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The 254 nm low intensity and 266 nm laser photochemistry of adenosine Effect of pH and concentration on the reactive precursors of the principal products, adenine and FAPyAde

Carlos E. Crespo-Hernández, Lydia Martínez, Aurea E. González-Sierra, Lizbeth Robles-Irizarry, Arnaldo Díaz-Vázquez, Rafael Arce*

Department of Chemistry, University of Puerto Rico, Río Piedras Campus, P.O. Box 23346, San Juan 00931-3346, Puerto Rico

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Abstract

The 254 nm low intensity steady-state photolysis of adenosine in aqueous solutions at different pHs and concentrations was studied. The quantum yield of photodestruction of adenosine decreases as the pH is increased: $(1.7 \pm 0.1) \times 10^{-2}$ (pH 2.6), $(1.20 \pm 0.04) \times 10^{-2}$ (pH 6.5) and $(0.29 \pm 0.01) \times 10^{-2}$ (pH 13.3). For the photodestruction of adenosine and the formation of adenine the quantum yield depends on the initial ground-state concentrations of adenosine and converges to a limiting value of the order of 10^{-3} and 10^{-4}, respectively. The effect of different substrates that react selectively with postulated reactive intermediates of adenosine was studied and the short-lived precursors for the formation of the major products are proposed. For adenine and 4,6-diamino-5-formamidopyrimidine, two of the products formed with the highest yields, their quantum yield of formation were determined under different experimental conditions. Contributions from electron adducts of the base play a major role in *N*-glycosidic bond cleavage for the nucleoside. Increases in pH and concentration result in a decrease in the yield of formation of adenine. The dependence on pH and the electron scavenger experiments provide further evidence for the radical anion or its protonated form to be one of the principal species involved in the process of base release at neutral pH. At an acidic pH a tautomer of the radical cation of adenosine is proposed as the precursor for adenine formation. The relative efficiency of the radical cation of adenosine for initiating the release of adenine at neutral pH is intrinsically low (35–43%) and this correlates with the efficiency reported for other adenine-containing polymers. Furthermore, the photoionization of adenosine using a 266 nm nanosecond laser excitation occurs through a combination of one- and two-photon processes under the experimental conditions used. Reactions of OH radicals and oxygen reactive species may also result in base release as shown in irradiations done in the presence of N_2O and O_2 as additives. For 4,6-diamino-5-formamidopyrimidine, the addition N_2O does not affect its yield, implying that the hydroxyl radicals are not involved in its formation. However, the presence of O_2, Tl^+, Ag^+, OH^-, H^+ or alcohols inhibits its formation.

Keywords: Quantum yield of adenosine's photodestruction; Quantum yield of product's formation; Adenine derivatives; Mechanism of formation; Concentration effects; pH effects; Laser flash photolysis; Photonicity determination

1. Introduction

Photochemical studies of the nucleic acid constituents have concentrated primarily on the reactions of the pyrimidine bases [1,2]. In these studies it has been assumed that the damaging effects of UV radiation on DNA are mainly a result of the chemical modification of these centers. However, in the last two decades, several purine photoproducts in UV-irradiated DNA that could lead to deleterious effects have been reported [3–13]. Except for the initial studies of Canzanelli et al. [14] and Kland and Johnson [15] most of the studies related to the photochemistry of Ade have been done with the base incorporated in dinucleosides or higher-order polymeric structures.

In terms of photoproduct identification, Canzanelli et al. [14] found evidence for the formation of urea and ammonia in the aerobic and anaerobic photolysis of Ado. Kland and Johnson [15] suggested that Ade is converted to uric acid following the sequence:

adenine → hypoxanthine → xanthine → uric acid

Abbreviations: UV, ultraviolet; HPLC, high performance liquid chromatography; Ade, adenine; Ado, adenosine; AMP, 5′-adenosine monophosphate; Guo, guanosine; Gua, guanine; FAPyAde, 4,6-diamino-5-formamidopyrimidine; DMU, 1,3-dimethyluracil; U, uridine

* Corresponding author. Tel.: +1-787-764-0000x2433; fax: +1-787-759-6885.
E-mail address: rarce@goliath.cnnet.clu.edu (R. Arce).

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and found evidence only for the formation of hypoxanthine. Compared with the decomposition of the purine ring, release of orthophosphate has been detected as a minor process in non-degassed solutions of 2′-AMP, 3′-AMP, 5′-AMP and 3′,5′-cyclic AMP upon exposure to far UV light [16]. It was suggested that direct absorption by the weakly absorbing ribose phosphate moiety is responsible for this process. More recently [17] we reported photodestruction yields for the 254 nm irradiation of Ado at room temperature as determined from changes in absorption at 260 nm or using high performance liquid chromatography (HPLC). At least eleven stable photoproducts were found under anaerobic conditions. The major product was isolated and characterized as Ade. Its formation yield was determined to be 4.5×10^{-4} as compared with an Ado photodestruction yield of 2.5×10^{-3}. This product yield is affected by the presence of oxygen and the initial concentration of Ado employed. Smaller Ado photodestruction (0.14×10^{-3}) and base release (0.18×10^{-4}) yields were reported later by Gurzadyan and Görner [18].

In this paper, we report on experiments in which we have determined the photodestruction yields of Ado and the formation yield of Ade and FAPyAde at different pHs and concentrations. In addition, base release and formation of FAPyAde from Ado in the presence of substrates that react selectively with previously identified or postulated short-lived intermediates [19,20] were studied. These results have allowed us to postulate reactive precursors and pathways for the base release process in the 254 nm photolysis of Ado. The results of experiments done with the purpose of clarifying the differences between the reported photodestruction and base release yields [17,18] and on the photonicity of these processes are included.

2. Materials and methods

2.1. Chemicals

Adenosine (Sigma Chemical Co., St. Louis, MO), adenine (Sigma Chemical Co.), 1,3-dimethyluracil (Sigma Chemical Co.), uridine (>99%, Sigma Chemical Co.), 4,5,6-triaminopyrimidine (Sigma Chemical Co.), formic acid (96%, Sigma Chemical Co.), argon (General Gases and Supplies Co., San Juan, Puerto Rico), thallium(I) sulfate (Fisher-certified reagent >99.9%, Cayey, Puerto Rico), silver sulfate (Aldrich Chemical Company Inc., Milwaukee, WI), nitrous oxide (Air Products of Puerto Rico, Guaynabo, Puerto Rico), and oxygen (General Gases and Supplies Co.) were used as received. FAPyAde was synthetized according to the method of Covalieri et al. [21].

