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Formation of 2′-deoxyuridine hydrates upon exposure of nucleosides to gamma radiation and UVC-irradiation of isolated and cellular DNA

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Received 13th February 2002, Accepted 24th May 2002
First published as an Advance Article on the web 13th June 2002

The two diastereoisomers of 6-hydroxy-5,6-dihydro-2′-deoxyuridine (dUrd hydrates) are produced upon deamination of the related 2′-deoxycytidine derivatives. Liquid chromatography coupled to tandem mass spectrometry was used to quantify dUrd hydrates. Their rate constant of formation within UVC-irradiated solutions of dCyd was first determined. Their formation was also shown to occur within solutions of either dUrd or dCyd exposed to γ-rays in the absence of oxygen. dUrd hydrates were then quantified within isolated and cellular DNA exposed to UVC light. In both cases, their yield of formation was found to be, at least, 2 orders of magnitude lower than the overall yield of bipyrimidine photoproducts such as cyclobutane dimers and (6–4) photoproducts.

Introduction

Ultraviolet radiation is one of the most studied physical agents in terms of formation of DNA lesions, mutagenic properties and DNA repair. The genotoxic effects of UV light strongly depend on the irradiation wavelength. In the UVA range, photons are absorbed by endogenous chromophores that may damage DNA through either electron abstraction from DNA or more likely by production of reactive singlet oxygen. Interestingly, both mechanisms lead to the predominant degradation of guanine. In addition, Fenton-type reactions triggered by the initial formation of superoxide anions may account for the production of hydroxyl radicals in low yield. As a result, UVA-induced damage to cellular DNA occurs mostly at guanine residues together with the formation of strand breaks and oxidised pyrimidine in much lower yield.[1,2] In the UVB range, DNA is the main absorbing molecule in the cell. This results in the excitation of DNA bases that undergo photochemical reactions involving both their singlet and triplet excited states. Pyrimidine dimerization is the major far-UV induced photoreaction yielding cyclobutane type dimers and pyrimidine (6–4) pyrimidinone photoproducts (for reviews see Refs. 3 and 4). The ratio between the two latter types of lesions has been found to depend strongly on the bipyrimidine site involved.[5] However, the latter dimeric photoproducts are not the only base damage generated upon exposure of DNA to UVB and UVC radiations. Indeed, photohydration of the double bond of pyrimidine bases has been one of the first identified far-UV induced photoreaction involving DNA components.[6–8] Extensive studies of 6-hydroxy-5,6-dihydrocytosine (cytosine hydrate), which exists as a mixture of enantiomers because of the presence of an asymmetric carbon, have been undertaken (for reviews see Refs. 3 and 9). A major observation, in addition to the high quantum yield (*ca.* 1%) of formation of cytosine hydrates within nucleosides,[10] is their low stability. Indeed, the latter photoproducts may either revert to the original base by loss of a water molecule or deaminate into the corresponding uracil hydrates.[7,11] Interestingly, the latter compounds are much more resistant to dehydration.

Information on the formation of cytosine and uracil hydrates within DNA is much more limited.[12] Most available data are based on studies involving the recognition of the latter lesions by the *Escherichia coli N*-glycosylase endonuclease III. The amount of cytosine hydrates may be determined following conversion of the initially generated abasic site into DNA strand break by the β-lyase activity of the repair enzyme.[13,14] It was thus observed that cytosine hydrates are produced in lower yield than pyrimidine dimeric photoproducts. A more specific approach, which allows the distinct measurement of cytosine and uracil hydrates, involves the quantification of the released bases from [^{3}H]-labelled poly(dG-dC).[15,16] Even though stability studies were carried out by using the latter assay, only relative levels of hydrates could be determined and the target of the photoreaction was not genomic DNA. In the present work, emphasis was placed on the accurate and specific quantification of 2′-deoxyuridine hydrates (6-hydroxy-5,6-dihydro-2′-deoxyuridine, oh^{6}hdUrd) by liquid chromatography coupled to tandem mass spectrometry. Their rate of formation was determined within solutions of nucleoside and isolated DNA exposed to UVC radiation. The production of oh^{6}hdUrd was also monitored within aqueous solutions of dCyd and dUrd exposed to gamma radiation in the absence of oxygen. Finally, oh^{6}hdUrd was measured within the DNA extracted from cells exposed to UVC radiation. This allowed the precise determination of the ratio between oh^{6}hdUrd and dimeric pyrimidine photoproducts.

