DOI: 10.1002/cbic.200700714

Polarity of Major Grooves Explored by Using an Isosteric Emissive Nucleoside

Renatus W. Sinkeldam, Nicholas J. Greco, and Yitzhak Tor*[a]

More than half a century after the double helical structure of DNA was revealed, our understanding of certain fundamental features of this magnificent macromolecular assembly is still lacking. In particular, the polarity of nucleic acid grooves remains rather illusive. Knowledge of the forces that operate in these cavities, where key biological-recognition events take place, is of fundamental as well as practical importance. A basic understanding of these unique environments, where multiple functional groups coalesce, is highly desirable as it can shed light on the interplay of weak molecular forces within confined spaces.[1–4] Practically, information on the local polarity of specific cavities in nucleic acids can facilitate the design of low molecular weight ligands that impact the structure and biological function of these key biopolymers.[5,6]

The polarity of DNA grooves has been experimentally interrogated by using environmentally sensitive fluorescent probes.[7–16] This approach is informative, but bears several predicaments: a) any probe placed within the cavity to be assessed inherently modifies the molecular architecture of the native environment and therefore taints the readout; b) most studies have utilized dielectric constant (ε, relative permittivity)—a parameter that defines bulk solvent property and not an anisotropic medium as its measure; c) relatively large fluorophores (e.g., dansyl), which are frequently connected by flexible linkers, have been employed; these possibly populate multiple conformers each of which senses a different microenvironment.[17] Collectively, these challenges are responsible, at least in part, for the dramatically different estimates so far reported for the dielectric constant of the major groove in nucleic acids, which range from about 40 to 70.[9–16]

For the most accurate readout of groove polarity by means of fluorescence spectroscopy, an "ideal" probe must meet the following requirements: 1) its size and shape must be such that only the groove is examined; a linker, if used, must be as short and rigid as possible; 2) its presence must not hamper Watson–Crick (WC) base pairing or native-helix formation; 3) its absorption maximum must allow for selective excitation; and 4) its fluorescence maximum must be sensitive to polarity changes while maintaining sufficient quantum yield under all conditions. To meet these requirements, we avoided the conjugation of large fluorophores, but rather developed new emissive nucleoside analogues in which a natural nucleobase fragment is an integral electronic element of the chromophore. In this fashion, small and minimally invasive probes, capable of engaging in normal WC base pairing within unaltered duplexes, were employed. Here, we report the application of an environmentally sensitive furan-containing deoxyuridine **1** for probing the groove microenvironment in B-, A-, and abasic-duplex DNA.

The emissive furan-containing nucleoside analogue **1** nicely fulfils the criteria listed above. It represents an isosteric nucleobase mimic of T that is capable of participating in WC base pairing with A to form stable duplexes (Figure 1).[18] The direct

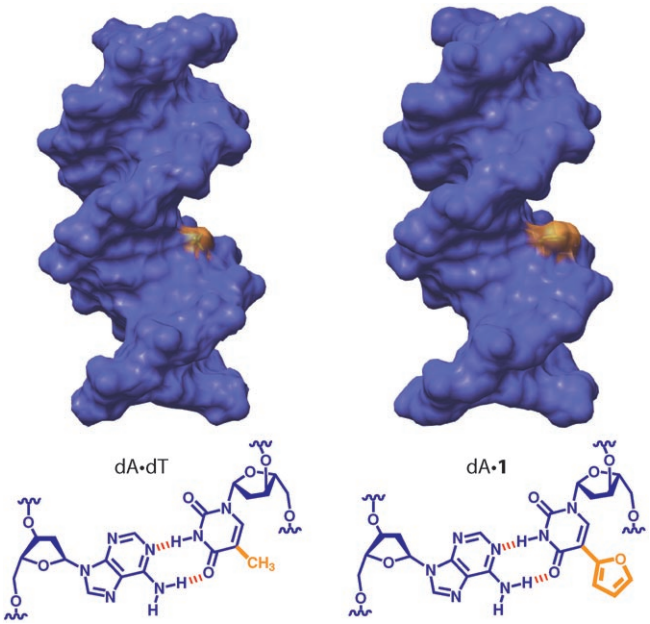

Figure 1. Models of DNA duplexes (blue) showing the location and surface area (orange) of the furan moiety of **1** (right) in comparison to the methyl group of T (left).

conjugation of the furan moiety to the pyrimidine core creates a biaryl chromophore, in which the pyrimidine nucleus is an integral component of the fluorescent probe.[19] The rigid modification at the 5-position projects toward the major groove with a well defined trajectory, while probing the groove's inner surface (Figure 1).

