beled by us as certain has had to be revised. Previous incorrect tentative assignments that are now revised and solid are AU7, AU12, GC11, and GC3. All other internal imino assignments are solid, in our opinion, except the following: two GC pairs GC1 and GC51 could be interchanged since they are based on melting data; T54-m^{1}A58 is in dispute; G19-C56 has not been located at all. Among noninternally bonded imino proton resonances only three out of eight are assigned, namely, Ψ55N3, Ψ39N3, and U59. Some of the other five may exchange too rapidly to be seen by NMR at neutral pH.

Thus, on the basis of our work on this tRNA and on yeast tRNA^{Asp} published elsewhere, and on the work cited from other laboratories at 500 MHz, we conclude that reliable assignments in these molecules can now be made relatively rapidly and in adequate numbers for use in studies of conformation change and mobility and interactions with proteins.

Acknowledgments

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References

Griffey, R. H., Poulter, C. D., Yamaizumi, Z., Nishimura, S., & Hurd, R. E. (1982) *J. Am. Chem. Soc. 104*, 5810–5811.

Hare, D. R., & Reid, B. R. (1982) *Biochemistry 21*, 1835–1842.

Heerschap, A., Haasnoot, C. A. G., & Hilbers, C. W. (1982) *Nucleic Acids Res. 10*, 6981–7000.

Hurd, R. E., & Reid, B. R. (1979) *Biochemistry 18*, 4017–4023.

Jardetzky, O., & Roberts, G. C. K. (1981) *NMR in Molecular Biology*, Academic Press, New York.

Johnston, P. D., & Redfield, A. G. (1978) *Nucleic Acids Res. 5*, 3913–3927.

Johnston, P. D., & Redfield, A. G. (1981a) *Biochemistry 20*, 1147–1156.

Johnston, P. D., & Redfield, A. G. (1981b) *Biochemistry 20*, 3995–4006.

Johnston, P. D., Figueroa, N., & Redfield, A. G. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3130–3134.

Patel, D. J., Pardi, A., & Itakura, K. (1982) *Science (Washington, D.C.) 216*, 581–590.

Reid, B. R. (1981) *Annu. Rev. Biochem. 50*, 969–996.

Roy, S., & Redfield, A. G. (1981) *Nucleic Acids Res. 9*, 7073–7083.

Roy, S., Papastavros, M. Z., & Redfield, A. G. (1982a) *Nucleic Acids Res. 10*, 8341–8349.

Roy, S., Papastavros, M. Z., & Redfield, A. G. (1982b) *Biochemistry 21*, 6081–6088.

Sânchez, V., Redfield, A. G., Johnston, P. D., & Tropp, J. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 5659–5662.

Tropp, J. S., & Redfield, A. G. (1981) *Biochemistry 20*, 2133–2140.

Cyclobutane-Type Pyrimidine Photodimer Formation and Excision in Human Skin Fibroblasts after Irradiation with 313-nm Ultraviolet Light†

Hugo J. Niggli and Peter A. Cerutti*

ABSTRACT: The formation and excision of 313-nm light-induced cyclobutane-type pyrimidine photodimers were determined in confluent cultures of human fibroblasts. A new method was developed for the resolution and determination of cytosine–thymine (CT) and thymine–thymine dimers (TT) by using sodium borohydride reduction and high-pressure liquid chromatography. This assay can detect as little as 1.8 TT or 5.6 CT per 10^8 daltons, levels induced in monolayers of human skin fibroblasts by doses of 1 and 2 kJ m^{-2} of 313-nm light, respectively. CT formation was 20% more efficient than TT formation in the physiological dose range of 2.25–15 kJ m^{-2} at 37 °C. Normal fibroblasts removed 61% TT within the first 8 h of incubation following a dose of 5.5 kJ m^{-2}. CT was removed approximately twice as efficiently as TT during the same time period following exposure to 10 kJ m^{-2}. The lack of removal of CT as well as TT observed in xeroderma pigmentosum fibroblasts indicates that the repair deficiency in these cells affects the repair of both classes of dimers.

Ultraviolet light in the range from 290 to 320 nm (UV-B)[1] is mostly responsible for sunlight-induced skin cancer (Blum, 1959; Urbach, 1975). DNA remains the most important target for the biological effects of UV light in this region of the spectrum, although its absorption decreases preciptously toward longer wavelengths (Setlow, 1974; Sutherland & Griffin, 1981). The major mechanism of the formation of DNA damage by UV-C in the region of the absorption maximum of DNA involves the electronic excitation of the heterocyclic

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[1] Abbreviations: CT, cyclobutane-type photodimer between cytosine and thymine; CC, cyclobutane-type cytosine–cytosine photodimer; DMEM, Dulbecco's modified Eagle's medium; HPLC, high-pressure liquid chromatography; $NaBH_4$, sodium borohydride; NF, normal human skin fibroblasts; PyT, cyclobutane-type pyrimidine dimer containing thymine; TT, cyclobutane-type thymine–thymine photodimer with cis-syn stereochemistry; UT, cyclobutane-type photodimer between uracil and thymine; UV, ultraviolet light; UV-B, UV light from 290 to 320 nm; UV-C, UV light of wavelength shorter than 290 nm; XPA, xeroderma pigmentosum skin fibroblasts of complementation group A; Me_2SO, dimethyl sulfoxide.

