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Exciplexes and conical intersections lead to fluorescence quenching in π -stacked dimers of 2-aminopurine with natural purine nucleobases[†]

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Abstract

Fluorescent analogues of the natural DNA bases are useful in the study of nucleic acids' structure and dynamics. 2-Aminopurine (2AP) is a widely used analogue with environmentally sensitive fluorescence behavior. The quantum yield of 2AP has been found to be significantly decreased when engaged in π -stacking interactions with the native bases. We present a theoretical study on fluorescence quenching mechanisms in dimers of 2AP π -stacked with adenine or guanine as in natural DNA. Relaxation pathways on the potential energy surfaces of the first excited states have been computed and reveal the importance of exciplexes and conical intersections in the fluorescence quenching process.

1. Introduction

The photostability of DNA and RNA is essential to the integrity of our genetic code. The naturally occurring purines and pyrimidines that make up DNA absorb ultraviolet (UV) radiation at wavelengths around 260 nm. After absorption, these chromophores return to the ground state through ultrafast internal conversion pathways.^{1–4} Consequently, the excited state lifetimes of the nucleic acid chromophores are on the subpico-second timescale and they exhibit fluorescence quantum yields of the order of 10^{-4} .^{4–8} These rapid modes of decay and short lifetimes allow the nucleobases to return to their ground states before deleterious photochemistry can take place. The fluorescence decays of the nucleobases are also complicated by interbase electronic coupling.^{7–9} While radiationless decay processes are essential to the photostability of these chromophores, they render the spectroscopic study of DNA problematic. Their complex fluorescence spectra and low quantum yields make them poor spectroscopic markers. In recent years, fluorescent base analogues have been developed in order to be utilized in the study of DNA dynamics.^{10–12} These analogues function as fluorescence probes and are designed with some structural similarity to natural DNA for facile incorporation into duplexes.

[†]Electronic supplementary information (ESI) available: Tables of vertical excitation energies with oscillator strengths, tables of redox potentials, figures of linear interpolations, figures of attachment/detachment densities, tables of bond lengths, and cartesian coordinates for all structures are given. See DOI: 10.1039/c3pp25449f

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2-Aminopurine (2AP) is one analogue that is often used in place of adenine (6-Aminopurine, (A)) in studies of proteins and nucleic acids. Adenine and 2AP are purine derivatives that are constitutional isomers of one another. The two bases differ only in the placement of the amino group substituent on the six membered ring, as shown in Fig. 1. Like adenine, 2AP can also form a base pair with thymine (T) and can be incorporated into a duplex without major perturbation of the nucleic acid duplex.¹³ It can be selectively excited at a longer wavelength (~300 to 320 nm) than the natural nucleobases and aromatic amino acids which absorb at ~260 nm and ~290 nm, respectively. The fluorescence quantum yield of 2AP riboside is significantly larger than its isomer at 0.68 and has a long excited state lifetime of 11.8 ns.^{14,15} The emission of the chromophore is enhanced in polar environments.¹⁵ In polar protic solvents such as water and ethanol, the quantum yield is reported to be as high as 0.85 and 0.55, respectively.¹⁶ In polar aprotic solvents, such as ethyl acetate, the quantum yield falls to as low as 0.05.¹⁶ Gas phase studies of 2AP show that two tautomers of the chromophore exist, 9H-2AP and 7H-2AP, although the former is dominant.¹⁷ Jet-cooled 2AP examined *via* laser induced fluorescence (LIF) and resonance enhanced two photon ionization (R2PI) show that 9H-2AP tautomer exhibits only weak fluorescence in the gas phase.^{18,19}

Despite the structural similarities between the two purines, adenine displays a distinct photophysical behavior from 2AP. Experimental studies have shown that adenine has a low quantum yield of 7×10^{-5} and subpicosecond lifetimes in aqueous media.⁶⁻⁸ The stark differences in the quantum yields can be explained by the differences in their potential energy surfaces (PES). Previous theoretical studies have shown that 2AP and adenine possess at least two conical intersection (CI) seams, which allow for ultrafast non-radiative excited state decay.²⁰⁻²² In 2AP, barriers on the S_1 surface hinder access to the nearby CIs.²⁰ In adenine, however, there are no high barriers to prevent access to the CIs, so ultrafast radiationless decay takes place much more readily.^{17,20,22}

When 2AP is incorporated into dinucleotides or large nucleic acid structures, the excitation energy becomes slightly redshifted and the fluorescence decay requires a multiexponential fit.²³⁻²⁵ Previous experimental work has shown that 2AP engaged with nucleotides exhibits four components of decay with lifetimes of <100 ps, 0.5 ns, 2 ns, and 10 ns.²³⁻²⁵ These multiple components have been attributed to conformations that have varying degrees of stacking between bases.²⁶⁻³³ In general, the long lived components have been attributed to conformations where interbase stacking is increased. Nevertheless, the persistence of the short lived decay components in complexes with less base stacking, such as short oligonucleotides, underscores that fluorescence quenching can still take place in environments lacking large degrees of π -stacking interactions.^{24,25,34,35} Although 2AP fluorescence has proven to be extremely sensitive to its local stacking environment, fluorescence quenching is not affected by hydrogen bonding effects, such as those that arise from base pairing interactions.²³

The fluorescence properties of 2AP, coupled with its facile incorporation into duplexes, make it an ideal spectroscopic probe. 2AP has provided scientists with great insight into processes such as base flipping, ribozyme folding, protein–DNA interaction, and electron transfer.^{27,36-43} Despite its far reaching utility, the precise mechanisms that contribute to the

fluorescence quenching of 2AP still remain an area of scientific debate. Diminished emission in 2AP arising from photo-induced electron transfer processes has been proposed as a quenching mechanism.^{43–47} These electron transfer processes often involve guanine, which readily oxidizes to behave like an electron donor, since its oxidation potential is the lowest among the natural nucleobases.^{43–46,48} Other mechanisms have also been proposed, specifically the involvement of dark states which can be accessed depending on the conformations in DNA.^{24,25,35,49}

