2-Aminopurine Optical Spectra: Solvent, Pentose Ring, and DNA Helix Melting Dependence

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2-Aminopurine (2AP) absorption and fluorescence excitation and emission spectra in a series of solvents have been measured to assess effects of solvent polarity. Emission spectra of the free base shift to the red in solvents of a higher dielectric constant, including water but excepting dioxane. Excitation spectra also red-shift, except in water. A change in dipole moment of 2.8 D upon excitation is obtained from a Bilot–Kawski plot which includes data from potentially anomolous solvents such as alcohols but which excludes dioxane and aqueous solvents. Attachment of ribose or 2′-deoxyribose causes 1 to 2-nm shifts in the position of fluorescence excitation and emission spectra of 2AP in water and little change in fluorescence yield. Melting of the DNA duplex d[CTGA(2AP)TTCAG]$_2$ yields a blue shift of the excitation and no shift of the emission spectrum—results consistent with increased exposure to water and formation of 2AP–water H bonds in the ground state. The spectral shift occurs 5°C or more below the helix melting temperature, implying a premelting structural change in the decamer.

KEY WORDS: 2-Aminopurine; nucleosides; fluorescence; absorption; spectroscopy; solvent effect; DNA melting.

INTRODUCTION

Conformational changes of proteins have been measured for many years using the fluorescence of the intrinsic chromophores tryptophan and tyrosine, in spite of the complicated nature of the transitions and their time dependence. Fluorescence lifetimes in proteins typically range from about 10^{-10} to 10^{-8} s, and the structural sensitivity of lifetime, spectral peak position, and polarization is great enough that denaturation, ligand binding, and other conformational changes can be easily monitored. DNA has no such intrinsic fluorophore. Roughly 90% or more of the room-temperature fluorescence of natural DNA decays in about 10^{-11} s, which makes large demands on the detector sensitivity and/or instrument response time if intrinsic fluorescence is to be used to monitor conformation [1–4].

2-Aminopurine is much like its normal sister base adenine (6-aminopurine), except that the fluorescence lifetime is up to three orders of magnitude larger [2,5–7]. It has long been recognized as a potential probe of nucleic acid structure, e.g., Refs. 8 and 9, but until recently synthetic techniques were not up to the task of specific incorporation of the modified base into DNA or RNA sequences [10–12]. Ward *et al.* [8] used a polymerase to produce RNA with 2AP incorporated at every other site. They proposed that 2AP base absorption and emission properties depended strongly upon location in the polymer: 2AP at the ends absorbed at 312–315 nm and emitted fluorescence; 2AP located in interior positions absorbed near 303 nm and did not fluoresce. (Subsequent work has shown that interior 2APs in DNA do emit, though with a quantum yield well below that of the free base [2,5–7,13,14].)

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During a series of investigations into the dependence of restriction endonuclease *Eco*RI-DNA recognition on the chemical structure of bases, McLaughlin *et al.* found that the substitution of 2-aminopurine (2AP) for adenine-5 in the d[CTGA*A*TTCAG]$_2$ duplex did not destroy recognition and cleavage efficiency and that the decamer appeared fluorescent on thin-layer chromatography plates (Ref. 10 and personal communication). We have therefore been using the fluorescent and natural decamer to investigate the sensitivity of 2-aminopurine fluorescence to DNA structure, dynamics, and interactions with this site-specific endonuclease [5–7,13–16]. In order better to interpret fluorescence lifetime, spectral and intensity changes of the oligomer as a function of temperature and binding, we have measured the optical spectra of 2-aminopurine as a function of the solvent polarity and as ribose and deoxyribose are attached to the base. We and others have also investigated the effects of base stacking interactions on steady-state and time-resolved spectroscopy of fluorescent bases in DNA [17–19]. Our approach is gradually to construct the type of environment which may be found around a fluorescent base in duplex DNA and observe spectroscopic effects of changes in each part of the environment. Smagowicz and Wierzchowski [20], Gryczynski and Kawski [21], and Kawski *et al.* [9] previously began such work and demonstrated in solvent- and temperature-dependent spectroscopic studies that parameters such as ground-state and excited-state dipole moments of 2-aminopurine and derivatives could be determined spectroscopically. We extend the measurements to additional solvents, including, besides water, more potential hydrogen-bonding or "anomalous" solvents such as propanol, methanol, DMSO, and dioxane [22], to find whether general principles governing 2AP fluorescence in solution (e.g., dielectric interactions) could be found or whether specific molecular interactions expressed themselves in each solvent. Further, we wanted to know whether the deoxyribose ring in 2AP deoxynucleoside would affect the 2AP spectrum. We find that increased solvent polarity shifts fluorescence emission spectra of 2AP free base (2AP) to the red in all tested solvents, including water but excepting dioxane. The Stokes shift of 2AP is described on Bilot–Kawski [23] or Lippert [24] plots for a series of solvents of varying polarity. Glycerol/water mixtures were also studied because of the common inclusion of glycerol in *Eco*RI-DNA preparations. The addition of ribose or 2′-deoxyribose to 2AP causes only small changes in fluorescence spectra and intensity. We then apply the lessons learned from the above studies to begin interpreting the spectral shifts of 2AP-containing DNA upon helix melting.

