AN ELECTRON PARAMAGNETIC RESONANCE STUDY OF THE EXCITED TRIPLET STATES OF ADENINE AND SOME OF ITS DERIVATIVES IN AQUEOUS GLASSES AT 77 K

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Summary

Electron paramagnetic resonance studies of the lowest triplet states of adenine (Ade), 2′-deoxyadenosine (dAdo), 2′-deoxyadenosine-5′-monophosphate (dAMP) and 2′-deoxyadenylyl-(3′,5′)-2′-deoxyadenosine (dApdA) in aqueous 12 M LiCl glasses at 77 K were performed. The triplet decay lifetimes of Ade, dAdo and dApdA are 2.9 s, 2.8 s and 2.9 s respectively, while the r.m.s. zero-field splitting parameter D^* is 0.133 cm^{-1} for Ade and dAdo and 0.134 cm^{-1} for dAMP and dApdA. The intersystem crossing quantum yields for the neutral molecules are 0.17, 0.051, 0.008 and 0.014 for Ade, dAdo, dAMP and dApdA respectively. The rates of intersystem crossing are lower for the cationic or anionic species of these adenine derivatives than for the neutral species.

1. Introduction

Purine bases have been recognized to participate in the light-induced reactions of nucleic acids [1 - 3]. Photoreactions of purines with alcohols, amines and ethers have been observed [1]. In these photoalkylation reactions the participation of the purine excited triplet states has been implied.

Phosphorescence spectra of deoxyribonucleic acid (DNA) at low temperature have been shown to consist of two components which originate mainly from neutral thymine and to a lesser degree from adenine (Ade) [4, 5]. This fact suggests that the triplet state of Ade, as well as that of thymine, is involved in the photochemistry of DNA. Steele and Szent-Gyorgyi [6] observed phosphorescence emission from Ade and its derivatives. From polarized emission studies, the phosphorescent triplet state in Ade was shown to be of π,π^* origin [7].

Phosphorescence decay lifetimes of 2.2 s, 2.5 s and 2.45 s have been measured for Ade, adenosine (Ado) and adenylic acid (AMP) respectively in ethylene glycol–water (1:1) glass at 77 K by Longworth *et al.* [8]. Kleinwachter [9] reported phosphorescence lifetimes of 2.3 s, 2.8 s, 2.7 s and

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2.6 s for Ade, Ado, AMP and adenylyladenosine (ApA) respectively under similar conditions. Progressive substitution of undissociated terminal phosphate groups has been reported [10] to increase phosphorescence lifetimes of Ade derivatives in acidic ethanol glasses. The phosphorescence lifetimes of the order of a few seconds indicate a π,π^* triplet state. Small variations in phosphorescence decay lifetimes have been observed for the neutral, cationic and anionic forms of Ade [8, 11] and Ado [12].

Phosphorescence quantum yields for Ade, Ado and AMP in neutral ethylene glycol–water (1:1) glass at 77 K were reported by Longworth *et al.* [8] to be 0.035, 0.008 and 0.004 respectively. Honnas and Steen [13] measured phosphorescence yields of 0.09, 0.09 and 0.010 for Ade, Ado and AMP under similar conditions. Higher phosphorescence yields have been reported in ethanol glass at 77 K for Ade (0.12), Ado (0.029) and AMP (0.04) by Gibson and Turnbull [10]. For the dinucleoside phosphate ApA a phosphorescence yield of 0.13 was reported by Kleinwachter and Kouldelka [14]. Protonation of Ade, Ado or AMP quenches their phosphorescence [8, 10], while deprotonation of Ade enhances it [8]. Quenching of fluorescence emission was also observed on substitution by ribose at the N-9 position of Ade [8, 10].

Rahn *et al.* [15, 16] have detected powerful electron paramagnetic resonance (EPR) signals from Ade, Ado, AMP and polyadenylic acid (poly A) in neutral ethylene glycol–water (1:1) glass at 77 K. From the $\Delta m_s = 2$ transitions of Ade and Ado a triplet decay lifetime of 2.6 s and a zero-field splitting parameter D^* of 0.126 cm^{-1} were determined for both compounds [15]. For AMP and poly A, the $\Delta m_s = \pm 1$ transitions were recorded [16]. The triplet decay lifetime for both compounds was 2.5 s, while the D^* parameters are 0.128 cm^{-1} and 0.125 cm^{-1} for AMP and poly A respectively. An intersystem crossing quantum yield of 0.02 was reported by Guéron *et al.* [17] for AMP in ethylene glycol–water (1:1) glass at 77 K. This value is 50% of the largest reported phosphorescence yield for AMP in ethanol glass. These results exemplify the large scatter in reported values for triplet state parameters of these molecules.

EPR signals due to photoexcited triplet states were also detected for Ado in ethanol solution at 77 K [18]. A D^* value of 0.129 cm^{-1} was reported for the $\Delta m_s = 2$ transition of Ado.

