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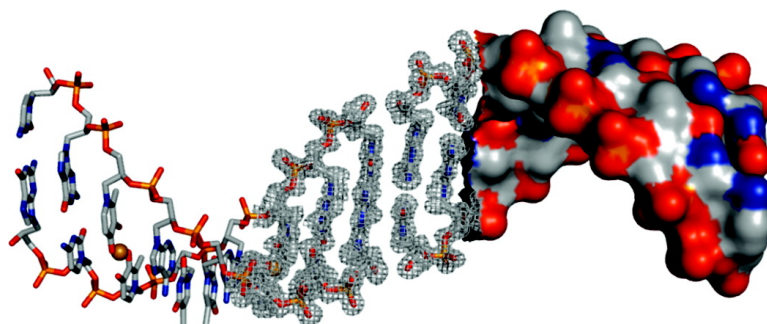
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Duplex Structure of a Minimal Nucleic Acid

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All forms of life use nucleic acids containing ribose or deoxyribose sugars as their genetic material. In order to understand the necessity for these structurally complicated pentose repeating units, researchers have investigated nucleic acids with sugar alternatives.[1–3] We recently reported a glycol nucleic acid (GNA) with an acyclic three-carbon propylene glycol phosphodiester backbone (Figure 1a).[4] GNA is structurally the most simplified solution for a phosphodiester-containing nucleic acid backbone and thus constitutes a promising candidate for initial genetic molecules of life.[5,6] In addition, due to its unique combination of high duplex stability, base pairing fidelity, and easy synthetic access of its nucleotides, GNA comprises an interesting scaffold for future nucleic acid nanotechnology.

We have now determined the crystal structure of an (*S*)-GNA duplex from the self-complementary strand 3′-CGHATHCG-2′ by X-ray crystallography. The GNA nucleotide H contains the artificial hydroxypyridone nucleobase which forms highly stable homobase pairs in the presence of copper(II) ions (see Figure 1b).[7,8] This metallo-base pairing scheme was utilized as a convenient handle to site-selectively introduce two heavy atoms per duplex for phasing the crystallograhic data.[9] In addition, we speculated that the high stability of duplexes containing such a metallo-base pairing scheme might be advantageous for growing high quality crystals. In fact, in the presence of 2 equiv of copper(II) ions, the self-complementary strand 3′-CGHATHCG-2′ forms a duplex with the astonishing high melting temperature of 78 °C (4 μM duplex, 10 mM phosphate buffer, 25 mM NaCl, pH 7.0). For comparison, the related DNA and GNA sequences 5′(3′)-CGAATTCG-3′(2′) display melting points of only 26 and 40 °C, respectively, under the same conditions.

Crystals which diffract to 1.3 Å were obtained from a chromatographically purified duplex using the sitting drop vapor diffusion method and 2-methyl-2,4-pentanediol as a precipitant. The asymmetric unit of the refined structure contains one single strand, one copper ion, and 86 water molecules (see Supporting Information for crystallographic data). Individual duplexes are coaxially stacked, thereby forming a continuous helix within the crystal (Figure 2).

The right-handed (*S*)-GNA double helix differs significantly from the canonical A- and B-form nucleic acid helices possessing a very large helical pitch of 60 Å with 16 residues per turn and a large helical rise (for a more detailed comparison with B- and A-DNA, see Table 1).[10–12] The base pairs are displaced from the helix axis (*x*-displacement) by 5.1 to 8.6 Å, resulting in a very large elliptic hollow core (Figure 2b). The GNA helix possesses only one large groove, corresponding to the canonical minor groove, whereas it lacks a major groove which is instead a convex surface. Therefore, the GNA helix structure might be best described as a helical ribbon loosely wrapped around the helix axis. With this, the GNA duplex resembles more the recently disclosed hexose containing nucleic acids HNA and homo-DNA rather than the canonical B- and A-form nucleic acids.[13,14]

All natural base pairs are engaged in standard Watson−Crick hydrogen bonding, whereas the two hydroxypyridone bases coor-
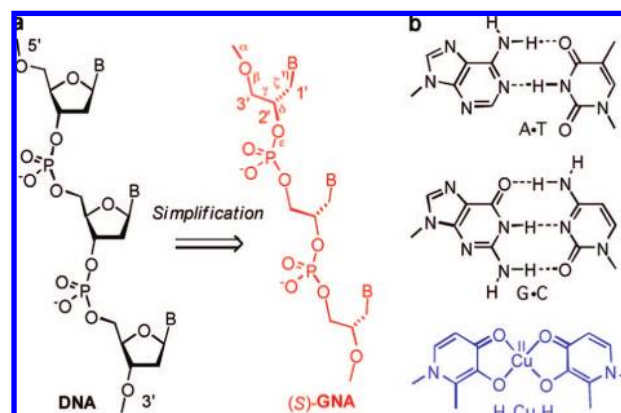

Figure 1. (a) Constitution of DNA and (*S*)-GNA oligonucleotides. (b) Comparison of Watson−Crick base pairs with the hydroxypyridone−Cu base pair used in this study.
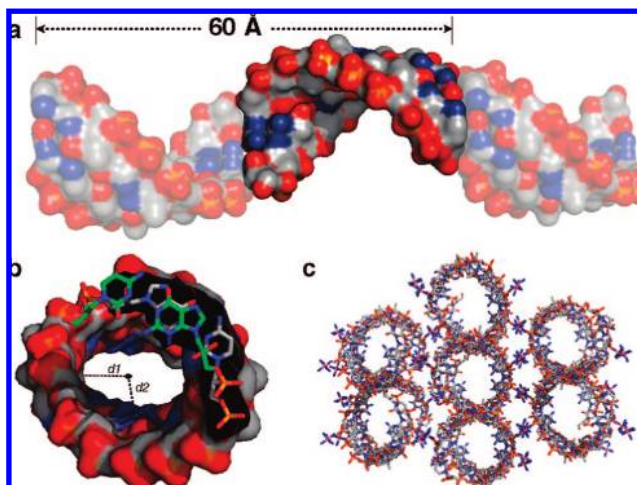

