Acid–base equilibria in aqueous solutions of 2-aminopurine radical cations generated by two-photon photoionization

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The acid–base equilibria of the nucleic acid analogue 2-aminopurine (2AP) and 2-aminopurine riboside (2-AP*r*) radicals generated by two-photon photoionization of the 2AP residues with intense 308 nm XeCl excimer laser pulses (fwhm = 12 ns, *ca.* 70 mJ cm^{-2} $pulse^{-1}$) have been investigated using transient absorption spectroscopy techniques. It is of interest to investigate the properties of these radicals as a function of pH because of their abilities to undergo proton-coupled electron transfer reactions with other nucleic acid bases. Pronounced differences in the 2AP and 2AP*r* radical absorption bands are observed in the 330–550 nm spectral region within the pH range of 1 to 12. These differences are attributed to the deprotonation of the 2AP and 2AP*r* radical cations, $2AP^{\bullet+}$(pK_{a1}^{r} = 2.8 ± 0.2), and $2APr^{\bullet+}$(pK_{a1}^{r} = 2.7 ± 0.2), with the concomitant formation of the neutral radicals, $2AP(-H)^{\bullet}$ and $2APr(-H)^{\bullet}$. At higher pH values, the $2AP(-H)^{\bullet}$ radical (pK_{a2}^{r} = 9.5 ± 0.2) loses a second proton at the N^9 position to form the radical anion $2AP(-2H)^{\bullet-}$; this deprotonation reaction does not occur in the case of the $2APr(-H)^{\bullet}$ radical because of the ribofuranosyl substitution at the N^9 position.

Introduction

2-Aminopurine is a mutagenic nucleic acid base analogue[1] which can substitute for adenine in double-stranded DNA without significantly altering the stability of the DNA duplexes.[2] While the normal DNA bases (A, C, G and T) are characterized by absorption maxima around 260 nm and negligible absorbances at wavelengths ≥300 nm, the absorption maximum of 2AP occurs at 305 nm.[3] Using intense 308 nm XeCl excimer laser pulses, we have recently shown that this spectral feature allows for the selective, two-photon-induced ionization of a single, site-specifically positioned 2AP residue in oligodeoxyribonucleotides.[4] The evolution in time of 2AP radicals generated by this mode of excitation has been monitored by transient absorption spectroscopy techniques; the results indicate that the 2AP radicals selectively oxidize nearby guanine residues within the oligonucleotides. Redox reactions of 2AP radicals with the other three nucleic acid bases (A, C and T) did not occur to any measurable extent.[4] The DNA lesions induced by photoionization of 2AP residues culminate in the formation of hot alkali-labile oxidized guanine residues that are revealed as strand breaks up to 8 bases from the site of the 2AP residue[4] (oxidative chemistry at a distance[5–9]).

A key question in such experiments is the nature of the reactive 2AP intermediates from which this long-distance chemistry is initiated. Using transient absorption techniques, we have previously shown that the two-photon excitation (308 nm) of 2AP gives rise to the radical cation $2AP^{\bullet+}$.[4] However, Steenken and co-workers[10] have demonstrated that in aqueous solutions, purine radical cations deprotonate rapidly, and that this effect is dependent on pH. In order to gain a better understanding of the oxidative reactions involving $2AP^{\bullet+}$ and its neutral, deprotonated product, $2AP(-H)^{\bullet}$, we studied the acid–base equilibria of the radical species generated by two-photon photoionization of 2-aminopurine and 2-aminopurine riboside (2-AP*r*, 2-amino-9-β-D-ribofuranosylpurine) in aqueous solutions. It is shown that, when the pH is increased from 1 to 4, a pronounced change in the transient absorption spectra of $2AP^{\bullet+}$ radical cations is observed. These observations are interpreted in terms of acid–base equilibria between $2AP^{\bullet+}$ or $2APr^{\bullet+}$ radical cations and $2AP(-H)^{\bullet}$ or $2APr(-H)^{\bullet}$ neutral radicals.

