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UV-induced Adenine Radicals Induced in DNA A-tracts: Spectral and Dynamical Characterization

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Abstract

Adenyl radicals generated in DNA single and double strands, (dA)₂₀ and (dA)₂₀•(dT)₂₀, by one- and two-photon ionization by 266 nm laser pulses decay at 600 nm with half-times of 1.0 ± 0.1 and 4 ± 1 ms, respectively. While ionization initially forms the cation radical, the radicals detected for (dA)₂₀ are quantitatively identified as N6-deprotonated adenyl radicals by their absorption spectrum, which is computed quantum mechanically employing TD-DFT. Theoretical calculations show that deprotonation of the cation radical induces only weak spectral changes, in line with the spectra of the adenyl radical cation and the deprotonated radical trapped in low temperature glasses.

Graphical Abstract

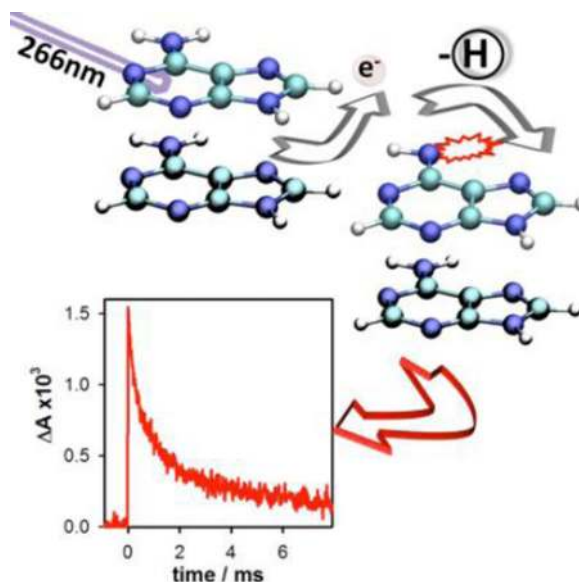
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Supporting Information: Details of time-resolved experiments; Additional transient absorption decays; Comparison of literature spectra; Computational details; Additional computational results; Methods for sample preparation for low temperature measurements; Characterization of adenine radicals by ESR spectroscopy.

The authors declare no competing financial interests.



Radicals generated via oxidation of nucleobases have been extensively studied since the 1960s and have elucidated mechanisms of damage to the genetic code by ionizing radiation.^{1,2} The discovery of charge transport in DNA³ has highly contributed to the renewal of this topic. The time evolution of various DNA base radicals has been investigated using pulsed electron beams or lasers.^{4–12} As guanine is known to be a trap for hole transfer,^{13–14} dynamical studies on DNA strands have been focused extensively on guanyl radicals. In contrast, no information is available in the literature about the lifetimes of adenyl radicals, i.e., the adenine cation radical ($A\bullet^+$), or its deprotonated neutral aminyl radical form ($A\text{-H}6\bullet$), either in DNA single strands (ss) or in DNA double strands (ds) to date. Here, we show that such adenyl radicals, generated by 266 nm laser pulses in the ss-A tract, $(dA)_{20}$, decay on the ms time-scale, and that base pairing prolongs their lifetime. Furthermore, we present the first characterization of the adenyl radical absorption spectra employing TD-DFT calculations. The computed spectral changes induced by deprotonation of adenine cation radicals are in excellent agreement with those determined in low temperature glasses, for which electron spin resonance (ESR) spectroscopic measurements allow clear identification of the radical species and their protonation states.¹⁵

