Communications

DNA Structures

yDNA: A New Geometry for Size-Expanded Base Pairs**

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Previous studies have explored whether it is possible to replace the information-encoding part of DNA-the bases and base pairs-with other molecular replacements, and whether such new base pairs might function in recognition and in replication processes, two of the defining characteristics of the natural genetic system.^[1-6] Many of these studies have been aimed at designing base pairs that can function within the content of the natural genetic system, for expansion of nature's genetic alphabet. However, recent studies have moved beyond the purine-pyrimidine framework of the natural system. Examples of this new approach include metal-bridged base pairs,^[4] nonpolar base pairs,^[2,4] and pairs containing more than the three different types of hydrogen bonds found in nature.^[5] We recently adopted a different strategy and described a molecular design in which the dimensions of the natural pairs are stretched by insertion of a benzene ring into the natural heterocycles, thus rendering base pairs 2.4 Å wider than the natural ones.^[6] Such broad alterations of the natural design are not necessarily expected to be compatible with the natural genetic system. Rather, such studies are useful as a test of researchers' understanding of how genetic systems function in general, and they may also lead to useful new applications in biotechnology and in selfassembling nanostructures.

Herein we evaluate whether size-expansion of DNA base pairs can be carried out using a new geometry. Our previous design of expanded DNA-like bases (xDNA) involved a linear extension of pyrimidines and purines by addition of a benzene ring to each of the four DNA base heterocycles.^[6] However, examination of models suggested that at least one other design strategy involving benzohomologation also appeared to allow for reasonable base-pair geometries and stacking with neighboring pairs. This new design, termed "yDNA" (an abbreviated form of "wide DNA"; Scheme 1) involves a different extension vector, but yields a similar degree of perturbation from the framework formed by the natural pair. Analogous designs for the other three nucleobases can be envisioned (not shown). In this initial study we

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Scheme 1. a) Structures of the free deoxyribonucleosides yA and natural A; b) proposed base-pair pattern for yA-T (left), compared with the natural A-T base pair (right). The arrow shows the extension vector for the yA base which adds about 2.4 Å to the length of natural adenine base.

tested the concept with one example, that of the expanded adenine base analogue (yA) and the corresponding deoxyriboside (dyA), both of which were previously unknown.

We developed a synthetic route to prepare the phosphoramidite derivative of the new nucleoside analogue dyA in 10 steps (6% overall yield on a gram scale, Scheme 2). Methylation of indole **1** at the 5 position was achieved by addition of three equivalents of methylmagnesium chloride to the unprotected indole **1**, followed by ring oxidation to restore aromaticity.^[7] The transformation of methylindole **3**^[8] to indole-5-carboxaldehyde **4** was a key step; tris(dimethylamino)methane converted **3** into the enamine intermediate which was then oxidized by KMnO₄ in one pot to afford compound **4**.^[9] The protected 2'-deoxyriboside **7** was formed in three subsequent steps from the indole-5-aldehyde **4**. Conventional methods were then used to convert the deoxyriboside **7** into the amidine-protected dyA phosphoramidite **11**.

Studies showed that the yA base is fluorescent, where as its natural congener is not. The yA free nucleoside was found to have absorption maxima at 262 and 355 nm in methanol. A fluorescence spectrum in methanol revealed that the nucleoside emits blue fluorescence ($\lambda_{max} = 433$ nm) with a quantum yield of 0.12 (Figure 1). Thus the yA fluorescence is redshifted by about 40 nm relative to the xA analogue.^[6]

We prepared a short oligomer of sequence 5'-TyAT-3' to confirm that dyA could be incorporated intact into a DNA by automated DNA synthesis. The expected trimer structure was confirmed by ¹H NMR spectroscopy and electron spray ionization (ESI) mass spectrometry after deprotection with concentrated ammonium hydroxide and partial purification by dialysis.

