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Effect of NH_2 rotation on the fluorescence of 2-aminopurine in solution

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ABSTRACT

Since the introduction of 2-aminopurine (2AP) in 1969 as a fluorescent analogue of adenine, its intense fluorescence in aqueous solution and the subsequent reduction of this intensity in DNA has been a powerful tool for studies of structural changes in DNA. Herein, we show that the unusual intense fluorescence of 2AP in water is attributed to the formation of a closed complex between one water molecule and 2AP in the excited state. This configuration restricts the rotation of the 2-NH_2 group which subsequently lowers the nonradiative decay rate. We supported this finding by attaching heavy masses to the amino group, dimethyl (2-($N(Me)_2$)) and diethyl (2-($N(Et)_2$)). By examining the fluorescence behavior in dioxane (an apolar, aprotic solvent), the lighter NH_2 group can rotate in the excited state more freely which enhances the nonradiative loss of fluorescence. On the other hand, this rotation slows down sharply in the two heavy-group derivatives, leading to a restoration of the fluorescence intensity and lifetime very close to that of 2AP in water. Depletion of fluorescence was observed in the 2AP derivatives in water and is attributed to the population of a twisted intramolecular charge transfer (TICT) state due to the strong electron donating power of the NR_2 groups, an effect that is absent for the parent 2AP.

1. Introduction

The potential use of 2-aminopurine (2AP) as a fluorescent analogue of adenine in DNA research was first suggested in 1969 by Stryer and co-workers [1]. The authors characterized the fluorescence of 2AP and reported a quantum yield of 0.68 in aqueous solution at pH 7.0 and a fluorescence maximum at ~370 nm. The excited state of 2AP is also red-shifted with respect to the natural bases, which allows for selective excitation. Upon incorporation of 2AP into an oligonucleotide, its fluorescence is significantly attenuated. This observation made it apparent that 2AP could be used to monitor changes in an oligonucleotide structure and environment. The molecule was then identified as a powerful tool for studies of DNA and has been used since then in various DNA investigations (for review, see refs. [2–5]).

Considerable progress has been made to explain the high fluorescence quantum yield of 2AP compared to adenine [6–9]. In adenine, fast nonradiative deactivation of the $\pi\pi^*$state (S_1 state) via a conical intersection to the ground state is accessible through a strong puckering of the six-membered purine ring with pyramidalization of the carbon atom in position 2 [6,7]. In 2AP, the presence of NH_2 in position 2 significantly lowers the energy of the $\pi\pi^*$ state, therefore the conical intersection occurs at higher energies than in adenine, giving stability to the fluorescent $\pi\pi^*$ state [6–9]. The close proximity of the $\pi\pi^*$ state to a nearby $n\pi^*$ state in adenine contributes also to the depletion of the former state via additional conical intersections to the latter state which in turn has a nonradiative reaction path to the ground state [6,8,9].

The fluorescence behavior of 2AP in different environment remains unclear. In particular, the drastic reduction in 2AP's fluorescence intensity and quantum yield upon incorporation into DNA is still ambiguous [10,11]. The most acceptable mechanisms for this fluorescence quenching are excited-state charge transfer [12] and the presence of dark states in the base stacking configuration [12–15]. In the present work, we explain the mechanism controlling the 2AP fluorescence in aqueous solution and we show that the internal rotation of the NH_2 group plays a major role in defining the fluorescence behavior of the molecule. This is not surprising since changing the position of the NH_2 group from the 6-position (adenine) to the 2-position (2AP) causes the considerable shift in fluorescence characteristics. We correlate the origin of the intense fluorescence of 2AP in aqueous solution to restriction of the NH_2 internal rotation. Easing of this restriction in aprotic solvents leads to increasing nonradiative decay that tends to deactivate the initially populated excited state. We support this finding by examining the fluorescence of two derivatives of 2AP in which the hydrogen atoms of NH_2 have been replaced by heavy groups. The chemical structures of N,N-dimethyl-2-aminopurine (2DMeAP) and N,N-diethyl-2-aminopurine (2DEtAP), along with the structure of 2AP, are shown in Fig. 1.