Theoretical studies can provide very useful insight into the mechanisms leading to the observed quenching. Such studies have been, however, limited.^{50–54} Earlier studies had focused on the vertical excitation of 2AP π -stacked with natural bases in a B-DNA conformation,^{50–52} while in our recent work, we performed a more extended study by going beyond the initial vertical excitations. We investigated the pathways on the excited states of dimers of 2AP π -stacked with pyrimidine nucleobases, which can lead to the observed fluorescence quenching.^{53,54} In the present work, we continue our theoretical investigation of the excited state behavior of 2AP in base stacked dimers with natural nucleobases, by broadening the scope of our studies to the interactions of 2AP with the purine bases. The two dimer systems studied are 2AP base stacked with adenine (A) or with guanine (G), where 2AP is placed at either the 5' (5'-2APA-3', 5'-2APG-3') or 3' (5'-A2AP-3', 5'-G2AP-3') terminus. The vertical excitation energies of the dimers in B-DNA conformation are calculated. Relaxation pathways on the S_1 surfaces of the dimers are also calculated to elucidate the behavior of the fluorescing state in each system as it moves beyond the Franck–Condon (FC) region. We report environmentally sensitive pathways of decay that may occur when 2AP is stacked with the natural purine bases. These results reveal the importance of exciplexes in these processes, as well as CIs between the ground and excited states.

2. Methods

The four dimer systems studied in this work are 5'-2APA-3', 5'-A2AP-3', 5'-2APG-3', and 5'-G2AP-3'. The initial geometries in a canonical B-DNA form were obtained from the ground state geometries reported by Thompson and Hardman, and are shown in Fig. 2a.⁵¹ In their work, the individual bases were optimized at the MP2/6-31G(d,p) level of theory and placed into truncated B-DNA conformations that were obtained *via* Spartan.⁵⁵

Vertical excitation energies of all geometries were calculated using the configuration interaction singles (CIS) method with second order perturbative correction using the resolution-of-identity approximation (RI-CIS(D)).^{56,57} Dunning's augmented correlation consistent polarized valence double zeta basis set (aug-cc-pVDZ) was used for these single point calculations.^{58,59} The natural orbitals describing the excited state transitions were obtained at the CIS/aug-cc-pVDZ level of theory. Some excited states were also visualized using attachment and detachment densities at the CIS/cc-pVDZ level.⁶⁰ In order to calculate the couplings between the different excited states, an Edmiston–Ruedenberg diabaticization procedure⁶¹ implemented in QChem at the CIS/cc-pVDZ level was used.

Optimizations on the S_1 excited state surfaces of the dimers and conical intersection searches were accomplished using the CIS(2) method, another variation of second order perturbative correction to CIS based on quasi-degenerate perturbation theory, which includes couplings between the ground and excited states as well.⁶² Since this method includes couplings with the ground state, it can be used for CI searches. The cc-pVDZ basis set was used in the optimization calculations. The CIS(2) method excluding the coupling with the ground state (denoted as CIS(2')) was also used to obtain more accurate oscillator strengths, since these are not given at the RI-CIS(D) level in the QChem software package. We have observed that the energies are more accurate when the coupling is not included, and for this reason single point calculations are performed without it. More details can be found in previous work.^{53,62} In the single point calculations, a modified version of the aug-cc-pVDZ basis set was used where the diffused functions were removed for more efficient computation, denoted here as cc-pVDZ+Diff.

The CIS(2)/cc-pVDZ method was also used to obtain the geometry of the 2AP monomer at its lowest bright $\pi\pi^*$ minimum. The geometry of the minimized monomer was then used in place of the ground state 2AP geometry in the dimers at B-DNA conformation to model the S_1 fluorescence maxima for the dimers.

Ten point linearly interpolated internal coordinate (LIIC) pathways were constructed as qualitative energy pathways from the initial B-DNA geometries to the final optimized minima and CIs. The excitation energies were calculated at each point in the LIIC pathway at both the CIS(2')/cc-pVDZ+Diff and RI-CIS(D)/aug-cc-pVDZ levels of theory. The LIIC pathways were constructed as an alternative to minimum energy paths, which are not implemented for these methods.

The ionization potentials (IPs) and electron affinities (EAs) of 2AP and the natural bases at their equilibrium geometries and at the conical intersection geometries were calculated at the DFT level. B3LYP/6-31++G(d,p) was used for the IPs and B3LYP/6-311++G(2df,p) for the EAs. The values were then used to obtain the oxidation and reduction potentials of the bases based on linear fitted expressions developed by Crespo-Hernandez *et al.*⁶³ These authors fitted the IPs vs. oxidation potentials and the EAs vs. reduction potentials of several bases and other organic molecules and obtained a linear expression which can be used for other similar molecules to obtain the redox properties after the IP and EA have been calculated. We used the same quantum chemistry methods for the IPs and EAs as these authors for best use of the fitted expressions. More details can be found in ESI[†] and in the original publication.⁶³

The Priroda computational package was used for the CIS(2) and CIS(2') calculations.⁶⁴ The natural orbitals calculations at the CIS level were accomplished using GAMESS.⁶⁵ The QChem quantum chemistry code version 4.0 was used for the RI-CIS (D) calculations, and for obtaining attachment/detachment densities and couplings of excited states.⁶⁶ Natural orbitals and geometries were visualized using MacMolPlt.⁶⁷

[†]Electronic supplementary information (ESI) available: Tables of vertical excitation energies with oscillator strengths, tables of redox potentials, figures of linear interpolations, figures of attachment/detachment densities, tables of bond lengths, and cartesian coordinates for all structures are given. See DOI: 10.1039/c3pp25449f

3. Results and discussion

3.1. Absorption

In this theoretical study, we focus on four unique dimer conformations. In these dimers, 2AP is affixed at either the 5' or 3' terminus. The systems under investigation are 5'-A2AP-3' and 5'-2APA-3', where 2AP is stacked with adenine, and the analogous dimers with guanine. The first several vertical excitation energies and transition characters of the dimers and their constituent monomers calculated at the RI-CIS(D)/aug-cc-pVDZ level of theory are shown in Fig. 3 and listed in Table S1.[†] While the energies of the optically excited states are obtained at the RI-CIS(D)/aug-cc-pVDZ level, the transition characters are assigned based on natural orbitals using CIS/aug-cc-pVDZ. The analogous table of monomer and dimer excitation energies and oscillator strengths at the CIS(2')/cc-pVDZ+Diff level of theory is also given in Table S2 in the ESI.[†]

