## Hybridization between "Six-Membered" Nucleic Acids: RNA as a Universal Information System

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## ABSTRACT

Within the polyA:polyT recognition system, cross-pairing between several nucleic acids with a phosphorylated six-membered carbohydrate (mimic) as repeating unit in the backbone structure has been observed. All investigated nucleic acids (except for  $\beta$ -homo-DNA) hybridize with RNA, leaving RNA as a versatile biopolymer for informational transfer.

On one hand, it is now commonly accepted that the RNAworld preceded the DNA-world. On the other hand, it is hard to believe that a  $\beta$ -D-*ribo*nucleotide, because of its structural and stereochemical complexity, was the first nucleotide formed by self-assembly of organic matter. The  $\beta$ -D*ribo*nucleotides are, most likely, themselves the end result of a stepwise constitutional metamorphosis process of nucleotide building blocks, finally leading to the selection of RNA as a biopolymer, which is able to generate life with a potential to evolve.<sup>1,2</sup> This molecular metamorphosis process has followed the rules of molecular assembly and reorganization, while retaining informational capacity.<sup>1,2</sup> This hypothetical view on life presumes that informational transfer between biopolymers to and from RNA (i.e. to DNA) is easily occurring.

In this study we investigated RNA's potential for hybridization with a series of distant related nucleic acids structures, containing a six-membered phosphorylated carbohydrate (mimic) backbone. The hybridization is studied within the simplest recognition mode (oligoadenylate-oligothymidy-late).

The modified nucleic acids that have been investigated on their base pairing properties have mostly a five-membered phosphorylated carbohydrate (mimic) as repeating unit of the backbone structure. These oligonucleotides are usually screened for cross-pairing with RNA (for antisense purposes or for information exchange with natural nucleic acids) and for self-hybridization (organization in higher molecular structures via base pairing). The conformational prerequisites of oligonucleotides with a five-membered carbohydrate (mimic) in the backbone structure for hybridization with RNA have been analyzed.<sup>3–4</sup>

Extensive information about the selectivity of hybridization of oligonucleotides with a six-membered carbohydrate (mimic) is lacking. It has been observed that potentially natural nucleic acid alternatives of the  $(6' \rightarrow 4')$  hexopyranosyl (including the  $\beta$ -homo-DNA model) and the  $(4' \rightarrow 2')$ pentopyranosyl families, where base pairing is orthogonal to that of the natural nucleic acids, do not cross pair with RNA and DNA.<sup>1</sup> In all these cases, the nucleobases are equatorially positioned on the pyranosyl chains. In cases were

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**Table 1.** Melting Points and Hyperchromicity (%) As Determined at 260 nm in 0.1 M NaCl Phosphate Buffer pH 7.5 with for Each Oligonucleotide at a Concentration of 4  $\mu$ M<sup>*a*</sup>

	β-homo-DNA	α-homo-DNA	β <b>-ΗΝΑ</b>	CNA	RNA
α-homo-DNA	36° (15 %) (2)	24° (22 %)	69° (31 %)	33° (23 %)	23° (38 %)
β-ΗΝΑ	62° (12 %)	64° (33 %)	78° (34 %)	62° (30 %)	33° (33 %)
	NS	35° (22 %)	72° (33 %)	56° (41 %)	52° (59 %)
α- <b>HNA</b> λ	49° (28 %)	NS	67° (10 %)	35° (4 %)	40° (14 %) (1)
	55° (5 %)	56° (28 %)	72° (33 %)	59° (33 %)	34° (32 %)
	NS	28° (25 %)	48° (30 %)	48° (38 %)	26° (32 %)

<sup>*a*</sup> All oligonucleotides are 13-mers. NS: complex not stable enough to be detected at 20 °C. (1) Melting point determined in 1 M NaCl (the  $T_{\rm m}$  at 0.1 M NaCl is approximately 20 °C). (2)  $T_{\rm m}$  of dissociation is 36 °C;  $T_{\rm m}$  of association is 33 °C.

the nucleobases are axially oriented on the six-membered ring, which is for example the case for hexitol-based nucleic acids,<sup>5</sup> base pairing with RNA and DNA is observed.

With equatorially oriented bases, quasilinear duplexes are formed.<sup>6</sup> With axially oriented bases, duplexes are formed that are similar in structure to natural nucleic acids helices.<sup>5</sup> However, recently we observed that intermediate structures may be formed upon hybridization of  $\alpha$ -homo-DNA with RNA.<sup>7</sup> In this case, hybrids are formed between nucleic acids having on the one strand equatorially oriented bases ( $\alpha$ homo-DNA) and on the other strand pseudoaxially oriented bases (RNA).