METHODS

2-Aminopurine free base was purchased from Sigma and used without further purification. 2-Aminopurine 2′-deoxynucleoside (2AP-dns) and 2-aminopurine riboside (2AP-rs) were generously provided by Dr. George W. Koszalka, Burroughs Welcome Co., Research Triangle Park, NC. They were prepared by reactions catalyzed by purified *Escherichia coli* nucleoside phosphorylases. Elemental analyses (C, H, N) were within 0.4% of expected values and mass spectroscopy and proton NMR confirmed the structures (Koszalka, personal communication). Organic solvents were reagent grade or better: 1-4 dioxane (Fisher Cert. ACS), 1-propanol (Fisher HPLC grade), methanol (Fisher HPLC grade), butanol (Fisher Cert. ACS), ethyl ether (Fisher Cert. ACS), DMSO (Fisher Cert. ACS), and ethyl acetate (Fisher HPLC grade). Water was distilled deionized water. A buffer consisting of 10 m*M* Tris–Cl, 0.1 *M* KCl, and 0.1 m*M* EDTA, pH 7.5, was used for the spectra of the 2AP nucleoside samples. Spectra were measured at 25°C, except as noted.

Solid 2-aminopurine free base was added to each solvent to produce 100 μ*M* stock solutions. Water is a relatively poor solvent for the free base, but complete dissolution was accomplished by slight warming. Stock solutions were diluted 10-fold for fluorescence experiments. Preparation of 2AP-dns and 2AP-rs solutions was similar except for the addition of buffer to water.

Absorption spectra were measured on Beckman DU-40 and Gilford Response II spectrophotometers, using a 1-nm bandwidth. Data were stored digitally and transferred to a computer for analysis and plotting. Fluorescence spectra were collected using Perkin-Elmer MPF-66 and LF-50 fluorometers. Excitation and emission bandwidths were 2 and 5 nm, respectively, for 2AP in pure water and the organic solvents and 2.5 and 2.5 nm for 2AP, 2AP-dns, and 2AP-rs in buffer. Data were again stored, converted to ASCII, and transferred to the computer for plotting. Background fluorescence from solvents was negligible compared to the strong emission from 2-aminopurine compounds. Raman emission from solvents amounted to about 2% of the maximum signal, was never within the full-width-at-half-maximum region of the spectra, and was not subtracted from data. Displayed excitation spectra are corrected. Fluorescence emission spectral peaks (Tables I and II) are corrected for detector wavelength sensitivity.

The shifts of spectra as a function of solvent polarity can be analyzed by means of Bilot–Kawski [22,23] or Lippert [24] equations:

Table I. Dependence of Spectral Maxima and Widths on Solvent Dielectric Constant, ϵ[a]

Solvent	ϵ	λ_{abs} (nm)	λ_{ex} (nm)	λ_{em} (nm)	$\Delta\lambda_{ex}$ (nm)	$\Delta\lambda_{em}$ (nm)
1,4-Dioxane	2.21	306	309	359	38.5	50.5
Ethyl ether	4.34	305.5	306	354	40.5	46
Ethyl acetate	6.02	305.5	306.5	354	39.5	48.5
Butanol	17.8	305	305	354	40	48
1-Propanol	20.1	308.5	313	366.5	40.5	51
Methanol	32.6	309	312	367.5	42	51.5
DMSO	45.0	311	317	369	40.5	52.5
Water	78.5	305	306	369	43.5	54
8% glycerol/H_2O	76.1	305	306	370	—	—
12% glycerol/H_2O	75.1	305	306	370		
26% glycerol/H_2O	71.4	306.5	307	370		
50% glycerol/H_2O	64.1	308	310	370		
90% glycerol/H_2O	47.8	311.5	314	370.5		

[a] $\Delta\lambda_{ex}$ and $\Delta\lambda_{em}$ are, respectively, the full width at half-maximum of excitation and emission spectra. Wavelengths rounded to nearest 0.5 nm.

