THE PHOTOCHEMISTRY OF ADENINE AND SOME OF ITS DERIVATIVES IN AQUEOUS GLASSES AT LOW TEMPERATURES: REACTIVE INTERMEDIATES AND QUANTUM YIELDS

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Summary

Reactive intermediates produced by UV irradiation of frozen aqueous glasses containing adenine, 2′-deoxyadenosine, 2′-deoxyadenosine-5′-phosphate and 2′-deoxyadenylyl-(3′,5′)-2′deoxyadenosine were detected and characterized by means of electron paramagnetic resonance, UV and visible absorption spectroscopy. In neutral (12 M LiCl) and basic (8 M NaOH) glasses at 77 K, photo-ionization occurs and is the principal photodestruction route. Photo-ionization is evidenced by the formation of trapped electrons and radical cations in yields of the order of 10^{-3} after 30 s of UV irradiation in both media. Prolonged irradiation induces partial bleaching of the trapped electron and recombination with its geminate cation. Photodestruction yields determined after 300 s irradiation are of the order of 10^{-4} in both glasses. The dinucleoside phosphate shows the largest photoreactivity yield of the adenine derivatives. UV irradiation of these purines in 12 M LiCl also results in reaction with the solvent producing Cl_2^- ions. Photo-ionization, as well as the reaction with the solvent, involves the excited triplet state. Neutral and negatively charged species of the adenine derivatives show similar photoreactivity with the exception of 2′-deoxyadenosine-5′-phosphate for which its negatively charged species is more photoreactive.

1. Introduction

Adenine, one of the main components and light-absorbing centers of deoxyribonucleic acid (DNA), has been considered to be photochemically inert and of no photobiological significance [1] since no evidence was obtained for the formation of photoproducts in UV-irradiated DNA. Pörschke [2, 3] reported on a specific adenine photoproduct formed in oligodeoxyadenylic acid chains, $d(pA)_n$, where $n \geqslant 2$, suggesting the possibility that a similar photoproduct may exist in irradiated DNA. Later, Rahn [4] demonstrated that adenine residues in DNA did not undergo reactions similar to those observed in polydeoxyadenosine. Recently, Bose *et al.* [5] have reported evidence for the formation of an adenine–thymine photoadduct in

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the deoxydinucleoside monophosphate d(TpA) and have presented evidence for the formation of this photoproduct in UV-irradiated DNA. These recent results have generated new interest in the photochemical studies of purine bases. In this paper we present results on the reactive intermediates, quantum yields and photochemical reactions of adenine, 2′-deoxyadenosine (dAdo), 2′-deoxyadenosine-5′-phosphate (dAMP) and the dinucleoside phosphate 2′-deoxyadenylyl(3′-5′)-2′-deoxyadenosine (dApdA).

In previous low temperature photochemical studies of adenine and some of its derivatives, electron paramagnetic resonance (EPR) spectroscopy has been employed to detect intermediate species. It was found that UV illumination of aqueous solutions of adenosine at 77 K ionizes the solute [6]. EPR spectra from irradiated glasses indicate the possible formation of trapped electrons and solute cations whose presence were not definitively established. In frozen adenosine solutions containing ethanol, UV irradiation induces photosensitization of ethanol yielding ethanol radicals in addition to trapped electrons [6]. The photo-ionization and photosensitization processes were explained in terms of a biphotonic absorption process. No quantum yields were reported for these competing processes. Frozen aqueous solutions of adenine, dAdo and dAMP (0.02 M) UV irradiated at 77 K and analyzed at 150 K have been found to yield an EPR signal at g values near 2.004 [7]. For adenine and dAdo singlets were observed at 150 K with linewidths of 14 G and 18 G respectively, and for dAMP a symmetric quintuplet of 90 G total width was reported. The same types of radical were observed in X-irradiated aqueous solutions. No assignment of the EPR spectra to a specific charged or neutral radical was attempted. Further studies by Lion and Van de Vorst [8] have been carried out on the reactions of electrons with adenine, adenosine, dAdo, ribose and 2′-deoxyribose in frozen 8 M NaOH solutions. Trapped electrons were generated by photo-oxidation of potassium ferrocyanide. It was reported that for these compounds hydrogen addition radicals are formed from unobserved anionic intermediates.

The π anion radicals of adenine have been investigated in 12 M LiCl (98% D_2O) solution at 77 K [9]. The term π cation or anion means base radicals produced by electron gain or loss from the π electron system, not the overall charge on the species which could already be charged by protonation or deprotonation reactions. The g values for the adenine cation (2.0043), anion (2.0034) and the composite cation–anion (2.0038) signal were determined. Protonation reactions of the adenine anion radicals in 8 M NaOH and 12 M LiCl (H_2O) glasses were also studied. No evidence for a protonation reaction was observed on warming to 170 K in 12 M LiCl glasses. When photolyzed samples in 8 M NaOH were warmed to 190 K, protonation of adenine anions was observed producing a 1:2:1 triplet with hyperfine splitting of about 41 G. The dAdo anion radicals in 12 M LiCl (D_2O) have also been generated and their EPR spectra consist of a singlet with a g value of approximately 2.0032 and a linewidth of 14.5 G [10]. Sevilla *et al.* [11] have also studied π cations produced by photo-ionization of dAMP, several dinucleoside phosphates and DNA. EPR spectra consisting of a singlet with a line-

width of 9 - 10 G and a g value of 2.0035 were assigned to dAMP and dApdA cation radicals in neutral glass. In basic 8 M $NaClO_4$ frozen solutions similar spectra were observed; however, two additional lines of 92 G separation were observed in the basic medium. These additional lines were attributed to a second radical located on the adenine base. The mechanism of production of this radical is reported to be photoprotonation of the adenine anions. No relative yields or photoreactivities of these adenine derivatives were reported.

Photoconversion quantum yields not exceeding 10^{-4} have been reported for adenine and the corresponding nucleosides and nucleoside 5′-phosphates in liquid and frozen aqueous solutions at 77 K by Ivanchenko *et al.* [12].

