UV Laser Photolysis of DNA: Effect of Duplex Stability on Charge-Transfer Efficiency

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Abstract: The distribution of the final base damage was determined within isolated DNA exposed to pulses of 266 nm laser light. Studied lesions included oxidation products arising from biphotonic ionization of DNA bases and pyrimidine dimeric photoproducts arising from monophotonic processes. The distribution of the latter class of damage was found to be correlated with the stability of the DNA duplex. The quantum yield for formation of 8-oxo-7,8-dihydroguanine was much higher than that of other oxidized nucleosides arising from the degradation of thymine and adenine. This observation, together with the shape of the intensity dependence curves, provided evidence for the occurrence of charge-transfer processes within DNA. In addition, increase in the ionic strength of the irradiated DNA and stabilization of the DNA duplex were found to induce a drastic decrease in the yield of thymine and adenine oxidation products. Concurrently, an increase in the yield of 8-oxo-7,8-dihydroguanine was observed. This was rationalized in terms of an increase in the overall charge-transfer efficiency. Therefore, it may be concluded that stabilization of the double-helix favors charge-transfer process toward guanine bases.

Introduction

One-electron oxidation of DNA is involved in processes such as the direct effect of ionizing radiation, type I photosensitization, and reactions with a wide array of oxidants. This leads to the formation of the radical cations of DNA bases. The reactivity of the latter transient species has been extensively investigated using monomeric model systems and isolated DNA.[1] In addition, the final lesions arising from the fate of the radical cation of the pyrimidine and purine bases have been identified upon isolation of the degradation products of bases and nucleosides.[2] The chemistry of base radical cations within double-stranded DNA is more complex particularly because of the occurrence of the well documented possibility of charge-transfer reactions within the highly organized biopolymer.[3] Guanine residues, especially when located at the 5′-end of G doublets or triplexes, are the main targets because they exhibit the lowest ionization potential among DNA bases and thus behave as traps for positive holes.[4] This may be visualized by sequencing gel electrophoresis of oligonucleotides following conversion of some of the damage into strand breaks upon alkaline treatment. Using this technique, favored degradation of guanine has been observed within DNA fragments under a variety of oxidative conditions including photoinduced electron abstraction,[3c,5] site-specific production of a 2-deoxyribose radical cation at the C4 position,[3d,6] and UV laser irradiation.[7] One limitation of the latter approach is the lack of structural information on the guanine oxidation products observed. This may be partially overcome by the use of bacterial DNA repair enzymes aimed at converting base damage into strand breaks. For instance, formamidopyrimidine DNA *N*-glycosylase (Fpg) has been used to visualize the predominant formation of 8-oxodGuo within oligonucleotides exposed to either 193[8] or 266 nm laser pulses.[9] Altogether, evidence has been provided for the occurrence of charge transfer within DNA. The exact mechanism is not fully understood but is likely to combine super-exchange through the π-stack of bases over a few base pairs[10] with long distance intra- and interstrand hopping

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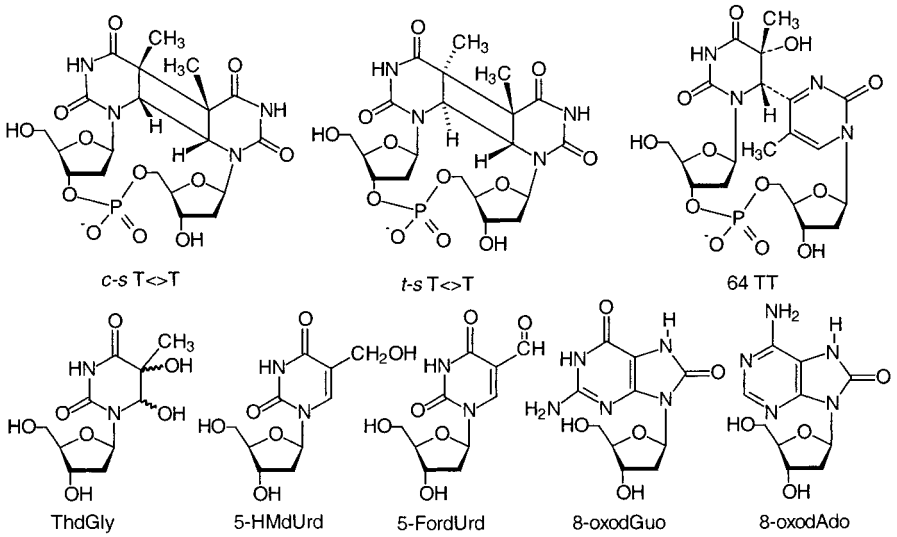
Scheme 1. Structure of the Lesions Studied[a]



