UVC induced oxidation of chloropurines: excited singlet and triplet pathways for the photoreaction

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Received 26th March 2008, Accepted 7th July 2008
First published as an Advance Article on the web 24th July 2008
DOI: 10.1039/b805149f

The phototransformation of 2-chloro, 6-chloro and 2,6-dichloropurines under UVC excitation (254 nm) has been studied and the major photoproducts have been identified using absorption spectroscopy, HPLC and mass spectrometry. It was shown that hydroxypurines were formed as the main products for all three investigated compounds both in the presence and absence of oxygen. In the case of 6-chloro- and 2,6-dichloropurine, a photodimer is also formed as a minor photoproduct in the absence of oxygen but is efficiently quenched in the presence of oxygen. Nanosecond photolysis experiments also revealed significant intersystem crossing to the triplet state of the chloropurines which has been characterized (transient absorption spectra, triplet formation quantum yields and rate constants of quenching by oxygen, Mn^{2+} ions and ground state). Experimental evidence allows to conclude that the triplet state is involved in photodimer formation whereas the hydroxypurine is formed from the reaction of the excited singlet state of chloropurines with the solvent (water addition) through heterolytic C–Cl bond rupture. Mass spectrometry and ^{1}H NMR results allowed to propose a chemical pathway for dimer formation in the case of 2,6-dichloropurine in a two-step process: first a homolytic rupture of C–Cl bond in the triplet state of the molecule with the formation of purinyl radicals, which subsequently react with an excess of ground state molecules and/or hydroxypurine primarily formed.

Introduction

Oxidatively generated damage is one of the main DNA lesions that can lead to mutagenesis and carcinogenesis;[1–3] it may result from ionizing radiation, UV light irradiation and/or reaction with other reactants present in the medium, such as enzymes or drugs or various kinds of toxic species, in particular reactive oxygen species (ROS).[4,5] Among these, OH radicals formed in biological media from Fenton-type reactions[6] have been shown to lead, among other products, to the formation of hydroxy derivatives when they react with DNA bases. In the case of pyrimidines, the hydroxy derivative formed is a stable final degradation product whereas in the case of purines it is an unstable intermediate which finally evolves toward a stable oxidation product.[7,8] Similar hydroxy derivatives are also formed as a result of enzymatic oxidation in the case of purine,[9] adenine[10] and 2-chloropurine.[11]

Halogeno-substituted pyrimidines and purines constitute useful tools to study hydroxy derivative formation of DNA components and their subsequent reactivity with the aqueous solvent, since the hydroxy derivative can be formed through dehalogenation by UV irradiation.[12–15] However, only few studies have been devoted to the UV photochemistry of these halogeno-substituted compounds and different mechanisms have been invoked, depending on the nature of the irradiated heterocyclic compound investigated but also on the halogen substitution position on the nuclear ring(s). For instance, in the case of 5-bromouracil and 5-iodouracil,[12] dehalogenation leads partly to uracil and to free radical formation (centered on the C5 position of the pyrimidine ring); in contrast, the photolysis of 6-chlorouracil generates photohydrates *via* an ionic mechanism in the excited state, along with elimination of HCl.[13]

Concerning halogeno-substituted nucleic acid purines, the photo-oxidation of 2-chloro-2′-deoxyadenosine (Cladribine, 2CldAdo) in aqueous solution under UV irradiation was reported to yield as the final photoproduct the keto tautomer of the 2-hydroxy derivative (2′-deoxyisoguanosine), *via* an ionic mechanism involving exclusively the excited singlet state.[14,15] Furthermore the study of different bromo-substituted deoxyadenosines[16] revealed that only the pyrimidine ring in halogeno-substituted adenines is photoreactive.

In this context, 2-chloropyrimidine (2ClPyr) was then investigated, as a simplified model for chloro-substituted adenines;[17] the mechanism of its photo-oxidation in water solution was studied in detail using steady UV irradiation and laser photolysis and shown to lead also to the hydroxy derivative (at the C2 position) and in addition to photodimer formation *via* two different excited state pathways.

To our knowledge, the photo-oxidation of chloro-substituted purines has not yet been investigated although hydroxy purines are also important reactive products in biology. The present study reports on the identification of the photoproducts formed upon steady UV (254 nm) irradiation of 2-chloropurine (2ClPu), 6-chloropurine (6ClPu), and 2,6-dichloropurine (2,6diClPu) (Scheme 1) and the characterization of their triplet states using

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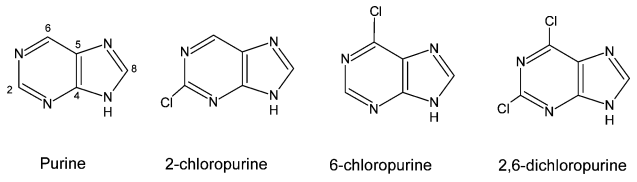


Scheme 1 Structures of purine and of the different studied chloropurines.

nanosecond laser photolysis; a mechanism involving both the excited singlet and triplet states is proposed for the photoreaction.