2.2. Actinometry and quantum yield determinations

The sample preparation, irradiation conditions and procedures for quantum yield determination have been previously described [17]. Previously [17], the incident photon fluence over the sample was measured using a 0.1 mM aqueous solution of 1,3-dimethyluracil (DMU) following the procedure of Shetlar et al. [22]. In this work, the precision of the measurement of the percent of photodestruction of DMU was improved by using HPLC techniques to follow the changes in DMU concentration. Using a calibration curve, the DMU concentration was calculated quantitatively before and after the irradiation. Therefore, in the calculation of the incident fluence intensity [22] the correction for the fraction of hydrate formed is not needed. The quantum yield values of Ado and Ade at neutral pH are presented in Table 1. The use of HPLC techniques in the actinometry determinations revealed that in those instances in which there was less than 20% photodestruction of DMU, the change in concentration can be directly determined by observing changes in absorbance without correction for the fraction of hydrate formed. At these low photoconversions, calculated photon fluence by both methods agrees. The photon fluence was also determined by using uridine following the decrease in its concentration by HPLC methods. The results were almost identical (data not shown). The same HPLC conditions used for the separation of Ado were employed (Section 2.4). All the quantum yields determined in this work were obtained using the dosimetry

Table 1
Room temperature quantum yields for Ado photodestruction and Ade and FAPyAde formation in the photolysis of Ado aqueous solution under different experimental conditions[a]

Additives	φ(Ado) $\times 10^2$	φ(FAPyAde) $\times 10^3$	φ(Ade) $\times 10^3$
N_2, [Ado] = 1 mM	0.30 ± 0.01	ND[b]	0.81 ± 0.02
N_2, [Ado] = 0.5 mM	0.42 ± 0.03	ND	0.76 ± 0.02
N_2, [Ado] = 0.1 mM	0.53 ± 0.01	ND	1.25 ± 0.02
N_2, [Ado] = 50 μM	1.20 ± 0.04	1.1 ± 0.5	2.1 ± 0.1
N_2, [Ado] = 10 μM	3.3 ± 0.1	ND	5.2 ± 0.3
[Ado] = 50 μM, 0.0013 M O_2	1.6 ± 0.4	0	1.3 ± 0.2
[Ado] = 50 μM, 0.027 M N_2O	1.08 ± 0.03	0.96 ± 0.04	1.6 ± 0.2
N_2, [Ado] = 50 μM, 0.05 mM Tl_2SO_4	0.45 ± 0.04	0	0.90 ± 0.08
N_2, [Ado] = 50 μM, 1 mM Tl_2SO_4	0.32 ± 0.03	0	0.73 ± 0.04
N_2, [Ado] = 50 μM, 0.085 mM Ag_2SO_4	0.37 ± 0.01	0	ND

[a] These values are the average of three independent determinations.
[b] Not determined.

method described by Johns [23] and explained elsewhere [24,25].

2.3. Laser irradiation conditions and data analysis

A Nd:YAG Q-switched Surelite II Laser from Continuum was used. Transient signals were recorded with a Le Croy Digital Oscilloscope (model 9360). Data acquisition and analysis were done with a National Instrument Lab View program and a Pentium/300 MHz PC. The net photoejection electron yield was determined using KI (0.16 M) as a standard ($\varphi = 0.36$) [26]. Due to the small dependence of the absorbance at 700 nm (due to the hydrated electron) with pulse energy at 266 nm, the net photoionization yield was calculated from the slope of a least square graph of the electron absorbance as a function of the pulse energy (10% estimated error, $r^2 = 0.99$). The laser irradiation conditions were established and data analysis conducted as reported previously [24].

2.4. Chromatographic analysis

Chromatographic analyzes of the photoproducts of Ado were achieved by injecting 150 μl of the photolyzed solution into a Waters HPLC model ALC-202 equipped with a solvent delivery system, model 501. A Rheodyne universal injector model 7125 with a loop of 100 μl was used. The absorption spectra of the photoproducts and the quantification of the fractions were obtained by using a Waters programmable photodiode array detector model 991 (Waters Associates, Milford, MA). This system was computer controlled by a Millennium chromatography workstation (Waters Associates, Milford, MA). A LC-18 Jupiter column (25.0 cm × 4.6 cm i.d., Phenomenex, CA) was employed using a solvent composition of 90% water and 10% methanol from 0 to 15 min and a 1 ml/min flow rate.

2.5. Emission spectra

Emission spectra were recorded using a PTI spectrofluorometer with FELIX 1.3 software. Front-face detection of the emission was used. The fluorescence intensities were not corrected for detector response, and spectra are presented as directly observed unless otherwise indicated.

3. Results

3.1. Quantum yields of photodestruction and main products formation: additives and concentration effects

To explain the differences in the reported photodestruction and base release quantum yields for Ado [17,18] the following experiments were conducted. Aqueous solutions of Ado, Guo, DMU and U (Table 2) were irradiated on the same day using an identical incident photon fluence as determined from an IL1700 radiometer (International Light Inc.). The concentration of the base at each irradiation time was determined by HPLC techniques. The solutions were degassed using purified nitrogen for 15 min before irradiation and continuously during the irradiation. The relative concentrations of Ado, Guo, DMU and U as a function of the irradiation time are presented in Fig. 1. As depicted in this graph, the initial slopes of the curves for the photodegradation of DMU and U actinometers are approximately five to six times larger than the corresponding slopes for Ado and Guo. The reported quantum yields for U and DMU photodestruction are 1.6×10^{-2} and 1.39×10^{-2}, respectively [27,28]. Thus, the observed differences between the slopes clearly show that the photodestruction yields for the purine bases are one-fifth or one-sixth of the reported yield for the actinometers and not a factor of 10^{-2} smaller as reported previously [18]. The calculated quantum yields using the actinometers are presented in Tables 1–3.

Table 2
Comparison of the quantum yields for the photodestruction of Ado, Guo, DMU and U in molecules/photon[a]

System	$\varphi \times 10^2$
[Ado] = 85 μM	0.25 ± 0.04
[Guo] = 85 μM	0.26 ± 0.04
[DMU] = 100 μM	1.39 ± 0.05[b]
[U] = 100 μM	1.6[c]

[a] These values are the average of at least four independent determinations.
[b] Taken from [28].
[c] Taken from [27].

Previously [17] we characterized the formation of Ade, one of the major product in the photolysis of Ado. Herein, the quantum yield of Ade formation in the presence of several additives using low intensity 254 nm irradiation was determined (Table 1). Furthermore, the absorption spectrum of the fraction with retention time 3.8 min (Fig. 2) was

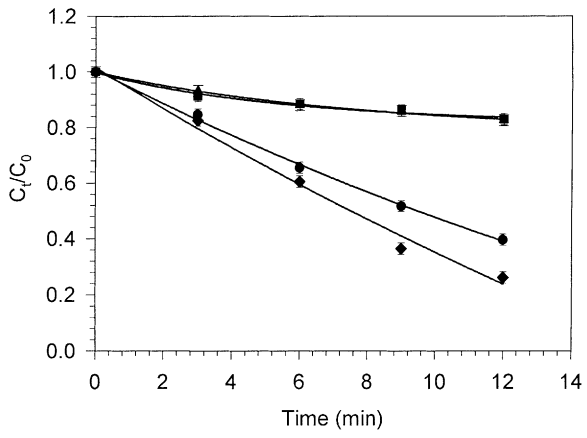


Fig. 1. Relative change in the concentration of (▲) Ado, (■) Guo, (●) DMU and (◆) U as a function of the irradiation time at 254 nm.

Table 3
Dependence of the quantum yields of Ado photodestruction and Ade and FAPyAde formation with pH in irradiated 50 μM aqueous solutions of Ado at 298 K[a]

pH conditions (N_2)	φ(Ado) × 10^2	φ(FAPyAde) × 10^3	φ(Ade) × 10^3
2.6	1.7 ± 0.1	0	4.3 ± 0.9
6.5	1.20 ± 0.04	1.1 ± 0.5	2.1 ± 0.1
13.3	0.29 ± 0.01	0	0

[a] These values are the average of three independent determinations.

identical to that of FAPyAde. The identity of this product was further confirmed by co-injection of FAPyAde to an irradiated solution of Ado; this resulted in an increase in the total area of the fraction with retention time of 3.8 min (data not shown). As for the case of Ade, its formation quantum yield under different conditions was determined (Tables 1 and 3).