[a] Only thymine dimeric photoproducts are shown. The level of related thymine−cytosine, cytosine−thymine, and cytosine−cytosine lesions were also determined.

of the charge.[11] A slightly different version of the latter mechanism involves the stabilization of the positive charge in a polaron-like species.[12]

Using specific chromatographic assays aimed at measuring a wide array of oxidative base damage, we also observed that guanine lesions were the major decomposition products upon type-I photosensitization of DNA.[13] This was partially attributed to charge-transfer processes from the T, C, and A radical cation toward guanine. However, it was not possible to rule out a preferential electron transfer from guanine to the photoexcited sensitizer, leading to a higher initial yield of guanine radical cation with respect to the three other bases. Biphotonic UV laser-induced photoionization of DNA[14] may be used as an alternative means to generate radical cations of the four bases in approximately equal amounts. Interestingly, a high-intensity UV laser has been used to generate DNA−protein cross-links in footprinting experiments.[15] In addition, direct measurements by high-performance liquid chromatography (HPLC) coupled to electrochemical detection have shown the induction of the 8-oxo purine derivatives upon exposure of DNA to UV laser pulses.[16] In the present work, the formation of a wider set of oxidative damage to guanine, thymine, and adenine (Scheme 1) was specifically measured by HPLC coupled to tandem mass spectrometry. In addition, the formation of dimeric pyrimidine lesions, arising from monophotonic processes,[17] was monitored to assess the effect of the laser pulse intensity on the efficiency of the depletion of excited pyrimidine bases by a second photon. The distribution of the latter lesions, that include cyclobutane dimers, (6−4) photoproducts and their related Dewar valence isomers, was also found to be dependent on the stability of the DNA duplex. Therefore, precise determination of the quantum yield of formation of these classes of monomeric and dimeric damage allowed us to show that stabilization of the DNA structure enhanced the efficiency of charge transfer toward guanine bases.

Results

Distribution of Dimeric Pyrimidine Photoproducts under Different Irradiation Conditions. The quantum yields of formation of the lesions upon high-intensity UV laser irradiation were compared with data obtained under conditions where only monophotonic processes are involved. For this purpose, control experiments were performed with a germicidal lamp that emits UVC light with a maximum emission wavelength at 254 nm. DNA solutions were used at a concentration of 75 μg/mL as for the laser experiments. This represented solutions 10 times more dilute than those in our previously reported works.[18] Emphasis was placed on the formation of dimeric pyrimidine photoproducts that are expected to be the main lesions. Quantum yields were calculated and expressed with respect to the overall DNA bases (Table 1). A major difference was observed between the distribution of lesions determined in experiments carried out at concentrations of 1 mg/mL and 75 μg/mL, respectively. The latter solution was prepared by dilution of the former in deionized water. Even though the quantum yield of the predominant *cis*−*syn* TT cyclobutane dimer remained almost unchanged, those of the TC photoproducts were at least 3 times lower in the most diluted samples. Similarly, the extent of formation of CC damage was drastically reduced, and the amount of the corresponding lesions was below the detection limit of the HPLC−MS/MS method. The most striking observation was the formation in relatively high yield of the *trans*−*syn* TT cyclobutane dimer. Indeed, this photoproduct is not expected to be produced within B form DNA because both adjacent thymine residues are in a preferred *anti* conformation. As a consequence, the *cis*−*syn* diastereoisomer is expected to

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Table 1: Quantum Yield (×1000) of Dimeric Pyrimidine Photoproducts[a] within DNA Exposed to the UVC Light Emitted by a Germicidal Lamp

concn	1 mg/mL[b]	75 μg/mL	75 μg/mL	75 μg/mL
additive	none	none	100 mM NaCl	1 mM Tris
6−4 TT	0.379 ± 0.005	0.307 ± 0.007	0.259 ± 0.006	0.255 ± 0.005
c,s T<>T	2.406 ± 0.206	1.801 ± 0.150	1.850 ± 0.0397	2.120 ± 0.113
t,s T<>T	n.d.[c]	0.177 ± 0.037	n.d.	n.d.
6−4 TC	1.477 ± 0.034	0.257 ± 0.025	1.350 ± 0.024	1.024 ± 0.037
c,s T<>C	1.208 ± 0.105	0.310 ± 0.038	0.915 ± 0.037	0.807 ± 0.089
6−4 CT	0.013 ± 0.001	0.019 ± 0.002	0.012 ± 0.003	0.011 ± 0.001
c,s C<>T	0.451 ± 0.036	0.286 ± 0.020	0.418 ± 0.011	0.359 ± 0.026
6−4 CC	0.111 ± 0.005	0.008 ± 0.001	n.d.	0.041 ± 0.004
dew CC	0.081 ± 0.010	n.d.	n.d.	0.033 ± 0.005
c,s C<>C	0.153 ± 0.025	0.022 ± 0.008	0.087 ± 0.017	0.109 ± 0.019

