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Electronic Excited-States Properties of the Purine Nucleoside Bases of DNA at Room Temperature

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Abstract: The effects of physical bulk properties on the electronic excited state properties of nucleoside purine bases of DNA (2`-deoxyadenosine (dA) and 2`-deoxyguanosine (dG)) were investigated. For this purpose, the intrinsic fluorescence spectra of solutions of these nucleosides in organic solvents, with various physical properties, were analyzed. The organic solvents used were: methanol, 2-propanol, n-propanol, isobutanol, n-butanol and acetonitrile. It was found that for solutions of dG, polar interactions affect both the spectral shifts and the fluorescence quantum yield: They tend to shift the fluorescence spectra to longer wavelengths, relative to the absorption spectra, and to quench their fluorescence. On the other hand, polarization effects (mainly dispersion interactions) enhance the fluorescence for solutions of dG. The tail in the fluorescence spectra of dG was also investigated. It was found that the tail-to-peak-ratio increases to some extent as the polarizability of the solvent increases and is decreased as the polarity is increased. Thus, solvent effects can alter the magnitude of the tail, even though, they don't seem to play a role in its origin. With regard to dA, neither polar interactions nor dispersion effects seem to contribute to the observed changes in their fluorescence spectra.

Keywords: 2`-deoxyadenosine, 2`-deoxyguanosine, purines, DNA, excited-state, fluorescence.

INTRODUCTION

The excited electronic states of the bases of DNA are of considerable interest. All four bases of DNA strongly absorb the ultraviolet (UV) radiation[1] which reaches the earth's surface from the solar irradiation in the wavelength range of 280–300 nm. This is of particular importance since this constitute the onset of absorption for many bases, especially the purines[2,3]. In fact, the DNA bases are the only nucleic components that can be electronically excited by sun light. Despite the very short lifetimes of the electronically excited states of the DNA base, which are of the order of pico-seconds[4,5], they undergo various types of photoinduced reactions that lead to mutagenic and carcinogenic consequences. It is now well established that solar UV radiation is the main cause of nonmelanome skin cancer[6]. Thus, understanding the effect of ultraviolet radiation on cells requires the understanding of the excited-state properties of nucleic acids. Information on the electronic structure of DNA bases is of crucial importance in understanding the photophysics and photochemistry of these bases as well as the DNA as a whole.

Fluorescence spectroscopy is inherently a sensitive technique for probing the electronic excited states and it has been very useful in the investigation of biophysical systems. When applied to the nucleic acid components, it provides information relevant to excitation dynamics leading to UV-induced genetic damage. However, due to the very low quantum yields of DNA bases[2], previous studies have either studied the DNA bases under very low temperatures, where the quantum yield is considerably increased as a result of the lack of mobility, or used modified DNA bases or chromophores. Recently[7], we have found that the purine bases exhibit interesting solavation effects in which the fluorescence properties are greatly affected by specific interactions between the purines and the surrounding environment[7]; these have great ramifications on gene expression and transcriptional activation as we have already shown[8].

In the present work we investigate the effect of the various bulk physical and chemical properties on the excited states of the purine nucleoside bases: 2`-deoxyadenosine and 2`-deoxyguanosine in an effort to understand the cause of their fluorescence quenching in DNA. This will be done by investigating their intrinsic steady-state fluorescence properties.

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MATERIALS AND METHODS

Materials and Sample Preparation: 2'-deoxyadenosine (dA) was obtained from USB Corporation (USA) and 2'-deoxyguanosine (dG) from Sigma (USA). Spectral grade methanol was a product of Malinkcrodt (USA). High purity acetonitrile, n-butanol, 2-propanol, and isobutanol were obtained from VWR Scientific (USA). HPLC grade n-propanol was obtained from Aldrich (USA). These solvents were chosen on the basis of their transmission in the UV region and the solubility of the nucleosides in them. The cut-off wavelengths, above which the solvent absorption is very small, for these solvents are (in nm): 191 for water, 190 for acetonitrile, 210 for methanol, 210 for 2-propanol, 215 for n-butanol, 210 for n-propanol, and 230 for isobutanol. All nucleosides and organic solvents were checked for fluorescence impurities and were used without further purification.