Experimental

Chemicals

2′-Deoxycytidine (dCyd) and 2′-deoxyuridine (dUrd) were obtained from Pharma-Waldhof. L-cysteine, desferroxamine mesylate and nuclease P1 were purchased from Sigma (St. Louis, MO). Alkaline phosphatase was a Roche Diagnostics product (Mannheim, Germany). The 6(*R*) and 6(*S*) diastereoisomers of dUrd hydrates were obtained by UVC irradiation of a 10 mM dUrd solution. They were then purified by reverse

phase HPLC and characterized by 200 MHz ^{1}H NMR. Isotopically labelled dUrd hydrates ([M + 3]-oh^{6}hdUrd) were obtained in a similar way from [1,3-$^{15}N_2$,2-^{13}C]-2′-deoxyuridine. The latter nucleoside was obtained by ribosylation of [1,3-$^{15}N_2$,2-^{13}C]-uracil (prepared from [$^{13}C_2$,^{15}N]-urea) by thymidine phosphorylase in the presence of 2-deoxyribose 1-phosphate (Sigma, St Louis, MO). The solutions of oh^{6}hdUrd and [M + 3]-oh^{6}hdUrd were calibrated by converting the compounds into uracil upon formic acid hydrolysis at 145 °C for 5 h. The resulting solutions were dried under vacuum and the obtained residues were solubilized in water. The resulting samples were calibrated on the basis of their UV absorption spectrum (uracil: λ_{max}: 260 nm; ε_{260}: 8994 mol^{-1} dm^3 cm^{-1}).

UVC irradiation of isolated DNA

Calf thymus DNA (Sigma, St Louis, MO) was exposed to UV light in aqueous solution (1 mg mL^{-1}, pH 6.5, 5 mL) in Petri dishes (3.5 cm diameter). The lamp (VL 215G, Bioblock Scientific, Illkirch, France), placed 10 cm above the DNA solution, was equipped with two 15 W tubes mostly emitting at 254 nm. The fluence, measured by a VLX 3W radiometer (Vilber Lourmat, Marne La Vallée, France) equipped with a CX 254 probe, was determined to be 0.235 J cm^{-2} min^{-1}. The DNA solutions were irradiated at room temperature for increasing periods of time, ranging between 0 and 20 min. Following irradiation, solutions of isolated DNA were incubated overnight at room temperature. Then, three aliquot fractions (100 µL) were collected in each sample and isotopically labelled oh^{6}hdUrd (50 pmol) was added.

UVC irradiation of cells

Cells (10 × 10^6), suspended in 15 mL of phosphate buffered saline (PBS) in a 8.5 cm plastic Petri dish, were irradiated with the UV lamp described above. Following irradiation, cell suspensions were centrifuged (260*g*, 5 min) and the supernatant discarded. The cellular membrane was lysed by addition of 2 mL of lysis buffer (320 mM sucrose, 10 mM TRIS, 5 mM $MgCl_2$, 0.1 mM desferroxamine mesylate, 1% Triton-100, pH 7.5). The samples were vigorously vortexed. They were then centrifuged (1500*g*, 5 min) and the supernatant was discarded. The resulting nuclear pellet was rinsed by addition of 1 mL of lysis buffer followed by a 5 min centrifugation (1500*g*). The nuclei were suspended in the extraction buffer (10 mM TRIS, 5 mM EDTA, 0.15 mM desferroxamine mesylate, pH 7) and left overnight at room temperature. Sodium lauryl sulfate (SDS) (10% in water, 35 µL) was then added and the samples vigorously vortexed to lyse the nuclear membrane. The samples were then incubated 15 min at 37 °C following addition of 30 µg of RNAseA (Qiagen, Mannheim, Germany) and 7 units of RNAse T1 (Sigma, St. Louis, MO). Then, proteinase was added (600 µg, Qiagen, Mannheim, Germany) and the samples were incubated 30 min at 37 °C. DNA was precipitated by addition of sodium iodide (1.2 mL, 7.6 M NaI, 40 mM TRIS, 20 mM EDTA, pH 8) and isopropanol (propan-2-ol) (2 mL). The samples were centrifuged (5000*g*, 15 min) and the supernatant discarded. The DNA pellet was then rinsed with 40% isopropanol (500 µL) and 70% ethanol (500 µL). The DNA sample was then solubilized in 100 µL of water to which [M + 3]-oh^{6}hdUrd (10 pmol) was added.