Experimental

The transient absorption spectra were recorded using a kinetic spectrometer system (~7 ns response time) described earlier.[4] Briefly, this system consists of a pulsed Lambda Physik EMG 160 MSC XeCl excimer laser excitation source (308 nm, fwhm = 12 ns, *ca.* 70 mJ $pulse^{-1}$ cm^{-2}, 10 Hz), and a 75 W xenon pulsed lamp as the transient absorbance probe flash. The wavelengths of the probe light flash were varied using a computer-controlled McPherson monochromator. The transient absorption signals were recorded at 5 nm intervals in the 260–750 nm spectral range. The probe flash signal was monitored by a Hamamatsu R928 photomultiplier, and its output was digitized and recorded using a Tektronix TDS 620 oscilloscope. The transient absorption spectra measured at fixed, different time intervals after the excitation flash, Δt, were constructed by sampling the amplitudes of each decay curve at the same time interval Δt. All experiments, including data collection and analysis, were controlled by a computer.

2-Aminopurine riboside and 2-aminopurine (99% purity) were obtained from Sigma Chemical Corp. (St. Louis, MO) and were used as received. The absorbances at 308 nm of the 2AP and 2AP*r* aqueous buffer solutions were generally ~0.6 cm^{-1}, corresponding to ~0.1 mM concentrations of 2AP residues (at pH = 7, the extinction coefficient of free 2AP,[11] ε_{305} = 6020 M^{-1} cm^{-1}). The sample solutions of 2AP or 2AP*r* were saturated with O_2 and, using positive O_2 pressure, were forced through a 0.5 ml quartz flow cell with an optical pathlength of 1 cm aligned parallel to the direction of the probe light beam[4] at a flow rate of 2–3 ml min^{-1}. The excimer laser light was focused with a quartz lens (focal length ≥1 m) and was passed through a 3 × 9 mm rectangular aperture to obtain a beam of uniform intensity (*ca.* 70 mJ $pulse^{-1}$ cm^{-2}) at the sample cell position. The transient absorbances of the sample solutions were probed by a light beam from the pulsed Xe lamp perpendicular

to the 308 nm laser light beam passing through a 3 mm aperture. All laser flash photolysis experiments were performed at 20 °C.

Results

Effects of pH on the transient absorption spectra of 2AP and 2AP*r* radicals

The photoexcitation of 2AP or 2AP*r* in aqueous solutions with intense nanosecond 308 nm excimer laser pulses results in a consecutive two-photon ionization of 2AP residues.[4] Absorption of the first photon results in the formation of the 2AP singlet excited state (the lifetime of ^{1}2AP is 10 ns[12]), and the absorption of the second photon causes photoionization to occur:

$$2AP \xrightarrow{h\nu} {}^1 2AP \xrightarrow{h\nu} 2AP^{\bullet +} + e_h^- \qquad (1)$$

In oxygen-saturated solutions, the hydrated electrons, e_h^- decay within ~200 ns after the actinic laser pulse. The decay rates of the 2AP and 2AP*r* radicals generated in reaction (1), are not affected by oxygen[13] and remain the sole transient species that decay on μs–ms time scales.

The transient absorption spectra of the 2AP or 2AP*r* radicals in oxygen-saturated aqueous buffer solutions were measured at a delay time, $\Delta t = 1$ μs. In acidic solutions, the photoionization efficiencies, and hence the radical yields, are lowered due to the protonation of the 2AP residues[14] ($pK_{a1} = 3.8$). In order to compensate for this effect, the spectra recorded at different pH values were normalized to one another at $\lambda = 310$ nm (Fig. 1). The typical transient absorption spectra thus obtained at $\Delta t = 1$ μs (pH = 2, 7 and 12), are characterized by bleaching of the 2AP (panel A) and 2AP*r* (panel B) absorbance bands near 310 nm, and the appearance of the 2AP and 2AP*r* radical absorption bands in the 260–300 nm and 330–600 nm spectral ranges.