In this paper, we report results on the low temperature photolysis of glassy aqueous solutions of adenine and some of its derivatives in different ionic forms: the nucleoside dAdo, the nucleotide dAMP and the dinucleoside phosphate dApdA which is considered as a model for adenine-containing polynucleotides. The purposes of this work were (1) to compare the photoreactivities of adenine and some of its derivatives with its negatively charged species in glassy solutions, (2) to identify by EPR and UV visible spectroscopy the reactive intermediates formed during the UV irradiation and to compare the photoreactivities of adenine and its derivatives on the basis of the yields of the reactive intermediates, (3) to compare the degree of photodestruction of these molecules and (4) to examine the possibility of an increase in photoreactivity of adenine when the base is incorporated in a polymeric chain. This information will enable us to understand the role of adenine and its derivatives in the photochemistry of nucleic acids.

2. Experimental details

2.1. Chemicals and sample preparation

Adenine (Aldrich; purity, 99%), dAdo (Sigma; purity, 98%), dAMP (Sigma; purity, 99%), dApdA (Sigma), NaOH (Fisher; certified reagent), LiCl (Fisher; certified reagent), galvinoxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals (Aldrich) were used as received. Aqueous glasses were produced by adding a high concentration of the inorganic salts to triply distilled water and cooling to 77 K.

Solutions of adenine or its derivatives with concentrations ranging from 1.0×10^{-4} to 5×10^{-4} M were used in the low temperature irradiations. These were prepared by analytical weighing and dilution of the weighed samples. The concentrations of the solutions were verified by measuring the absorption at the wavelength of maximum absorption and using the Beer–Lambert law to determine the concentration from the known molar absorption coefficient. An aliquot of the solution was introduced into a square Suprasil cell with an optical path length of 3 mm. The cell was connected to a vacuum line and the solution was degassed by several freeze–thaw cycles using liquid nitrogen for the LiCl glasses and a (dry ice)–acetone mixture for the NaOH glasses. After the sample had been degassed it was sealed under vacuum and kept refrigerated below 273 K until used.

2.2. Irradiation conditions, actinometry and quantum yield determinations

The procedures followed to determine the incident light intensity and the radical concentrations from EPR data and to calculate the quantum yields have been described previously [13 - 15]. Irradiation of the samples at 77 K was performed using a low pressure mercury (254 nm) helical lamp (Hanovia) with a Vycor tube inside as a filter.

3. Results and discussion

3.1. Molar absorption coefficients of adenine and its derivatives

The absorption band at 260 nm in neutral solutions of adenine and its derivatives has been assigned to π,π^* transitions on the basis of fluorescence polarization studies [16]. The molar absorption coefficients of adenine, dAdo, dAMP and dApdA were determined both in 12 M LiCl and in 8 M NaOH solutions at room temperature (Table 1). A red shift in the wavelength of maximum absorption of adenine occurs in the alkaline medium. In basic solution adenine is deprotonated at the N(9) position. On deprotonation the highest occupied orbital, which was a π orbital, is substituted by a non-bonding orbital. The lowest transition is then of n,π^* type, which occurs at a lower energy. For the other adenine derivatives at pH values greater than 6 the secondary phosphate dissociates and becomes doubly charged whereas above pH 12 hydroxyl groups of the sugar moiety become negatively charged. For these charged species no shift in the absorption band was observed.

The molar absorption coefficient per base for dApdA in both solvents is lower than the corresponding coefficients of the nucleoside component dAdo in the same solvent. This decrease in absorption of light by a chromophore or hypochroism has been observed in all common ribo and deoxyribo nucleoside phosphates [17]. With respect to the individual nucleo-

TABLE 1

Molar absorption coefficients of adenine and its derivatives

Compound	*Solvent*	λ_{max} (nm)	$\epsilon_{max} \times 10^{-4}$ (M^{-1} cm^{-1})
Adenine	12 M LiCl	260	1.31
Adenine	8 M NaOH	269	1.22
dAdo	12 M LiCl	260	1.47
dAdo	8 M NaOH	261	1.30
dAMP	12 M LiCl	260	1.45
dAMP	8 M NaOH	261	1.36
dApdA	12 M LiCl	260	2.44
dApdA	8 M NaOH	261	2.11

side units, the hypochroism of dApdA was calculated to be 17% in 12 M LiCl and 19% in 8 M NaOH. Cantor *et al.* [17] calculated 11% hypochroism for dApdA in dilute aqueous solution.

3.2. Photochemistry in neutral media

UV illumination of adenine solutions in 12 M LiCl glass for 30 s produces a faint blue color. The visible spectra of the irradiated solutions consist of a weak band (absorbance, about 0.04) with maximum absorption at 585 nm. This absorption disappeared completely when the sample was photobleached with visible–IR light or when the sample was warmed to room temperature and has been assigned to absorption by trapped electrons. A similar band has been assigned to solvated electrons in the pulse radiolysis of 18 M LiCl solutions [18]. The visible absorption is weaker in irradiated dAdo solutions, stronger in dApdA and absent in dAMP solutions. The UV spectra of irradiated frozen solutions of adenine and its derivatives in 12 M LiCl were also recorded to detect the possible destruction and/or formation of intermediate species or stable photoproducts not detectable by visible or EPR spectroscopy. The UV spectra taken after 30 s photolysis were almost identical with those recorded before irradiation. Although some decreases in the absorbance at 260 nm were observed for adenine and dApdA, they were too small to be measured accurately. For these two compounds a very weak absorption band appears around 340 nm (absorbance, about 0.05), which remains after the photobleaching but disappears when the sample is warmed to room temperature. After longer irradiation periods (60 s or more) with 254 nm light, adenine solutions showed a larger decrease in absorbance at 260 nm and a stronger absorption occurred at 340 nm as shown in Fig. 1. Photobleaching with visible–IR light for 10 min caused partial regeneration of the 260 nm band (30%) while warming the solution to room temperature completely regenerated the 260 nm band and bleached the 340 nm band. Similar changes were observed in the UV region of dAdo and dApdA after 600 s irradiation. No photodestruction or appearance of the 340 nm band occurred after 600 s illumination of dAMP solutions.