Experimental

6ClPu, 2,6diClPu 2-hydroxypurine (2OHPu) and hypoxanthine (6OHPu) were Sigma products. 2-Chloropurine (2ClPu) and 2-chloro-hypoxanthine (2Cl6OHPu) have been synthesized as described previously.[18,19] Other chemicals used in experiments were of analytical grade. The concentrations of investigated compounds were determined spectrophotochemically (using a Cary Model 300 spectrophotometer) on the basis of their molar absorption coefficients.[20,21] The solutions were deaerated or saturated with oxygen by purging with argon (purity 99.9%) or oxygen (purity 99.5%), respectively, for 30 min before irradiation. All experiments were carried out at room temperature.

Photochemical reactions of chloropurines were performed in aqueous, non-buffered solutions using a low-pressure Hg 8 W linear bactericidal lamp (Spectronics Corporation, Model EF-180, 1.9 mW cm^{-2} at 25 cm) as a source of continuous UVC radiation (254 nm). The solutions were stirred continuously during irradiation. The progress of photochemical reaction was monitored by UV absorption spectra recorded by a UV-VIS spectrophotometer Cary 300.

Photosensitization experiments have been done in deaerated solutions of chloropurines containing 25% v/v acetone, exposed to irradiation by a medium-pressure Hg 75 W lamp (Philips, model TQ-150) emitting mainly at 313 nm. However, the emission profile of the lamp revealed relative intensities of a few percents down to 270 nm allowing some direct excitation of the different chloropurines (see below).

Irradiated and photosensitized samples of chloropurines were analyzed by HPLC analysis (Spectra-Physics Instrument). The solutions were injected into an analytical RP column (Supelco-LC-18T) using a linear gradient of methanol in 0.1 M KH_2PO_4 (pH = 6.0) from 0–100% within 20 min and analyzed by a Spectra 100 UV monitor at wavelengths corresponding to the absorption maxima of the photoproducts.

The nanosecond flash photolysis was carried out by using Nd/YAG laser with fourth harmonic generation (266 nm, pulse width 2 ns). A flash lamp synchronized with the laser pulse was used as the analysis light source in the crossed-beam configuration for measuring light transmission variations at ~2 ns time resolution across the laser irradiated samples at wavelengths selected by means of a monochromator. The fluence of the laser pulse was monitored using a pyroelectric joulemeter receiving light deviated from the laser beam.[22]

A determination of the product of the triplet quantum yield (ϕ_T) and triplet molar absorption coefficient (ε_T) of the chloropurines was obtained by comparing the absorption changes due to the chloropurine triplet population measured at the end of the laser pulse (ΔA_T) with those measured for naphthalene in cyclohexane at the same laser fluence and the same absorbance of the solutions at the laser wavelength (266 nm). Under these conditions, the following equation can be used:

$$(\Delta A_T)/(\Delta A_{T'}) = (\varepsilon_T \phi_T)/(\varepsilon_{T'} \phi_{T'}) \quad (1)$$

where the primes refer to naphthalene. According to literature values, $\varepsilon'_T = 2.45 \times 10^4$ $M^{-1}cm^{-1}$ and $\phi'_T = 0.75$ for naphthalene in cyclohexane.[23]

Results

Continuous irradiation

The oxygen-saturated and deaerated aqueous solutions of 2ClPu, 6ClPu and 2,6diClPu were exposed to continuous irradiation at 254 nm. For the three investigated compounds significant changes of absorption spectra were observed during the course of photolysis (Fig. 1–3). In contrast, only insignificant phototransformation

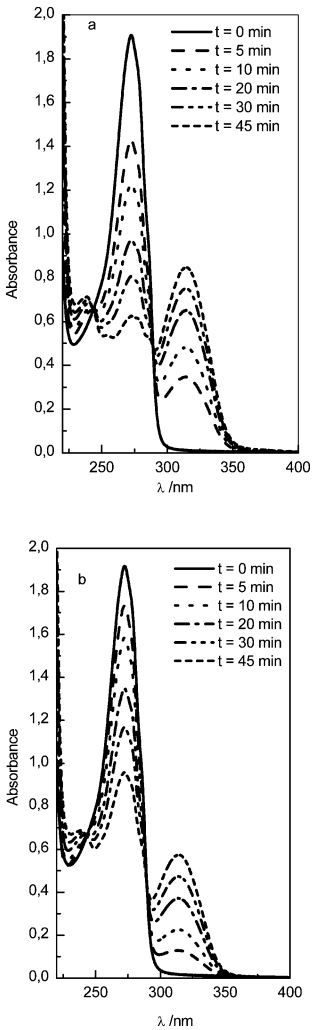
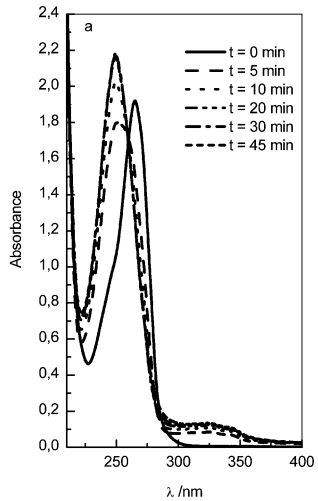


Fig. 1 Absorption spectra of 2.4 × 10^{-4} M 2ClPu before irradiation (thick line) and after irradiation at 254 nm during 5, 10, 20, 30 and 45 min respectively (thin lines): (a) in deaerated solution (under argon atmosphere); (b) in oxygen-saturated solution.