For aqueous solutions of AMP at 300 K, Lamola and Eisinger [19] determined an intersystem crossing quantum yield of 3.7×10^{-4}, using the Eu^{3+} method after excitation of the nucleoside at 265 nm. A triplet lifetime of 8×10^{-6} s was estimated under these conditions. From nanosecond laser UV photolysis experiments, Nikogosyan *et al.* [20] reported an intersystem crossing quantum yield of 2.3×10^{-3} for Ade in aqueous solutions at room temperature.

In the present work the properties of the triplet states of Ade and its derivatives 2′-deoxyadenosine (dAdo), 2′-deoxyadenosine-5′-monophosphate (dAMP) and 2′-deoxyadenylyl-(3′,5′)-2′-deoxyadenosine (dApdA) were studied in order to compare them and to initiate studies on the effect of

deoxyribose compared with ribose substitution on the triplet excited states of purine nucleoside and nucleotides, since this effect has not been explored in detail [21]. The triplet quantum yields were measured to determine whether any correlation exists between these yields and the low temperature photoionization and photodestruction yields of these molecules [22].

2. Experimental details

2.1. Materials and sample preparation

Ade (99%; Aldrich), dAdo (98%; Sigma), dAMP (99%; Sigma), dApdA (Sigma), NaOH (Fisher; certified reagent), LiCl (Fisher; certified reagent), H_3PO_4 (ortho, 85%; Fisher; certified reagent) and galvinoxyl free radical (Aldrich) were used as received. Aqueous glasses were produced by adding a high concentration of the inorganic compounds to triply distilled water and cooling to 77 K.

Solutions of Ade or its derivatives of known concentration in the range 0.02 - 0.04 M were prepared using standard weighing and volumetric techniques. In this concentration range the solutions absorb a greater fraction of UV light of wavelengths longer than 260 nm, at which wavelengths the intensity of the excitation of the Hg–Xe lamp is greatest. These solutions showed optical densities larger than or at least equal to unity for wavelengths of less than 295 nm. An aliquot of the solution was introduced into a square Suprasil cell of optical path 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 mixture of dry ice–acetone for the other glasses to avoid cell cracking. After degassing, the sample was sealed under vacuum.

2.2. Irradiation conditions, actinometry and quantum yield determination

The samples were irradiated using similar conditions to those previously described [22, 23]. For Ade samples, a high intensity monochromator (Bausch and Lomb, 33-86-79) was used to irradiate at a wavelength of 280 nm. For the other Ade derivatives, a combination of a Corning 7-54 filter and a nickelous sulfate solution (path length, 2 cm) was used instead of the monochromator, to obtain a larger incident light intensity and therefore a higher triplet molecule concentration. To compare intersystem crossing yields obtained with the filter assembly with those measured using the monochromator, the triplet yield of Ade was determined by also illuminating the samples with a lamp plus filter arrangement. The values obtained by both methods agree within 20%.

The procedure followed to determine the incident light intensity and the triplet concentration from EPR data and to calculate the intersystem crossing quantum yields has been described previously [22, 23].

3. Results and discussion

On UV illumination of 0.035 M solutions of Ade, dAdo, dAMP or dApdA in neutral aqueous 12 M LiCl glasses, EPR signals were detected in the range 1300 - 1500 gauss. These signals consisted of asymmetric singlets of 25 - 40 gauss linewidth, extending over 85 - 100 gauss. No such EPR signal was detected during UV irradiation of the LiCl glass alone. The decay lifetimes for the species yielding the EPR signals were measured from the rate of disappearance of the signal after the UV illumination had been stopped. The decay lifetimes determined were 2.9 ± 0.1 s, 2.08 ± 0.2 s and 2.9 ± 0.2 s for Ade, dAdo and dApdA respectively. The signal intensity resulting from dAMP was too weak to allow measurement of its decay time. Since these values are similar to the phosphorescence decay lifetimes reported for Ade [8, 9, 11] in neutral ethylene glycol–water (1:1) glasses or in ethanol glass at 77 K [10], the EPR signals were assigned to transitions of the triplet excited states of these molecules. From the observed resonance magnetic fields, the transitions are identified as the $\Delta m_s = 2$ transitions of the excited triplet states. The $\Delta m_s = 1$ transitions could not be detected for any of these molecules under the specified conditions even at spectrometer sensitivities ten times higher than those required to detect the $\Delta m_s = 2$ triplet state signals. The $\Delta m_s = 1$ transitions have been observed for AMP and poly A [16] in ethylene glycol–water (1:1) glass.

Since the $\Delta m_s = \pm 1$ transitions are highly anisotropic, their absence in Ade derivatives in LiCl glass could imply a more distorted LiCl matrix which prevents proper orientation of the triplet states with respect to the external magnetic field.

