Hybrids of RNA and Arabinonucleic Acids (ANA and 2′F-ANA) Are Substrates of Ribonuclease H

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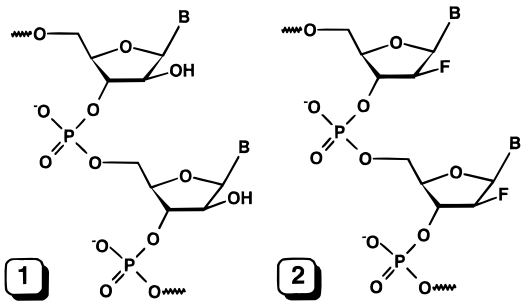
Antisense oligonucleotides (AON) have attracted intense interest and are becoming increasingly important in therapeutic strategies against a range of human diseases including cancer and infectious diseases.[1] The formation of a duplex between AON and cellular RNA prevents the translation of such RNA both by "translation arrest" and, most importantly, by activation of ribonuclease H (RNase H), an endogenous enzyme that specifically degrades the target RNA in the antisense oligonucleotide/RNA duplex.[2] The biological activity of AONs that activate RNase H is generally superior to that of AONs which do not activate this enzyme.[1a,3] Over 60 types of modified AON have been studied since 1994,[4] but none of them, apart from the DNA phosphorothioate, phosphorodithioate, and boranophosphate, have the ability to activate RNaseH when hybridized to a target RNA.[5] Although oligonucleotide phosphorothioates are performing well in clinical trials and are moving rapidly toward new drug applications (NDA), sequence-nonspecific biological effects are often encountered in the evaluation of these compounds.[6]

We[7] and others[8] have been interested in the synthesis and hybridization properties of arabinonucleic acid (ANA, **1**), the 2′-stereoisomer of RNA based on D-arabinose instead of the natural D-ribose. Pfleiderer[8] has described the chemical synthesis of a "transfer ANA" molecule, and several groups[9] have reported on the hybridization properties of duplexes incorporating one or two β-D-arabinonucleotide units. Little is known, however, about the biochemical properties of oligoarabinonucleotides of mixed-base composition. For this purpose, various monomers for solid-phase synthesis were prepared which allow for the synthesis of ANA as well as of 2′F-ANA (2′-deoxy-2′-fluoro-β-D-arabinonucleic acid, **2**).[10,12a] The behavior of these compounds with respect to their base-pairing properties with RNA, nuclease stability, and their ability to induce RNaseH activity was also evaluated.



Synthesis of ANA Strands. ANA and 2′F-ANA monomers namely, 5′-monomethoxytrityl-2′-OAc (or 2′-F)-3′-*O*-(β-cyanoethyl-*N*,*N*-diisopropylphosphoramidite) derivatives of A (N^6-Bz), C (N^4-Bz), U/T, and G (N^2-*i*-Bu, O^6-*p*-nitrophenylethyl) were prepared by variations of the published procedures.[7,8,10] Oligomers were prepared as described previously for homopolymeric ANA sequences,[7b] and characterized by PAGE and MALDI-TOF mass spectrometry.

ANA/RNA and 2′F-ANA/RNA Duplexes. First, the binding affinity of various arabinonucleic acids to their complementary RNA targets was evaluated in a buffer containing 140 mM KCl, 1 mM $MgCl_2$, 5 mM Na_2HPO_4 (pH 7.2), which is representative of intracellular conditions.[11] T_m values obtained from UV (260 nm) absorption data for these duplexes and for DNA and DNA-thioate/RNA duplexes containing the same sequence are reported in Table 1. In all cases, ANA(2′-OH)/RNA hybrids had a lower value of T_m, compared to the control DNA/RNA hybrids. This destabilization is presumed to derive from steric interference by the β-C2′-OH group, which is oriented into the major groove of the helix, causing slight local deformation (unstacking).[9] The hybrid stability of ANA/RNA duplexes also seems to be influenced by the base sequence, since ara$(Ap)_7$A/poly-rU and deoxy$(Ap)_7$A/poly-rU exhibit similar thermal stability, whereas ara$(Up)_7$U/poly-rA is unstable.[7b] Replacing the ara-2′-OH group by a 2′-F atom resulted in a marked increase in duplex melting temperature (Table 1). For example, the 2′F-ANA (**XII**)/RNA duplex had a higher value of T_m (65 °C) compared to the corresponding hybrids formed by ANA **XIII** (32 °C), thioate S-DNA **XIV** (38 °C), and DNA **XV** (51 °C). The significantly higher value of T_m for 2′F-ANA/RNA relative to that for ANA/RNA hybrids may reflect reduced steric interactions of fluorine atoms (versus OH groups), whereas the higher value of T_m of 2′F-ANA/RNA relative to that of DNA/RNA hybrids may result from a higher pre-organization state of 2′F-ANA relative to DNA (a β-2′-F atom is expected to stabilize the C2′-endo sugar pucker).[12]

The circular dichroic (CD) spectra of ANA (**I**)/RNA and 2′F-ANA (**IV**)/RNA (140 mM KCl, 1 mM $MgCl_2$, 5 mM Na_2HPO_4, pH 7.2) closely resembles that of the corresponding DNA/RNA hybrids, suggesting that they share the same helical conformation (see Supporting Information). The spectral features observed are characteristic of an A-like helix,[13] a structure that appears to be important in the recognition of DNA/RNA substrates by RNaseH.[14]