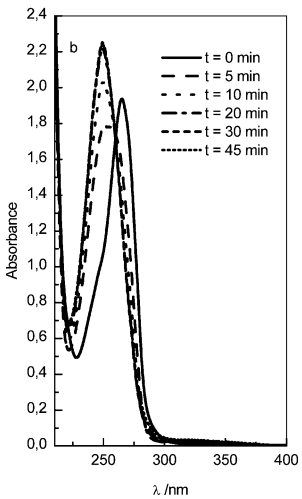
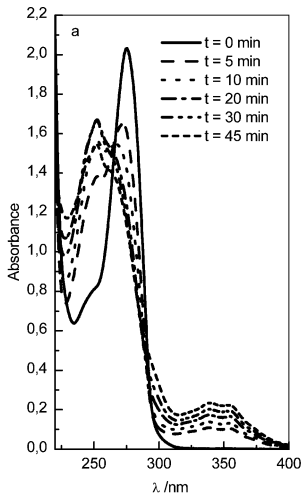


Fig. 2 Absorption spectra of 2.4×10^{-4} M 6ClPu before irradiation (thick line) and after irradiation at 254 nm in similar conditions as described in Fig. 1.



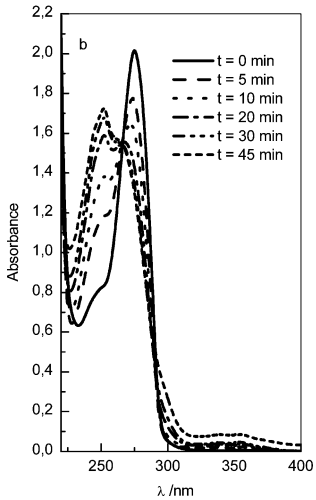


Fig. 3 Absorption spectra of 2.75×10^{-4} M 2,6diClPu before irradiation (thick line) and after irradiation at 254 nm in similar conditions as described in Fig. 1.

was observed in the case of unsubstituted purine under the same experimental conditions (data not shown).

In both deaerated and oxygenated media, the absorption peak characteristic for 2ClPu ($\lambda_{max} = 270$ nm, $\varepsilon_{max} = 8000$ M^{-1} cm^{-1}) decreases with the irradiation time and a new prominent band appears with a maximum at 314 nm (Fig. 1a and b) consistent with the known 2-hydroxypurine (2OHPu) absorption spectrum ($\lambda_{max} =$ 314 nm, $\varepsilon_{max} = 4700$ M^{-1} cm^{-1}).[20] This assignment is supported by the results of HPLC analysis (see below). The existence of isobestic points shows that photodegradation of this photoproduct is negligible at this level of irradiation or not observable in this wavelength range. Comparison of the photolysis spectra at the same irradiation times in the absence and in the presence of O_2 reveals a 2.5 times higher quantum yield for 2OHPu formation in deaerated medium by comparison with oxygenated condition (Fig. 1b).

In the case of 6ClPu and 2,6diClPu, hydroxy derivatives were also formed following irradiation at $\lambda = 254$ nm. The yields of the respective hydroxy derivatives, 6OHPu ($\lambda_{max} = 249.5$ nm, $\varepsilon_{max} =$ 10700 M^{-1} cm^{-1}) (Fig. 2) and 2Cl6OHPu ($\lambda_{max} = 252$ nm, $\varepsilon_{max} =$ 9600 M^{-1} cm^{-1}) (Fig. 3) and their rates of formation were found to be similar under argon or oxygen atmosphere. Furthermore, additional broad absorption bands are observed in the wavelength range (320–360 nm) with maxima at 325 nm and 342 nm in the case of 6ClPu and 338 nm and 358 nm for 2,6diClPu respectively. This result suggests the formation of a new photoproduct not observed for 2ClPu. Its relative yield is significantly reduced in the presence of O_2 (Fig. 2b and 3b).

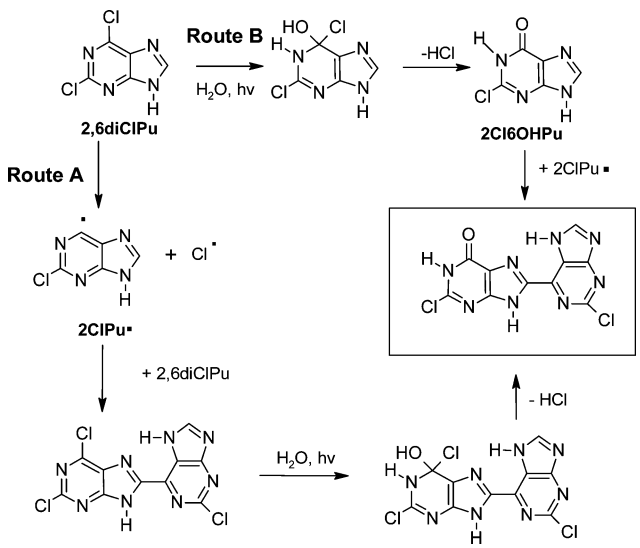
Identification of photoproducts

HPLC analysis of the irradiated solutions of 2ClPu in argon, air and oxygen atmospheres respectively revealed a single chromatographic peak, with an absorption maximum at 314 nm and a retention time of 4.4 min (the observation wavelength was 314 nm corresponding to the maximum of the absorption band of the photoproduct). The compound thus eluted was identified as 2-hydroxypurine by comparison with the HPLC peak obtained in the same conditions for a reference 2OHPu sample. The intensity decrease of HPLC peaks observed from argon to air and oxygen

atmosphere after 1 h of irradiation is in agreement with the absorption spectral evolution described above.