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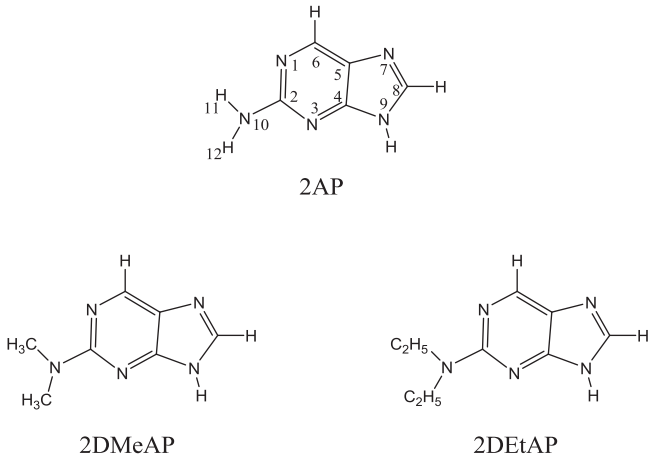

Fig. 1. Structures of 2AP and its derivatives. Conventional numbering is shown. The structure of 2AP is the predominant amino-N9H tautomer.

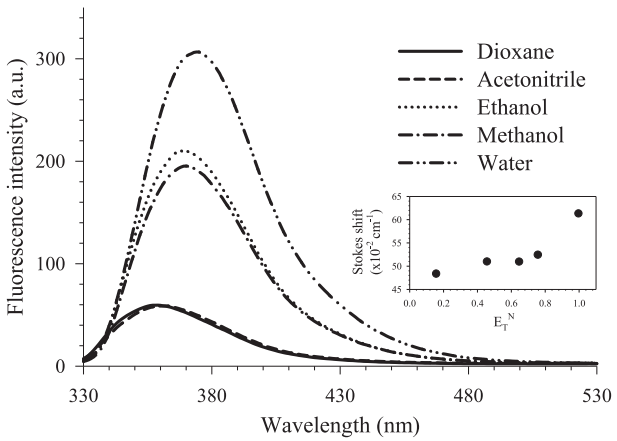

Fig. 2. Corrected fluorescence spectra of 2AP in different solvents as indicated in the graph. λ_{ex} = 310 nm. The inset shows the Stokes shift versus the empirical polarity parameter E_T^N.

2. Experimental and theoretical methods

2AP (99%) was obtained from Sigma. (2DMeAP) and (2DEtAP) were custom-made by GlycoTeam GmbH, Germany. The purity of both derivatives was estimated to be ≥98%. Acetonitrile (spectroscopic grade), anhydrous 1,4-dioxane and methanol were obtained from Sigma-Aldrich Chemical Co. Anhydrous ethanol was received from Acros Organics. Deionized water (Millipore) was used. The concentration of all materials was kept at 0.02 mM.

Absorption spectra were obtained with an HP 845x Diode Array spectrophotometer. Fluorescence spectra were recorded on a Shimadzu RF-5301 PC spectrofluorophotometer. The fluorescence intensities were corrected for the difference in optical density at the excitation wavelength and inner filter effect by using the following equation [16]:

$$F_{cor} = F_{obs} \times \text{antilog}\left(\frac{OD_{ex} + OD_{em}}{2}\right) \quad (1)$$

where F_{cor} and F_{obs} are the corrected and observed fluorescence intensities, respectively. OD_{ex} and OD_{em} are the optical densities at the excitation and emission wavelengths, respectively.

Lifetime measurements were performed using a TimeMaster fluorescence lifetime spectrometer obtained from Photon Technology International. Excitation was at 310 nm using a light-emitting diode. The system response time as measured from the scattered light was estimated to be approximately 1.5 ns. The measured transients were fitted to multi-exponential functions convoluted with the instrument response function (IRF). The fit was judged by the value of the reduced chi-squared (χ^2). The experimental time resolution (after deconvolution) was approximately 100 ps, using stroboscopic detection [17]. In all the experiments, samples were contained in a 1 cm path length quartz cell and the measurements were conducted at 23 ± 1 °C.

Geometry optimization was carried out using the GAMESS program [18] at the second-order Moller-Plesset (MP2)/6-31++G(d,f) level of calculation.

3. Results and discussions

3.1. Characterization of 2AP in different solvents

The steady-state fluorescence spectra of 2AP dissolved in different solvents are shown in Fig. 2. Table 1 summarizes the spectroscopic parameters along with the measured lifetime values in the corresponding solvents. The results are in substantial agreement with previously reported results for some of the solvents [1,11,19,20].

The fluorescence behavior cannot be directly correlated to solvent polarity. In order to understand the fluorescence behavior, we need to discuss the solvent properties beyond the average values of their dielectric constants (ε), particularly in terms of their hydrogen bond ability. Table 1 shows the ε values, along with the empirical parameters of solvent polarity (π^* and E_T^N) [21].