[a] The formation of TT, TC, and CT Dewar valence isomers was not observed. [b] Results are expressed with respect to the overall DNA bases (mean ± standard error). [c] n.d.: no detectable formation.

be predominantly produced. However, t,s T<>T has been detected within denatured DNA[19] and DNA exposed to very high doses of UVB light, under conditions where the overall degradation yield of pyrimidine bases was close to 1%.[18a] The level of photoproducts was significantly lower in the present experiment. In addition, the level of t,s T<>T was found to be linear with respect to the applied UV laser dose. This is strongly indicative that the generation of t,s T<>T was not a consequence of a partial denaturation of DNA induced by the accumulation of other photoproducts. Interestingly, the distribution of damage obtained within DNA irradiated at a concentration of 1 mg/mL was similar to that obtained in diluted solution (75 μg/mL) in which the ionic strength was raised by the addition of either 100 mM NaCl or 1 mM Tris. This was shown by the increased yield in TC and CC damage together with the lack of formation of t,s T<>T.

Effect of UV Laser Intensity on the Distribution of Lesions. The first series of experiments with UV laser involved exposure of an aqueous aerated solution of DNA to pulses of increasing intensity ranging between 18 and 347 mJ/cm^2. No salt was added to the solution. To avoid a heterogeneous distribution of photoinduced damage in the sample, the chosen concentration of the solution was 75 μg/mL to obtain an optical density of 1.5. In addition, the solution was stirred during the irradiation. Dimeric pyrimidine lesions and base oxidation products were quantified by HPLC−MS/MS. The yield of 8-oxodGuo upon exposure of DNA to the light emitted by the germicidal lamp was at least 1 order of magnitude lower than that of c,s T<>T. This oxidation may be partly accounted for by the formation of singlet oxygen from excited guanine residues.[20] No formation of the other oxidized bases quantified was observed upon exposure of DNA to 254 nm light. All UV laser-induced DNA base lesions were found to be generated in a linear way with respect to the total UV irradiation dose. This showed that all lesions were primary photoproducts and that no secondary reactions, such as the degradation of highly oxidizable 8-oxodGuo, occurred. The quantum yield of TT and TC cyclobutane dimers and (6−4) photoproducts was found to decrease with increasing laser pulse intensity (Figure 1). No Dewar valence isomer was detected. Interestingly, the ratio between the *cis*−*syn* and *trans*−*syn* thymine cyclobutane dimers was constant over the range of pulse intensities investigated. It was similar to that determined in the control experiments

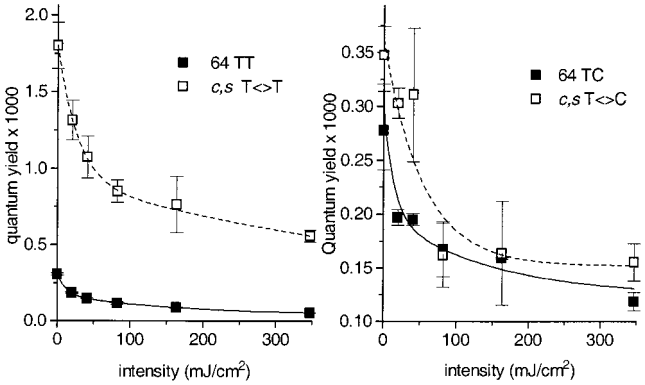

Figure 1. Effect of the laser pulse intensity on the quantum yield of formation of dimeric photoproducts at (A) TT and (B) TC sites within isolated DNA (75 μg/mL). Values for the lowest intensity were inferred for experiments using a 254 nm-emitting germicidal lamp.

