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SYNCHROTRON EXCITATION OF DNA FLUORESCENCE

DECAY TIME EVIDENCE FOR EXCIMER EMISSION AT ROOM TEMPERATURE

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The first lifetime measurements of DNA fluorescence are reported. Natural and synthetic DNA have been excited by 1.76 ns pulses of synchrotron ultraviolet radiation (270 nm) and the time profile of the fluorescence has been measured by synchronous single-photon counting. A post-pulse exponentially decaying emission has been observed with a lifetime of 2.9 ± 0.4 ns for calf thymus DNA and 3.0 ± 0.3 ns for poly(dA-T); this is most likely an excimer fluorescence.

1. Introduction

Despite its obvious importance for understanding the photochemistry and photobiology of DNA, the fluorescence of DNA remains incompletely characterized and consequently is poorly understood. The reason for this stems from the low quantum efficiency which is only 5×10^{-3} at 77 K and decreases to 2×10^{-5} at room temperature. Spectral measurements are difficult with conventional xenon-arc excitation and lifetime measurements at room temperature have so far been impossible to carry out using standard kilohertz flashlamp (N_2 or $^2H_2$) excitation. For some time time now we have been involved in developing the use of synchrotron ultraviolet radiation as an excitation source for studying very weak emitters such as DNA. Synchrotron radiation has many attractive features, viz., a brightness many times greater than xenon arcs and flashlamp sources, yet not so high that multiphoton effects are troublesome, the emission is continuous in wavelength and very simply tuned, the excitation pulse is almost Gaussian in time (i.e., without a troublesome 'tail') and lastly the beam has a remarkable stability over a period of hours. With this exciting beam, together with spectroscopic and data-processing equipment described below, we have been able to make the first measurements of the time profile of the fluorescence emission from DNA at room temperature, which we are now reporting.

The significance of these lifetime measurements arises as follows. The basic problem in understanding the excited-state behavior of DNA is the relationship between the excited states of its monomer components (A, G, T, C) and their interactions in the helical stacked structure of DNA. Emissions by the individual bases have band envelopes well characterized both at 77 K and at room temperature. Emission from interacting (stacked) bases should be 'excimeric' in nature, with an unresolved band envelope red shifted from the individual bases ('monomeric' emission); this feature has been observed at 77 K [1–3]. However, at room temperature, results are ambiguous. The earliest report [4] showed an emission from buffered aqueous solution with $\lambda_{max} \approx 350$ nm quite similar to the low-temperature work, indicating that the emitting state might similarly be excimeric at room temperature. This spectrum has

been confirmed by Anders [5] using narrow-band microsecond pulsed-laser excitation. However, in other experiments somewhat different spectra have been observed, i.e., narrower [6,7], with $\lambda_{max} \simeq 340$ nm [7a] or 330 nm [7b,8], and with a weaker broad emission appearing at approx. 440 nm [7a,7b,8]. The latter feature is similar to the behavior observed and discussed for ApA and poly(rA), CpC and poly(rC) [9–13] and is observed for ApC, CpA and TpdA [7a]. Our present concern is with the ultraviolet fluorescence band of DNA and it is apparent from the foregoing that at room temperature the emission band shape is not a clear criterion for distinguishing between monomer and excimer emissions which may indeed be considerably overlapped. Accordingly, we have turned to lifetime measurements.

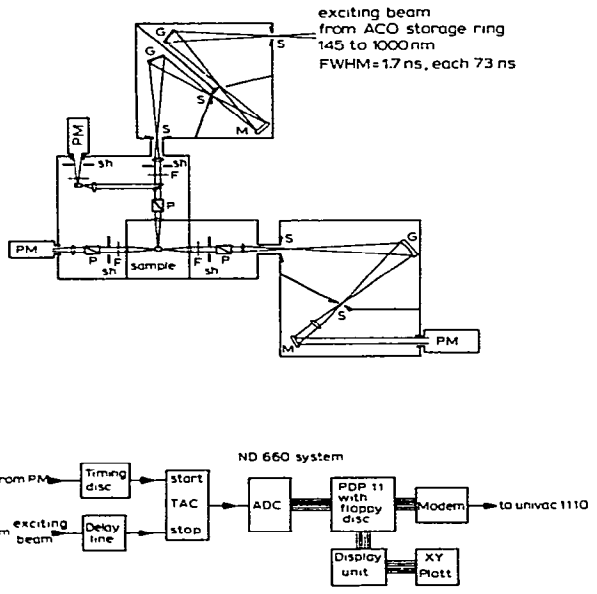

Fig. 1. Experimental setup used for luminescence decay measurements using the synchrotron radiation source ACO at LURE (Orsay, France). (Upper part) Schematic diagram of the SLM spectrofluorometer. (Lower part) Electronics for photon counting (Ortec modules coupled with Nuclear Data 660).

