PHOSPHOROTHIOATE OLIGONUCLEOTIDES AS APTAMERS OF RETROVIRAL REVERSE TRANSCRIPTASES

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1. Introduction

Retroviral reverse transcriptases catalyze the synthesis of a double-stranded DNA copy of the RNA genome for integration into host chromosome. These enzymes possess three enzymatic activities essential for retrovirus replication: an RNA-dependent DNA polymerase, a DNA-dependent DNA polymerase which synthesizes the second strand of the proviral DNA, and an RNase H which degrades RNA template after the synthesis of the first strand of the proviral DNA. The importance of reverse transcriptase (RT) in the life cycle of retroviruses (e.g. HIV) makes the enzyme a preferred target for antiviral strategies [1].

Among many agents which are being considered to inhibit reverse transcriptase activity there are phosphorothioate analogues of oligonucleotides (PS-oligos) [2-6]. They can influence the RT activity by oligonucleotide sequence-independent [2, 3] and/or sequence-dependent mechanism [4-6]. A proposed mechanism for sequence-dependent antiviral activity of PS-oligos involves their hybridization to viral RNA and formation of substrates for RNase H associated with RT enzyme. This ribonuclease cleaves RNA fragments involved in the formation of RNA/PS-oligos duplexes and inhibits full-length cDNA synthesis [4-6]. For explanation of sequence-independent antiviral effect, the direct interaction of PS-oligos with reverse transcriptase has been postulated [3]. Phosphorothioate oligonucleotides bind to the enzyme with high affinity and, therefore, can competitively inhibit the synthesis of cDNA. This increased affinity is probably a result of the fact that, at least for some proteins, the dissociation rate of the PS-oligo/protein complex is much lower than that for the corresponding PO-oligo [7]. It has been suggested that the sequence-independent inhibitory effect of PS-oligos relies upon the total number of internucleotide phosphorothioate linkages rather than the oligonucleotide length or the position of modified bonds within the oligomer [8]. The sequence-independent inhibition by PS-oligos has been reported not only for reverse transcriptase but also for human DNA polymerases and human RNase H [8]. It should be noticed that in these studies PS-oligos have been used as mixtures of $2^n$ diastereomers, where $n$ is a number of internucleotide phosphorothioate linkages.

Our earlier results have indicated that the direct binding of PS-oligos to the RT protein and their inhibitory effect against the enzyme can depend upon a sequence of oligonucleotide as well as upon the absolute configuration at P-atoms of internucleotide phosphorothioate bonds. We have demonstrated that the stereoregular PS-oligonucleotide d[AAG CAT ACG GGG TGT] containing phosphorothioate internucleotide functions of [Rp]-configuration effectively inhibits the AMV RT although this oligomer is not complementary to the RNA template and, therefore, cannot activate RNase H. We have suggested sequence-dependent inhibition by PS-oligos.
aptameric interaction of this oligonucleotide with the AMV RT enzyme [9]. The sequence-selective mode of RT inhibition by PS-oligonucleotides was earlier postulated also by Tamura et al. [10].

In this report we present a more detailed data on the sequence-dependent inhibition of AMV and HIV reverse transcriptases by stereoregular PS-oligos. We have examined several oligonucleotides of different nucleotide sequences. Some of them contain contiguous four G bases which can be responsible under in vitro conditions for the formation of tetraplex structures of oligonucleotides [11].

2. Inhibition of AMV Reverse Transcriptase

The oligonucleotides listed in Table I were used for the studies on the inhibition of retroviral reverse transcriptases by PS-oligos. Polyribonucleotide PO-6 (475 nt) used as a template for RT enzymes was obtained by in vitro transcription using plasmid PTI-7 containing the interleukin-2 (II-2) gene [9, 12]. Because the template PO-6 does not contain any fragment complementary to the oligonucleotides 1-4, the presence of these oligomers in the reaction mixture allowed us to test their ability to block the reverse transcription only by binding to the RT enzyme. At the first stage of our studies we used as potential inhibitors of the AMV RT the oligonucleotide d[T]19 (PO-1) and its phosphorothioate analogues (pS-la-c), while unmodified oligomer (PO-5) of the sequence d[AAA GGT AAT CCA TCT GTT CA] was used as a primer for the enzyme. Inhibitory effect of the oligonucleotides 1 was studied at their concentration ranging from 65 nM to 1.2 μM. Reverse transcription of RNA template (PO-6) catalyzed by AMV RT (used at 5 nM concentration) gave the predicted cDNA fragment of 437 nucleotides. However, PAGE analysis of resulting products indicated that the intensity of the band corresponding to this full-length product was strongly influenced by the presence of some phosphorothioate oligonucleotides in the reaction mixture. The phosphorothioate analogues of the oligomer 1 (1a-c) caused 50% inhibition of the AMV RT activity at the 400 nM concentration, independently of the absolute configuration at the phosphorus atoms of their phosphorothioate internucleotide functions.

Phosphorothioate analogues of the oligonucleotides 2 and 3, especially their [all-Rp]-isomers, appeared to be much stronger inhibitors of the AMV RT activity than the oligonucleotides 1a-c. The oligonucleotides 2a and 3a have inhibited the enzyme with IC₅₀ (50% of inhibition) at conc. 20-30 nM. Under the same conditions (30 nM conc. of the oligonucleotide) [all-Sp]-isomers did not show any inhibitory effect. The increase of their concentration to 130 nM gave 90% inhibition of the RT activity (Figs. 1 and 2). Under the same conditions unmodified phosphodiester oligonucleotides PO-2 and PO-3 did not cause significant inhibition of the enzyme activity. This observation allows to suggest that the presence of several phosphorothioate linkages within oligonucleotide chain is a prerequisite for high affinity binding of PS-oligonucleotides to the RT protein. This is in agreement with observation of Gao et al. that chimeric PO/PS oligonucleotides containing 18 or 27 phosphorothioate linkages were stronger inhibitors of human DNA polymerases than the oligomers containing only 9 phosphorothioate linkages [8].