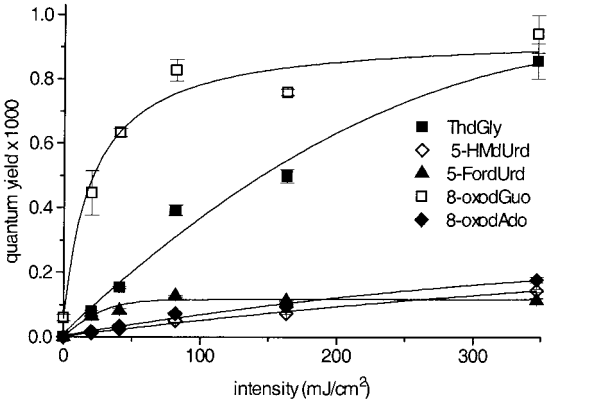

Figure 2. Effect of the laser pulse intensity on the quantum yield of formation of oxidized nucleosides within isolated DNA (75 μg/mL). Values for the lowest intensity were inferred for experiments using a 254 nm emitting germicidal lamp.

involving the use of the low-intensity UVC lamp. In contrast to pyrimidine dimeric photolesions, the quantum yield of oxidized bases increased with increasing intensity of the laser pulse (Figure 2). However, it must be emphasized that the yield of 8-oxodGuo rapidly reached a plateau, while those of ThdGly, 5-HmdUrd, and 8-oxodAdo were still increasing at the highest intensity. For instance, the ratio between the yield of ThdGly and 8-oxodGuo was 0.12 and 0.85 for intensities of 18 and 347 mJ/cm^2, respectively. The increase in the yield of formation of 5-FordUrd with increasing intensity was lower than that of ThdGly and 5-HmdUrd. Indeed, while the ratio between the two latter lesions remains constant over the range of intensity studied (ca. 6.4 ± 0.4), the ratio between the yield of 5-FordUrd

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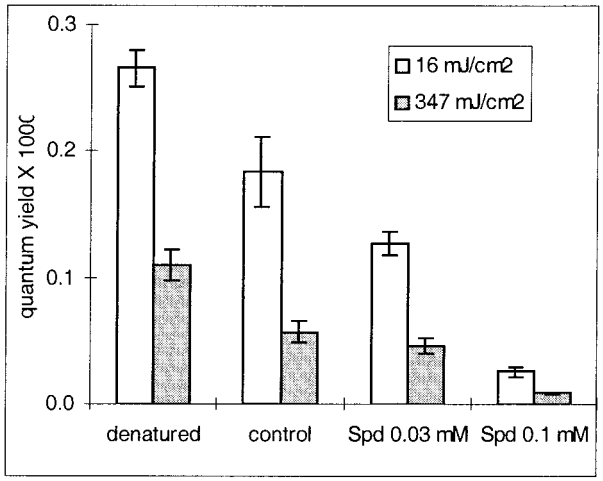

Figure 3. Effect of the irradiation conditions on the quantum yield of formation of the *trans*−*syn* thymine cyclobutane dimer within isolated DNA exposed to laser pulses of either 16 or 347 mJ/cm^2 intensity. The DNA concentration was 75 μg/mL for all samples. The DNA samples were either used without further treatment, heated at 100 °C prior to the irradiation, or mixed with spermidine to reach final concentrations of 0.03 and 0.1 mM in the latter compound.

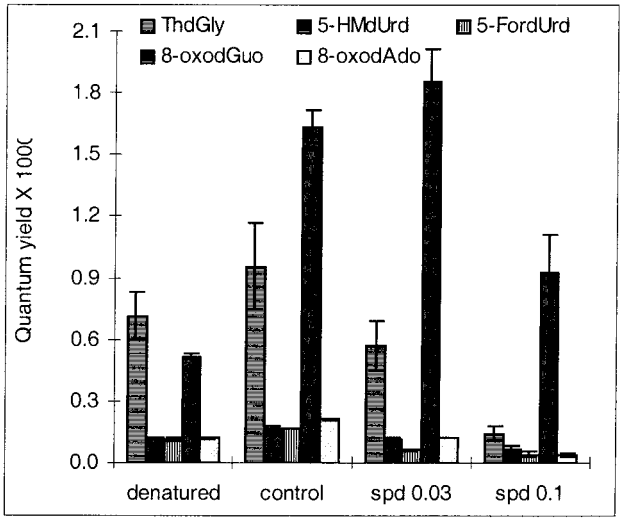

Figure 4. Effect of the irradiation conditions on the yield of formation of oxidized nucleosides within isolated DNA exposed to UV pulses of 347 mJ/cm^2. The DNA concentration was 75 μg/mL for all samples. They were either used without further treatment and heated at 100 °C prior to the irradiation, or mixed with spermidine to reach final concentrations of 0.03 and 0.1 mM in the latter compound.

and 5-HmdUrd decreased from 4 to 1. However, the ratio between the yield of 5-FordUrd and 8-oxodGuo constantly increased. It should be mentioned that an increase in the yield of oxidized nucleosides with increased laser intensity was also observed under higher ionic strength conditions. Indeed, the yield of 8-oxodGuo determined within DNA samples containing 10 mM Tris was 7 times higher upon irradiation at 354 mJ/cm^2 than at 16 mJ/cm^2. A value of 8 was found for the combined yield of all oxidized nucleosides quantified.

