Tautomerization Dynamics of a Model Base Pair in DNA

Osama K. Abou-Zied, Ralph Jimenez, and
Floyd E. Romesberg*

Department of Chemistry, The Scripps Research Institute 10550 North Torrey Pines Road, La Jolla, California 92037

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Structural and dynamic properties of DNA base pairs are virtually always discussed in terms of the keto-amine tautomers, which are thought to be the dominant forms of the base pairs in duplex DNA.[1] However, each base pair may be converted to its minor, but often reasonably stable enol-imine tautomer by a double proton transfer (prototropic tautomerism, Figure 1a).[2] The presence of rare tautomers in DNA may be difficult to detect due to the fast time scale and minimal heavy atom motion, as well as the reversibility inherent to the proton-transfer reaction. Nonetheless, the rapid interbase proton transfer may contribute to the physical properties of DNA, including duplex flexibility, protein recognition, or fidelity during DNA replication, by providing a dipolar coupling between flanking base pairs.[3] Proton transfer tautomerization may therefore contribute to DNA dynamics in the same way that heavy atom motion contributes to electron transfer processes. Despite the potential importance, tautomerization in DNA has not been well characterized experimentally because, in addition to the difficulties inherent to studying the ultrafast and reversible proton transfer, it has not been possible to selectively initiate proton transfer between a specific base pair.

In an effort to facilitate the study of tautomerization, we have developed a model DNA base pair that can be selectively induced to tautomerize. We have recently reported that when incorporated into DNA, 2-(2′-hydroxyphenyl)benzoxazole (HBO) is a reasonable mimic of a natural Watson−Crick base pair (Figure 1).[4] Prototropic tautomerization of HBO may be selectively and efficiently induced by photoexcitation, which leads to excited-state intramolecular proton transfer (ESIPT)[5] allowing for the study of tautomerization in DNA. We report here the sequence and structure dependence of the model base pair ground state and its proton-transfer dynamics.

To address the effects of sequence and helix formation, HBO was synthesized and incorporated into DNA oligonucleotides, 5′-CGTTTCXTTCTC and 5′-GAGAAXGAAACG at the positions labeled X. To examine helix formation, each oligonucleotide was hybridized to a complementary oligonucleotide containing an abasic site at the position opposite HBO, resulting in duplexes **1** and **2**, respectively. The purine rich oligonucleotide was also used to characterize the model base pair in single stranded DNA (ssDNA).

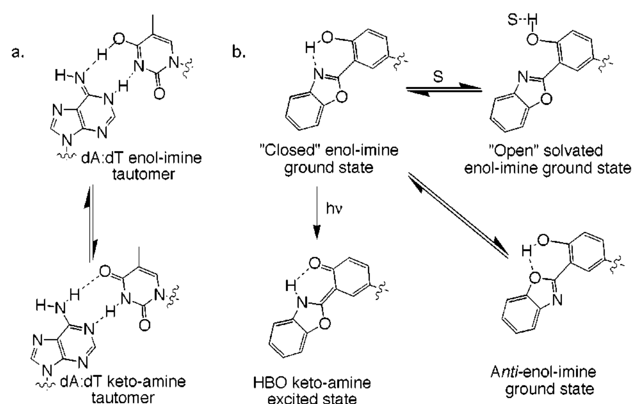

Figure 1. (a) Prototropic tautomerization in natural dA:dT base pair. (b) Equilibrium of HBO ground states and photoinduced tautomerization.

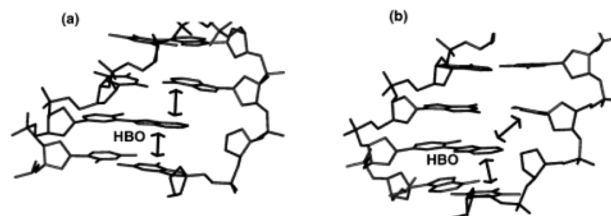

Figure 2. Simulated structure of (a) duplex **1**[4] and (b) duplex **2**. Base overlap is emphasized by the addition of arrows.

