sensitive sites, were the major lesions led us to more thoroughly investigate the formation of bipyrimidine lesions, i.e., CPDs and (6−4) and Dewar photoproducts. The induction of such lesions has been previously assessed in CHO cells exposed to UVB, SSL, or UVA, using a calibrated immuno-dot-blot assay (*15*). However, this technique, as well as other immunological methods and most of those based on the use of repair enzymes, cannot reveal the distribution of the lesions arising at the four possible bipyrimidine sequences. In contrast, the recently developed HPLC−MS/MS assay (*18*, *19*) used below allows the simultaneous and individual quantification of CPDs, (6−4) photoproducts, and Dewars at TT, TC, CT, and CC sites.

In UVB-irradiated cells (Figure 3), the main bipyrimidine photoproducts were observed at TT and TC sequences, and the overall ratio of CPDs to (6−4) photoproducts was 3:1. The proportion of TT photoproducts is slightly lower than that previously reported in human monocytes (*19*). This is due to the use of a short-UVB wavelength cutoff filter in this work. The shift of the emission toward longer wavelengths favors the excitation of cytosine with respect to thymine, leading to an increase in the yield of cytosine-containing photoproducts (*27*). Dewars were not detected, and cyclobutane dimers at CC were rather infrequent.

This distribution profile is in great contrast to that observed upon UVA irradiation. Only thymine-containing CPDs, with a large majority of CPDs at TT sites (Figure 3), were detected in the DNA of UVA-irradiated cells. Interestingly, (6−4) photoproducts, whose formation involves photocycloaddition of a pyrimidine base in its singlet excited state with an adjacent thymine or cytosine residue, are not produced by UVA under our conditions. The lack of formation of (6−4) photoproducts and Dewars rules out direct excitation of DNA bases by UVA photons as a major process in the formation of CPDs. This point is strengthened by the lack of emission of the UVA lamp used at wavelengths below <320 nm that could have been absorbed by DNA bases (Figure 1). These observations together with the almost exclusive production of thymine-containing CPDs are rather evocative of a DNA damaging process *via* photosensitization. Indeed, it has long been known that UVA irradiation of DNA in the presence of chromophores, such as aromatic ketones, induces the formation of CPDs through triplet energy transfer (*28*, *29*). In this case, thymine is the major target, because of its lower triplet state energy with respect to the other bases. To further investigate such a possibility, we exposed calf thymus DNA to UVA in the presence of acetophenone or benzophenone, and analyzed the production of the diverse CPDs by HPLC−MS/MS. CPDs at CC sites were not observed. TT, TC, and CT cyclobutane pyrimidine dimers were produced in 68:17:15 and 66:14:20 ratios upon acetophenone- and benzophenone-mediated UVA photosensitization, respectively (Table 1). In the case of UVA-irradiated CHO cells, the ratio was 87:9:4. Even though this similarity in CPD distribution is not absolute proof, our data strongly suggest a photosensitized triplet energy transfer from an excited chromophore to thymine as a major process in the formation of CPDs in UVA-irradiated cells. The UVA absorbing chromophore that is involved remains to be identified. However, it may already be anticipated that it is tightly bound to DNA and in the vicinity of the bases to favor energy transfer. The latter feature of the putative photosensitizer is likely to explain why thymine-containing CPDs could be detected, though to a lesser extent than within DNA of irradiated cells, in crude commercially available calf thymus DNA (likely to be contaminated by proteins and other compounds) exposed to high doses of radiation from our UVA lamp (data not shown).

Table 1: Yields of Formation of Thymine-Containing CPDs within Isolated DNA Photosensitized to UVA by Benzophenone or Acetophenone[a]

photosensitizer	< > TT	< > TC	< > CT
acetophenone	0.109 ± 0.007	0.027 ± 0.002	0.025 ± 0.002
benzophenone	0.022 ± 0.002	0.005 ± 0.001	0.006 ± 0.001

[a] The yields of formation (expressed as CPDs per 10^4 bases per kilojoule per square meter ± standard error) were obtained by linear regression of the UVA dose−response curve ($n = 12$). Neither CC cyclobutane pyrimidine dimer nor any of the (6−4) and Dewar photoproducts were detected.

UVA Photons within Sunlight Convert (6−4) Photoproducts into Dewar Isomers. The unexpected damage distribution observed for UVA led us to search for the formation of the bipyrimidine photoproducts induced by simulated sunlight, which comprises a ratio of UVA to UVB energy of ~95:5. As observed for UVB, SSL induced damage at all bipyrimidine sites and both CPDs and (6−4) photoproducts were produced (Figure 4). However, significant differences were observed. First, the proportion of cyclobutane dimers at TT sites was higher in SSL- than in UVB-exposed cells. This likely reflects the UVA contribution, even though the UVB component which represents no more than 5% of the solar UV energy is expected to induce most of the damage as a result of the relatively strong absorption of DNA at these wavelengths as compared to UVA.