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basis. This mechanism continues to be important in the UV-B region, but in addition, photosensitization (Kaneko et al., 1979; Sutherland & Griffin, 1980) and indirect action contribute to damage formation (Elkind & Han, 1978; Netrawali & Cerutti, 1979). Cyclobutane-type dimerization represents the predominant photoreaction of DNA even in the UV-B region. However, different relative amounts of dimer subspecies, i.e., TT, CT, CC, may form in the UV-C and UV-B regions (Ellison & Childs, 1981), and we have recently observed that their distribution in chromatin is wavelength dependent (Niggli & Cerutti, 1982). Changes in the photochemistry of chromatin and in lesion repairability may be responsible for the observed differences in the biological effects of UV-C and UV-B. In particular, wavelength-dependent changes in the formation of CT relative to TT might have important consequences. Dimers containing cytosine may be of particular biological importance because they can spontaneously deaminate to form a uracil-containing dimer (Wacker, 1963; Freeman et al., 1965; Setlow et al., 1965; Hariharan & Johns, 1968). Photoreactivation of such a dimer would leave mutagenic lesions in the DNA. Studies on UV mutagenesis in procaryotes indicate that the presence of pyrimidine dimers may be a prerequisite for a mutagenic event (Schaaper & Glickman, 1982). In addition, a substantial fraction of mutation induced by UV irradiation in the absence of photoreactivation occurs as a C to T transition (Drake, 1963; Person et al., 1974; Coulondre & Miller, 1977) which may be due to a cytosine-containing pyrimidine dimer or a pyrimidine–pyrimidine (6–4) photoproduct (Brash & Haseltine, 1982). Therefore, we were interested in studying the efficiency of formation and the excision repair of CT as well as TT induced by 313-nm light in human skin fibroblasts. In order to be able to use physiological doses, we have improved the procedures for the chromatographic determination of cyclobutane-type pyrimidine dimers. The differences in the chemical properties of the dimers and their parent bases were augmented by reduction with $NaBH_4$ before separation by HPLC. We found that CT dimers were formed more efficiently than TT dimers at 313 nm and that they were exicised more rapidly during posttreatment incubation.

Materials and Methods

Cell Culture and Irradiation Conditions. Skin fibroblasts from normal individuals (CRL 1221 and CRL 1222) and from a patient with XP12BE of complementation group A (CRL 1223) were obtained from the American Type Culture Collection. They were grown in monolayers in 6-cm petri dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. They were labeled with [^{3}H]thymidine for 12–24 h at a concentration of 2 μCi mL^{-1} (specific activity 43 Ci mmol^{-1}) and then grown for 5–6 days in nonradioactive medium during which time the cultures became confluent. Prior to irradiation, the monolayers were washed with serum-free DMEM from which phenol red, riboflavin, and tryptophan had been omitted. The cultures were irradiated through a thin layer of the same medium (1.2 mL per 6-cm petri dish) on a rotating platform at 37 °C with 313-nm light from our Schoeffel GM250 high-intensity quarter meter monochromator. The entrance and exit slits were 3 mm, and the half-bandwidth at these settings was 10 nm. The culture dishes were covered with a Kodacel plastic sheet in order to diminish short-wavelength stray light. The dose rate under these conditions varied from 6.9 to 9.2 J m^{-2} s^{-1}, depending on the age of the lamp, and was monitored with an IL 770A germicidal/erythemal radiometer equipped with an SEE 400 photodetector (International Light, Newburyport, MA) and ferrioxalate actinometry. The cultures were then incubated for 0–48 h in fresh complete media in a CO_2 incubator at 37 °C.

