Induction of Strand Breaks in Single-Stranded Polyribonucleotides and DNA by Photoionization: One Electron Oxidized Nucleobase Radicals as Precursors

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Abstract: As a model for the *direct effect* of ionizing radiation, time-resolved changes of light-scattering intensity (LSI) of aqueous, oxic solutions of single-stranded DNA samples (calf thymus, *Micrococcus lysodeikticus*, *Clostridium perfingens*), polyC, polyU, and polyA at pH 7.5−8 have been studied following pulse irradiation with 193-nm laser light. A comparison of LSI changes (an index of single-strand break formation) with transient optical absorption changes (an index of nucleic acid base radical chemistry) following pulsed 193-nm irradiation shows that strand breakage occurs at rates similar to those for the decay of the nucleic acid base radical species for DNA, polyC, and polyU but not for polyA. With the exception of polyA, 193-nm light leads to a reduction in the LSI over ∼0.2 s, indicating that strand breakage occurs by a nucleobase radical-mediated process, whereby the nucleobase radical site is transferred to the sugar moiety. With polyU and polyC, significant changes in LSI also occur on a much faster time scale, (within 3 ms). In DNA, the one electron oxidized sites become localized predominantly at guanine and the resulting one electron oxidised guanine radicals lead to strand breakage in competition with major, non-strand-breakage pathways.

Introduction

Exposure to ionizing radiation causes biologically, deliterious effects, such as cellular inactivation, transformation, and mutagenesis.[1] After exposure, numerous chemical modifications within the DNA helix are formed; of these important events, strand breakage is one such lesion. The primary events leading to DNA damage occur from direct ionization of DNA or as a result of attack on DNA by radicals formed from ionization of the immediate environment.[2,3] These radicals are mainly derived from water, the most damaging being the hydroxyl radical.[4−6] The contribution of direct ionization[6] to DNA damage is estimated to be ≥35%; this contribution is dependent on the ionizing density of the radiation (e.g., γ compared with α radiation).

Study of the direct ionization processes within DNA in dilute aqueous solutions, using conventional ionization sources, is hampered because of the high yields of water radicals formed. Irradiation of DNA at low temperatures in frozen, aqueous solutions has indicated that direct ionization produces two types of radicals, electron gain and electron loss centers.[7−10] Migration of the oxidized "radical species" occurs with localization at the more easily oxidized nucleobase, guanine, so that damage at this site within DNA would be predicted.[8]

One approach to study the properties of the radical species produced by ionization of homopolynucleotides or DNA in aqueous solution at room temperature has utilized the known one-electron oxidant $SO_4^{\bullet-}$.[11,12] Strand breakage is induced within 100 μs on reaction of $SO_4^{\bullet-}$ with these polynucleotides and DNA. With purines, this reaction proceeds via one-electron oxidation[13,14] whereas with pyrimidines, $SO_4^{\bullet-}$ adducts are formed.[11] From these differences in reaction pathway, the approach of using $SO_4^{\bullet-}$ to mimic the *direct effects* of ionizing radiation in DNA may not be informative.

An alternative approach is to utilize 193-nm laser light, since photoionization of DNA and its constituents in aqueous solutions occurs by a monophotonic process.[3,15−18] Photoionization of DNA or polynucleotide results predominantly in oxidation of the nucleic acid base moieties.[15,17−19] Ionization at each of the nucleic acid base moieties within a DNA strand is assumed to occur with an efficiency that is dependent on their extinction coefficients at the wavelength used and the quantum yield of photoionization. The quantum yield of photoionization of polynucleotides and DNA is in the range of 0.03−0.11.[15,17,18] A large proportion of the initial ionization of DNA by 193-nm light is predicted to occur at the guanine residue, which has the lowest ionization potential.[20] Further, migration of the oxidative damage to the guanine residue was suggested to occur.[17−19] Our

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recent studies using various single-stranded oligonucleotides of differing nucleic acid base composition indicate that migration of oxidative damage does take place between neighboring bases.[19]