An electron scavenger, KNO_3, at 0.01 M concentration [19] was added to the solutions in 12 M LiCl prior to the 600 s UV irradiation of the frozen solution. The solution remained colorless (no absorption at 585 nm) after irradiation and showed increases in light absorption in the 290 - 410 nm region and 100% increase in the intensity of absorption at 340 nm compared with irradiated solutions not containing the scavenger. Manganous sulfate, a triplet quencher [20], was added at a concentration of 5×10^{-3} M to 12 M LiCl solutions containing adenine or its derivatives and subsequently the solution was UV illuminated for 600 s; after irradiation the solution was colorless and the extent of photodestruction as well as the intensity of the absorption band at 340 nm decreased almost to zero. Thus, the excited triplet state of adenine is involved in the photodestruction mechanism of adenine and in the process which leads to the formation of the 340 nm band. At the quencher or scavenger concentrations used in these experiments

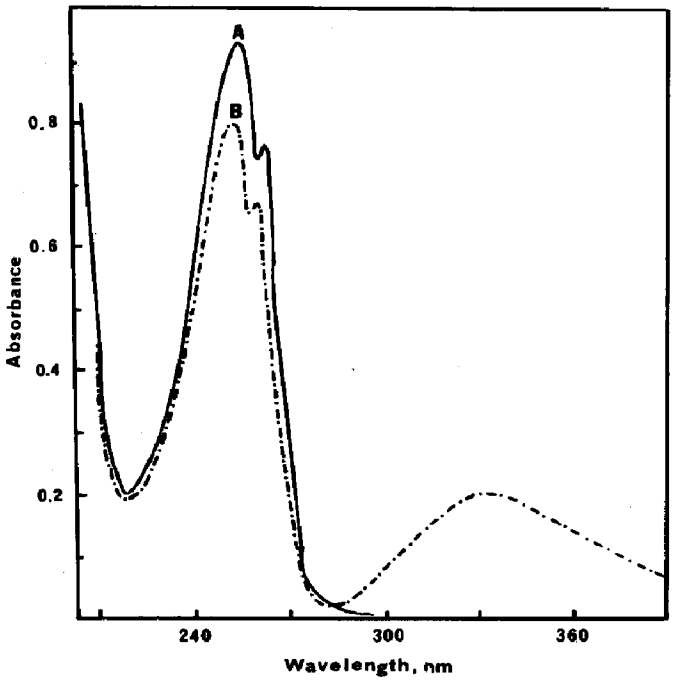

Fig. 1. UV absorption spectra of a 2×10^{-4} M adenine solution in 12 M LiCl glass: spectrum A, prior to irradiation; spectrum B, irradiated for 300 s with 254 nm light.

there was no interference with the UV absorption band of the purine. Broad singlets at low magnetic fields (1420 - 1440 G) were observed during UV illumination for adenine, dAdo and dApdA; these were assigned to the $\Delta m_s = 2$ transition of the purine triplet state on the basis of their decay lifetimes, which are similar to the reported phosphorescence decay lifetimes, and on the basis of the r.m.s. zero-field splitting parameter of the transition [21]. Intersystem crossing quantum yields of 0.17, 0.051, 0.008 and 0.014 were determined for adenine, dAdo, dAMP and dApdA respectively.

Examination of the UV-irradiated solutions by EPR presents an unresolved singlet with a g value of 2.0017, which grows into a more complex spectrum as the time of irradiation increases as shown in Fig. 2. The EPR spectrum obtained after 15 s irradiation consists of a singlet with a linewidth of 23.5 G and an extension of 78 G. After the sample had been photobleached for 5 min, the intensity of the main central peak decreased to about 8% - 14% of the original intensity. The spectrum remaining after the photobleaching is characterized by a g value of 2.0049 and a linewidth of 18 G. These changes in the g value and linewidth are evidence of the existence of different paramagnetic species before and after the photobleaching. The peaks at low field or with a higher g value than the main central peak grow with irradiation time while the intensity of the main peak as well as that of the blue coloration decrease. Two outer peaks separated by 500 G and with a linewidth of 11 G are also observed. These are attributed to trapped hydrogen atoms.

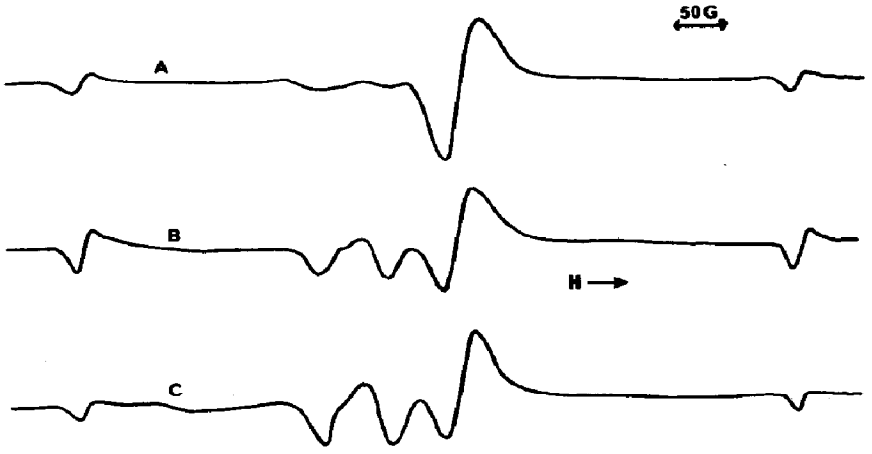

Fig. 2. EPR spectra of a 2×10^{-4} M adenine solution in 12 M LiCl glass after UV irradiation for 30 s (spectrum A), 300 s (spectrum B) and 900 s (spectrum C) (spectrometer conditions: modulation ampltiude, 10 G; gain, 8×10^4; microwave power, 0.5 mW).

Since the absorption band in the visible region evidences the presence of trapped electrons, these intermediates as well as their geminate radical cations must contribute to the observed EPR spectrum (Fig. 2). Trapped electrons in 12 M LiCl glass were generated by UV irradiation of 0.01 M potassium ferrocyanide in the glass for 20 s [22]. Their EPR spectrum is characterized by a g value of 1.9987, a linewidth of 23 G and an extension of 79 G. A comparison of the shape and parameters of the EPR spectrum obtained after 30 s irradiation of adenine in 12 M LiCl glass with those of the trapped electron spectrum suggests that the spectrum obtained after 30 s photolysis is due mainly to trapped electrons, radical cations and/or radical anions. Reduction of the area of the EPR spectrum on photobleaching is due to mobilization of trapped electrons by visible light followed by recombination with adenine radical cations to yield neutral molecules, as is confirmed by the partial regeneration of the 260 nm band or by reaction with hydronium ions from the solvent matrix.

