

Insight into the High Duplex Stability of the Simplified Nucleic Acid GNA**

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More than 50 years after Watson and Crick unraveled the secondary structure of the canonical B-form DNA duplex, the function of the pyrimidine–purine nucleobase pairing and the importance of the charged phosphodiester backbone are well established.^[1] However, the chemical and evolutionary necessity for the rather complicated deoxyribose and ribose moiety in the backbone of DNA and RNA, respectively, is still uncertain.^[2] To address this question, researchers have investigated nucleic acids with alternative sugar residues.^[3–5] In the course of searching for structurally simplified nucleic acid backbones, we discovered recently that a glycol nucleic acid (GNA) with an acyclic propylene glycol phosphodiester backbone can form stable antiparallel duplexes in a Watson–Crick fashion.^[6–9] The constitution of the GNA backbone as well as a newly published GNA-duplex structure are shown in Figure 1.^[9] The glycol nucleotide building blocks contain just three carbon atoms and one stereocenter, and are connected by phosphodiester bonds. GNA combines structural simplicity and atom economy with a high duplex stability that significantly exceeds the stabilities of analogous duplexes of DNA and RNA. These features not only make GNA a possible genetic molecule for initial life on Earth but also an interesting scaffold for nucleic acid derived nanotechnology.

For almost the last two decades, it had been widely assumed that nucleic acid analogues containing a phosphodiester backbone need to be cyclic to produce the required conformational preorganization of the individual strands for the formation of a stable duplex.^[10,11] The high duplex stability of GNA therefore appears very surprising. In fact, GNA is to date the only known phosphodiester-based nucleic acid with an acyclic backbone that is capable of forming stable duplexes. Herein we present data that resolve this apparent discrepancy between the acyclic nature of the GNA backbone and the high stability of GNA duplexes.

To gain insight into the reason for the high duplex stability of GNA, we began by determining the thermodynamic parameters of duplex formation. Accordingly, the values of ΔG (Gibbs free energy), ΔH (change in enthalpy), and ΔS (change in entropy)

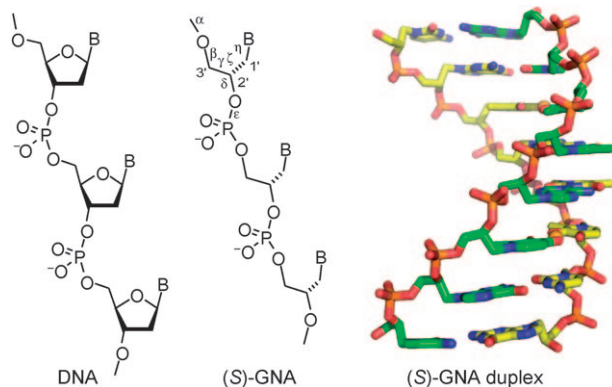


Figure 1. Constitution of DNA and the *S* enantiomer of GNA, and recently solved structure of an 8-mer (*S*)-GNA duplex (reference [9], image generated with PyMOL).

were obtained from van't Hoff plots by charting the reciprocal of the melting temperature, T_m , against the natural logarithm of varying duplex concentrations for three GNA duplexes and comparison with DNA duplexes with the same sequences (Table 1). As expected, the higher thermal stabilities of GNA duplexes correlate with higher thermodynamic stabilities

Table 1: Thermodynamic and thermal stabilities of (*S*)-GNA and DNA duplexes (brackets).

Entry	Duplex ^[a]	T_m [°C]	ΔG (298 K) ^[b] [kcal mol ⁻¹]	ΔH ^[b] [kcal mol ⁻¹]	$-T\Delta S$ (298 K) ^[b] [kcal mol ⁻¹]
1 ^[c]	CACATTATTGTTGTA GTGTAATAACAACAT	70 (47)	–21.1 (–15.2)	–95.6 (–103.3)	74.4 (88.1)
2 ^[d]	AATATTATTATTTTA TTATAATAATAAAAT	59 (41)	–16.2 (–12.4)	–77.0 (–86.5)	60.8 (74.1)
3 ^[d]	CGAATTTCG GCTTAAGC	54 (36)	–12.2 (–9.4)	–54.0 (–59.4)	41.8 (49.9)

[a] Upper strand: 3'→2' direction for GNA and 5'→3' direction for DNA. Bottom strand: 2'→3' direction for GNA and 3'→5' direction for DNA. [b] ΔG , ΔH , and ΔS were estimated from van't Hoff plots. Each data point was determined in triplicate, and the mean value was taken. [c] Measured in 10 mM sodium phosphate (pH 7.0) with 100 mM NaCl. [d] Measured in 10 mM sodium phosphate (pH 7.0) with 500 mM NaCl.