The ground-state absorption spectrum of **1**, which has a maximum at 316 nm when isolated from the native nucleobases, is virtually insensitive to changes in polarity, while its emission spectra are significantly impacted by the environment.[18–20] In addition to these attractive photophysical features, nucleoside **1** is prepared in only one step from available

[a] *Dr. R. W. Sinkeldam, Dr. N. J. Greco, Prof. Dr. Y. Tor*
Department of Chemistry and Biochemistry
University of California San Diego, 9500 Gilman Drive
La Jolla, CA 92093-0358 (USA)
Fax: (+1) 858-534-0202
E-mail: ytor@ucsd.edu

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

precursors, and can be sequence-specifically incorporated into oligonucleotides by using standard solid-phase phosphoramidite-based chemistry.[18–20]

Regardless of the probe's identity, estimating the polarity of DNA grooves under native conditions is always referenced to values determined for the isolated chromophore in solvent mixtures of known polarity. To generate an expanded polarity scale for nucleoside **1**, its absorption and emission spectra were measured in methylcyclohexane/isopropanol and dioxane/water mixtures—two solvent systems that cover a wide, yet considerably overlapping polarity range (Figure 2 A).[21] For

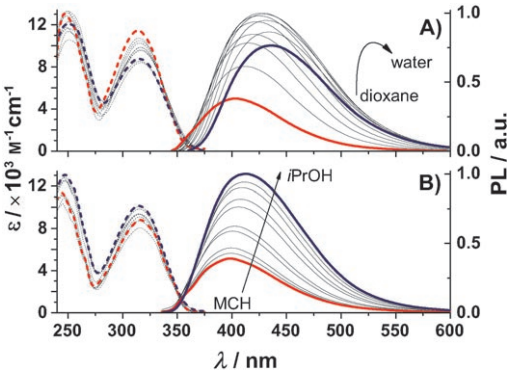

Figure 2. Absorption (dashed lines) and emission (solid lines) spectra of A) **1** in dioxane (red) and water (blue) mixture; B) the diacetate of **1** in methylcyclohexane (red) and isopropanol (blue) mixtures.[21]

each solution, the Stokes shift ($\nu_{abs}-\nu_{em}$) was then calculated and the associated microscopic solvent-polarity value $E_T(30)$ was experimentally determined;[22,23] this resulted in an amalgamated reference scale (Figure 3).

To probe the polarity of major grooves in nucleic acids, a nonself-complementary oligonucleotide **2**, that contained **1** in a central position, was prepared.[21] It was then hybridized to

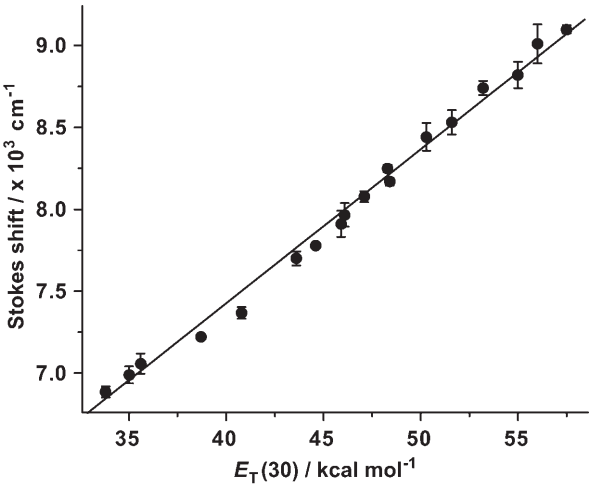

Figure 3. Correlation between Stokes shifts and microscopic polarity $E_T(30)$ for **1** and its diacetate. The averaged data points (●) are shown with error bars and a linear fit (——).

different single-stranded oligonucleotides, including a perfect complement **3**, an identical RNA complement **4**, and a THF-containing oligonucleotide **5** (Figure 4).

2 5'-GCG—ATG—**1**GT—AGC—G-3'
3 5'-CGC—TAC—ACA—TCG—C-3'
4 5'-CGC—UAC—ACA—UCG—C-3'
5 5'–CGC—TAC—**Y**CA—TCG—C-3'
6 5'–GCG—ATG—TGT—AGC—G-3'

Figure 4. Single-stranded oligonucleotides used in this study, where **Y** is a THF (abasic) residue.