The chromatogram obtained for a photolyzed 50 μM aqueous solution of Ado after 20 min of irradiation under anaerobic conditions using a 254 nm low intensity mercury lamp is presented in Fig. 2a. For comparison, the HPLC

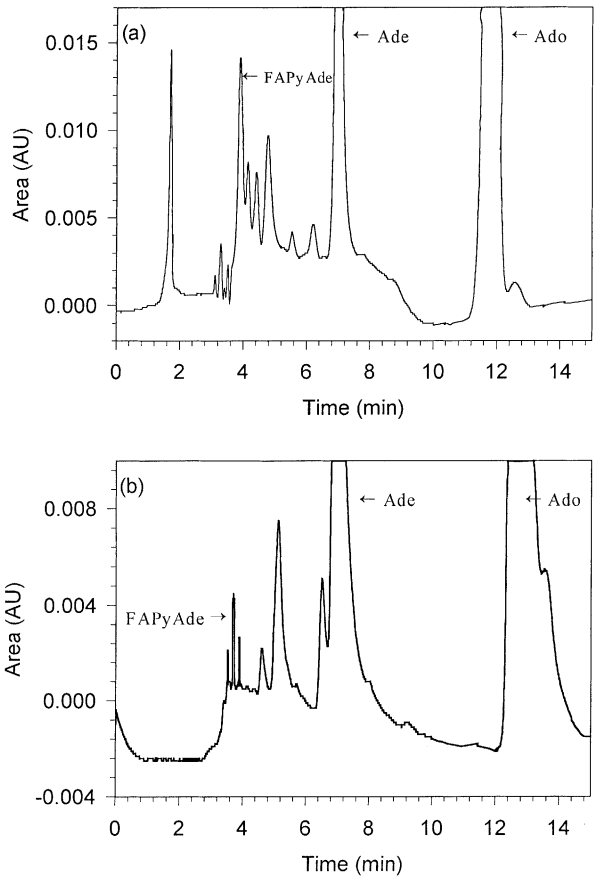


Fig. 2. (a) HPLC chromatogram (detection at 260 nm) obtained from the 254 nm photolysis of a 50 μM Ado aqueous solution at neutral pH after 20 min of low intensity steady-state irradiation under nitrogen saturated conditions. (b) Chromatogram obtained for the photodegradation of Ado after 15 min of 266 nm laser irradiation using a pulse energy of 10 mJ.

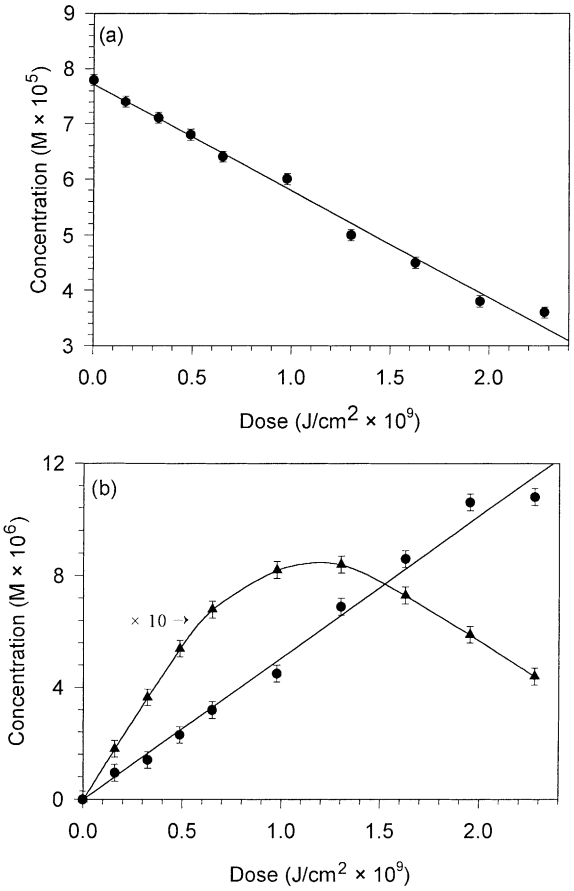


Fig. 3. Dependence of the concentration of (a) 79 μM Ado aqueous solution and (b) formation of Ade (●) and FAPyAde (▲) on the 266 nm laser radiation dose using a pulse energy of 10 mJ and a repetition rate of 10 Hz (11 ns pulses).

chromatogram obtained from the 266 nm laser photolysis (at ≈10 mJ per pulse) of a 50 μM aqueous solution of Ado at neutral pH after 15 min of irradiation at a pulse rate of 10 Hz under nitrogen saturated conditions is presented in Fig. 2b. From these chromatograms one can conclude that the principal products formed by laser excitation are similar to those produced in the 254 nm steady-state experiments. Fig. 3 shows that the dependence of the concentration of Ado and Ade on 266 nm laser radiation doses follow a linear relation under the experimental conditions used in this work. For FAPyAde, an initial linear increase is observed at laser irradiation doses up to 5×10^8 J/cm^2 followed by a depletion at higher doses. The above results imply that the photochemical processes occurring at the photon fluences used in the steady-state excitation at 254 or 266 nm laser irradiation of Ado are similar and lead to the same major photoproducts.

The effect of O_2, N_2O, Tl^+ and Ag^+ on the yields of photodestruction of Ado and on the formation of Ade and FAPyAde are summarized in Table 1. At concentrations in the range of 0.05–1 mM, Tl^+ and Ag^+ will scavenge the hydrated electron ($k = 5.4 \times 10^{10}$ and 3.2×10^{10} M^{-1} s^{-1},

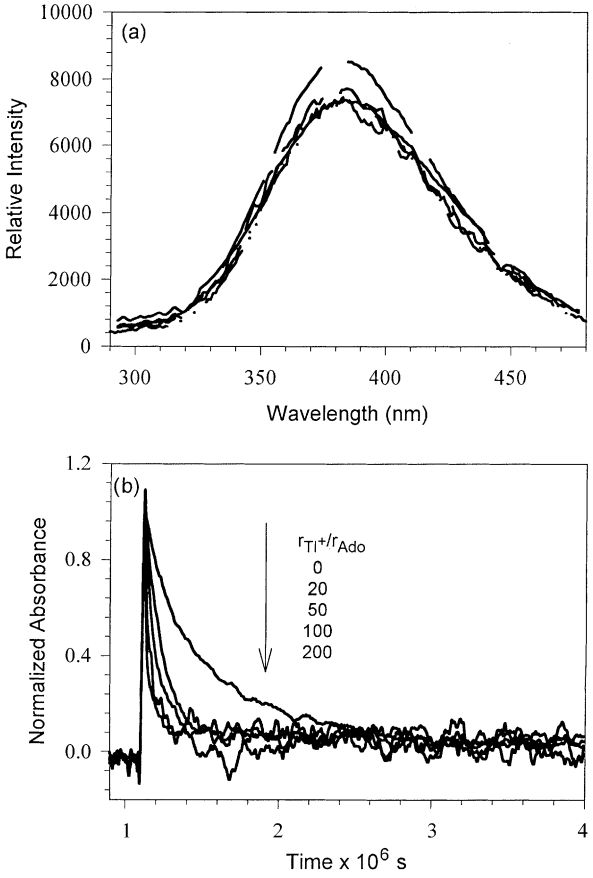


Fig. 4. Effect of Tl^+ concentration: (a) on the 50 μM AMP emission in aqueous solution; (b) on the transient decays of the hydrated electron (700 nm) of a 50 μM Ado aqueous solution upon the 266 nm nanosecond laser flash excitation.