The S_1 state of 2AP monomer is of $\pi\pi^*$ character and has a vertical excitation energy of 4.45 eV. The first excited state in all the dimers is localized on 2AP, since this state is considerably redshifted from the guanine or adenine excited states, and a small coupling is expected between them. The vertical excitation energy of S_1 for 5'-A2AP-3' and 5'-2APA-3' is redshifted relative to 2AP by 0.08 eV and 0.06 eV, respectively. The S_1 state of the guanine containing dimers is less perturbed than their adenine analogues, with the S_1 state of 5'-G2AP-3' exhibiting no energetic change whereas the analogous state in 5'-2APG-5' is redshifted by just 0.02 eV. Small shifting of the absorption is in agreement with experimental observations.^{68–71}

The oscillator strengths show that the S_1 states in the dimers diminish in brightness relative to 2AP monomer. The oscillator strengths for 5'-A2AP-3', 5'-2APA-3', and 5'-G2AP-3' decrease by ~26%, going from 0.19 to 0.14. The reduction in oscillator strength is slightly more pronounced in 5'-2APG-3' where it decreases from 0.19 to 0.13 (~30%). Similar reductions in oscillator strengths are also observed for the higher states (see Tables S1 and S2[†]). The reduction in oscillator strengths when nucleobases are incorporated in DNA is the well known hypochromism in polynucleotides,⁷² where as much as a 40% reduction of the absorption is observed.

The higher excited states of the dimers S_n are also often dominated by local excitations on one monomer, but now more coupling is expected with the neighboring bases, as the excitation energies are comparable. In Fig. 3, they are color-coded so that the color represents the monomer that the excitation is predominantly on. It is seen that the states are primarily localized on the monomers although they are shifted compared to those of the monomers, indicating that some coupling is taking place. Our results show that $\pi\pi^*$ states in adenine and guanine are in many cases shifted by 0.1 eV compared to the monomer. $\pi\sigma^*$ states shift even more, often more than 0.2 eV.

The shifts in the energies and the reduced oscillator strengths compared to the monomer values are a measure of the interactions between the bases. These interactions can be divided into two effects: either the excitation energy of one base is affected by the environment produced by the other base (similar to a solvatochromic shift), or there is coupling between

the excited states of the two bases. Both of these effects can and do occur at the same time, and they are responsible for the shifts we observe. The shifts in the $\pi\sigma^*$ and $n\pi^*$ states are most likely because of the environmental effects. The $\pi\sigma^*$ states are very diffuse and delocalized and are affected by the environment the most. $n\pi^*$ states are affected because they often have dipole moments that are very different from those in the ground state, and this causes the excited state to have different interactions with the environment compared to the ground state. The shifts in the $\pi\pi^*$ states are more likely because of the couplings. The coupling will also cause delocalization between the molecules and possibly energy transfer. Energy transfer between 2AP and natural bases based on the coupling between them has been observed and discussed in the literature.⁶⁸ In order to obtain a more quantitative picture of the coupling in these dimers we performed additional calculations where we diabatized the excited states through a recently developed localization procedure by Subotnik *et al.*⁶¹ The first five excited states at the CIS level were used in the calculations. The diabatization procedure gave values for the couplings which were between 0.03 and 0.08 eV. These values are certainly non-negligible, so the coupling is expected to play a role even for the S_1 state.

Besides the mostly-localized states, new states can also exist. These are charge-transfer (CT) states where an electron is excited from the HOMO of one monomer to the LUMO of the other. These states have been discussed extensively in 2AP containing DNA,^{50–52} since it is believed that electron transfer is responsible for the fluorescence quenching. Our calculations show that the CT states are high in energy. In adenine containing dimers they do not appear at all within 1 eV of the S_1 state. In guanine containing dimers, for 5'-G2AP-3' a CT state appears about 1 eV above S_1 , while for 5'-2APG-3' there is a mixed state with CT mixing about 0.6 eV above S_1 . The energies of CT states are greatly affected by any polar environment, and our results, which are in the gas phase, most likely overestimate these energies compared to what they are in solution or other environments. As discussed above, the energies of the $n\pi^*$ and $\pi\sigma^*$ states are also expected to be affected by the external environment.

It should also be noted that when calculating the CT states we had to take into consideration the fact that CIS overestimates the energy of these states.^{73,74} For this reason we had to include many more states at the CIS level (30 states) to ensure that the CT states were included and could be corrected based on perturbation theory at the RI-CIS(D) level. We also noticed that some $n\pi^*$ states were also overestimated at the CIS level, and RI-CIS(D) stabilized them considerably compared to other states. For example, the second excited state in 5'-2APG-3', which is an $n\pi^*$ state on 2AP, was state 11 at the CIS level, and became state 2 after the perturbation correction.

3.2. Relaxation on the S_1 surface

The topography of the S_1 potential energy surface plays a large role in determining the modes and mechanisms of excited state decay. Thus, investigation of the S_1 surface is of great pertinence towards understanding the excited state decay of the 2AP containing dimers. The first bright state of the four dimer systems was minimized and two distinct and environmentally dependent pathways of relaxation were discovered. When 2AP is placed at the 5' terminus, relaxation leads to a conical intersection seam between the ground and first

excited states. Relaxation on the S_1 surfaces of dimers with 2AP at the 3' terminus leads to minima that involve charge transfer character and diminished oscillator strengths. The pathways found in this work and discussed below are not expected to be the only possibilities to be found in π -stacked dimers of 2AP with purine bases, especially when they are in their natural environment including the backbone and solvent. These results however highlight possibilities that can explain some experimental findings and they theoretically show the involvement of charge transfer states and conical intersections, which had never been calculated before.