The HPLC analysis of irradiated 6ClPu and 2,6diClPu solutions has been performed at 270 nm and 330 nm, corresponding to the absorption maxima of the photoproducts. Under air and oxygen atmospheres, chromatograms recorded at 270 nm exhibited peaks characteristic of hypoxanthine (6OHPu, retention time 5.2 min) and 2-chlorohypoxanthine (2Cl6OHPu, retention time 5.4 min) which are produced with the same amount under both conditions as reported above from spectrometric measurements. Under deaerated conditions, a second photoproduct, exhibiting an absorption maximum at 330 nm was eluted with retention time of 16.9 min for 6ClPu and 17.7 min for 2,6diClPu.

The yield of formation for this unidentified photoproduct from 2,6diClPu was sufficient to allow further analysis by mass spectrometry and ^{1}H NMR measurements. A pseudomolar ion at $m/z = 323$ was detected, a result which strongly suggests that this photoproduct is a dimer issued from two molecules of 2,6diClPu with loss of two Cl atoms. Besides, ^{1}H NMR spectrum showed three dominant peaks at 8.89 ppm (s, 1H, H_8), 12.56 ppm (s, 1H, pyrimidine NH) and 13.29 ppm (s, 2H, imidazole NH) corresponding to the three positions of the protons in the proposed dimer structure (Scheme 2): one imidazole CH_8 proton, one pyrimidine NH proton and two imidazole NH protons. These ^{1}H NMR results are consistent with the formation of a C2–C8 covalent bond between the two purine monomers. In the case of 6ClPu, the amount of dimer was significantly lower so that mass spectroscopy measurements were impossible. We can only postulate that dimer formation also occurs by comparison of the absorption band location and retention time of the photoproduct which are very similar to those observed for 2,6diClPu.



Scheme 2 2,6diClPu dimer formation.

No other products were detected by HPLC with the exception of traces of subsequent photolysis products found in the 2,6diClPu samples (with a retention time of 5.9 min) in agreement with the observation of ill defined isobestic points (Fig. 3).

Analysis of continuous irradiation spectra

Both spectral changes upon irradiation and HPLC analysis revealed the formation of 2OHPu as single photoproduct in 2ClPu photolysis. The quantum yield (ϕ) of 2-hydroxypurine formation was determined in deaerated solution using the photohydration reaction of 2ClPyr as a reference. The latter reaction was reported to have a quantum yield of 0.01 ($\pm$ 10%) for the formation of the 2OHPyr.[17] The results gave a quantum yield value for the formation of 2OHPu of 0.015 ($\pm$ 10%). From the analysis of the absorption spectra shown in Fig 2a and b, the corresponding ϕ value in oxygen atmosphere is lower by a factor 2.5 ($\phi = 0.006 \pm$ 10%).

It was also possible to obtain reconstructed photolysis spectrum of 2ClPu down to 225 nm by using the known literature absorption spectrum of the hydroxy derivative 2OHPu (λ_{max} = 314 nm, ε_{max} = 4700 M^{-1} cm^{-1}).[20] Fig 4 shows a very close agreement between this reconstructed spectrum and the experimental one for 30% conversion of the chloropurine (corresponding to 5 min of irradiation).

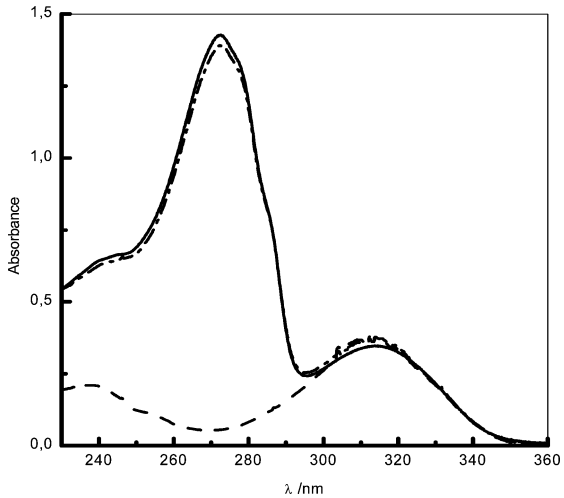


Fig. 4 Absorption spectra of 2OHPu (dashed line), of 2ClPu measured after 5 min of irradiation (thin line) and the reconstructed spectrum corresponding to 70% of 2ClPu and 30% of 2OHPu (thick line) which superimposed well that obtained after 5 min of irradiation of 2ClPu.

This spectral analysis was not achievable for 6ClPu and 2,6ClPu due to overlapping absorption spectra of starting compounds and photoproducts (hydroxy and dimer compounds).