Irradiation of single- or double-stranded DNA with 193-nm light results in strand breakage by a monophotonic process in extremely low yields (<2% of the total photoejected electron yield).[15,18,21,22] It was suggested that the minor yield of single-strand break (ssb) caused by the direct ionization of DNA using 193-nm light is due to photoionization products of the phosphate only and that ionization of the base moieties only yields base modifications. Our recent studies indicate that the major sites for 193-nm light-induced ssb in double-stranded DNA are guanine.[18] Since cleavage of labile, damaged sites within the DNA could occur during the detection by electrophoresis, it was not possible to ascertain whether the strand breakage at the guanine moiety occurs by a frank process originating from a guanine radical. Recently, it was inferred[23] that one electron oxidized guanine products, produced from biphotonic photoionization of oligonucleotides with 248-nm light, are not precursors of frank strand breaks.

Detection of changes in the intensity of Rayleigh light scattering (RLS), which reflects changes in the molecular weight of the biopolymer, enables direct measurement of the time course of strand-breakage processes.[24,25] This technique has previously been used to show that ssb induced in DNA by hydroxyl radicals results from transfer of the radical site from the nucleic base moiety to the sugar backbone by a hydrogen abstraction to yield frank strand breakage.[24−27] Together with transient optical absorption spectroscopy, RLS has been used to evaluate the time course of ssb induction by 193-nm light. The question arises as to whether strand breakage of DNA caused by exposure to 193-nm light occurs as a result of a prompt radical process and/or involves a "labile" diamagnetic product of guanine. Further, since there is little information known about the DNA radicals ultimately responsible for strand breakage caused by the direct effect of ionizing radiation, the following study was undertaken to assess the mechanism of ssb following the exposure of single-stranded polyribonucleotides polyA, -C, and -U and three DNA samples from calf thymus, *micrococcus lysodeikticus*, and *Clostridium perfringens* to a 20-ns pulse of 193-nm light.

Experimental Section

The polydeoxynucleotides, polycytidylic acid (polyC), polyadenylic acid (polyA), and polyuridylic acid (polyU), were purchased from Pharmacia Biotech and used without further purification. DNA samples of *M lysodeikticus* (ML), *C perfingens* (CP), and calf thymus (CT) were purchased from Sigma (guanine/cytosine mole fraction percentage: CT = 42%, ML = 72%, CP = 31%). All solutions were prepared with water purified from a Milli-Q system (Millipore). The samples were dissolved overnight at 277 K in an aqueous solution containing 10^{-2} mol dm^{-3} sodium perchlorate and then adjusted by dilution to give a concentration such that the optical density at 193 nm was unity. All solutions of the polymers contained sodium perchlorate, since it maximizes the light scattering from the polynucleotides and DNA samples, while being transparent at the excitation wavelength. Where necessary, the samples were made single-stranded by heating (363 K) in a water bath and quickly cooled over ice. The pH was adjusted if required with NaOH or $HClO_4$. Prior to photolysis, the solutions were saturated with oxygen (British Oxygen Co., BOC) for 30 min or aerated.

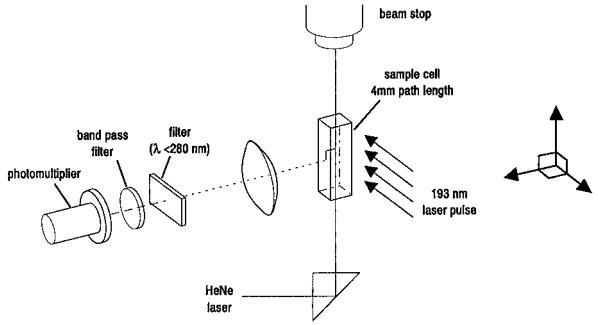

Figure 1. Schematic diagram of the setup for determination of changes in RLS produced by 193-nm photolysis.

The 193-nm laser flash photolysis and the detection system used for transient absorption spectroscopy has been described previously.[18] To determine the time limit for observation of the transient change in light scattering intensity (LSI) and optical absorption, where diffusion resulting in dilution of the photoionized products might play a role in the decay, a tetranitromethane solution ($OD_{193} = 1$) was irradiated and the absorbance at 350 nm probed since the resulting nitroform anion is stable. This absorbance was stable up to 500 ms, after which time a gradual decrease in absorbance was detected. This decay is not due to a chemical decay of the nitroform anion but reflects a concentration decrease through diffusion out of the window of observation within the cell.