A solvent such as dioxane, which appears to be nonpolar according to its static dielectric constant (ε = 2.21), has a high solvent polarity parameter (π^* = 0.49) and an E_T^N value of 0.16 [21]. Dioxane has two CH_2-O-CH_2 groups opposite to each other which results in a net zero dipole moment. Hence, it is considered a nondipolar solvent. However, dioxane exhibits a large quadrupole moment [22,23] which is reflected in its π^* parameter that mainly takes into consideration the polarizability and the dipolarity of the solvent [24]. The corresponding E_T^N value indicates that dioxane exhibits only 16% of the solvent polarity of water which classifies dioxane as an apolar, non-hydrogen-bond donor solvent [25]. Among the solvents used in this study, we observed in dioxane the lowest fluorescence intensity of 2AP with the largest blue shift, and the shortest fluorescence lifetime (1.68 ns).

In a polar, aprotic solvent such as acetonitrile, the fluorescence behavior of 2AP is similar to that in dioxane. The results point to the fact that polarity is not the dominant factor in changing fluorescence. The slight increase in the Stokes shift ($\Delta\tilde{\nu}$) and fluorescence lifetime in acetonitrile compared to those in dioxane (see Table 1) may be attributed to a relative stability of the excited state due to the large polarity of acetonitrile that tends to solvate polar groups through its lone electron pairs [21].

In polar, protic solvents, the behavior of 2AP in the excited state is different and the increase in the Stokes shift is much more pronounced as the polarity increases, accompanied by a drastic increase in intensity and much longer lifetimes. In ethanol, for example, the measured Stokes shift is similar to that in acetonitrile, although the polarity of ethanol is much lower than that of acetonitrile [25,26]. The fluorescence intensity and lifetime of 2AP in ethanol are more than three times larger than those in acetonitrile. The trend is much more pronounced in water as shown in Table 1. The drastic change in the fluorescence characteristics of 2AP in protic solvents indicates a different mechanism of solvation that must derive from intermolecular hydrogen bonding interaction between 2AP and the protic solvent in the excited state potential energy surface [19]. This mechanism is expected to stabilize the excited state which is evidenced in the larger Stokes shifts compared to other

Table 1
Spectroscopic data for 2AP and its derivatives in different solvents.

	Solvent	ε[a]	π^*[a]	E_T^N [a]	λ_{abs}^{max} (nm)	λ_{em}^{max} (nm)[b]	$\Delta\nu$ (cm^{-1})	τ (ns)
2AP	Dioxane	2.21	0.49	0.16	306	359	4825	1.68[c]
	Acetonitrile	35.94	0.66	0.46	305	361	5086	2.06[c]
	Ethanol	24.55	0.54	0.65	310	368	5084	6.75[d]
	Methanol	32.66	0.60	0.76	310	370	5231	6.82[d]
	Water	78.30	1.09	1.00	305	375	6120	10.91[e]
2DMeAP	Dioxane				326	390	4968	9.35[e]
	Water				332	429	6810	3.58[c]
2DEtAP	Dioxane				330	387	4463	9.63[e]
	Water				336	429	6452	1.92[c]

[a] Obtained from reference 21 at 25 °C.
[b] λ_{ex} = 310 nm.
[c] ±0.05.
[d] ±0.10.
[e] ±0.13.

solvents. It is worth noting that the Stokes shift for 2AP in the solvents studied here correlates with E_T^N as shown in the inset in Fig. 2. Similar correlation was obtained for the total decay rate of 2AP in the same solvents (data not shown). This correlation was not obtained for the other parameters (ε and π^*) which confirms the major effect of hydrogen bonding on fluorescence.

Protic solvents are both hydrogen bond donors and acceptors [21]. The vicinal position of the H-bond donor and acceptor sites in 2AP allows the formation of closed complexes with the solvent molecules. The ability of such solvents to form hydrogen bonds with both the positively polarized hydrogens of the NH_2 group at the 2-position and the lone pair of electrons of the N-heteroatom at the 1-position (or at the 3-position) of 2AP is expected to hinder the internal rotation of the NH_2 group. The rotational motion of the NH_2 group acts as an efficient nonradiative decay channel that tends to deactivate the initially populated excited state. It is this configuration that we propose to be the factor causing the increase in the fluorescence intensity and lifetime of 2AP.

A protic solvent like water, a strong hydrogen bond donor/acceptor, can lock the NH_2 rotation by forming two strong hydrogen bonds as discussed above. In order to greatly restrict the NH_2 rotation, a strong interaction between one or more water molecules with the 2AP moiety is necessary in the close proximity of NH_2. This configuration can be achieved through short-range hydrogen bonding interaction in which an explicit interaction with a limited number of water molecules could restrict the NH_2 motion. This ability is weaker in alcohols, which explains the lower fluorescence intensities and shorter lifetimes in methanol and ethanol. From the spectral changes in dioxane/water binary mixtures, we found that one water molecule is responsible for the fluorescence increase (details are included in the Supplementary Information).