Photosensitization

Photosensitization experiments have been performed to study the involvement of the triplet state in the phototransformations of chloropurines. Acetone was used as a photosensitizer, for which the triplet state is populated efficiently on irradiation at 313 nm. The photoproducts formed under these conditions are expected to originate mainly from the triplet state of chloropurines.

The HPLC analysis of the acetone-photosensitized solutions of 6ClPu and 2,6diClPu revealed that the dimers of chloropurines are the main photolysis product in these conditions. The hydroxy derivatives are also formed to some extent but their yields of formation represent only 30% and 25% of those of the dimers for

6ClPu and 2,6diClPu respectively. These results have to be compared with the hydroxy derivative/dimer ratios found on direct photolysis ($\lambda_{exc} = 254$ nm) for the two chloropurines which were about 2.6 for 6ClPu and about 3.0 for 2,6diClPu. Furthermore, we have controlled that the residual hydroxy derivative formation still observed in the photosensitized reactions is consecutive to direct excitation of the chloropurines due to the residual emission (down to 270 nm) from the UV lamp.

In the case of 2ClPu, the yield of formation of the hydroxy derivative upon photosensitization experiments was also drastically reduced (by ~70%) by comparison to that obtained on direct photolysis (taking into account the 2OHPu concentration formed upon direct excitation of the 2ClPu with the residual emission of the lamp below 313 nm). Furthermore, an additional HPLC peak with a retention time of 14.9 min was observed at 314 nm, indicating dimer formation also in this case.

Laser photolysis results

Under laser excitation at 266 nm of deaerated solutions of 2ClPu, 6ClPu and 2,6diClPu, transient absorption bands have been detected at the end of the laser pulse (10 ns) for all the investigated chloropurines both in the 300–500 nm spectral range and around 700 nm. The latter absorption band with 200 ns lifetime was assigned to the hydrated electron (e_{aq}), since it was scavenged very efficiently by oxygen and N_2O.

The apparent yields of photoionization (defined as the ratio between the e_{aq} and the chloropurine concentrations) were significantly higher for 2ClPu (*ca.* 7%) than for the other chloropurines 6-ClPu and 2,6diClPu (*ca.* 1%).

The photoionization process is essentially biphotonic (the slope of the linear fit of the ratio OD due to e_{aq}/laser energy *versus* laser energy is equal to 0.93), which is not unexpected since the gas phase ionization potential of purines is 9.5 eV[24] and the energy of a 266 nm photon is 4.66 eV only (going from gas phase to solution would not lower the ionization potential by more than 2–2.5 eV).

The results presented below for 2ClPu were obtained at fluence levels that minimized the occurrence of the photoionization process; for the other two compounds the transient absorption signals were too low to allow similar fluence conditions. Thus, while the experiments were performed under N_2O (hydrated electron scavenger) conditions, the cationic radicals formed upon photoionization and the subsequent products derived from these radicals gave rise to a residual long lived absorption. On the microsecond time scale, the absorption of 2OHPu and of the photodimers of 6ClPu and 2,6diClPu formed upon irradiation in deaerated conditions (see above) also contribute to the transient absorption signal. Thus, the microsecond transient spectra have been subtracted from the transient spectra measured at the end of the laser pulse. The resulting spectra (Fig. 5), with maxima centered at 400 nm for 6ClPu and 360 nm for both 2ClPu and 2,6diClPu and with a lifetime of a few microseconds (<6 μs) in deaerated conditions were assigned to the absolute triplet state spectra of the chloropurines (in the spectral range above 300 nm, there is no absorption of the ground states of the studied compounds). Indeed, these transient absorptions were quenched by both oxygen (at a diffusion-controlled rate: $k_q = 5 \times 10^9$ M^{-1} s^{-1} for 2ClPu and 2.9×10^9 M^{-1} s^{-1} for both 6ClPu and 2,6-diClPu) and paramagnetic ions Mn^{2+} (although not diffusion-controlled,

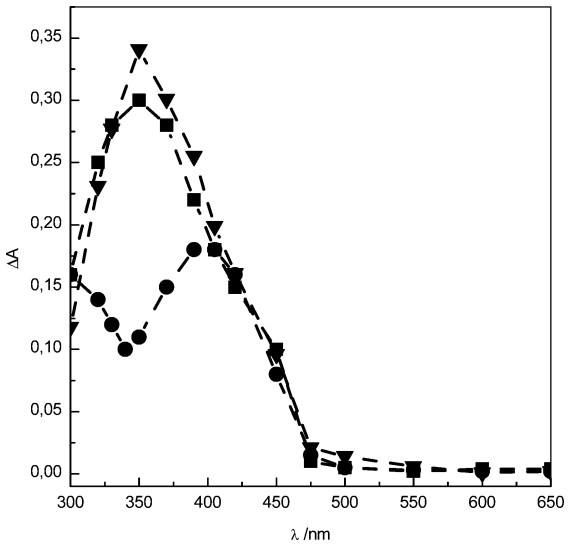


Fig. 5 Absorption triplet spectra of chloropurines determined as described in the text. (▼) 2ClPu, (■) 6 ClPu and (●) 2,6diClPu. For the sake of clarity, the ordinate scale for 6 ClPu and 2,6diClPu was multiplied by a factor 10.

