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LUMINESCENCE CHARACTERISTICS OF ADENOSINE AND ITS PHOSPHATES

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Summary

Singlet and triplet excited state parameters of adenine, adenosine and adenosine phosphates are determined and compared. Progressive substitution of undissociated terminal phosphate groups increases triplet lifetimes in the order τ_p(adenosine 5′-triphosphate) > τ_p(adenosine 5′-diphosphate) > τ_p(adenosine 5′-monophosphate) > τ_p(adenosine) > τ_p(adenine).

1. Introduction

The importance of adenosine and its phosphates in biological energy transfer processes has stimulated interest in the excited states of these derivatives of adenine. In particular, we are led to ask to what extent are the excited states of the adenine nucleus affected by the ribose moiety and the attached phosphate groups?

The work described here represents an experimental study of this question. With this aim we have determined and compared the absorption and emission characteristics of adenine, adenosine and adenosine phosphates. Earlier fragmentary contributions in this field have been handicapped by instrumental limitations.

2. Experimental

Adenine was supplied by Koch-Light Laboratories Ltd., adenosine by BDH, and the adenosine phosphates by Sigma Chemical Company Ltd. Ethanol which was used as a solvent was purified by distillation through a Widmer fractionating column at a reflux ratio of 20:1 (the first and last 20% of the charge were discarded).

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The spectrofluorimeter used has been described previously [1]. The system utilizes a 2 kW xenon arc lamp (Mazda XE/D), two 500 mm grating monochromators (Bausch and Lomb) and an EMI 9558 QB photomultiplier tube in a cooled housing at 203 K. An excitation wavelength of 280 nm and slit widths of 3 mm (5 nm bandpass) were used throughout. For ambient temperature measurements samples were mounted in a cell block thermostatically controlled at 298 K. Low temperature measurements were made at 77 K by use of a cylindrical quartz sample tube mounted in a quartz Dewar flask containing liquid nitrogen. A rotating can phosphoroscope attachment inserted around the Dewar flask was used to eliminate fluorescence when phosphorescence emission was being studied. Phosphorescence lifetimes were determined by photographing the cathode ray oscilloscope trace of the phosphorescence decay at the wavelength of maximum phosphorescence intensity.

Fluorescence lifetime measurements were made by using a time-correlated single-photon counting system employing a thyratron-controlled gated lamp. The excitation pulse had a typical width at half height of about 4 ns and a repetition rate of 20–50 kHz. The method of calculating the decay time from the experimental data has been described previously [2].

3. Quantum yield determinations

Luminescence quantum yields were determined by the comparative method, using Chen's value of 0.13 for L-tryptophan in water as a reference standard at 298 K [3] and Ermolaev's value of 0.74 for benzophenone at 77 K [4]. Additionally, it was assumed that the change in optical density with temperature was not significant between different samples. The quantum yield ϕ is calculated from the relation

$$\phi_x = \phi_{st} \frac{I_x}{I_{st}} \frac{A_{st}}{A_x} \frac{\theta_{st}}{\theta_x} \frac{n_x^2}{n_{st}^2} \quad (1)$$

where I is the area under the corrected emission curve, A the absorbance at the exciting wavelength, θ the relative photon output of the excitation system at the exciting wavelength and n the refractive index; the subscripts refer to the standard (st) and the unknown (x).

4. Procedure

For fluorescence and phosphorescence measurements solutions of concentration 10^{-4} M were used in order to eliminate distortion caused by inner filter effects. For low temperature measurements solutions in clear ethanolic glasses were used, which were degassed on a vacuum line by the cyclic freeze–pump–thaw technique to minimize oxygen quenching and to reduce the frequency of cracking of the glass. The spectra were corrected for

the spectral response of the emission monochromator and photomultiplier by the method of Melhuish [5].