The importance of the position of the base and of the neighboring base likely arises from the differences in the degree of base overlap and specific interactions when 2AP is either above or below its base partner. The deviations in base overlap created by stacking order are shown in Fig. 2a where the bases are viewed from the reference of the $\text{HN}^9(\text{X})\text{--N}^9\text{H}$ (2AP) angle where X is the natural purine base (indicative of the twist between bases in B-DNA). The importance of the position of the base at either the 5' or the 3' terminus has been observed experimentally and discussed extensively by O'Neill and Barton,⁷⁵ referring to this as directional asymmetry. Our findings and comparisons with the experimental work are discussed in detail below.

3.2.1. 2AP at the 3' terminus: charge transfer states/exci-plex formation—In order to have a more overall picture of fluorescence we need to know how the 2AP localized S_1 state in the dimers behaves beyond the FC region. What happens to the excited state wavepacket as it moves along the potential energy surface and what processes take place to reduce the quantum yield? In this study, we find that the outcome depends on the location of 2AP. Minimizing the energy of the S_1 states of 5'-A2AP-3' and 5'-G2AP-3' leads to minima on their respective surfaces where the excited state is delocalized over both bases (denoted as $S_1(\text{Min})$). The energy of $S_1(\text{Min})$ relative to the ground state energy at the initial geometry is 3.42 eV and 3.01 eV for 5'-A2AP-3' and 5'-G2AP-3', respectively. These results are summarized in Table 1. The oscillator strength of 5'-A2AP-3' at this minimum decreases from 0.1406 (the value at the initial B-DNA geometry) to 0.0859. While the brightness of the state has decreased by about 40%, the magnitude of the oscillator strength is still significant. In 5'-G2AP-3', the oscillator strength is decreased from 0.1388 to 0.0267, a more severe 80% reduction. Linear interpolation internal coordinate (LIIC) paths were constructed from the FC geometries to the minimized geometries on the S_1 surfaces for 5'-A2AP-3' and 5'-G2AP-3'. The LIIC paths at the CIS(2')/cc-pVDZ+Diff level are shown in Fig. 4a,b, while those at the RI-CIS(D)/aug-cc-pVDZ are shown in ESI.[†] The absence of any barriers alludes to the energetic accessibility of the located minima.

The natural orbitals describing the S_1 state are shown in Fig. 4c,d. The natural orbitals shown are those with occupation numbers closest to one, as they represent single electron excitations. In the FC region at B-DNA conformation, the S_1 transition is a $\pi\pi^*$ transition localized purely on 2AP. At the S_1 minimum of the 5'-A2AP-3' dimer, some molecular orbital density is present on adenine, but most of the density remains on 2AP. In 5'-G2AP-3', a degree of charge transfer character dominates the excited state where charge that is localized on guanine becomes localized on 2AP after the excitation. The degree of CT transfer in these minima explains the reduction of oscillator strength. In both of these cases,

we have found a new minimum on the excited state that is stabilized because of the interactions between the chromophores, is not present in the monomer, and has some degree of CT character. Such a minimum represents the formation of an exciplex.

The mixing of CT character with the local 2AP $\pi\pi^*$ character occurs slowly and gradually along the path. This can be seen in the LIIC paths which show four excited states, where the crossings are strongly avoided, resulting in a smooth adiabatic S_1 surface.

The exciplex character is also evident in the geometries. We compare the geometries of 2AP in the FC region, at the S_1 minimum of 2AP monomer, and also at the $S_1(\text{Min})$ in the dimers. These data are shown in Table S4.[†] The geometry of 2AP at the S_1 minima for both dimers is more similar to the S_1 minimum of the monomer 2AP than to its initial ground state geometry. This reflects the fact that the excitation has significant 2AP $\pi\pi^*$ character. There are differences however. The absolute deviations of all bond lengths in 5'-G2AP-3' and 5'-A2AP-3' compared to the monomer 2AP $\pi\pi^*$ minimum are 0.017 Å and 0.007 Å, respectively. The deviation is larger for 5'-G2AP-3', as one would expect. The minimum in 5'-G2AP-3' has much more CT character and thus it is reasonable that 2AP will be very different from the monomer like minimum. On the other hand, 5'-A2AP-3' is much more localized on 2AP, and the geometry reveals that as well.

The changes in the bond lengths of the natural purine bases between the FC region and the $S_1(\text{Min})$ also reflect the extent of their roles in excited state decay. The bond lengths of adenine and guanine are also shown in Table S4.[†] The bonds in adenine do not undergo large degrees of stretching or contraction with an average absolute deviation of 0.008 Å. In guanine, the average absolute deviation over the analogous ring system is 0.023 Å. The greater bond deviations in guanine show that the purine plays a bigger role in the relaxation of 5'-G2AP-3' than adenine does in 5'-A2AP-3'.

Besides the changes in the bonds within each base, there are also significant changes in the parameters describing the relative position of one base with respect to the other. The geometries of 5'-A2AP-3' and 5'-G2AP-3' in B-DNA and at the $S_1(\text{Min})$ are shown in the right panels of Fig. 2b. The dimers have undergone geometrical changes from their initial B-DNA conformations to access the minima. In 5'-A2AP-3', the $\text{HN}^9(\text{A})\text{--N}^9\text{H}(2\text{AP})$ dihedral angle, representative of the twist in B-DNA, changes by 8° from the canonical B-DNA value of 36° to 44°, whereas in 5'-G2AP-3', the analogous angle changes by 7° from 35° to 42°. The base overlap between the six membered rings in each dimer increases. The bases also come closer in the dimers, going from 3.4 Å to 2.9 Å and 2.8 Å in 5'-G2AP-3' and 5'-A2AP-3', respectively. In both systems, the bases remain relatively planar although the C^6 atom in the six membered ring of 2AP is slightly lifted up from the plane of the base. The out-of-plane distortions are described by the $\text{C}^2\text{N}^1\text{C}^5\text{C}^6$ dihedral angle, which is -165° in both systems. The C^6 atom in 2AP moves above the plane by 15°, closer to its natural purine base partner. In 5'-G2AP-3', the amino group in guanine is pyramidalized with the hydrogens angled in the direction of 2AP. The amino group in 2AP is also pyramidalized which may be due to the electrostatic interaction of the substituents between the two bases. No pyramidalization of the amino group in 2AP is observed in 5'-A2AP-3' although it occurs in adenine, with the hydrogens angled towards 2AP.