The resulting double-stranded oligonucleotides represented a perfect B-form duplex DNA (**2·3**), an A-form DNA·RNA mixed duplex (**2·4**), and an abasic-containing B-form DNA duplex (**2·5**), in which the fluorescent nucleoside was placed opposite to the defect position. Thermal denaturation experiments confirmed that the emissive oligonucleotides were fully hybridized

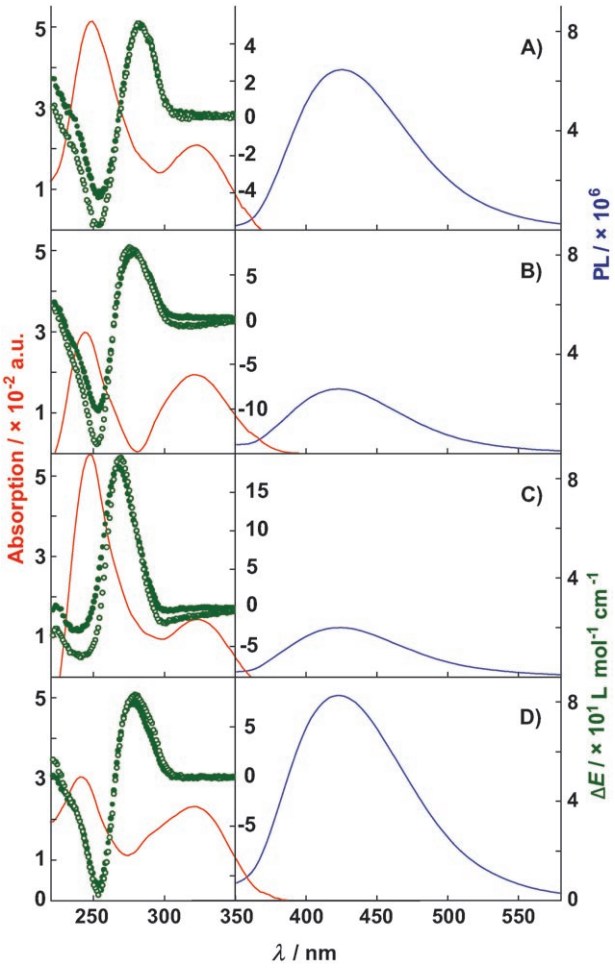

Figure 5. Ground-state absorption (——)[24] and emission (——) spectra for A) single strand **2**, B) **2·3** duplex, C) **2·4** duplex, and D) **2·5** duplex. Also shown are CD spectra for modified (○) and control oligonucleotides (●): A) **2** vs. **6**, B) **2·3** vs. **6·3**, C) **2·4** vs. **6·4**, and D) **2·5** vs. **6·5**.[21]

under the experimental conditions used for photophysical evaluation, and CD spectroscopy confirmed the presence of the expected duplex polymorphs (Figure 5).[21]

The single-stranded oligonucleotide **2** and the corresponding duplexes **2·3**–**2·5** were then photophysically evaluated for their absorption and emission maxima, and Stokes shifts were calculated (Table 1).[21,24] As expected, single-stranded **2** showed the largest Stokes shift; this indicates that the environmentally sensitive nucleoside is relatively exposed to a polar aqueous environment. Its "absolute" polarity ($E_T(30) = 48.3$ kcal mol^{-1}) was significantly lower than that of water ($E_T(30) = 63.1$ kcal mol^{-1}); this suggests partial shielding of **1** by neighboring nucleobases and is supported by a CD spectrum that shows a noteworthy secondary structure (Figure 5 A).[18] Upon perfect duplex formation, a significant drop in Stokes shift suggests a more apolar environment ($E_T(30) = 46.2$ kcal mol^{-1}), which is consistent with encapsulation of the probe within a double helical B-form DNA. Hybridization of **2** to its RNA complement **4**, which forced A-form duplex formation (Figure 5 C), yielded a lower major-groove polarity ($E_T(30) = 44.8$ kcal mol^{-1}). This is consistent with concealment of the probe in a much deeper major groove found in A-form duplexes. Interestingly, placement of the fluorescent probe opposite an abasic site showed the smallest Stokes shift; this corresponds to an apolar environment ($E_T(30) = 44.6$ kcal mol^{-1}) similar to that of A-form DNA. This is consistent with our previous proposal, which suggested that in such abasic-containing duplexes **1** assumes a *syn* conformation and is stacked between neighboring base pairs.[18,25–27]