5. Results and discussion

5.1. Adenine

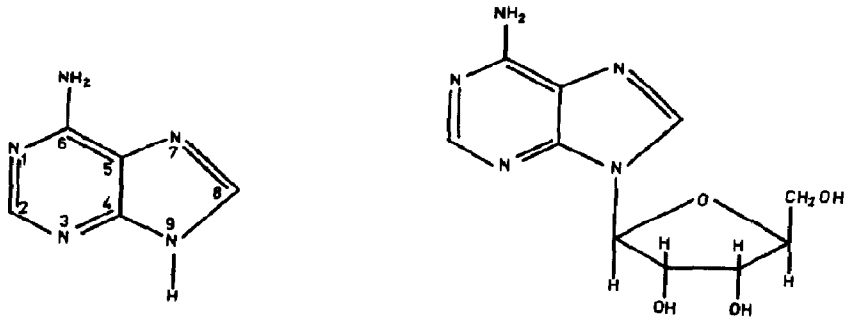
The ultraviolet absorption spectrum of adenine (Fig. 1, I) in neutral ethanol at 298 K takes the form of a single unresolved peak (λ_{max} = 260 nm; ϵ_{max} = 14 500; Fig. 2, curve B). In aqueous solution [6, 7] this spectrum is modified somewhat with the appearance of a slight but significant shoulder at 267 nm. At low temperature (150 K in ethylene glycol mixtures) further resolution was observed [8] in the form of a second shoulder at 280 nm.

The high intensity of the absorption peak at 260 nm indicates that this is most probably a $\pi-\pi^*$ transition. The inflections observed in the resolved spectra are thought to represent vibrational bands in this electronic transition. There are indications of the presence of $n-\pi^*$ transitions in adenine but these tend to be hidden under the main absorption band [9].

The fluorescence emission spectrum of adenine in ethanol at 298 K takes the form of a single peak (λ_{max} = 335 nm) (Table 1) which corresponds in shape to the absorption peak (λ_{max} = 260 nm). The transition is designated $\pi-\pi^*$ but the observed Stokes shift Δ_{max} (75 nm) is large. The fluorescence intensity ϕ_f at 298 K is extremely weak (2.7×10^{-4}) (Table 1) and the fluorescence lifetime could not be determined with our equipment. The fluorescence quantum yield is in agreement with data published elsewhere [10] and the calculated fluorescence lifetime τ_f based on the Strickler–Berg [11] equation is 1.5×10^{-3} ns. The last value is obtained by assuming that the entire low energy absorption band is responsible for the emission and must therefore be regarded as a lower limit. Lamola and Eisinger [12], using the europium ion energy transfer method, have estimated the fluorescence lifetime of adenine in aqueous solution as 6.5×10^{-3} ns.

At 77 K the fluorescence emission increases (ϕ_f = 0.19) showing some fine structure with peaks at λ_{max} of 296 and 308 nm (Fig. 2, curve B) in agreement with the data of Eastman [13]. Attempts by Eastman and Borresen [14] to invoke tautomeric shifts to account for the fluorescence characteristics of adenine are not supported by the later work of Callis *et al.* [15]. The observed blue shift Δ_{max} (3933 cm^{-1}) is consistent with normal Frank–Condon emission in highly viscous media. The fluorescence lifetime determined under these conditions gave a value of τ_f = 2.1 ns. The foregoing fluorescence characteristics would be associated with $\pi-\pi^*$ singlet emission. Fluorescence polarization measurements [16] tend to confirm this assignment.

The observed phosphorescence spectrum (Fig. 2, curve B and Table 1) shows some fine structure (λ_{max} = 365, 383 and 401 nm), a high quantum yield (ϕ_p = 0.12) and a long phosphorescence lifetime (τ_p = 2.1 s). The emis-



I II

Fig. 1. Chemical structures of (I) adenine and (II) adenosine.