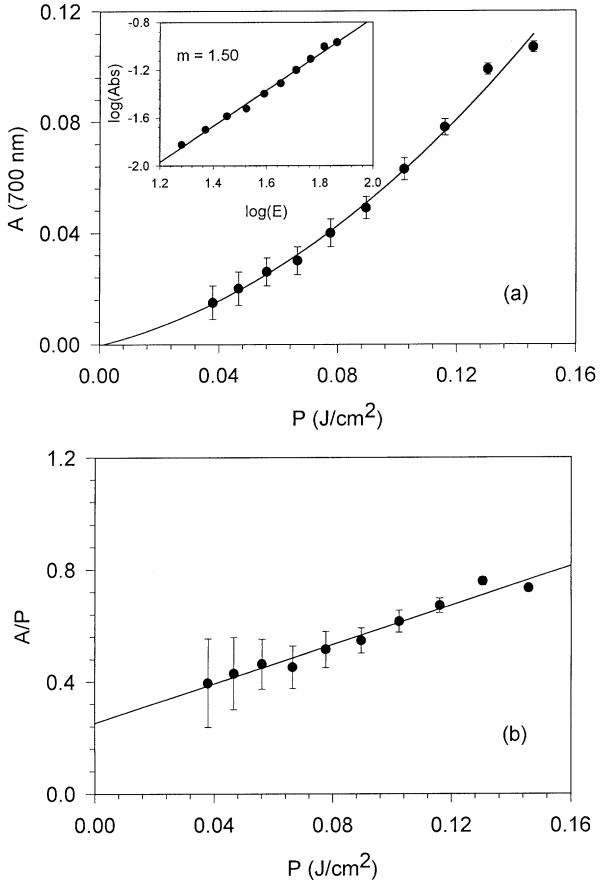


Fig. 5. (a) Hydrated electron absorbance at 700 nm as a function of the pulse energy (λ_{exc} = 266 nm, 11 ns pulses); inset, hydrated electron absorbance as a function of the laser energy in a logarithmic scale. (b) Absorbance of the hydrated electron divided by the pulse energy (A/P) as a function of the pulse energy.

respectively [29]) at a rate of 100–200 times higher than Ado ($k = 1.2 \times 10^{10}\,M^{-1}\,s^{-1}$) [29]. At the highest concentration used, the absorbance for both additives at 254 nm was less than 0.04. An increase in the Tl^+ concentration did not have an appreciable effect on the emission spectra of 50 μM AMP solution, implying that this additive does not quench the singlet excited state of Ado (Fig. 4a). The nucleotide was used since the fluorescence yield of Ado in aqueous solutions is too small to conduct the measurements. Thus, the observed decrease in the formation yield of Ade in the presence of an electron scavenger (Tl^+) was assumed to be due to a decrease in the precursor of the species that leads to the base release. In O_2 saturated solutions a 25% increase in the photodestruction of Ado was observed. The quantum yields of photodestruction for Ado and of formation for Ade obtained in the presence of N_2O, an efficient electron scavenger were, respectively, three- and two-fold higher than those obtained for Tl^+. This suggests the participation of other species such as the OH radicals (Eq. (1)) in the photodestruction of Ado and in the formation of Ade at neutral pH in the presence of N_2O. At the concentration of N_2O employed, this scavenger would react with the hydrated electron at a relative efficiency of 200:1 compared with Ado:

$$N_2O + e^-_{aq} + H_2O \rightarrow N_2 + OH^\bullet + OH^- \quad (1)$$

The electron participation in the photochemistry of Ado was reconfirmed by doing 266 nm laser irradiations. The effect of the Tl^+ concentration on the electron decay rates at 700 nm of 50 μm Ado aqueous solution is presented in Fig. 4b corroborating its participation as an efficient electron scavenger. The photonicity of the electron photoejection process for Ado was determined from the slope of a plot of the initial electron absorbance (after the laser pulse) as a function of the laser dose (Fig. 5a). As a first approximation, a linear function could fit the data ($r^2 = 0.99$), although it does not pass through zero. A photoionization yield of 0.023 ± 0.003 was obtained.

Further analysis of the hydrated electron data showed that these fit an equation of the form [30]

$$\frac{A}{P} = a + bP \quad (2)$$

where A is the absorbance of the hydrated electron, P the pulse energy, the coefficient a depends on the quantum yield

of the one-photon process while *b* depending on the molar absorption coefficients and yields of the intermediate species in the consecutive two-photon process [31]. Fig. 5b shows that a mixture of mono- and biphotonic pathways are involved in the formation of the hydrated electrons (note the non-zero intercept). From the intercept, a one-photon quantum yield of 0.006±0.004 at neutral pH was determined. The calculated monophotonic yield for Ado at 266 nm is in very good agreement with that estimated under the assumption that the decrease in the quantum yield of photodestruction in the presence of the scavengers can be related to the electron yield at 254 nm (0.008 ± 0.002). At the pulse energies used, the main component is due to a biphotonic process (see also Fig. 5a, inset). This result corroborates that in the 266 nm photoionization of Ado a monophotonic pathway [17,19,20] is present, although a principal biphotonic component is also observed [32–35]. Mixtures of a one- and two-photon mechanisms have also been reported for several organic molecules [31,36,37]. In the low intensity 254 nm steady-state irradiations, the only pathway from which the photoionization can occur is through the monophotonic component.

The formation of FAPyAde was not observed when O_2, Tl^+ or Ag^+ were present in the solution of Ado. In addition, its formation appears not to be affected by the presence of N_2O. Similar results have been obtained recently [38] for the formation of FAPyAde and other monomeric photoproducts of DNA upon 254 nm irradiation in the presence of other hydroxyl radicals scavengers. Addition of 0.5 M methanol or isopropanol inhibits the formation of FAPyAde in aqueous solution of Ado under anaerobic conditions.

An increase in the concentration of the purine bases results in a dramatic decrease in the quantum yield of photodestruction of the bases [17,24]. This reduction in the yield was explained in terms of self-quenching and aggregate effects that deactivate the bases' excited states. Similarly, the photodestruction of Ado and the formation of Ade decrease with an increase in the Ado concentration (Table 1). Experiments were not done to test if the formation of FAPyAde is affected by an increase in Ado concentration.

3.2. Effect of pH on the photodestruction of adenosine, the formation of adenine and FAPyAde, and the changes in the absorption spectra during steady-state photolysis

The effects of pH on the quantum yield of photodestruction of Ado and on the formation of Ade and FAPyAde are summarized in Table 3. At these different pHs, Ado can exist in the neutral, protonated ($pK = 3.45$) or deprotonated ($pK = 12.5$) forms [39]. A decrease in the photodestruction yield of Ado is observed with an increase in pH. A decrease in the quantum yield of Ade formation is noticeable as the pH increases, dropping to zero at pH of 13.3. Interestingly, the formation of FAPyAde is only observed at a neutral pH.