$k_q = 3 \times 10^8$ M^{-1} s^{-1} for 2ClPu and 2×10^7 M^{-1} s^{-1} for 6ClPu and 2,6diClPu (Fig. 6a). It must be noted that the location of the absorption maxima in the visible of these triplet state spectra are consistent with published reports on unsubstituted purine[24] and purine nucleotides (AMP, GMP).[25]

The rate of decay of the triplet was also measured for two chloropurine concentrations differing by a factor three. The results obtained in deaerated solutions (Fig. 6b) showed that the 360 nm absorption decay increased linearly with the chloropurine concentration. The kinetic analysis revealed for all investigated compounds that the triplet state reacts with the chloropurine ground states, however more efficiently for 2ClPu ($k_q = 2.2 \times 10^9$ M^{-1} s^{-1}) than for 6ClPu ($k_q = 5 \times 10^8$ M^{-1} s^{-1}) or 2,6diClPu ($k_q = 1.5 \times 10^8$ M^{-1} s^{-1}).

The product $\varepsilon_{T(max)}\Phi_T$ was determined for 2ClPu using eqn (1). A value of 1.08×10^4 M^{-1} cm^{-1} (estimated error ± 10%) was obtained at $\lambda = 360$ nm. No data are available for the $\varepsilon_{T(max)}$ values for chloropurines. Literature values only concern $\varepsilon_{T(max)}$ of purine and pyrimidine nucleotides (AMP and GMP) which lie in the range 4×10^3–1.3×10^4 M^{-1} cm^{-1} and appear to be higher for purine than for pyrimidine nucleotides.[25] By setting also a value of $\varepsilon_{T(max)} = 10^4$ M^{-1} cm^{-1} for 2ClPu, one obtains a Φ_T value close to unity, which agrees with an earlier study of Murgida *et al.*[26] for unsubstituted purine in aqueous solution (Table 1). Assuming similar $\varepsilon_{T(max)}$ values for 6ClPu and 2,6diClPu, much lower values of triplet formation quantum yields close, to 0.1 were obtained for these two chloropurines (Table 1).

Discussion

The major photoproducts formed under UV irradiation at 254 nm of the different chloropurines investigated in this study are the hydroxy derivative, both in the presence and in the absence of oxygen and a photodimer, formed essentially in the absence of oxygen. In the case of 2ClPu, this dimer is only detected, but

Table 1 Triplet state properties of the different chloropurines by reference to unsubstituted purine. Estimates of the triplet quantum yields are within ±20%

Compound	λ_{max}/nm	Φ_t
Pu	400	0.88[a]
6-ClPu	400	0.1
2-ClPu	360	1
2,6-diClPu	360	0.15

[a] From ref. 26, a value obtained using a laser-induced optoacoustic spectroscopy technique.

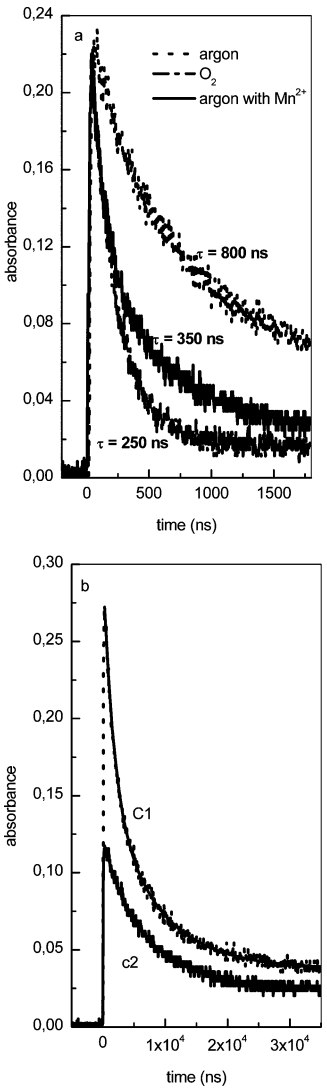


Fig. 6 (a) Triplet decay kinetics measured at 375 nm after exposure of a 2.4×10^{-4} M 2ClPu solution at 266 nm in deaerated solution (thick line), oxygen-saturated solution (dotted line) and in the presence of 10^{-2} M Mn^{2+} ions (---) respectively. (b) Dependence of triplet decay rate on 2ClPu concentration in N_2O-saturated solution measured at 375 nm. (c_1) 2.4×10^{-4} M 2ClPu; (c_2) 8×10^{-5} M 2ClPu.

with a low concentration, in the acetone sensitized photolysis experiments in deaerated solutions.