The most striking observation was the high relative yield of Dewar photoproducts at the expense of their (6−4)

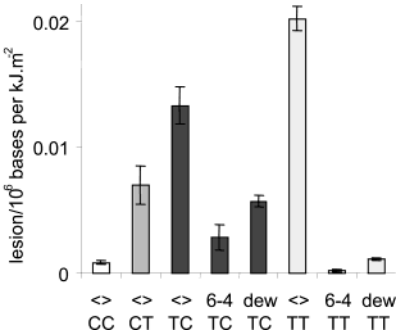

FIGURE 4: Distribution of bipyrimidine photoproducts within the DNA of SSL-irradiated CHO cells: < >, cyclobutane dimers; 6−4, (6−4) photoproducts; and Dew, Dewar valence isomers. Results represent the yield of formation (±standard error) obtained by linear regression of the level of lesion with respect to the applied dose ($n = 12$).

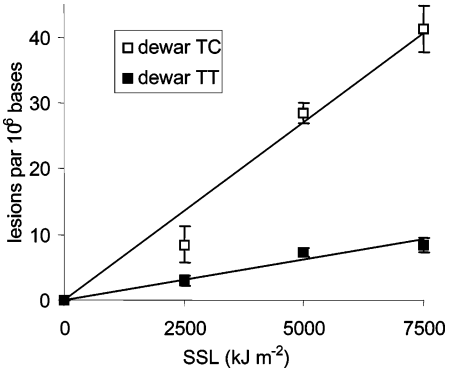

FIGURE 5: Formation of TT (■) and TC (□) Dewar valence isomers upon exposure of CHO cells to simulated solar light (SSL). Results represent the average (±standard deviation) of three independent experiments.

photoproduct precursors (Figure 4), as previously reported using immunological assays (*15*, *30*). UVB photons present in SSL can be ruled out as the radiation involved in the formation of the Dewar photoproducts. First, these latter lesions were not detected using a pure UVB source, at similar damaging doses (Figure 3). Second, the extent of formation of Dewar photoproducts in SSL-irradiated cells was found to be linear with respect to the dose (Figure 5), while the extent of formation of Dewar isomers upon exposure of DNA to UVB light is not expected to be linear, as we recently observed within isolated DNA (*18*, *19*). Indeed, even though the quantum yield of photoisomerization of (6−4) photoproducts is 20 times higher than that for their formation (*31*), the rate of induction of Dewar isomers by UVB light is limited by the competition between (6−4) photoproducts and normal bases for the absorption of the incident photons. As a result, a significant photoisomerization is only observed at a sufficient level of (6−4) photoproducts. Accordingly, only 10% of the (6−4) photoproducts were photoisomerized for UVB doses generating approximately one (6−4) photoproduct per 10^3 bases within isolated DNA (*18*, *19*). In the work presented here, the total amount of (6−4) and Dewar photoproducts formed in cells exposed to the largest SSL dose was at least 20 times smaller than the amount mentioned above. These overall observations thus clearly show that UVB cannot be involved in the photoisomerization of (6−4) photoproducts in SSL-irradiated cells and that the incident photons responsible for the formation of Dewar isomers are not efficiently absorbed by normal bases. Such

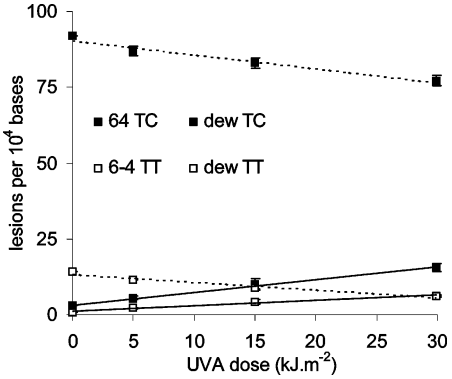

FIGURE 6: Photoisomerization of the TT (■ with the dashed line) and TC (□ with the dashed line) (6−4) photoproducts into their respective Dewar isomers (■ and □ with solid lines) within UVC-irradiated isolated DNA exposed to UVA. Results represent the average (±standard deviation) of three independent experiments.

a consideration led us to propose that the UVA portion of the SSL spectrum is responsible for the photoisomerization of the (6−4) photoproducts.