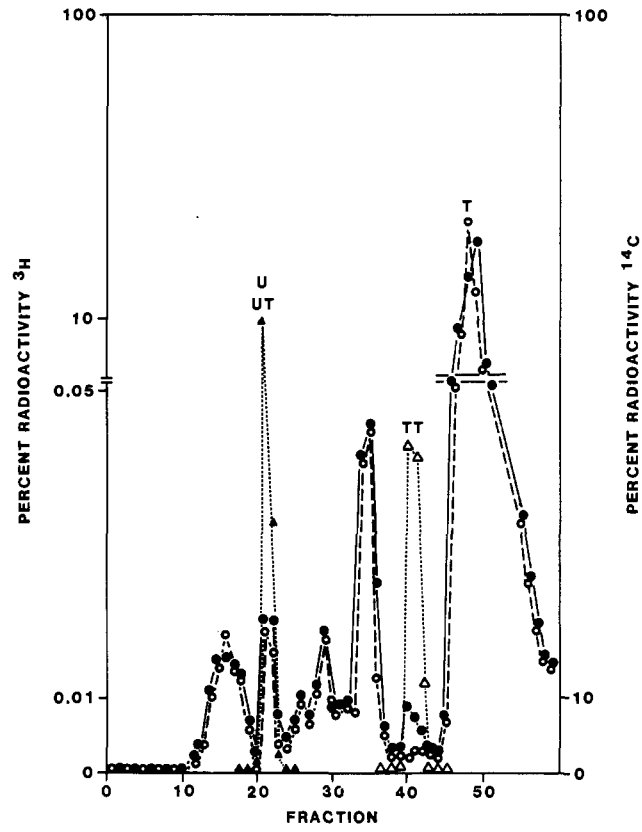

FIGURE 1: HPLC analysis of acid hydrolysates of DNA from [^{3}H]thymidine-labeled human fibroblasts exposed to 5.5 kJ m^{-2} of 313-nm light. Two μBondapak C_{18} columns in tandem were eluted with 2% aqueous methanol: (O---O) unirradiated controls; (●—●) irradiated with 313-nm light at 37 °C; (▲---▲) authentic [^{14}C]UT marker; (△---△) authentic [^{14}C]TT marker.

DNA Isolation and Determination of TT by HPLC. DNA was isolated by our filter elution method (Leadon & Cerutti, 1982) by using DNase I to release the DNA from the filter. The samples were flash evaporated to dryness, treated with 98% formic acid at 170 °C for 90 min, and again flash evaporated, and the residues were dissolved in 2% aqueous methanol. The samples were chromatographed on a Waters ALC/GPC-204 liquid chromatograph with a Model 440 absorbance detector set at 254 nm (Waters Associates, Milford, MA) Two μBondapak C_{18} columns connected in tandem were used at ambient temperature with 2% aqueous methanol as an isocratic solvent system at a flow rate of 1 mL min^{-1}. The fraction size was 0.5 mL. A small steel precolumn packed with pellicular μBondapak C_{18} material was used to protect the analytical columns from resinous material. A typical elution profile in which the positions of thymine, TT, and UT are indicated is shown in Figure 1. Authentic samples of [^{14}C]TT and [^{14}C]UT were prepared by the irradiation of frozen solutions of [2-^{14}C]thymine and of equimolar [2-^{14}C]thymine–uracil with 20 kJ m^{-2} of 254-nm light. The markers were purified by paper chromatography according to Carrier & Setlow (1971) and by HPLC according to Cadet et al. (1980). The six fractions eluting directly before thymine which contained TT were pooled, evaporated to dryness, and rechromatographed under the conditions described above but with 1% aqueous Me_2SO as eluent. The radioactivity content of the fractions was determined after the addition of 6 mL of Aquassure in a Beckman LS9000 scintillation system.

Table I: Data Evaluation for the Determination of PyT by the $NaBH_4$ Reduction Procedure

dimer type	[^{14}C]PyT markers: input (cpm)	reduction (cpm)	yield (%)	fractions containing reduced PyT	yield (cpm) of [^{3}H]PyT isolated from DNA: unirradiated control	irradiated with 5.5 kJ m^{-2}	cpm found in control subtracted	yield corrected to 100%	total thymine radioactivity (cpm)	% radioactivity in PyT region	% PyT corrected[a]	PyT/10^8 daltons[b]
UT	200	102	51	38–44	40	160	120	235	1 970 000	0.012	0.024	11.1
TT	190	152	80	77–85	0	321	321	401	1 970 000	0.020	0.020	9.2

[a] Corrected percentage of UT dimers, after accounting for the fact that only the thymine of a UT dimer could be labeled. [b] Calculated by using the corrected PyT percentage, assuming that in human DNA 30% of the bases are thymine (Scherrer et al., 1962) and the average molecular weight of a nucleotide is 325.

Determination of TT and UT after $NaBH_4$ Reduction by HPLC. The formic acid hydrolysates of DNA from [^{3}H]-thymidine-labeled irradiated cells were prepared and chromatographed on μBondapak C_{18} columns as described above. Fractions 19–24 and 39–44 containing UT plus uracil and TT, respectively, were pooled, evaporated to dryness, redissolved in 40 μL of 1 mM Na_2CO_3 buffer, pH 9.5, and reduced with 100 μg of $NaBH_4$ in 10 μL of 0.1 M NaOH. To the reaction mixture were added small amounts (usually 150–200 cpm) of [^{14}C]TT and [^{14}C]UT for the internal determination of the reduction yield in each individual sample. The reduction was allowed to proceed overnight at room temperature and terminated by the addition of glacial acetic acid. The samples were evaporated to dryness, redissolved in methanol, and again evaporated in order to remove volatile boranes. The reaction mixture was chromatographed by using the Waters Associates radial compression separation system. Two RCM100 modules with radial PAK A cartridges containing 10-μm reverse-phase C_{18} filling material connected in tandem were used with 2% aqueous methanol as the isocratic solvent system. Fractions of 0.6 mL (fractions 1–50) and of 1 mL (fractions 51–100) were collected at a flow rate of 2 mL min^{-1}, and their ^{14}C and ^{3}H contents were determined as described above. A typical elution profile derived from XPA cells which had been exposed to 5.5 kJ m^{-2} of 313-nm light is shown in Figure 2.