The instrumental equipment used for the RLS experiments is represented in Figure 1 and is based on that previously described.[24,25] Light from a helium−neon (HeNe) laser (Spectra Physic; λ 632.8 nm, $E_{max} = 10$ mW) was aligned through the base (*y*-direction) of a five-windowed quartz Suprasil cuvette (1 × 0.4 × 3 cm) parallel to the walls. A pulse of 193-nm light (Questek series ArF excimer laser, 20 ns FHW) was used to irradiate as much of one face (*x*-axis) of the cuvette (path length 0.4 cm) as possible (beam height ∼2.5 cm × width ∼2 cm). Scattered 632-nm light from the central portion of the sample was collected by a lens (5-cm focal length, 10-cm diameter) set perpendicular to both the laser light sources. The light was then imaged onto the face of the photomultiplier (EMI, RF/QL-30F, type 59698QB) with a second lens (30-cm focal length, 10-cm diameter). Prior to the photomultiplier window, the scattered light was passed first through a cutoff filter (WG280) and then a band-pass filter (632 nm) to attenuate 193-nm light. LSI changes were detected by the photomultiplier. The signals from the photomultiplier were fed into a transient digitiser (Tektronix 2432A), which was interfaced to a PC486. In-house software was used to process the data. Processes occurring within 4 ms could not be distinguished from within the rise time of the system. Single-stranded polymer solutions were introduced into the cuvette by filtering through Millipore filters (0.45 μm). The air-saturated sample was irradiated with a single pulse of 193-nm UV laser light at an energy of ∼70 mJ pulse^{-1} as measured with a joulometer (Genetek). Under aerobic or oxic conditions, the photoejected electron becomes hydrated and is scavenged by oxygen, yielding superoxide anion. Any electron adducts of the nucleic acid bases within the polymer formed upon interaction with hydrated electrons will be oxidized, in the presence of oxygen, to their original base. The scattering yields cannot be compared between sample types, since all of the polymers have different structured forms and molecular weights and different extinction coefficients at 193 nm.

Results

PolyC. The transient optical absorption spectra at 8 and 150 ms after photolysis, with 193 nm, of an oxygen-saturated solution of single-stranded polyC (pH 8) are displayed in Figure 2. The change in optical absorption at 300 nm with time is shown in Figure 3a. The initial component of the optical

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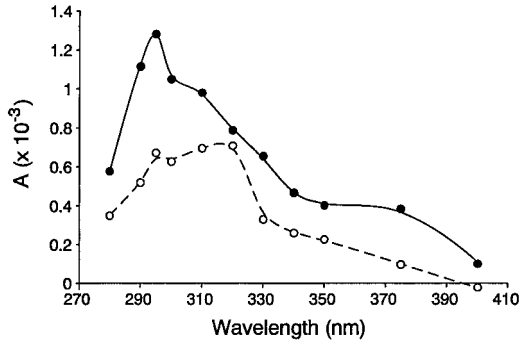


Figure 2. Transient optical absorption spectra determined 8 (●) and 150 ms (○) after 193-nm photolysis of an oxygenated, aqueous solution of polyC at pH 8.

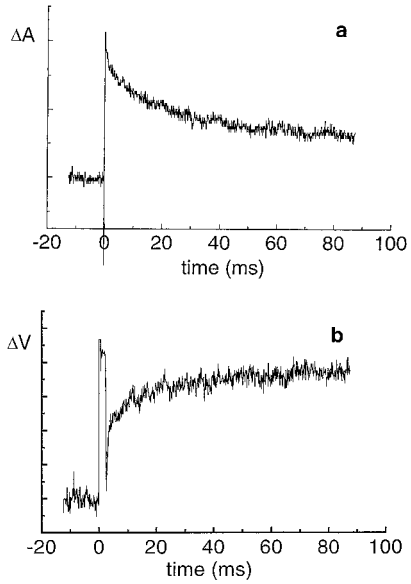


Figure 3. Time dependence for changes in LSI and transient optical absorption at 300 nm (a) and LSI (b) on 193-nm photolysis of an oxygenated, aqueous solution of polyC at pH 8.0.