The ss and ds DNA oligomers $(dA)_{20}$ and $(dA_{20})\bullet(dT_{20})$ (Eurogentec Europe; HPLC purified) were dissolved in phosphate buffer ($0.15 \text{ mol}\cdot\text{L}^{-1} \text{ NaH}_2\text{PO}_4$, $0.15 \text{ mol}\cdot\text{L}^{-1} \text{ Na}_2\text{HPO}_4$; pH=7) and were studied at room temperature. As previously reported for various DNA systems,^{16–17} laser excitation at 266 nm of ss and ds A-tracts leads to electron detachment via one- and two photon ionization.¹⁸ However, the spectral and dynamical features of the resulting radicals have not been properly studied. An important difficulty arises from the fact that, in addition to the radical species resulting from ionization of A-tracts, we also have the formation of adenine¹⁹ or thymine dimers²⁰ which may affect both the spectrum profile and its dynamics. A key point in our time-resolved experiments was the quantification of the various UV-induced species. Thus, at the highest excitation intensity used, 3 MWcm^{-2} , the total concentration of the species generated by each laser pulse is

much lower than the oligomer concentration (SI-Section 1). Another important issue is the photo-ionization of water.^{4, 6} Under our experimental conditions, no hydrated electron was detected for the aqueous solvent alone (Figure 1a). Therefore, reactions between the studied oligomers and the ejected electrons (e^-) or the hydroxyl radicals ($\bullet\text{OH}$), generated via two-photon ionization of water, encountered in experiments employing shorter wavelength⁶ and/or higher intensity⁴ excitation pulses are minimized in the present study.

The decay of the hydrated electron was detected at 700 nm with a time-resolution of 30 ns, which is at least one order of magnitude faster than that used in previous flash photolysis studies on adenylyl radicals.^{4, 6, 18} They were fitted with an exponential function $y=y_0+ae^{-t/\tau}$ (Figure 1a), with $\tau = 0.4 \mu\text{s}$. Such a short lifetime is due to the reaction of hydrated electrons with phosphate ions.¹ The concentration of ejected electrons, $[e^-]$, was determined from the zero-time absorbance y_0 , associated to a molar absorption coefficient of $22\,700 \text{ molL}^{-1}\text{cm}^{-1}$.²¹ The dependence of $[e^-]$ on the absorbed photons, $[h\nu]$, (Figure 1b) was fitted with the function $[e^-]/[h\nu]=\phi_1 + \alpha[h\nu]$, where ϕ_1 corresponds to the one-photon ionization quantum yield and α is associated with the two photon ionization quantum yield, $\phi_2 = \alpha[h\nu]$. The ϕ_1 values determined for the single-strand $(\text{dA})_{20}$, $(1.0\pm 0.1)\times 10^{-3}$, and the double-strand $(\text{dA})_{20}\bullet(\text{dT})_{20}$, $(1.1\pm 0.3)\times 10^{-3}$, are quite similar. While ϕ_2 values vary with excitation intensity, they are proportional to the slope α of the fitted curve and differ for the ss and ds oligomers. We find that α is nearly twice as high for the ss oligomer (0.040) compared to the ds oligomer (0.023). As seen in Figure 1b, at higher excitation intensities, two photon e^- yields far outweigh single photon e^- yields for both systems.

Figure 2a shows the transient absorption spectra recorded for $(\text{dA})_{20}$ at 0.1, 1 and 10 ms. The absorption band peaking at 600 nm decays within a few ms with a half-life of 1.1 ± 0.1 ms (Figure 3a). Its intensity scales with the number of ejected electrons (inset in Figure 2a) confirming that the species absorbing at 600 nm result from a photo-ionization process. An increase in the ratio of the electrons $[e^-]_{h\nu 1}/[e^-]_{h\nu 2}$ resulting from one- and two-photon ionization from 0.38 to 0.68 does not modify the decay kinetics (SI; Section 2), showing that the radicals stemming from the two types of ionization processes exhibit similar lifetimes. Argon bubbling in the solution to remove air does not induce any noticeable effect neither in the intensity (<10%) nor in the shape of the spectra. This result is in agreement with the literature that the adenylyl radicals (cation or deprotonated) do not react with molecular oxygen.¹ It also shows that the fingerprint of superoxide anion radical, produced from the reaction of oxygen with hydrated electrons and absorbing at wavelengths shorter than 300 nm,^{22, 24} is not detected in the transient spectra. This is due to the partial quenching of the hydrated electron by the phosphate buffer.¹ Finally, no noticeable modification of the decays was observed upon replacing the phosphate buffer by a NaCl solution while keeping the same ionic strength, in order to maintain base stacking.