The fluorescence maxima of 5'-A2AP-3' and 5'-G2AP-3' were calculated by replacing the 2AP base in the B-DNA geometry with a minimized geometry of 2AP at the $\pi\pi^*$ minimum. This geometry of the dimer is denoted as $S_1(\text{LMin})$ to distinguish from the optimized $S_1(\text{Min})$. Since the S_1 state at absorption is localized on 2AP, fluorescence would also occur from a 2AP localized dimer geometry depending on how much stacking is present. It is believed that the observed fluorescence in 2AP single and double strands occurs from the unstacked or not as well stacked conformations. The adiabatic gap in 5'-A2AP-3' is 4.06 eV while the fluorescence maximum is at 3.71 eV. In 5'-G2AP-3', the adiabatic gap is 4.13 eV with a fluorescence maximum of 3.79 eV. The adiabatic gap corresponds roughly to the origin of a transition, and the difference between them is the zero point vibrational energies. The magnitude of the oscillator strength at this local $S_1(\text{LMin})$ is slightly smaller than for absorption (see Table 1).

It should be noted that this $S_1(\text{LMin})$ minimum was never found in the optimizations for the dimers. This implies that at the B-DNA geometries we are using, the π -interactions between the bases are already strong enough and they lead to the alternative fluorescence quenching pathways rather than the local bright minimum. In other words we are using stacked bases, so we should not expect to see the bright fluorescent state which dominates in the not as well stacked conformations. Since our work does not include the environment of the backbone or the solvent, how the strength of the stacking interactions competes with all the other interactions is not represented by our calculations, and this competition could have an effect on when the local bright state is expected to be dominating the fluorescence.

3.2.1.1. Experimental relation to our findings: Experimentally, 2AP containing duplexes excited between 300 and 320 nm show a fluorescence band that is similar to that of 2AP in solution. It has been shown, however, that if 2AP in DNA is excited at the longer wavelength of ~360 nm, 2AP exhibits both short and long wavelength emission emitting at 370 nm and 450 nm.^{32,76,77} The emission intensity from the longer wavelength is roughly 10% of the short wavelength emission. The more intense short wavelength emission is assigned to a transition local on 2AP. Long wavelength emission was speculated to occur from preformed conformations within the DNA duplex where 2AP achieves a greater degree of π -stacking with its neighbor.

While our theoretical results cannot be expected to duplicate the exact experimental numbers, they may explain qualitatively some experimental findings. The fluorescence maximum predicted at the $S_1(\text{LMin})$ should correspond to the intense short wavelength observed fluorescence. The 5'-A2AP-3' system has a fluorescence maximum that is equivalent to 334 nm while in 5'-G2AP-3' the predicted fluorescence maximum occurs at 327 nm. The fluorescence maximum for monomer 2AP is 328 nm according to our calculations. So, our numbers agree with the experiment that the fluorescence in the dimers is very similar to the monomer, and our numbers in general differ from the experimental ones by about 40 nm.

The experimentally observed long wavelength emission could correspond to our calculated exciplex-like minima. Given the increased stacking between 2AP and adenine at this minimum, emission would correspond to long wavelength fluorescence. For 5'-A2AP-3'

the calculated emission maximum corresponding to the vertical excitation at the S_1 minimum is at 423 nm (2.93 eV, see Table 1). The oscillator strength is reduced by 40% at the minimum, which agrees with the weak fluorescence intensity observed experimentally. The difference between short and long wavelength fluorescence found theoretically is ~89 nm, which is not very different from the experimental difference of 80 nm.

Experimentally the red-shifted emission is only seen when exciting at longer wavelengths.^{32,76,77} This implies that absorption at longer wavelengths leads directly to the exciplex states according to our results, but absorption at shorter wavelengths does not. Even though the motion from the FC region to the exciplex states seems to be downhill and barrierless according to our LIIC paths, the existence of the backbone and other environmental interactions will probably create some barriers, inhibiting the direct access to the exciplexes. Absorption at longer wavelengths where the conformations are in the right place can lead directly to the exciplex minima. A similar wavelength dependence has been observed in AT duplexes and A single strand systems, where it has been found that fluorescence spectra of (dA)₂₀ and (dA)₂₀·(dT)₂₀ are strongly dependent on excitation wavelengths, and fluorescence spectra recorded upon UVA excitation show maxima at long wavelengths.⁷⁸ Recent theoretical studies found the presence of charge-transfer exciplexes in adenine tetramers, which can explain the experimental findings.⁷⁹

The energy at the S_1 minimum geometry of 5'-G2AP-3' is equivalent to 571 nm (2.17 eV, see Table 1) and does not correlate well with the magnitude of the redshift for the long wavelength fluorescence found experimentally. The oscillator strength of 5'-G2AP-3' is also significantly darker at the minimum compared to 5'-A2AP-3'. The decrease in state brightness at the S_1 minimum arises from the charge transfer character observed. Guanine is well known to have the lowest reduction potential of the natural nucleobases and is documented in the literature to behave as a good electron donor.

Since our calculations are done in free dimers without the backbone that is present in DNA and oligonucleotides, there is a question about the feasibility of the observed conformational changes in DNA. The rotational parameter between the bases in our final geometries is not very distorted, and the main distortion is the close proximity between the two π -stacked bases. In a recent work⁸⁰ we have addressed this particular point using MD simulations for a poly(dA)₂₀·(dT)₂₀ B-DNA strand. Assuming that the distributions we found for adenine oligomers will be similar to strands with 2AP incorporated, our results show that the probability of two bases coming closer to a distance of less than 3.1 Å is 0.2%. This value is quite small, but one has to remember that MD simulations use the ground state forces, while the processes described here occur on the excited state. It is quite possible that the forces in the excited states will increase the probability of reaching these close distances, particularly since the excited states have charge transfer character which increases the Coulomb interactions between the bases. In order to properly address this issue, we plan to carry out QM/MM calculations in the future, which can use the excited state forces within a single or double stranded system.