Previously, it was shown that duplex **1** is virtually as stable as a fully native duplex, while duplex **2** is significantly less stable (T_m = 38, 34, and 39 °C respectively for **1**, **2**, and the same duplex containing a dA:dT at the analogous position).[4] By CD spectroscopy, **1** showed an ellipticity consistent with an A-form helix, while **2** showed little ellipticity relative to ssDNA.[4] Presumably, the structure and stability of **1** results from the good overlap of the benzoxazole fragment with flanking pyrimidines (dA and dG) of the opposite strand, while the overlap with purines within **2** is less stabilizing. To further define these issues, a model of **2** was generated and is compared with the previously reported model of **1** in Figure 2. The structure of **2** represents the average structure from a 2 ns molecular dynamics simulation (Supporting Information). It is apparent that HBO in duplex **1** is well packed by flanking bases, while distortions in duplex **2** significantly unpack HBO and expose it to solvent. These differences in the simulated structures of **1** and **2** are in excellent agreement with the theremal, UV/vis, and CD data reported previously.[4]

The dynamic behavior of HBO may be understood by considering the conformational equilibrium between the *syn*- ("closed"), *anti*-, and "open-" enols depicted in Figure 1b. ESIPT is only possible in the closed-enol, resulting in the excited keto, which gives rise to an emission band at ~500 nm due to an anomalously large Stokes shift (~10 000 cm^{-1}). ESIPT is not possible within the solvated open-enol, because the H-bond required for ESIPT has been disrupted, or in the *anti*-enol, due to an insufficient change in the benzoxazole oxygen pK_a.[5]

The absorption and emission spectra of **1** were previously characterized.[4] In this sequence context HBO exists exclusively in the closed *syn*-enol configuration based on the 338 nm absorption maximum and a single, strongly Stokes-shifted fluorescence band at 480 nm. This implies that the major groove environment of DNA is dominated by interbase packing effects and not by solvation effects. The steady-state emission spectra of **2**, ssDNA, and HBO in hexane are shown in Figure 3a. The emission spectrum of **1** is included for comparison. The steady-state emission spectra of **2** and ssDNA were similar, but

* To whom correspondence should be addressed. Phone: (858) 784-7290. Fax: (858) 784 7472. E-mail: floyd@scripps.edu.

(1) Sanger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.

(2) (a) Chattopadhyaya, S.; Ikuta, K.; Grzeskowiak, R. E. *Nature* **1988**, *334*, 175. (b) Lippert, B.; Schöllhorn, H.; Thewaldt, U. *J. Am. Chem. Soc.* **1986**, *108*, 6616. (c) Sponer, J.; Sponer, J. E.; Gorb, L.; Leszczynski, J.; Lippert, B. *J. Phys. Chem. A* **1999**, *103*, 11406. (d) Nishio, H.; Ono, A.; Matsuda, A.; Ueda, T. *Nucleic Acids Res.* **1992**, *20*, 777.

(3) Strazewski, P.; Tamm, C. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 36.

(4) Ogawa, A. K.; Abou-Zied, O. K.; Tsui, V.; Jimenez, R.; Case, D. A.; Romesberg, F. E. *J. Am. Chem. Soc.*, **2000**, *122*, 9917.

(5) (a) Woolfe, G. J.; Melzig, M.; Schneider, S.; Dörr, F. *Chem. Phys.* **1983**, *77*, 213. (b) Krishnamurthy, M.; Dogra, S. K. *J. Photochem.* **1986**, *32*, 235. (c) Arthen-Engeland, Th.; Bultmann, T.; Ernsting, N. P.; Rodriguez, M. A.; Thiel, W. *Chem. Phys.* **1992**, *163*, 43. (d) Rios, M. A.; Rios, M. C. *J. Phys. Chem.* **1995**, *99*, 12456. (e) Mosquera, M.; Penedo, J. C.; Ríos Rodríguez, M. C.; Rodríguez-Prieto, F. *J. Phys. Chem.* **1996**, *100*, 5398.

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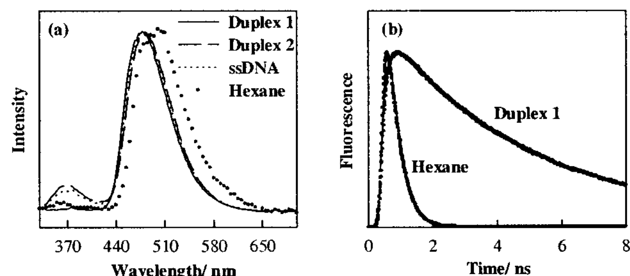

Figure 3. (a) Steady-state emission spectra of HBO in duplex **1**, duplex **2**, ssDNA, and hexane. (b) Fluorescence decay curves of HBO in duplex **1** and hexane.

qualitatively different from that of **1** (Figure 3a). In addition to emission at 485 nm, a strong fluorescence band was observed at 370 nm, indicating that there is a significant concentration of the open- or *anti*-enol ground states.[5] To further quantitate the conformational equilibrium in **1** and **2**, excitation-dependent fluorescence spectra of each duplex were examined (Supporting Information). Upon varying the excitation wavelength from 310 to 350 nm, emission was only detected at 480 nm in **1**, demonstrating that only the internally H-bonded closed-enol was present at a significant concentration. However, the excitation-dependent spectra of **2** revealed that maximum fluorescence at 370 nm follows excitation at 318 nm, consistent with the open- or *anti*-enol of HBO. This shift in the equilibrium of the model base pair in **2** and ssDNA may result from solvation or rotation of HBO to the *anti*-enol. In either case, the absence of the open- and *anti*-enols in **1** appears to require stable duplex formation.