Table 1. Photophysical data and estimated polarities of modified oligonucleotides.[a]

Oligonucleotide	λ_{abs} [cm^{-1}]	λ_{em} [cm^{-1}]	Stokes shift [cm^{-1}]	$E_T(30)$ [kcal mol^{-1}]
2	31 218	23 050	8168	48.3 ± 0.44
2·3	31 088	23 121	7976	46.2 ± 0.78
2·4	31 056	23 220	7836	44.8 ± 0.73
2·5	30 992	23 113	7879	44.6 ± 0.65

[a] λ_{abs} and λ_{em} (corrected) were averaged over three independent measurements.[21]

To put these results in perspective, we compared our observations to previously reported values for the polarity of the DNA major groove (Figure 6).[28] Ganesh and Barawkar reported an ε value of 55 for a DNA duplex with a dansyl probe placed close to the center of a self-complementary dodecamer.[10] This corresponds to 75% water in dioxane, which is equivalent to $E_T(30) \sim 57$ kcal mol^{-1}.[29] Majima and co-workers reported a major-groove dielecric constant of 61,[12] which relates to ~83% water in dioxane; this corresponds to $E_T(30)$ value of about 58 kcal mol^{-1}. Saito et al. estimated the major groove to be even more polar, with an ε value of 70,[14] which on their reference scale equals 61% water in ethylene glycol, or an $E_T(30)$ value of approximately 57 kcal mol^{-1}.[30] As all probes, except **1**, are connected through relatively long and flexible linkers, they interrogate an environment farther away from the groove wall. The relatively rigid and linkerless furan-containing probe **1** is located deeper in the major groove, and its readout suggests a rather apolar environment. This is consistent with a relatively low polarity proposed for the interior of the groove by distant-dependent dielectric constant correlations that show a steep increase in polarity as one moves away from the groove wall toward the groove exterior.[3,4,31]

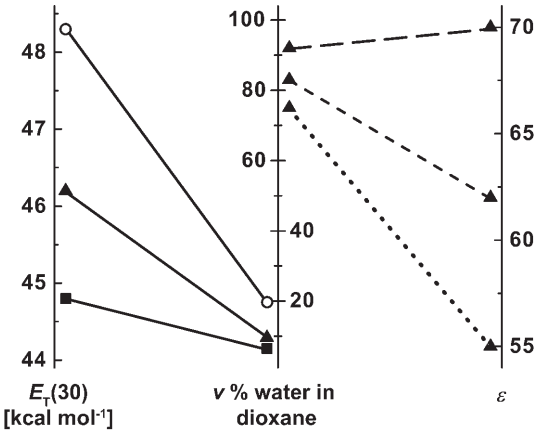

Figure 6. The major-groove polarity as determined by using **1** was compared to reported values. For perspective, both $E_T(30)$ values (left, this work) and published work (right, reported as ε) are converted to v% water in dioxane (center).[28] Key: single strand (○), perfect duplex (▲), A-form DNA (■), data presented herein (——), Barawkar and Ganesh[10] (•••••), Majima et al.[12] (-----), and Saito et al.[14] (– – –).

Although seemingly straightforward, the experimental evaluation of confined biomolecular environments with fluorescent probes is a laden and challenging task. The probe's intrinsic features, such as size, shape, and polarizability, inevitably evoke changes within the biomolecular cavity that might taint the readout. Infinitesimally small probes are clearly unrealistic, but small and minimally perturbing new fluorophores, such as **1**, when judiciously placed and systematically applied in conjunction with the expression of polarity based on $E_T(30)$ are likely to shed new light on fundamentally important questions regarding biopolymeric microenvironments.

Acknowledgements

We thank the National Institutes of Health (GM 069773) for support. We are grateful to Prof. Dr. C. Reichardt for a gift of penta-tert-*butyl substituted pyridinium*-N-*phenolate betaine dye.*[32,33]

Keywords: fluorescence · groove polarity · nucleic acids · sensors

[1] B. Jayaram, K. A. Sharp, B. Honig, *Biopolymers* **1989**, *28*, 975–993.
[2] J. Mazur, R. L. Jernigan, *Biopolymers* **1991**, *31*, 1615–1629.
[3] G. Lamm, G. R. Pack, *J. Phys. Chem. B* **1997**, *101*, 959–965.
[4] L. H. Wang, B. E. Hingerty, A. R. Srinivasan, W. K. Olson, S. Broyde, *Biophys. J.* **2002**, *83*, 382–406.
[5] X. F. Lu, J. M. Heilman, P. Blans, J. C. Fishbein, *Chem. Res. Toxicol.* **2005**, *18*, 1462–1470.