The nucleosides were added (in small amounts) to a vial containing the solvent and were hand-shaken until dissolved. When necessary, vortexing was also used. The absorption was checked and the solution was diluted in the cuvette until an absorbance *A* between 0.05 and 0.06 at 265 nm (which was the wavelength of excitation) was reached. This very low absorbance was used in order to insure that the fluorescence intensity is proportional to the sample concentration. The absorbance *A* and the concentration are related by[9]:

$$A = \log(I_0/I) = \varepsilon c d \qquad (1)$$

Here, *Io* and *I* are the incident and the transmitted light intensities, respectively, *d* is the optical path of the sample (in cm), ε is the molar extinction coefficient (in M^{-1} cm^{-1}), and *c* is the molar concentration (in M). The concentration of the samples for the fluorescence measurements carried out in the present study was of ~ 5×10^{-6} M. For the absorption spectral measurements, concentrations of about one order of magnitude larger than this were used.

Measurements and Data Analysis: All absorption measurements were performed using a Shimadzu UV/Vis - 254 spectrophotometer (Shimadzu , Japan). Fluorescence measurements were made at a right angle to the incident light using a spectrofluorometer that consists of: a 1 kW Xe-Hg lamp, a 0.5 m focal length excitation monochromator, sample compartment (chamber) which holds the cuvette that contains the sample, quartz cuvettes that had all four sides polished, a 0.32 m focal length emission monochromator, a thermoelectrically cooled photomultiplier, a preamplifier, and a photon counter. The software supplied with the system was used for the acquisition of the data.

In all measurements, the excitation wavelength was 265 nm because the Hg-Xe lamp has a strong line at that wavelength and the absorption spectra of the nucleosides have their maximum at about that line[2]. The excitation and emission bandwidths were kept at 5 nm and 1.7 nm, respectively. The entrance/exit slit widths of the excitation monochromator were 4 mm/ 3 mm, whereas those for the emission monochromator were 2 mm / 2 mm, respectively. Both the sample and the reference were scanned in the range of 300 nm - 500 nm in steps of 0.2 nm. When necessary, scanning from 290 nm was made. For each sample, the measurements were repeated several times and the obtained spectra were averaged. The uncertainty in the fluorescence intensity was determined from the standard deviation of the average spectra and found to be ~ 7%. The widths of the fluorescence spectrum as well as that of the absorption spectrum, designated as the full-width-at-half-maximum (FWHM) $\Delta\bar{\nu}$ (in cm-1) were calculated from:

$$\Delta\bar{\nu} = (\Delta\lambda)\frac{10^7}{\langle\lambda\rangle^2} \qquad (2)$$

where Δλ .is the bandwidth (in nm) at half maximum and < λ > is the average wavelength of the band (in nm). For dG, the full-width-at-two-third-maximum (FWTTM) was used to calculate $\Delta\bar{\nu}$ in order to eliminate contributions from the long tail of the fluorescence spectrum. Thus, in Eq. 2 for this case, Δλ. is the bandwidth at two third maximum and < λ > is the average wavelength of this band. The calculation of the FWTTM was also made for the absorption spectrum of dG. The magnitude of the long tail in the fluorescence spectrum of dG is quantified by calculating the tail-to-peak-ratio γ from the following formula:

$$\gamma = \frac{I_{460}}{I_P} \qquad (3)$$

Here, I_p is the fluorescence peak intensity and I_{460} is the intensity at λ = 460 nm. The estimated uncertainty in the wavelength of the fluorescence spectral peak was ± 2 nm, and that in the wavelength of the absorption spectral peak was ± 0.25 nm.

The fluorescence quantum yield φ *F* of the sample of interest was calculated using[10].

$$\phi_F = \phi_1 \frac{A_1 n_2^2 \int I_2(\lambda)\,d\lambda}{A_2 n_1^2 \int I_1(\lambda)\,d\lambda}$$

Here, φ_r is the quantum yield of the reference, n_s and n_γ are the refractive indices of the solvent in the sample of interest and in the reference sample, respectively, A_s and A_r are the corresponding absorbances, and $I_s(\lambda)$ and $I_r(\lambda)$ are the corresponding fluorescence intensities at wavelength λ . Each integral in the above formula is carried over the whole spectrum range and it represents the area under the fluorescence spectrum.