Table I contains the results of an actual experiment and illustrates the data evaluation and corrections which were used.

Results

Formation of TT by 313-nm Light in Human Fibroblasts: Determination by HPLC. An improved method was developed for the chromatographic determination of TT in DNA from [^{3}H]thymidine-labeled cells which had been irradiated with physiological doses of UV. The improvements relative to previous procedures are on two levels: (1) the DNA was purified by our rapid filter elution procedure (Leadon & Cerutti, 1982) before it was subjected to formic acid hydrolysis; (2) HPLC replaces thin-layer or paper chromatography, and conditions were chosen where TT elutes earlier than thymine. As a consequence of these changes, the nonspecific background radioactivity in the TT region of the chromatogram is reduced to 0.0015 ± 0.0010% of the radioactivity content of the thymine peak (of the first HPLC run; see Materials and Methods). Column recovery from μBondapak C_{18} was quantitative, and the counting efficiency of the liquid HPLC fractions was considerably higher than that for scrapings from thin-layer chromatograms. The maximal sensitivity of our procedure lies at approximately 1.8 TT per 10^8 daltons (equivalent to 0.004% TT) which corresponds to a dose of 2 J m^{-2} of 254-nm light or 1 kJ m^{-2} of 313-nm light (of the monochromatic purity specified under Materials and Methods). This represents an improvement relative to earlier chromatographic procedures

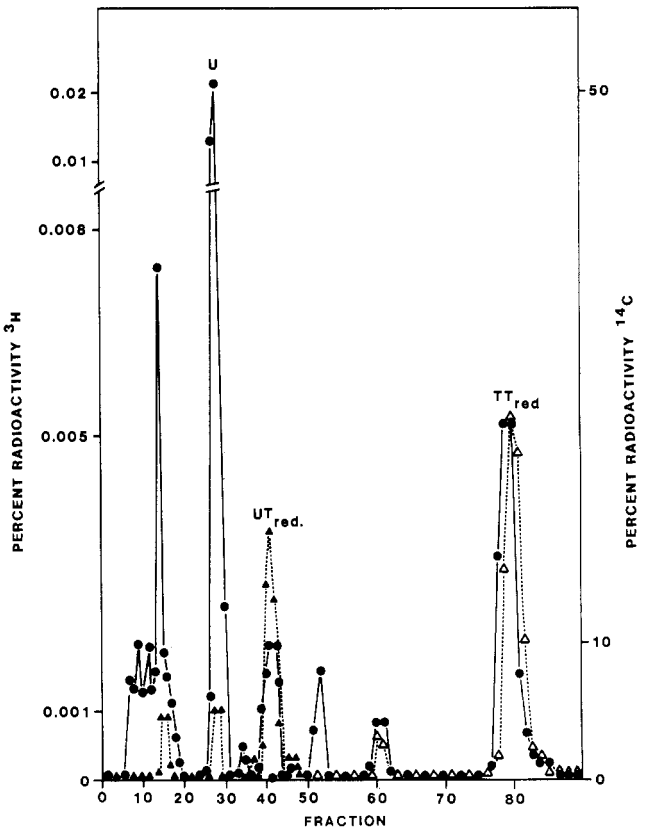

FIGURE 2: HPLC analysis of sodium borohydride reduced UT and TT from [^{3}H]thymidine-labeled human fibroblasts exposed to 5.5 kJ m^{-2} of 313-nm light. Two RCM100 modules containing radial PAK A cartridges in tandem were eluted with 2% aqueous methanol. Fractions 19–24 and 39–44 shown in Figure 1 were supplemented with authentic [^{14}C]UT and [^{14}C]TT markers and reduced with sodium borohydride: (●—●) irradiated with 313-nm light at 37 °C; (▲---▲) reduced [^{14}C]UT; (△---△) reduced [^{14}C]TT.

(Carrier, 1981; Reynolds et al., 1981) of a factor of 3–5. The dose–response curve for the formation of TT at 313 nm in the DNA of confluent monolayers of NF is shown in Figure 3. A linear relationship is obtained, and the rate of TT formation derived from the slope of the linear regression curve is 1.68 TT per 10^8 daltons per kJ m^{-2}.

High radioactivity backgrounds did not allow the determination of CT in the physiological dose range by this procedure, on the other hand. A new method using $NaBH_4$ reduction was developed for this purpose and is described below.