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[**] GNA = glycol nucleic acid.

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(298 K).^[8] However, surprisingly, for all three duplexes we found that GNA-duplex formation is less exothermic than DNA-duplex formation, but at the same time entropically significantly less unfavorable. This entropic advantage is counterintuitive, since one would expect that an acyclic backbone is more flexible and thus entropically unfavorable for duplex formation.

A less unfavorable entropic contribution to the duplex formation of GNA may indicate a strongly preorganized conformation of the GNA single strands.^[11,12] Therefore, we investigated the temperature-dependent conformation of GNA single strands by CD spectroscopy. Figure 2a shows

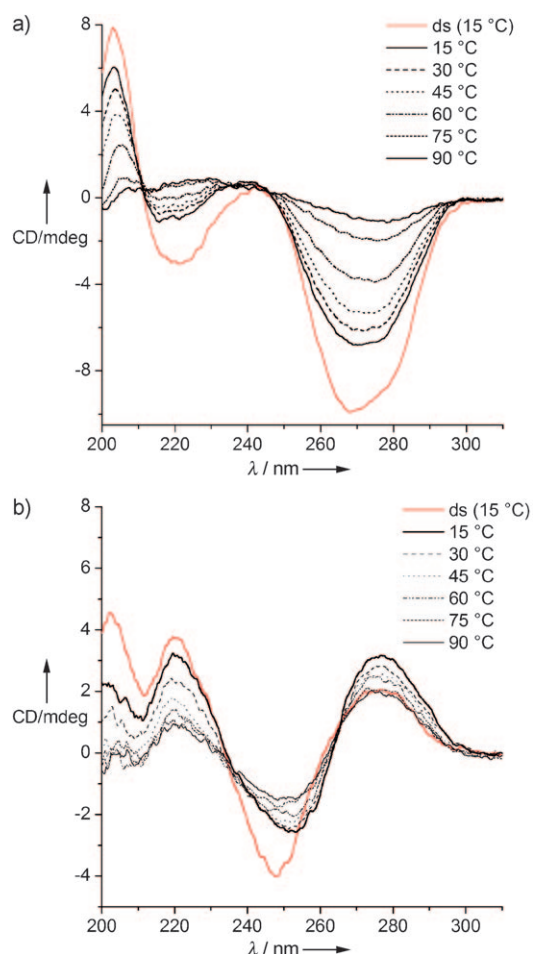


Figure 2. Melting of the a) GNA and b) DNA single strands CACAT-TATTGTTGTA (3'→2' direction for GNA and 5'→3' direction for DNA) as monitored by circular dichroism (CD). The measurements were made in 10 mM sodium phosphate (pH 7.0) with 100 mM NaCl at a concentration of 20 μ M. The CD spectra of the corresponding duplexes (10 μ M per single strand, red lines) are shown for reference.

that a 15-mer single strand (20 μ M) displays significant Cotton effects that are only slightly less pronounced than for the corresponding duplex at the same overall single-strand concentration (10 μ M per strand). The CD signals disappear gradually upon an increase in temperature from 15 to 90 °C, which indicates that the GNA single strand adopts a defined helical structure that melts upon heating. Furthermore, since the maxima at around 205 and 240 nm and minima at 220 and 270 nm are at the same wavelengths in the spectrum of the corresponding duplex, it can be concluded that the conformation of the single strand resembles the conformation of the individual strands within the duplex. For comparison, the analogous CD melting experiments with DNA single strands are not as conclusive and show a less marked temperature-

dependent melting behavior (Figure 2b). Thus, although acyclic, the GNA backbone apparently leads to an ideal preorganization of the single strands for duplex formation.