The r.m.s. zero-field splitting parameters $D^* = (D^2 + 3E^2)^{1/2}$ which measure the interaction energy of the two spins along the molecular z axes are given in Table 1. The D^* for the four molecules are essentially identical; thus it can be concluded that no change in the zero-field splitting of the triplet sublevels of Ade is produced by substitution of the N-9 hydrogen atom by a deoxyribose or deoxyribose phosphate group even if the energy

TABLE 1

R.m.s. zero-field splitting parameters D^* for adenine and derivatives in aqueous 12 M LiCl glass at 77 K

Molecule	H_{min} (gauss)	D^* (cm^{-1})
Ade	1420	0.133
dAdo	1425	0.133
dAMP	1440	0.134
dApdA	1423	0.134

$D^* = (D^2 + 3E^2)^{1/2} = \{3/4(h\nu)^2 - 3(g\beta H_{min})^2\}^{1/2}$ where $h\nu$ is the microwave energy, $g = 2.0023$, β is the Bohr magneton and H_{min} is the magnetic field at the maximum of the triplet absorption spectrum.

of the triplet state is shifted by 9-ribose substitution as deduced from a large red shift in the phosphorescence emission [10]. The D^* parameters as well as the magnitude of the decay lifetimes indicate that the lowest triplet state of Ade and of its deoxyribose derivatives at neutral pH is a π,π^* state. The D^* measured differ somewhat from $D^* = 0.126$ cm^{-1} reported by Rahn *et al.* [15] for Ade in neutral ethylene glycol–water (1:1) glass at 77 K. The small variation observed between these D^* values can be attributed to different solvent–solute interactions in different host media [24], such as charge transfer interactions [25] from the triplet Ade molecule to the solvent or hydrogen bonding [26] to the Ade aza nitrogens. These interactions increase charge delocalization in the Ade molecule and decrease the dipolar interaction energy in the triplet state. The higher D^* parameter for Ade in 12 M LiCl glass compared with the ethylene glycol–water glass indicates that the magnitude of the solvent–solute interactions in the LiCl glass is smaller than that in the other glass. The difference in D^* parameters between AMP and dAMP is accounted for in terms of different solvent–solute interactions in different host media instead the effect of the deoxyribose phosphate on the interaction energy of the spins being different from that of the ribose phosphate. Arce and Rivera [22] measured $D^* = 0.152$ cm^{-1} for purine in 12 M LiCl glasses which is 20% smaller than the value measured in neutral ethylene glycol–water (7:3) glass. The smaller D^* value obtained for Ade compared with purine free base in the same LiCl glass is explained in terms of a greater electron delocalization in the Ade triplet through the amino group attached at the C-6 position.

Intersystem crossing quantum yields were determined for Ade and the three derivatives in the neutral glass at 77 K and are shown in Table 2. The intensities of the dAMP triplet EPR signals were too low to allow integration of the areas of the spectra. In this case the intersystem crossing yields were only estimated by comparing the signal height with the height of the signal from an Ade sample in 12 M LiCl glass, for which $\phi_{isc} = 0.17$, and making corrections for differences in intensity of light absorbed and spectrometer sensitivities. The triplet yields decrease in the order Ade >

TABLE 2

Intersystem crossing quantum yields ϕ_{isc} for 0.035 M adenine and its derivatives in neutral 12 M LiCl glass at 77 K[a]

Molecule	[T] × 10^5 (M)	$I_{abs} \times 10^{-17}$ (photons cm^{-3})	ϕ_{isc}
Ade	0.82	0.281	0.17 ± 0.04
dAdo	2.1	1.95	0.051 ± 0.009
dAMP	0.79	5.6	0.008 ± 0.001
dApdA	1.9	8.6	0.014 ± 0.001

[a]Values correspond to average values of at least five determinations.

dAdo > dApdA > dAMP. No intersystem crossing quantum yield values at 77 K for these compounds are available in the literature. For AMP, ϕ_{isc} has been reported to be 0.02 [17]. However, the conformational effects that substitution of a ribose by a deoxyribose could produce on the excited state properties of this molecule are unknown. Differences in the circular dichroism of the deoxy dimers from the corresponding ribose compounds have been explained in terms of marked differences in the average conformation [21]. No such differences were found in the circular dichroism of the monomers. For Ade the triplet yield is 0.17 while for purine free base in the same glass it is 0.37 [22]. Thus the introduction of an amino group in the C-6 position of purine decreases the efficiency of triplet state population by a factor of 2.