[6] J. Szekely, K. S. Gates, *Chem. Res. Toxicol.* **2006**, *19*, 117–121.
[7] R. Jin, K. J. Breslauer, *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 8939–8942.
[8] G. Q. Tang, N. Tanaka, S. Kunugi, *Bull. Chem. Soc. Jpn.* **1999**, *72*, 1129–1137.
[9] D. A. Barawkar, K. N. Ganesh, *Biochem. Biophys. Res. Commun.* **1994**, *203*, 53–58.
[10] D. A. Barawkar, K. N. Ganesh, *Nucleic Acids Res.* **1995**, *23*, 159–164.
[11] V. R. Jadhav, D. A. Barawkar, K. N. Ganesh, *J. Phys. Chem. B* **1999**, *103*, 7383–7385.
[12] T. Kimura, K. Kawai, T. Majima, *Org. Lett.* **2005**, *7*, 5829–5832.
[13] T. Kimura, K. Kawai, T. Majima, *Chem. Commun.* **2006**, 1542–1544.
[14] A. Okamoto, K. Tainaka, I. Saito, *Bioconjugate Chem.* **2005**, *16*, 1105–1111.
[15] A. Okamoto, K. Tainaka, Y. J. Fujiwara, *J. Org. Chem.* **2006**, *71*, 3592–3598.
[16] K. Tainaka, K. Tanaka, S. Ikeda, K. Nishiza, T. Unzai, Y. Fujiwara, I. Saito, A. Okamoto, *J. Am. Chem. Soc.* **2007**, *129*, 4776–4784.
[17] Most studies calculate the Stokes shifts from the emission and excitation maxima and do not extract the actual ground state absorption maximum of the probe within the specific duplex.
[18] N. J. Greco, Y. Tor, *J. Am. Chem. Soc.* **2005**, *127*, 10784–10785.
[19] N. J. Greco, Y. Tor, *Tetrahedron* **2007**, *63*, 3515–3527.
[20] N. J. Greco, Y. Tor, *Nat. Protoc.* **2007**, *2*, 305–316.
[21] See the Supporting Information for procedures, spectra and additional data.
[22] C. Reichardt, *Chem. Rev.* **1994**, *94*, 2319–2358.
[23] For the benefits of using microscopic solvent polarity scales, compared to dielectric constants, see: R. W. Sinkeldam, Y. Tor, *Org. Biomol. Chem.* **2007**, *5*, 2523–2528.
[24] To accurately determine the ground state absorption maximum of the emissive nucleoside within the hybridized duplexes, the absorption spectrum of a control, unmodified oligonucleotide that contained the same base composition, was subtracted from the absorption of the modified duplex.
[25] J. T. Stivers, *Nucleic Acids Res.* **1998**, *26*, 3837–3844.
[26] E. B. Brauns, M. L. Madaras, R. S. Coleman, C. J. Murphy, M. A. Berg, *J. Am. Chem. Soc.* **1999**, *121*, 11644–11649.
[27] M. M. Somoza, D. Andreatta, C. J. Murphy, R. S. Coleman, M. A. Berg, *Nucleic Acids Res.* **2004**, *32*, 2494–2507.
[28] See the Supporting Information for conversion of reported values to *v*% water in dioxane.
[29] The relation between $E_T(30)$ and dioxane:water ratio is included in the Supporting Information.
[30] R. D. Skwierczynski, K. A. J. Connors, *Chem. Soc. Perkin Trans. 2* **1994**, 467–472.
[31] Experiments to evaluate the generality of these observations as well as the impact of sequence, composition, and conditions on groove polarity are underway.
[32] C. Reichardt, E. Harbusch-Goernert, *Liebigs Ann. Chem.* **1983**, 721–743.
[33] C. Reichardt, S. Lobbecke, A. M. Mehranpour, G. Schafer, *Can. J. Chem.* **1998**, *76*, 686–694.

Received: November 22, 2007
Published online on February 19, 2008