In acidic solutions (pH 2), the absorption maxima of the $2AP^{\bullet +}$ (panel A) and $2APr^{\bullet +}$ (panel B) radical cations are observed at 355 nm. When the pH is increased, a pronounced shift of the $2AP^{\bullet +}$ absorption band (panel A) to longer wavelengths occurs. Absorption maxima are observed at 385 nm (pH 7), and at 400 nm (pH 12). However, in the case of 2AP*r*, the analogous red shifts are smaller. At pH 7, the absorption maximum of the 2AP*r* radicals occurs at 365 nm (panel B); however, in contrast to 2AP radicals, the absorption spectra of 2AP*r* radicals at pH 7 and pH 12 are very similar (data not shown). Thus, at least three spectroscopically distinct 2AP and two distinct 2AP*r* radical species, can be recognized. Previous studies on acid–base equilibria of the purines adenosine and guanosine radicals[10] suggest that these species can be tentatively identified as the $2AP^{\bullet +}$ and $2APr^{\bullet +}$ radical cations (acidic solutions), the $2AP(-H)^{\bullet}$ and $2APr(-H)^{\bullet}$ neutral radicals (neutral solutions), and the $2AP(-2H)^{\bullet -}$ radical anion species dominant at high pH values.

The equilibria between the $2AP^{\bullet +}$ or $2APr^{\bullet +}$ radical cations and the $2AP(-H)^{\bullet}$ or $2APr(-H)^{\bullet}$ neutral radicals were characterized by the dependences of the absorbance on pH measured at $\lambda = 355$ nm. The molar extinction coefficients of these radical species are quite different from one another at this wavelength (Fig. 1). Fig. 2A depicts the dependences of the 355 nm absorbance on pH; the shape of these curves is sigmoidal with inflection points at pH = 2.8 ± 0.2 ($2AP^{\bullet +}$), and pH = 2.7 ± 0.2 ($2APr^{\bullet +}$). The equilibrium between the $2AP(-H)^{\bullet}$ and the $2AP(-2H)^{\bullet -}$ radicals was characterized by the dependence of the absorbance on pH measured at $\lambda = 525$ nm (A_{525}). The largest change in absorbance is observed at this particular wavelength for the reversible interconversion between $2AP(-H)^{\bullet}$ and the $2AP(-2H)^{\bullet -}$ radicals (Fig. 1A). The plot of A_{525} *vs.* pH also exhibits a sigmoidal profile with an inflection point at pH = 9.5 ± 0.2 (Fig. 2B).