Moan and Kaalhus [23] observed a seven-line EPR spectrum from 9 M HCl solutions exposed to X-rays at 77 K. This spectrum has been assigned to Cl_2^- ions. These ions also show a characteristic absorption band with a maximum at 340 nm as observed in this work. The central part of the Cl_2^- spectrum is very similar to the spectrum obtained after 15 min UV irradiation of the 12 M LiCl glass containing adenine. Thus, the EPR spectra observed after prolonged irradiation of frozen 12 M LiCl solutions of adenine are attributed to the overlap of the spectra of trapped electrons, adenine radical cations, adenine radical anions and Cl_2^- ions. Illumination of 12 M LiCl glasses at 77 K for time intervals up to 15 min did not yield any significant EPR signals; thus the production of the trapped electron and Cl_2^- requires the presence of an excited state of adenine or its derivatives or the presence of one of the photo-ionization products.

Sevilla and Mohan [9] measured *g* values for adenine radical cations and anions generated by photochemical methods in 12 M LiCl (D_2O) glass. They reported values for the *g* factor of 2.0043 and 2.0034 for the cation and the anion respectively and linewidths of 12 G and 9 G. For a composite radical cation–anion signal a *g* value of 2.0038 and a linewidth of 11 G were reported. In UV-irradiated aqueous adenine solution frozen to 77 K and analyzed at 150 K, Lacroix and Van de Vorst [7] reported a singlet with a linewidth of 14 G and a *g* value close to the DPPH *g* value (2.0036). Since this singlet is observed after warming the irradiated solution, it could originate from an intermediate resulting from a deprotonation reaction of the radical cation or from a radical produced by a reaction of detrapped electrons and adenine molecules. On the basis of the reported *g* values and linewidths for the adenine ions, we assume that the EPR spectra observed after the UV irradiation of adenine solutions are mostly due to the presence of trapped electrons, radical cations and Cl_2^- ions. After photobleaching of the solutions the species remaining are radical cations and in a lesser percentage radical anions. The *g* values and linewidths measured in this work for the combined spectra of cations and anions are larger than those reported by Sevilla and Mohan [9] because of contributions from Cl_2^- radicals ($g = 2.04$ [24]). However, these researchers do not report the formation of Cl_2^- radicals during photolysis of LiCl glasses containing adenine.

The EPR spectra of irradiated dAdo solutions are similar to those of adenine solutions, but their intensities are lower and longer irradiation periods (60 s or more) are required to observe the multiplet assigned to Cl_2^- ions. The *g* value of the central main peak after 30 s UV irradiation was determined to be 2.001 with a linewidth of 22.6 G. The spectra were assigned to trapped electrons and dAdo radical cations. Photobleaching produced almost complete disappearance of the signal. For UV-irradiated dAdo frozen aqueous solutions a singlet with a linewidth of 18 G was observed at 150 K [7].

Photolysis of dApdA solutions yields EPR spectra similar in shape and intensities to those produced by adenine solutions with a *g* value for the main central peak of 2.0011 and a linewidth of 23.4 G. After photobleaching of the solutions the *g* value increased to 2.0065 and the linewidth decreased to 19.8 G, indicating the possible presence of radical anions in addition to the radical cations.

No phosphorescence or permanent blue color develops on UV illumination of frozen LiCl glasses containing dAMP. Only after 5 min or longer irradiation times do very weak multiplet signals appear at a *g* value of around 2. A weak doublet due to trapped hydrogen atoms also appears and remains with a constant intensity on further irradiation. Thus, no significant photoionization or formation of Cl_2^- radical ions is produced during UV irradiation of dAMP solutions under these experimental conditions. The formation of hydrogen atoms indicates possible photo-ionization of dAMP. The triplet yield of dAMP was also found to be the smallest of the adenine derivatives; thus this is further evidence that the triplet state is involved in the photo-

ionization and photodestruction mechanism of these purines. Sevilla *et al.* [11] observed a singlet with a g value of 2.0035, which is less than that found previously in 12 M LiCl, for the photo-ionization of dAMP and dApdA in neutral 8 M $NaClO_4$ glass, whereas Lacroix and Van de Vorst [7] observed a symmetric quintuplet with a linewidth of 18 G in UV-irradiated aqueous frozen solutions of dAMP analyzed at 150 K.

Quantum yields for the production of radical intermediates and trapped electrons and for the photodestruction were determined from the doubly integrated areas of the EPR spectra or from changes in the absorbance intensity at different wavelengths. In the trapped electron yield determinations (Table 2) it was assumed that the total initial concentration of spins results from equal contributions from the trapped electrons and radical cations and that the contribution of Cl_2^- to the central peak is small. These assumptions are supported by photoelectron yields obtained from optical measurements.

The order observed for intersystem crossing yields in the same glass [21] is adenine > dAdo > dApdA > dAMP. Since the triplet state is involved in the photo-ionization process, the differences in the order of reactivity toward intersystem crossing and photo-ionization (dApdA > adenine > dAdo > dAMP) arise from differences in the rates of other decay processes from the triplet state such as phosphorescence, energy transfer to the solvent and/or other triplet photoreactions.

Quantum yields for production of radical cations and anions after photobleaching of the solutions were also calculated (Table 3). These yields decrease in the same order as do the photodestruction yields. The order for the percentage of radical remaining after the photobleaching is dAdo (34%) > dApdA (21%) > adenine (11%), indicating that the probability of radical anion formation for dAdo is highest at this low temperature. The yield of radical cations and anions is higher for dApdA than for adenine by a factor of 2.3, while the yield of trapped electrons is higher for dApdA than for adenine by a factor of only 1.25, indicating that dApdA radicals are more stable in 12 M LiCl glass than are those of adenine.

