Quadruplex Topologies

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Topological Characterization of Nucleic Acid G-Quadruplexes by UV Absorption and Circular Dichroism**

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The emergence of nucleic acid four-stranded architectures, denominated G-quadruplexes, as a prolific area of research has led to an interest in the development of inexpensive methods for the rapid assessment of their structural characterization in solution. Research in this area is motivated by their potential impact on regulation of biological mechanisms and technological applications.[1] For many applications, light absorption spectroscopic methods, such as circular dichroism (CD) and UV spectroscopy, have been sufficient to discriminate the quadruplex fold from other architectures.[2] CD spectroscopy is also useful to discriminate a single quadruplex topology from all other 25 generic folding topologies.[3] Herein we demonstrate the use of these techniques for the characterization of three different types of G-quadruplex topologies classified through the sequence of glycosidic bond angles (GBA) adopted by guanosines of the G-quadruplex stem.

Light absorption by the quadruplex stem can be measured with the help of unpolarized (UV absorption),[2a] or circularly polarized light (CD).[2b,c] The quadruplex stem can be viewed as a system of uninterrupted overlapped guanosine molecular orbitals that are affected by the relationship between the base and its sugar (Figure 1).[4,5] The remaining question is: to what extent are the molecular orbitals affected? To answer this question, we consider the effect of variations in the extent of base stacking within the quadruplex stem caused by the glycosidic bond angle adopted by the guanosine.

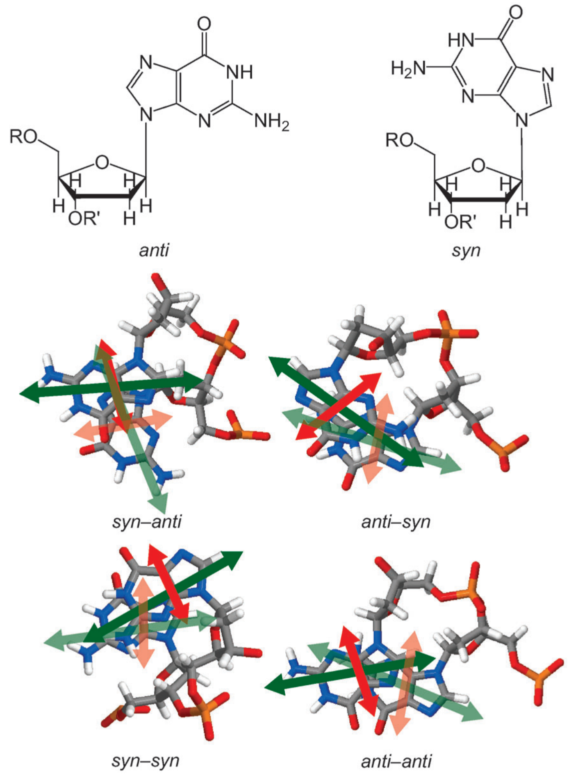

Figure 1. Top: chemical structures of *anti* and *syn* glycosidic bond angles. Bottom: transition dipole moments at 248 and 279 nm in green and red, respectively, for stacked guanines.

A stable G-quadruplex stem is thus a system of stacked guanosines that may adopt either an *anti* or a *syn* glycosidic bond angle, Figure 1.[3] We have previously grouped quadruplex topologies according to the sequence of GBA within a quadruplex stem.[6] A quadruplex stem with strands that progress in the same direction, parallel-stranded quadruplexes, is characterized by guanosines of the same GBA (group I). Antiparallel quadruplexes can be characterized as belonging to two different types. In the first type (group II), they can be characterized by containing both, sequences of guanosines of GBA of the same type (such as *anti–anti* and *syn–syn*), as well as of different type (such as *syn–anti* and *anti–syn* steps). The other grouping (group III) consists of antiparallel quadruplexes with consecutively stacked guanosines of distinct GBA. These three groups of quadruplexes include all possible types of overlap based on GBA irrespective of the number of stacking tetrads, and are thus representative of all possible quadruplex topologies.

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To demonstrate the feasibility of distinguishing these three groups through their light-absorption properties, we selected ten representative atomic-detail structures determined in potassium or sodium cation solutions (see Table S1). In Figure 2, we show their schematic topologies. The data set was further enriched by comparison with CD spectra recorded in this work and/or reported in literature (Table 1).