We investigated the solvation sites around the NH_2 group by calculating the possible optimum geometries of one molecule of water in the vicinity of NH_2 by ab initio methods at the MP2/6-31++G(d,f) level of theory. For a better description of the long-range interaction of H-bonding, we employed sets of orbitals that possess sufficient diffuseness and angular flexibility. Therefore, an extension of the basis set with the diffuse functions (++) is used in the current calculations. The structures have been fully optimized without any symmetry constraint. The calculations were initially carried out for the 2AP bare molecule (without water) in order to predict the most stable structure in the ground state. The calculated structure is planar with the two hydrogen atoms of the NH_2 group tilted with respect to the plane of the molecule by angles of 19° (N1-C2-N10-H11) and 21° (N3-C2-N10-H12). The results agree with previously reported calculations [27,28]. The calculated pyramidal geometry of the amino group in the S_0 state was reported to be planarized in the first excited singlet state ($\pi\pi^*$) [29,30].

Two hydrogen bonding sites around the NH_2 group were then considered when placing a single water molecule. The results of the optimized geometries are shown in Fig. 3. In each site, the close proximity of the H-bond donor and acceptor atoms allows the formation of a closed complex. Site 1 is more stable than site 2 by 4.0 kJ/mol. This is in agreement with previously reported calculations at different levels of theory [31,32].

In a related theoretical study, Ludwig et al. [33] used sequential Monte Carlo quantum mechanics with a solvation model to show that upon $\pi\pi^*$ excitation, a barrier in the potential energy surface to access a conical intersection to the ground state increases in aqueous environment (compared to gas phase), hence increasing fluorescence in water. At a molecular level, Leutwyler and coworkers observed two isomers of 2AP:$(H_2O)_1$ [31]. Isomer A was the most stable one in which the water molecule forms two hydrogen bonds with N3 and N9-H (see Fig. 1). The second isomer (isomer B) is equivalent to our site 1 conformation shown in Fig. 3. Their resonance two-photon ionization spectra of isomer A exhibit low-frequency out-of-plane overtone and combination bands, which the authors interpret as a coupling of the optically excited $\pi\pi^*$ state to the lower-lying $n\pi^*$dark state. In contrast, these bands are much weaker for isomer B, implying that the $\pi\pi^*$ state in this isomer is planar and decoupled from the $n\pi^*$ state.

The above reported results are in line with our finding that restricting the internal rotation of the NH_2 group is the main source of increasing fluorescence in aqueous environment. We provide the following support for this point. First, although isomer A was reported to be the most stable structure for 2AP:$(H_2O)_1$ [31], its lack of solvating the NH_2 group leads to increasing the efficiency of the nonradiative decay channel. This was correlated to the observation of several out-of-plane vibrations that involve the NH_2 internal rotation besides other modes [29,31]. These vibrations are the means to reach the conical intersection to either the $n\pi^*$ state [29,31] or the ground state [20] via vibronic coupling.

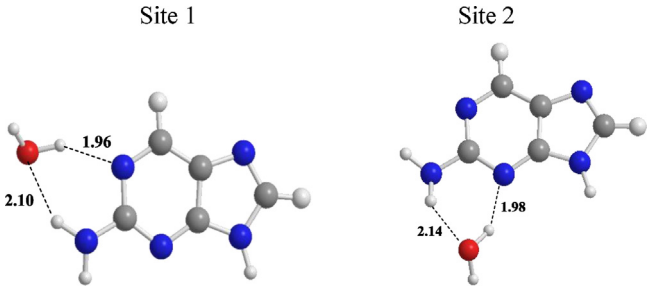

Fig. 3. The calculated structures for the possible sites of the solvation of the NH_2 group by one water molecule. Site 1 is more stable than site 2 by 4.0 kJ/mol. The hydrogen bonds are displayed in Å.

Second, the presence of a water molecule in site 1 (Fig. 3), or isomer B in ref. 31, stabilizes the already planar NH_2 group in the excited $\pi\pi^*$ state through a closed hydrogen bond structure which reduces population of the out-of-plane vibrations. This is in agreement with the experimental observation in which these vibrations are much weaker for isomer B [31].