Trapped electron quantum yields were also obtained from electron concentrations determined from the Beer–Lambert law by measuring the absorption at 585 nm of irradiated solutions (Table 2). For this calculation the molar absorption coefficient of trapped electrons in 12 M LiCl was determined from EPR and visible absorption data. From a combination of trapped electron EPR concentrations with the absorbance measured at 585 nm, on the assumption that Beer's law holds, a molar absorption coefficient of $(1.7 \pm 0.5) \times 10^4$ M^{-1} cm^{-1} was calculated. The large uncertainty in the absorption coefficient is a result of the low absorption intensity (absorbance, between 0.05 and 0.15) of the visible absorption spectra and of cracks formed in the glass on freezing. In aqueous solutions at room temperature the ratio of the molar absorption coefficients [18] for solvated electrons at the wavelength of maximum absorption in 15 M NaOH and 15 M LiCl solutions is 1.33. On the assumption that the value of the molar absorption

TABLE 2

Comparison of photoelectron quantum yields in 12 M LiCl glasses containing adenine or its derivatives as determined by electron paramagnetic resonance or visible spectroscopy after 30 s UV irradiation[a]

Compound	*EPR*[b]			*Visible spectroscopy*			
	$[e^-_t] \times 10^5$ (M)	$I_{abs} \times 10^{-17}$ (photons $cm^{-3} s^{-1}$)	$\phi(e^-_t) \times 10^3$	*Absorbance at 585 nm*	$[e^-_t] \times 10^6$ (M)	$I_{abs} \times 10^{-17}$ (photons $cm^{-3} s^{-1}$)	$\phi(e^-_t) \times 10^3$
Adenine	0.87	1.64	1.1 ± 0.3	0.036	7.1	1.62	0.88
dAdo	0.31	2.02	0.30 ± 0.06	[c]	—	—	[c]
dApdA	1.15	1.56	1.5 ± 0.2	0.042	8.2	1.68	0.98

[a] Average value of three to five determinations.
[b] On the assumption that $[e^-_t]$ is one-half of the total radical concentration.
[c] Absorbance is too low to be determined.

TABLE 3

Quantum yields for production of solute radical cation and anion in 12 M LiCl glasses containing adenine or its derivatives after 30 s UV irradiation and subsequent photobleaching[a]

Compound	[R] $\times 10^6$ (M)	$I_{abs} \times 10^{-17}$ (photons $cm^{-3} s^{-1}$)	ϕ(radical) $\times 10^4$
Adenine	2.17	1.62	2.7
dAdo	2.33	2.02	2.3
dApdA	5.11	1.68	6.1

[a] Average values of three to five determinations.

TABLE 4

Photodestruction quantum yields of adenine and its derivatives in 12 M LiCl glasses after 300 s irradiation[a]

Compound	$-\Delta A$ *at* λ_{max}	$-\Delta C \times 10^5$ (M)	$I_{abs} \times 10^{-17}$ (photons cm^{-3} s^{-1})	ϕ(destruction) $\times 10^4$
Adenine	0.11	2.85	1.71	3.3
dAdo	0.05	1.13	1.78	1.3
dApdA[b]	0.07	0.96	1.29	2.9

[a]Average values of three to five determinations.
[b]Photodestruction yield per base.

coefficients in frozen glassy 8 M NaOH and 12 M LiCl is also 1.33 and using the known value of the coefficient in 8 M NaOH (2.0×10^4 M^{-1} cm^{-1}), the molar absorption coefficient for trapped electrons in 12 M LiCl would be 1.5×10^4 M^{-1} cm^{-1}. This result is very close to the value determined in this work and can be considered as a measure of the precision of the radical concentration measured by EPR methods. The yields determined by EPR measurements were 20% - 30% larger than those obtained through optical measurements because of the small contribution of Cl_2^- ions to the total radical concentration. A photo-ionization yield of 2×10^{-3} was determined for purine free base [14] in 12 M LiCl glass under similar conditions.

The photodestruction quantum yields in LiCl glass (Table 4) vary in the order adenine > dApdA > dAMP. These yields were calculated in the early stages of irradiation to avoid possible interference from photoproduct absorption at the wavelength of maximum absorbance of the purines. Absorption by photoproduct could have resulted in a decrease in the observed yields. For adenine and dApdA the destruction yields after 300 s photolysis are smaller than the ionization yields at 30 s irradiation by factors of 3.8 and 5.3 respectively, whereas for dAdo the photodestruction yield is slightly larger than the photo-ionization at 30 s. These results indicate that there is a high probability of recombination of electrons with their geminate cations when the samples are UV irradiated for prolonged periods. These destruction yields are very low compared with the intersystem crossing quantum yields and with the photodestruction yield of purine free base (5×10^{-3}) [14].

3.3. Photochemistry in basic media

On UV irradiation of a solution of adenine, dAdo, dAMP or dApdA in 8 M NaOH glass at 77 K, the sample acquires a permanent blue color of greater intensity than that observed in 12 M LiCl glasses. The visible spectrum of the illuminated solution consists of a broad band with maximum absorption at 585 nm. After 60 s of irradiation a weak absorption appears around 340 nm and some photodestruction is evident from a decrease in absorption at 269 nm. These changes in absorption at the maximum of the band are too small

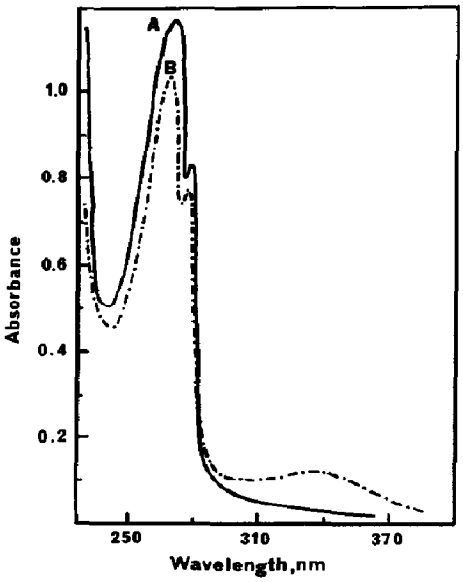

Fig. 3. UV absorption spectra of a 2×10^{-4} M adenine solution in 8 M NaOH glass: spectrum A, before irradiation; spectrum B, after 300 s irradiation with 254 nm light.