To bring further support to this finding, we explored the ability of UVA radiation to photoisomerize (6−4) photoproducts in calf thymus DNA. Exposure of UVC-irradiated DNA to various doses of UVA radiation leads to a decrease in the level of (6−4) photoproducts with a concomitant increase in the amount of Dewar photoproducts formed (Figure 6). At the highest dose of UVA radiation, 51% of TT (6−4) photoproducts were converted into their Dewar valence isomers, while only 17% of the TC (6−4) photoproducts were isomerized. The ratio of 3 observed between the yields of photoisomerization of TT and TC (6−4) photoproducts into Dewar photoproducts is exactly that obtained in SSL-irradiated cells (Figure 4). The more efficient photoisomerization of (6−4) TT with respect to (6−4) TC cannot be explained by the quantum yields of the photoreaction, since they are similar in both cases (*31*). It may be better explained by the shift of the maximum UV absorption toward longer wavelengths in the case of (6−4) TT versus (6−4) TC (325 and 315 nm, respectively) (*32*, *33*). These features point again to a major role of UVA in the photoisomerization process since UVA is not directly absorbed by purines and pyrimidines and may be absorbed by (6−4) photoproducts.

Biological Relevance. In summary, we have shown that, in mammalian cells, the contribution of oxidative lesions, mostly 8-oxoGua, to the DNA damage induction by solar UVA radiation is minor in comparison to the contribution of bipyrimidine photoproducts. Thymine−thymine cyclobutane dimers, likely formed by photosensitization, are the predominant lesions produced by pure UVA radiation; 8-oxoGua is quantitatively the second most important lesion, while (6−4) and Dewar photoproducts are not detectable. As opposed to CPDs, 8-oxoGua is rapidly and efficiently repaired in mammalian cells (*34*, *35*). When unremoved, 8-oxoGua is expected to cause GC to TA transversion (*36*). Such mutations are however poorly recovered either in mutation spectra generated in mammalian cells irradiated with UVB, UVA, and SSL (*5*, *6*, *17*) or at the mutated tumor suppressor p53 gene in skin tumors (*2*, *37*). In contrast, there is evidence to support a preeminent role in solar mutagenesis for the CPDs, which are repaired much more slowly, relative

to (6−4) photoproducts (*38*, *39*). In addition, a large majority of the mutations induced by UVA in mammalian cells occur at bipyrimidine sites (*5*, *6*, *17*), including at TT sites (*5*). This observation further supports a role for CPDs in UVA mutagenesis. Besides the significant induction of CPDs at doses compatible with human exposure, UVA radiation may also contribute to the biological effect of sunlight by its involvement, rather than UVB, in the photoisomerization of (6−4) photoproducts. UVA converts the efficiently and quickly repaired (6−4) photoproducts into poorly repaired (*15*) and highly mutagenic (*40*, *41*) Dewar valence isomers. Taken together, the data presented here establish new and major pathways for UVA genotoxicity that should now be investigated in human skin cells.

ACKNOWLEDGMENT

We are grateful to Dr. D. Grierson (CNRS-IC UMR 176) for critical reading of the manuscript. We thank Didier Boucher (CNRS-IC UMR 2027) for his help with the quantification of strand breaks.