For further investigation of the hybridization potential of DNA with a six-membered ring in the backbone structure, we selected several dideoxy nucleic acids as examples. The selection of dideoxy nucleic acids is based on the observation that the presence of free hydroxyl groups on the sugar moiety of the nucleic acids may weaken base pairing due to steric hindrance, as was demonstrated for  $\beta$ -D-glucopyranosyl nucleic acids.<sup>8</sup> The hydroxyl groups may, likewise, be involved in intramolecular interactions and induce a conformational preorganization maladjusted for base pairing, as was demonstrated with D-mannitol nucleic acids.<sup>9</sup> By select-

ing dideoxy nucleic acid analogues, we avoid that interpretation of the results is hampered by these "secondary" effects.

In the oligothymidylate series,  $\beta$ -homo-DNA,  $\alpha$ -homo-DNA,  $\beta$ -HNA, and CNA were selected (Table 1). These oligonucleotides were available from previous research projects.<sup>7,10–12</sup> In the oligoadenylate series,  $\beta$ -homo-DNA is removed because of its very strong self-pairing properties,<sup>13</sup> and  $\alpha$ -HNA and CeNA are added (Table 1).<sup>14</sup> The synthesis of the  $\alpha$ -HNA phosphoramidite is given in Scheme 1. This amidite was used for the synthesis of a  $\alpha$ -HNA 13-mer from which a correct MS analysis was obtained. The synthesis of the nucleoside itself was previously described by following a different scheme.<sup>15</sup>

The base-pairing landscape of these artificial oligonucleotides is given in Figure 1. All oligonucleotides are used as 13-mers. Several of the associations show low hyperchromicity, suggesting the absence of intensive stacking interactions (Table 1). This low hyperchromicity is observed for the hybrids formed between  $\beta$ -homo-DNA or  $\alpha$ -HNA and all other six-membered nucleic acids (except for the  $\beta$ -homo-

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**Scheme 1.** Synthesis of the Phosphoramidite of 1,5-Anhydro-2,3-dideoxy-2-(*N*<sup>6</sup>-benzoyladenin-9-yl)-D-glucitol (the Building Block for α-HNA-synthesis)<sup>*a*</sup>



<sup>*a*</sup> (i) pNO<sub>2</sub>BzOH, Ph<sub>3</sub>P, DEAD, THF; NH<sub>3</sub> MeOH, 85%; (ii) pTosCl, pyridine; 80% HOAc, 80 °C, 52%; (iii) PivCl, pyridine, 85%; (iv) adenine, LiH, 12-crown-4, DMF, 60 °C, 25%; (v) NaOH 1N, dioxane, 60 °C, 55%; (vi) TMSiCl, pyridine, BzCl, NH<sub>4</sub>OH, 80%; (vii) MMTrCl, pyridine, 75%, (viii) (<sup>*i*</sup>Pr)<sub>2</sub>NP(Cl) (OCH<sub>2</sub>CH<sub>2</sub>CN); (<sup>*i*</sup>Pr)<sub>2</sub>NEt, CH<sub>2</sub>Cl<sub>2</sub>, 85%.

T: $\alpha$ -HNA-A association itself).<sup>16</sup> This was, likewise, observed for  $\beta$ -homo-A self-association (5–10% hyperchromicity).<sup>13</sup> Low hyperchromicity is observed when one of the pairing partners is an all-equatorial system (i.e. the three substituents on the six-membered ring are equatorially oriented). High hyperchromicity values are indicative for triple helix formation as was observed for CNA(A):RNA-(U) and CNA:CNA.<sup>12</sup>

 $\beta$ -Homo-DNA,  $\alpha$ -homo-DNA, and RNA have a 1,3 relationship between the base moiety and the phosphorylated



**Figure 1.** Melting points as determined in 0.1 M NaCl, phosphate buffer pH 7.5. The base-pairing landscape shows that the strongest pairing occurs for complexes involving  $\beta$ -HNA.