The 600 nm band was also detected for $(\text{dA})_{20}\bullet(\text{dT})_{20}$ at $2 \mu\text{s}$.¹⁸ Its decay (Figure 3b) is noisier than that of $(\text{dA})_{20}$ obtained for the same excitation intensity because of the lower two-photon ionization yield of the ds (Figure 1b) and its higher photo-sensitivity (SI, Section 1). The ds signal disappears with a half-life of 4.0 ± 1.0 ms, revealing that base-pairing of A-tracts increases the lifetime of this transient species by about a factor of four.

The UV part of the spectra in Figure 2a exhibits a more complex time-evolution compared to the visible part. This is due to the contribution of the azetidinium intermediate, which is created from a singlet excited state and leads to the formation of adenine dimers at times longer than 0.1 sec.¹⁹ Based on this knowledge, we subtracted the azetidinium spectrum determined at 20 ms, when adenylyl radicals have decayed, from the transient spectrum recorded at 0.1 ms.¹⁹ The resulting difference spectrum is shown in Figure 2b. The molar absorption coefficient, obtained assuming that the radical concentration at 0.1 ms equals the concentration of ejected electrons (inset Figure 2a) matches perfectly that determined upon 193 nm excitation of 5'-dAMP.⁶ The discrepancy below 300 nm is due to the higher ground state absorption of oligomer compared to the monomer in this spectral region, affecting the shape of the transient differential spectra (inset in Figure 2b).²³ Interestingly, a less intense UV band, compared to that of the monomer, was also observed in the adenylyl radical spectrum obtained by 193 nm ionization of an RNA polymer, poly(A)²⁴ (Figure SI-2a).

The UV transient absorption of (dA)₂₀•(dT)₂₀ also contains dimerization fingerprints. They arise from thymine (6-4) adducts,²⁰ whose spectrum peaks at 325 nm.^{25–26} Their formation from the oxetane intermediate in thymine single strands (dT)₂₀ was shown to take place within 4 ms,²⁷ a time comparable with the decays of the adenylyl radical. For these reasons, and also because of the higher photosensitivity of (dA)₂₀•(dT)₂₀ due to the formation of cyclobutane thymine dimers with a high quantum yield (0.022),²⁰ refined determination of the radical spectrum at the short wavelength range is delicate and challenging for the duplex.

In Figure 2b we notice a very good quantitative agreement between the spectrum of the adenylyl radical from (dA)₂₀ and that reported by Candeias and Steenken for (A-H6)• from 5'-dAMP.⁶ For both the monomer and the oligomer spectra in Figure 2b the intensity ratio between the high and the low energy bands is 3.4, suggesting that we deal with localized radicals. This is in agreement with already reported ESR²⁸ and TD-DFT studies²⁹ which show that deprotonation of A•⁺ in stacked monomers or in dinucleoside phosphates leads to localization of the radical site on to a single adenine. A recent pulse radiolysis study on A-tracts, reported that hole localization, associated with deprotonation of A•⁺, takes place on the ns time-scale.³⁰ Yet, the spectra provided in that study for the deprotonated radical (A-H6)• (Figure SI-2b) differ from those shown in Figure 2b. More importantly, the spectrum of UV-induced adenylyl cation radical, A•⁺, the precursor of the deprotonated radical, (A-H6)•, is not available in literature to date. Further, it cannot be determined without the use of additional electron scavengers,^{1,30} which the present study tried to avoid. This knowledge is very important not only for the study of DNA oxidative processes but also for the investigations on hole delocalization processes in A-tracts, which recently has attracted significant interest.^{3, 28, 31} On this ground, we decided to better characterize the absorption spectra of adenylyl radicals.