Firstly, we investigated their absorption properties by UV spectroscopy under the same or under similar solution conditions as for the structure determination. The difference in the UV absorption before and after melting (thermal difference spectrum, TDS) is a "fingerprint" spectrum currently used to distinguish different classes of higher-order architectures, including quadruplexes.[2a]

A representative example of a TDS spectrum for each of the three quadruplex groups is shown in Figure 3b. In the spectral region of interest (220–320 nm) there are at least four distinct positive/negative bands centered at 243, 255, 273, and 295 nm, respectively. We probed the relative intensities of these TDS spectral regions over a temperature range below their melting point. We defined TDS factors as the absolute values of $\Delta A_{240\,\mathrm{nm}}/\Delta A_{295\,\mathrm{nm}}$, $\Delta A_{255\,\mathrm{nm}}/\Delta A_{295\,\mathrm{nm}}$, and $\Delta A_{275\,\mathrm{nm}}/\Delta A_{295\,\mathrm{nm}}$, where ΔA_λ is the difference, at a given λ, between the absorbance above the melting and at a lower temperature T. They all include the band at 295 nm that is known to be highly responsive to quadruplex folding.[7] The analysis of these TDS factors demonstrated a marked difference between the magnitude of parallel quadruplexes (group I) and antiparallel quadruplexes (groups II and III). This effect can be attributed to the substantially different stacking of electronic transition dipole moments (hypochromicity). In Figure 3 we show a representative TDS factor; that for the

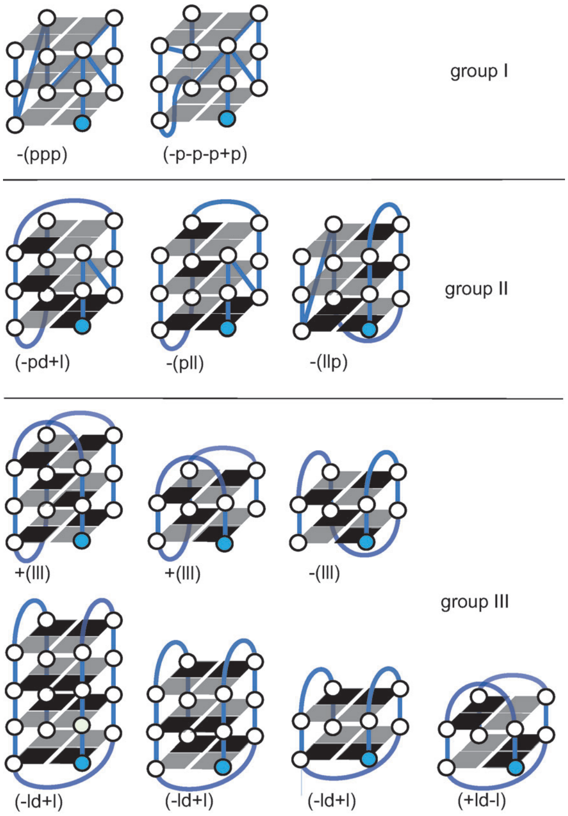

Figure 2. Schematic representations for structurally determined G-quadruplex topologies studied and reviewed herein. The 5′-end is indicated by the gray shaded circle. Guanosines in black are *syn*, and those in gray are *anti*. The sequences of the loops are reported in brackets and are defined as described in references [3, 6] (see also the Supporting Information). The topologies are described as pertaining to one of the three quadruplex structural groups.

Table 1: Categorization of structurally determined G-quadruplex topologies according to their CD profile.

Name	Looping sequence[a]	CD profile at λ [nm][b]			Group
		240	270	295	
I-1XAV	−(ppp)	−	+	n/a	I
I-2O3M	(−p−p−p+p)	−	+	n/a	
II-JSL	−(llp)	−	+	+	II
II-2GKU	−(pll)	−	+	+	
II-11a	(−pd+l)	−	+	+	
II-2HY9	−(pll)	−	+	+	
II-2JPZ	−(llp)	−	+	+	
II-2KZD	−(llp)	−	+	+	
II-186D	−(llp)	−	+	+	
III-201D	(−ld+l)	+	−	+	III
III-143D	(−ld+l)	+	−	+	
III-2KKA	(−ld+l)	+	−	+	
III-6a	−(lll)	+	−	+	
III-2KM3	+(lll)	+	−	+	
III-148D	+(lll)	+	−	+	
III-2KOW	(+ld−l)	−	+	+	
III-2KF8	(−ld+l)	−	+	+	

[a] Sequence of loops as described in the Supporting Information. [b] Positive (+) or negative (−) dichroic band.