REFERENCES

1. Setlow, R. B. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3363−3366.
2. Brash, D. E., Rudolph, J. A., Simon, J. A., Lin, A., McKenna, G. J., Baden, H. P., Halperin, A. J., and Ponten, J. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 10124−10128.
3. Gasparro, F. P. (2000) *Environ. Health Perspect. 108* (Suppl. 1), 71−78.
4. Tyrrell, R. M., and Keyse, S. M. (1990) *J. Photochem. Photobiol., B 4*, 349−361.
5. Drobetsky, E. A., Turcotte, J., and Chateauneuf, A. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 2350−2354.
6. Stary, A., Robert, C., and Sarasin, A. (1997) *Mutat. Res. 383*, 1−8.
7. Ravanat, J.-L., Douki, T., and Cadet, J. (2001) *J. Photochem. Photobiol., B 63*, 88−102.
8. Cadet, J., and Teoule, R. (1978) *Photochem. Photobiol. 28*, 661−667.
9. Ravanat, J.-L., Saint-Pierre, C., Di Mascio, P., Martinez, G. R., Medeiros, M. H., and Cadet, J. (2001) *Helv. Chim. Acta 84*, 3702−3709.
10. Treadway, C. R., Hill, M. G., and Barton, J. K. (2002) *Chem. Phys. 281*, 409−428.
11. Giese, B. (2000) *Acc. Chem. Res. 33*, 631−636.
12. Schuster, G. B. (2000) *Acc. Chem. Res. 33*, 253−260.
13. Kvam, E., and Tyrrell, R. M. (1997) *Carcinogenesis 18*, 2379−2384.
14. Kielbassa, C., Roza, L., and Epe, B. (1997) *Carcinogenesis 18*, 811−816.
15. Perdiz, D., Grof, P., Mezzina, M., Nikaido, O., Moustacchi, E., and Sage, E. (2000) *J. Biol. Chem. 275*, 26732−26742.
16. Douki, T., Perdiz, D., Grof, P., Kulucsics, Z., Moustacchi, E., Cadet, J., and Sage, E. (1999) *Photochem. Photobiol. 70*, 184−190.
17. Sage, E., Lamolet, B., Brulay, E., Moustacchi, E., Chateauneuf, A., and Drobetsky, E. A. (1996) *Proc. Natl. Acad. Sci. U.S.A. 93*, 176−180.
18. Douki, T., Court, M., Sauvaigo, S., Odin, F., and Cadet, J. (2000) *J. Biol. Chem. 275*, 11678−11685.
19. Douki, T., and Cadet, J. (2001) *Biochemistry 40*, 2495−2501.
20. Grof, P., Ronto, G., and Sage, E. (2002) *J. Photochem. Photobiol., B 68*, 53−59.
21. Sutherland, B. M., Bennett, P. V., and Sutherland, J. C. (1999) *Methods Mol. Biol. 113*, 183−202.
22. Helbock, H. J., Beckman, K. B., Shigenaga, M. K., Walter, P. B., Woodall, A. A., Yeo, H. C., and Ames, B. N. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 288−293.
23. Kuluncsics, Z., Perdiz, D., Brulay, E., Muel, B., and Sage, E. (1999) *J. Photochem. Photobiol., B 49*, 71−80.
24. Pouget, J.-P., Douki, T., Richard, M.-J., and Cadet, J. (2000) *Chem. Res. Toxicol. 13*, 541−549.
25. Peak, M. J., Peak, J. G., and Carnes, B. A. (1987) *Photochem. Photobiol. 45*, 381−387.
26. Churchill, M. E., Peak, J. G., and Peak, M. J. (1991) *Photochem. Photobiol. 54*, 639−644.
27. Ellison, M. J., and Childs, J. D. (1981) *Photochem. Photobiol. 34*, 465−469.
28. Lamola, A. A. (1970) *Pure Appl. Chem. 24*, 599−610.
29. Charlier, M., and Hélène, C. (1972) *Photochem. Photobiol. 51*, 527−536.
30. Clingen, P. H., Arlett, C. F., Roza, L., Mori, T., Nikaido, O., and Green, M. H. L. (1995) *Cancer Res. 55*, 2245−2248.
31. Lemaire, D. G. E., and Ruzsicska, B. P. (1993) *Photochem. Photobiol. 57*, 755−769.
32. Franklin, W. A., Doetsch, P. W., and Haseltine, W. A. (1985) *Nucleic Acids Res. 13*, 5317−5325.
33. Rycyna, R. E., and Alderfer, J. L. (1985) *Nucleic Acids Res. 13*, 5949−5963.
34. Friedberg, E. C., Walker, G. C., and Siede, W. (1995) *DNA repair and mutagenesis*, ASM Press, Washington, DC.
35. Klungland, A., Rosewell, I., Hollenbach, S., Larsen, E., Daly, G., Epe, B., Seeberg, E., Lindahl, T., and Barnes, D. E. (1999) *Proc. Natl. Acad. Sci. U.S.A. 96*, 13300−13305.
36. Shibutani, S., Takeshita, M., and Grollman, A. P. (1991) *Nature 349*, 431−434.
37. Dumaz, N., Stary, A., Soussi, T., Daya-Grosjean, L., and Sarasin, A. (1994) *Mutat. Res. 307*, 375−386.
38. Mullenders, L. H., Hazekamp-van Dokkum, A. M., Kalle, W. H., Vrieling, H., Zdzienicka, M. Z., and van Zeeland, A. A. (1993) *Mutat. Res. 299*, 271−276.
39. You, Y.-H., and Pfeifer, G. P. (2001) *J. Mol. Biol. 305*, 389−399.
40. Horsfall, M. J., and Lawrence, C. W. (1993) *J. Mol. Biol. 235*, 465−471.
41. Lee, J.-H., Bae, S.-H., and Choi, B.-S. (2000) *Proc. Natl. Acad. Sci. U.S.A. 97*, 4591−4596.

BI034593C