DNA hydrolysis

DNA was hydrolyzed by sequential incubation at 37 °C with nuclease P1 (10 units in 10 µL of buffer containing 300 mM ammonium acetate, 1 mM $ZnSO_4$, pH 5.5) for 2 h and alkaline phosphatase (2 U in 12 µL of buffer containing 500 mM TRIS, 1 mM EDTA, pH 8) for 1 h. Then, the pH was set to 5.5–6 by addition of 8 µL of 0.1 M HCl and proteins were precipitated by addition of 30 µL of chloroform. The sample was vigorously vortexed and then centrifuged (5000*g*, 5 min). The aqueous layer was then transferred into HPLC injection vials.

UVC and gamma-irradiation of nucleosides

A solution of dCyd (3 mL, 1 mM) was exposed at room temperature for 20 min to the UVC light emitted by the germicidal lamp described above. The irradiation was carried out in solution either in pure water, TRIS buffer (50 mM, pH 8) or ammonium acetate buffer (30 mM, pH 5.5). The extent of hydration (20–25%) was determined by reverse-phase HPLC analysis with UV detection set at 230 nm. An aliquot fraction was transferred into an HPLC injection vial together with 500 pmol of a mixture of 6(*R*) and 6(*S*) diastereoisomers of [M + 3]-oh^{6}hdUrd. The resulting solution was injected several times on the HPLC-MS/MS system to determine the content in either dCyd hydrates (injection volume 2 µL) or dUrd hydrates (injection volume 20 µL). The data were then used to calculate dCyd hydrates overall decomposition and deamination rate constants with the assumption that the latter reactions were of first order. The rate constant for dehydration of oh^{6}hdCyd was inferred from the difference between the two latter values. Three independent experiments were performed. The yield of formation of dUrd hydrates was also determined in samples of dCyd and dUrd (1 mM) exposed to gamma radiation in the presence or the absence of cysteine (1 mM). Prior to irradiation, samples were purged with argon for 60 min to remove most of the solubilized oxygen. They were then exposed in sealed Pyrex tubes to the gamma radiation emitted by a ^{60}Co source immersed in a water pool. The applied doses were 0, 15, 75, 150 and 300 Gy. Following irradiation, samples were left overnight at 37 °C and [M + 3]-oh^{6}hdUrd (50 pmol) was added. Samples were analysed in triplicates by HPLC-MS/MS.