RESULTS AND DISCUSSIONS

In the present study, the electronic excited-state properties of the purine nucleosides in solution of organic solvents were investigated. A number of organic solvents were selected such that they transmit well in the UV region, have different physical and chemical properties and that the purine nucleosides have sufficient solubility in them. Table 1 lists some physical properties of the solvents used: acetonitrile, n-butanol, methanol, 2-propanol, n-propanol, and isobutanol. The fluorescence spectra of the nucleosides (dA and dG) in these organic solvents are shown in Figures 1 - 2, respectively, and the corresponding spectral parameters are listed in Tables 2 - 3. Also listed in these tables are the parameters for the absorption spectra (which are not shown).

Spectral Shifts: We will first investigate the shifts in the wavelengths of the spectral peaks. These may be the result of the following interactions[11]: (i) solvent polarity, (ii) solvent polarizability, or (iii) specific. Since shifts in the fluorescence maxima might be related to shifts in the absorption maxima (see Eq. 5 below), the difference in the peak wavelengths between the absorption and the fluorescence spectra, rather than just the fluorescence peak, is investigated. Moreover, differences in the wavenumbers (1/wavelength), rather than wavelengths, are often used because of the proportionality between energy and wavenumber. A simplified theory, in which the solvent is regarded as a continuum, predicts that the difference between the wavenumbers of the maxima of the absorption and fluorescence spectra (V_a V_f) depends on the refractive index n and the dielectric constant å of the solvent in the form[10].

$$V_a - V_f = \frac{2(\mu_g - \mu_e)^2}{hca^3}\left[\frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1}\right] + \text{constant} \quad (5)$$

Where μg and μe are the permanent dipole moments of the ground and excited states, respectively, h is Planck's constant, c is the speed of light in vacuum, and a is the radius of the cavity in which the fluorophore resides. Equation 5 is called the Lippert's equation and the function in brackets is called the Lippert's function .f. This function is taken to represent a macroscopic measure of the solvent polarity[10]. It should be noted here that for nonpolar solvents $\varepsilon = n^2$ and, hence, Δf vanishes, whereas for polar solvents, $\Delta f > 0$ (Table 1). Plots of the spectral shifts V_a V_f as a function of Δf for the two nucleosides, dA and dG, (data not shown) reveal that there is no correlation between the spectral shifts and the solvent polarity expressed in terms of Δf for any of the nucleosides. However, this finding alone does not rule

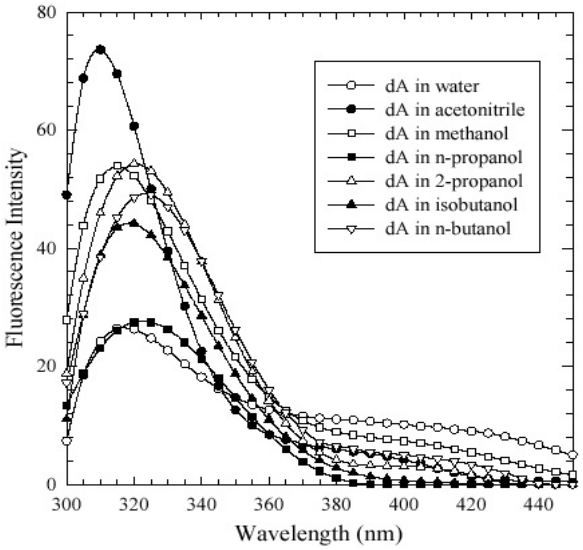

Fig. 1: Fluorescence spectra of 2'-deoxyadenosine (dA) in the organic solvents used in the present study and in water.

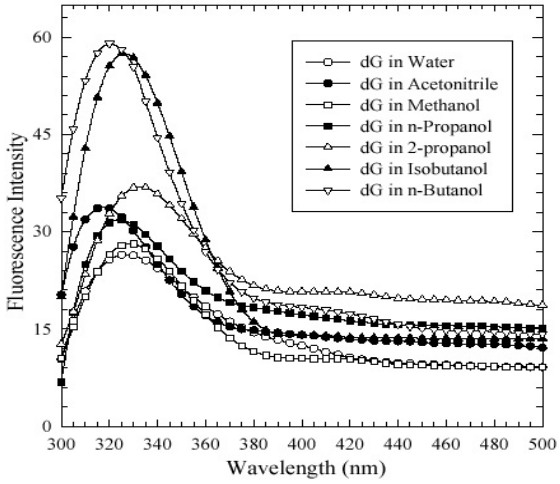

Fig. 2: Fluorescence spectra of 2'-deoxyguanosine (dG) in the organic solvents used in the present study and in water.