We computed the absorption spectrum of the cation radical of adenosine (A•⁺) and of its deprotonated neutral aminyl radical derivative (A-H6)• using PCM/TD-M052X/6-31+G(d,p) calculations in which we explicitly included 5 water molecules of the first solvation shell. This computational approach has been already applied to study the optical properties of dA and A-tracts.^{19, 23, 32–34} The calculated spectra, red-shifted by 0.55 eV (see SI, Sections 4 and 5 for a more detailed discussion), are in very good agreement with the experimental

spectra. In particular, the computed spectrum of (A-H6)• (Figure 4a) is extremely close to that shown in Figure 2b, providing a broad peak around 620 nm and another peak at *ca.* 300 nm, three times more intense than the former. The spectrum computed for A•⁺ is similar to that of (A-H6)•, but the peak in the UV region is slightly red-shifted with respect to its counterpart of (A-H6)•; it is about four times more intense than the peak in the visible region. At this point we stress that, in contrast to the computed spectra, the experimental ones contain the bleaching of the ground state which leads to a decrease in the intensity and red-shift of the UV band. As shown in the SI, the spectra computed for the A•⁺ and (A-H6)• in (dA)₂ dinucleotide are very similar to that shown in Figure 2b and 4a, thereby confirming the localization of the radical site.

Though the deprotonation reaction of A•⁺ is strongly favored in aqueous solution at room temperature,^{1,6} it has been possible to follow it by ESR in low temperature homogeneous aqueous (H₂O or D₂O) glasses (7.5 molL⁻¹ LiCl)^{15, 28, 35} allowing observation of both A•⁺ and its conjugate base (A-H6)• by ESR spectroscopy.^{15,28} The ESR studies show that in aqueous glasses at pH \leq 5, A•⁺ converts to (A-H6)• either via annealing to higher temperatures (*ca.* 165 K) or via annealing at lower temperature (*ca.* 150 K) at higher pH (pH *ca.* 12). Although we observe conversion of A•⁺ to (A-H6)• at higher annealing temperatures (*ca.* 165 K), the ESR signal intensity decreases owing to the radical-radical recombination.²⁸ As a result, (A-H6)• obtained by this method leads to a poor absorption spectrum of this radical at 77 K, due to its much lower concentration in the sample. On the other hand, samples at a higher pH (*ca.* 12) allow for production of (A-H6)• at lower temperatures which maintains its signal intensity and results in improved UV-visible spectra of both A•⁺ and (A-H6)•. The detailed procedure for the preparation of the glassy samples and their ESR characterization is given in the SI-Section 6. The ESR spectra of A•⁺ and (A-H6)• are quite distinct as the loss of the proton from N6 (A-H6)• leads to a large N6 nitrogen coupling that is apparent in the wings (SI, Section 7).¹⁵ The UV-visible spectra corresponding to A•⁺ and (A-H6)• recorded at 77 K for the same samples as were used for the ESR spectra are shown in Figure 4b. We observe that the UV-visible spectra of the two radicals are quite similar. Yet, below 330 nm, the spectrum of (A-H6)• is clearly more intense than that of A•⁺, in agreement with the computational results, showing a blue-shift of the UV band upon deprotonation (Figure 4a).

In conclusion, we have shown that A-tracts undergo one-photon ionization at energies lower by *ca.* 3.5 eV compared to the adenine ionization potential,^{36–37} generating the equivalent concentration of deprotonated adeny radicals, which have been characterized by their absorption spectrum. The decays of these radicals determined on the ms time-scale are not governed by the presence of non-biologically relevant species, such alcohols serving as •OH scavenger, which are used in pulse radiolysis experiments.¹⁰ The lifetime of (A-H6)• radicals increases upon base pairing proving that the helix conformation plays a key role in their reactivity. Our transient spectra have not revealed any fingerprint of species appearing simultaneously with the radical disappearance. This means that the reaction products resulting from the deprotonated radicals absorb below 300 nm. This is the case, for example, of 8-oxo-7,8-dihydro-2'-deoxyadenosine and strand breaks, which have been detected upon high intensity 266 nm excitation of RNA single stranded poly(A).³⁸ We hope that this study will incite determination of the final oxidative products by analytical techniques. Moreover,