Formation of TT and CT by 313-nm Light in Human Fibroblasts: Determination by HPLC following $NaBH_4$ Reduction. The high radioactivity backgrounds in the region of UT (derived from CT by deamination) could not be reduced under a variety of conditions of HPLC. It consisted mostly

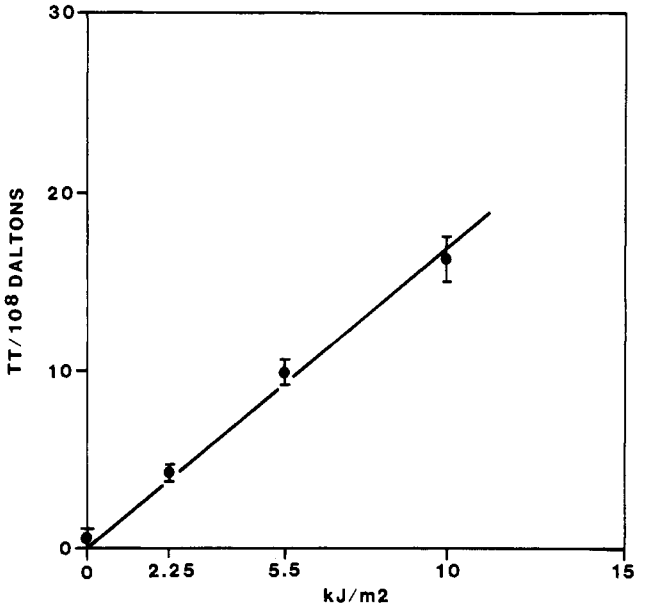

FIGURE 3: Formation of thymine–thymine dimers (TT) in DNA of normal human fibroblasts upon irradiation with 313-nm light at room temperature. Mean values of at least two analyses with standard deviations are given. The correlation coefficient calculated by linear regression of mean values is 0.998.

of [^{3}H]uracil which was formed from [^{3}H]cytosine in the DNA upon formic acid hydrolysis. The deoxy[^{3}H]cytidine content in the DNA of cells grown in the presence of [3H]thymidine was due to cellular metabolism rather than impurity of the label. The principal of the $NaBH_4$ method is to selectively reduce UT and TT but not uracil or thymine to ring-opened derivatives (Kunieda & Witkop, 1967). The chemical properties of the reduced dimers are sufficiently different from uracil and other background radioactivities to allow their separation and quantitation by HPLC on RCM100 modules. The reduction yields were for TT 72 ± 10% and for UT 48 ± 8%. They were determined internally in every sample by the addition of a known amount of authentic ^{14}C-labeled UT and TT. Limitation of the $NaBH_4$ method is estimated at 5.5 CT or 1.8 TT per 10^8 daltons (equivalent to 0.012% CT and 0.004% TT).

Dose–response curves for TT and CT formation in confluent monolayers of XPA by 313-nm light at 37 °C are given in Figure 4. Both types of dimers are formed linearly as a function of dose. CT formation is more efficient than TT formation, the rates derived from the linear regression curves being 2.06 CT per 10^8 daltons per kJ m^{-2} and 1.66 TT per 10^8 daltons per kJ m^{-2}. The latter value obtained with the $NaBH_4$ method is in excellent agreement with that reported above for direct chromatography of TT.

Kinetics of Excision of Photodimers Induced by 313-nm Light. The kinetics of excision of TT from the DNA of confluent monolayers of NF were determined following exposure to 5.5 kJ m^{-2} of 313-nm light at 37 °C. As shown in Figure 5, TT removal was rapid during the first 8 h and resulted in the removal of 61% of the initial lesions. It then continued at a much slower pace. After 48 h of incubation, only 17% of the initial TT remained in the DNA. For comparison, the kinetics of removal of TT which had been induced by 254-nm light are included in this figure. No significant difference is discernible at the two wavelengths.

For the precise determination of the kinetics of removal of CT in NF, it was necessary to increase the dose of 313-nm light to 10 kJ m^{-2}. The DNA hydrolysates were reduced with $NaBH_4$ before separation by HPLC as described in the previous section. Figure 6A,B shows the results for two different strains of NF. It is evident that CT dimers were removed considerably faster during early incubation in comparison to TT dimers; e.g., after 8 h, only 38% (CRL 1222) and 30% (CRL 1221) CT remained in the DNA relative to 68% and 71% TT, respectively. Neither TT nor CT induced with 313-nm light was excisable in the XPA strain (data not shown).

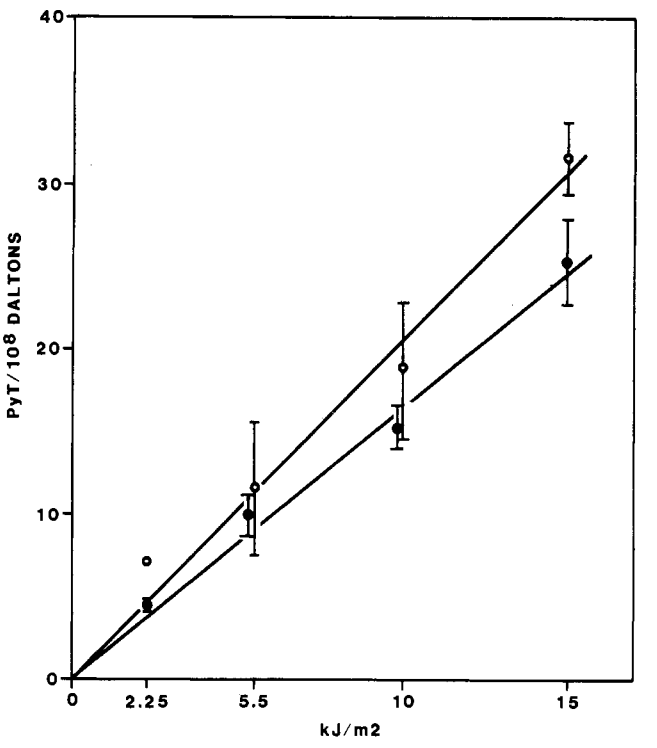

FIGURE 4: Formation of cytosine–thymine (CT) and thymine–thymine (TT) dimers in DNA of xeroderma pigmentosum skin fibroblasts of group A upon irradiation with 313-nm light at 37 °C. Mean values of at least two analyses with standard deviations are given. (○) CT induced with 313-nm light; (●) TT induced with 313-nm light. The correlation coefficient calculated by linear regression of mean values is 0.993 and 0.998, respectively.