HPLC-MS/MS analysis

Samples were injected (20 µL) onto a HPLC system consisting of a 7100 Hitachi-Merck pump (Merck, Darmstadt, Germany) associated to a SIL-9 automatic injector (Shimadzu, Tokyo, Japan). The column was an Uptisphere ODB (150 × 2 mm, 5 µm particle size) octadecylsilyl silica gel column (Interchim, Montluçon, France). The mobile phase was a gradient of 2 mM ammonium formate, which was prepared from ACS reagent grade formic acid and 99.9% ammonium hydroxide (Aldrich, Steinheim, Germany), and acetonitrile (HPLC grade, Carlo Erba, Milan, Italy). The flow rate was 0.2 mL min^{-1}. The proportion of acetonitrile rose from 0 to 2% within 8 min and reached 20% after 28 min. Methanol was added at the outlet of the column prior to the inlet of the mass spectrometer at a flow rate of 0.1 mL min^{-1}. The API 3000 spectrometer (Perkin-Elmer/SCIEX, Thornhill, Canada) was operated in the negative electrospray ionisation mode for the quantification of oh^{6}hdUrd. The specific transition 245 ⟶ 158 was monitored, together with the corresponding signal (248 ⟶ 160) for the [M + 3] isotopically labelled oh^{6}hdUrd used as internal standard. The quantification of oh^{6}hdCyd was performed in the positive mode. The selected transition was 246 ⟶ 130. An external calibration was performed by injecting a UVC-irradiated dCyd solution in which the hydration yield had been determined by HPLC-UV. The 6(*R*) and 6(*S*) diastereoisomers of both dCyd and dUrd hydrates were well resolved on the HPLC system. The retention time of the two pairs of peaks were 3.5 and 4.5 min for oh^{6}hdCyd, and 5.7 and 6.8 min for oh^{6}hdUrd, respectively. Dimeric pyrimidine photoproducts were quantified as previously reported.[5]

Results and discussion

Decomposition of dCyd hydrates

The 6(*R*) and 6(*S*) diastereoisomers of dCyd hydrates undergo two decomposition reactions in aqueous solution. Dehydration

leads to the restitution of the starting dCyd nucleoside while deamination generates the dUrd hydrates. These two reactions have been extensively studied and were used here to check the reliability of the developed HPLC-MS/MS assay. This assay involved the use of the specific and sensitive multiple reaction monitoring mode based on the detection of a specific fragment of the pseudo-molecular ions of the compounds of interest. dCyd hydrates, analysed in the positive mode, were found to undergo the loss of the 2-deoxyribose unit upon fragmentation. In contrast, deprotonated dUrd hydrates underwent ring opening and fragmentation (Fig. 1). The rate constants for the deamination and the dehydration of dCyd hydrates were determined in water (Fig. 2) and under the acidic and alkaline conditions

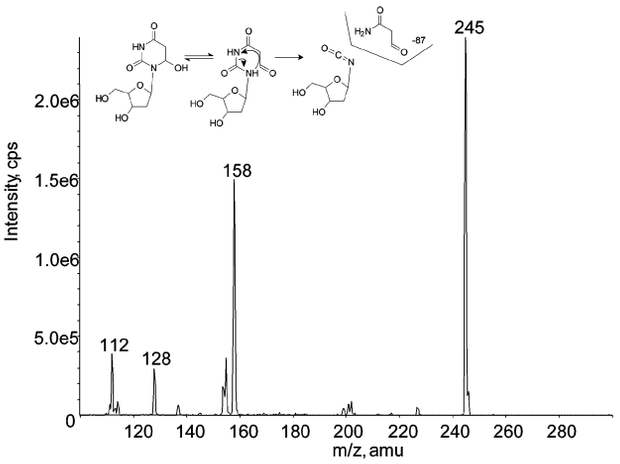

Fig. 1 Fragmentation mass spectrum of the mixture of 6(*R*) and 6(*S*) diastereoisomers of dUrd hydrates. The parent ion was set at 245 ($[M - H]^-$). The proposed fragmentation pathway is in agreement with the presence of two labelled atoms in the main daughter ion obtained upon fragmentation of [1,3-$^{15}N_2$,2-^{13}C]-oh^{6}hdUrd.

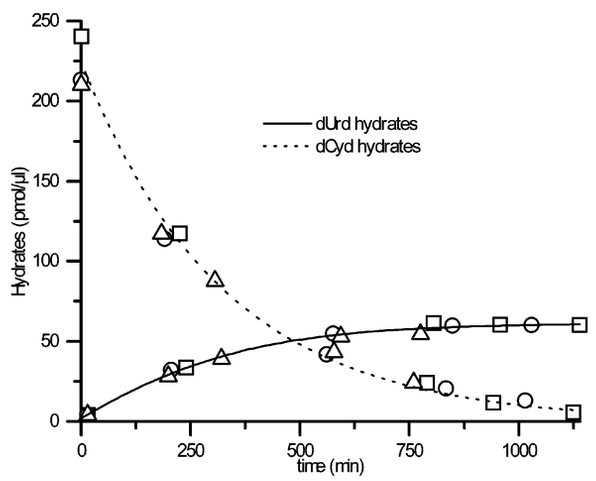

Fig. 2 Decomposition of the dCyd hydrates and formation of dUrd hydrates in water following exposure of a 1 mM dCyd solution to UVC light. Results represent the compilation of three independent experiments (symbols △, □ and ○).