The changes in the absorption spectra at different pHs during the 254 nm photolysis of Ado are presented in Fig. 6. At neutral pH (6.5) the absorption spectra show a decrease

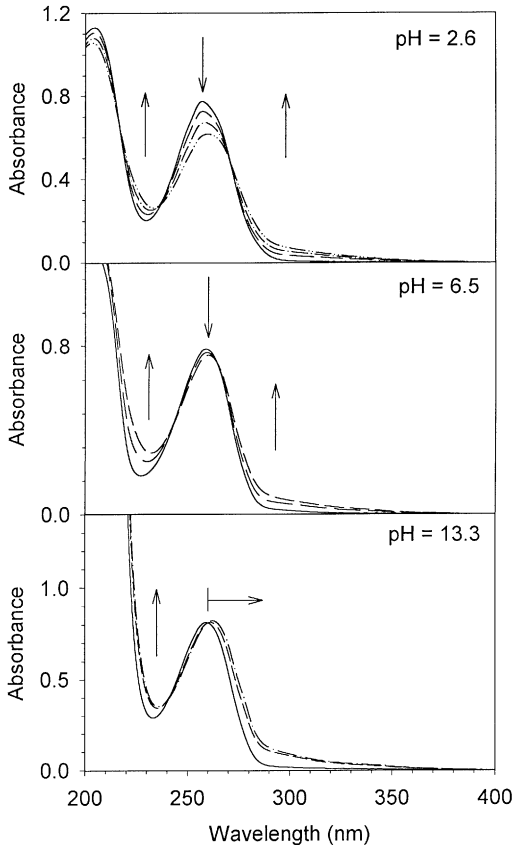


Fig. 6. Changes in the absorption spectra observed during the 254 nm irradiation of a 50 μM Ado aqueous solution under anaerobic conditions at different pHs.

in the intensity of the absorption band at 260 nm with an increase in the irradiation time. The increase in absorption at several wavelength regions and the formation of isosbestic points are considered evidence of the phototransformation of Ado into products that absorb at the same wavelength region. Irradiated solutions at pH = 2.6 show a decrease in the intensity of the 260 nm band with irradiation time and an increase in absorbance at 220–240 and 275–375 nm wavelength regions. At a basic pH (13.3), the wavelength of maximum absorbance shifts to a higher wavelength with an increase in irradiation (Fig. 6). Based on the changes in the absorption spectra, the number of products observed, and the differences in their absorption properties at different pHs, as determined from the HPLC analysis of Ado-irradiated solutions (data not shown), major differences in the photochemical behavior of Ado at different pHs can be expected.

4. Discussion

Absorption of light at 254 nm by Ado excites both the (π, π^*) and the (n, π^*) transitions of the bases [40,41]. The decrease in the main absorption band during the 254 nm steady-state photolysis implies the photodegradation of the

conjugated π system of Ado as one destruction path. The increase in absorption in certain wavelength regions and the presence of three isosbestic points is explained in terms of the formation of absorbing products at these wavelengths. Results from the HPLC measurements provide evidence of the similarity in absorption properties of many stable products of Ado (data not shown), suggesting that in most of the transformations the aromatic character and main chromophore characteristics are retained.

In addition to adenine ($R_t = 7.1$ min) another major photoproduct in the 254 nm photolysis of Ado is the one with a retention time of 3.8 min (Fig. 2). This product was characterized as FAPyAde through standard addition with an authentic sample of FAPyAde and based on its absorption spectra. The formation of FAPyAde has been observed in aqueous solutions of Ado [42] and Ade [43,44] subjected to ionizing radiation and as a monomeric photoproduct in photolyzed DNA solutions with 254 nm low intensity radiation [38]. Ade and FAPyAde are two of the major photoproducts in the 254 nm photochemistry of Ado since their formation yields correspond to 27% of the photodestruction yield of Ado at neutral pH. If the other products have similar molar absorption coefficients at 254 nm, these account for another 72% of the destruction of Ado.

Our laser photolysis results (Fig. 5) show that the 266 nm nanosecond laser excitation of Ado aqueous solutions leads to its photoionization through a combination of one- and two-photon processes. The contribution from the monophotonic pathway to the photoejected electron is 25%. In addition, data from the room temperature microsecond flash photolysis of various Gua and Ade derivatives and from 266 nm nanosecond laser flash photolysis [19,20,24] are evidence of the photoionization of these bases as one of the primary process and the participation of the solvated electron in the photodestruction mechanism (Eqs. (3) and (4)):

$$\text{Ado} + nh\nu \rightarrow \text{Ado}^{\bullet+} + e^-_{aq}, \quad n = 1 \text{ or } 2 \tag{3}$$

$$\text{Ado} + e^-_{aq} \rightarrow \text{Ado}^{\bullet-} \tag{4}$$

Several investigators have presented evidence for the photoionization of DNA and RNA polymeric structures using 266 nm nanosecond laser excitation and concluded that this occurs through a multiphotonic or biphotonic mechanism [32–35]. Nonetheless, there is already evidence that the formation of various adenine, guanine, and thymine monomeric products in DNA, typical of nucleic acids radical cations can be produced by exposure to low intensity 254 nm radiation [38]. Also, theoretical calculations have shown that the ionization threshold of 5′-dGMP$^-$, 5′-dAMP$^-$, 2′-dThd$^-$, 5′-dTMP$^-$, Urd and 5′-dCMP$^-$ bases in aqueous solution are between 4.7 ± 0.5 and 5.5 ± 0.5 eV [45]. These are close to the energy of a 266 nm photon (4.66 eV), thus supporting the possibility of a monophotonic ionization of these bases. The formation of FAPyAde, which has been proposed to be formed through a free radical mechanism in DNA solutions subjected to ionizing radiation, could also suggest the photoionization of Ado at low intensity UV radiation [46,47].

The rate constant for the reaction of Ado with the hydrated electron (Eq. (4)) was determined, from the electron's decay curve, to be 1×10^{10} M^{-1} s^{-1} [29], close to a diffusion controlled reaction of the hydrated electron. Using conductance and optical detection with nanosecond time resolution, it has been shown [48,49] that the electron adduct of Ado is protonated by water in 5 ns ($k \geq 1.4 \times 10^8$ s^{-1}), Eq. (5):

$$\text{Ado}^{\bullet-} + H_2O \rightarrow \text{Ado}(+H)^{\bullet} \tag{5}$$

The competition experiments using Tl^+ or Ag^+ as electron scavengers suggest that at neutral pH, the electron adduct of Ado is one of the principal precursors in its photodestruction mechanism. The participation of this electron adduct is estimated to be 68%, leaving only 32% for the participation of the radical cation, the triplet state of Ado or other intermediates in the phototransformation process.

A possible route for the formation of the radical species in Eqs. (3) and (4) is via an intramolecular electron transfer from an excited Ado. However, the results from this work and those previously reported [17,24,25] are not consistent with this possibility. For example, an increase in the concentration of Tl^+ from 0 to 0.01 M in a 5.5×10^{-5} M AMP solution does not significantly decrease the fluorescence intensity of AMP (<15%). This shows that quenching of the singlet excited state of AMP by Tl^+ is not occurring. Furthermore, the 266 nm laser photolysis experiments show the presence of the electron formed through a mixture of one- and two-photon processes. The results of the experiments in which electron scavengers were added confirmed the presence of the electron and their reaction with it. Also, in the presence of these additives a decrease in the photodestruction yield is observed corroborating the participation of the hydrated electron in these phototransformation processes. In terms of the effect of increasing the Ado concentration on the photodestruction yield, one would expect that the increase in concentration of Ado will favor an intramolecular electron process instead of the processes depicted in Eqs. (3) and (4). Nonetheless, the observed effect is a decrease in the yield (Table 1), suggesting a lower degree of participation of the charged radicals.