Table II. Bilot–Kawski Equation Parameters and Measured Spectral Shifts

Solvent	ϵ	n	f[a]	$\Delta\bar{\nu}=\bar{\nu}_a-\bar{\nu}_{am}$ (cm^{-2})
1,4-Dioxane	2.21	1.420	0.043	4680
Ethyl ether	4.43	1.352	0.377	4490
Ethyl acetate	6.02	1.370	0.490	4480
Butanol	17.7	1.397	0.754	4790
1-Propanol	20.1	1.390	0.776	5140
Methanol	32.6	1.330	0.854	5150
DMSO	45.0	1.330	0.882	5040
H_2O	78.5	1.331	0.913	5670
8% glycerol/H_2O[b]	76.1	1.346	0.908	5760
12% glycerol/H_2O	75.1	1.351	0.906	5760
26% glycerol/H_2O	71.4	1.372	0.898	5600
50% glycerol/H_2O	64.1	1.404	0.884	5480
90% glycerol/H_2O	47.8	1.455	0.850	5110

[a] Bilot–Kawski parameter, defined in text.
[b] Percentage by volume. ϵ and n data in glycerol/water mixtures from Miner and Dalton [25].

$$\Delta\bar{\nu} = 2(\Delta\mu)^2 f/(hca^3) + \text{const}$$
$$f(\text{Lippert}) = d(\epsilon) - d(n^2)$$
$$f(\text{Bilot-Kawski}) = [d(\epsilon) - d(n^2)]/[1 - d(n^2)]^2[1 - d(\epsilon)]$$
$$d(x) = (x - 1)/(2x + 1)$$

where $\Delta\bar{\nu}$ is the Stokes' shift in cm^{-1}, $\Delta\mu$ is the difference between excited-state and ground-state dipole moments (in esu-cm; 1 D = 10^{-18} esu-cm), h is Planck constant (6.63×10^{-27} erg-s), c is the speed of light (3.00×10^{10} cm/s), a is the cavity radius of fluorophore in solvent (cm), ϵ is the solvent dielectric constant, and n is the solvent index of refraction. The Bilot–Kawski analysis is presented in this paper. Lippert analysis differs by neglecting the (presently unknown) polarizability of the 2AP molecule, rather than approximating it as $1/2a^3$. Characteristic spectral wavenumbers were determined from peak locations. An attempt to use mean, rather than peak, wavenumbers resulted in increased uncertainty because of the overlap of excitation bands in the 250- to 270-nm region. Polarity data for glycerol-water mixtures were taken from Miner and Dalton [25].

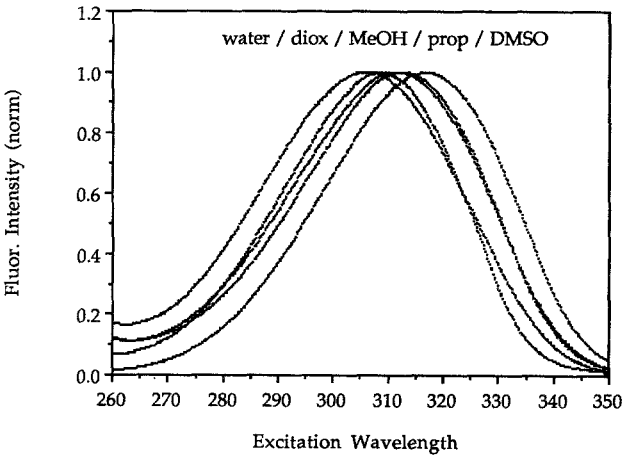

Fig. 1. Fluorescence excitation spectra of 2-aminopurine (free base). Solvents are water, 1,4-dioxane, methanol, 1-propanol, and dimethyl sulfoxide, left to right, respectively. Emission wavelength, 380 nm; bandwidths, 2 and 5 nm, respectively, for excitation and emission. Base concentration, 10 μM. Recorded on MPF-66 flurometer. Amplitudes normalized to 1.0.

RESULTS

Absorption spectra of 2-aminopurine in a variety of solvents show a progressive shift to longer wavelengths with increasing solvent polarity, except for water, which has the highest energy ground- state-to-first- excited-state transition (Table I). Excitation spectra of 2AP (Fig. 1) show the same trend, with excitation maxima shifted 1 to 6 nm to the red of the corresponding absorption peaks. The width of both types of spectra (nm) also depends upon the solvent and generally increases with increasing solvent polarity.