2. Experimental

Experiments have been carried out using the synchrotron radiation facility at LURE (Laboratoire d'Utilisation du Rayonnement Electromagnetique), Orsay, France. The exciting beam (fig. 1) consists of near-Gaussian pulses, 1.76 ns (full-width at half-maximum) in duration, at a frequency of 14 MHz. These are monochromatised with an SLM double monochromator with concave holographic gratings. Scattered radiation and emission is collected at right angles through a single SLM monochromator (also concave holographic) and detected by a synchronous single-photon timing system using a Phillips XP2020 photomultiplier. Data are collected in an ND 660 multichannel analyzer system and analyzed on a Univac 1100 where deconvolutions are performed using a weighted nonlinear least-squares program, together with inspection of the autocorrelation function of the residuals.

3. Results and discussion

It has long been apparent, from considerations of quantum yield and intrinsic radiative lifetimes, that the fluorescence lifetimes of the DNA bases ('monomers') should be in the low or subpicosecond range. Consequently, with an exciting pulse of 1.76 ns duration, the temporal fluorescence profile should be identical with the exciting pulse profile. This has been found to be so for three of the four major nucleosides, an example, thymidine, being shown in fig. 2A. The exception is adenosine which has a significant long-lived tail (fig. 2B). We have strong evidence that this is most likely due to some residual aggregation as similar but stronger effects are seen in ApA and poly(rA) [14] and time-resolved spectroscopy shows a delayed emission [15] spectrally similar to low-temperature excimer fluorescence. When we examine poly(dA-T) and DNA (fig. 3A and B) there is clear evidence that part of the emission is decaying more slowly than the exciting pulse. Quantitative treatment of these results shows that they can be described well by two exponential decays (fig. 4 and table 1).

Before we discuss the interpretation of these

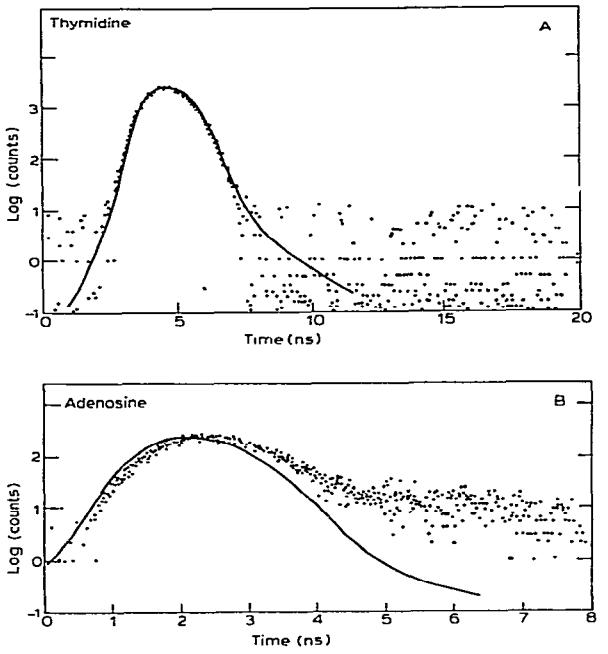

Fig. 2. Fluorescence decays of nucleosides at room temperature. (A) Thymidine. (B) Adenosine. The decays (·····) are recorded under the same conditions with an excitation wavelength $\lambda_{exc} = 270$ nm. The solvent is 0.15 M phosphate buffer, pH 7. Its contribution to the fluorescence decay has been subtracted, taking into account the absorbances of the solutions at the excitation wavelength, which are, respectively, 0.95 for thymidine and 0.62 for adenosine. The scattering pulse from the exciting beam (———) has been recorded using the solvent cuvette with the excitation wavelength set at the same wavelength as the emission wavelength ($\lambda_{exc} = \lambda_{em} = 330$ nm).

findings, it is necessary to examine the possibility that the delayed emissions arise from some impurity centers. This we discount on various grounds. First, X-ray fluorescence analysis of the DNA (Calbiochem, calf thymus) shows a virtual absence of heavy metals as well as Sr, Rb and K [16]. Second, poly(dA-T), being a synthetic product, has the potential for being contaminated with residual blocking agents, but its emission spectrum [6] shows no evidence of this. Third, there may be residual nucleoprotein attached to the DNA; this should give rise, by energy transfer, to tryptophan fluorescence having a lifetime in the range of that

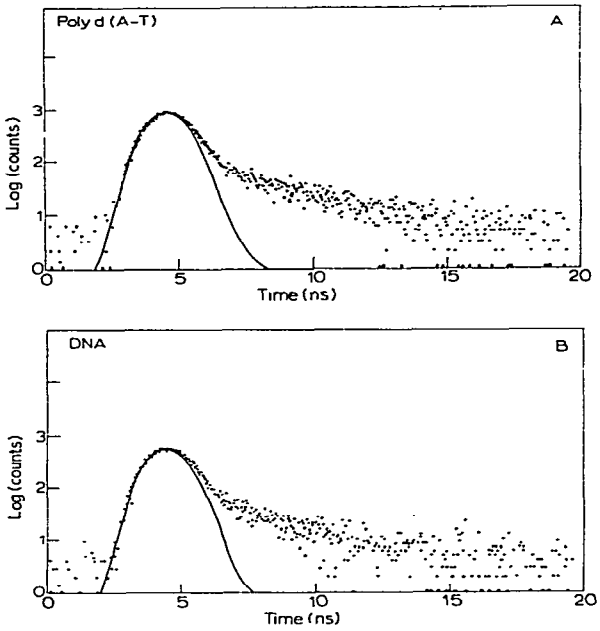

Fig. 3. Luminescence decay of poly(dA-T) and DNA at room temperature in 10^{-2} M Tris-NaCl, pH 7.5, buffer. Solvent background has been subtracted taking into account the absorbance (1.0) at the exciting wavelength. Counting time is 1000 s for each decay. $\lambda_{exc} = 260$ nm (bandwidth $\Delta\lambda = 16$ nm) and $\lambda_{em} = 330$ nm (bandwidth $\Delta\lambda = 16$ nm).