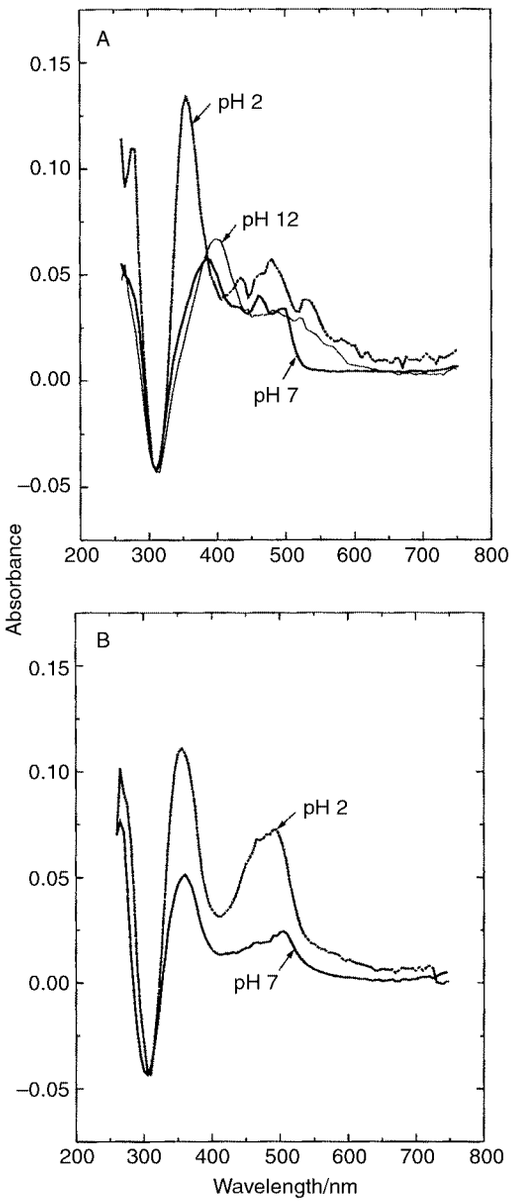


Fig. 1 Transient absorption spectra of 2-aminopurine and 2-aminopurine riboside (0.1 mM) in oxygenated (1 atm partial pressure†) buffer solutions measured at the delay time, $\Delta t = 1$ μs after 308 nm XeCl excimer laser pulse excitation (70 mJ pulse^{-1} cm^{-2}). The different spectra were normalized to one another at 310 nm. The transient spectra are attributed predominantly to $2AP^{\bullet +}$ (pH 2), $2AP(-H)^{\bullet}$ (pH 7), $2AP(-2H)^{\bullet -}$ (pH 12), and $2APr^{+\bullet}$ (pH 2), $2APr(-H)^{\bullet}$ (pH 7).

Kinetics of interconversion between differently protonated species of 2AP radicals

A typical kinetic response curve measured at 525 nm at pH 9.5 following a 308 nm laser flash, is shown in the insert in Fig. 3. The initial prompt rise (<100 ns) in the absorbance is attributed to the formation of $2AP^{\bullet +}$ radical cations and hydrated electrons (*ca.* 80% of the signal amplitude). In oxygen-saturated solutions, the lifetime of e_h^- is ~40 ns because of the rapid reaction[15] of e_h^- with O_2 to form O_2^-. The hydrated electrons thus no longer contribute to the absorbance at 525 nm on time scales beyond ~400 ns under the conditions of the experiment shown in the insert in Fig. 3.

The deprotonation of $2AP^{\bullet +}$ radicals with the formation of $2AP(-H)^{\bullet}$ neutral radicals is complete within $\Delta t < 100$ ns.[4] The

† 1 atm = 101325 Pa.