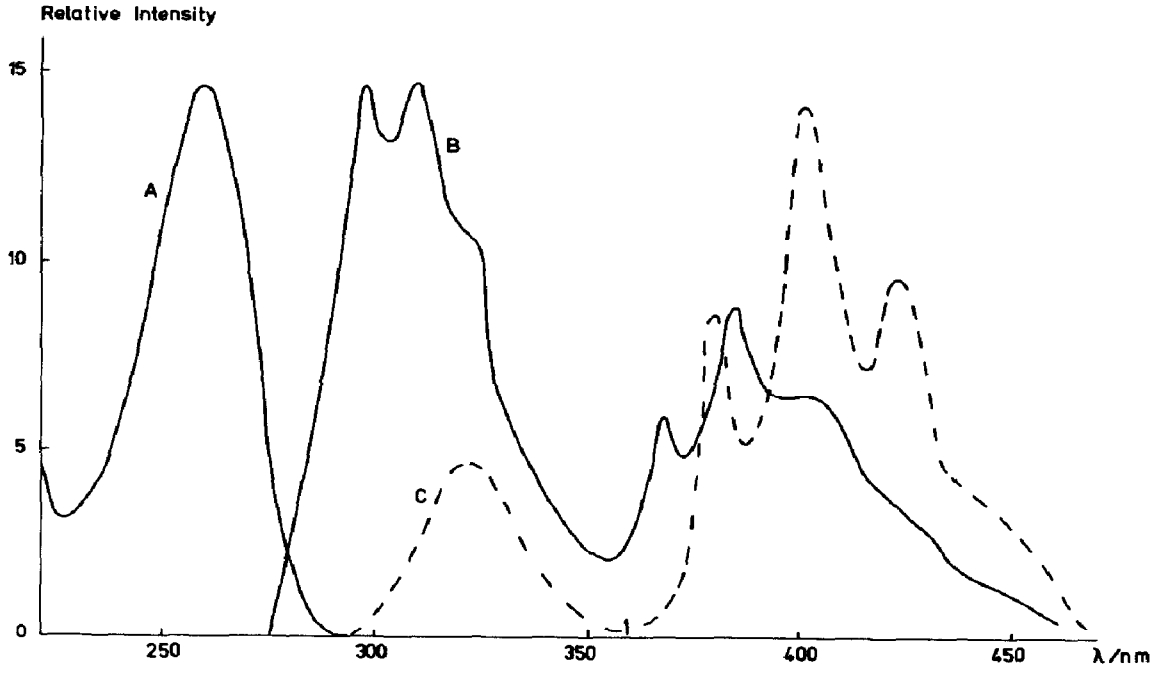


Fig. 2. Absorption spectrum (A) of adenine in neutral ethanol at 298 K and corrected luminescence spectra of adenine (B) and adenosine (C) in neutral ethanol at 77 K. The phosphorescence of adenosine is multiplied by 10.

sion maxima and the phosphorescence lifetime agree with those previously reported [17] although our quantum yield as measured in ethanol is significantly larger. The total luminescence quantum yield is 0.31 showing that radiationless deactivation processes are important even at 77 K. The large triplet–singlet split ($\lambda_{max}(296) - \lambda_{max}(365) = 6400\ cm^{-1}$) together with the long phosphorescence lifetime observed indicates that the emitting triplet state is $\pi-\pi^*$. The emission spectra of adenine in acidic ethanol (Table 2) are consistent with this assignment. The observations that fluorescence quantum yields at 77 K and phosphorescence lifetimes are decreased on protonation confirms that $n-\pi^*$ states are probably not involved.

TABLE 1

Fluorescence and phosphorescence emission characteristics of adenine derivatives in ethanol

Compound	Fluorescence (298 K)		Fluorescence (77 K)			Phosphorescence (77 K)		
	λ_{max} (nm)	ϕ_f	λ_{max} (nm)	ϕ_f	τ_f (ns)	λ_{max} (nm)	ϕ_p	τ_p (s)
Adenine	335	2.7×10^{-4}	296, 308	1.9×10^{-1}	2.1	365, 383, 401	1.2×10^{-1}	2.1
Adenosine	345	2.5×10^{-4}	320	1.6×10^{-2}	1.2	382, 403, 425	2.9×10^{-2}	3.2
5'-AMP	360	5.4×10^{-5}	340	1.7×10^{-2}	0.9	395, 410	4.0×10^{-2}	3.6
5'-ADP	365	1.1×10^{-4}	360	3.4×10^{-2}	0.9	385, 403, 425	6.5×10^{-2}	2.8
5'-ATP	360	9.5×10^{-5}	370	2.6×10^{-2}	1.1	415	1.9×10^{-2}	2.5

TABLE 2

Fluorescence and phosphorescence emission characteristics of adenine derivatives in acidic ethanol

Compound	Fluorescence (298 K)		Fluorescence (77 K)			Phosphorescence (77 K)		
	λ_{max} (nm)	ϕ_f	λ_{max} (nm)	ϕ_f	τ_f (ns)	λ_{max} (nm)	ϕ_p	τ_p (s)
Adenine	365	5.6×10^{-4}	303	1.9×10^{-2}	1.3	383, 402, 420	1.8×10^{-2}	2.1
Adenosine	375	6.8×10^{-4}	320	3.0×10^{-3}	1.2	385, 405, 430	4.0×10^{-3}	2.6
5'-AMP	375	8.6×10^{-4}	300	5.0×10^{-3}	1.1	387, 405	7.0×10^{-3}	3.1
5'-ADP	365	5.6×10^{-4}	360	1.2×10^{-2}	1.2	400	1.7×10^{-2}	3.6
5'-ATP	365	7.3×10^{-4}	360	5.0×10^{-3}	1.0	415	3.0×10^{-3}	4.1