Based on both the steady photolysis and ns laser photolysis results, the following mechanism is proposed to account for the experimental results obtained for the three chloropurines investigated.

$$ClPu + h\nu \rightarrow {}^1ClPu \quad (2)$$

$${}^1ClPu \rightarrow ClPu \quad (3)$$

$${}^1ClPu \rightarrow {}^3ClPu \quad (4)$$

$${}^3ClPu \rightarrow ClPu \quad (5)$$

$${}^3ClPu + ClPu \rightarrow ClPu + ClPu \quad (6)$$

$${}^3ClPu + O_2 \rightarrow ClPu + {}^1O_2 \quad (7)$$

$${}^1ClPu + H_2O \rightarrow OHPu + HCl \quad (8)$$

$${}^3ClPu \rightarrow Pu^{\bullet} + Cl^{\bullet} \quad (9)$$

UV-irradiation at 254 nm of the chloropurines studied, takes the molecules into their excited singlet state (reaction 2). Chloropurines have very low fluorescence quantum yields, particularly the 6-chloroderivative,[21,27] similar to the case of other 6-substituted purines like adenine (6-aminopurine).[27] Since the photochemical quantum yield is very small as well, the major deactivation pathways for the excited singlet state are radiationless deactivation to the ground state (reaction 3) and/or intersystem crossing to the triplet sate (reaction 4). The present laser photolysis results clearly demonstrate an efficient population of the triplet state in the case of 2ClPu with a quantum yield of triplet formation (Φ_T) close to unity. This high Φ_T value is consistent with previous literature reports for unsubstituted purine,[26] pyrimidine[28] and 2ClPyr,[17] but is quite different from those obtained in this study for 6ClPu and 2,6diClPu ($\Phi_T \sim 0.1$). Thus, substitution at the 6 position of purine by a nucleophilic group such as the amino group or Cl strongly decreases the triplet formation efficiency, as in the case of adenine[27] and 2ClAdo.[15] This marked dependence of Φ_T on substituent position (2- or 6-) may be a consequence of the effect of the substitution position of the Cl atom in the pyrimidine ring on the relative energies of the sets of π–π* states and n–π* excited states of the molecule, favorable or unfavorable to the intersystem crossing process according to the El-Sayed's rule.

The results of this study show that the triplet state once populated may undergo intersystem crossing to the ground state (reaction 5) and/or more or less efficient bimolecular reaction with an other chloropurine molecule in its ground state (see results) (reaction 6). This self-quenching reaction of the triplet state has already been observed for both pyrimidine and purine derivatives and its efficiency varies within a wide range for such compounds in water solution. Rate constants for AMP and GMP triplet self-quenching of 3.3×10^8 M^{-1} s^{-1} and 0.67×10^8 M^{-1} s^{-1} respectively have been obtained by Gutman *et al.*[25] A value of 4.4×10^8 M^{-1} s^{-1} has been reported for 2ClPyr in water solution.[17]

The chloropurines triplet states are quenched by O_2, probably with formation of singlet oxygen (reaction 7) as already reported in the case of purine bases.[29] The HPLC analysis clearly reveals that no significant photodegradation of the molecules is observed in the presence of O_2 in the wavelength range studied. Furthermore, an isobestic point was observed on the photolysis absorption spectra as a function of time irradiation. These results suggest

that singlet oxygen reaction with purines does not significantly occur and/or that the photoproducts formed absorb below 200 nm. Previous studies have shown that pyrimidine rings are not prone to react with singlet oxygen,[30,31] however the imidazole ring may be attacked. Such singlet oxygen reactions have been postulated to occur in the UV photochemistry of some guanine derivatives, adenosine and adenine, however without any clear cut identification of the photoproducts formed.[8,32]

Besides these photophysical processes, hydroxy derivatives and dimers are formed upon photolysis of the different chloropurines studied and the question arises, what are the chemical reactions leading to their formation?

The photosensitization experiments in deaerated conditions strongly suggest that the hydroxy derivatives of chloropurines are formed from the excited singlet state of the molecules. However, the case of 2ClPu must be distinguished from those of 6ClPu and 2,6ClPu. For the latter two compounds, the hydroxy derivative/dimer ratio is significantly lower in the triplet photosensitized reaction than in direct photolysis of the molecules. Furthermore, the finding that the yields of hydroxy derivatives formed are independent of the presence of air and oxygen (under 1 atm) in the solution, speaks in favor of the excited singlet state as the precursor state of hydroxypurine formation; otherwise, the quenching of the triplet state by oxygen would be expected to decrease drastically the hydroxy derivative yield in these conditions.

In the case of 2ClPu, the photosensitization results also reveal a drastic decrease of the hydroxy derivative formation in deaerated conditions confirming the involvement of the excited singlet state in this photoreaction. However, the yield of 2OHPu formation is significantly higher for deaerated than for oxygenated (1 atm) solutions (Fig. 1). This feature, not observed for 6ClPu and 2,6ClPu could indicate that due to its efficient population, the 2ClPu triplet state could also be involved in 2OHPu formation. According to this hypothesis, since the triplet state is quenched efficiently by O_2, the yield of 2OHPu formation should decrease steeply with the dissolved oxygen concentration. However, HPLC analysis revealed that after any irradiation time, 2OHPu concentration formed in direct photolysis decreased by approximately the same amount in aerated and in oxygen-saturated solutions. Thus, a triplet state pathway for the formation of 2OHPu has to be disregarded. More probably, an oxidation of 2OHPu is expected to occur in the presence of oxygen with the formation of products which cannot be identified because their absorption presumably lies below 200 nm.