Phosphorescence quantum yields for Ade in ethylene glycol–water glass range from 0.035 [8] to 0.06 [13] and 0.12 [10], suggesting a high probability (65% - 79%) for non-radiative processes of the Ade triplet state in ethylene glycol–water glass, such as non-radiative return to the singlet ground state or processes leading, via the triplet state, to photoionization of the solute or energy transfer to the solvent or other solute molecules in the matrix. Photoionization of these molecules is evidenced by the formation of trapped electrons and radical cations in yields of the order of 10^{-3} [27]; reactions with the solvent are also evidenced by the formation of Cl_2^- ions. Both processes involve a purine triplet excited state since they are absent in the presence of $MnSO_4$ (0.005 M), which is a triplet quencher [27]. The difference of a factor of 10 or higher between the intersystem crossing yields and the photoionization or destruction yields of the corresponding Ade derivatives implies that a large rate for decay processes from the triplet state of the purines does not lead to its photoionization or destruction.

Differences in the order of reactivity toward intersystem crossing (Ade > dAdo > dApdA > dAMP) and photoionization (dApdA > Ade > dAdo > dAMP [27]) arise from differences in the rate of decay processes from the triplet state such as phosphorescence, energy transfer to the solvent and other triplet reactions. The sixfold difference between the intersystem crossing for dAdo and dAMP follows a similar trend to that observed by Honnas and Steen [13] for the fluorescence quantum yield ϕ_f of Ado and AMP (ϕ_f(Ado) = 10ϕ_f(AMP)). On the assumption that dAdo and dAMP follow a similar trend in their ϕ_f, their difference in intersystem quantum yield can be explained in terms of a difference in intersystem crossing rates and not from internal conversion rate differences.

Fluorescence quantum yields for Ade in neutral ethylene glycol–water (1:1) glass at 77 K are of the order of 0.1 - 0.2 [8, 10, 13]. Recently, the fluorescence from Ade has been attributed to a minor tautomeric (7-H) form as shown by comparative fluorescence excitation spectra from Ade and 7-methyladenine [28]. The possibility of this minor tautomer contributing to the triplet yield of Ade is of interest and is presently under study in our laboratory. The total fluorescence and intersystem crossing yields account

for only 27% of the excited singlet state species, suggesting again a large participation of non-radiative processes in the decay of the first excited state at 77 K. No luminescence yields have been reported for dAdo, dAMP or dApdA.

The presence of the deoxyribose and of the deoxyribose phosphate groups in the N-9 position of an Ade molecule seems to provide additional pathways for the degradation of the excited singlet state. Incorporation of another nucleoside unit into the dAMP molecule as in dApdA almost doubles its triplet state yield. This effect can be attributed to base stacking in dinucleoside phosphates. It has been proposed [29] that base interaction in stacked nucleotides may lead to population of the mononucleotide triplet through intersystem crossing plus a lowering in energy of the triplet state.

Ade and its derivatives exist as cationic species at low pH [30]

$$AdeH_2^+ \rightleftharpoons AdeH + H^+ \qquad pK_a = 4.15 \tag{1}$$

and as anionic species at high pH [30]

$$AdeH \rightleftharpoons Ade^- + H^+ \qquad pK_a = 9.8 \tag{2}$$

In 12 M LiCl glasses containing 0.035 M Ade and 0.025 M NaOH (estimated pH 10), the triplet EPR peak height decreased by 25% compared with the height of the EPR signal observed in the neutral LiCl glass containing Ade. During UV irradiation of 8 M NaOH glasses containing 0.035 M Ade, dAdo, dAMP or dApdA, no EPR signals could be detected in the low field range (1000 - 2000 gauss), even at very high spectrometer sensitivities. However, a strong long-lived luminescence was observed in these cases resulting from the decay of excited species formed through the recombination of photoejected electrons and radical cations [27]. These effects suggest a lower intersystem crossing rate for the deprotonated Ade compared with the neutral species.

During UV irradiation of 6 M H_3PO_4 glasses containing 0.035 M Ade, a shorter-lived luminescence was observed but no EPR signals that could be attributed to triplet states could be recorded. The phosphorescence and fluorescence yields for Ade as a function of pH in neutral glass at 77 K were studied by Longworth *et al.* [8]. Both yields decrease in the order $Ade^- > AdeH > AdeH_2^+$. Since the present results indicate that the intersystem crossing yield is higher for AdeH than for either Ade^- or $AdeH_2$, the rate of internal conversion for Ade^- must be lower than that for AdeH or $AdeH_2^+$.

4. Conclusions

The triplet states of Ade and its derivatives dAdo, dAMP and dApdA in neutral aqueous glasses at 77 K show similar decay lifetimes and zero-field splitting parameters D^*. These excited states participate in photo-destruction and photoionization processes of these molecules.

Substitution of a deoxyribose or a deoxyribose phosphate group in Ade results in a decrease in intersystem crossing yield. Deprotonation or protonation of Ade also produces a reduction in the triplet yield. Previously reported fluorescence yields and the determined intersystem crossing yields account for less than 0.3 of the species in excited singlet states.

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