used in the DNA hydrolysis protocol (pH 5.5 and 8, respectively). The obtained values (Table 1) agree very well with previously reported data.[17] In addition, the good stability of dUrd hydrates in aqueous solution was confirmed. These results clearly show that dCyd hydrates cannot be accurately quantified within DNA upon immediate enzymatic hydrolysis because of their instability. In addition, dUrd hydrates produced upon decomposition of dCyd hydrates only represent a minor fraction of the initial amount of the latter compounds. Therefore, the strategy applied was to quantify dUrd hydrates following deamination of dCyd hydrates in the whole DNA. Indeed, this allows the quantification of stable dUrd hydrates that can be quantitatively released from DNA. It should also be mentioned that the life-time of dUrd hydrates in DNA is much longer than that of their cytosine precursors[15,16,18] and might therefore be more relevant in terms of deleterious cellular effects. In a first step, the extent of deamination of dCyd hydrates was determined by quantifying dUrd hydrates within DNA that were enzymatically digested after increasing periods of time following exposure to UVC radiation. As shown in Fig. 3, a plateau was reached after 5 h. Then, dUrd hydrates

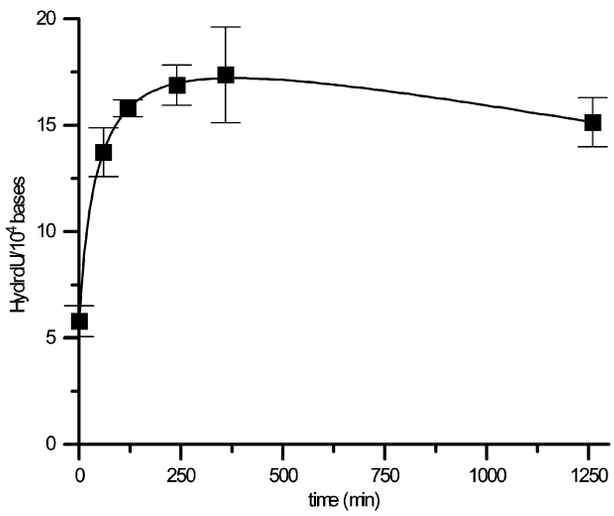

Fig. 3 Level of dUrd hydrates measured within DNA samples enzymatically hydrolyzed following increasing period after exposure to UVC light. Analyses were performed in triplicate and the results represent mean ± standard deviation.

undergo only minor degradation. These observations are in agreement with those previously reported within [^{3}H]-labelled poly(dG-dC) using an enzymatic assay.[16] The actual deamination rate of dCyd hydrates within DNA could not be calculated because, in contrast to the work at the nucleoside level, their initial amount within DNA could not be determined. It should be mentioned that a half-life of 23 h has been reported for cytosine hydrates within [^{3}H]-poly(dG-dC).[15,16] This high value seems to contrast with the relatively fast appearance of dUrd hydrates, the level of which remains unmodified after 5 h of incubation following irradiation.