Table 1: Values of the dielectric constant ε, the polarizability α (in Å^3), the dipole moment μ (in D), the refractive index *n*, the empirical parameter of solvent polarity E_T^N T E , and the Lippert's function Δ*f* for the solvents used in the present study.

Solvent	ε[21]	α (Å^3)[21]	μ(D)[21]	*n*[21]	E_T^N [2]	Δ*f*[a]
Water	80.1	1.45	1.86	1.333	1	0.320
Acetonitrile	36.6	4.40	3.93	1.344	0.46	0.305
Methanol	33.0	3.29	1.7	1.378	0.762	0.309
n-Propanol	20.8	6.70	1.55	1.385	0.617	0.275
2-propanol	20.2	7.61	1.58	1.378	0.546	0.277
Isobutanol	17.9	8.90	1.64	1.396	0.552	0.266
n-Butanol	17.8	8.88	1.66	1.385	0.586	0.264

[a] The values of the Lipperts function were calculated form the bracketed function in Eq. 5.

Table 2: Fluorescence and absorption spectral parameters for 2'-deoxyadenosine (dA) in the solvents used in the present study: the relative fluorescence quantum yield φ_F, the wavelength of the fluorescence and absorption spectral peaks $\lambda_{p,f}$ and $\lambda_{p,a}$, respectively, and the full-width- at-half-maximum of the fluorescence and absorption spectra, $FWHM_f$ and $FWHM_a$, respectively.

Solvent	φ_F (*x10 $^{-4}$*)	$\lambda_{p,f}$ (nm)	$\lambda_{p,a}$ (nm)	$FWHM_f$ (cm $^{-1}$)	$FWHM_a$(cm $^{-1}$)
Water	0.6	319	260	4570	4470
Acetonitrile	0.84	310	258	3430	4570
Methanol	0.84	315	260	4240	4520
n-Propanol	0.42	322	260	4850	4470
2-propanol	0.78	320	260	4300	4520
Isobutanol	0.6	318	261	4130	4440
n-Butanol	0.84	322	261	4530	4520

Table 3: Fluorescence and absorption spectral parameters for 2'-deoxyguanosine (dG) in the solvents used in the present study: the relative fluorescence quantum yield ö F, the wavelength of the fluorescence and absorption spectral peaks ë p,f and ë p,a , respectively, the full-at-two-third- maximum for the fluorescence and absorption spectra, FWTTMf and FWTTMa, respectively, and the fluorescence spectrum tail-to-peak-ratio γ.

Solvent	φ_F (*x10 $^{-4}$*)	$\lambda_{p,f}$ (nm)	$\lambda_{p,a}$ (nm)	$FWHM_f$ (cm $^{-1}$)	$FWHM_a$(cm $^{-1}$)	γ
Water	1.0	327	252	5040	4980	0.36
Acetonitrile	1.2	317	254	4160	4710	0.38
Methanol	1.0	329	254	4290	4710	0.33
n-Propanol	1.4	324	255	4690	4710	0.49
2-propanol	1.7	333	255	4950	4710	0.53
Isobutanol	1.8	324	256	4690	4750	– [a]
n-Butanol	2.0	319	255	4150	4850	0.25

[a] γ could not be determined in this solvent because of relatively high impurity fluorescence at λ = 460 nm.

out the role polarity might play in the observed spectral shifts, and other measures of polarity need to be considered (see below).

Empirical scales for solvent polarity have been used in the literature due to the difficulty involved in defining it in terms of a single physical parameter of the solvent, such V_a V_f as its dipole moment or its dielectric constant[12]. Among them, the E_T^N scale (Table 1) is the one most widely used[13]. Figure 3 shows the spectral shifts as a function of E_T^N for dG. It is seen that the spectral shifts of dG have the tendency to increase with increasing polarity, but the correlation is rather weak. Thus, it seems that polar interactions tend to shift the fluorescence spectra dG to

