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One- and Two-Photon Ionization of DNA Single and Double Helices Studied by Laser Flash Photolysis at 266 nm

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The ionization of the DNA single and double helices $(dA)_{20}$, $(dT)_{20}$, $(dAdT)_{10}(dAdT)_{10}$ and $(dA)_{20}(dT)_{20}$, induced by nanosecond pulses at 266 nm, is studied by time-resolved absorption spectroscopy. The variation of the hydrated electron concentration with the absorbed laser intensity shows that, in addition to two-photon ionization, one-photon ionization takes place for $(dAdT)_{10}(dAdT)_{10}$, $(dA)_{20}(dT)_{20}$ and $(dA)_{20}$ but not for $(dT)_{20}$. The spectra of all adenine-containing oligomers at the microsecond time-scale correspond to the adenine deprotonated radical formed in concentrations comparable to that of the hydrated electron. The quantum yield for one-photon ionization of the oligomers (ca. 10^{-3}) is higher by at least 1 order of magnitude than that of dAMP, showing clearly that organization of the bases in single and double helices leads to an important lowering of the ionization potential. The propensity of $(dAdT)_{10}(dAdT)_{10}$, containing alternating adenine−thymine sequences, to undergo one-photon ionization is lower than that of $(dA)_{20}(dT)_{20}$ and $(dA)_{20}$, containing adenine runs. Pairing of the $(dA)_{20}$ with the complementary strand leads to a decrease of quantum yield for one photon ionization by about a factor of 2.

The ionization potential (IP) of DNA bases is a key factor in the oxidative damage of the double helix induced by various types of radiation and oxidizing agents.[1] During the past decade, several theoretical studies have shown that the presence of sugar, phosphate groups, counterions, water molecules, as well as base stacking and base pairing lead to a decrease of the IP of the bases with respect to the gas-phase values.[2−6] Experimental evidence was brought by various methods, such as the study of gas-phase clusters[7] or the analysis of oxidation products formed upon weak UV irradiation at different wavelengths.[8] In double helices, all the factors mentioned above are expected to act simultaneously and be tuned by base sequence effects. Direct information about the ionization process can be obtained by transient absorption spectroscopy which allows the quantification of the generated hydrated electrons (e_{aq}^-) and the identification of the resulting cations and/or derived radicals of nucleobases.[9−16] The sparse experiments of this type performed for DNA oligomers and polymers in neutral pH did not detect one photon ionization for excitation wavelengths greater than 210 nm.[11,15−17]

Here we report a time-resolved study dealing with the ionization of $(dA)_{20}$, $(dT)_{20}$, $(dAdT)_{10}(dAdT)_{10}$ and $(dA)_{20}(dT)_{20}$. The choice of these base sequences was guided by previous photophysical and photochemical studies carried out in our group which allowed us to overcome experimental and conceptual difficulties related to the fragility and complexity of these systems.[18−21] We show that, upon excitation at 266 nm, one-photon ionization is observed only for the adenine-containing oligomers, leading, in all cases, to the formation of deprotonated adenine radicals. The quantum yield for one-photon ionization of these oligomers (ca. 10^{-3}) is at least 10 times higher than that of the mononucleotide 2′-deoxoadenosine monophosphate (dAMP).

Our experiments were performed using the fourth harmonic (266 nm) of a Nd:YAG laser delivering 8 ns pulses at a repetition rate of 2 Hz. The absorbed laser intensity (I) varied from 0.13 to 1.3×10^6 W/cm^2; in this range I exhibited a linear dependence as a function of the exciting intensity. The laser energy was measured by an energy ratiometer (cf. Supporting Information). A crucial element of the experimental protocol was the use of a flow-through quartz cell (10 mm × 1 mm) allowing the circulation of 60 mL of solution. If this condition was not fulfilled, successive decays recorded during signal averaging were clearly different due to the excitation of reaction products. The compounds were dissolved in phosphate buffer (0.15 M), and the absorbance at 266 nm was adjusted to 0.24 (1 mm), corresponding to a helix concentration of about 10^{-5} M. The solutions were continuously purged by nitrogen during the measurements, which were carried out at 20 ± 1 °C.

The transient absorption spectra of all the oligomers at the nanosecond time-scale are dominated by the broad absorption band of e_{aq}^-, peaking at 700 nm, which disappears with a time constant of 0.38 ± 0.02 μs. The latter value is shorter than the lifetime of e_{aq}^- in pure water because of a reaction with the dihydrogen phosphate ions of the buffer.[22] The efficiency of this reaction prevents the attack of nucleic acids by hydrated electrons, as proved by the fact that the lifetime of e_{aq}^- in oligomer solutions is also 0.38 μs.