3.2.2. 2AP at the 5' terminus: conical intersections—Conical intersections play an important role in the radiationless decay of all the natural nucleobase monomers. In all of

these molecules, there are CI seams between the ground state and S_1 which facilitate ultrafast decay to the ground state. Access to these CIs is accompanied by large geometrical changes, most notably severe out-of-plane deformations of the six-membered rings in the purine and pyrimidine bases.

Very recently we discovered that CIs between S_0 and S_1 exist not only in the monomers but also in π -stacked dimers. Our results showed that the 5'-C2AP-3' and 5'-T2AP-3' dimers involved similar out-of-plane deformations to access CIs between the S_1 and S_0 states.^{53,54} The present study shows that CIs exist also in the dimers between 2AP and purine bases, specifically in 5'-2APG-3' and 5'-2APA-3'. The characteristics of these CIs and the comparison with the ones in previous work will be discussed in more detail here.

3.2.2.1. Monomer-like conical intersections: The most obvious reason why CIs can be found in dimers is that the CIs present in the monomer nucleobases can also exist when the bases are π -stacked, in which case the neighboring base just causes a small perturbation. Previous QM/MM studies addressed that point, and showed that the monomer-like CIs are still present in oligonucleotides, but their energies and accessibility may be perturbed.^{81–84}

In this work, optimization on the S_1 surface of 5'-2APG-3' leads to a CI similar to the one found in guanine. Even though the optimization on a single surface cannot converge in this case, we performed a CI search which located the actual CI. LIIC paths connecting the FC region to the CIs are shown in Fig. 5 and establish that the CI is reached through a small barrier. Since LIIC paths are not minimum energy paths, the actual barrier height or even the presence of the barrier cannot be obtained from these calculations. The origin of the barrier seen is an avoided crossing with a higher state. The energy of the CI for 5'-2APG-3' is 3.17 eV above the ground state initially, while the CI in monomer guanine has been reported in previous work to be about 3.7 eV above the ground state.⁸⁵ Given the fact that different methods were used in the two calculations, the perturbation from 2AP on G seems to be mild. The orbitals describing the excited state at this geometry are shown in Fig. 5c. Although initially the excitation is localized on 2AP, at the CI the orbitals involved are almost exclusively on guanine, showing that the excitation has transferred on guanine leading to the CI. The avoided crossing at points 3,4 of the LIIC is responsible for the change of character from the 2AP localized state to the G localized state. The G localized state, which is originally higher in energy, is stabilized along the path, while the 2AP localized state is destabilized, leading to the avoided crossing. An actual conical intersection can most likely be found if one includes more than the simple one-dimensional cut we have in Fig. 5. This change of character corresponds to an energy transfer from 2AP to G.

The structure of the dimer is shown in Fig. 2b, where it is seen that guanine has distorted significantly. This structure is very similar to the CI reported in the literature for guanine with C^2-N^3 twisting and forcing the amino group out of the plane of the molecule.^{85–87} A comparison of the bond lengths in 5'-2APG-3' between the initial B-DNA geometry and the CI geometry is shown in Table S5.[†] This comparison further demonstrates that the structure of guanine is very much distorted at the CI while that of 2AP is distorted very little. The minute deviations in the 2AP monomer of 5'-2APG-3' indicate that 2AP does not play a large part in accessing the CI. The rotation between the bases, represented by the $HN^9(G)-$

N^9H (2AP) dihedral angle, is very large, approaching 90° . This value is very different from the twist in natural B-DNA, so it appears that access to this CI would require a complete destruction of the rigidity of the DNA backbone, and it should not be possible in regular DNA.

3.2.2.2. Bonded exciplex: Most interesting are CIs that exist in the dimers but do not exist in the monomers. The CI found in $5'-2APA-3'$ is such a CI, and is starkly different from the one found in $5'-2APG-3'$. Here, the two bases have come very close to each other and they interact forming a bond between the C^6 atoms on each base. The distance between $C^6(2AP)$ and $C^6(A)$ is 1.62 Å, close enough for a bonding interaction to occur. This situation is very similar to our previously reported CIs in 2AP base stacked with pyrimidine bases.

Besides the two bases approaching each other, their structure changes within each base as well. A comparison of the bond lengths between the B-DNA structure and the CI geometry for $5'-2APA-3'$ is shown in Table S6.[†] Both bases show significant distortions at the CI. In both adenine and 2AP the largest changes occur in the C^6N^1 and C^5C^6 bonds, which stretch by ~ 0.09 – 0.10 Å. The extension of these bonds indicates that double bonds in the six membered rings are transitioning to single bonds and the C^6 atoms in both molecules change from sp^2 to sp^3 hybridization. In summary, both monomers in $5'-2APA-3'$ are instrumental in accessing the CI between the S_1 and S_0 surfaces.

The natural orbitals describing the S_1 transitions at the CIs for the dimers are shown in Fig. 5d. Initially, the S_1 state is a localized $\pi\pi^*$ transition on 2AP. The transition at the CI shows charge transfer character from 2AP to adenine and persistent density between the C^6 atoms of the two bases. The charge transfer character of the complexes is also confirmed by the partial charges derived from Mulliken population analysis on the S_1 state, which show an overall negative charge for adenine and positive for 2AP.

The CI found in $5'-2APA-3'$ here, and the CIs found in $5'-C2AP-3'$, $5'-T2AP-3'$ in previous work,^{53,54} all have some common characteristics. All the structures have been collected in Fig. 6, where it is seen that in all these three cases, there is a bonding interaction between the two bases. In all cases the C^6 of 2AP is bonded with a carbon on the other base; C^4 in cytosine, C^5 in thymine, and C^6 in adenine. Furthermore, in all cases there is some degree of charge transfer between the bases at the CI. Similar structures have also been reported for adenine dimers by other researchers, with the C^2 carbons on each adenine approaching each other, as well as on a dimer of adenine and thymine.^{88,89}

Analyzing the direction of the charge transfer at the CI structures can be tricky, especially if one concentrates on visualizing the transition. The problem occurs both because we are close to a CI, and because the two bases are very close to each other and there is very strong overlap between them. Fig. S3[†] shows the attachment/detachment densities for the CIs found in $5'-C2AP-3'$, $5'-T2AP-3'$ and $5'-2APA-3'$. According to these results, for $5'-T2AP-3'$ and $5'-2APA-3'$ an electron moves from 2AP to T and A, respectively, while for $5'-C2AP-3'$ an electron moves from C to 2AP. It should be pointed out that these calculations are done using CIS, where the energies are not degenerate but have a small gap between them. CIS is not expected to describe the CI region well. Similar results are

obtained if one uses natural orbitals to visualize the excitation. At the CI geometry, however, and even at previous geometries along the LIIC paths, there is charge transfer character in both the ground and excited states. For 5'-C2AP-3' both the ground and excited states have dipole moments pointing towards C, indicating more negative charge on this molecule (see Fig. S4[†]). The fact that the excitation shows that negative charge moves from C to 2AP is because there is less negative charge on C for the excited state compared to the ground state.

