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Influence of Base Dynamics on the Conformational Properties of DNA: Observation of Static Conformational States in Rigid Duplexes at 77 K

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The dynamic behavior of the DNA bases plays an important role in processes that are critical to the maintenance and function of the duplex. These include electron transport along the duplex and many fundamental DNA−enzyme interactions. The conformational properties of the DNA duplex can be probed using the fluorescent adenine analogue, 2-aminopurine (AP), shown in Figure 1. When AP is substituted for a natural base in duplex DNA, its fluorescence is strongly quenched. A primary mechanism of this quenching is charge transfer between excited AP (AP^*) and neighboring bases, most favorably electron transfer from guanine (G) to AP^*. In a series of studies elucidating the influence of base stack structure and dynamics on charge transport in DNA, Barton, O'Neill, and co-workers have used the fluorescence intensity of AP as a probe of the yield of electron transfer from G to AP^*.[1] In a recent study, they have reported a dramatic increase in fluorescence intensity when AP-labeled duplexes are rendered rigid in a frozen matrix at 77 K, confirming the crucial role of base motion in mediating charge-transfer quenching of AP^*.[2]

Time-resolved fluorescence studies of AP-labeled DNA show that the duplex exists in a multiplicity of conformational states, manifested by a complex decay that can be described by the sum of four exponential components[3] with typical lifetimes of <100 ps, ~0.5 ns, ~2 ns, and ~10 ns. It is generally accepted that the very short component (<100 ps) corresponds to a highly stacked conformation in which AP^* is efficiently quenched by interbase charge transfer.[4] The long lifetime, ~10 ns, is attributed to an unstacked conformation in which AP protrudes from the duplex structure.[3,5] The existence of intermediate lifetimes indicates the existence, in the excited state, of conformational structures intermediate between the two extremes, but the number and nature of these conformational states remains unknown. If the ensemble of conformational states in DNA were static, on the time scale of the excited-state lifetime, the measured decay times would reflect the different intrinsic nonradiative decay rates of distinct ground-state conformations, with their amplitudes proportional to the equilibrium populations of these conformations. However, base dynamics during the excited-state lifetime may contribute substantially to the measured decay, as proposed recently for AP in oligodeoxynucleotide trimers.[6] In this picture, those structures that are closely stacked at the moment of excitation result in the fast decay component, while the slower components reflect the rate of exchange between unstacked (unquenched) and stacked (rapidly quenched) states.

To investigate the nature of the conformational states that give rise to the heterogeneous decay of AP^* and the role of base dynamics in populating these states, we have examined the fluorescence decay of rigid AP-labeled duplexes at 77 K. We have adopted the methodology of O'Neill and Barton[2] to freeze AP-labeled duplex oligodeoxynucleotides in 10 M aqueous LiCl, to give a stable, transparent glass at 77 K, and have recorded their fluorescence decays using time-correlated single-photon counting (Supporting Information). Three duplexes (Table 1) were examined, each containing a single AP in a different sequence context: GPG, CPC, and TPA, where AP is designated P. In GPG, AP is paired with G. In CPC and TPA, AP is paired with T. The base pair structures are shown in Figure 1.

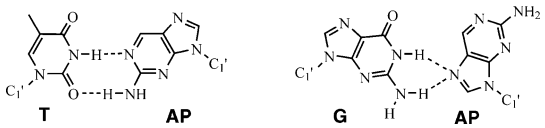

Figure 1. Base pair structures of AP with thymine (left) and AP with guanine (right). Further details are given in the Supporting Information.

In LiCl at 293 K all three duplexes show four exponential decays (Table 2), with parameters similar to those observed in aqueous buffer (Supporting Information). The high concentration of LiCl appears to cause some perturbation to the base-stacking interaction (changes in the magnitude and amplitude of the short decay components), and the difference in extrahelical environment is apparent as a shortening of the longest component in all cases (Supporting Information). Freezing the duplexes at 77 K has a dramatic effect on the decay functions. In all cases the shortest decay component is eliminated, and for TPA the second subnanosecond component also vanishes. The corresponding changes in fluorescence quantum yield (Table 2) are consistent with those reported previously on the basis of intensity measurements.[2]

We shall first consider duplexes CPC and GPG which show similar behavior. The absence of the shortest decay component, τ_1, in the frozen matrix at 77 K demonstrates conclusively that rapid charge-transfer quenching is entirely the consequence of base dynamics and is eliminated when the bases are static, even when AP is stacked and paired with G. The conformational structure that is subject to rapid quenching can be accessed only by thermal fluctuations of the bases at room temperature; it is not a minimum-energy geometry on the ground-state potential energy surface but a vibrationally excited-state in which the optimal stacked structure for rapid charge transfer is attained. This supports the principle of conformational gating of charge transfer proposed by Barton et al.[1a,2,4] and confirms previous suggestions[6,7] that the lowest energy conformation does not correspond to the fastest quenching rate.