the first spectral characterization of the adeny radical cation, which is extremely short-lived, will provide the grounds for the interpretation of future time-resolved studies on DNA with high time resolution. Finally, our study does not challenge the deprotonation of adenine cation radicals occurring on the ns time-scale,³⁰ rather, our study shows that adeny radical spectra are more reproducible when obtained by direct UV ionization of DNA than via multistep reactions involving a series of additives.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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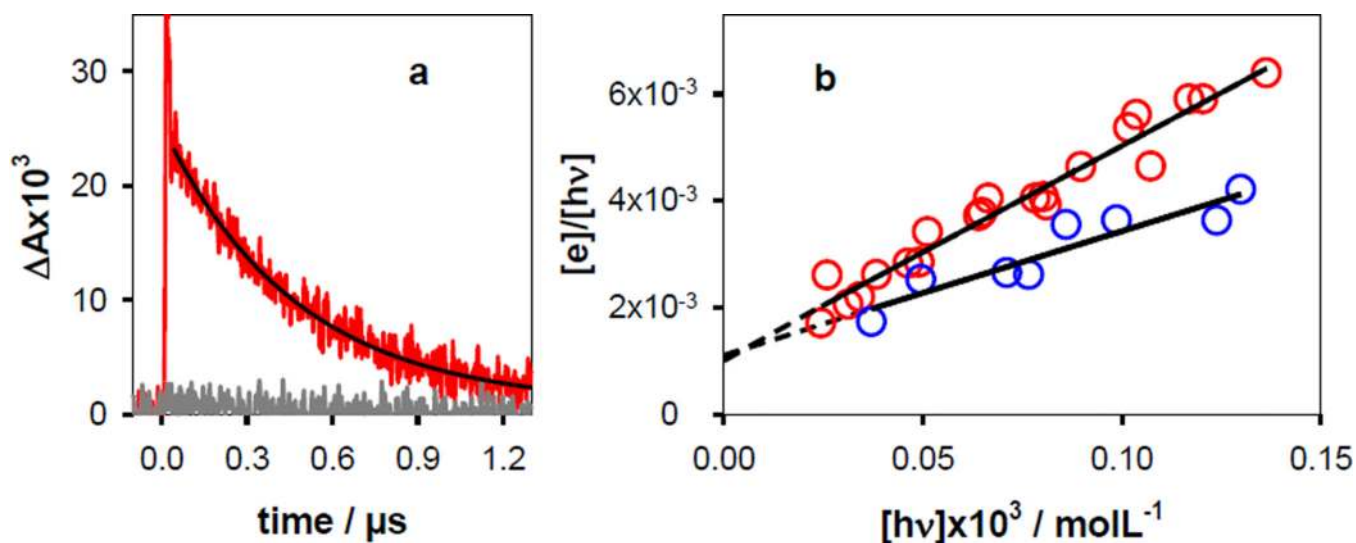


Figure 1.

(a) Transient signals recorded at 700 nm for the buffer alone (grey) and a deaerated $(dA)_{20}$ solution (red) with an excitation intensity of 3 MWcm^{-2} ; the dark line corresponds to the fit with the function $y = y_0 + ae^{-t/\tau}$, ($\tau = 0.4 \mu\text{s}$). (b) Electron $[e^-]$ ejected as a function of the photons $[hv]$ absorbed by $(dA)_{20}$ (red) and $(dA)_{20} \cdot (dT)_{20}$ (blue). Experimental points (circles) are fitted with the function $[e^-]/[hv] = \phi_1 + \alpha[hv]$ (solid lines).