The emission spectrum peak shifts to the red as the solvent polarity increases except in dioxane, as Fig. 2 and Table I show. This holds also for water and glycerol/

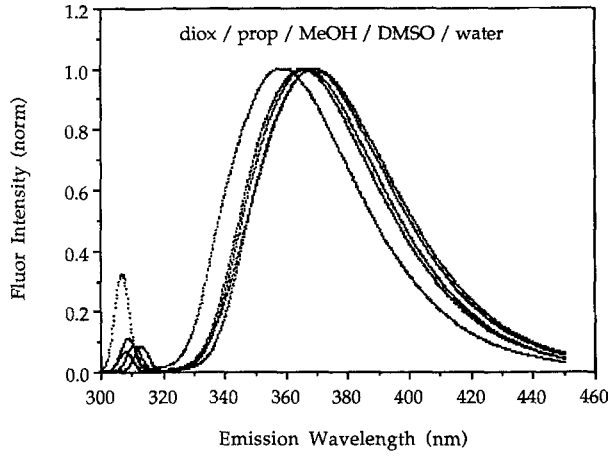

Fig. 2. Fluorescence emission spectra of 2-aminopurine (free base). Solvents are dioxane, 1-propanol, methanol, dimethyl sulfoxide, and water, left to right, respectively. Elastic scattering peaks at left indicate excitation wavelength. Bandwidths, 2 and 5 nm, respectively, for excitation and emission. Base concentration, 10 μM. Recorded on MPF-66 fluorometer. Amplitudes normalized to 1.0.

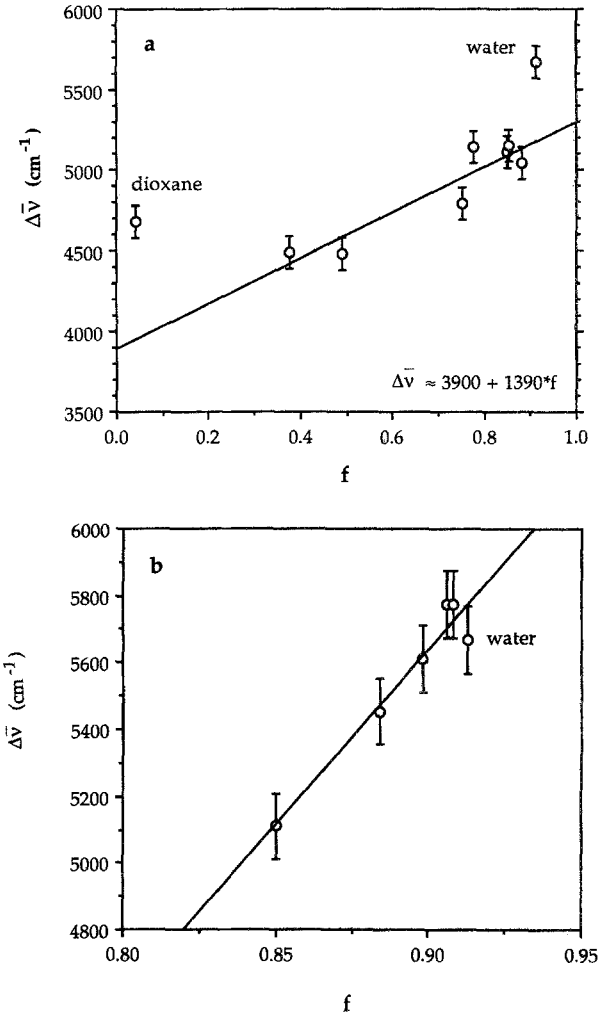

Fig. 3. Bilot–Kawski equation plot of solvent effect on 2AP free base absorption and emission spectra in solvents of varying dielectric constant and refractive index. (a) Solvents include dioxane, ethyl ether, ethyl acetate, 1-butanol, 1-propanol, methanol, DMSO, water, and 90% glycerol. Water and dioxane data points were excluded in the linear fit. (b) Glycerol–water mixtures, 90, 50, 26, 12, 8, and 0% (by volume) left to right. $\bar{\nu}_{em}$, the emision peak position, is almost a constant in this data (Fig. 4).

water mixtures. The maximal shift is about 12 nm. This shift is small compared to the typical wavelength spectral widths (FWHM) of 47–54 nm, which also increase with polarity.