Effect of UV Laser Irradiation Conditions on the Yield of Oxidative Base Damage. In a subsequent step, UV laser irradiations were performed at an intensity of either 16 or 347 mJ/cm^2 under different conditions. First, spermidine was added to a 1.5 OD DNA solution to increase the stability of the DNA duplex. In addition, irradiations were performed in water either at room temperature or immediately after warming the sample to 100 °C for 1 min to partially denature DNA. Denaturation of the sample by heating leads to an increase in the yield of t,s T<>T, while addition of spermidine induced an opposite effect (Figure 3). It must be emphasized that the combined yield of dimeric pyrimidine photoproduct was not modified by the addition of spermidine in the DNA solution. The relative yield of ThdGly, 5-HMdUrd, 5-FordUrd, and 8-oxodAdo with respect to that of 8-oxodGuo decreased when the DNA duplex was stabilized by addition of spermidine. (Figure 4). An opposite trend was observed upon heating the solution. The effect of the ionic strength was also investigated. For this purpose, DNA solutions containing increasing concentration of Tris were exposed to laser pulses at the highest intensity (347 mJ/cm^2). The quantum yield of formation of 8-oxodGuo increased upon addition of Tris while the yield of the other four oxidative lesions decreased until the Tris concentration was 1 mM (Figure 5). For the highest Tris concentrations (3 and 10 mM), the values slightly increased. However, the relative yield of ThdGly, 5-HMdUrd, 5-FordUrd, and 8-oxodAdo with respect to 8-oxodGuo decreased over the whole range of Tris concentrations. A similar trend was observed at lower laser intensity (data not shown), although the formation of ThdGly, 5-FordUrd, 5-HmdUrd, and 8-oxodAdo were minor processes. The presence of Tris in the DNA solution did not interfere with the formation of dimeric pyrimidine photoproducts. Indeed, the combined

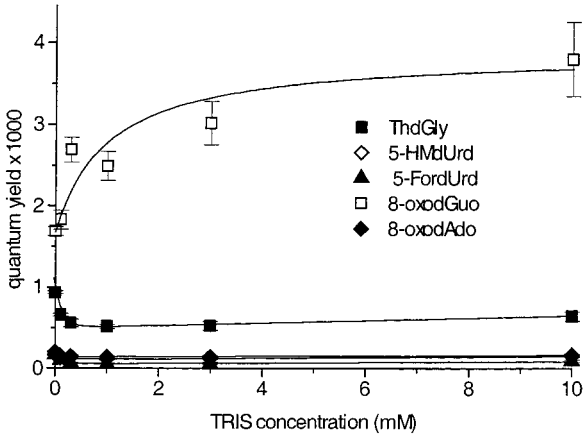

Figure 5. Effect of increasing the concentration of Tris on the yield of the formation of oxidized nucleosides within isolated DNA (75 μg/mL) exposed to UV pulses of 347 mJ/cm^2.

quantum yield for the formation of TT and TC photoproducts was found to be 1.11×10^{-3} and 1.17×10^{-3} within DNA sample exposed to 354 mJ/cm^2 laser pulses in the presence of 0 and 10 mM Tris, respectively.

Discussion

Stability of the DNA Duplex and Distribution of UVC-Induced Photoproducts. We have recently reported the individual quantification of the level of cyclobutane dimers, (6−4) adducts, and the related Dewar valence isomers for each of the four possible bipyrimidine sites within UVB- and UVC-irradiated DNA.[18] Concentrated solutions of DNA (1 mg/mL) were used in the latter works. As a control for the present study of UV laser-induced DNA damage, more diluted DNA samples were exposed to the UV light emitted by a 254 nm germicidal lamp. A major difference was observed in the relative yield of the photoproducts under the two different conditions. A first significant effect of the dilution of the sample was the decrease in the yield of formation of TC and CC photoproducts. Another striking result was the formation of the *trans*−*syn* thymine cyclobutane dimer in significant yield, while it was expected