An increase in the pH of solutions of Ado decreased its photodestruction quantum yield (Table 3) contrary to the observed behavior for Guo [24]. A possible explanation for this difference could be the following. Hug and Tinoco have shown that for Ado and Guo the orders of the B_{1u} and B_{2u} transitions in the wavelength interval of 220–280 nm are inverted in neutral and in basic pHs (11.1) [50]. For example, at a basic pH, the order of the two transitions for Ado is (B_{1u}, B_{2u}) while for Guo it is (B_{2u}, B_{1u}). If it is assumed that a much higher probability for intersystem crossing to the triplet excited state from a B_{1u} transition than from the B_{2u} transition occurs, this increase in the yield of the triplet intermediate will facilitate a biphotonic ionization process in the purine bases. The participation of a triplet state in

the 266 nm nanosecond laser photodestruction mechanism of Ado has been postulated at neutral pH [17,20] but not for Guo [24]. This can be considered a indirect evidence of this difference in the probability of intersystem crossing from the B_{1u} and B_{2u} transitions. In addition, these assumptions could explain the formation of the hydrated electron by a combined one- and two-photon pathway at 266 nm nanosecond laser excitation of Ado. On the other hand, a decrease in the probability of the B_{1u} transition or an increase in the B_{2u} transition could lead to an increase in the monophotonic ionization through a non-relaxed singlet state. Thus, since the B_{2u} transition is assumed to be populated with higher probability (contrary to the case of Guo), a lower monophotonic photoionization yield at 254 nm is expected for Ado at basic pH. This leads to a decrease in the photodestruction of Ado with an increase in the pH of the solution. It should be stressed that we do not have direct evidence to support these assumptions.

One of the effects of photoionization at 266 nm is the breakage of the *N*-glycosidic bond or base release of Ado (Fig. 2b) and this process occurs through a one-photon mechanism (Fig. 3b). Recently similar results were obtained [25] for various Gua derivatives. For example, an equal extent of electron adduct participation for Ade and Gua [24] is observed. Also an increase in pH of the solution results in a decrease of the formation yield of Ade and Gua. Thus, a similar mechanism may be operating for base release. These results also support the hypothesis that the proposed photoionization of the base even with low intensity 254 nm irradiation is an important primary process [17,24,25]. If all the electrons generated react with Ado, 76% of the reactions of the electron adducts formed results in the breaking of the *N*-glycosidic bond, the other 24% leads to the formation of other products or to restitution reactions. The 61% decrease in the release of Ade in the presence of Tl^+, an efficient electron scavenger [24], further implies the participation of an electron adduct of the base in the cleavage of the *N*-glycosidic bond.

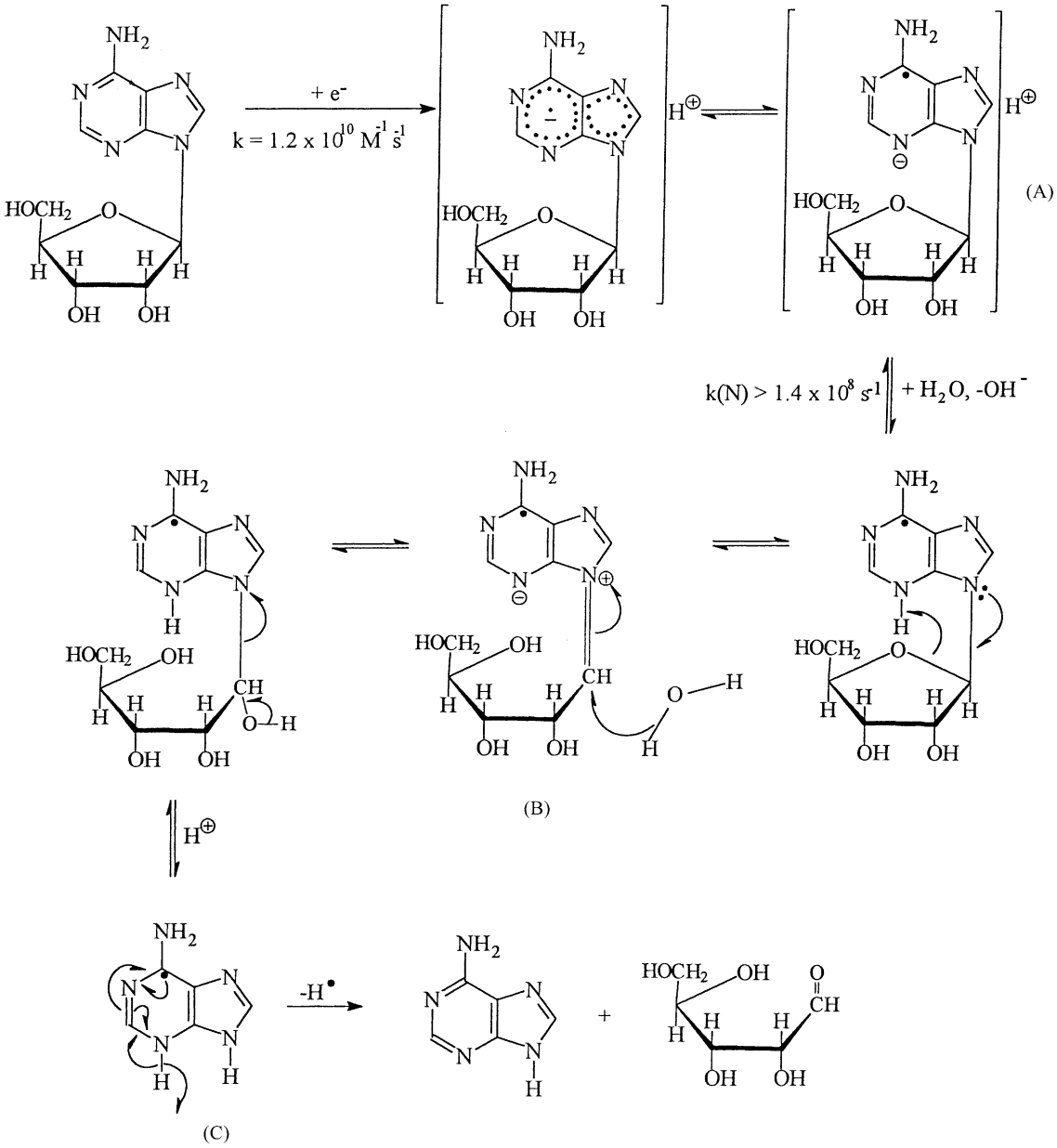
The dependence of the photodestruction quantum yield on pH (Table 3) and the competition experiments with different electron scavengers (Table 1) suggest the participation of the radical anion of Ado in these processes. A probable mechanism of formation of Ade from Ado is presented in Scheme 1. Once the electron adduct is formed, charge stabilization at positions 3 and 6 on the pyrimidine ring leads to the formation of species **A**. Because of the high electron charge density on the nitrogen, this is rapidly protonated ($k > 1.4 \times 10^8\ s^{-1}$) [48,49]. It is known that the presence of a proton near the base that can be easily transferred to the sugar ring, facilitates the breakage of the *N*-glycosidic bond [39], thus forming species **B**. After hydrolysis, the neutral radical **C** rearranges to form the free base due to aromatic stabilization processes. A similar mechanism for the formation of base release from the electron adduct of various Gua derivatives was presented recently [25]. In these terms, we suggest that the mechanism of base release from the purine bases could be considered as a general one, having as precursors similar intermediate species.