Support for the helical preorganization of GNA single strands was obtained from a comparison of crystallographic data for a GNA duplex structure with the conformation of GNA nucleosides. In the recently reported crystal structure of an 8-mer GNA duplex, all natural nucleotides adopt the same staggered conformation with an *anti* orientation of the nucleobase and a *gauche* conformation with respect to the vicinal C–O bonds (Figure 3).^[9] A comparison of this back-

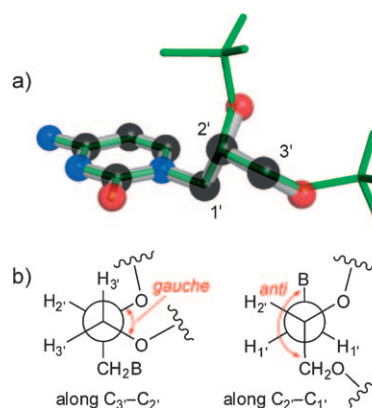


Figure 3. a) (S)-1-(2,3-Dihydroxypropyl)cytosine (colored balls and sticks) superimposed with the conformation of a cytosine nucleotide within a GNA duplex (green sticks). b) The corresponding preferred conformations of the natural nucleotides within the GNA backbone are shown as Newman projections.

bone conformation with the conformation of crystallized single nucleosides reveals that this conformation is already favored in the single nucleosides, as can be seen by the superimposition of the crystal structure of 1-(2,3-dihydroxypropyl)cytosine with the conformation of a cytosine nucleotide within a GNA duplex (Figure 3a).^[7,9] Furthermore, an NMR spectroscopic investigation with 1-(2,3-dihydroxypropyl)cytosine in both organic and aqueous solutions is in agreement with the preferred *anti*-C₁–C_{2'} and *gauche*-C₂–C_{3'} conformation in the crystal structures (see the Supporting Information for details).^[13]

A further reason for the relative entropic advantage of the formation of GNA duplexes over DNA-duplex formation may result from differences in stacking interactions. Stacking interactions contribute to an increase in entropy through partial dehydration of the nucleobases during the transition from single strands to duplexes. In fact, a comparison of base-pair stacking interactions in GNA and DNA revealed significant differences. Whereas base stacking in B-form DNA is predominately due to intrastrand base–base interactions, the main contacts in GNA can be found between bases of opposite strands (see Figure 1).^[9] To assess the contribution of base stacking to the stability of GNA duplexes, we analyzed the effects of dangling nucleobases.^[14] Single nucleotides were added at the 2' or 3' end of the self-complementary 8-mer duplex 3'-CGAATTCG-2', and thermodynamic parameters for duplex formation were deter-

mined from van't Hoff plots. The data obtained (Table 2) reveal strong directional effects: Whereas overhanging nucleosides at the 3' end do not provide any increase in the thermal and thermodynamic stability of the resulting GNA

(Table 1). Averaging of the thermodynamic effects observed in each of the individual experiments with dangling nucleotides (with each pyrimidine and purine nucleotide overhanging on each end of the single strands) demonstrates an

average stabilization by dangling nucleotides of $0.36 \text{ kcal mol}^{-1}$ per nucleotide in DNA and $0.69 \text{ kcal mol}^{-1}$ per nucleotide in GNA. Thus, from these experiments it can be concluded that nucleobase stacking is thermodynamically more favorable for GNA-duplex formation than for DNA-duplex formation.

Several factors probably contribute to this favorable base stacking in GNA duplexes.^[15] First, the experiments with dangling residues measure the thermodynamically driven equilibrium between single strands and the duplex. One therefore has to consider the change in stacking interactions in the course of the duplex formation. On the basis of our CD experiment, NMR spectroscopic data, and crystallographic data (Figures 2 and 3), the extreme backbone–nucleobase

inclination observed in the GNA duplex must already be present in the preorganized GNA single strands. As a consequence, GNA nucleobases cannot optimize their intra-strand stacking interactions in the single strand and therefore should profit more from conversion into the duplex form. Second, the extensive zipperlike interstrand stacking interactions should contribute to the duplex stability of GNA as a result of attractive interstrand van der Waals forces.^[16] Third, the structure of the GNA duplex (Figure 1) reveals that the C1'H₂ group of the GNA backbone serves as an extension of the base pairs and increases hydrophobic interactions with neighboring nucleobases. Such nucleobase–backbone interactions are not present in DNA duplexes. Lastly, the high conformational preorganization of the GNA single strands should also be partly responsible for more favorable stacking interactions.