3.2. Effect of NR_2 substituents on fluorescence

Different N-substituents are expected to affect the barriers to internal rotation of the amino group. The effect can be described in terms of the electron donor property of the amino nitrogen towards the unsaturated molecular system of purine. The extent of a partial double bond character of the N10-C2 bond (see Fig. 1) should depend on the basicity of the nitrogen atom: the higher the basicity of the NR_2 group the greater the double bond character which leads to a higher barrier to internal rotation. A second factor that is expected to affect the barrier to rotation is steric interactions induced by the presence of bulky R groups attached to the nitrogen atom. With this in mind, we substituted the two hydrogen atoms of NH_2 by two methyl groups (2DMeAP) and two ethyl groups (2DEtAP). The structures of the new compounds are shown in Fig. 1.

The absorption and fluorescence spectra of 2DMeAP and 2DEtAP dissolved in dioxane and water are shown in Figs. 4 and 5, respectively. The corresponding spectra for 2AP are shown for comparison. In order to reflect the correct fluorescence intensities, the fluorescence spectra were measured for excitation at 323 nm (a near isosbestic point in Fig. 4) and the spectral intensities were corrected using Eq. (1).

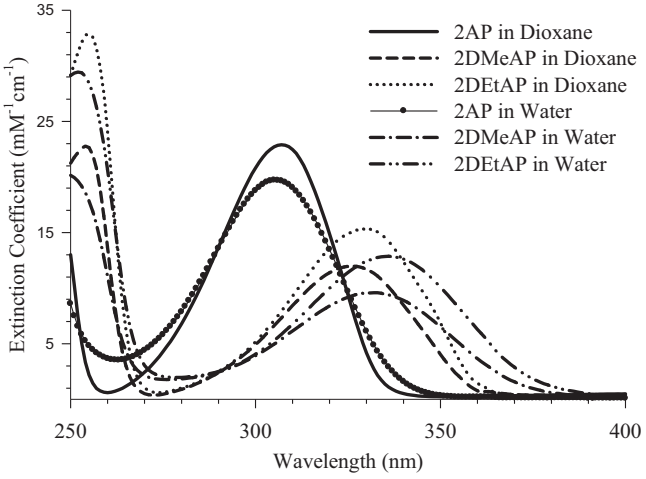

Fig. 4. Absorption spectra of 2AP, 2DMeAP, and 2DEtAP in water and dioxane.

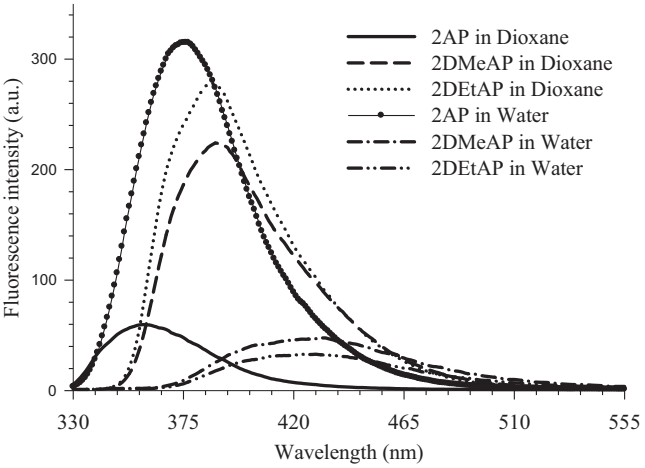

Fig. 5. Corrected fluorescence spectra of 2AP, 2DMeAP, and 2DEtAP in water and dioxane. λ_{ex} = 323 nm.

For 2AP, the two solvents (dioxane and water) represent the lower and upper limits for the change in the fluorescence intensity as discussed above for Fig. 2. As shown in Fig. 5, the fluorescence intensities of the 2AP derivatives in dioxane approach that of 2AP in water. The results point to a structural effect on the fluorescence behavior when attaching heavy masses to the 2-amino group similar to the effect of water on the 2AP parent molecule. The fluorescence results in dioxane for 2AP and its derivatives can be explained by the much slower rotational motion of the NR_2 groups in 2DMeAP and 2DEtAP compared to that of the NH_2 group in 2AP.

The fluorescence results indicate that the effect of one water molecule on the internal rotation of the NH_2 group is similar to attaching heavy masses to NR_2: both lead to a reduction in the nonradiative decay rate. This is confirmed by measuring the fluorescence lifetimes of 2DMeAP and 2DEtAP in dioxane. As shown in Table 1, the lifetimes of both derivatives are close to that of 2AP in water. The increase in the fluorescence intensity and lifetime is thus due to a reduction in the internal rotation of the NR_2 group as a result of the presence of heavy masses.