Lippert or Bilot–Kawski equation plots are helpful in separating the general dependence of absorption and emission spectra on solvent dielectric constant and refractive index from dependence due to specific chromophore–solvent interactions. The linear dependence of the Stokes' shift $\bar{\nu}_a - \bar{\nu}_{em}$ with the Bilot–Kawski parameter f is predicated on the absence of specific interactions such as hydrogen bonding, which may occur in water and alcohols. Table II and Figs. 3a and b show the dependence of Stokes' shift on f for seven solvents and glycerol–water mixtures. Dioxane and water clearly lie off the straight line which is fitted to the data excluding these two solvents. Glycerol–water mixtures also do not lie on this line, as Figs. 3a and b show. The plot in Fig. 3b cannot, of course, be interpreted in terms of Lippert or Bilot–Kawski parameters, since the water in the mixture has a specific interaction with 2AP. The plot does show, however, that the progression from normal to anomalous behavior occurs in proportion to the fraction of water in the mixture, i.e., water does not exhibit any partitioning around or away from 2AP.

The addition of glycerol to aqueous solutions of 2AP shifts absorption and fluorescence excitation spectra to the red while not appreciably affecting the emission spectrum (Fig. 4). The effect of the added glycerol on the Bilot–Kawski plot is to bring the water data point in Fig. 3a down to the line which fits the data recorded in non-aqueous solvents. Although not designed to determine fluorescence yield, the measurements on the glycerol/water mixtures also showed that glycerol quenches 2AP fluorescence. The intensity of fluorescence of 2AP in 90% glycerol was an order of magnitude less than that in water (data not shown).

The attachment of ribose or 2′ deoxyribose to the 2AP base has a small but measurable effect on fluores-

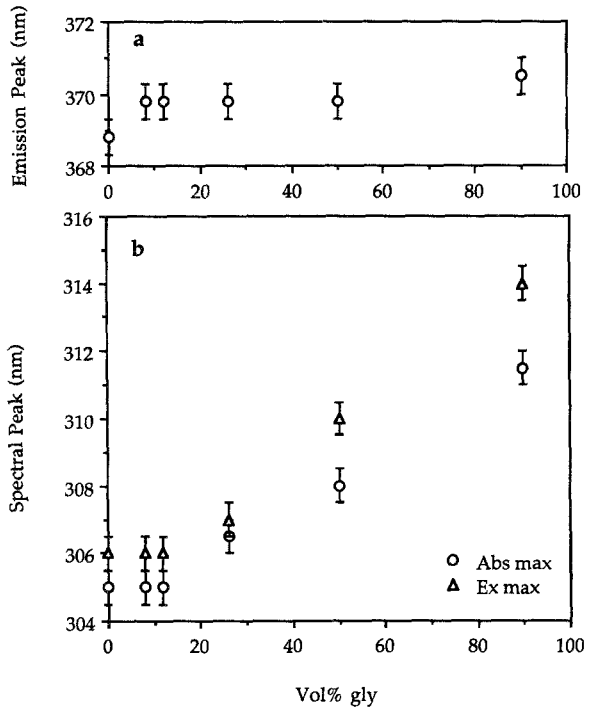

Fig. 4. Dependence of absorption, fluorescence excitation, and fluorescence emission spectral maxima on percentage glycerol (by volume) in glycerol/water mixtures. $T = 20°C$.