In conclusion, we have shed light on the reasons for the surprisingly high duplex stability of the simplified nucleic acid GNA.^[17] Thermodynamic measurements demonstrated that the entropic penalty for duplex formation is significantly smaller for GNA than for DNA. This result is consistent with a strong conformational preorganization of GNA single strands as well as especially favorable stacking interactions in the corresponding GNA duplex. Thus, GNA can be viewed as a preorganized zipper with highly favorable duplex-formation properties.

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Table 2: Thermodynamic and thermal stabilities of (S)-GNA and DNA duplexes with overhanging nucleotides.^[a]

Entry	Overhanging nucleotide X ^[b]	<i>T</i> _m [°C]	X CGAATTCG GCTTAAGC X		<i>T</i> _m [°C]	CGAATTCG X X GCTTAAGC	
			ΔT_m [°C]	$\Delta\Delta G^\circ$ (298 K) [kcal mol ^{−1}]		ΔT_m [°C]	$\Delta\Delta G^\circ$ (298 K) [kcal mol ^{−1}]
GNA duplexes							
1	none	54.3	0	0	54.3	0	0
2	A	55.2	0.9	−0.2	68.9	14.6	4.0
3	T	55.5	1.2	0.1	64.9	10.6	2.4
4	G	54.8	0.5	−0.6	66.8	12.5	3.6
5	C	55.4	1.1	−0.6	62.6	8.3	2.3
DNA duplexes							
6	none	35.5	0	0	35.5	0	0
7	A	45.0	9.5	1.8	39.1	3.6	0.6
8	T	41.1	5.6	0.9	36.8	1.3	0.0
9	G	42.6	7.1	1.3	39.4	3.9	0.7
10	C	40.1	4.6	0.6	36.9	1.4	−0.1

[a] UV melting experiments with the duplexes at a concentration of $2 \mu\text{M}$ in 10 mM phosphate buffer with 500 mM NaCl at pH 7.0. [b] Sequence of the upper strand in the direction 5'→3' for DNA and in the direction 3'→2' for GNA.

duplexes, overhanging nucleotides at the 2' end lead to an exceptionally strong stabilization. For example, an extra A nucleotide at the 2' ends improves the melting temperatures of the duplex by 14.6°C with an increase in the thermodynamic stability by $4.0 \text{ kcal mol}^{-1}$. This directionality can be explained on the basis of the strong backbone–nucleobase inclination in GNA, which prevents the stacking of overhanging nucleotides at the 3' end (Figure 4b) but enables efficient stacking of extra nucleobases at the 2' end (Figure 4a). A comparison with analogous experiments with dangling residues in DNA reveals that stabilizing effects of single overhanging bases are less pronounced in DNA

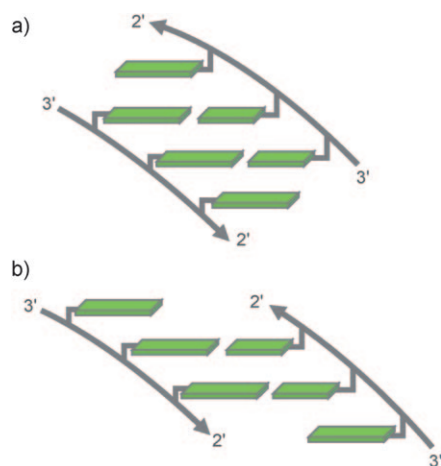


Figure 4. Expected stacking of dangling nucleotides at the a) 2' and b) 3' ends of GNA duplexes.

Keywords: biomimetic structures · duplex stability · nucleic acid analogues · supramolecular chemistry · thermodynamics

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