On the contrary, the fluorescence intensity and lifetime of the 2AP derivatives show a large reduction in water (see Fig. 5 and Table 1). The fluorescence intensity of 2DMeAP is reduced in water, compared to that in dioxane, by almost five times and its fluorescence lifetime is about three times shorter in water. The effect is more pronounced in 2DEtAP. In a related study (the only available work to our knowledge on one of the 2AP derivatives studied here), the ability of 2DMeAP to dimerize was ruled out as the reason for this reduction [19]. We also rule out here the proposed mechanism of intersystem crossing in depleting the $\pi\pi^*$ singlet state [19] since the excited states of 2DMeAP and 2DEtAP are located at the same energy, but the reduction in fluorescence is much more pronounced in 2DEtAP. The reduction in fluorescence must then stem from another effect on the excited state.

Considering the chemical structures of the two derivatives, we propose that this new deactivation channel arises because of the involvement of a non-fluorescent twisted-intramolecular charge transfer (TICT) state as an intermediate. The TICT state is formed as a result of the electron donating property of the amino nitrogen towards the unsaturated molecular system. This leads to the formation of a partial double bond character to the N10-C2 (see Fig. 1). The extent of the double bond character and the formation of a TICT state should depend on the basicity of the nitrogen atom. The latter is expected to rise in the presence of more electron donating power of the NR_2 groups. This power is more in 2DEtAP than in 2DMeAP which explains the greater reduction in the fluorescence intensity and lifetime in the former. The formation of a TICT molecule results in a charge separation within the molecule and the production of a zwitterionic structure, and is thus expected to stabilize in highly polar solvents. This explains the absence of the TICT state in a solvent such as dioxane.

The present results indicate that the TICT state is populated in water for 2DMeAP and 2DEtAP, but not for the 2AP parent molecule. This observation can be explained by the fact that for 2AP in water, the basicity of the nitrogen atom of the NH_2 group is not strong enough to form the TICT compound due to the weak electron-donating ability of the 2-NH_2 group. A similar conclusion has been reported for compounds with a weak electron-donating NH_2 group in which a TICT state is formed upon substitution of the two hydrogen atoms by two alkyl groups [34]. In the case of 2AP, the planarity of the NH_2 group in the excited $\pi\pi^*$ state is expected to give the state some intramolecular charge transfer (ICT) character in which a partial charge is transferred from the NH_2 group to the purine

moiety. This ICT character explains the large increase in the dipole moment of 2AP in the excited $\pi\pi^*$ state by ~ 50% compared to that of the ground state [35–37]. Support for the ICT character also comes from the red shift in the fluorescence maximum of 2AP upon increasing the solvent polarity of the protic solvent (Fig. 2 and Table 1). The fluorescence maximum in acetonitrile is close to that in dioxane which supports the role of the closed-structure of 2AP:(protic solvent), involving the NH_2 group, in increasing the ICT character of the excited state. The current results indicate that the ICT → TICT deactivation channel is blocked in 2AP which is reflected in the rich fluorescence properties.

Pervious measurements have shown that the quantum yield of fluorescence of 2AP depends on the solvent used [1,11,19]. In general, it has been found that the fluorescence quantum yield decreases in nonpolar and aprotic solvents. The effect of closed hydrogen bonding on the fluorescence properties of 2AP is also evidenced in the quantum yield measurements. The relative quantum yield was measured to be 5-fold greater in water than in dioxane [11]. Our lifetime measurements give a ratio of 6.5 (water/dioxane). On the other hand, the molar absorptivity ratio at 310 nm is 0.9 (from Fig. 4). These data imply, as pointed out by Rachofsky et al. [11], that the changes in quantum yield and lifetime should arise primarily from changes in nonradiative decay rates. In the light of the above discussion, these observations can be explained by more freedom for internal rotation of the NH_2 group in dioxane.

While the fluorescence behavior of 2AP in neat solvents reflects a homogeneous environment with a single exponential decay component, its incorporation into dinucleotides and larger DNA fragments shows a heterogeneous environment. The latter is evidenced in multi-exponential fluorescence decays that are best represented by four lifetime components ranging from a few picoseconds to several nanoseconds [10–14]. The multi-lifetime components were attributed to interbase stacking [15,38] and interaction between 2AP and flanking bases that are proposed to lead to different quenching mechanisms such as charge transfer and/or electron transfer [10–14]. The complex multi-exponential fluorescence decay nature of 2AP in DNA has also been attributed to the existence of different conformational states [11]. Various conformations can be reached via excited-state dynamics which may involve the NH_2 rotation. In the light of the present results, a degree of unrestricted rotation of the NH_2 group is vital to reach the conical intersection region between the excited state and the ground state, leading to a fluorescence reduction of 2AP in DNA.