**Figure 2.** Thermal dissociation curves (temperature versus absorbance at 260 nm) in 0.1 M NaCl of RNA-A with RNA-U ( $\Rightarrow$ ), CNA-T ( $\triangle$ ),  $\alpha$ -homo-T ( $\Box$ ), and  $\beta$ -HNA-T ( $\bigcirc$ ).

hydroxymethyl group.  $\alpha$ -HNA,  $\beta$ -HNA, CNA, and CeNA have a 1,4 relationship between both functionalities. Except for  $\beta$ -homo-T:CNA,  $\beta$ -homo-T:RNA, and  $\alpha$ -homo-T: $\alpha$ -HNA, stable associations are formed; the distance between the base moiety and the hydroxymethyl group cannot be considered as a decisive factor influencing base pairing.

 $\beta$ -Homo-DNA and  $\alpha$ -HNA are all-equatorial systems. In  $\beta$ -HNA, the base is axially oriented and the two other substituents are equatorially oriented. In  $\alpha$ -homo-DNA (when pairing with RNA), the base moiety is equatorially oriented and the hydroxyl and hydroxymethyl groups are axially oriented.<sup>7</sup> The situation is less clear for CNA (both might be possible)<sup>12</sup> and for CeNA (that is a more flexible system).<sup>14</sup> As most of these nucleic acids do cross pair, base orientation seems, likewise, not to be a decisive factor for hybridization. The strongest pairing, however, is observed for complexes involving  $\beta$ -HNA.

All the six-membered oligonucleotides (except for  $\beta$ -homo-DNA) do hybridize with RNA. RNA seems to be an oligonucleotide system of excellent adaptability when considering cross-pairing with other informational pairing systems. The observation that  $\beta$ -homo-DNA does not hybridize with RNA seems to be an exception in the pairing behavior of "six-membered" artificial nucleic acids. DNA is much less versatile in its pairing behavior than RNA. No hybridization of DNA (oligo dT) at 0.1 M NaCl was observed with  $\alpha$ -homo-DNA or  $\alpha$ -HNA, and no hybridization of DNA (oligo dA) at 0.1 M NaCl was observed with  $\beta$ -homo-DNA and  $\alpha$ -homo-DNA (data not shown). RNA, the bioisosteric cyclohexene congener, and its conformationally restricted artificial congener  $\beta$ -HNA are the most universal pairing partners, the latter giving the most stable complexes (Figures 2 and 3 and Supporting Information).We realize that polyA:polyT is a weak base pairing system and

<sup>(16)</sup> The probability that  $\alpha$ -HNA might pair with  $\beta$ -homo-DNA was also predicted by A. Eschenmoser and communicated to Piet Herdewijn.



**Figure 3.** Thermal dissociation curves (temperature versus absorbance at 260 nm) in 0.1 M NaCl of RNA-U with RNA-A ( $\stackrel{()}{\propto}$ ), CNA-A ( $\stackrel{()}{\sim}$ ),  $\alpha$ -homo-A ( $\square$ ),  $\beta$ -HNA-A ( $\bigcirc$ ), and CeNA-A ( $\diamond$ ) and with  $\alpha$ -HNA-A (at 1.0 M NaCl) ( $\bigtriangledown$ ).

that the pairing landscape may be different with heterobasic systems. However, within this simple system, base pairing is possible between most six-membered oligomers, whether orthogonal to each other or not. The structure of most of these complexes has not been determined yet, but on the basis of experiments with  $\alpha$ -homo-DNA,<sup>7</sup> these results suggest that the universe of stable (supra)molecular associa-

tions of nucleic acids is more extended than what is presently described (natural nucleic acids helices and ladder-like structures). Indications for this are the different UV-spectra obtained for some of the complexes (data not shown).

In conclusion, these data confirm that the structural and conformational properties of the carbohydrate moiety have an important influence on hybridization strength. Extensive cross-pairing between oligonucleotides with a six-membered carbohydrate moiety within the polyA:polyT mode is occurring. Although none of the artificial systems studied can be considered to be a potentially natural nucleic acid alternative, the observation that RNA (and its conformational rigid analogues that mimic A-form nucleic acid duplexes) is an universal pairing partner suggests that molecular evolution to and from RNA is easily possible and that the A-form duplex is probably the most stable helical association form in the nucleic acid field.

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Supporting Information Available:  $T_{\rm m}$  curves of  $\beta$ -HNA-A, CNA-A, and CeNA-A with RNA-U, CNA-T,  $\alpha$ -homo-T, and  $\beta$ -HNA-T. Experimental section for the synthesis of protected 1,5-anhydro-2,3-dideoxy-2-( $N^6$ -benzoyl-adenin-9-yl)-D-glucitol. This material is available free of charge via the Internet at http://pubs.acs.org.

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