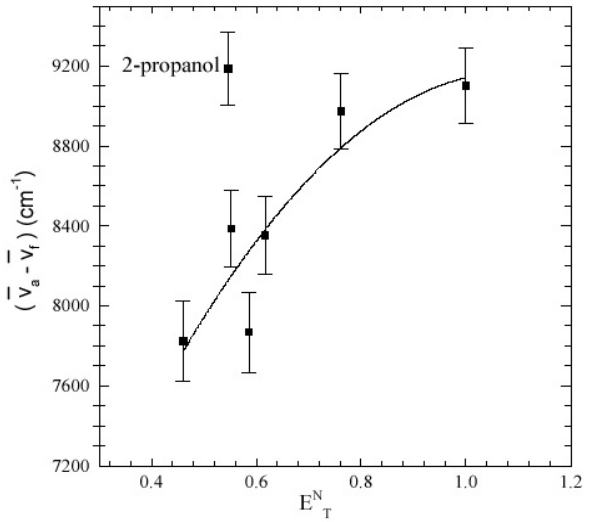

Fig. 3 Plot of the difference between the wavenumbers of the maxima of the absorption and fluorescence spectra V_a V_f of 2'-deoxyguanosine (dG) as a function of the empirical parameter of solvent polarity. E_T^N The bars indicate the standard deviation.

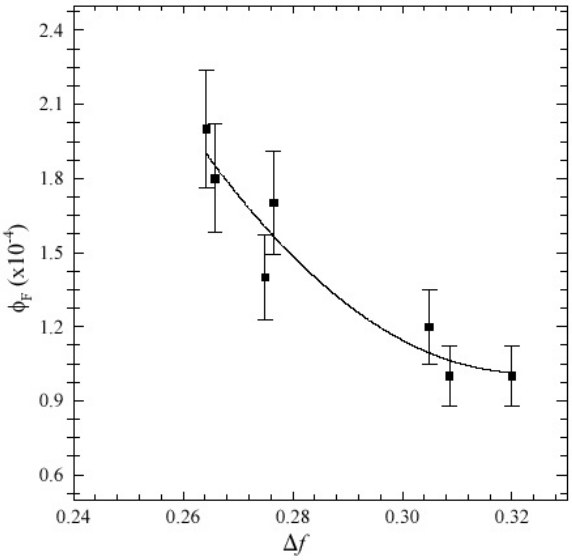

Fig. 4: Plot of the fluorescence quantum yield φ_F of 2'-deoxyguanosine (dG) as a function of the solvent polarity according to Lippert's function Δf. The bars indicate the standard deviation.

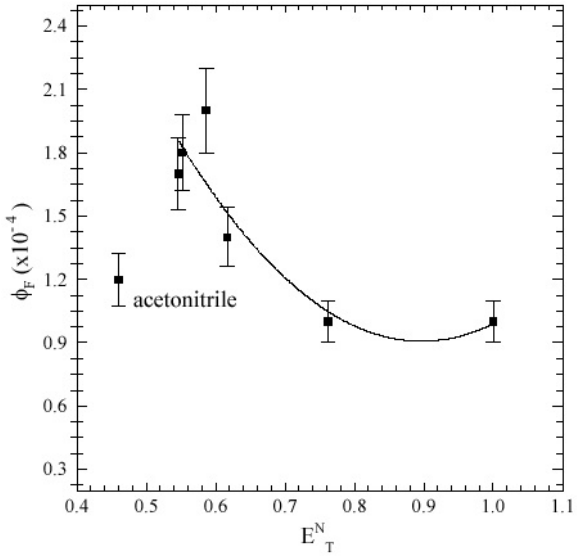

Fig. 5: Plot of the fluorescence quantum yield φ_F of 2'-deoxyguanosine (dG) as a function of the empirical parameter of solvent polarity. E_T^N The bars indicate the standard deviation.

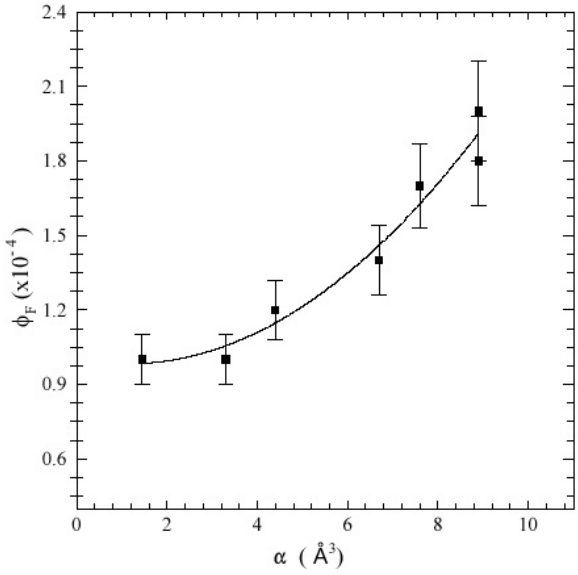

Fig. 6: Plot of the fluorescence quantum yield φ_F of 2'-deoxyguanosine (dG) as a function of the solvent polarizability α . The bars indicate the standard deviation.

longer wavelengths. As for the other purine, dA, no correlation was observed between the spectral shifts and the empirical scale for solvent polarity.