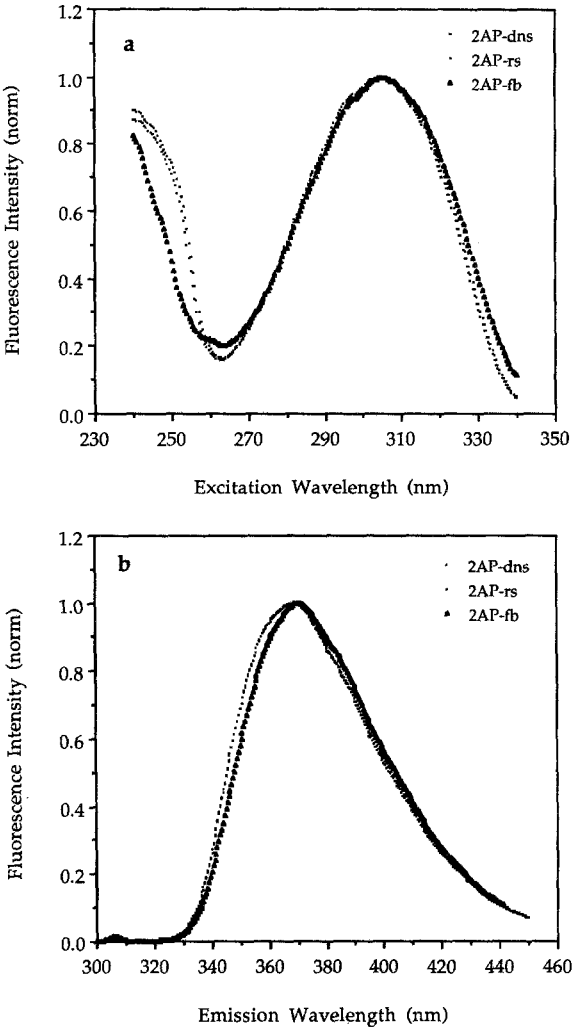

Fig. 5. Fluorescence spectra of 2AP 2′-deoxynucleoside, 2AP riboside, and 2AP free base. (a) Excitation spectra. Emission wavelength, 375 nm. (b) Emission spectra. Excitation wavelength, 306 nm. Emission and excitation bandwidths, 2.5 nm. Buffer: 10 m*M* Tris–Cl, 0.1 *M* KCl, 0.1 m*M* EDTA, pH 7.5. Base concentration, 10 μ*M*. The deoxynucleoside and riboside data are almost indistinguishable. Recorded on LS-50 fluorometer. Amplitudes normalized to 1.0.

cence (Fig. 5). The absorption and excitation spectra of 2AP, 2AP-rs, and 2AP-dns are almost identical in the 270- to 340-nm region. The nucleoside excitation spectra are shifted about 1 nm to the blue compared to the free base. A 1.5- to 2-nm blue shift in the emission spectrum occurs upon attachment of ribose or 2′ deoxyribose to 2AP, along with a slight spectral narrowing. The two nucleoside emission spectra are virtually indistinguishable. Ward *et al.* (8) previously demonstrated that attachment of phosphate (2AP monophosphate) to the nucleoside had no spectral effect in polyribonucleosides. We also noted that the fluorescence yield of the nucleosides was the same as that of the free base to within ± 15% (data not shown). The spectra of the nucleosides, like all others presented here, show no structure which might be indicative of two or more distinct electronic or vibrational states.

If transfer from a lower-polarity solvent to water changes the energy levels of free 2AP in solvents, it would be expected that a DNA oligomer containing 2AP in a double-helical region would change spectral properties upon melting of the helix. This is indeed observed in Fig. 6. The excitation spectrum of the decamer d[CTGA[2AP]TTCAG]$_2$ shifts to the blue, from 308.5 to 306 nm, as the temperature is raised past the melting point, about 23°C under the concentration and salt conditions in Fig. 6 [10,12]. The high-temperature excitation peak matches that of the free base in water. Almost the entire blue shift occurs between 2 and 23°C; further increase in temperature, to 50°C, has little effect. The emission spectrum, in contrast, does not appreciably change with temperature over the temperature range 4 to 50°C (data not shown). Neither emission nor excitation spectra of 2AP (free base) in water change with temperature. The intensity of decamer fluorescence also

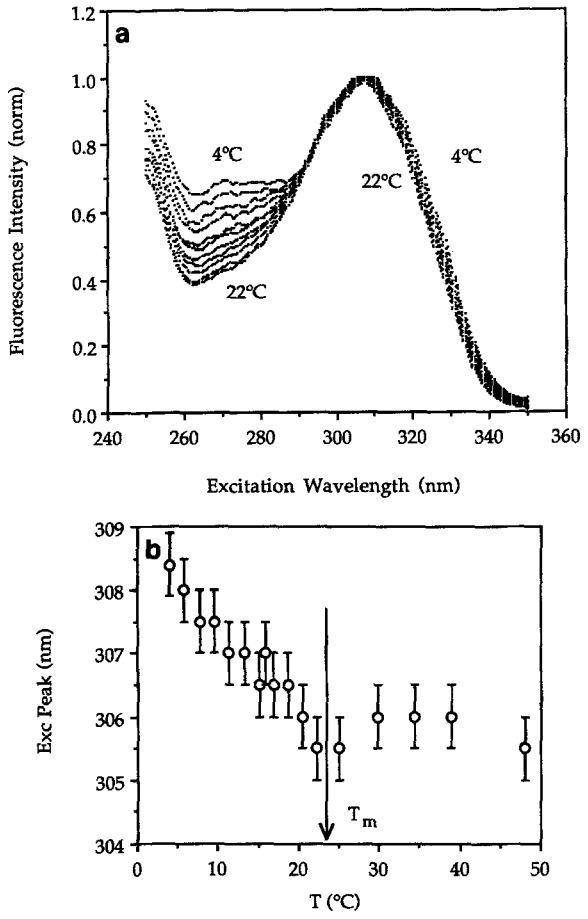

Fig. 6. Blue shift of d[CTGA[2AP]TTCAG]$_2$ DNA decamer fluorescence excitation spectrum upon melting of the helix. The peak of the high-temperature spectrum coincides closely with that of the 2AP monomer in water (Table I, Figs. 1 and 5). (a) Spectra normalized to amplitude 1. Temperatures approximately equally spaced. (b) Plot of excitation spectral shift vs temperature. T_m is the melting temperature determined from absorbance (260 or 330 nm) vs temperature (data not shown).

changes with temperature, as has been noted [6,10,13] and will be explored further in a forthcoming publication.