The heterogeneity of the AP decay function persists at 77 K, and the observation of three decay components indicates the existence of a number of discrete, static conformational states that can be characterized by three distinguishable intrinsic decay times. For simplicity we will refer to these as three conformations, although as discussed below, each decay time is representative of a family of several geometrical structures with similar nonradiative decay rates. These conformations correspond to minima on the potential energy surface, and the dynamic conformational population

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Table 1. Base Sequences of the Duplexes

Duplex	Sequence
GPG	5′ -ACTGGTACAGTATCAGG**P**GCTGACCCACAACA TCCG-3′
	3′ -TGACCATGTCATAGTCCGCGACTGGGTGTTGT AGGC-5′
CPC	5′ -CACGGGCCTAACGATATCGTGCGTACGAGC-3′
	3′ -GTGCCCGGATTGCTATAGC**P**CGCATGCTCG-5′
TPA	5′ -CACGGGCCT**P**ACGATATCGTGCGTACGAGC-3′
	3′ -GTGCCCGGATTGCTATAGCACGCATGCTCG-5′

Table 2. Fluorescence Decay Parameters and Relative Quantum Yields for Duplexes GPG, CPC and TPA in 10 M LiCl

Sample	τ_1/ ns (A_1)[a]	τ_2/ ns (A_2)	τ_3/ ns (A_3)	τ_4/ ns (A_4)	Φ_{rel}[b]
GPG 293K	0.17 (0.56)	0.97 (0.23)	3.9 (0.13)	8.5 (0.08)	0.19
GPG 77K	-	0.76 (0.33)	3.6 (0.51)	10.1(0.16)	0.40
CPC 293K	0.06 (0.67)	0.34 (0.16)	2.0 (0.07)	7.9 (0.10)	0.10
CPC 77K	-	0.79 (0.40)	3.7 (0.34)	9.9 (0.26)	0.51
TPA 293K	0.22 (0.69)	0.94 (0.15)	2.0 (0.09)	7.9 (0.07)	0.15
TPA 77K	-	-	2.9 (0.14)	11.3 (0.86)	1.21

[a] Fluorescence decays were acquired at 3 emission wavelengths and analyzed globally to yield the reported lifetimes lifetimes (τ). Amplitudes (A) showed little variation with emission wavelength and are reported for 370-nm emission. (Full decay data are shown in Supporting Information). [b] Quantum yield relative to free AP-riboside under the same conditions. (Relative quantum yields were determined from the decay parameters; their values are consistent with the observed increase in steady-state fluorescence intensity on cooling to 77 K).

that exists at 293 K is frozen into these static structures when the duplex is cooled to 77 K.

The similarity of each of the lifetimes τ_2 and τ_3, respectively, at 77 and 293 K implies that the intermediate decay times measured at 293 K are essentially the intrinsic lifetimes of conformational states whose populations remain constant on the time scale of the excited-state decay. In duplex GPG, AP is in close proximity to guanine bases, and quenching of AP* in these conformations does not rely on base dynamics. Indeed, in this duplex, τ_2 and τ_3 become shorter at 77 K, implying that thermal excursions from the equilibrium geometry access structures in which quenching is less efficient. In CPC, there is some lengthening of τ_2 and τ_3 at 77 K, suggesting that vibrational motion of the bases at 293 K does enhance quenching of these conformations when AP is not stacked directly with G. The longest decay time, τ_4, characteristic of AP* free from interbase quenching, remains similar to that of free AP* at 77 K.

The large amplitude of the shortest decay component (A_1) at 293 K (Table 2 and Supporting Information) shows that a large proportion of duplexes (60% or more) attains this highly quenched geometry within picoseconds (or less) of excitation, faster than the time resolution of the present measurements. It follows that the majority of duplexes must exist in, or close to, this highly stacked conformational structure in the ground state at the moment of excitation. Although this is not the equilibrium geometry (lowest energy structure) of the ground state, it appears to be the "normal" (most populated) structure of the duplex at 293 K. It is evident from the large thermal population that this is not a single conformation but a collection of conformations that have in common certain critical structural coordinates that facilitate efficient charge transfer.

We now turn to duplex TPA for which both subnanosecond decay components, τ_1 and τ_2, are eliminated at 77 K and the predominant decay time of 11.3 ns (τ_4) is characteristic of unquenched AP*. Thus, in this duplex, base motion is required to access any conformation in which AP* is subject to rapid nonradiative decay. The significant difference of TPA from the other two duplexes is the absence of neighboring Gs and hence the greater conformational motion required to facilitate charge transfer from G to AP* through intervening bases.[1] This supports the assertion that electron transfer from G is the major channel for quenching of AP* and suggests that this may be the only channel for nonradiative decay of AP* on the subnanosecond time scale. However, the persistence of τ_3 shows that nonradiative decay on the nanosecond time scale can still occur when AP* is remote from G in the rigid duplex.

Although the decay parameters of TPA at 77 K are markedly different from those of GPG and CPC, we do not infer from this that the conformational structures adopted by the TPA duplex are significantly different from those of the other two. In fact, the similarity of the decay parameters of all three duplexes at 293 K suggest that their conformational behavior is similar. Our interpretation is that the decay time of AP* in frozen duplexes of similar conformational structure can be quite different, depending on the relative location of AP and G. Thus, the absence of τ_2 in TPA at 77 K does not indicate the absence of conformations of geometry similar to those that display lifetimes τ_2 in GPG and CPC, but that AP* in these frozen conformations of TPA is inaccessible to electron transfer from G and exhibits a longer lifetime. At 293 K, base motion allows these conformations of TPA to access charge-transfer active structures[1a] characterized by an AP* lifetime τ_2. By a similar argument, the large amplitude of the 11.3-ns component does not indicate a large population of a conformation with AP extrahelical, but shows that in ~80% of the multiplicity of structures represented by τ_2 and τ_3 in GPG and CPC, AP* in TPA is free from quenching in the rigid duplex.

It is clear that base dynamics profoundly influence the populations and properties of the conformational states of the duplex. In particular, the highly stacked geometry that gives rise to the very short decay time of AP-labeled DNA can be attained only through thermal motion of the bases. This conformation does not, therefore, correspond to the duplex geometry that we perceive from low-temperature crystal structures. Nevertheless, this appears to be the predominant form of the duplex in solution at room temperature.

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Supporting Information Available: Experimental details; AP-G and AP-T base pair structures; fluorescence decay parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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