Narayanan *et al.* have reported the oxidative and reduction potentials of the excited state of 2AP in order to determine the ability of excited 2AP to reduce or oxidize the neighboring bases.⁴⁸ Based on the values they obtained, the excited state of 2AP can oxidize guanine but it will reduce cytosine and thymine. The situation for adenine is less clear since either oxidation or reduction can occur based on the free energies.⁴⁸

The experimental redox values apply to the initial B-DNA structures. In order to examine the driving forces for electron transfer at the distorted CI geometries, we obtained redox potentials theoretically. We calculated the ionization potentials and electron affinities of each base in the dimers at their equilibrium geometry and also at the geometry they have at the conical intersections. These results are further used to calculate redox potentials using the fitted expressions of Crespo-Hernandez *et al.*⁶³. The results are shown in Table S3.[†] Comparison of the values initially and at the distorted geometries allows us to see more clearly how the driving forces for electron transfer change at the CIs. In particular, the electron affinities for all bases change dramatically between the initial geometry and the distorted geometry, and they show a great stabilization of the anions. The values change sign from negative to positive, indicating that the anion is more stable than the neutral at these geometries. Based on the redox values, oxidation of 2AP is predicted to be the favored process in all cases at the CI. The driving force for oxidation in the distorted geometries is higher than initially. Our results agree with these predictions, if we consider the charge transfer at both the ground and excited states as described above.

The basic characteristics observed here, where a charge transfer state forms a CI with the ground state of interacting dimers while a bond is formed between the two monomers, can be explained with the concept of “bonded exciplex”, which has been used earlier to explain quenching reactions of excited electron acceptors with pyridine.^{90,91} The bonded exciplex model is demonstrated in Fig. 7b. Fig. 7a shows a regular exci-plex, where the excited state is stabilized at some distance between the two monomers. This situation is what we saw in 5'-A2AP-3' and 5'-G2AP-3'. In the bonded exciplex model the stabilization of the exciplex becomes very strong by covalent bonding interactions between the monomers. A regular exci-plex has some charge-transfer character, and in the extreme case there are distinct radical ions with unpaired electrons. If the unpaired electrons can spin-pair to form a covalent bond, then the covalent bonding interaction can lower the energy of the charge transfer state. If the stabilization is strong it will lead to a CI of this exciplex state with the ground state, which is what we observe in our calculations. Although the original reports discuss avoided crossings with the ground state, our work demonstrates that they can actually be real CIs where the gap between S_0 and S_1 is zero.

Another way to visualize these CIs is by comparing to reaction intermediates in the [2 + 2] cycloaddition reactions. This photochemical reaction is very common between two thymines and is the one responsible for the most common photochemical product in DNA. The photocycloaddition in the thymine dimer has been found to proceed in a concerted mechanism *via* a CI. For the ground state, however, the mechanism is stepwise with a transition state involving the formation of a single bond first.^{92,93} The structures seen in the CIs here resemble that transition state and could also be thought of as intermediates for a stepwise cycloaddition, where the second bond never forms. Instead, after passing through the CI to the ground state the first bond breaks and the structure returns to its original form.

We have now studied all π -stacked dimers of 2AP with natural bases. Out of the eight dimers we have studied (four here and four in our previous study^{53,54}), only three of them exhibited the bonded exciplexes. In guanine dimers, the CI found was not a bonded exciplex, but rather a monomer like one. In this case twisting of the bond did not lead to a bonding formation between the bases. Steric interactions between the bases prevent this. We cannot exclude the possibility that bonded exciplexes can be found in other dimers. The ones we found are very easily accessible. There could be other ones that are more difficult to access. There seems to be a delicate balance between steric effects, electrostatic interactions, stacking interactions, and relative conformations that can determine the formation and accessibility of the bonded exciplexes.

3.2.3. Final remarks on experimental comparisons—The results found in this work and in our previous publication highlight a very interesting phenomenon, the fact that the position of 2AP on either the 5' or 3' side has a great impact on the outcome of the dynamical fate of its excited state. This phenomenon has been observed previously experimentally as well.⁷⁵ O'Neill and Barton observed that the fluorescence quantum yields of oligonucleotides which incorporated 2AP were affected by whether 2AP was at the 3' or the 5' side with respect to guanine. This directional asymmetry agrees with our work. In the experimental study it was found that there is more quenching when 2AP is on the 5' side, and this was interpreted as there being more charge transfer when 2AP is on the 5' side. If one, however, considers that there can be more modes for quenching and not just charge transfer, then our results are not in disagreement with the experimental results. According to our results charge transfer exciplex states are just one pathway for quenching. Other pathways involve CIs, or as we discussed in our previous work, other dark states localized on 2AP. In fact there have been experimental studies based on transient absorption spectroscopy on dinucleotides consisting of 2AP and a natural base that propose quenching mechanisms based on the presence of a dark state.^{24,35,49} These workers have proposed that there are two states present, a radiative and a non-radiative, and in the dinucleotides, the non-radiative dominates while in 2AP the radiative dominates. The model has also been extended to include more than two states²⁵ and the authors discussed the idea that ground or excited state dynamics can be important in the observed multiexponential decay times. We favor this interpretation, as it seems natural that many different mechanisms can be operative in these complicated systems. Our results show that there is a variety of pathways that can be responsible for the decay.