At pH 2.6, the yield of formation of Ade is two-fold of that observed at neutral pH. This suggests that Ade is produced from an intermediate species formed in higher yields under acidic conditions. At this pH, if Ado is photoionized at the same rate as at neutral pH, the ejected electron will react preferentially with the hydronium ions. This implies a decrease in the yield of electron adducts and that the destruction path originating from these species would not be available. The observed increase in the yield of Ade is explained by the fact that its precursor under acidic conditions could be a tautomeric species of the Ado radical cation, $Ado(-H)(+H)^{\bullet +}$, formed in higher yields under acidic conditions.

Using high intensity laser radiation, Budovskii et al. [51] observed free bases resulting from the splitting reaction of the *N*-glycosidic bond in Ado and dAdo. This reaction is very efficient since more than half of the degradation products of the nucleosides were the free bases. Base release and strand breakage have also been observed in the 248 nm high intensity laser excitation of poly-A and other polynucleotides [32,34]. In addition, a quantum yield for Ade release of 1.1×10^{-3} in the anaerobic 193 nm laser photolysis of Ado and poly-A has been reported by Gurzadyan and Görner [52].

For polynucleotides, hydrated photoelectrons are assumed not to be involved in the reactions leading to strand breakage since no effect on the quantum yield is observed in the presence of electron scavengers [34]. In part, this could be explained in terms that the incorporation of Ade residues into polymeric structures reduces the rate of reaction of the hydrated electron with the base by 10-fold [29]. Depending on the conformation of the polynucleotide, the accessibility of the scavenger to the immediate surroundings of the base would not allow the scavenger to trap the electron, allowing for the formation of the polynucleotide electron adducts (i.e. hydrophobic or hydrophilic effects for water soluble scavengers). In addition, in these structures the nucleotides are close to one another, and these interactions result in modifications of the excited states [53]. Proton-transfer, energy-transfer and electron-transfer reactions are likely to cause a change in the participation of different intermediate species in higher-order polymeric nucleic acids as compared with the monomeric units in which these transfer processes are less probable. These reactions could result in an increase in the participation of other reactive intermediates such as the radical cation or the triplet state in photochemical reactions. Nevertheless, the evidence presented in this work strongly suggests that electron adducts of Ado lead to the release of Ade more efficiently than the radical cation at neutral pH.

In O_2 saturated conditions, a 44% increase in the formation of Ade was obtained as compared with solutions containing Tl^+. This result suggests that oxygen is not only acting as an electron scavenger in the formation of Ade but also that oxygen reactive species could react with Ado through not yet understood reactions that yield base release.



Scheme 1. A probable mechanism of Ade formation from Ado at neutral pH.

Further evidence for the possible oxygen reactive species reactions with Ado come from the fact that in the presence of oxygen the photodestruction quantum yield of Ado increases by 25% relative to anaerobic conditions (Table 1).

Employing N_2O as an electron scavenger (Eq. (1)) resulted in a two-fold increase in the formation of Ade as compared when using Tl^+. This enhancement should result if one considers the generation of purine-OH radicals as intermediates by Eqs. (6) and (7):

$$Ado^{+\bullet} + H_2O \rightarrow AdoOH^{\bullet} + H^+ \quad (6)$$

$$Ado + HO^{\bullet} \rightarrow AdoOH^{\bullet} \quad (7)$$

or by the formation of radicals at the sugar moiety produced by the direct reaction of OH radicals with this moiety [54].

Recently [38], it was shown that the hydroxyl radicals do not participate in the formation of FAPyAde upon UV irradiation of DNA. Similar results were obtained in this work in the presence of N_2O. This result can be considered additional evidence to the suggestion that the mechanism of formation of FAPyAde upon UV irradiation does not involve the initial reaction of Ado with the OH radicals. Also, the addition of Tl^+, O_2, Ag^+, H^+, OH^- or alcohols inhibits the formation of FAPyAde. The lack of formation of FAPyAde in the presence of O_2 and H^+ have been observed before [42–44]. In acidic solution, deformylation has been suggested to explain the lack of observation of FAPyAde [42]. At basic pH, the absence of FAPyAde could be due to

the observed decrease in ring opening with an increase in pH for Ado [55]. The observation of FAPyAde in N_2O but not in the presence of Tl^+ and Ag^+ suggests that FAPyAde is not formed through the participation of the radical cation or anion of Ado. Furthermore, no production of FAPyAde was observed in experiments where the radical cation of Ado was generated by the reaction of SO_4^- [56] (produced from the UV photolysis of S_2O_8) with Ado (data not shown). In terms of these results we cannot speculate on the nature of the intermediate species precursor to this product. Although the singlet state has been suggested as a candidate for its formation [38], the lack of formation of FAPyAde in the presence of Tl^+ that does not quench the singlet state of AMP, appears to rule out this possibility.

In this work, the dose-dependent increase in FAPyAde was linear up to 5×10^8 J/cm^2 and subsequently deviated from linearity due to its photodestruction at higher doses (Fig. 3b) using 266 nm laser pulse irradiation. For DNA upon irradiation with 254 nm low intensity light, a linear increase of FAPyAde was observed up to a dose of 0.02 J/cm^2; at higher doses its photodegradation was suggested [38]. However, the photolysis was done under an air atmosphere and it is known that the formation of FAPyAde is inhibited under these conditions [42,44]. Thus, the loss of linearity at a much lower dose in the case of DNA could be a consequence of the reaction of the precursors of FAPyAde with oxygen or to the differences in the systems studied.

5. Conclusions

The evidence presented suggests that the electron adducts of Ado release free Ade more efficiently than the radical cation of the base. Pertaining to the photochemistry of DNA, this result implies that in these biomolecules where electron–hole migration occurs over several bases and in which the electron will be trapped mainly by a pyrimidine, the efficiency of Ade release should be intrinsically low. The opening of the imidazole ring and base release are the main UV radiation-induced photodegradation pathway of Ado in deareated aqueous solution. It should be noted that the formation of FAPyAde and other monomeric base damage photoproducts in the low intensity irradiation at 254 nm of DNA could be biologically important components responsible for the deleterious effect of UV radiation, as suggested recently [38].

The 266 nm laser excitation promotes the photoionization of Ado through a mixture of one- and two-photon mechanisms; the biphotonic pathway been the predominant (75%). The calculated photoionization yield from the one-photon component at 266 nm laser excitation is in very good agreement with the one obtained at 254 nm low intensity irradiation. Photoejection of the electron accounts for 68% of the destruction of the base, and reactions of the electron to form an Ado electron adduct promote its degradation. The electron adducts of Ado account for 61% of the Ade produced. The unassigned 39% of the formation of Ade could result from the contributions of the radical cation, ^{3}Ado and/or the excited singlet state. Thus, the contribution of the radical cation of Ado to the release of free Ade is not greater than 39% efficiency per ionizing event. Reactive species of O_2 may participate in the photodestruction of Ado and the formation of Ade. For FAPyAde, further investigation of the mechanistic aspects of its formation are needed.

Concerning the photochemistry of the purine bases, at a similar concentration (5×10^{-5} M), Ado and Guo show similar photodestruction yields (see also Fig. 1). Also, their electron adducts are one of the principal species participating in their photodestruction mechanism. An increase in concentration of these purine bases decreases their photodestruction and base release yields, suggesting that the formation of aggregates and self-quenching effects at the concentrations used could deactivate their excited states. For both bases, the relative efficiency of the radical cation for initiating the release of the free base depends on the pH of the solution, decreasing with an increase in pH. Furthermore, the formation of base release for Ado and Guo seems to have similar precursors, suggesting that the mechanism of base release for the purine bases could be generalized. In addition, the participation of the electron adduct in the formation of base release in Ado is equal to that observed for Guo (61%). All these observations suggest that the photochemistry of the purine bases at neutral pH and with irradiation at 254 nm are similar and their principal phototransformations could be generalized for both bases.