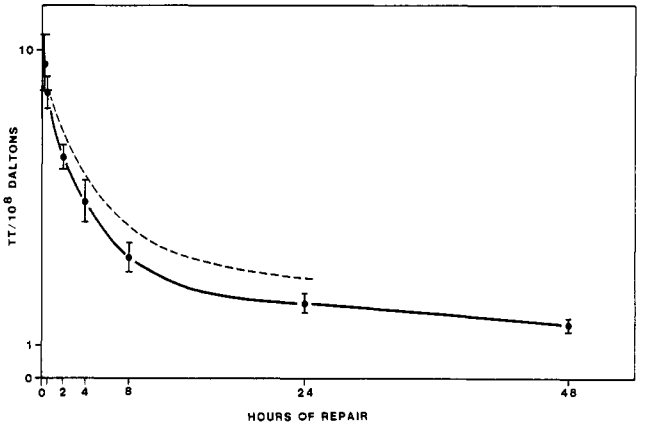

FIGURE 5: Kinetics of excision of TT from DNA of normal human fibroblasts after irradiation with 5.5 kJ m^{-2} of 313-nm light at 37 °C. The data are presented as the mean and standard deviation of two to six independent experiments for each time point. (●) TT remaining after 0–48-h posttreatment incubation. The dashed line represents the kinetics of excision repair at 254-nm light irradiated with 8.25 J m^{-2} for comparison.

Discussion

Table II compares dimer yields obtained in eukaryotic cells in the UV-B region reported in the literature to our present

Table II: Synopsis of the Efficiency of Cyclobutane-Type Pyrimidine Dimerization by Near-UV Light in Mammalian Cells

organism	light source[a]	irradiation conditions[b]	assay[c]	PyT per 10^8 daltons per kJ m^{-2}	ref
hamster HEC	313, M	PBS, RT monolayer	ESS	0.5	Doniger et al. (1981)
human HeLa	313	Hank, 0 °C suspension	TLC	5.2	Hariharan & Cerutti (1977)
human CRL 1221, 1223	313, K	DMEM, 0 °C monolayer	HPLC	2.2	Niggli & Cerutti (1982, 1983)
frog ICR 2A	313, M, PD	PBS, RT monolayer	ESS	0.9	Rosenstein & Setlow (1980)
human skin	sunlamp	in vivo	ESS	5.0	Sutherland et al. (1980)
hamster V79	sunlamp	PBS, RT monolayer	PC	80.8	Suzuki et al. (1981)
hamster V79	sunlamp, PD	PBS, RT monolayer	PC	2.6	Suzuki et al. (1981)
hamster CHO	sunlamp, PD	PBS monolayer	PC ESS	24.9 27.0	Zelle et al. (1980)
hamster CHO	sunlamp, PTF	PBS monolayer	ESS	0.1	Zelle et al. (1980)
human CRL 1223	313, K	DMEM, 37 °C monolayer	HPLC	3.6	this report

[a] UV-B was provided either from a monochromater (313) or from a Westinghouse sunlamp. [For reduction of contamination with short-wavelength UV light, various filters were used: Kodacel filters (K) remove light below 280 nm, polystyrene petri dishes (PD) light below 290 nm, Mylar filters (M) light below 305 nm, and polyethylene terephthalate films (PTF) light below 310 nm.] [b] Irradiation of cultured cells was carried out in monolayers through a thin layer of phosphate-buffered saline (PBS) or Dulbecco's modified Eagle's medium without serum, riboflavin, tryptophan, and phenol red (DMEM). HeLa cells were irradiated in a suspension of Hank's medium. Irradiation temperatures were as indicated: 0 °C, 20–25 °C (RT), or 37 °C. [c] For the determination of PyT, UV-endonuclease-susceptible sites (ESS), high-pressure liquid chromatography (HPLC), paper chromatography (PC), or thin-layer chromatography (TLC) was used. Chromatographic procedures detect thymine-containing pyrimidine dimers while the ESS assay includes CC.