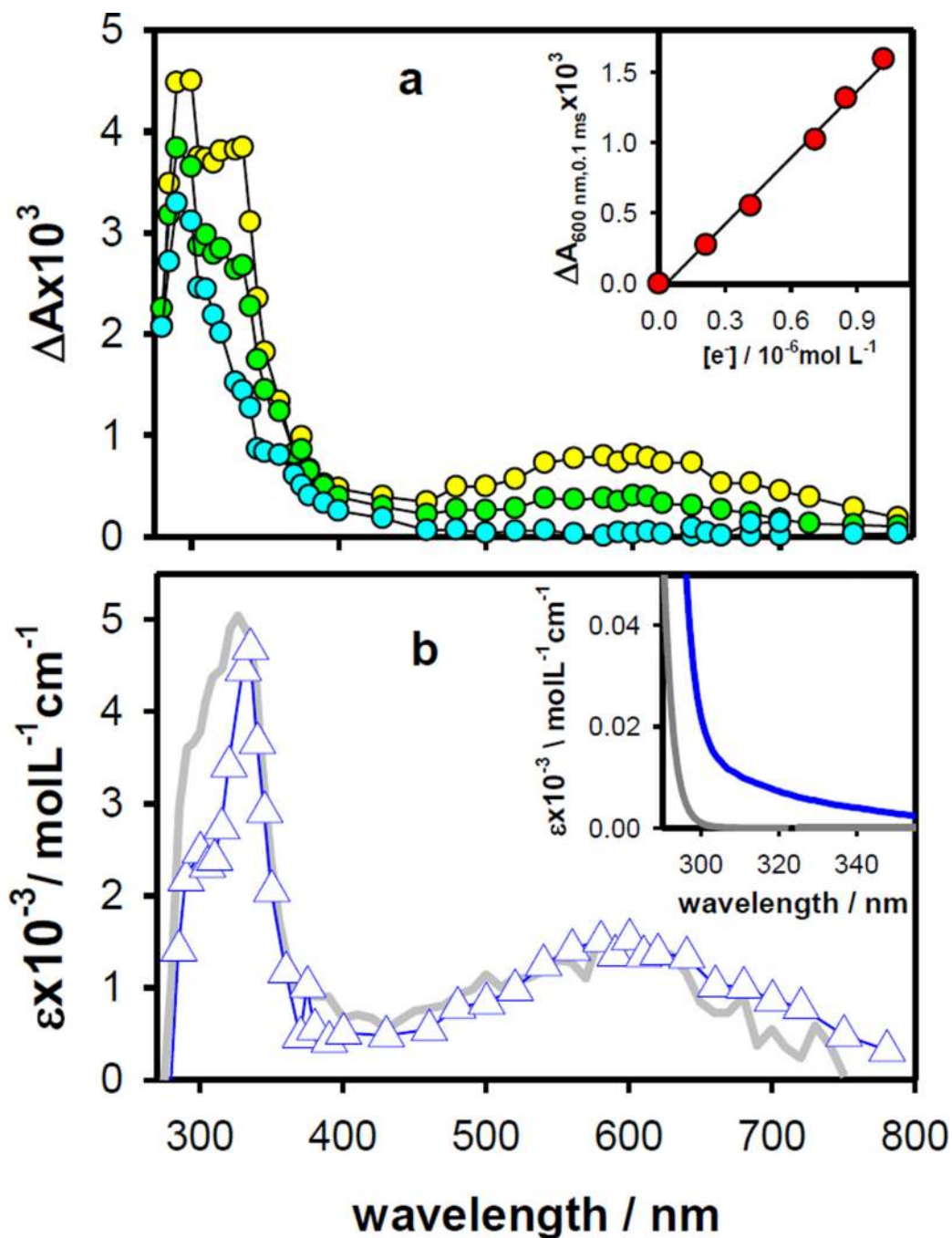


Figure 2.

(a) Transient absorption spectra recorded for the ss oligomer (dA)₂₀ at 0.1 ms (yellow), 1 ms (green) and 10 ms (cyan). (b) Triangles: difference of the (dA)₂₀ spectra recorded at 0.1 and 20 ms, associated with adenine dimerization;¹⁹ solid line: spectrum obtained upon 193 nm excitation of 5'-dAMP at 300 ns.⁶ Inset in (a): Correlation of transient absorbance detected at 600 nm and 0.1 ms with the concentration of ejected hydrated electrons. Inset in (b): ground state absorption spectra of 5'-dAMP (grey) and (dA)₂₀; the molar absorption coefficient is given per base.²³