absorption decays with a half-life of ~7 ms to yield a species, the optical absorption of which is shown in Figure 2. This latter species decays more slowly (measured up to 500 ms). The decrease of LSI with time upon 193-nm irradiation of polyC, under conditions identical to those for absorption changes, is shown in Figure 3b. These changes represent at least two pathways to ssb. The proportion of strand breakage that occurs via the "prompt" process is 50% and it occurs within 4 ms. There is no significant change in optical absorption at early times corresponding with this prompt formation of strand breakage. The slow component of the strand breakage, as detected by RLS, occurs at a rate similar to that for the decay of the optical absorption at 300 nm, as shown in Table 1.

PolyU. The time-dependent changes in LSI obtained on irradiation of an oxygenated, aqueous solution of polyU (pH 7.9) with 193-nm light is displayed in Figure 4a. Strand breakage occurs by at least two distinct processes from the observation of more than one kinetic component. For simplicity, these changes have been separated into two components, a prompt component (60%) that occurs within 4 ms and a slow component (40%) with a half-life of 40 ms. The change in optical absorption for polyU at 290 nm under identical conditions for LSI changes is displayed in Figure 4b. The half-life for this optical absorption change is 45 ms, which, within error, is the same as that for the slow component of the LSI change. In contrast to the observations with polyC, there is no significant

Table 1. Contribution of the Fast Component and the Half-Life of the Slower Component of LSI Change following 193-nm Photoionization of Various ss- Polynucleotides and DNA Samples with Different Base Compositions

		$\tau_{1/2}$ (ms)		
polynucleotide	fast component (%)	TRLS	optical	ϕ_{ssb}/ϕ_e[a]
PolyC	<50	6−7	7.0	0.32
PolyU	60	40	45	
PolyA	>90			0.015
DNA ML (72% G:C)	<10	40		
DNA CT (43% G:C)	<10	46	40	0.011
DNA CP (31% CP)	<10	40		

[a] ϕ_{ssb}, from ref 15.

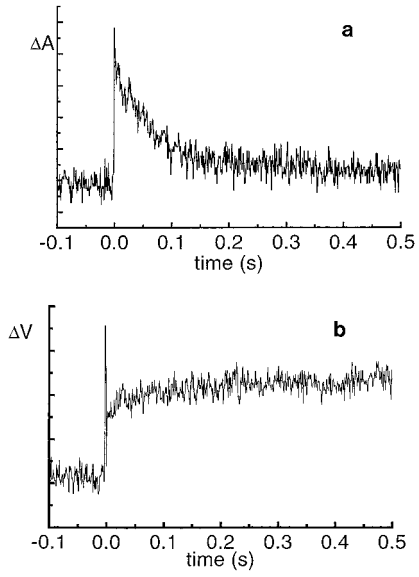


Figure 4. Time-dependent change in transient optical absorption at pH 7.9 (a) and in LSI (b) on 193-nm photolysis of an oxygenated, aqueous solution of polyU at pH 7.9.

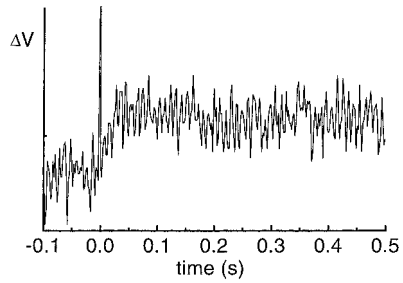


Figure 5. Time-dependent changes of LSI on 193-nm photolysis of an oxygenated, aqueous solution of polyA at pH 8.

residual absorption in the wavelength region 300−400 nm following the decay of the optically absorbing species.

PolyA. Figure 5 displays the time dependence for LSI changes detected upon 193-nm photolysis of an oxygenated polyA solution (pH 8). Strand breakage occurs mainly by a prompt process (>90%). A polyA solution similar to that used for the Rayleigh light scattering experiments was irradiated with 193-nm light. The optical absorption spectrum of the resulting transient species is similar to that obtained previously from 193-nm photoionization[17] and from reaction of the oxidizing sulfate radical anion with polyA[13,14] and is assigned to the deprotonated radical cation of adenine base. At 330 nm, the absorption maximum for the adenine radical, the optical absorption was found to decay slowly. There is no change in optical absorption corresponding with that in RLS, indicating that strand breakage does not involve the major adenine radical species within polyA.