Figure 2. Overall GNA duplex structure. (a) Continuous packing of octamer duplexes along the crystallographic *z*-axis. (b) View along the *z*-axis with approximate distances *d*1 = 7.0 Å and *d*2 = 4.5 Å (defined from the helix axis to the center of the closest atom). (c) Packing of duplexes within the crystal. The structure has been deposited in the Protein Data Bank under PDB code 2JJA.

dinate to a central copper(II) ion in a square planar fashion with a slight propeller twist of 15°. The metallo-base pair appears to fit well into the overall helix structure without any major distortions even though the $C_{1'}-C_{1'}$ distance of 12.7 Å is expanded by 2.0 Å compared to the standard Watson−Crick base pairs (Figure 3).

Within the crystallized 8-mer duplex, the propylene glycol nucleotides adopt two different conformations with respect to the torsional angles between $C_{2'}-O$ and $C_{3'}-O$ (Figure 4b). Whereas nucleotides of Watson−Crick base pairs maintain a *gauche* conformation with an average torsional angle γ of 70°, the glycol at the hydroxypyridone nucleotides assume an *anti* conformation

Table 1. Comparison of Average Helical Parameters for GNA, B-DNA, and A-DNA[a]

	(S)-GNA	B-DNA	A-DNA
helical sense	right	right	right
residues per turn	16	10	12
helical pitch (Å)	60	34	34
helical rise (Å)	3.8	3.4	2.9
x-displacement (Å)	−7.0	0.1	−4.2
tilt (°)	0	−0.1	0.1
roll (°)[b]	−2.8	0.6	8.0
twist (°)[b]	22.9	36	31
slide (Å)[b]	−3.4	0.2	−1.5
P−P distance (Å)[c]	5.4	7.0	5.9

[a] Data for GNA were calculated using the program Curves (ref 12). Data for B- and A-DNA were taken from refs 10 and 11. [b] Local base pair step parameters. [c] Average intrastrand P−P distances.
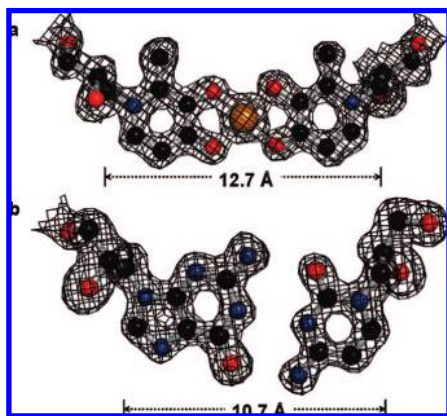

Figure 3. Electron density of the H−Cu−H base pair (a) and the terminal G−C base pair (b). The $C_{1'}-C_{1'}$ distances are indicated.
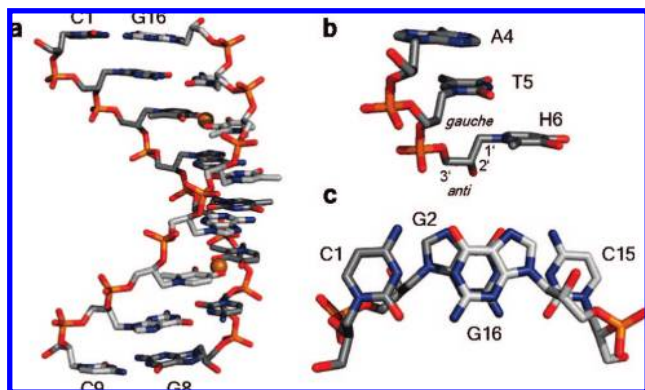

Figure 4. Details of the GNA duplex structure. (a) A single GNA octamer duplex. (b) The backbone conformation with *gauche* and *anti* referring to the torsional angle between $C_{2'}-O$ and $C_{3'}-O$. (c) Interstrand stacking of two adjacent base pairs.

(γ = 165 and 172°) (Figure 1a for annotations, Figure 4). As expected for such a simplified backbone, the distances between intrastrand phosphates are quite short, with an average of 5.4 Å, compared to around 7 Å for B-DNA and 5.9 Å for A-DNA.

Maybe the most interesting feature of this GNA duplex structure is the large average slide between neighboring base pairs of 3.4 Å (Figure 4). This is a consequence of the large backbone-base inclination, ranging for this duplex from 42 to 50° as compared to 0° for B-DNA but similar to the unnatural hexose nucleic acids.[13,14] This inclination results in an almost complete absence of intrastrand base−base stacking, which is the predominant stacking interaction in A- and B-form nucleic acids, but extensive interstrand base−base stackings. In order to compensate for the solvent-exposed base resulting from the large base pair slide, the CH_2 group of the propylene glycol backbone is participating in packing against nucleobases of the same strand. Thus, in this simplified GNA double helix, the backbone is directly involved in hydrophobic interactions with the π-system, which might contribute to the high duplex stability of GNA.

In conclusion, the here presented GNA duplex structure reveals how a minimal nucleic acid backbone can support antiparallel duplex formation in a Watson−Crick fashion. With its helical ribbon structure, the GNA double helix differs significantly from the canonical A- and B-form nucleic acids. Particularly intriguing are the extensive interstrand base−base stacking interactions and the participation of the GNA backbone in hydrophobic packing against nucleobases. Efforts to learn from this structure about the intrinsic reasons for the high duplex stability are in progress.

Supporting Information Available: Experimental details, spectroscopic data, and crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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