As discussed above, the formation of a TICT configuration of 2AP in water is not possible. This is also true for 2AP attached to a ribose or 2′-deoxyribose as evidenced by the absence of any significant red shift in the fluorescence peak [39] or any change in the fluorescence quantum yield [39] or lifetime [9] compared to that of 2AP in buffer. These results indicate that the presence of a sugar unit attached to N9 of 2AP has no significant role in affecting the dynamics or solvation of the NH_2 group. On the other hand, the presence of 2AP in a simple DNA composed of only dinucleotide shows a blue shift and a large reduction in fluorescence intensity [14]. This is a manifestation of a local environment that lacks water. A degree of unrestricted rotation of the NH_2 group is then expected in DNA. Recently, the characteristics of quadruplex formation was monitored by incorporating 2AP in different positions in order to detect parallel versus antiparallel folding [40,41]. Interestingly, the fluorescence of 2AP was restored to a similar level as that of its free state in solvent for the parallel folding with a fluorescence peak position similar to that of 2AP in buffer. Although the 2AP base is immobilized in the DNA, the complete exposure of the loop nucleotide to solvent regained 2AP its high fluorescence properties.

The aforementioned discussion indicates that it is very difficult to point to one effect as the major fluorescence deactivation channel when 2AP is incorporated inside DNA. In duplex DNA, the 2-NH_2 group is reported to participate in base pairing with a complementary thymine or cytosine [10–15,38]. However, similar dynamics were observed when 2AP complexed with a derivative of thymine (3-methylthymidine) where the only H-atom that could act as a hydrogen bond donor to 2AP is replaced by a methyl group [12]. The authors indicate that the fast dynamics of 2AP are not very sensitive to structural changes of the pairing base and are not caused by specific hydrogen-bonding interactions between 2AP and the base. This will leave the only possible hydrogen bond acceptor in thymine(the C=O group) to interact with the NH_2 group of 2AP. This interaction can be viewed as the interaction of a polar, aprotic solvent such as acetonitrile with 2AP. As shown in this work, although the polarity of acetonitrile is large, the effect on the fluorescence behavior of 2AP in acetonitrile is similar to that in dioxane (an apolar, aprotic solvent) due to the lack of the ability to form a closed complex between 2AP and the solvent molecule. Rotation of the 2-NH_2 group is then not expected to be largely restricted as in water when 2AP pairs with a natural base.

4. Conclusions

In summary, the intense fluorescence of 2AP in aqueous solution is attributed to restriction of the NH_2 rotation in the excited state by forming a closed complex between one water molecule and 2AP. This configuration restricts the rotation of the 2-NH_2 group which subsequently lowers the nonradiative decay rate. This was confirmed by attaching heavy masses to the amino group, dimethyl (2-($N(Me)_2$)) and diethyl (2-($N(Et)_2$)) in which fluorescence was restored in dioxane (an apolar, aprotic solvent) very close to that of 2AP in water. Depletion of fluorescence was observed in the 2AP derivatives in water and is attributed to the population of a TICT state due to the strong electron donating power of the NR_2 groups, an effect that is absent for the parent 2AP.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jphotochem.2013.03.007.

References

[1] D.C. Ward, E. Reich, L. Stryer, Journal of Biological Chemistry 244 (1969) 1228–1237.
[2] L.M. Wilhelmsson, Quarterly Reviews of Biophysics 43 (2010) 159–183.
[3] K.B. Hall, Methods in Enzymology 469 (2009) 269–285.
[4] T.M. Nordlund, Photochemistry and Photobiology 83 (2007) 625–636.
[5] V. Shafirovich, N.E. Geacintov, Topics in Current Chemistry 237 (2004) 129–157.
[6] L. Serrano-Andres, M. Merchan, A.C. Borin, Proceedings of the National Academy of Sciences of the United States of America 103 (2006) 8691–8696.
[7] K. Feng, G. Engler, K. Seefeld, K. Kleinermanns, ChemPhysChem 10 (2009) 886–889.
[8] A. Broo, Journal of Physical Chemistry A 102 (1998) 526–531.
[9] A. Holmen, B. Norden, B. Albinsson, Journal of the American Chemical Society 119 (1997) 3114–3121.
[10] J.M. Jean, K.B. Hall, Biochemistry 41 (2002) 13152–13161.
[11] E.L. Rachofsky, R. Osman, J.B. Ross, Biochemistry 40 (2001) 946–956.
[12] T. Fiebig, C. Wan, A.H. Zewail, ChemPhysChem 3 (2002) 781–788.
[13] M.A. O'Neill, C. Dohno, J.K. Barton, Journal of the American Chemical Society 126 (2004) 1316–1317.
[14] O.J.G. Somsen, A. van Hoek, H. van Amerongen, Chemical Physics Letters 402 (2005) 61–65.
[15] J. Liang, S. Matsika, Journal of the American Chemical Society 133 (2011) 6799–6808.
[16] J. Lakowicz, Principles of Fluorescence Spectroscopy, Springer Verlag, New York, 2006.