Gamma-radiation induced formation of dUrd hydrates

Formation of hydrates upon exposure of dCyd to hydroxyl radicals produced by gamma radiolysis of water is much less well documented than following UV irradiation. Formation of the related thymidine hydrates has been reported to occur in aerated solution in low yield, as the result of the initial addition of H$^\bullet$ to the C5 position, followed by addition of oxygen and decomposition of the resulting peroxide.[19] The yield of formation of thymidine hydrates is higher under oxygen-free conditions.[20–22] This might first be accounted for by the initial formation of a 5,6-dihydrothymine radical arising from addition of either an hydrogen atom or a solvated electron followed by a protonation step. Oxidation of the 5,6-dihydrothymine radical has been shown to yield a carbonium ion that may react with water to yield thymine hydrates.[23] However, this reaction only occurs in significant yield below pH 5. At neutral pH, deprotonation of the 5,6-dihydrothymine cation results in the restitution of thymine. An alternative mechanism for the formation of thymine hydrates upon exposure to ionizing radiation in the absence of oxygen is the addition of an hydroxyl radical to the C6 position, followed by reduction of the resulting transient. Similar reactions with dCyd would yield dCyd hydrates

Table 1 Reaction rate constants/min^{-1} for the decomposition, the deamination and the dehydration of 6-hydroxy-5,6-dihydro-2′-deoxycytidine under various conditions. The values were obtained from three independent determinations

Conditions	Ammonium acetate	Water	TRIS
pH	5.5	*ca.* 6.5	8
Decomposition	0.0112 ± 0.0007	0.0029 ± 0.0003	0.0047 ± 0.0003
Deamination	0.0008 ± 0.0001	0.0009 ± 0.0001	0.0014 ± 0.0003
Dehydration	0.0105 ± 0.0006	0.0021 ± 0.0001	0.0034 ± 0.0001

Table 2 Radiolytic yield of formation (in nmol J^{-1}) of dUrd hydrates within deaerated solutions of dCyd and dUrd exposed to gamma radiation in the presence or the absence of cysteine. The values represent the slope of the linear regression ± the standard error

Sample	dCyd	dCyd + cysteine	dUrd	dUrd + Cysteine
Yield	0.43 ± 0.05	0.76 ± 0.04	5.92 ± 0.35	9.96 ± 0.17

that may be converted into their dUrd analogs. We thus quantified the formation of the latter modified nucleosides in aqueous solutions of dCyd and dUrd exposed to the gamma radiation emitted by a ^{60}Co source in the absence of oxygen. Both dCyd and dUrd were found to yield dUrd hydrates upon irradiation (Table 2). The yield was found to be lower for dCyd than dUrd because the corresponding hydrates undergo both deamination and dehydration (*vide supra*). Therefore, not all dCyd hydrates were recovered as dUrd hydrates. To determine whether a reduction step was involved in the formation of the hydrates upon γ-irradiation in the absence of oxygen, the experiments were repeated in the presence of cysteine. Addition of the latter reducing species was found in both cases to increase the yield of hydrates by a factor of 2, in agreement with a mechanism involving initial addition of $^{\bullet}$OH rather than $^{\bullet}$H or e^{-}_{aq}.

Formation of dUrd hydrates within UVC-irradiated isolated DNA

2′-Deoxyuridine hydrates were first quantified within isolated DNA exposed to UVC radiation in aqueous solution. The level of pyrimidine dimeric photoproducts, including *cis,syn* cyclobutane dimers (c,s <>), (6–4) photoproducts ((6–4)) and their Dewar valence isomers (Dew) produced at TT and TC sites, was also determined. Two separate analyses were performed because different chromatographic conditions were used. Indeed, resolution of the mixture of dimeric pyrimidine photoproducts requires a solution of triethylammonium formate as the mobile phase while dUrd hydrates are separated using ammonium formate. It should be mentioned that the sensitivity of the HPLC-MS/MS technique is almost one order of magnitude lower for hydrates than for dimeric photolesions. This explains why higher doses than those used in our previous works on UVC-induced DNA damage were applied.[5,24] The 6(*R*) and 6(*S*) diastereoisomers of oh^{6}hdUrd were detected in similar amounts in UVC-irradiated solutions of isolated DNA. However, this might not reflect the actual ratio between the two diastereoisomers within DNA because of the conversion of one oh^{6}hdUrd diastereoisomer into the other through ring-chain tautomerism.[25,26] In addition, the formation of dUrd hydrates was found to be linear with respect to the applied UVC dose (Fig. 4). In contrast, the formation of the TT and TC dimeric photoproducts was not linear for doses higher than 0.5 J cm^{-2} due to the photoreversion of the cyclobutane dimers and the conversion of the (6–4) adducts into their Dewar valence isomers, as previously reported.[5,24] The quantum yields of formation of the lesions, expressed with respect to the overall DNA bases, were calculated in the linear part of their formation (Table 3). Interestingly, the quantum yield of oh^{6}hdUrd was almost 2 orders of magnitude lower than that of the *cis,syn* cyclobutane thymine dimer. This value is in agreement with an estimation made by using DNA repair enzymes for the measurement.[13] Only deamination products were quantified.