Thus, for the different chloropurines studied, the formation of the hydroxy derivatives must be due to a concerted reaction of the chloropurine excited singlet state with water molecules with elimination of an HCl molecule (reaction 8). The participation of the solvent in this photoreaction was supported by the observation of a significant decrease in the kinetics upon changing the solvent from H_2O to D_2O (*e.g.* a decrease by a factor of 1.6 in the case of 2,6diClPu). These results are similar to those already reported in the case of 2ClPyr,[17] 2ClAdo[15] and its 2-and 8-bromoderivatives.[16]

Considering the chloropurine dimers, the triplet–triplet photosensitization study shows that their formation is markedly enhanced compared with direct photolysis (for 2ClPu, dimers are only detected in photosensitization experiments). Furthermore, dimer concentration is drastically decreased in the presence of oxygen. These results thus lend support to the participation of the triplet state of chloropurines into dimer formation. This is also consistent with their excited singlet state properties (their lifetimes are too short to allow encounter between two molecules in solution[21]) which precludes their involvement in such dimer formation.

The mass spectroscopy and ^{1}H NMR results only obtained for 2,6diClPu emphasize that the corresponding dimer structure implies the loss of two Cl atoms and the formation of a C2–C8 covalent bond between the two purine monomers. This precludes a direct reaction between chloropurine triplet and ground states which would lead to a C6–C8 linked dimer with three Cl atoms. One has therefore to assume the involvement of a transient species formed from the triplet state of 2,6diClPu. Previous literature data report the detection of purinyl radicals upon UV irradiation of 2,6-dichloropurines.[33] It was also demonstrated for 2ClPy, that the triplet state is the precursor of a pyrimidinyl radical.[17] On the basis of these results, we propose that the purinyl radical (Pu$^\bullet$) of 2,6diClPu is also generated through homolytic C–Cl bond breaking in the triplet state (reaction 9). This Pu$^\bullet$ radical is expected to react in turn with an other 2,6diClPu ground state molecule with elimination of HCl leading to the dimer formation (route A, Scheme 2). However, an other pathway involving a reaction between Pu$^\bullet$ and 2Cl6OHPu primarily formed from the excited singlet state (route B, Scheme 2) is also feasible, but presumably less efficient in view of the low quantum yield of hydroxy derivative formation. Indeed, the irradiation of 2Cl6OHPu in the presence of 2,6diClPu was found to lead to an increased amount of dimers compared with the irradiation of 2,6diClPu alone (data not shown).

The same type of reaction probably also occurs for the formation of 2ClPu and 6ClPu dimers. In this case, two types of structures are possible, C2–C8 or C2–C6 linked dimers which could not be identified in this study due to the small amounts formed.

In the presence of oxygen, it was observed that dimer formation from photoexcited 6ClPu and 2,6diClPu is strongly decreased. This suggests that in these conditions, competition between the reactions of Pu$^\bullet$ with the chloropurine ground state and oxygen under 1 atm would then be dominated by reaction with oxygen, a hypothesis which is supported by previous studies on pyridine and chloropyrimidine showing that O_2 is an efficient scavenger of pyridyl radicals.[34]

The present study also reveals that the dimer formation is more efficient for 6-substituted than for 2-substituted chloropurines, a result which may be ascribed to the much greater reactivity of the Cl atom when substituted in the 6 position on the purine ring instead of the 2-position as already reported.[35]

Summarizing, the common photoreaction mechanism invoked for the chloropurines presently investigated is similar to that previously reported for 2ClPyr,[17] involving in both cases excited singlet and triplet states pathways. The hydroxy derivative is formed exclusively from the excited singlet state, through reaction with the aqueous solvent, as occurs in photohydrate formation of pyrimidines[36] or halogeno-substituted pyrimidines[13,17] and 2′-deoxyadenosine.[15] In contrast, photodimer formation is complex. It takes place from the triplet state through (i) purinyl radical formation and elimination of a Cl atom and (ii) subsequent reactions with either chloropurines or hydroxy derivatives. Such photochemical reaction has been essentially reported in literature up to now for pyrimidine bases but is new finding for

purines. For example, cyclobutane-type dimers are known to be the major stable photoproducts in the case of uracil and thymine.[37,38] Photochemical transformations of 5-iodo substituted uracil and cytosine also lead to dimer formation (5,5′-diuracil type) with radical formation following rupture of the C–I bond.[12] Concerning purines, the study of 2CldAdo ascertained that due to the absence of triplet state formation for purine nucleic acid bases, such photodimers were not detected.[15] It must be noted that dimer formation of adenine under UV irradiation has been reported by Kumar *et al*.[39] as a minor process; however, the nature of the excited state involved was not discussed in that study.

Halogeno-substituted purines have useful biological and medical applications. For example, 2,6-dichloropurines have been shown to exhibit both antiviral[40] and antineoplastic activities.[41] Bridged bis-(6-chloropurines) have been proposed as potential DNA bis-intercalating antitumor agents.[42] The present study reveals that such compounds may give rise to hydroxy derivatives which may be implied in their metabolism and may contribute to their cytotoxicity. Furthermore, chloropurines present analogies with pyrimidine nucleic acid bases and undergo photochemical reactions also *via* their triplet state, thus leading to potential damage to DNA. Such a reactivity has to be taken into account in clinical applications.

Acknowledgements

The present study was supported by the Polonium Contract of Exchange. One of the authors (E. B.) gratefully acknowledges the opportunity given by the University Paris-Sud to stay at Orsay as a visiting professor.

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