to be produced in very low amount.[18a] A likely explanation is a lower stability of the DNA duplex in diluted with respect to concentrated solutions. Indeed, it is well established that an increase in ionic strength leads to the stabilization of the DNA duplex as shown by the increase in the melting temperature.[21] It is therefore likely that, through a dynamic process, DNA locally adopts a denatured conformation making possible the formation of t,s T<>T. A complete denaturation into single-stranded DNA is very unlikely since the melting temperature of genomic DNA of various sources is around 75 °C.[21] Increase in the ionic strength by addition of either NaCl or Tris led to the prevention of the formation of t,s T<>T together with the increase in the yield of TC and CC photoproducts. The relative yields of the photolesions obtained under these conditions were similar to those observed in concentrated DNA solutions. The latter observation suggests that dilution of the 1 mg/mL DNA solution with pure water induces a decrease in the ionic strength, leading to the destabilization of the duplex. As a conclusion, it can be proposed to use the formation of t,s T<>T as a marker of the stability of the DNA duplex. This will be an important piece of information in the following study on the laser-induced degradation of DNA. It can be added that the monophotonic oxidation of DNA bases upon exposure to low-intensity UVC radiation is a minor process that will be neglected in the following discussion.

Effect of the Laser Pulse Intensity on the Damage Distribution. Exposure of DNA to UV laser pulses induces monophotonic processes leading to the formation of pyrimidine photoproducts through the excitation of DNA bases. In addition, absorption of a second photon by an excited base provides enough energy to allow its ionization.[14] The probability for the occurrence of such a biphotonic process increases with increasing intensity of the UV laser pulse. This was unambiguously shown by the decrease in the yield of dimeric pyrimidine photoproducts arising from monophotonic excitations as the pulse intensity increased. Similar results have been reported for c,s T<>T and 6−4 TC upon exposure of DNA to 266 and 248 nm laser pulses, respectively.[14,22] However, only two intensities that differed by 4 orders of magnitude were used in the latter work. Comparisons between the DNA damage induced by continuous UVC light and 193 nm laser pulses have also been previously reported.[23] These results cannot be compared with the present data because 193 nm photons are able to ionize DNA bases through a monophotonic process. It should be emphasized that, in the present work, the yields of both cyclobutane dimers and (6−4) adducts were found to decrease to a similar extent at both TT and TC sites. This means that the lifetime of at least a part of the transient excited species giving rise to these photoproducts is similar or higher than the laser pulse duration (5 ns). The singlet excited state can be ruled out because its lifetime lies within the picosecond range in DNA.[14,24] These results could also be accounted for by the involvement of the triplet excited state. Indeed, cyclobutane dimers may be produced from the triplet excited state, as shown by photosensitized energy-transfer experiments.[25] However, (6−4) photoproducts could not be detected under the latter conditions. This was confirmed by the highly sensitive HPLC−MS/MS assay (Douki and Cadet, unpublished results). Therefore, triplet excited state can be ruled out as the intermediate in the formation of (6−4) adducts. An alternative explanation could be that the photoproducts (or at least a significant fraction) are formed through singlet excited state complexes (exciplexes) exhibiting nanosecond lifetime. The possible occurrence of such transient species has already been discussed.[26] There is thus an obvious need for a deeper understanding of the photoexcitation processes of nucleobases within DNA.

Evidence for the Occurrence of Charge Transfer within DNA. In contrast to the formation of monophotonic dimeric pyrimidine photoproducts, the overall yield of oxidized bases increased with increasing intensity of the laser pulse, as already observed for the level of Fpg-sensitive sites in oligonucleotides and 8-oxodGuo within isolated DNA.[16] This is expected because an increase in the intensity leads to a higher probability for a base in its triplet excited state to absorb a second photon and to become ionized. Strikingly, the distribution of oxidation products was found to be dependent on the intensity of the laser pulse. Indeed, at the lowest intensity, 8-oxodGuo was produced in a yield at least 1 order of magnitude higher than those of ThdGly, 5-HMdUrd, 5-FordUrd, and 8-oxodAdo. However, as the intensity of the laser pulse increased, the relative yield of the latter lesions with respect to that of 8-oxodGuo increased. This shows that the biphotonic ionization of guanine reaches a saturation level (equilibrium between the formation of molecules in their triplet excited state and their excitation) for intensity lower than the other DNA bases. The maximum quantum yield of biphotonic ionization is first limited by the extent of the triplet-state population. However, the intersystem-crossing yield is lower for guanine than for the other DNA bases.[14] In addition, thymine and not guanine would be expected, on the basis of the energy level of the triplet excited states, to be the main target of triplet energy-transfer processes. Altogether, the present observations cannot be accounted for by a larger population of guanine triplet excited state. Therefore, we propose that the observed intensity dependence of the formation quantum yield of oxidative base damage is a manifestation of hole migration within DNA and trapping at guanine moieties. It can be added that for a defined UV laser intensity the ratio between the yield of guanine modifications on one hand and thymine, cytosine, and adenine damage on the other hand depends on the distance of the positive hole migration and the efficiency of trapping by guanine bases. Indeed, a decrease in the rate of the latter process leads to an increase in the lifetime of thymine, adenine, and cytosine radical cation. As a result, the level of products arising from the latter transient species increases. Therefore, the ratio between the yields of 8-oxodGuo and those of damage at other bases is a marker of the charge-transfer efficiency. However, it should be kept in mind that other guanine degradation products are produced, such as the formamidopyrimidine derivative and oxidation products arising from the deprotonation of the guanine radical cation.[27]