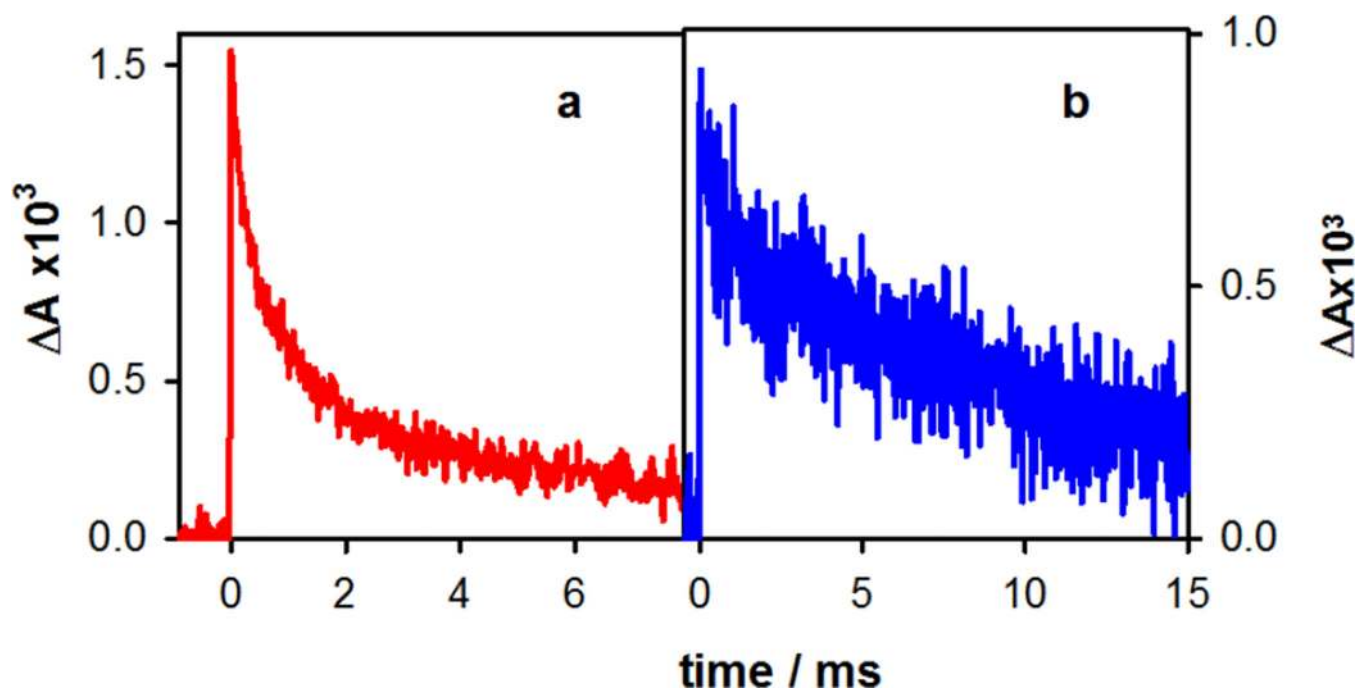


Figure 3. Transient absorption signals recorded at 600 nm for $(dA)_{20}$ (a) and $(dA)_{20} \bullet (dT)_{20}$ (b). Excitation intensity: 3 MWcm^{-2} .