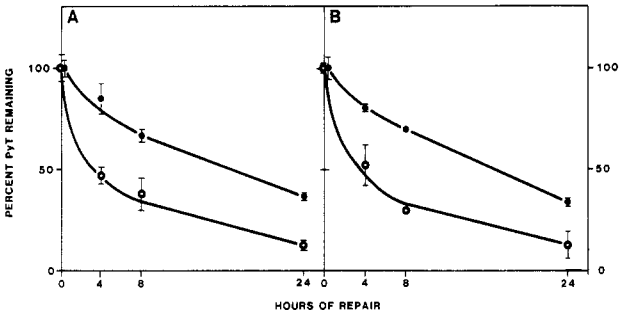

FIGURE 6: Kinetics of excision of cytosine–thymine (CT) and thymine–thymine dimers (TT) from DNA of human fibroblasts after irradiation with 313-nm light at 37 °C. Mean values of at least two analyses with standard deviations are given. (○) CT remaining after 0–24-h posttreatment incubation; (●) TT remaining after 0–24-h posttreatment incubation. (A) Kinetics of dimer removal in normal human CRL 1222 fibroblasts after irradiation with 10 kJ m^{-2}. (B) Kinetics of dimer removal in normal human CRL 1221 fibroblasts after irradiation with 10 kJ m^{-2}.

results. Where there are large differences, they can be mostly accounted for by the contribution of light below 300 nm in the emission spectrum of a particular light source. Because of the very steep change in the absorption of DNA in the UV-B region, light in the short-wavelength end of UV-B is much more efficient for photodimerization than light at the long-wavelength end. The relevance and usefulness of different light sources to UV-B photobiology depend on the question which is being asked and are debated by different investigators. Polychromatic sunlamps yield results which are close to the practical situation of solar exposure. The irradiation with highly pure monochromatic light produces precise data, but the particular relevance of a specific wavelength to photobiology is not clear a priori. In the present work, we attempted a compromise in using monochromatic light at 313 nm with a relatively large half-bandwidth of 10 nm combined with a Kodacel plastic filter which removes scattered light below approximately 290 nm (20% residual transparency). Other investigators have used monochromatic light of higher purity in conjunction with a Mylar plastic sheet which removes light below 300 nm (Rosenstein & Setlow, 1980; Doniger et al., 1981). Besides the light source, the dimer yields are influenced by the irradiation temperature possibly because of temperature-dependent differences in DNA and chromatin conformation (Niggli & Cerutti, 1983). We found that the dimerization rates at 313 nm were 30–50% higher at 37 °C than those at 0 °C. We also discovered that the nucleosomal structure of chromatin affected the dimer distribution. At 313 nm, the nucleosomal linker DNA was enriched with dimers relative to the nucleosomal core DNA while dimer distribution was random at 254 nm (Niggli & Cerutti, 1982). It is evident that results obtained with free DNA and bacteria cannot be simply extrapolated to mammalian cells. Skin optics and differences in cell type, culture conditions (e.g., monolayer or suspension cultures), and the irradiation medium are further factors which can alter the photochemical results. Our finding that CT dimerization was somewhat more efficient than TT dimerization with physiological doses of 313-nm light in human fibroblasts is in agreement with the results of Ellison & Childs (1981) obtained at much higher doses with free DNA. Qualitatively similar results were also obtained by Suzuki et al. (1981) with monolayers of rodent fibroblasts irradiated with a filtered sunlamp.

The exact kinetics of PyT removal induced by 254-nm light in human cells have been controversial. While Amacher et al. (1977) reported 50–70% dimer removal in 1 h, Regan et al. (1978) and Ehman et al. (1978) observed no excision. Our results at 313 nm agree best with those of Konze-Thomas et al. (1979) at 254 nm, who found 70% excision in a 6-h incubation. Fast dimer removal (endonuclease-sensitive sites) was also observed in human skin (D'Ambrosio et al., 1981). In our experiment, the excision kinetics are strongly dose dependent. As shown in Figure 5, 56 ± 6.6% TT remained in the DNA after 4-h incubation, 38 ± 4% TT after 8-h incubation, and 24 ± 3% TT after 24-h incubation following a dose

of 5.5 kJ m^{-2} while the corresponding values for a dose of 10 kJ m^{-2} were 83 ± 5%, 68 ± 3%, and 35 ± 2% (data not shown). The enzymatic repair system is removing the same number but not the same proportion of TT. Figure 5 also contains the kinetics of removal of TT induced by 8.25 J m^{-2} 254-nm light which induced a TT concentration comparable to 5.5 kJ m^{-2} of 313-nm light. It is evident that the excision kinetics after 254- and 313-nm irradiation are essentially the same. Other lesions such as DNA single-strand breaks and products of the 5,6-dihydroxy-5,6-dihydrothymine type which are induced with much higher relative efficiency at 313 nm did not affect the excision kinetics of TT. The observation that CT dimers were removed much faster than TT dimers (see Figure 6A,B) is intriguing in view of the chemical similarity of these photoproducts. Both are cis-syn cyclobutane-type dimers and would be expected to produce the same structural distortion in DNA. It is conceivable that the excision pathways differ for these classes of dimers. However, our data with XPA cells show that both CT as well as TT dimers remained unexcised, indicating that the repair function which is deficient in these cells is common for both types of dimers. The distribution of TT and CT could be different in chromatin and affect lesion excisability. This question could not be resolved in the physiological dose range of our present experiments.