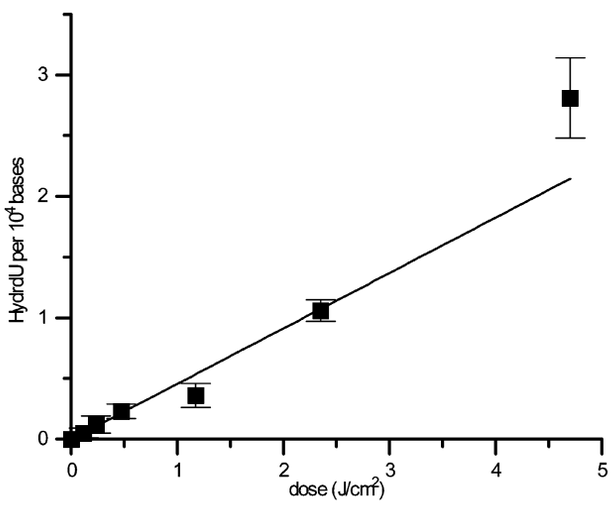

Fig. 4 Level of dUrd hydrates measured within DNA upon exposure to increasing doses of UVC light. Analyses were performed in triplicate and the results represent mean ± standard deviation.

However, they represent more than one third of the initial cytosine hydrates, at least at the nucleoside level. The reported values for the dehydration rate of cytosine hydrates within DNA is much lower than within nucleosides.[15,16] Therefore, within DNA, oh^{6}hdUrd might represent an even larger fraction of the cytosine hydrates initially produced. It thus appears reasonable to conclude that photohydration of cytosine is a minor photochemical process with respect to dimerization at bipyrimidine sites.

Formation of dUrd hydrates within human cells exposed to UVC radiation

The formation of dUrd hydrates was then monitored within the DNA of cultured human monocytes exposed to UVC light. Because of the low yield of formation and the relatively low sensitivity of the assay, doses as high as 7 J cm^{-2} were applied. Under these conditions, the formation of *cis,syn*-thymine cyclobutane dimer was found to be in equilibrium with its photoreversion, as shown by the plateau in its level with increasing doses (Fig. 5). Similarly, the photoconversion of the corresponding (6–4) photoproduct into its Dewar valence isomer was found to be efficient (Fig. 5). Interestingly, the level of dUrd hydrates was found to be proportional to the UVC dose (Fig. 6). As observed within isolated DNA, the level of oh^{6}hdUrd was much lower than that of the TT cyclobutane dimer. The ratio was likely to be underestimated in the present experiment because, even for the lowest dose, an extensive photoreversion of c,s <> TT occurred. We have previously reported the yield of TT photoproducts within the same cell

Table 3 Quantum yield of formation (Φ) of dUrd hydrates and dimeric photoproducts (× 1000) upon exposure of isolated DNA to UVC light

Lesion	c,s <> TC	(6–4) TC	c,s <> TT	(6–4) TT	oh^{6}hdUrd
Φ × 1000	0.765 ± 0.073	0.843 ± 0.040	1.407 ± 0.040	0.136 ± 0.008	0.017 ± 0.001