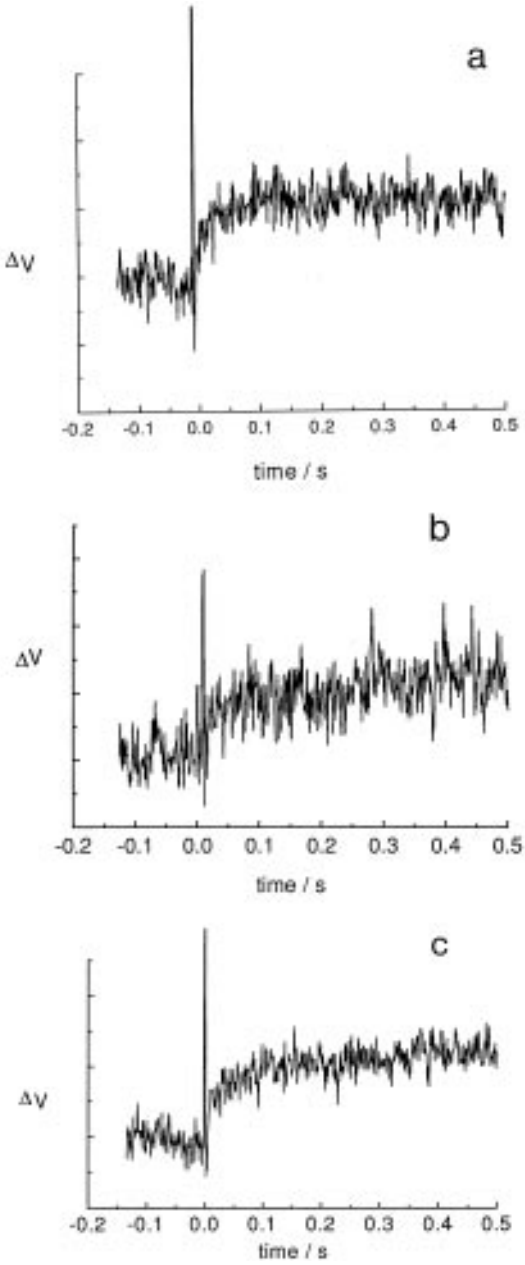


Figure 6. Time-dependent changes in LSI on 193-nm photolysis at pH (7.6−8) of an oxygenated, aqueous solution containing CP (a), CT (b), and ML (c) DNA.

DNA. Three different DNA samples (CP, CT, ML) were chosen to assess whether there is an influence of base composition on ssb induction within 0.5 s. The single-stranded DNA samples (pH 7.6−8) in oxygenated aqueous solution were irradiated with a short pulse of 193-nm laser light, and the resulting LSI changes with time are displayed in Figure 6. There is no significant difference in the time response of overall LSI changes for the three different DNA samples. From the time dependence of these LSI changes, strand breakage occurs by at least two processes: a prompt process and a slower process. The proportion of strand breaks that occurs by the prompt process is <10% and occurs within 8 ms. The half-lives for the slow component of strand breakage of the DNA samples are determined to be between 40 and 50 ms.

The transient optical absorption spectra at 10 μs for the DNA samples (CT, ML, CP) on 193-nm photolysis under oxic conditions are similar to those reported previously.[17,18] The loss of optical absorption at 320 nm following photoionization of the DNA samples occurs with a half-life of 40 ms, similar to

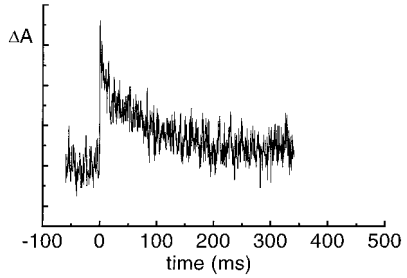
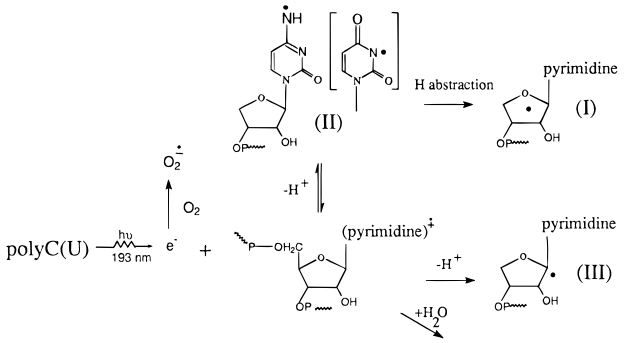