5.2. Adenosine

The absorption spectrum of adenosine (Fig. 1, II) in neutral ethanol is identical with that of adenine [6 - 8]. The emission spectra (Table 1) show significant differences, however. The fluorescence spectrum of adenosine in ethanol at 298 K is shifted to the red* (λ_{max} = 345 nm) but the intensity is virtually unchanged ($\phi_f = 2.5 \times 10^{-4}$). At 77 K the normal low temperature blue shift in the fluorescence peak is observed (λ_{max} = 320 nm) (Fig. 2, curve C). The fluorescence quantum yield at 77 K ($\phi_f = 1.6 \times 10^{-2}$) is significantly lower than that of adenine and the fluorescence lifetime (τ_f = 1.2 ns) is correspondingly reduced. It is evident that the 9-ribose substituent has a quenching effect on the fluorescence of the adenine moiety. The quenching of fluorescence observed in adenosine most probably indicates a perturbation of the excited singlet in adenine by the ribose substituent. Our observation that the quantum yield of phosphorescence is not increased would indicate that the effect of the ribose substituent is predominantly upon internal conversion as opposed to intersystem crossing. The phosphorescence spectrum of adenosine at 77 K (Fig. 2, curve C and Table 1) shows well-defined peaks at λ_{max} of 382, 403 and 425 nm. The large red shift ($\Delta\lambda_{max}$ = 1200 cm^{-1}) in the highest energy triplet emission band compared with adenine is noteworthy. The phosphorescence lifetime τ_p of adenosine (3.2 s) is significantly increased and ϕ_p is reduced, which is consistent with a decrease in the phosphorescence radiative rate constant. The large red shift in the phosphorescence emission indicates that the 9-ribose substituent has appreciably altered the energy of the triplet state, which is consistent with the Taft correlations presented by King [18].

5.3. Adenosine phosphates

The absorption spectrum of adenosine 5′-monophosphate (5′-AMP) is identical with that of adenosine but the emission characteristics (Table 1) are significantly different. The fluorescence spectrum of 5′-AMP at 298 K shows a red shift to λ_{max} = 360 nm ($\phi_f = 5.4 \times 10^{-5}$). At 77 K the fluorescence emission (λ_{max} = 340 nm; $\phi_f = 1.7 \times 10^{-2}$) shows a red shift of 1850 cm^{-1} compared with adenosine, and the fluorescence lifetime (τ_f = 0.9 ns) is slightly shorter. The phosphorescence emission (Table 1; λ_{max} = 395 and 410 nm) is slightly more intense ($\phi_p = 4.0 \times 10^{-2}$) than that of adenosine and the lifetime (τ_p = 3.6 s) is somewhat longer.

The emission spectra of adenosine 5′-diphosphate (5′-ADP) and 5′-triphosphate (5′-ATP) show similar characteristics to those of 5′-AMP. The emission spectra in acidic ethanol solution (Table 2) presents a similar but more consistent pattern. The phosphorescence lifetimes are progressively lengthened by substitution of phosphate groups: τ_p(5′-ATP) = 4.1 s > τ_p(5′-ADP) = 3.6 s >(5′-AMP) = 3.1 s > τ_p(adenosine) = 2.6 s > τ_p(adenine) = 2.1 s, indicating an absence of spin–orbit perturbation of the excited

*This shift indicates increased vibrational relaxation in adenosine compared with adenine.

adenine nucleus by the phosphate groups. It is significant that nuclear magnetic resonance studies on these adenosine phosphates have shown that the ribose phosphate exists in solution in the extended conformation [19]. The increase in triplet lifetimes indicates a decrease in vibrational coupling between the excited molecule and the solvent matrix, possibly associated with a greater degree of hydrogen bonding. The non-radiative rate constants calculated from τ_p and ϕ_p are consistent with this trend. The unusually long lifetime of the triplet state of ATP is certainly interesting but its biological significance remains to be investigated.

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