The lifetimes of the excited open-/*anti*-enol tautomer and the excited keto tautomer (formed by ESIPT from the excited closed-enol) were measured by picosecond fluorescence decay as described in the Supporting Information. In hexane and duplex **1**, the emission at 370 nm was extremely weak (but observable) due to the very small equilibrium concentration of the open model base pair. The fluorescence decay rate of the excited open-/*anti*-enol was not strongly sensitive to the environment, as the measured rate was approximately the same in **1**, **2**, ssDNA, and hexane ($(6.2-7.3) \times 10^8\ s^{-1}$). As mentioned above, the steady-state data are consistent with the 370 nm emission band arising from the open- or *anti*-enol HBO. However, the lifetime of the excited solvated open-enol is known to be solvent dependent, while the lifetime of the excited *anti*-enol is insensitive to solvent.[6] We therefore conclude that in **2** and in ssDNA, HBO exists in an equilibrium between the internally H-bonded closed *syn*-enol and the *anti*-enol. The assignment of the species emitting at 370 nm as the *anti*-enol is also consistent with the absorption maximum of 318 nm, mentioned above.[5a] In contrast to the excited enol, the fluorescence lifetime of the excited keto was strongly dependent on its environment (Figure 3b). In hexane, the excited keto fluorescence decays with a rate of $3.4 \times 10^9\ s^{-1}$. The fluorescence decay of the keto in **1**, **2**, and ssDNA was indistinguishable ($2.0 \times 10^8\ s^{-1}$) but 17-fold slower than that in hexane.

(6) Abou-Zied, O. K.; Thompson, E.; Millar, D.; Romesberg, F. E. Unpublished results.

In interpreting the data it is important to keep in mind the strengths and weaknesses of the model. Some structural and electronic properties of HBO in DNA are expected to be different from a Watson−Crick base pair due to the fact that the phenyl and benzoxazole rings of HBO are connected by one covalent and one H-bond, while the natural pyrimidine and purine rings are connected only by H-bonds. However, as a model of a DNA base pair, HBO has several advantages. HBO is easily incorporated into duplex DNA, and in this regard is a better model of a DNA base pair than is 7-aza indole, which has received much attention as a nucleobase model, but which may not be incorporated in DNA. Moreover, HBO may be incorporated into DNA without a strong perturbation of the duplex. Additionally, the change in magnitude and orientation of the HBO dipole moment upon phototautomerization is expected to be similar to that which occurs during natural tautomerization.[5a] Therefore, HBO is expected to be a reasonable probe of how fluctuating dipoles might communicate within flanking base pairs. As reported in this communication, the selective stabilization of the proton-transfer product tautomer of HBO by the DNA environment suggests that flanking bases may be strongly coupled electronically. Such interbase communication is expected to have important effects on the physical properties and dynamics of DNA.

In DNA, the HBO model base pair exists in a structure-dependent equilibrium between the *anti*- and *syn*-enols, which is strongly shifted toward the *syn* species upon helix formation. Increased rigidity, a change in the electronic nature of the flanking base pairs upon helix formation, or solavation effects may contribute to the selective stabilization of the *syn*-enol. The *syn*-enol forms an internal H-bond that results in ultrafast phototautomerization to the excited keto state. The increased lifetime of the excited keto state in DNA, relative to hexane, likely results, at least in part, from an increase in the energy gap between the keto excited and ground states in DNA. This is consistent with the 15−20 nm blue shifted emission band in DNA relative to that in hexane (Figure 3a). Since the increased lifetime is observed in **1** and **2**, as well as in the single stranded oligonucleotides, it must derive from through-space interactions between the model base pair and the intrastrand flanking bases. The structure-independent, but selective interactions of the DNA environment with only the keto state may imply that these interactions play a role in nucleic acid function, for example, by stabilizing the critical, keto-amine tautomers in template DNA to facilitate high-fidelity DNA replication. Experiments to further define the sequence-dependent behavior of this model base pair, as well as the effects of DNA binding proteins such as DNA polymerases, are currently in progress.

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Supporting Information Available: Experimental details along with the structure of duplex **2** and fluorescence excitation spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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