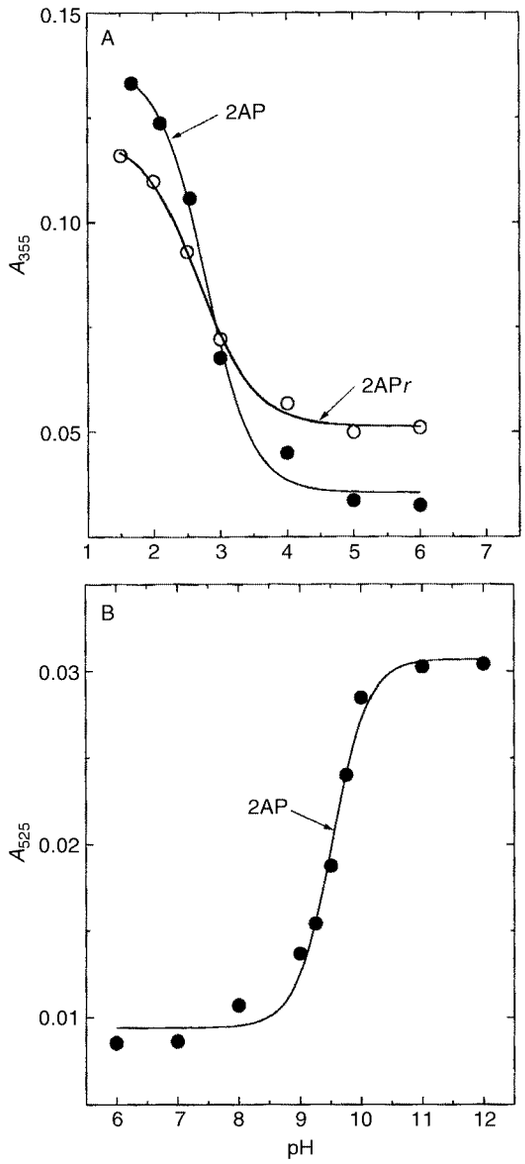


Fig. 2 pH Dependence of the normalized absorbances measured at a delay time of $\Delta t = 1$ μs after the 308 nm XeCl excimer laser pulse excitation (70 mJ pulse^{-1} cm^{-2}) of 2-aminopurine and 2-aminopurine riboside (0.1 mM) in oxygenated buffer solutions. The solid lines are shown for visualization only. (A) $2AP^{+\bullet}$ at pH 2, $2AP(-H)^{\bullet}$ at pH 7, and $2AP(-2H)^{\bullet -}$ at pH 12. (B) $2APr^{+\bullet}$ at pH 2, $2APr(-H)^{\bullet}$ at pH 7.

absorbance at 525 nm increases within the time interval from ~200 ns to ~3 μs, and decays slowly on still longer time scales (insert in Fig. 3). We attribute the rise in the absorbance in this time interval to the deprotonation of $2AP(-H)^{\bullet}$ radicals resulting in the formation of $2AP(-2H)^{\bullet -}$ radical anions (rate constant k_1). On time scales ≥3 μs, the decrease in absorbance is attributed to the decay of the $2AP(-2H)^{\bullet -}$ radical (rate constant k_2).

The kinetic absorbance response curves within the time domain of 0.4–8 μs can be approximated by a sum of two exponential terms:

$$A_{525}(t) = A \exp(-k_1 t) + B \exp(-k_2 t) \qquad (2)$$

The solid line shows the best fit of eqn. (2) to the experimental data points (Fig. 3, Insert). The effect of OH^- ion concentration on the k_1 and k_2 values is shown in Fig. 3. The value of k_1 increases linearly with increasing OH^- concentration. In contrast, the value of k_2 appears to be nearly independent of $[OH^-]$, exhibiting only a modest decrease with increasing OH^- concentration.

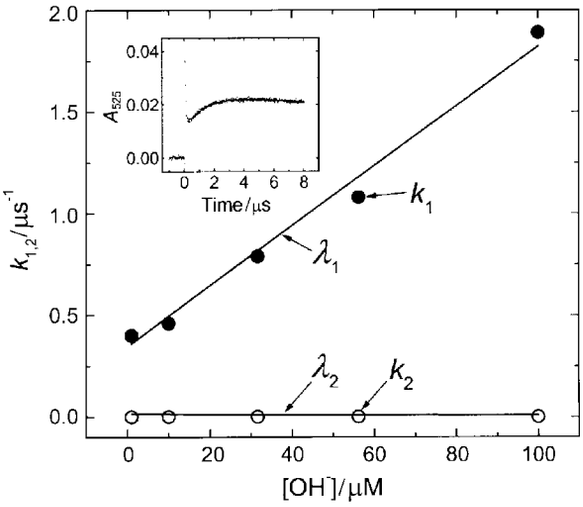
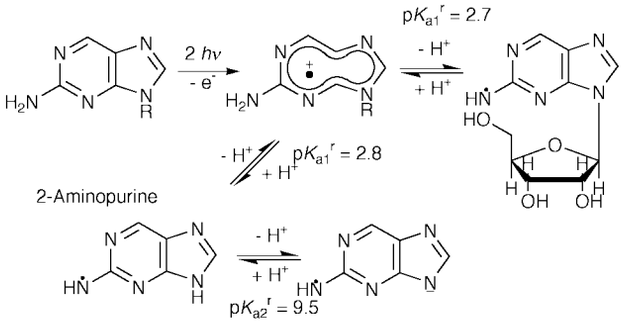


Fig. 3 Relationship between the experimental rate constants, $k_{1,2}$, and the parameters $\lambda_{1,2}$, at the different OH^- concentrations. The solid lines show the best fits to the k_1 (closed circles), k_2 (open circles) data points using the parameters $\lambda_{1,2}$ calculated from eqn. (6). The insert shows the kinetics of the transient absorbance at 525 nm induced by 308 nm XeCl excimer laser pulse excitation (70 mJ pulse^{-1} cm^{-2}) of 2-aminopurine in oxygenated 20 mM borate buffer solution (pH 9.5).