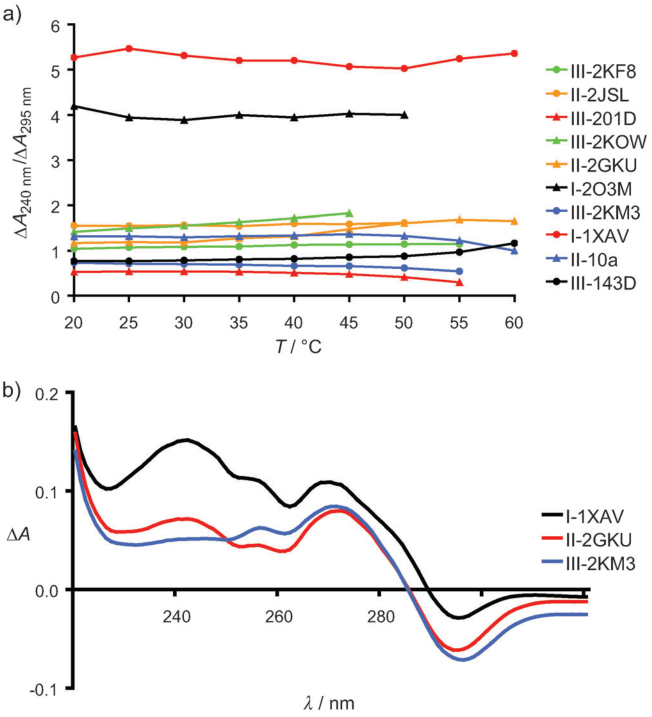

Figure 3. UV TDS parameters for a selection of topologies structurally characterized by solution NMR studies. a) UV TDS factors $\Delta A_{240\,\mathrm{nm}}/\Delta A_{295\,\mathrm{nm}}$ over a temperature range. b) Representative UV TDS spectra ($A_{95\,^\circ\mathrm{C}}$–$A_{20\,^\circ\mathrm{C}}$) for groups I through III.

magnitude of $\Delta A_{240\text{nm}}/\Delta A_{295\text{nm}}$. For parallel quadruplexes, the magnitudes for this TDS factor appear above 4, and for antiparallel quadruplexes below 2. With currently available data on atomic-detail structures, we cannot find statistical significance differentiating group II from group III antiparallel quadruplexes.

It is thus feasible to correlate the type of GBA sequence in the quadruplex stem with the magnitude of the TDS factor. Although the causes for this relationship remain unexplained in their details, the TDS factor appears to indicate a causal relationship to GBA that allows for a phenomenological interpretation.

We also investigated the absorption properties of the ten G-quadruplex standards by CD spectroscopy. Currently, this technique is widely used for distinguishing a single topology, the parallel-stranded (group I), from all others (antiparallel quadruplexes). Examples of typical CD spectra are shown in Figure 4a–c. The spectra show at least three distinctive features in the spectral range of interest, in which guanine absorbs: bands centered at 235–245 nm, 264–270 nm, and 280–297 nm. As for group I (Figure 4a), the difference in absorbance in the right-handed and left-handed circularly polarized light for parallel quadruplexes containing three (I-1XAV) or four (I-2O3M) loops has the same characteristics: an intense CD band at approximately 264 nm, and a negative band at approximately 245 nm, while the dichroic signal around 290 nm is negligible.

In contrast to group I, both group II and group III antiparallel quadruplexes show a positive band at 290 nm (Figure 4b and c). However, whilst group II shows a negative band for 240 nm and a positive band for 264, group III shows the reverse (Table S2). A spectroscopic interpretation of the three different CD-spectroscopic features observed for quadruplexes that belong to the different groups can come from the excitonic origin of such spectra (see Supporting Information).[2c,8]

Quadruplexes may be part of higher-order architectures with additional supramolecular features such as double helix, triads, further stacking of any base, and mismatches. How well does CD spectroscopy explain the spectra of higher-order architectures that include a quadruplex stem? In the Supporting Information, we describe why the CD signature for III-2KF8 and III-2KOW fit the spectral properties for group II quadruplexes (Figure 4d). These examples illustrate that caution should be exercised in assigning an antiparallel topology to group III from a CD spectrum in systems for which extensive base stacking is expected.