for reliable values of the photodestruction yields to be calculated. Prolonged illumination (300 s or more) produced an increase in absorption of the 340 nm band and a decrease in the base absorption band at 269 nm (Fig. 3). Photobleaching with visible–IR light for 5 - 10 min causes the simultaneous disappearance of the 585 nm band and of the 340 nm band and almost total recovery of the 269 nm band. In the presence of 0.01 M KNO_3, UV-irradiated solutions of adenine in 8 M NaOH show an increase in the absorption at 340 nm and in the region from 290 to 370 nm and total disappearance of the 585 nm band. On the basis of these two observations, the 340 nm band is attributed to adenine radical cations. On the assumption that the radical cation concentration is equal to the concentration of trapped electrons, the molar absorption coefficient of the radical cation is estimated as $(2.0 \pm 0.7) \times 10^4$ M^{-1} cm^{-1}. During the X-irradiation of adenine and other purines at a concentration of 0.05 M in alkaline glasses at 77 K, Lion and Van de Vorst [8] attributed a band at 330 nm, whose intensity varied directly with the adenine concentration, to an absorption of the radical anion. The intensity of this band increased on photobleaching of the trapped electron band. In the present work the radical anion absorption band could not be observed because of the low yield of anions which resulted from a low initial concentration of trapped electrons and adenine molecules.

Photolysis of the three adenine derivatives for 300 s did not produce light absorption at 340 nm, although the samples turned blue and the derivatives were partially photodestroyed.

Examination by EPR of irradiated solutions of adenine in 8 M NaOH glass yields the spectrum shown in Fig. 4, spectrum A. This consists of an unresolved singlet at a g value of 2.0008 with a linewidth of 15.1 G,

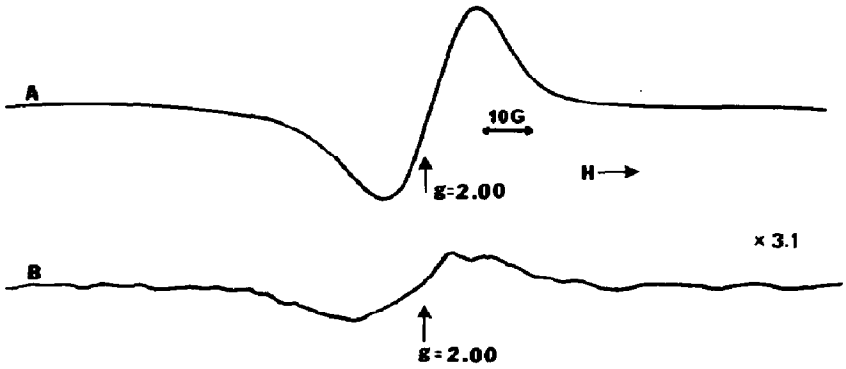

Fig. 4. EPR spectra of a 2×10^{-4} M adenine solution in 8 M NaOH glass after 60 s UV irradiation (spectrum A) and after subsequent photobleaching for 600 s (spectrum B) (spectrometer conditions: modulation amplitude, 10 G; gain, 8×10^4; microwave power, 0.5 mW).

extending over about 70 - 80 G. No change occurred in the linewidth or in the g value of the singlet when irradiation was prolonged from 10 to 60 s. When the irradiated sample was photobleached for 10 min with visible–IR light, the blue color disappeared and the intensity of the signal decreased to about 10% of the original intensity. The g value of the remaining singlet increased to 2.0046, while its linewidth increased to 19 G (Fig. 4, spectrum B). UV irradiation of 8 M NaOH glass alone did not produce coloring of the glass nor yield any EPR signal.

Trapped electrons in 8 M NaOH glass at 77 K were generated by irradiation for 20 s of a 0.01 M solution of potassium ferrocyanide. The singlet obtained has a g value of 1.9995 and a linewidth of 13.5 G. Radical cations were produced without interference from photoejected electrons by adding 0.01 M potassium ferricyanide [9], as an electron scavenger, before irradiation of the solution at 77 K. The EPR spectrum consisted of a singlet with a g value of 2.0045 and a linewidth of 12.3 G. The adenine radical anion was produced by photo-ionization of 0.01 M potassium ferrocyanide solution containing 10^{-3} M adenine in 8 M NaOH. The solutions were UV irradiated for 50 s and subsequently photobleached for 5 min. The species produced in this way has a g value of 2.0058 and a linewidth of 19.0 G.

Adenine radical anions in 8 M NaOH generated either by X-irradiation [8] or photochemically [9] exhibit EPR signals consisting of a singlet. Illumination of the radical anions with light of wavelengths greater than 320 nm [8] or warming to 180 K [8, 9] transforms the adenine anion into a hydrogen adduct radical with an EPR spectrum consisting of a 1:2:1 triplet with hyperfine coupling of 41 G, $g = 2.0042$ and a linewidth for the central peak of 19 G.

Under our experimental conditions the intensity of the signal of the radical anion was too small for its hyperfine structure to be resolved. How-

ever, the linewidth of the central peak is 19 G; thus it is possible that the species generated instead of the anion is the hydrogen adduct of the adenine radical anion.

On the basis of the *g* values, the linewidths and the optical evidence already presented, the EPR signal obtained after UV irradiation of adenine frozen solutions (2×10^{-4} M) in 8 M NaOH is attributed to the overlap spectra of trapped electrons and adenine radical cations and possibly to a small concentration of radical anions and/or neutral adenine radicals. The *g* value of this spectrum (2.0008) is larger than that for the trapped electrons mainly as a result of the contribution of radical cations with a *g* value of 2.0043. The EPR spectrum that is left after the photobleaching (Fig. 4, spectrum B) is considered to be the overlap of the spectra of adenine radical cations and neutral adenine radicals, on the basis of the large linewidth (19 G) and the *g* value (2.0046) which are not compatible with the presence of only radical anions. The neutral radicals are produced by reaction of adenine radical anions with water molecules from the medium.