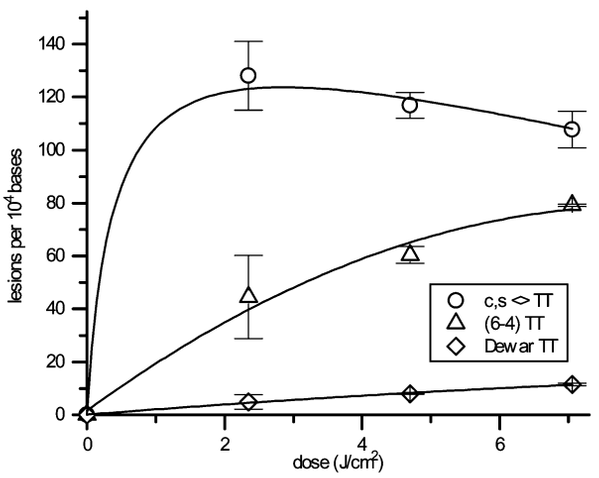

Fig. 5 Formation of thymine dimeric photoproducts within the DNA of cultured human monocytes exposed to high doses of UVC light. Irradiations were performed in triplicate and the results represent mean ± standard deviation.

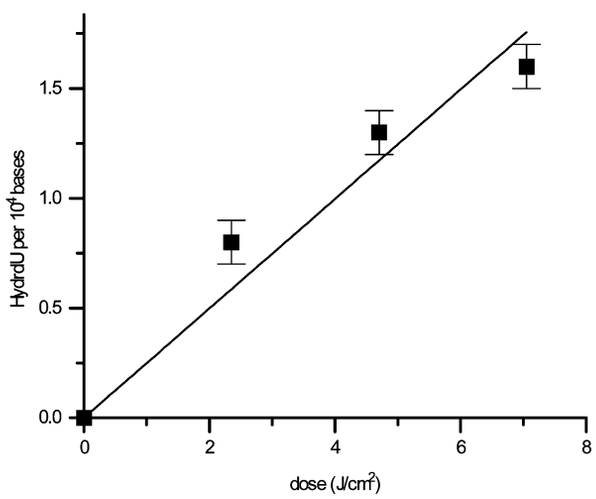

Fig. 6 Formation of dUrd hydrates within the DNA of cultured human monocytes exposed to high doses of UVC light. Irradiations were performed in triplicate and the results represent mean ± standard deviation.

type exposed to UVC light under similar experimental conditions but at much lower doses.[24] Using the values obtained then (240 c,s <> TT/10^4 bases per J cm^{-2}), the ratio between the yield of c,s <> TT and dUrd hydrates may be estimated to be close to 1000. This result further emphasises the very low yield of photohydration of cytosine within double-stranded DNA. Interestingly, the formation of dUrd hydrates with respect to <>TT is ten times lower within cellular DNA with respect to isolated DNA. This may be accounted for by a more rigid structure of the DNA compacted within the cell nuclei which reduces the reactivity of the excited cytosine with the surrounding water molecules.

Conclusion

The reported analytical approach provides new insights into the formation of cytosine hydrates within UV-irradiated DNA. Indeed, the HPLC-MS/MS assay represents a more direct technique than the methods used in previously published works based on the use of indirect enzymatic assays. Interestingly, both approaches lead to the same conclusion that cytosine photohydration is a minor process within double-stranded DNA. It may thus be concluded that the contribution of dCyd and dUrd hydrates to the overall UV genotoxicity is likely to be limited. Indeed, these lesions are produced in very low amount and are efficiently repaired by *N*-glycosylases.[27–29] Interestingly, the HPLC-MS/MS showed that dUrd hydrates are $^{\bullet}$OH radical-mediated degradation products of cytosine. In addition, evidence was provided that the formation of dCyd hydrates following addition of $^{\bullet}$OH to the C5–C6 double bond involves a reduction step. We have previously shown that, according to the distribution of guanine damage, DNA seems to be in a more reducing context in cells than in aqueous solution.[2] The relevance of the formation of dCyd hydrates in cellular DNA would be interesting to determine. However, the sensitivity of the assay presented above is not high enough to allow the quantification of oh^{6}hdUrd following exposure of cells to ionizing radiation. The level of dUrd hydrates within γ-irradiated DNA remains to be determined.

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