Figure 7. Time-dependent changes in the transient optical absorption at 320 nm on 193-nm photolysis of oxygenated, aqueous solutions containing DNA.

Scheme 1



that observed for the slower LSI changes. The optical absorption decays to yield a species that absorbs in the wavelength region 300−400 nm.

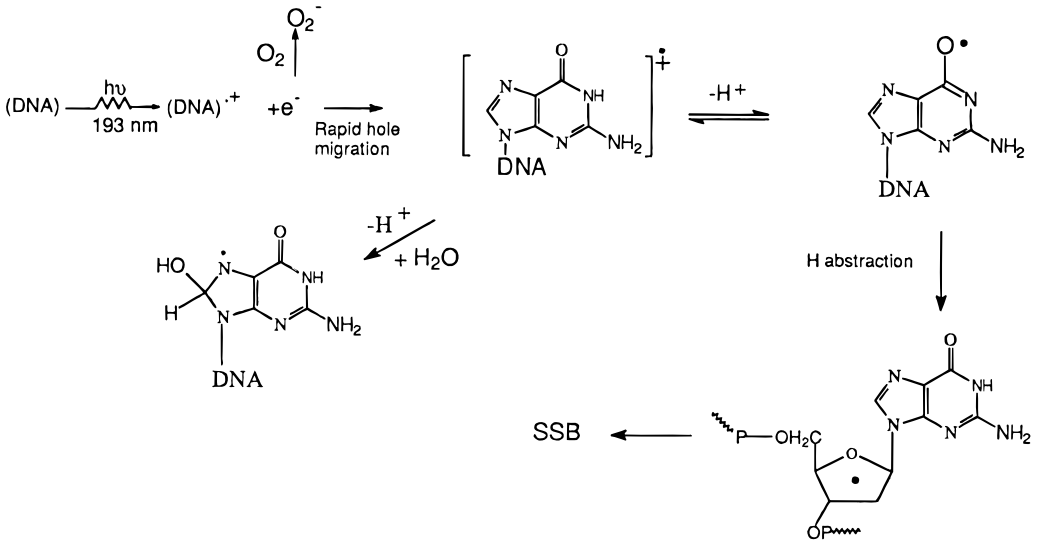
All observations reported above for the polynucleotides and DNA samples were found to be independent of the laser power (up to 70 mJ pulse $^{-1}$) and the concentration of oxygen (0.28−1.38 mmol dm^{-3}). The percentage of strand breakage that occurs by the prompt process and the first half-life for the change of LSI and decay of optical absorption for all the biopolymers are given in Table 1. The efficiency of strand breakage per photoelectron previously determined is also included in Table 1.

Discussion

The 193-nm photolysis of polyC, polyU, and the three different ssDNA samples induces, within 0.5 s, ssb by at least two processes: a prompt and a slow component. With polyA only prompt strand breakage occurs. Since photoionization of polyU, polyA, and ssDNA has previously been shown to produce their corresponding one electron oxidized species,[17,18] the initial species produced on photoionization of polyC is therefore assigned to its one electron oxidized base radical. From the similarity of the time scale of the decay of these one electron oxidized base radicals and of the slower component of ssb formation, the one-electron oxidized nucleobase radicals are identified as precursors to the slower process of strand breakage. Interactions involving two radicals leading to strand breakage may also be ruled out since the rate of decay of the precursors to strand breakage is independent of the laser power and therefore their concentrations. For polyA, however, there is no indication that a nucleic acid base radical acts as a precursor for strand breakage within the measurement timescale of 0−0.5 s.