When 8 M NaOH glasses containing dAdo or dAMP are irradiated with 254 nm light, a blue color also develops in the sample, and EPR spectra are obtained which are very similar to those obtained from adenine under identical conditions. The EPR spectrum obtained from irradiated frozen dApdA solutions appears in Fig. 5, spectrum A. This spectrum seems to be the overlap of a triplet signal and a stronger central singlet peak. The appearance of this complex spectrum occurs even after only 10 s irradiation. Photobleaching of irradiated samples of dAdo, dAMP or dApdA removes the blue color of the samples. Examination of the photobleached samples by

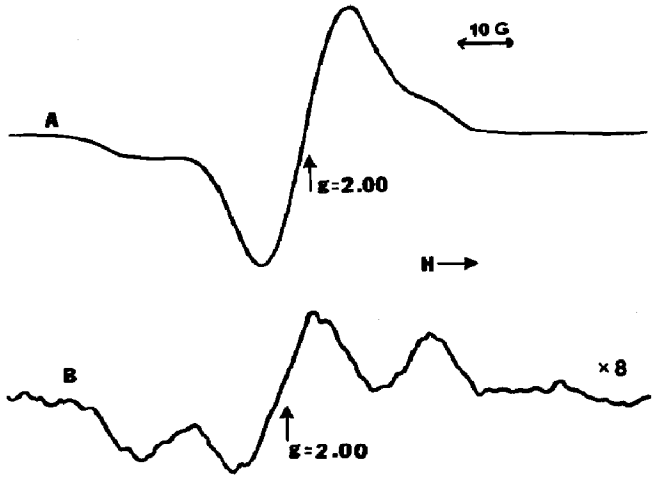

Fig. 5. EPR spectra of a 2×10^{-4} M dApdA solution in 8 M NaOH glass after 60 s UV irradiation (spectrum A) and after subsequent photobleaching for 600 s (spectrum B) (spectrometer conditions: modulation amplitude, 10 G; gain, 8×10^4; microwave power, 0.5 mW).

EPR reveals low intensity broad signals which, for dAdo and dAMP, are not very well defined, suggesting the existence of a hyperfine structure. For dApdA, the signal is a triplet with 1:2:1 intensities, a linewidth of 14 G for the central peak and a hyperfine splitting of 40 G (Fig. 5, spectrum B). This triplet is attributed to the hydrogen adduct of the dApdA radical anion.

EPR spectra attributed to the hydrogen addition radicals have been reported for dAdo [8], dAMP [11, 25] and for dApdA [11]. These spectra consist, as for adenine, of 1:2:1 triplets with hyperfine splittings of about 40 G and linewidths of 14 G. The triplet structure of the radical spectrum indicates hyperfine coupling with two equivalent β protons, which is consistent with hydrogen addition to an unsaturated C=N double bond at the C(2) or C(8) atoms of the adenine moiety [25].

The g values of species produced during UV photolysis of the four compounds in 8 M NaOH are presented in Table 5. The g values and linewidths of the irradiated samples are somewhat larger than those of trapped electrons, implying that these spectra correspond to trapped electrons plus other radicals with higher g values.

TABLE 5

Electron paramagnetic resonance spectral parameters of species produced by photolysis of 8 M NaOH glasses containing adenine or its derivatives[a]

Compound	*Stage of photolysis*	*g value*	*Linewidth* (G)
Adenine	60 s UV irradiation	2.0008	15.1
Adenine	60 s UV irradiation + 10 min bleaching	2.0046	19.0
dAdo	60 s UV irradation	2.0007	14.6
dAdo	60 s UV irradiation + 10 min bleaching	2.0041	—
dAMP	60 s UV irradiation	2.0005	14.5
dAMP	60 s UV irradiation + 10 min bleaching	2.0045	—
dApdA	60 s UV irradiation	2.0004	14.3
dApdA	60 s UV irradiation + 10 min bleaching	2.0045	—

[a]Average of four or more determinations; the estimated uncertainty in the g values is ±0.0006; the absence of data means that the spectra were too weak for the parameters to be measured.

The radical ions of dAdo, dAMP and dApdA were generated by the same methods used to produce the adenine radical ions. The EPR parameters are included in Table 6. As for adenine, the hydrogen adducts were obtained instead of the anions, as can be deduced from the measured g values and linewidths. However, the observed spectra for dAdo and dAMP hydrogen adducts consist of weak singlet signals with broad shoulders, instead of a well-defined 1:2:1 intensity triplet signal reported for these radicals.

TABLE 6

Electron paramagnetic resonance spectral parameters for radical species produced in UV-irradiated 8 M NaOH glasses containing adenine or its derivatives in the presence of ferrocyanide or ferricyanide ions

Solute	*Species*	*g value*[a]	*Linewidth* (G)
Adenine + $K_4Fe(CN)_6$	Adenine H adduct	2.0058	19.0
Adenine + $K_3Fe(CN)_6$	Adenine radical cation	2.0045	12.3
dAdo + $K_4Fe(CN)_6$	dAdo H adduct	2.0056	19.3
dAdo + $K_3Fe(CN)_6$	dAdo radical cation	2.0049	13.3
dAMP + $K_4Fe(CN)_6$	dAMP H adduct	2.0060	19.5
dAMP + $K_3Fe(CN)_6$	dAMP radical cation	2.0045	15.1
dApdA + $K_4Fe(CN)_6$	dApdA H adduct	2.0055	19.3
dApdA + $K_3Fe(CN)_6$	dApdA radical cation	2.0050	16.0

[a]Average of four or more determinations; the uncertainties in the *g* values are estimated as ±0.0006.