With regard to polarizability effects on the spectral shifts, no correlation was observed (data not shown) for either dA or dG. It should be noted here that polarizability effects include: (i) dispersion interactions between an instantaneous dipole in one molecule and the dipole it induces in another molecule and (ii) interactions in which a permanent dipole moment in one molecule induces a dipole in another molecule. The latter interaction is, however, weak relative to the former[11,14]. Thus, dispersion interactions do not seem to contribute significantly to the observed spectral shift for any of the two nucleosides

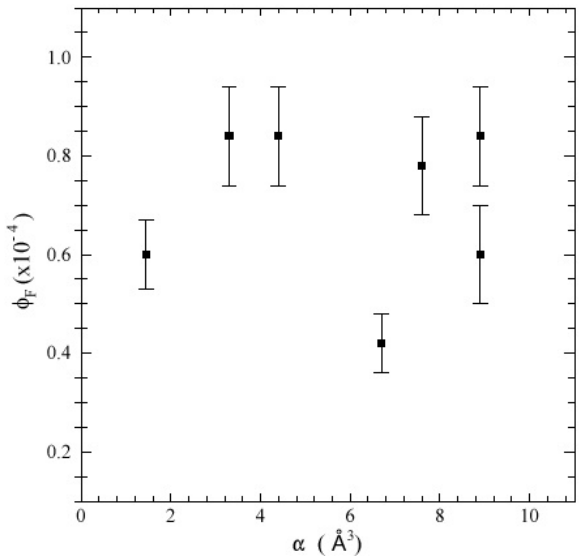

Fig. 7: Plot of the fluorescence quantum yield φ_F of 2'-deoxyadenosine (dA) as a function of the solvent polarizability α . The bars indicate the standard deviation.

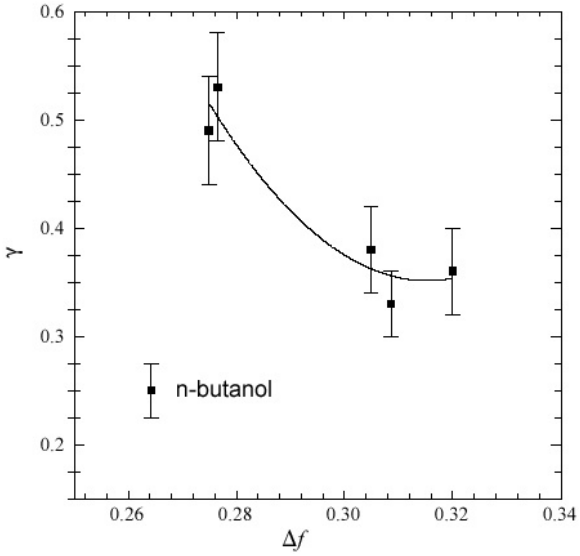

Fig. 8: Plot of the tail-to-peak ratio γ of the fluorescence spectrum of 2'-deoxyguanosine (dG) as a function of the solvent polarity according to Lippert's function Δ*f*. The bars indicate the standard deviation.

As for specific effects, shifts to shorter wavelengths relative to a nonhydrogen bonding solvent are observed when the strength of the hydrogen bond is greater in the ground electronic state than in the excited state[15,16]. On the other hand, shifts to longer wavelengths correspond to stronger hydrogen bonding in the excited state. Unfortunately, none of the two purine nucleosides is soluble in non-hydrogen