[17] D.R. James, A. Siemiarczuk, W.R. Ware, Review of Scientific Instruments 63 (1992) 1710–1716.
[18] M.W. Schmidt, K.K. Baldridge, J.A. Boatz, S.T. Elbert, M.S. Gordon, J.H. Jensen, S. Koseki, N. Matsunaga, K.A. Nguyen, S.J. Su, T.L. Windus, M. Dupuis, J. A. Montgomery, Journal of Computational Chemistry 14 (1993) 1347–1363.
[19] J. Smagowicz, K.L. Wierzchowski, Journal of Luminescence 8 (1974) 210–232.
[20] S.K. Pal, J. Peon, A.H. Zewail, Chemical Physics Letters 363 (2002) 57–63.
[21] C. Reichardt, Solvents and Solvent Effects in Organic Chemistry, 3rd ed, VCH, Weinheim, Germany, 2003.
[22] P. Suppan, Photochemistry and Photobiology A 50 (1990) 293–330.
[23] P. Suppan, Journal of Photochemistry and Photobiology A 33 (1985) 29–32.
[24] C. Reichardt, Chemical Reviews 94 (1994) 2319–2358.
[25] From idealized theories, the solvent dielectric constant (i.e. the relative permittivity ε) is often predicted to serve as a quantitative measure of solvent polarity. However, this approach is often inadequate since these theories regard solvents as a non-structured isotropic continuum, not composed of individual solvent molecules with their own solvent/solvent interactions. They also do not take into account specific solute/solvent interactions such as hydrogen-bonding, which often play a dominant role in solute/solvent interactions. See reference [21] for more details.
[26] The reported value of E_T^N for ethanol is larger than that for acetonitrile. This is because of the way the empirical parameter E_T^N was formulated which is based on classifying solvents into protic, hydrogen-bond donors (E_T^N ca. 0.5. . .1.0), dipolar non-hydrogen-bond donors (E_T^N ca. 0.3. . .0.5) and apolar non-hydrogen-bond donors (E_T^N ca. 0.0. . .0.3). See reference [21] for more details.
[27] A.C. Borin, L. Serrano-Andrés, V. Ludwig, K. Coutinho, S. Canuto, International Journal of Quantum Chemistry 106 (2006) 2564–2577.
[28] A. Broo, A. Holmén, Chemical Physics 211 (1996) 147–161.
[29] S. Lobsiger, R.K. Sinha, M. Trachsel, S. Leutwyler, Journal of Chemical Physics 134 (2011) 114307/1–114307/14.
[30] R.-B. Zhang, X.-C. Ai, X.-K. Zhang, Q.-Y. Zhang, Journal of Molecular Structure (THEOCHEM) 680 (2004) 21–27.
[31] R.K. Sinha, S. Lobsiger, M. Trachsel, S. Leutwyler, Journal of Physical Chemistry A 115 (2001) 6208–6217.
[32] R. Ramaekers, L. Adamowicz, G. Maes, European Physical Journal D 20 (2002) 375–388.
[33] V. Ludwig, M. Serrou do Amaral, Z.M. da Costa, A.C. Borin, S. Canuto, L. Serrano-Andres, Chemical Physics Letters 463 (2008) 201–205.
[34] S. Nad, H. Pal, Journal of Physical Chemistry A 105 (2001) 1097–1106.
[35] G. Kodali, K.A. Kistler, S. Matsika, R.J. Stanley, Journal of Physical Chemistry B 112 (2008) 1789–1795.
[36] E.L. Rachofsky, J.B.A. Ross, M. Krauss, R. Osman, Journal of Physical Chemistry A 105 (2001) 190–197.
[37] J.M. Jean, K.B. Hall, Journal of Physical Chemistry A 104 (2000) 1930–1937.
[38] R.A. Hochstrasser, T.E. Carver, L.C. Sowers, D.P. Millar, Biochemistry 33 (1994) 11971–11979.
[39] K. Evans, D. Xu, Y. Kim, T.M. Nordlind, Journal of Fluorescence 2 (1992) 209–216.
[40] B.I. Kankia, Analytical Biochemistry 409 (2011) 59–65.
[41] J. Johnson, R. Okyere, A. Joseph, K. Musier-Forsyth, B. Kankia, Nucleic Acids Research 41 (2012) 220–228.