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References

[1] S.Y. Wang (Ed.), Pyrimidine bimolecular photoproducts, in: Photochemistry and Photobiology of Nucleic Acids, Vol. 1, Academic Press, New York, 1976, pp. 295–356.

[2] J. Cadet, P. Vigny, The photochemistry of nucleic acids, in: H. Morrison (Ed.), Biorganic Photochemistry, Wiley, New York, 1990, pp. 1–272.

[3] S. Elad, Products of purines, in: S.Y. Wang (Ed.), Photochemistry and Photobiology of Nucleic Acids, Vol. 1, Academic Press, New York, 1976, pp. 357–378.
[4] J. Cadet, L. Voituriez, A. Grand, F.E. Hruska, P. Vigny, L.S. Kan, Biochimie 67 (1976) 277.
[5] D. Porschke, Proc. Natl. Acad. Sci. U.S.A. 70 (1973) 2683.
[6] D. Porschke, J. Am. Chem. Soc. 95 (1973) 8440.
[7] D. Porschke, Nucl. Acids Res. 2 (1975) 1621.
[8] R.O. Rahn, Nucl. Acids Res. 3 (1976) 879.
[9] R.J.H. Davies, S. Bose, S.K. Sethi, J.A. McCloskey, Science 220 (1983) 723.
[10] R.J.H. Davies, S. Bose, S. Kumar, S.K. Sethi, J.A. McCloskey, Nucl. Acids Res. 12 (1984) 7929.
[11] F.P. Gasparro, J.R. Fresco, Nucl. Acids Res. 14 (1985) 4239.
[12] S. Kumar, N.D. Sharma, R.J.H. Davies, D.W. Phillipson, J.A. McCloskey, Nucl. Acids Res. 15 (1987) 1199.
[13] N.D. Sharma, R.J.H. Davies, J. Photochem. Photobiol. B: Biol. 3 (1989) 247.
[14] A. Canzanelli, R. Guild, D. Rapport, Am. J. Physiol. 167 (1951) 364.
[15] M.J. Kland, L.A. Johnson, J. Am. Chem. Soc. 79 (1957) 6187.
[16] J.T.H. Goosen, J.G. Kloosterboer, Photochem. Photobiol. 27 (1978) 703.
[17] R. Arce, L. Martínez, E. Danielsen, Photochem. Photobiol. 58 (1993) 318.
[18] G.G. Gurzadyan, H. Görner, Photochem. Photobiol. 60 (1994) 323.
[19] R. Arce, G. Rodríguez, K. Singmaster, Photochem. Photobiol. 38 (1983) 631.
[20] R. Arce, Photochem. Photobiol. 45 (1987) 713.
[21] L.F. Covalieri, A. Bendich, J. Am. Chem. Soc. 71 (1949) 533.
[22] M.D. Shetlar, J. Christensen, K. Hom, Photochem. Photobiol. 39 (1984) 125.
[23] H.E. Johns, Quantum yields and kinetics of photochemical reactions in solution, in: A.A. Lamola (Ed.), Creation and Detection of the Excited State, Vol. I, Part A, Marcel Dekker, New York, 1971, pp. 123–172.
[24] C.E. Crespo-Hernández, S. Flores, C. Torres, I. Negrón-Encarnación, R. Arce, Photochem. Photobiol. 71 (2000) 534.
[25] C.E. Crespo-Hernández, R. Arce, Photochem. Photobiol. 71 (2000) 544.
[26] D.F. Bryant, R. Santus, L.I. Grossweiner, J. Phys. Chem. 79 (1975) 2711.
[27] G.J. Fisher, H.E. Johns, Pyrimidine photohydrates, in: S.Y. Wang (Ed.), Photochemistry and Photobiology of Nucleic Acids, Vol. 1, Academic Press, New York, 1976, pp. 169–224.
[28] R.O. Rahm, H.G. Sellin, Photochem. Photobiol. 30 (1979) 317.
[29] G.V. Buxton, C.L. Greenstock, W.P. Helman, A.B. Ross, J. Phys. Chem. Ref. Data 121 (1990) 513.
[30] U. Lahish, A. Shafferman, G. Stein, J. Chem. Phys. 64 (1976) 4205.
[31] S. Monti, G. Köhler, G. Grabner, J. Phys. Chem. 97 (1993) 13011.
[32] J. Opitz, D. Schulte-Frohilnde, J. Photochem. 39 (1987) 145.
[33] D.N. Nikgosyan, Int. J. Radiat. Biol. 57 (1990) 233.
[34] M. Wala, E. Bothe, H. Görner, D. Schulte-Frohilnde, J. Photochem. Photobiol. A: Chem. 53 (1990) 87.
[35] E. Bothe, H. Görner, J. Opitz, D. Schulte-Frohilnde, Photochem. Photobiol. 52 (1990) 946.
[36] G. Grabner, N. Getoff, Ts. Gantichev, D. Angelov, M. Shopova, Photochem. Photobiol. 54 (1991) 673.
[37] S. Sortino, J.C. Scaiano, Photochem. Photobiol. 70 (1999) 590.
[38] P.W. Doetsch, T.H. Zatawny, A.M. Martin, M. Dizdaroglu, Biochemistry 34 (1995) 737.
[39] R. Baker, in: L. Hager, F. Wald (Eds.), Organic Chemistry of Biological Compounds, Prentice-Hall, Englewood Cliffs, NJ, 1971, pp. 281–339.
[40] L.B. Clark, I. Tinoco Jr., J. Am. Chem. Soc. 87 (1965) 11.
[41] V. Kleinwachter, J. Drobnik, L. Augenstein, Photochem. Photobiol. 6 (1967) 133.
[42] G. Hems, Radiat. Res. 13 (1960) 777.
[43] J.J. Conlay, Nature 197 (1963) 555.
[44] J.J. Van Hemmen, J.F. Bleichrodt, Radiat. Res. 46 (1971) 444.
[45] H. Fernando, G.A. Papadantonakis, N.S. Kim, P.R. LeBreaton, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 5550.
[46] R. Téoule, Int. J. Radiat. Biol. 51 (1987) 573.
[47] M. Dizdaroglu, Mutat. Res. 275 (1992) 331.
[48] K.J. Visscher, M.P. De Haas, H. Loman, B. Vojnovic, J.M. Warman, Int. J. Radiat. Biol. 52 (1987) 745.
[49] K.J. Visscher, M. Hom, H. Loman, H.J.W. Spoelder, J.B. Verberne, Radiat. Phys. Chem. 32 (1988) 465.
[50] W. Hug, I. Tinoco Jr., J. Am. Chem. Soc. 96 (1974) 665.
[51] E.I. Budovskii, N.A. Simukova, A.M. Tikhonova, T.N. Menshoukova, L.B. Rubin, Sov. J. Quantum Electron. 11 (1981) 1602.
[52] G.G. Gurzadyan, H. Görner, Photochem. Photobiol. 56 (1992) 371.
[53] M. Guéron, R.G. Shulman, Annu. Rev. Biochem. 37 (1968) 571.
[54] C. von Sonntag, Adv. Carbohyd. Chem. Biochem. 37 (1980) 7.
[55] A.J.S.C. Viera, S. Steenken, J. Am. Chem. Soc. 112 (1990) 6986.
[56] L.P. Candeias, S. Steenken, J. Am. Chem. Soc. 111 (1989) 1094.