DISCUSSION

Specific interactions of solvent with a fluorescent solute molecule are usually indicated by spectral shape changes or in nonlinear behavior of the Stokes' shift with f [22,26]. Water and dioxane do interact differently with 2-aminopurine than do the other solvents, as can be seen directly in Table I. The energy of the absorption maximum of 2AP in water is at a shorter wavelength than in any other solvent, even though the trend in the other solvents is for energy to decrease as dielectric constant increases. The emission spectrum of 2AP in water, however, fits into the trend in other solvents, with a shift to the red with increasing dielectric constant. Suppan and others have noted that this behavior can be expected from proton-doning solvents if the ground-to-first- excited-state transition transfers electronic charge away from the amino group to the aromatic ring [22,27]. In contrast to the case of water, it appears that the alcohols methanol, 1-propanol, butanol, and glycerol do not act as proton donors to 2-aminopurine, as Smagowicz an Wierzchowski observed for 2AP in ethanol [20]. Dioxane has been noted to deviate from Bilot–Kawski-type plots for many fluorophores [22].

The slope of the Bilot–Kawski or Lippert equation plot can be used to calculate the difference between the excited-state and the ground-state dipole moments if no specific interactions are present [22,26]. The slope is

$$\text{slope} = 2\Delta\mu^2/hca^3 \qquad (1)$$

where $\Delta\mu$ is the difference between excited-state and ground-state dipole moments (esu-cm; 1D = 10^{-18} esu-cm), h is the Planck constant (6.63×10^{-27} erg-s), c is the speed of light (3.00×10^{10} cm/s), and a is the cavity radius of the fluorophore in solvent (cm). Gryczynski and Kawski [21], using mixed-solvent experiments, determined $\Delta\mu$ = 2.9 D for 2AP, assuming a cavity radius of 3.8 Å, while the ground- and first- excited-state dipole moments were 2.3 and 4.6 D, respectively. Smagowicz and Wierzchowski [20], measuring spectra in a variety of solvents, found $\Delta\mu$ = 1.6 D. Various 2AP derivatives have had observed $\Delta\mu$ values in the range 2.5–3.8 D [9,20,21]. The slope of the line fitted to our data points (assuming a = 3.8 Å; excluding dioxane and glycerol–water mixture below 90%) results in a dipole moment change of 2.8 ± 0.4 D, close to the value found by Gryczynski and Kawski. Use of the Lippert analysis for the same data gives a $\Delta\mu$ value close to 5 D. Our fits to the two theories are about equally good. Past work has applied B-K theory [9,21], which had been observed to provide better data fits [27].

The Stokes' shift for 2AP in 90% glycerol lies close to the line describing the Bilot–Kawski theory, which suggests that (i) glycerol does not hydrogen-bond or otherwise specifically interact with 2AP and (ii) solvent relaxation in glycerol is more rapid than fluorescence decay. From our observation that the fluorescence yield in glycerol is an order of magnitude less than in water, we estimate that the decay in glycerol is about 1 ns. The

data of McDuffie and Litovitz [28] show the average dielectric relaxation time of 90% (vol) glycerol at 20°C to be 0.5 to 0.7 ns, which indeed is somewhat shorter than our estimate of the fluorescence decay time. It is clear, however, that many relaxation effects of organic molecules in viscous solvents occur on time scales considerably faster than dielectric relaxation times (e.g., Refs. 29 and 30). The question of the rate of 2AP excited-state relaxation cannot be clearly resolved until detailed time-resolved spectra of 2AP in glycerol and other solvents are measured.

We have noted that glycerol greatly reduces the fluorescence yield of 2AP, while the other solvents and attachment of ribose or deoxyribose do not. We will present in a separate paper a study of yield and fluorescence decay on solvent and chromophore molecular structure. It appears at this point that the highly nonexponential decay and lower fluorescence yield of 2AP incorporated into DNA oligomers [6,7,13] is not due to rotameric structures of the base with respect to deoxyribose but, rather, a combination of quenching by specific molecular groups and molecular motion.