Stabilization of the DNA Duplex and Charge Transfer. The stability of the DNA duplex can be estimated by the yield of t,s T<>T and the efficiency of charge transfer inferred from the relative yield of 8-oxodGuo with respect to that of other

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oxidized bases. Both aspects were studied within samples of DNA exposed to either low- or high-intensity laser pulses under various experimental conditions. The first involved the stabilization of the DNA duplex by the polyamine spermidine. This class of small polycationic molecules is known to tightly bind DNA, leading to its compaction.[28] In addition, it has been shown that polyamines do not modify the chemistry of DNA base radicals even though they very efficiently protect DNA against the attack of hydroxyl radicals by decreasing the accessibility of the reactive sites.[29] To further assess the effect of the destabilization of the DNA duplex, laser irradiations were also performed on heated DNA samples which were thus expected to be partially denatured. As anticipated, the quantum yield of formation of t,s T<>T was higher in the denatured samples, while it decreased with increasing spermidine concentration. The relative yield of 8-oxodGuo with respect to that of the other measured oxidized bases behaved in an opposite way. The former lesion was produced in a much higher amount than ThdGly, 5-HMdUrd, 5-FordUrd, and 8-oxodAdo when the duplex was stabilized (presence of spermidine) than within denatured DNA (heated samples). These results were confirmed by a last series of experiments involving exposure to laser pulses in the presence of an increasing amount of Tris. This aimed at increasing the ionic strength of the solution and thus stabilizing the DNA duplex. As expected, addition of Tris led to an increase in the relative yield of 8-oxodGuo within DNA exposed to high-intensity laser pulses. Altogether, the results reported above strongly suggest that the stabilization of the duplex is associated with an increase in the charge-transfer efficiency. The effect of stability on the charge transfer within DNA has already been predicted by Schuster and co-workers in the phonon-assisted polaron-like mechanism.[3e,5e,12] These authors rationalize the transfer of a positive charge within DNA as a series of hoppings. Between each hopping step, the positive charge spreads over several bases, inducing a local distortion of the DNA. This type of process is likely to be dependent on the flexibility of the duplex. Indeed, theoretical calculations have shown that the stability of a polaron within DNA increases with increasing stability of the base stacking.[30] It can thus be expected that the efficiency of the charge migration is favored when the duplex is less flexible. It should be mentioned that an opposite conclusion was made from theoretical calculations based on a charge-transfer mechanism involving electron coupling.[31] It might thus be concluded that the latter process is only responsible for a minor fraction of charge transfer.

Conclusions

Several groups have used a series of physical and chemical tools in order to investigate charge-transfer processes within DNA. In the present work, precise quantification of the distribution of UV laser-induced base damage led to the assumption that the efficiency of charge transfer is enhanced by an increased stability of the DNA duplex. This represents a new piece of information in this widely studied field. The strong ionic strength effect reported points out that differences in experimental conditions should be considered when comparing different systems. In addition, the presently reported results may have consequences in terms of sequence effect. Indeed, the DNA structure may locally vary and exhibit a lower stability and different reactivity, as previously reported.[9] It might be of interest to extend the global approach developed in this work on DNA to specific investigations at the sequence level on defined oligonucleotides. Such an approach would likely allow a precise quantification of the qualitative observations made in the present work. However, such studies require the development of specific and sensitive tools for the quantification of defined lesions at the nucleotide resolution. The array of available assays including piperidine treatment and *N*-glycosylase digestion should thus be extended.