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Table 1. Melting Temperatures (T_m) for the Arabinonucleic Acid Duplexes and Control DNA/RNA and S-DNA/RNA Duplexes[a]

sequence and backbone type		T_m, °C (RNA target)
(i) AGC UCC CAG GCU CAG AUC		
I	ANA	44
II	S-DNA	63
III	DNA	72
(ii) TTT TTT TTT TTT TTT TTT		
IV	2′F-ANA	44
V	ANA	*c*
VI	S-DNA	21
VII	DNA	39
(iii) AAA AAA AAA AAA AAA AAA		
VIII	2′F-ANA	30
IX	ANA	26
X	S-DNA	∼33[d]
XI	DNA	25
(iv) TTA TAT TTT TTC TTT CCC		
XII	2′F-ANA	65
XIII	ANA[b]	32
XIV	S-DNA	38
XV	DNA	51

[a] Aqueous solutions 2.8×10^{-6} M in each nucleotide, 140 mM KCl, 1 M $MgCl_2$, 5 mM Na_2HPO_4 buffer (pH 7.2). [b] ANA strand contained uracil instead of thymine. [c] Not observed. [d] Very broad transition.

RNaseH Assays. The similar helical conformations of ANA (2′F, or 2′OH)/RNA and DNA/RNA hybrids prompted us to compare their susceptibility to cleavage by HIV-1 RT-associated RNaseH and *Escherichia coli* RNaseH. The latter enzyme is the most readily available and the best-characterized and exhibits very similar cleavage properties to the eukaryotic enzyme.[15,1] Indeed, both HIV-RT and *E. coli* RNaseH were able to degrade hybrids of arabinonucleic acids and RNA (see Figure 1, and Supporting Information). As expected, neither the RNA/RNA, 2′F-RNA/RNA, or the RNA alone served as substrate of RNaseH.[14] Moreover, *E. coli* RNaseH had a higher capacity for degrading the RNA component of the hybrids than does HIV-RT RNaseH, and higher RNaseH induction occurred with Mn^{2+} than with Mg^{2+} as cofactor.[2b] To our knowledge, there are no previous examples of RNaseH induction resulting from association of RNA with a *uniformly sugar-modified* AON. Also significant is the finding that the susceptibility of the 2′F-ANA/RNA hybrids to RNaseH cleavage (*E. coli*) is similar to that observed for DNA/RNA and DNA-thioate/RNA hybrids (Figure 1). ANA (2′OH)/RNA hybrids (e.g., ANA **I**/RNA, Supporting Information) were the least susceptible to cleavage, which can be attributed to the lower thermal stability of these duplexes (i.e., less duplex present). The ability of RNaseH to degrade RNA in ANA/RNA hybrids (2′F or 2′OH) may result from (a) the similarity of structure of these hybrids to that of the normal DNA/RNA substrate and (b) the fact that the 2′-OH or F substituents of the arabinose sugar ring projects into the major groove of the helix,[9] at a site where it should not interfere with the binding and catalytic processes of RNaseH.[16] Preliminary results show that arabinonucleic acids exhibit superior nuclease-resistance to serum and cellular nucleases compared to DNA strands (see also refs 7b and 10), although less than phosphorothioate S-DNA. However, unlike

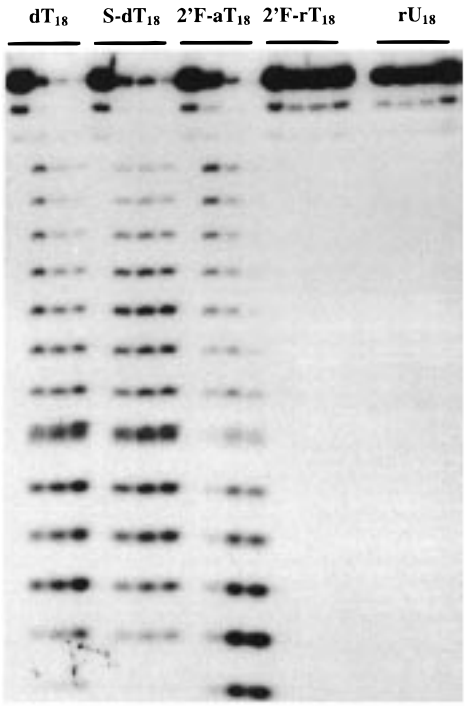

Figure 1. PAGE gel showing RNaseH mediated cleavage of duplexes. 1 pmol of target 5′-[^{32}P]-rA_{18} and 8 pmol of test AON in 60 mM Tris-HCl (pH 7.8) containing 2mM dithiothreitol, 60 mM KCl, and 2.5 mM $MgCl_2$. Reactions were started by the addition of *E. coli* RNaseH (22 °C; lanes: 0, 10, 20, 30 min).

S-DNA, arabinonucleic acids show little nonspecific binding to cellular proteins (data not shown), a property that may result in a significantly improved interaction with cellular RNA in vivo. These combined properties establish that arabinonucleic acids may serve as excellent models of antisense agents and as valuable tools for studying and controlling gene expression in cells and organisms. The 2′-arabino modification may also prove useful in other applications such as enhancing the RNA hybridization properties (affinity) of S-DNA drugs while retaining the ability to elicit RNaseH activity. These and other applications such as the synthesis of L-/D-ANA chimera[17] to enhance nuclease resistance are being investigated in our laboratories.

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Supporting Information Available: CD spectra, melting curves, and PAGE gels of RNaseH-mediated cleavage of arabinonucleic acid duplexes (9 pages, print/PDF). See any current masthead page of ordering information and Web access instructions.

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