The spectra recorded for $(dAdT)_{10}(dAdT)_{10}$ and $(dA)_{20}(dT)_{20}$ at 2 μs for an intensity of 0.4×10^6 W/cm^2 are presented in Figure 1. They are characterized by a peak at 330 nm and a low intensity broad band around 600 nm. These spectral features do not depend on I and are also present in the spectra recorded for $(dA)_{20}$. They resemble the spectrum of the adenosine deprotonated radical A(−H)$^\bullet$, known to be formed following

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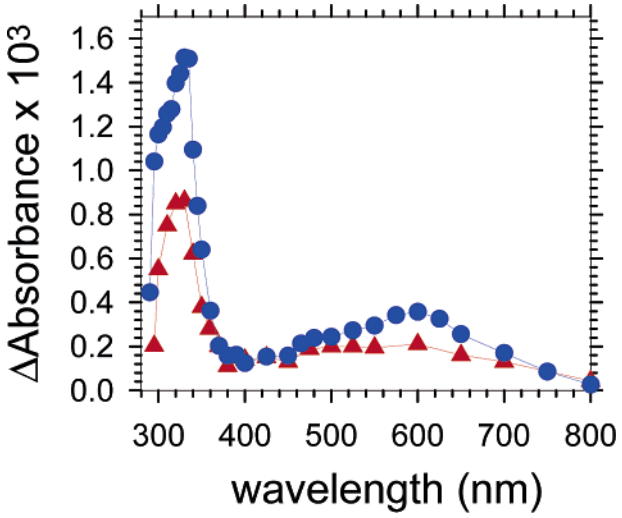

Figure 1. Transient absorption spectra recorded for nitrogen purged buffer solutions (pH = 6.6) of $(dAdT)_{10}(dAdT)_{10}$ (blue circles) and $(dA)_{20}(dT)_{20}$ (red triangles) at 2 μs. Double strand concentration: 10^{-5} M. Excitation conditions: 266 nm, 8 ns pulses, $I = 0.4 \times 10^6$ W/cm^2.

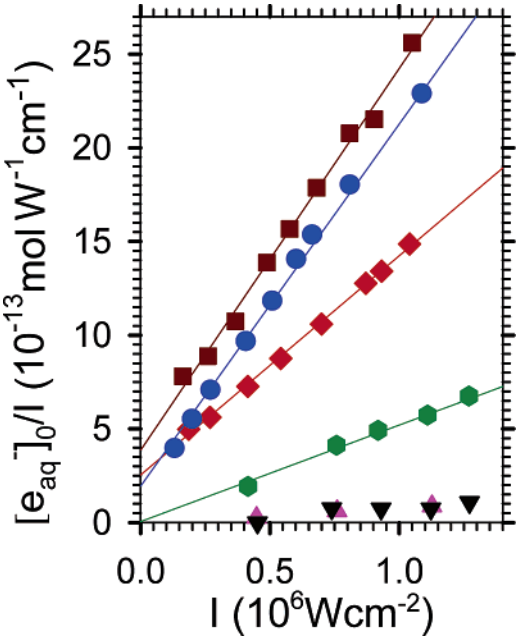

Figure 2. Hydrated electron concentration at zero time $[e_{aq}^-]_0$ divided by the absorbed laser intensity (I) as a function of I recorded for nitrogen purged buffer solutions (pH = 6.6) of $(dA)_{20}$ (brown squares), $(dAdT)_{10}$-$(dAdT)_{10}$ (blue circles), $(dA)_{20}(dT)_{20}$ (red diamonds), $(dT)_{20}$ (green hexagons). For comparison, the signals obtained for dAMP in buffer (pink triangles) and the buffer alone (black triangles) are also shown. The experimental points are represented by circles. Straight lines correspond to fits with the function $aI + b$; the fitting parameters are given in Supporting Information.

excitation at 193 nm.[10] Similarly, the typical absorption band of the deprotonated thymidine radical[10] T(−H)$^\bullet$ is present in the spectra of $(dT)_{20}$ at the microsecond time scale. It is worth noticing that the absorption band of the thymidine triplet,[19,23,24] peaking at 360 nm, is not observed in the spectra of $(dA)_{20}(dT)_{20}$ and $(dAdT)_{10}(dAdT)_{10}$ recorded at the microsecond time scale. The absence of triplet absorption is corroborated by the fact that the signals, which decay at the millisecond time scale, do not undergo any change when oxygen is bubbled into the solutions. It is also supported by the results of a recent theoretical study suggesting that the lowest triplet sate in these types of double helices is a charge transfer state and corresponds to an electron transfer from the adenine to the thymine.[25]