In conclusion, we utilized the formalism of quadruplex folding to build on the general understanding of the precise origins of the light absorption properties of a G-quadruplex stem. The developments described herein represent a more informed and inexpensive characterization of G-quadruplex topologies in solution.

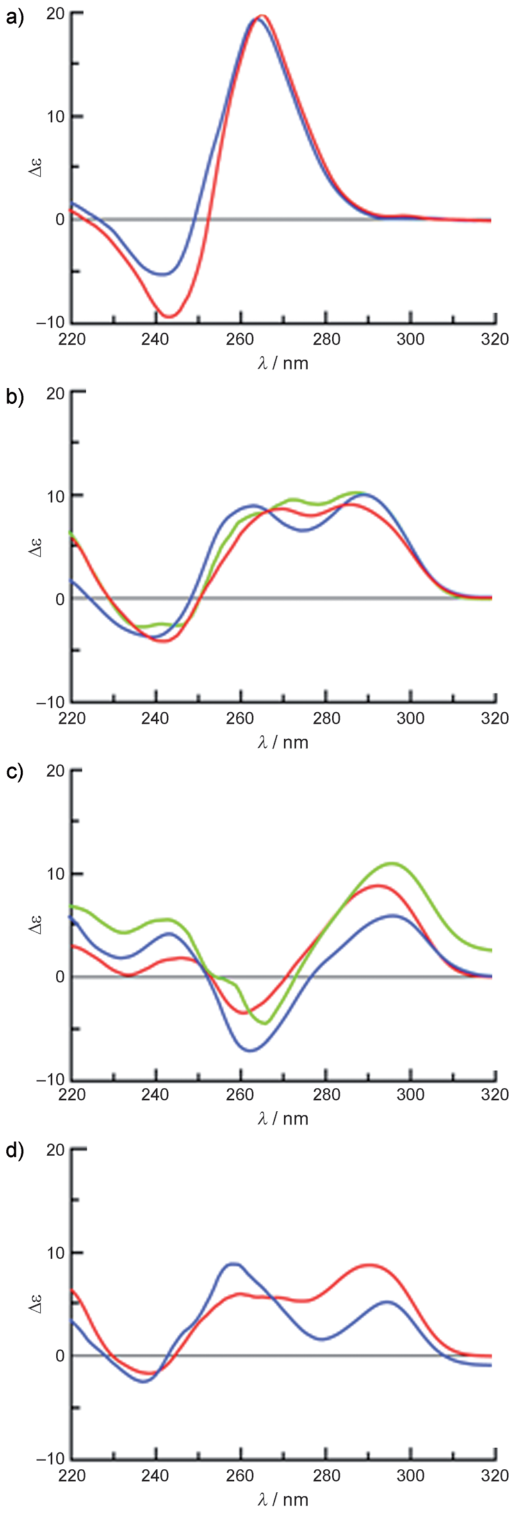

Figure 4. CD spectra for a set of standard structurally characterized quadruplex topologies. a) Spectra for parallel stranded quadruplexes I-1XAV (red) and I-2O3M (blue; group I). b) CD spectra for group II quadruplexes: II-JSL (green), II-2GKU (red), II-11a (blue). c) CD spectra for group III quadruplexes: III-201D (green), III-143D (blue), III-2KM3 (red). d) CD spectra of III-2KF8 (red) and III-2KOW (blue).

Experimental Section

All oligonucleotides were ordered as 40 nM desalted syntheses from Eurogentec (Belgium). Oligonucleotides were prepared at 100 μM concentration, in the presence of 70 mM KCl, buffered with 20 mM KAc at pH 6.8, with the exception of sample III-201D (folded in the presence of 70 mM NaCl buffered with 20 mM NaAc at pH 6.8). Samples I-1XAV, II-11a were prepared at 50 μM concentration in the presence of 100 mM KCl buffered with 20 mM KAc at pH 6.8. Sample III-143D was prepared at 50 μM concentration in the presence of 100 mM NaCl buffered with 20 mM KAc at pH 6.8. Further details are given in the Supporting Information.

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