Discussion

Acid–base equilibria and redox reactions in aqueous solutions of 2AP and 2APr radicals

Electron removal from purines greatly increases their Brønsted acidity.[10] The purine radical cations generated by photoionization, or by reaction with strong oxidants (*e.g.*, $SO_4^{\bullet -}$, Tl(III), $Br_2^{\bullet -}$), rapidly deprotonate in neutral aqueous solutions.[10] For instance, removal of one electron from adenines (6-aminopurines), induces a rapid deprotonation of the radical cation and the formation of an N^6-centered neutral radical.[10a–c] These deprotonation reactions are generally accompanied by changes in the absorption spectra. However, in the case of the adenosine neutral radicals, $A(-H)^{\bullet}$, no change in absorbance is observed in the pH range 1–13, suggesting that the pK_a^r of the adenosine radical cation, $A^{\bullet +}$, is ≤1.[10c] Correspondingly, the $2AP^{\bullet +}$ and $2APr^{\bullet +}$ radical cations should also deprotonate thus forming the neutral radicals $2AP(-H)^{\bullet}$ and $2APr(-H)^{\bullet}$ (Scheme 1).



Scheme 1

From changes in the transient absorption spectra, and from the first inflection points in the plots of A_{355} *vs.* pH (Fig. 2A), the pK_{a1}^r values of 2.8 ± 0.2 ($2AP^{\bullet +}$) and of 2.7 ± 0.2 ($2APr^{\bullet +}$) were determined for the deprotonation of the aromatic 2-aminopurine radical cations. Thus, $2AP^{\bullet +}$ and $2APr^{\bullet +}$ are weaker Brønsted acids than the adenosine radical cation, $A^{\bullet +}$.

The $2AP(-H^{\bullet})$ radical, in contrast to the the $2APr(-H)^{\bullet}$ radical, has a proton at the N^9 position. We propose here that deprotonation from this N^9 position of $2AP(-H^{\bullet})$ (Scheme 1) gives rise to the second acid–base equilibrium with pK_{a2}^r =

9.5 ± 0.2 (Fig. 2B). Consistent with this interpretation, this acid–base transition is not observed in the case of the 2AP*r*(−H)$^{\bullet}$ radical (Scheme 1).

The reaction of hydrated electrons [eqn. (1)] with molecular oxygen, generates the radicals[15,16] $HO_2^{\bullet}$ or $O_2^{\bullet -}$. The 2AP$^{\bullet +}$ or 2AP*r*$^{\bullet +}$ radical cations could, in principle, react with $HO_2^{\bullet}$ or $O_2^{\bullet -}$. However, the pK_{a1}^{r} values of the 2AP$^{\bullet +}$ and 2AP*r*$^{\bullet +}$ radical cations are ~2.8, while the pK_a^{r} value of the $HO_2^{\bullet}$ radical is 4.69.[16] These acid–base properties do not favor the reactions between the 2AP$^{\bullet +}$ and 2AP*r*$^{\bullet +}$ radical cations and the $O_2^{\bullet -}$ radical anions. However, in the pH range of 4.7 < pH < 9.5 reactions on μs–ms time scales between the 2AP(−H)$^{\bullet}$ and 2AP*r*(−H)$^{\bullet}$ neutral radicals and $O_2^{\bullet -}$ and oxygen[13] are possible and can serve as a source of hydroperoxides. In oligonucleotides, their possible contributions to the complex chemistry that leads to the low-yield, long-distance strand cleavage,[4] needs to be evaluated. However, this topic was beyond the scope of this work.