The concentration of hydrated electron initially formed by ionization of the double helices $[e_{aq}^-]_0$ was calculated from the zero time differential absorbance ΔA at 700 nm using a molar extinction coefficient of 18 200 M^{-1}cm^{-1}.[22] The $[e_{aq}^-]_0$ generated upon ionization of $(dAdT)_{10}(dAdT)_{10}$ and $(dA)_{20}(dT)_{20}$, at $I = 0.4 \times 10^6$ W/cm^2, corresponding to the spectra in Figure 1, is $(3.8 \pm 0.4) \times 10^{-7}$ M and $(2.5 \pm 0.1) \times 10^{-7}$ M, respectively. Taking into account the ground-state concentration of the double helices (ca. 10^{-5} M), we find that less than 5% of the double helix population is ionized. The concentration of A(−H)$^\bullet$ at 2 μs, [A(−H)$^\bullet$], determined using the molar extinction coefficients reported in ref 10, is $(3.6 \pm 1.0) \times 10^{-7}$ for $(dAdT)_{10}(dAdT)_{10}$ and $(1.8 \pm 1.0) \times 10^{-7}$ for $(dA)_{20}(dT)_{20}$. These values match those found for $[e_{aq}^-]_0$. Such an agreement shows that there is a correlation between hydrated electrons and adenosine radical cations, which are the precursors of [A(−H)$^\bullet$].[9]

To check whether the hydrated electrons are produced via one- or two-photon ionization, we have plotted in Figure 2 the quantity $[e_{aq}^-]_0/I$ as a function of I. For comparison, the values obtained for dAMP and the buffer alone are also shown. We observe that the $[e_{aq}^-]_0$ generated from the mononucleotide cannot be distinguished from that generated from the solvent and is negligible with respect to the $[e_{aq}^-]_0$ obtained from the oligomers. Taking into account the precision of our measurements, we conclude that the quantum yield for one photon ionization ($\phi_{1h\nu}$) of dAMP does not exceed 10^{-4}. This upper limit is 30 times lower than the value found for monophotonic ionization of dAMP in ultrapure water from experiments performed with higher laser intensities without using a flow-through cell.[26]

The plot corresponding to each oligomer in Figure 2 are fitted correctly by the function $aI + b$. In all cases it is found that two photon ionization takes place ($a \neq 0$). This is not surprising since the decays of the singlet excited states of such single and double strands are longer than the decays of dAMP,[20,27] allowing the absorption of successive photons. Moreover, exciton−exciton[20] interaction could also be responsible for two-photon ionization in these multichromophoric systems. A nonzero intercept ($b \neq 0$), corresponding to one-photon ionization is observed for $(dAdT)_{10}(dAdT)_{10}$, $(dA)_{20}(dT)_{20}$, and $(dA)_{20}$ but not for $(dT)_{20}$. This difference in the behavior of $(dT)_{20}$ compared to the adenine-containing oligomers is not surprising since the gas phase IP of hydrated thymine is higher than that of the hydrated adenine.[7] It has also been reported that, in contrast to 2′deoxyadenosine, only two-photon ionization was observed upon irradiation of thymidine aqueous solutions by 193 nm laser pulses.[9] Finally, $(dA)_n$ strands are known to stack better[28] than $(dT)_n$ strands,[29,30] and base stacking is expected to promote the decrease of IP.

The quantum yield for one-photon ionization $\phi_{1h\nu}$ found from the plots in Figure 2 is $(1.1 \pm 0.1) \times 10^{-3}$, $(1.4 \pm 0.1) \times 10^{-3}$, and $(2.2 \pm 0.2) \times 10^{-3}$ for $(dAdT)_{10}(dAdT)_{10}$, $(dA)_{20}(dT)_{20}$, and $(dA)_{20}$, respectively. These values are comparable to the quantum yield found for the formation of (6−4) thymine dimers in $(dT)_{20}$.[19] The $\phi_{1h\nu}$ of all three oligomers is higher by at least 1 order of magnitude compared to that of dAMP, demonstrating that organization of the bases in single and double helices leads to a lowering of the ionization potential. This is a combined effect resulting not simply from base stacking or base pairing but also from differences in the ionic atmosphere and specific solvation of the bases. The geometrical arrangement determined

by the base sequence and the base pairing has a smaller but still detectable effect. Thus, the propensity of $(dAdT)_{10}(dAdT)_{10}$, containing alternating adenine−thymine sequences, to undergo one-photon ionization is lower than that of $(dA)_{20}(dT)_{20}$ and $(dA)_{20}$, containing adenine runs. Finally, pairing of the $(dA)_{20}$ with the complementary strand leads to a decrease of $\phi_{1h\nu}$ by about a factor of 2.

So far the experimental studies concerning the lowering of ionization potential of bases in DNA have been focused on guanines which constitute traps for hole migration.[31] Our findings suggest that the primary event of radical cation formation could also take place under relatively mild conditions (in terms of photon energy or redox potential) in adenine-rich regions.

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Supporting Information Available: Details on compounds, melting curves, experimental setup and procedure, fitting parameters, determination of quantum yields. This material is available free of charge via the Internet at http://pubs.acs.org.

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