We then investigated the stacking and pairing properties of dyA in the context of natural DNA oligonucleotides. Thermodynamic data from thermal denaturation studies (Table 1) showed that yA (like xA)^[6] stacks more strongly than natural A at the terminus of the duplex formed by 5'-

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Scheme 2. Reagents and conditions: a) 1. CH₃MgCl,THF,-25 °C; 2. Pb(OAc)₄, CH₂Cl₂, 42%; b) 1. NaOH, CH₂Cl₂; 2. ClSO₂Ph, 87%; c) 1. DMF, CH(NMe₂)₃, 105 °C; 2. KMnO₄, THF/H₂O 10:1, 70%; d) Na₂S₂O₄, THF/H₂O = 2:1; e) MeCONMe₂, guanidine carbonate, 150 °C, 61% over two steps; f) 1. NaH, CH₃CN, RT; 2. Hoffer's chlorosugar, 0 °C, 92%, $\beta/\alpha > 20:1$; g) NaOMe, MeOH, 91%; h) CH(OMe)₂NMe₂, pyridine; i) DMTrCl, pyridine, DIPEA, 62% over two steps; j) chlorodiisopropyl-cyanoethylphosphoramidite, CH₂Cl₂, DIPEA, 70%. DMTr = 4,4'-dimethoxytriphenyl-methyl, DIPEA = N,N-diisopropylethylamine, Tol = tolyl.



Figure 1. Emission and excitation spectra of the free nucleoside yA in methanol.

CGCGCG-3'. This result may be a consequence of the expanded surface area and higher hydrophobicity of the new analogue. We then tested the pairing properties of yA opposite natural bases in DNA (Table 1, entries 4–15). With a single substitution in DNA, yA was found to be destabilizing to some extent, which may be a result of the distortion of the DNA backbone caused by the increased size of yA relative to A. Interestingly, when yA was paired with T, the duplex was less destabilized by 1.5–2.1 kcalmol⁻¹ than when yA was paired opposite A,G, and C. This result suggests that, despite some destabilization, yA may be able to form a selective hydrogen-bonded pair with T in the natural duplex.

Although the yA·T pair was found to be destabilizing in natural DNA, probably because of backbone distortions, we expected that such strain might be avoided if all pairs were expanded in a homologous way. Thus, further studies were performed to test whether it is possible to construct fully expanded helices composed of yA·T and T·yA base pairs. This was tested in two contexts. The first one contained a selfcomplementary sequence (Table 1, entry 16) and the second one (Table 1, entry 18) contained a non-self-complementary pair of oligonucleotides. Both assays showed distinct cooperative transition curves in thermal denaturation experiments (see Supporting Information). In the first case, the nonnatural helix displayed a higher melting temperature (T_m, by) 25°C) and more favorable free energy (by 4.1 kcalmol⁻¹ at 37°C) relative to the natural helix of the analogous sequence. Similarly, the helix formed with the second sequence gave a $T_{\rm m}$ value 23 °C higher and a free energy 4.9 kcal mol⁻¹ higher than the natural helix. We hypothesize that the added stability of these helical complexes arises from the strong stacking of the larger base pairs; further experiments will be needed to better understand this.

Circular dichroism spectra of the duplex formed in the second case (Table 1, entry 18) suggest an overall helical form resembling that of B-DNA of the analogous sequence (see Supporting Information), but with positive and negative bands red-shifted by 5–20 nm, presumably as a consequence of the extended π system of yA. Interestingly, the single component strands of the putative yDNA duplex also show similar spectra, which is indicative of strong helical stacking in the single-stranded state.