Our previous studies[4] have shown that 2AP(−H)$^{\bullet}$ is an oxidant which selectively oxidizes guanines (as expected for a radical with an electron spin density on the nitrogen atom[17]), but does not oxidize any of the other nucleic acid bases (A, C and T). For instance, 2AP(−H)$^{\bullet}$ oxidizes guanine derivatives in solutions of free nucleotides, or in 2AP modified oligonucleotides; in oligonucleotides these processes culminate in a G-selective alkalilabile strand cleavage at a distance.[4]

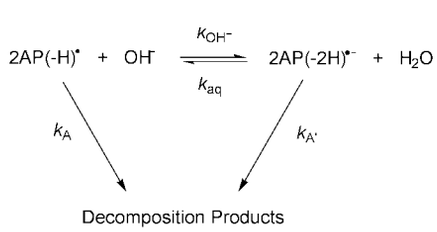
The analysis of the kinetics of guanine oxidation by 2AP(−H)$^{\bullet}$ has shown that, at pH 7, the redox potential of 2AP(−H)$^{\bullet}$, $E_7 = 1.23$ V *vs.* NHE. The dependence of the redox potential on pH, E_{pH}, is given by eqn. (3),[18] where p$K_{a1} = 3.8$,

$$E_{pH} = E^0 + 0.059 \log \{(K_{a1}K_{a2} + K_{a1}10^{-pH} + 10^{-2pH})/(K_{a1}^{r}K_{a2}^{r} + K_{a1}^{r}10^{-pH} + 10^{-2pH})\} \quad (3)$$

and p$K_{a2} = 9.9$ for 2AP,[11] and p$K_{a1}^{r} = 2.8$ and p$K_{a2}^{r} = 9.5$ for the 2AP radicals. Using these values and eqn. (3), a value of $E^0 = 1.34$ V *vs.* NHE is obtained. This value of E^0 for the 2AP$^{\bullet +}$/2AP redox couple is less than the $E^0 = 2.03$ V *vs.* NHE value for the A$^{\bullet +}$/A redox couple.[19] Hence, the electron affinity of the adenosine radical cation is larger than the electron affinity of the 2AP radical cations. This decrease in the electron affinity correlates with the reduction in the Brønsted acidity of the 2AP radical cations (see above).

Analysis of the reaction kinetics of the deprotonation of 2AP(−H)$^{\bullet}$ radicals

The effect of OH^- concentration on the deprotonation rates of 2AP(−H)$^{\bullet}$ radicals is typical of reversible reactions with hydroxy anions and can be described by Scheme 2. In this



Scheme 2

Scheme, 2AP(−H)$^{\bullet}$ radicals can react with OH^- anions with a bimolecular rate constant, k_{OH^-}, and the 2AP(−2H)$^{\bullet -}$ radicals formed can be hydrated with a pseudo-first order rate constant, k_{aq}. Both the 2AP(−H)$^{\bullet}$ and the 2AP(−2H)$^{\bullet -}$ radicals can also decay by other, competitive reactions, with pseudo-first order rate constants k_A and $k_{A'}$, respectively. Based on Scheme 2, the decay rate constants of 2AP(−H)$^{\bullet}$ and 2AP(−2H)$^{\bullet -}$ are defined by $X = k_A + k_{OH^-}[OH^-]$, and $Y = k_{A'} + k_{aq}$, respectively.

The solution of the standard differential equations derived from Scheme 2, yield analytical expressions for the time dependence of the radical concentrations following excitation with a laser pulse at $t = 0$; the width of the laser pulse is assumed to be short relative to the time scale of evolution of the radical concentrations. These expressions are given by eqns. (4) and (5), with eigenvalues given by eqn. (6).

$$[2AP(-H)^{\bullet}] = A_1 \exp(-\lambda_1 t) + B_1 \exp(-\lambda_2 t) \quad (4)$$

$$[2AP(-2H)^{\bullet -}] = A_2 \exp(-\lambda_1 t) + B_2 \exp(-\lambda_2 t) \quad (5)$$

$$\lambda_{1,2} = [X + Y \pm \{(Y - X)^2 + 4 k_{aq} k_{OH^-}[OH^-]\}^{1/2}]/2 \quad (6)$$

Eqns. (4) and (5) can be rewritten as eqns. (7) and (8), where $[2AP(-H)^{\bullet}]_{t=0} = [2AP(-H)^{\bullet}]_0$, and $[2AP(-2H)^{\bullet -}]_{t=0} = 0$ are the initial concentrations of the radicals.