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Registry No. TT, 3660-32-0; CT, 10457-03-1; UT, 28171-56-4.

References

Amacher, D. E., Elliot, J. A., & Lieberman, M. W. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1553–1557.

Blum, H. F. (1959) *Carcinogenesis by Ultraviolet Light*, Princeton University Press, Princeton, NJ.

Brash, D. E., & Haseltine, W. A. (1982) *Nature (London) 298*, 189–192.

Cadet, J., Voituriez, L., Hahn, B. S., & Wang, S. Y. (1980) *J. Chromatogr. 195*, 139–145.

Carrier, W. L. (1981) in *DNA Repair* (Friedberg, E. C., & Hanawalt, P. C., Eds.) Vol. 1, Part A, pp 3–10, Marcel Dekker, New York.

Carrier, W. L., & Setlow, R. B. (1971) *Methods Enzymol. 21*, 230–237.

Coulondre, C., & Miller, J. H. (1977) *J. Mol. Biol. 177*, 525–567.

D'Ambrosio, S. M., Slazinski, L., Whetstone, B. S., & Lowney, E. (1981) *J. Invest. Dermatol. 77*, 311–313.

Doniger, J., Jacobson, E. D., Krell, K., & DiPaolo, J. A. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 2378–2382.

Drake, J. W. (1963) *J. Mol. Biol. 6*, 268–283.

Ehman, U. K., Cook, K. H., & Friedberg, E. C. (1978) *Biophys. J. 22*, 249–264.

Elkind, M. M., & Han, A. (1978) *Photochem. Photobiol. 27*, 717–724.

Ellison, M. J., & Childs, J. D. (1981) *Photochem. Photobiol. 34*, 465–469.

Freeman, K. B., Hariharan, P. V., & Johns, H. E. (1965) *J. Mol. Biol. 13*, 833–848.

Hariharan, P. V., & Johns, H. E. (1968) *Photochem. Photobiol. 8*, 11–22.

Hariharan, P. V., & Cerutti, P. A. (1977) *Biochemistry 16*, 2791–2795.

Kaneko, M., Matsuyama, A., & Nagata, C. (1979) *Nucleic Acids Res. 6*, 1177–1187.

Konze-Thomas, B., Levinson, J. W., Maher, V. M., & McCormick, J. J. (1979) *Biophys. J. 28*, 315–326.

Kunieda, T., & Witkop, B. (1967) *J. Am. Chem. Soc. 89*, 4234–4235.

Leadon, S. A., & Cerutti, P. A. (1982) *Anal. Biochem. 120*, 282–288.

Netrawali, M. S., & Cerutti, P. A. (1979) *Biochem. Biophys. Res. Commun. 87*, 802–810.

Niggli, H. J., & Cerutti, P. A. (1982) *Biochem. Biophys. Res. Commun. 105*, 1215–1223.

Niggli, H. J., & Cerutti, P. A. (1983) *Photochem. Photobiol.* (in press).

Person, S., McCloskey, J. A., Snipes, W., & Bockrath, R. C. (1974) *Genetics 78*, 1035–1049.

Regan, J. E., Carrier, W. L., Smith, D. P., Waters, R., & Lee, W. H. (1978) *Natl. Cancer Inst. Monogr. No. 50*, 141–143.

Reynolds, R. J., Cook, K. H., & Friedberg, E. C. (1981) in *DNA Repair* (Friedberg, E. C., & Hanawalt, P. C., Eds.) Vol. 1, Part A, pp 11–21, Marcel Dekker, New York.

Rosenstein, B. S., & Setlow, R. B. (1980) *Photochem. Photobiol. 32*, 361–366.

Schaaper, R. M., & Glickman, B. W. (1982) *Mol. Gen. Genet. 185*, 404–407.

Scherrer, K., Latham, H., & Darnell, J. E. (1962) *Proc. Natl. Acad. Sci. U.S.A. 49*, 240–248.

Setlow, R. B. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3363–3366.

Setlow, R. B., Carrier, W. K., & Bollum, F. J. (1965) *Proc. Natl. Acad. Sci. U.S.A. 53*, 1111–1118.

Sutherland, B. M., Harber, L. C., & Kochevar, I. E. (1980) *Cancer Res. 40*, 3181–3185.

Sutherland, J. C., & Griffin, K. P. (1980) *Radiat. Res. 83*, 529–536.

Sutherland, J. C., & Griffin, K. P. (1981) *Radiat. Res. 86*, 399–409.

Suzuki, F., Han, A., Lankas, G. R., Utsumi, H., & Elkind, M. M. (1981) *Cancer Res. 41*, 4916–4924.

Urbach, F. (1975) in *Climatic Impact Assessment Program* (Nachtwey, D. S., Ed.) Part 1, Chapter 7, pp 19–28, Department of Transportation, Washington, DC.

Wacker, A. (1963) *Prog. Nucleic Acid Res. 1*, 369–399.

Zelle, B., Reynolds, R. Y., Kottenhagen, M. J., Schuite, A., & Lohman, P. H. M. (1980) *Mutat. Res. 72*, 491–509.