Our study has demonstrated that the new geometric design for benzo-fused bases is successful for at least one such base analogue (yA), and the data show that $yA \cdot T$ and $T \cdot yA$ pairs can self-assemble into cooperative helices that are considerably more stable than natural DNA. Thus, yDNA

Communications

Table 1: Melting temperatures and free energies for DNA helices containing yA bases and base pairs.			
	Duplex ^[a]	<i>T</i> _m [°C] ^[b]	$-\Delta G^{^{\circ}}_{_{37}}[ext{kcal mol}^{^{-1}}]^{[ext{cl}]}$
1	5 ' – ^x A C G C G C G 3 ' – G C G C G C ^x A	64.4	13.2±0.5
2	5 ' – A C G C G C G 3 ' – G C G C G C A	52.6	10.2 ± 0.1
3	5 ' – C G C G C G 3 ' – G C G C G C	43.8	$9.2\!\pm\!0.4$
4	5 ' – С Т Т Т Т С ^У А Т Т С Т Т 3 ' – С А А А А С Т А А С А А	38.7	8.8±0.1
5	5 '	29.2	6.8±0.1
6	5 ' – С Т Т Т Т С ^У А Т Т С Т Т 3 ' – С А А А А С С А А С А А	31.0	7.1±0.1
7	5 ' – С Т Т Т Т С ^У А Т Т С Т Т 3 ' – G А А А А С С А А G А А	32.2	7.3 ± 0.1
8	5 ' – C T T T T C ^y A T T C T T 3 ' – G A A A A G 🖗 A A G A A	33.9	7.7±0.1
9	5 ' –	35.2	7.9±0.1
10	5 ' – А А G А А ^X A G А А А А G 3 ' – ТТС ТТ А С ТТ ТТ С	25.9	5.8±0.1
11	5 ' – А А С А А ^Х А С А А А А С 3 ' – ТТС ТТ С С ТТТТ С	27.5	6.3±0.1
12	5 ' – А А С А А ^У А С А А А А С 3 ' – Т Т С Т Т С С Т Т Т Т С	28.0	6.4±0.1
13	5 ' – Α Α G Α Α ^X Α G Α Α Α Α G 3 ' – Τ Τ C Τ Τ	27.5	6.3±0.1
14	5 ' – А А G А А А G А А А А G 3 ' – Т Т С Т Т Т С Т Т Т Т С	41.4	9.5 ± 0.1
15	5 ' – ААСАА ^ї АСААААС 3 ' – ТТСТТ ^ї АСТТТТС	35.4	8.0±0.1
16	5 ' – ἌΤ ἌἌΤἈΤΤἈΤ 3 ' – ΤἈΤΤἈΤΤἈΆΤἈ	44.7	8.7±0.1
17	5 ' — АТ ААТАТТАТ 3 ' — ТА ТТАТААТА	19.5	4.6±0.2
18	5 ' – ĂŢ ĂĂŢŢŢĂĂŢ 3 ' – TĂ ŢŢĂĂĂŢŢĂ	44.1	9.5 ± 0.3
19	5' — АТ ААТТТААТ 3' — ТА ТТАААТТА	20.9	4.6±0.2

[a] ϕ in entries 8 and 13 denotes an abasic tetrahydrofuran site. [b] Entries 1, 2, 15, 16: the buffer contained 1 m NaCl and 10 mm Na₂HPO₄ at pH 7.0; DNA concentration: 5.0 µm; error in the T_m value: \pm 0.5 °C or less. Entries 3–14, 17, 18: buffer contained 100 mm NaCl, 10 mm MgCl₂, and 10 mm sodium 1,4-piperazinediethanesulfonate at pH 7.0; DNA concentration: 5.0 µm. Error in the T_m value: \pm 0.5 °C or less. [c] Data were obtained by averaging free energies from curve fits and a van't Hoff plot. The van't Hoff data were obtained by plotting 1/ T_m versus ln(C/4) (where C is the total oligonucleotide concentration) with data from five concentrations. Curve fits data were averaged from fits of five denaturation curves.

may be a candidate for a new genetic system, distinct from the recently described xDNA design.^[6,10] Like xDNA, it appears that the sugar–phosphate backbone in yDNA can tolerate the stretched size of bases and the altered pairing direction, while retaining the structure of the duplex. However, beyond the hypothesized base pairing, the structure of yDNA is unknown and needs to be studied. Also unknown is whether other analogously expanded bases and base pairs could be constructed; studies are underway to test these.

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