In discussing the mechanistic pathways of this slow component of strand breakage for polyU and polyC, it is assumed that their one electron oxidized base radicals leads to strand breakage under oxic conditions as shown in Scheme 1. The pyrimidine radical cation initially produced may deprotonate to give the heteroatom-centered radicals **II** or the C(1)′ radical **III**, hydrate,

Scheme 2



or transfer the radical site to the sugar by H-atom transfer yielding radical **I**. Depending on the site of H-atom abstraction, the resulting sugar radicals lead to strand breakage via a non-rate-determining, heterolytic elimination of the phosphate ester bond. Of the sugar radicals **I** produced, the resulting C(4)′ radical, in the presence of oxygen, is reported to be the major sugar radical which leads to immediate strand cleavage.[28−31] Recently, it was suggested that the initial strand cleavage, involving the C(4)′ radical as the precursor, occurs prior to its interaction with oxygen.[29] Formation of C(1)′ sugar radicals does not result in strand cleavage but an alkali-labile site.[28−31] Recent studies have presented evidence for formation of the C(1)′ radical (**III**) from the radical cation of cytosine.[32] One of the mechanisms of base release is initiated by deprotonation of the C1′ hydrogen from the sugar moiety (radical **III**).[28,33,34] From the percentage yield of strand break per photoelectron via the slow component (Table 1), the occurrence of competitive non-strand-breaking reactions of the radical cation is significant. It is inferred from these results that ssb from 193-nm photoionization of polyC and polyU or DNA does not occur via processes common to either OH radical-[3,26,27] or $SO_4^{\bullet-}$ [11]-initiated strand breakage. Hydration of the radical cations of polyU and polyC are proposed to yield predominantly the 6-hydroxy-5-yl radical and not their corresponding 5-hydroxy-6yl radicals, the main precursor to strand breakage by OH radicals.[3] On photooxidation of 2′-deoxycytidine and thymidine, hydration is shown to occur predominantly at C(6) of the radical cation.[35,36]

The question arises with polyC as to the identity of the species that is present following strand breakage and absorbs at $\lambda >$ 300 nm, as shown in Figure 2. Addition of oxygen to radical **III** may occur, but in a non-rate-determining step, to yield peroxyl radicals that absorb only weakly at $\lambda >$ 300 nm. It is therefore suggested that the radical species remaining following strand breakage is radical **II**, since it is assumed that a heteroatom-centered radical is relatively stable in the presence of oxygen.

The slow component to strand breakage in DNA is more difficult to resolve in terms of the one electron oxidized base(s) which acts as the precursor(s). However, our recent results from sequence gel electrophoresis of small fragments induced by 193-nm radiation of double-stranded DNA show that the majority of frank strand breakage occur 3′ to the guanine molecule.[18] In fact, migration of the electron hole from neighboring bases to guanine has recently been demonstrated in a series of model oligonucleotides.[12,19] Furthermore, the one electron oxidized guanine radical has recently been identified[17,18] as the major species present at early times of 193-nm photoionization of these three different DNA samples. Although the yield of ssb in ds DNA is low,[15] the majority of the transient optical absorption of the DNA radicals decays by a process(es) that occurs on the same timescale as strand breakage. Since strand breakage for all DNA samples (CP, CT, ML) follows similar kinetics (0−0.5 s), it is concluded that strand breakage is not affected significantly, within experimental error, by the neighboring base to the guanine radical.

The proposed pathway to strand breakage in DNA is shown in Scheme 2. The guanine radical cation formed by 193-nm photolysis of DNA or by migration of the hole to guanine is in equilibrium with its deprotonated form (pK_a 3.9),[37] which does not react rapidly with oxygen. The radical cation of guanine may hydrate in competition with its deprotonation to yield its deprotonated form, which undergoes H-atom abstraction from the sugar moiety. If H-atom abstraction occurs at C(4)′ of the sugar moiety, the sugar radical yields a strand break by non-rate-determining, heterolytic elimination of the phosphate ester bond.[28−31]

Kasai *et al.*[38] showed that the radical cation of guanine within a DNA matrix undergoes a hydration reaction to yield 8-hydroxyguanine. In view of the large yields of base damage excised by the Fpg protein from a DNA sample photolysis with 193-nm light,[39] some products are expected to be hydration products of the radical cation including 8-hydroxyguanine. In

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the monomeric guanine nucleotide, there is little or no evidence for interaction of the guanine radical with water.[40] This may reflect the importance of bimolecular processes for the monomeric species and/or the equilibrium of the proton between the strands in ds DNA so that the guanine radical is "partially" protonated at pH 7.[41,42] Biomolecular processes are less efficient for radicals formed within a polymer matrix where the lifetimes of radicals will be extended, enabling different chemical pathways to become available for base radicals in the biopolymer.