Photoelectron yields after 60 s irradiation obtained by visible spectroscopy and by EPR are given in Table 7. The yields obtained by EPR were calculated on the assumption that the concentration of trapped electrons is one-half of the total radical concentration measured in the irradiated sample before photobleaching. The order of reactivity with respect to photo-ionization observed by either method is the same: dApdA > dAdo > adenine > dAMP. The large discrepancy between yields calculated by visible spectroscopy and EPR measurements results from the assumption that one-half of the total radical concentration corresponds to trapped electrons. This difference can be accounted for if in the alkaline media the photoejected electrons react rapidly to form radical anions or secondary radicals in which case the total radical concentration consists of contributions from trapped electrons, radical cations, anions and/or neutral radicals. The relative order of the electron yields may indicate the relative probability of photo-ionization of these adenine derivatives or could reflect the stability of trapped electrons and radicals in the presence of these adenine derivatives in NaOH glass. The radical yields measured after photobleaching of the solutions follow the same order as the yields found after UV photolysis and in the four cases the intermediates are photobleached to the same extent. Quantum yields of photoelectrons were also determined at 30 s photolysis for comparison with the results obtained in LiCl glass (Table 8). In general, the quantum yields for total radicals or trapped electrons are higher in the alkaline matrix than in the neutral medium. The difference is most dramatic for dAMP while the effect is smallest for adenine. Similar results were observed in studies on the UV photo-ionization of purine free base at 77 K [15]. The photo-ionization yield of purine free base in 8 M NaOH glass was found to be one order of magnitude higher than in neutral glass. At the high pH of the 8 M NaOH glass these molecules exist as the anions as a result of deprotonation at the N(9) position in adenine or deprotonation in the deoxyribose and phosphate

TABLE 7

Comparison of photoelectron quantum yields measured in 8 M NaOH glasses containing adenine or its derivatives determined by electron paramagnetic resonance or visible spectroscopy after 60 s UV irradiation[a]

Compound	*EPR*			*Visible spectroscopy*			
	$[e^-_t] \times 10^5$ (M)	$I_{abs} \times 10^{-17}$ (photons $cm^{-3} s^{-1}$)	$\phi(e^-_t) \times 10^3$	*Absorbance at 585 nm*	$[e^-_t] \times 10^5$ (M)	$I_{abs} \times 10^{-17}$ (photons $cm^{-3} s^{-1}$)	$\phi(e^-_t) \times 10^3$
Adenine	2.76	1.86	1.5 ± 0.2	0.075	1.20	1.98	0.6 ± 0.1
dAdo	3.72	1.95	1.9 ± 0.2	0.094	1.60	1.71	0.9 ± 0.1
dAMP	2.59	1.84	1.4 ± 0.2	0.060	1.00	1.88	0.54 ± 0.05
dAdpA	3.69	1.61	2.3 ± 0.1	0.155	2.58	2.07	1.3 ± 0.1

[a] Average values of three to five determinations.

TABLE 8

Comparison of trapped electron quantum yields of adenine and its derivatives in 8 M NaOH and 12 M LiCl glass determined from electron paramagnetic resonance measurements after 30 s UV irradiation

Compound	$\phi(e^-_t) \times 10^3$ *determined after 30 s*	
	8 M NaOH	*12 M LiCl*
Adenine	1.9	1.1
dAdo	2.9	0.3
dAMP	1.9	≃0
dApdA	4.2	1.5

groups of the derivatives. The higher yields observed in 8 M NaOH compared with those in 12 M LiCl glass are due to the higher photoreactivity of the anionic species than the neutral molecules as well as to slower rates of recombination of electrons and radical cations in the alkaline medium. In 8 M NaOH no EPR spectrum due to the excited triplet state of these compounds was detected. Furthermore, gradual addition of NaOH to solutions of adenine in 12 M LiCl produced a continuous decrease in the triplet yield although the trapped radical and electron yields increased. This indicates that in the alkaline medium the photo-ionization process does not go through an excited triplet state.

Photodestruction and trapped electron quantum yields were determined after 300 s of UV irradiation in the same samples and are included in Table 9. No significant differences in the yield of photodestruction or production of trapped electrons is observed among these compounds, implying that photo-ionization of adenine or its derivatives is the principal photodestruction mechanism. It should be noted that trapped electron yields determined after 30 or 60 s photolysis are larger than those determined at longer irradiation periods (300 s). This means that spontaneous reactions

TABLE 9

Photodestruction and photoelectron quantum yields of adenine and its derivatives in 8 M NaOH glass after 300 s irradiation[a]

Compound	$-\Delta A$ *at* λ_{max}	$-\Delta C \times 10^5$ (M)	$I_{abs} \times 10^{-17}$ (photons cm^{-3} s^{-1})	ϕ(destruction) $\times 10^4$	$\phi(e^-_t) \times 10^4$
Adenine	0.08	2.2	1.61	2.7	2.4
dAdo	0.10	2.6	1.83	2.8	2.6
dAMP	0.11	2.7	1.86	2.9	2.2
dApdA[b]	0.05	0.79	1.60	2.0	2.6

[a] Average value of three to five determinations.
[b] Photodestruction yield per base.

and/or photobleaching of the trapped electrons occur during the UV illumination. Except for dAMP, photodestruction yields in 8 M NaOH glass are of the same order of magnitude as the photodestruction yields in 12 M LiCl glass. However, the photoreactivities of dAdo and dAMP are higher in the alkaline glass than in the neutral medium, while adenine and dApdA are more photoreactive in the neutral solvent.

4. Conclusions

Photo-ionization of adenine, dAdo, dAMP and dApdA has been observed in neutral and basic glasses at 77 K and appears to be the principal photodestruction path. The intermediate species detected during UV photolysis of adenine and its derivatives in neutral glass at low temperatures are radical cations, trapped electrons, anions and excited triplet state molecules. Photosensitization or charge transfer reactions to the solvent are also observed, leading to Cl_2^- ions. Both photo-ionization and reactions with the solvent involve an excited triplet state of adenine or its derivatives. In the neutral glass the most reactive derivative with respect to photo-ionization and solvent reaction is the dinucleoside phosphate.

Intermediate species produced in UV-irradiated solutions of adenine or its derivatives in alkaline glasses include, in addition to trapped electrons, radical ions and hydrogen addition radicals resulting from reactions of radical anions with water. The dinucleoside phosphate is the most reactive derivative with respect to photo-ionization. The results indicate that incorporation of adenine into a deoxyadenylic acid chain may reduce its photoresistance.

Except for the negatively charged species of dAMP, which is much more photoreactive in the basic than in the neutral glass, the photoreactivity of the other adenine derivatives as measured from the yield of their intermediate is similar in both media.

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