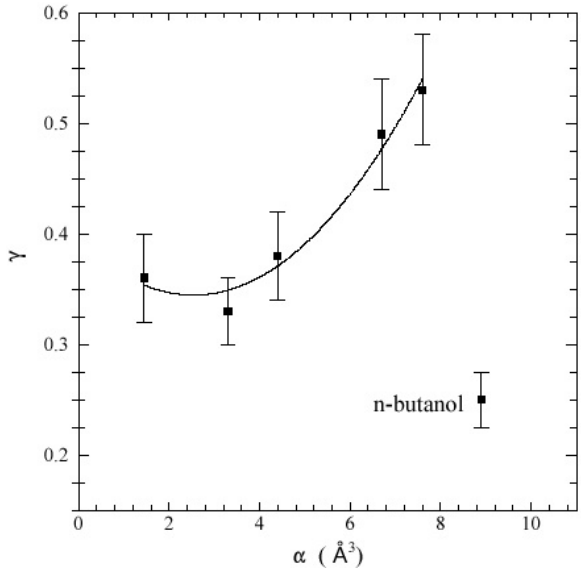

Fig. 9: Plot of the tail-to-peak ratio γ of the fluorescence spectrum of 2'-deoxyguanosine (dG) as a function of the solvent polarizability α. The bars indicate the standard deviation.

bonding solvents (e.g. hexane), and this does not allow conclusions to be drawn regarding the change in the strength of hydrogen bonding following electronic excitation.

The Fluorescence Quantum Yield: We now shift our attention to solvent effects on the fluorescence quantum yield φ_F. Figures 4 -5 show plots for the fluorescence quantum yields of dG as a function of the Lippert's function (Δ*f*) and the empirical parameter for solvent polarity (E_T^N), respectively. Form these figures, it is seen that the quantum yield of dG decreases with increasing polarity, according to both the Lippert's function Δ*f* and the empirical parameter of solvent polarity E_T^N, with the plots exhibiting a fair correlation. However, the value for acetonitrile in Figure 5 exhibits considerable deviation from the fit; a similar observation was previously made for the pyrimidines nucleoside (dT) and was attributed to an intrinsic underestimation of the value of E_T^N for this solvent[17]. As for dA, there is no correlation observed between the fluorescence quantum yield and the solvent polarity according to either Δ*f* or E_T^N (data not shown). Thus, solvent polarity does not seem to play a role in the observed changes in the fluorescence quantum yield for dA. With regard to polarizability effects on the quantum yield, a good correlation is found for dG as shown in Figure 6 with the quantum yield increasing as the polarizability increases and no correlation is observed for dA (Figure 7).

We also inquired about a correlation between the empirical parameter of solvent polarity E_T^N and the polarizability. We found that E_T^N tends to decrease as the polarizability is increased (not shown). However, the correlation is not strong. Therefore, the effects of these two parameters can be considered to operate independently. Hydrogen bonding effects on the quantum yield are also expected, but the quantification of these effects is known to be in general not straightforward[18].

Tail in the dG Spectra: We have also examined whether there is a correlation between the tail in the fluorescence spectra of dG and the solvent properties. Figures 8 - 9 show plots of the tail-to-peak- ratio γ as a function of the Lippert's function, and the polarizability δ , respectively. It is found from these figures that γ exhibits a good correlation with the solvent polarity according to Lippert's function (but not according to E_T^N (not shown)) and the solvent polarizability (with γ decreasing as Δf is increased and increasing as α is increased), with the value in–butanol exhibiting a significant deviation from the fit in both plots. The trends observed in these two figures are similar to those observed for the quantum yield (Figures 4 and 6).

We should note that the origin of the long tail in the fluorescence spectrum of dG is not clear. It is rather unlikely that it is the result of a solvent-induced reversal of the electronic energy levels because it is present not only in water as well as in organic solvents, but also in the double-stranded polynucleotide poly(dG-dC). poly(dG-dC)[20] in which the bases are shielded from the solvent to a good extent. As we discussed before, the tail-to-peak-ratio γ increases to some extent as the polarizability of the solvent increases. Thus, although solvent effects do not appear to be the origin of the tail, nevertheless, they can alter its magnitude. Broad electronic potential energy surfaces would appear to constitute a likely cause of the spectral tail.

Finally, it should be noted that, unlike the pyrimidines, the purines have two ππ* electronic states in their long-wavelength absorption band[2]. The energy gap between the two states is much smaller for adenine. Georghiou and co-workers resolved the two absorption bands of 7-methyl guanine[19]. The data in aqueous solution were found to be consistent with a model that involves conversion of the upper-energy state to the lower-energy state with 100% efficiency and emission exclusively from the lower-energy state. At the excitation wavelength of 265 nm employed in the present study, both states of dG are being excited. (This takes into account a spectral shift of 10 nm to longer wavelengths for 7-methyl guanine.) It is conceivable that the order of the two states may be dependent on the nature of the organic solvent.

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