For the prompt component of strand breakage, observed to varying degrees for the polynucleotides and DNA, a corresponding decay of the radicals that absorb at >300 nm was not observed. There are several possible precursors to this prompt strand breakage, namely, strand breakage initiated by a weakly absorbing radical or from one electron oxidized phosphate radicals. Weakly absorbing radicals resulting from ionization could include C(1)′ sugar radicals resulting from deprotonation of the base radical cation. Sugar radicals at C(4)′ and C(2)′ are not expected to be produced directly by 193-nm light but via a base radical transfer as discussed above. Model systems indicate though that C(1)′ does not yield immediate ssb.[28,31] It was previously suggested[43] that strand breakage involves an oxidized phosphate moiety (Table 1). It is therefore proposed that prompt strand breakage mainly arises from a one electron oxidized phosphate radical as precursor, since phosphate could contribute a relatively large component to the photoejected electron yield, especially with polyU and polyC (see Table 2). These predicted contributions are based on the assumption that the quantum yields of photoionization and the extinction coefficients of the polymers are similar to those of the individual components, e.g., the base and phosphate. Once a one electron oxidized phosphate is formed, competitive pathways include (i) transfer of the electron hole to a more easily oxidized base moiety depending on the ease of oxidation of the base, (ii) transfer of phosphate radical site via a hydrogen atom abstraction to the sugar moiety, or (iii) direct photocleavage of the phosphate ester bond. Both (ii) and (iii) would lead to strand breakage that is not mediated by an interaction of the nucleic acid base moiety. At present, a competition is proposed between pathway ii leading to ssb in competition with pathway i leading to base oxidation, which depends upon the oxidation potential of the neighboring nucleobase. It is suggested that the importance of pathway ii on the oxidation potential of the base is reflected from comparison of the relative Φ_{ssb}^{prompt} for prompt ssb with polyC and polyA, shown in Table 2.

Table 2 Estimated Fraction (×100) of the Number of Photoelectrons Arising from Phosphate (Φ_e^{phos}) and the Yield of Prompt ssb (Φ_{ssb}^{prompt}) with Respect to the Overall Yield of Electrons (Φ_e^{total})

sample	$\Phi_e^{phos}/\Phi_e^{total}$	Φ_e^{prompt} [a]$/\Phi_e^{total}$
polyA	13	1.5
polyC	28	19
polyU (assuming monophotonic)	45	nd[b]
DNA (CP) (31% GC)	6	<0.1
DNA (CT) (43% GC)	5.5	<0.1
DNA (ML) (75% GC)	4	<0.1

[a] Φ_{ssb}^{prompt}, from percentage fast component in Table 1 and ϕ_{ssb}.[15] [b] nd, not determined.

Conclusions

Photoionization of DNA and polynucleotides leads to strand breakage by two processes within the observation time of 0.5 s. One electron oxidized nucleic acid base radicals formed by 193-nm photoionization of DNA and polynucleotides are precursors to the slower process leading to strand breakage. In DNA, the deprotonated radical cation of guanine is the precursor to the slower process leading to strand breakage. This strand breakage is in competition with major, nonstrand-breaking processes. The faster process to strand breakage is suggested to involve ionization of the phosphate backbone whereby the efficiency of strand breakage depends on the absorption cross section of the individual components and the oxidation potential of the nucleobases. One might predict therefore that the *direct effects* of ionizing radiation (e.g., X-rays or ^{60}Co γ-rays) leading to frank strand breakage in double-stranded DNA involve predominantly ionization of the sugar phosphate backbone. "Frank" single-strand breakage arising from the one electron oxidized guanine radical through transfer of the radical site to the sugar moiety is only a minor contribution.

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