The ultimate test of the utility of a fluorescence probe is whether the behavior when in place in a biomolecule can be understood from the results of the model system studies. The effect of helix melting on the spectrum of 2AP incorporated into $d[CTGA[2AP]TTCAG]_2$ shows effects expected from the solvent studies. Upon melting, the 2AP in position 5 in the decamer shifts its excitation spectrum to the blue, until its spectral maximum corresponds to that of the 2AP monomer in water. This is expected if the 2AP becomes exposed upon melting and forms hydrogen bonds with water. The decamer has been shown to be double helical below about 15°C [5,6,10,12,13] and the interior location of 2AP assures that it is not subject to end fraying of the double helix. The 2AP should then make a transition from a relatively low dielectric constant which characterizes the interior of the helix to $\epsilon \approx 80$. If only a dielectric constant effect were involved, however, the spectrum should shift to the red. Since a blue shift is seen, H bonding from water must occur. The 2AP amino group is presumed to form a hydrogen bond with thymine in the helix [10,12]. Assuming this to be true, the blue shift occurring upon melting shows that the H bond in the helix must be weak in terms of its spectroscopic signature.

The spectrum of 2AP in the $d[CTGA[2AP]TTCAG]_2$ decamer is substantially as predicted by the early work of Ward *et al* on DNA polymers with alternating 2AP bases [8]. They concluded that 2AP in DNA was nonfluorescent unless located at a helix end. The fluorescence yield of $d(CTGA[2AP]TTCAG]_2$ is significantly below that of free 2AP, though not zero [13]. Ward *et al.* found a fluorescence excitation peak at 315 nm and concluded that this excitation/emission was due to 2AP at the helix ends. The excitation maximum for our interior 2AP is at 309 nm.

If hydrogen bonding of the 2-amino group generally results in a blue-shifted excitation spectrum, one may ask why 2AP in the decamer at low temperatures does not have an excitation peak farther to the blue. One possibility is that the 2AP is, in fact, not H-bonded to thymine, in spite of the evidence for the B-helical form of the decamer at 20°C. In order to form the H bond from the 2, rather than from the 6, position of the purine, the geometry must be somewhat distorted, though a B-type helix is still probable [13]. NMR measurements of the 2AP in this decamer [5,13] show either that the 2AP is quite mobile or that its position is different than that of the adenine which it replaces. Molecular dynamics simulations and fluorescence anisotropy decay confirm the enhanced mobility [13].

The 2-aminopurine energy levels are perturbed by three main factors in the present study: the interaction of the 2AP dipole moment with the dielectric medium, solvent or other relaxation interactions which occur between the time of excitation and that of fluorescence emission (about 10 ns) and after transition to the ground electronic state, and hydrogen-bonding interactions. All these interactions are negative (lower the relevant energy level). The red shift of 2AP excitation spectra in higher-ϵ solvents (excluding water) is the result of an increase in the dipole moment upon excitation. The red shift of 2AP emission in higher-ϵ solvents likewise results from the increased dipole moment and may be augmented by any solvent relaxation occurring in the excited state. The blue excitation peak of the 2AP free base in water shows that the reduction in ground-state energy by H bonding is greater than the reduction in excited-state energy due to increased dielectric interaction. Likewise, in the melted DNA helix, the H bonding to water dominates. The lack of any emission spectral shift of the DNA decamer with temperature shows that the effects of H bonding on the ground state of 2AP in the melted helix is about equal to the reduction of the excited state energy by the combination of dielectric and relaxation effects. One must then conclude that excited-state relaxation of 2AP is reduced in the DNA decamer compared to that of the free base in water.

SUMMARY

1. Generally, a higher solvent dielectric constant shifts the emission spectrum of 2-aminopurine to the red,

reflecting the lowering of the excited-state energy by the interaction of its larger dipole moment with the dielectric environment. Dioxane is an exception.

2. A higher solvent dielectric constant tends to shift the absorption and fluorescence excitation peaks of free 2AP to the red, except in water, where lowering of the ground-state energy by H bonding occurs.

3. A Bilot–Kawski plot of the spectral data from five solvents results in $\Delta\mu = 2.8 \pm 0.4$ D, a value similar to that of Gryczynski and Kawski [21]. Lippert analysis results in a value of 5 D.

4. Melting of the DNA decamer helix causes a blue shift of the excitation spectrum, probably because of hydrogen bonding of water to the 2-amino group ground state in the unfolded helix. We observe this shift to take place 5°C or more below the helix melting temperature.

5. The moderate sensitivity to environmental polarity, the strength and simplicity of the time-resolved free base fluorescence, the separation of the absorption spectrum from those of the normal bases, and the absence of large DNA structural perturbative effects [10,13] make 2-aminopurine a superior probe of DNA conformation and interactions.

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