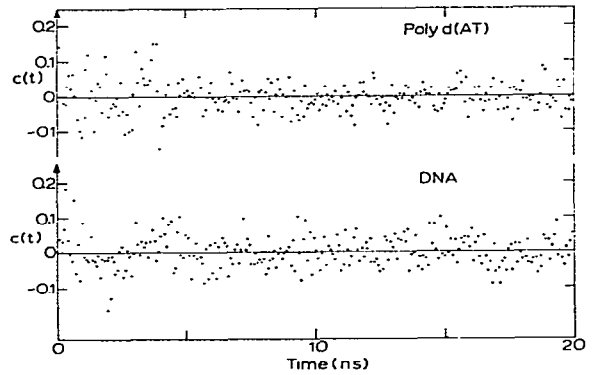

Fig. 4. Autocorrelation function $c(t)$ of the residuals, for two-component fit to the luminescence decays.

Table 1

Two-component analysis of decays of adenosine, poly(dA-T) and DNA

Adenosine	81 ± 7%	of	$\tau_1 < 0.1$ ns
	19 ± 8%	of	$\tau_2 = 2.4 \pm 0.8$ ns
Poly(dA-T)	78 ± 10%	of	$\tau_1 < 0.1$ ns
	22 ± 3%	of	$\tau = 3.0 \pm 0.3$ ns
DNA	80 ± 10%	of	$\tau < 0.1$ ns
	20 ± 3%	of	$\tau_2 = 2.9 \pm 0.4$ ns

observed here. However, in the case of energy transfer, the decay should not be exponential. Furthermore, examination of the DNA phosphorescence shows no sign of the vibronic structure of triplet tryptophan. Lastly, if the DNA emission were to arise from tryptophan then of course it would be absent from poly(dA-T) and one would expect the lifetimes of these two species to be different, but the results for DNA and poly(dA-T) in table 1 are essentially identical. In summary, we find no positive evidence for the emissions from poly(dA-T) and DNA being from impurity centers and we consider the results of fig. 3 and table 1 to refer to intrinsic emissions.

In discussing the nature of the observed emissions from DNA and poly(dA-T) we point out that, as the limit of detectable emission decay time in the apparatus is at best 100 ps, the present experiments can only give us an upper limit for emission of monomer fluorescences, which have at most low or subpicosecond lifetimes. Consequently, any monomer fluorescence (by which we mean fluorescence from any excited constituent base emitting independently) will have the exciting pulse profile. On the other hand, the existence of a longer-lived emission is undeniable and the question arises as to whether it is an 'excimer' type (by which we mean emission from a more or less delocalized exciton state, hence implying coupling between the bases) or whether it is a delayed monomer fluorescence. Criteria for the latter depend on the process by which the emission is delayed; if the excited but delayed monomer arises as a result of triplet-triplet annihilation, then its decay can be exponential and equal to one-half the triplet state lifetime. Triplet lifetimes under the present condition are in the microsecond range [17] and this would be too long, by two orders of magnitude, to account for the results observed here. Furthermore, there is no indication of any nonlinear dependence on exciting beam intensity which the triplet-triplet mechanism would require. Delay of emission due to energy migration or transfer would in general lead to a nonexponential decay function. Arguments favoring the assignment as an excimer type are its long lifetime (relative to the monomers) and its exponential decay, and in view of the above considerations, the observed emission is best assigned as excimer fluorescence. The essential identity of the DNA decay with that from poly(dA-T), which similarly is considered to be excimeric and to result from stacked (AT)* states, strongly suggests that such states are involved in the DNA excimer fluorescence, thus corroborating an earlier suggestion based on excitation spectra studies [6].

There are a number of questions which this work now raises and to which we presently do not have the answers, such as (1) what is the mechanism whereby some of the excitation energy appears as monomers emission while the rest is channeled into excimer emission, (2) which of the bases A, G, T and C are responsible for the rapid monomer fluorescence, and (3) what is the precise molecular nature of the excimer emission in DNA? The present experiments show a strong correlation with AT excimers but others must be investigated. These questions are the subject of a continuing investigation, the results of which will be communicated in due course.

In conclusion, our experiments provide evidence that most of the fluorescence from DNA is a very fast decaying emission which may be assigned to monomers. They also demonstrate the existence of a long-lived fluorescence which is most likely excimeric, and is very similar to that from poly(dA-T).

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