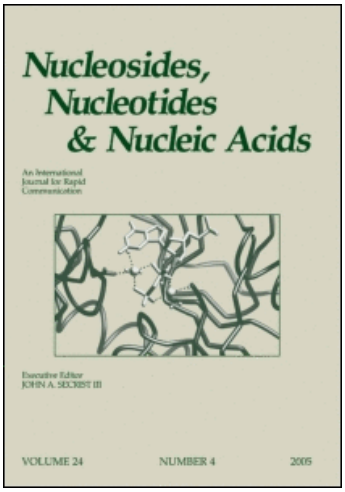

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Properties and Anti-HIV Activity of Circular Sense and Antisense Oligonucleotides

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PROPERTIES AND ANTI-HIV ACTIVITY OF CIRCULAR SENSE AND ANTISENSE OLIGONUCLEOTIDES

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ABSTRACT: The circularization of a 48 mer oligonucleotide was investigated by using enzymatic ligation with T4 DNA ligase. This method yielded 30% circular molecules. The circular oligonucleotides are molecules with no free ends and are therefore more resistant to exonuclease attack. The circular oligonucleotide has remarkably increased Tm values as compared to the nicked and double stranded DNAs. However, the RNase H activity is lower than that with a single stranded DNA. We also describe the anti-HIV activity of a circular oligonucleotide.

INTRODUCTION

The therapeutic use of the oligonucleotides as sense and antisense agents poses several problems, including the problem of molecular stability. Antisense oligonucleotides undergo nucleolytic degradation and are mainly sensitive to exonucleases. The first generation of antisense oligonucleotides, including phosphorothioates, has been used to inhibit viral as well as cellular gene expression. However, phosphorothioate oligonucleotides are eventually degraded, primarily from the 3'-end. Recently, several workers have proposed stabilization methods, including the circularization of the oligonucleotides via the 3'- and 5'-ends.[1,2] A major interest in circular oligonucleotides concerns their use as antisense compounds, due to their increased resistance to degradation by cellular exonucleases. In this paper, we describe the characterization and anti-HIV activity of circular sense and antisense oligonucleotides.

EXPERIMENTAL

The linear oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer, Model 392. The 5'-phosphorylated oligonucleotides were synthesized using 5'-phosphate on cyanoethyl phosphoramidite as the phosphorylating agent. The oligonucleotide derivatives were purified by polyacrylamide gel electrophoresis. Ligated oligonucleotide dumbbells (Figure 1) were obtained by ligation of the corresponding 5'-phosphate oligonucleotides with T4 DNA ligase.[2] The identity of the ligated dumbbells was verified by incubation with phosphodiesterase protection mapping.

Thermal denaturation profiles

Thermal transitions were recorded at 260 nm using a Shimadzu UV-2200A spectrometer. The insulated cell compartment was warmed from 25°C to 90°C, with increments of 1°C and equilibration for 1 min after attaining each temperature, using a temperature controller SPR-8 (Shimadzu). Samples were heated in masked, 1 cm path length quartz cuvettes fitted with Teflon stoppers. Each thermal denaturation was performed in 10 mM sodium phosphate buffer (pH 7.0) and 10 mM NaCl, containing 1 μM of each strand. The mixture of duplex and single strands was kept at 90°C for 10 min, and then cooled to 4°C.

Nuclease sensitivity of circular oligonucleotides

Each oligonucleotide (0.2 OD unit) was incubated with 200 μl of culture medium containing 10% fetal bovine serum for 16 h at 37°C. An aliquot (20 μl) was removed, and extracted with phenol-chloroform. The samples were analyzed by electrophoresis on a 20% polyacrylamide gel containing 8 M urea.

RESULT AND DISCUSSIONS

The nuclease sensitivities of nicked (nick-1, nick-1(S)), circular (circular-1), and antisense oligonucleotides were studied (Figure 1). Two enzymes, snake venom phosphodiesterase (SVPD) and nuclease S1 were used in comparative digestion studies of the nicked and circular oligonucleotides. The antisense oligonucleotide was digested extensively by SVPD within 43 min, whereas the nick-1, -1(S), and circular-1 oligonucleotides were more resistant. The nick-1, -1(S), and circular-1 oligonucleotides were more stable than the antisense oligonucleotide. The endonuclease activity of S1 digested the antisense oligonucleotide to mononucleotides in 30 min, whereas the nick-1, -1(S), and circular-1 oligonucleotides were very slowly digested. Similar results were obtained when the antisense, nick-1, and circular-1 oligonucleotides were studied for their nuclease sensitivity in fetal bovine serum (Figure 2). The antisense oligonucleotide was digested extensively, whereas the nick-1 and circular-1 oligonucleotides were digested slowly.