4. Conclusions

Four π -stacked dimer systems containing 2AP and the purine bases (5'-A2AP-3', 5'-G2AP-3', 5'-2APA-3', and 5'-2APG-3') were studied, with interest in understanding the mechanisms that contribute to the experimentally observed decreased emission of 2AP when incorporated in π -stacking arrangements. Particular focus was placed on the S_1 excited state of the dimers, as photophysical features on this surface dictate the mode of decay after absorption. Our results reveal the importance of exciplexes and CIs in the fluorescence quenching. In 5'-A2AP-3' and 5'-G2AP-3', local minima on the excited surface with charge transfer character were found which can be interpreted as exciplexes. In 5'-2APA-3' and 5'-2APG-3', CIs between S_1 and S_0 were found which may lead to fast radiationless decay and fluorescence quenching. Quite interestingly, the CI structure for 5'-2APA-3' has bonding interactions between the monomers and shows charge transfer character. We have found similar structures for 2AP dimers with pyrimidine bases cytosine and thymine in previous work.^{53,54} These features are interpreted as bonded exciplexes, which differ from regular exciplexes by the bonding interaction which stabilizes the S_1 state greatly, causing it to cross with the ground state.

The present study is limited, since the calculations are done on two bases in the gas phase, whereas in order to relate to DNA, the backbone and aqueous environment should be included. Consequently, we cannot claim direct relevance to experimental results. Nevertheless, the results point to possibilities that can also exist in the DNA environment, as long as the right conformations can be achieved. The value of the rotation between the bases in most of our results (excluding 5'-2APG-3') does not distort much from the proper value, while the bases come much closer to each other compared to the 3.4 Å distance in B-DNA. The probability of whether the current conformations will be reached in DNA depends on the exact sequence and flexibility of the oligomers in which 2AP is incorporated, as well as the dynamics of both the ground and the excited state. Furthermore, our study is not comprehensive, in the sense that we do not claim that these are the only pathways that occur in these dimers. Our results, however, point to very interesting features that had not been explored before. Mainly, our results indicate that there may be more than one mechanism for the fluorescence quenching. The nature and position of the neighboring bases have a big effect on the participation of the different mechanisms.

The importance of CT states in the quenching of 2AP has often been proposed in experimental studies. The present study, along with our previous study on 2AP dimers with pyrimidine bases,^{53,54} provides a theoretical validation that indeed CT character is crucial in the mechanisms for fluorescence quenching. Nevertheless, one has to be cautious that there can be more than one way that CT can contribute. A complete electron or hole transfer can occur, or a partial charge transfer, such as in the exciplexes and bonded exciplexes discussed here. Experimental evidence of the electron transfer mechanisms is based on correlating the driving force for electron (or hole) transfer with the rate constants. Such correlations can be suggestive of a mechanism but they cannot provide the whole story.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Structures of 2-aminopurine, adenine, guanine, cytosine, and thymine, with labeling of the atoms.

Fig. 2. Geometries and dihedral angles of 5'-2APA-3', 5'-A2AP-3', 5'-2APG-3', and 5'-G2AP-3', in (a) the initial B-DNA structure and in (b) S_1 minimized conformations. Tables with more details of geometries can be found in ESI.[†]

Fig. 3. Vertical excitation energies of the monomers and dimers in this study at the RI-CIS(D)/aug-cc-pVDZ level of theory.

Fig. 4.

Energies of the S₀ through S₃ states at the CIS(2')/cc-pVDZ+Diff level along the LIIC path connecting B-DNA to S₁(Min) for (a) 5'-A2AP-3' and (b) 5'-G2AP-3'. Natural orbitals describing the S₁ state at the FC region and at the S₁ optimized minima for (c) 5'-A2AP-3' and (d) 5'-G2AP-3' are also shown.

Fig. 5.

Energies of the S_0 through S_3 states at the CIS(2')/cc-pVDZ+Diff level along the LIIC path connecting B-DNA and CI for (a) 5'-2APG-3' and (b) 5'-2APA-3'. The energies of S_0 and S_1 are not necessarily equal here since a different method has been used for the CI optimization. Natural orbitals describing the S_1 state of (c) 5'-2APG-3' and (d) 5'-2APA-3' at the FC region and at the S_1 - S_0 CI are also shown.

Fig. 6. Geometries of the conical intersections found in (a) 5'-2APA-3', (b) 5'-T2AP-3', and (c) 5'-C2AP-3'. The structures of (d) 2AP, (e) A, (f) T, and (g) C are also shown. A red filled circle indicates the atoms that participate in the bond formation. The geometries of 5'-T2AP-3' and 5'-C2AP-3' are taken from ref. 53 and 54.

Fig. 7.

Cartoon showing (a) a regular exciplex, and (b) a bonded exciplex. (a) Summarizes our findings for 5'-A2AP-3' and 5'-G2AP-3' while (b) summarizes our finding for 5'-2APA-3'. LE stands for locally excited state, in this case on 2AP, and CT stands for charge-transfer state.

Table 1

Summary of important energies in eV at the RI-CIS(D)/aug-cc-pVDZ level and oscillator strengths in parentheses at the CIS(2')/cc-pVDZ+Diff level. The vertical excitation energy at B-DNA geometry is given (B-DNA), as well as the energies for S₁(Min), S₁(LMin), and S₁/S₀ CIs relative to the ground state at B-DNA. Also shown are vertical emission energies corresponding to the S₁(Min) geometry and long wavelength (Fluor. Max. long), and to the S₁(LMin) geometries and short wavelength (Fluor. Max. short). The energies at the conical intersection reported are the average of the S₀ and S₁ energies at that geometry, since, because a different method was used for the location of the CI, these energies are not exactly degenerate

	5'-A2AP-3'	5'-G2AP-3'	5'-2APA-3'	5'-2APG-3'
B-DNA	4.37 (0.1406)	4.45 (0.1388)	4.39 (0.1436)	4.43 (0.1287)
S ₁ (Min)	3.42 (0.0859)	3.01 (0.0267)		
Fluor. Max. long	2.93	2.17		
S ₁ (LMin)	4.06 (0.1339)	4.13 (0.1363)		
Fluor. Max. short	3.71	3.79		
S ₁ /S ₀ CI			3.59	3.17