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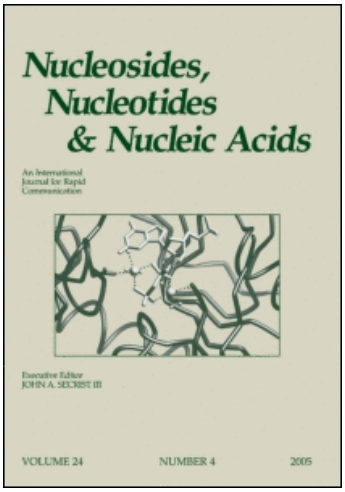

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BINDING OF CHEMICALLY-MODIFIED OLIGONUCLEOTIDES TO THE DOUBLE-STRANDED STEM OF AN RNA HAIRPIN.

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ABSTRACT : We monitored the binding of triplex-forming oligopyrimidines to the double-stranded stem of the RNA hairpin responsible for the *gag-pol* frameshift in HIV-1. Whereas the substitution of 5,propynyl-C for C had a limited effect, the use of a Peptide Nucleic Acid 12mer led to a drastic reduction in the stability of the oligomer/RNA complex.

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In HIV, the translation of the polycistronic message results in the synthesis of a polyprotein which is further processed by a protease to give rise to the viral proteins and enzymes. As the *gag* and *pol* genes are in a different frame the synthesis of the polyprotein requires a frame-shift event before the ribosome reaches the stop codon of the upstream *(gag)* gene in order for the downstream *(pol)* gene to be translated [1]. Ribosomal frame-shifting is triggered by a heptanucleotide sequence located upstream of a hairpin structure [2]. It is assumed that this RNA structure causes the ribosome stalling, increasing the probability of -1 frame-shifting on the heptanucleotide slippery sequence. This is a very controlled event with a low occurence (5-10 %) which tunes the relative production of *gag* and *pol* products [3]. It is likely that any ligand which would perturb the ribosomal frame-shifting efficiency would have antiviral properties.

The *gag-pol* frame-shifting RNA structure is a 4nt loop and a 12 bp stem hairpin. The regions on each side of the stem show some complementarity which might extend the hairpin structure under some conditions (Fig. 1). The 5'strand of the stem is purine-rich,

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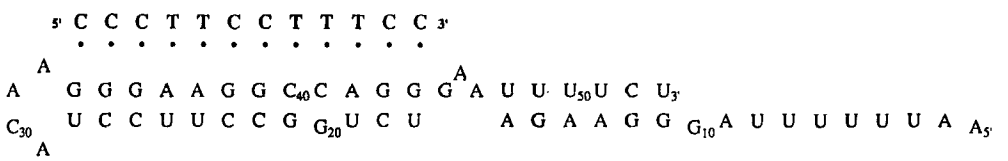

Figure 1 : HIV-1 RNA sequences of the gag-pol hairpin and of the pyrimidine triplex-forming oligonucleotide 12Py. Modified analogues containing 5, propynyl, cytosine (12PyPrC) or with peptide nucleic acid backbone (12PyPNA) were also synthesized.

making this double-stranded region a reasonable target for triplex-forming oligonucleotides (ODNs) [4]. Such ODNs will have to read two consecutive inverted G-C pairs in the lower part of the stem. We investigated the interaction of dodecamer oligopyrimidines (in which Ts were introduced to read the inverted GCs) with the RNA fragment, 53 nt long, corresponding to the *gag-pol* hairpin of HIV-1 (Fig. 1). The triplex forming ODNs were synthesized either unmodified (12Py) or with 5, propynyl,cytosine (PrC) residues. In addition the peptide nucleic acid (PNA) homologue of 12Py was also tested. These modifications are known to modulate the stability of triple-stranded DNA structures [5, 6].

We first monitored the binding of these 12mers to the RNA hairpin by electrophoretic mobility shift assays, at 4°C in a 50 mM Tris acetate buffer (pH 7.0) containing 10 mM magnesium acetate. The addition of the oligomers to the ^{32}P 5' end-labelled RNA resulted in the appearance of a low mobility species which can be ascribed to RNA-ODN complexes (not shown). Titrations were performed with the three different 12 mers. The dissociation constants were evaluated to be 1-5 nM for 12 Py and its PrC-containing derivative. In contrast 12PyPNA was a poor ligand of the RNA hairpin with a Kd of about 250 nM.

We then performed RNase V1 footprinting experiments to compare the behavior of 12Py and 12PyPNA with respect to the target RNA hairpin. RNase V1 which is an endonuclease specific for double-stranded regions yields major products corresponding to the cleavage of the ^{32}P 5'end-labelled RNA in the region C_{23}-C_{25} (Fig. 2). Minor fragments corresponding to cleavage at G_{12}-G_{15} and C_{18}-G_{20} were also observed. The

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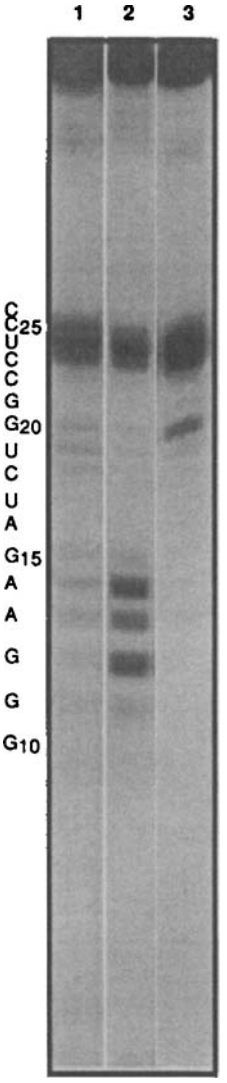

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Figure 2 : Pattern of RNase V1 digestion of the HIV-1 gag-pol RNA hairpin alone (lane 1) or in the presence of the oligonucleotide 12Py (lane 2) or 12PyPNA (lane 3). Labelled RNA (0.02 pmole) and oligonucleotides (10 μM) were mixed in 50 mM Tris acetate buffer adjusted at pH 7.0, containing 10 mM magnesium acetate and 10 mM potassium acetate. The mixture was heated for 10 min at 65°C and then let 3 h at room temperature. Yeast tRNA and RNase V1 (10^{-2} U) of enzyme per μg were added. The digestion was performed for 5 min at room temperature and quenched on dry ice. The samples were then extracted with a phenol/chloroform/isoamyl alcohol (50:49:1) mix before ethanol precipitation. Samples were then loaded on a 20 % polyacrylamide gel containing 7 M urea in TBE buffer. Electrophoresis was run at 50 V/cm and gels were analyzed by autoradiography.

addition of 12Py induced a protection of the former regions (C_{23}-C_{25} and C_{18}-G_{20}), *i.e.* at the expected binding site of 12Py and an enhanced sensitivity of the latter one (G_{12}-A_{14}). No such effects were seen with 12Py PNA. In contrast, increased cleavage was observed at G_{20} and C_{23}-C_{25} indicative of a different structure.

Therefore we demonstrated that an oligodeoxypyrimidine was able to bind to a double-stranded RNA region and that a PNA analogue was not a good alternative ligand. However the oligonucleotide 12Py did not induce any effect on the translation of a mRNA in which the HIV hairpin was inserted in front of a reporter gene (not shown), in contrast to what was observed for the *gag-pro* frame-shift hairpin of HTLV-I (Le Tinévez, Chabas and Toulmé, unpublished).

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