The thermal stability of the base-stacked circular-1 molecule was compared with that of nick-1, -1(S), and double stranded DNA (Table 1). The nick-1 oligonucleotide has a Tm=47°C, whereas the circular-1 oligonucleotide gives an estimated Tm=73°C, an increase of 26°C. However, the melting temperature of the nick-1 oligonucleotide was 7°C higher than the Tm of the double stranded DNA. These results suggest that the increase in the

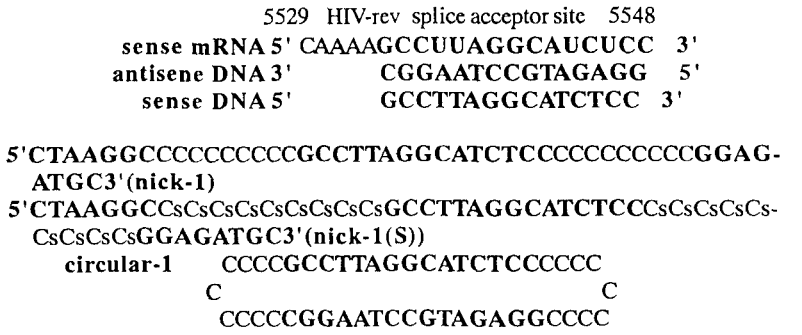

Figure 1. The structure and sequence of the oligonucleotides used in this study, as described in the text.

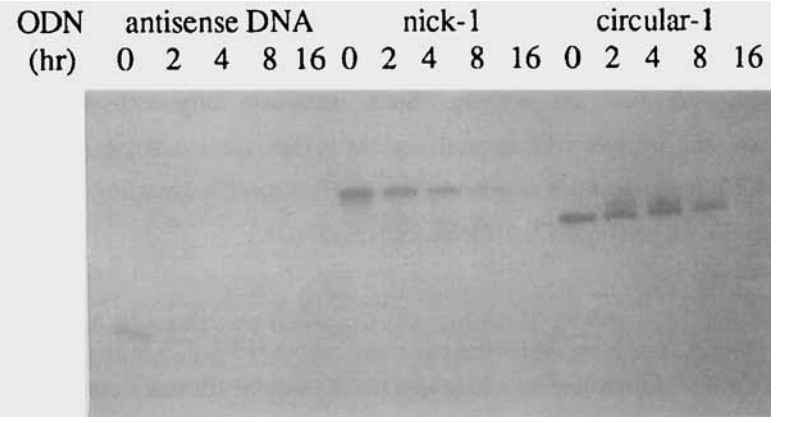

Figure 2. Digestion of the antisense, nick-1, and circular-1 oligonucleotides in the presence of 10% calf serum at 37°C for 16 h.

Table 1. Melting temperature of oligonucleotides.

Sequences	Tm (°C)[a] -RNA	+RNA
antisense DNA	-	55
double stranded DNA	40	41, 53
nick-1	47	51
nick-1(S)	48	50
circular-1	73	55, 73

a) Values were obtained in 10mM sodium phosphate buffer and 10mM NaCl at pH 7.0.

stability depended on the circularization of the oligonucleotide containing the 3'- and 5'-ends in the hairpin loop region. However, the binding of circular oligonucleotides to complementary nucleic acids suffers from interference. The circular-1 DNA only partially bound to the complementary RNA. This result was also confirmed by cleavage of the RNA at specific sites in the presence of RNase H, and by a mobility shift assay.

Finally, we analyzed the anti-HIV activity. The nick-1 (S) oligonucleotide containing phosphorothioate bonds was active, causing more than 75% inhibition at 2 μM, without cytotoxicity, in acute HIV-1 infected cells[3], but the circular-1 and nick-1 oligonucleotides showed no activity. Such antisense oligonucleotides may be multifunctional and interact with several regions which are not adjacent to the target mRNA. These oligonucleotides may be used to interact specifically which protein factors having an affinity for certain RNA or DNA sequences.

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