Experimental Section

Chemicals. Calf thymus DNA, Tris, spermidine, nuclease P1, phosphodiesterase I, phosphodiesterase II, and sodium chloride were obtained from Sigma (St. Louis, MO). Alkaline phosphatase was purchased from Roche Molecular Biochemicals (Mannheim, Germany). Oxidized nucleosides and their related isotopically labeled derivatives were synthesized as previously reported.[32] Bipyrimidine photoproducts were prepared from synthetic dinucleoside monophosphates. The cyclobutane dimers were obtained by benzophenone photosensitization, while the corresponding (6−4) photoproducts were prepared by UVC photolysis. The Dewar valence isomers were obtained by UVB photolysis of the purified (6−4) adducts. All products were purified by reverse phase HPLC.

Irradiations. All irradiations were performed in a 3 mL quartz cell (optical path 1 cm, width 1 cm). The sample was under constant magnetic stirring during the experiment. UVC irradiations of 2 mL of DNA solution were performed with a germicidal lamp emitting at 254 nm at a dose rate of 50.4 $mJ \cdot cm^{-2} \cdot min^{-1}$. Periods of exposure ranged between 2 and 10 min. Laser pulses were provided by the fourth harmonic of a Nd:YAG laser (Surelite II, Continum, U.S.A.). The pulses lasted 5 ns at a frequency of 1 Hz. The energy was measured by a pyroelectric detector using the 8% reflection of a quartz beam splitter. The intensity variation was achieved by detuning the fourth harmony generator crystal. The irradiation procedure was automated by a microcomputer. Either 1 or 2 mL of solution were irradiated. Total irradiation energies ranging between either 50 and 200 mJ or 100 and 400 mJ were applied, respectively. Following irradiation, DNA was precipitated by addition of NaCl (final concentration 0.1 M) and 2.5 vol of cold ethanol. DNA was then solubilized at a final concentration of 1 mg/mL. The DNA sample was subsequently hydrolyzed by sequential incubation with a mixture of phosphodiesterase II and nuclease P1 at pH 5.5 and a mixture of phosphodiesterase I and alkaline phosphatase at pH 8.[18a,32b] The digested solution was neutralized by addition of 0.1 M HCl, and proteins were precipitated by addition of chloroform. The aqueous phase was collected and transferred into HPLC injection vials. Isotopically labeled internal standards (50 pmol of each) were added prior to the quantification of oxidized nucleosides.

HPLC−MS/MS Analyses. Oxidized nucleosides and pyrimidine dimeric photoproducts were quantified by HPLC coupled to an API 3000 tandem mass spectrometer (Perkin-Elmer/SCIEX, Toronto, Canada) used in the multiple reaction monitoring mode as previously described.[18,32b] Briefly, the detection of 8-oxodGuo, 5-HMdUrd, 5-FordUrd, ThdGly, and 8-oxodAdo involved a separation on an ODB Uptisphere (2 × 150 mm, particle size 5 μm) octadecylsilyl silica gel column. A gradient of acetonitrile (maximum proportion: 10%) in 2 mM ammonium formate was used. Each nucleoside was quantified under optimized conditions, together with its isotopically labeled derivative used as the internal standard. Pyrimidine photoproducts were

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analyzed as modified dinucleoside monophosphates. They were injected onto an ODB Uptisphere (2 × 150 mm, particle size 3 μm) reverse phase column. A gradient of acetonitrile in 2 mM triethylammonium acetate was used. The amount of detected material was inferred from an external calibration. Either positive (8-oxodGuo, 8-oxodAdo) or negative ionization (all other lesions) was used for the analyses.

Calculation of the Quantum Yields. For each experiment, three different irradiation doses were independently applied to DNA samples. The level of dimeric pyrimidine photoproducts and oxidized bases was determined by HPLC−MS/MS. These values, together with those determined in the nonirradiated sample, were used to calculate the quantum yield of formation by linear regression. The error on the quantum yield was inferred from the standard deviation of the slope. Because both dimeric and monomeric lesions were quantified, quantum yields were expressed with respect to the overall DNA bases. For pyrimidine photoproducts, this contrasts with previous convention that only took into account the number of bipyrimidic sites within DNA. To convert our values into this system, the presently reported quantum yields have to be divided by the product of the abundance of the two pyrimidine bases of interest. For instance, the abundance of thymine is 27% in calf thymus DNA. Therefore, the quantum yields for the formation of the TT photoproducts have to be divided by 0.0729 (0.27 × 0.27) to be expressed as in the previous convention. Similarly, the quantum yield of formation of the oxidative lesions from the initial base could be obtained by dividing the reported values by either 0.23 (cytosine and guanine) or 0.27 (thymine and adenine).

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