$$[2AP(-H)^{\bullet}] = \{[2AP(-H)^{\bullet}]_0/(\lambda_2 - \lambda_1)\}\,[(\lambda_2 - X)\exp(-\lambda_1 t) + (X - \lambda_1)\exp(-\lambda_2 t)] \quad (7)$$

$$[2AP(-2H)^{\bullet -}] = \{k_{OH^-}[OH^-][2AP(-H)^{\bullet}]_0/(\lambda_2 - \lambda_1)\}\,[\exp(-\lambda_1 t) - \exp(-\lambda_2 t)] \quad (8)$$

The transient absorbance at 525 nm is given by eqn. (9), where ε_A and $\varepsilon_{A'}$ are the molar extinction coefficients of 2AP(−H)$^{\bullet}$ and 2AP(−2H)$^{\bullet -}$ radicals at 525 nm. Using eqns. (7) and (8), eqn. (9) is rearranged to give eqn. (10).

$$A_{525}(t) = \varepsilon_A [2AP(-H)^{\bullet}] + \varepsilon_{A'} [2AP(-2H)^{\bullet -}] \quad (9)$$

$$A_{525}(t) = \{[2AP(-H)^{\bullet}]_0/(\lambda_2 - \lambda_1)\}\,\{\varepsilon_A(\lambda_2 - X) + \varepsilon_{A'} k_{OH^-}[OH^-]\}\exp(-\lambda_1 t) + \{[2AP(-H)^{\bullet}]_0/(\lambda_2 - \lambda_1)\}\,\{\varepsilon_A(X - \lambda_1) - \varepsilon_{A'} k_{OH^-}[OH^-]\}\exp(-\lambda_2 t)\} \quad (10)$$

Eqn. (10) indicates that the transient absorbance is described by the sum of two exponential terms. Therefore, the simplified eqn. (2) is suitable for describing the experimentally observed transient absorbance curves at 525 nm.

The effect of OH^- concentration on the experimental rate constants, $k_{1,2}$, obtained by fitting eqn. (2) to the experimental kinetic curves, is shown in Fig. 3. The relationships between the experimentally measured rate constants, $k_{1,2}$ [eqn. (2)], and the parameters $\lambda_{1,2}$ [eqn. (6)], are that k_1 corresponds to λ_1 [the plus sign in eqn. (6)], and that k_2 corresponds to λ_2 [the minus sign in eqn. (6)]. The parameters $\lambda_{1,2}$ include four rate constants, k_A, $k_{A'}$, k_{OH^-} and k_{aq}. The rate constants $k_A = (3 \pm 0.5) \times 10^4$ s^{-1} and $k_{A'} = (1 \pm 0.2) \times 10^4$ s^{-1} were estimated from the experimental kinetic curves recorded at pH values where the equilibrium is shifted towards the 2AP(−H)$^{\bullet}$ radical (pH 7) or the 2AP(−2H)$^{\bullet -}$ radical (pH 12). These k_A and $k_{A'}$ values were used in the fitting procedures. The values $k_{OH^-} = (1.4 \pm 0.2) \times 10^{10}$ M^{-1} s^{-1} and $k_{aq} = (3.4 \pm 0.5) \times 10^5$ s^{-1} were obtained from the best fit of eqn. (6) (solid line) to the experimental data points, $k_{1,2}$ (see, Fig. 3). The value of p$K_{a2}^{r} = 9.4$ calculated from the ratio of k_{OH^-}/k_{aq} is close to p$K_{a2}^{r} = 9.5$ estimates from the second inflection point of the pH curve (Fig. 2B).

Conclusion

The acid base equilibria of radical cations and neutral radicals of 2AP and 2AP*r* formed by deprotonation of the radical cations can be studied by transient absorption spectroscopy techniques. The radical cations can be conveniently generated by two-photon induced photoionization of the aromatic residues employing 308 nm laser pulses ~12 ns in duration. These results are useful for studying the redox properties of 2-aminopurine residues incorporated site-specifically into oligonucleotides.

Acknowledgements

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