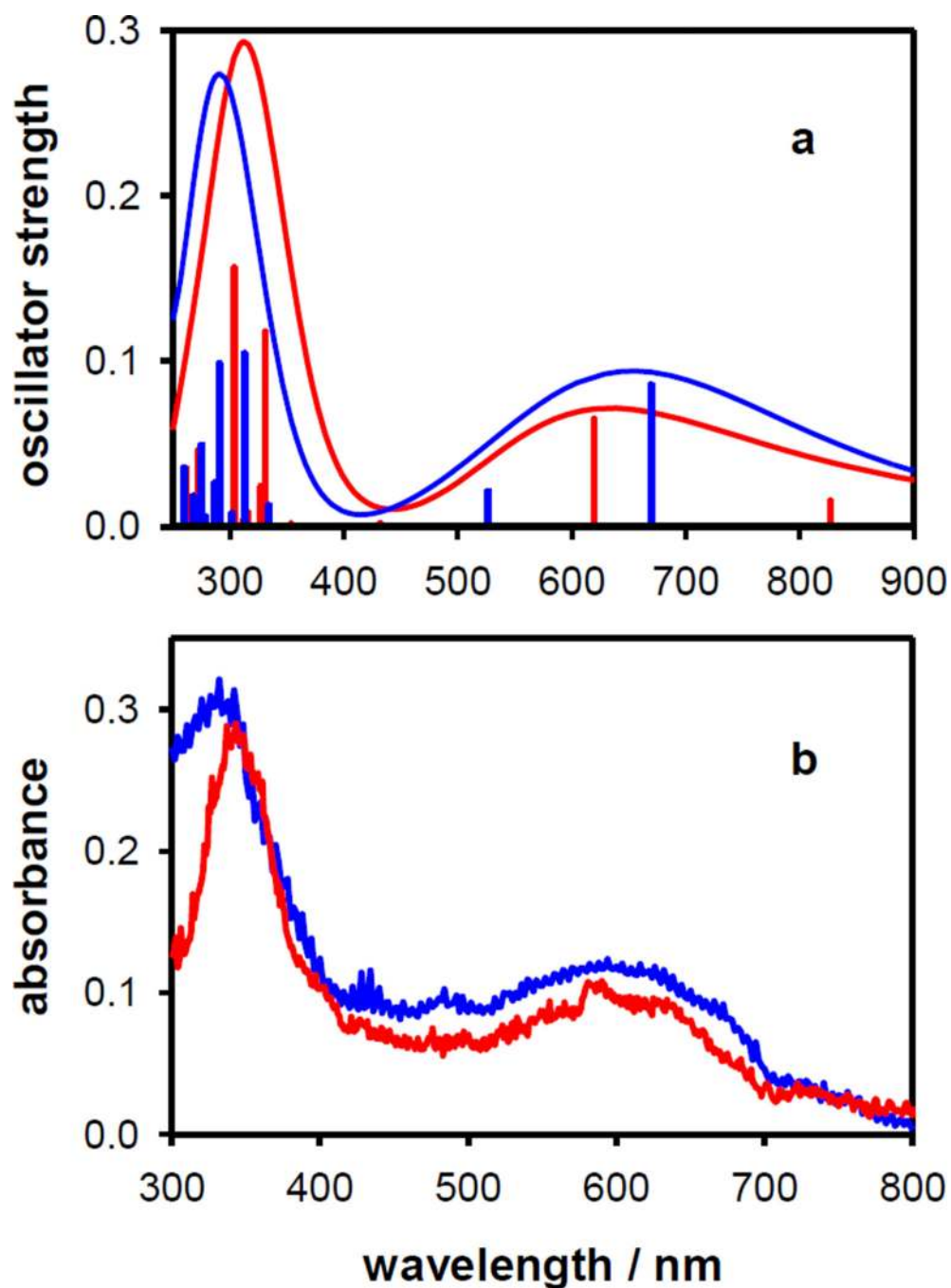


Figure 4. Electronic absorption spectra determined for the radical cation (red) and the deprotonated radical (blue) of dA. (a) PCM/TD-M052X/6-31+G(d,p)// PCM/M052X/6-31+G(d,p) level including five water molecules. Energies are homogeneously decreased by 0.55 eV. Spectral shapes were obtained by convoluting each transition by a Gaussian curve with a width of 0.4 eV (FWHM). The electronic transitions are shown as sticks with their oscillator strength. (b)

UV-visible spectra of $A^{\bullet+}$ (red) and its N-6 deprotonated radical, $(A-H6)^{\bullet}$ (blue) at 77K produced by $Cl_2^{\bullet-}$ one electron oxidation in a 7.5 molL^{-1} LiCl aqueous glass.

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