Review article

4'-THIODNA: UNEXPECTED BEHAVIOUR

IJCRR
Section: Healthcare
Sci. Journal
Impact Factor
4.016

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ABSTRACT

This viewpoint briefly discusses the impact of 4'-thiosubstitution on oligonucleotides behaviour. The study in the reviewed articles has shown unexpected A-form formation, and unexpected RNA-like behaviour of the 4'-thioDNA. This has been confirmed by the unexpected interaction of 4'-thioDNA with Lividomycin A, a RNA major groove binder, and by resistance to cleavage by DNase I, which is a DNA-specific endonuclease. 4'-ThioDNAwas also recognized by RNase V1 which is a RNA-specific endonuclease. These all findings will trigger the main focus of this viewpoint.

Key Words: Nucleic acids, ThioDNAs, 4'-ThioDNAs Structure and Properties

INTRODUCTION

Irrational unpredictable changes may lead to dramatic effect on people, nature and the world. Changes can be political, environmental, personal or chemical. What chemical changes can do to the behaviour of certain molecules? This leads me to ask the following questions: What a dramatic change will the replacement of oxygen atom by sulphur have on oligonucleotide structure and properties? Shall it really change the structural behaviour of those thio-oligonucleotides (thioONs)? Will they really exhibit any resistance to enzymatic cleavage? In this viewpoint, I will try briefly to answer the above mentioned questions through the discussion of the findings of the two papers on 4'-thioDNAs that were published by both Matsuda [1] and Katahira [2] groups.

DISCUSSION

Matsuda group researchers were working on developing 4'-thionucleic acids as functional oligonucleotides. These oligonucleotides consist of 4'-thionucleosides, a sugar-modified nucleoside analogue, as building blocks (Figure 1) [1, 3, 4]. Although there have been investigations by Walker and his co-workers on the synthesis and properties of 4'-thioDNA (Figure 2) [5], the ONs prepared on those studies were only partially modified with 2'-deoxy-4'-thiopyrimidine nucleosides [6-9]. However, the preliminary results have shown high hybridization to the complementary RNA and promising endonuclease (nuclease S1) resistance [8].Matsuda group carried out more investigations on 4'-thioDNAs, oligonucleotides batch 1, ONs1 (DNA1, thioDNA1 and RNA1) followed by a series of the complementary oligonucleotides batch 2, ONs2 (DNA2, thioDNA2 and RNA2) (Figure 3) [1].

Ultraviolet melting experiments were used to measure the thermal stabilities of the complementary duplexes. The homo duplex of thioDNA1:thioDNA2 showed a higher T_m value (65.2 ± 0.2_C) than that of DNA1:DNA2 and it was similar to that of RNA1:RNA2. The hetero duplex RNA1:DNA2 (T_m ¼ 51.6 ± 0.2_C) was less thermally stable than the corresponding homo duplexes (DNA1:DNA2 and RNA1:RNA2). When RNA1 was changed to thioDNA1, the corresponding T_m value was nearly the same (thioDNA1:DNA2 ¼ 48.3 ± 0.2_C) as that of the RNA1:DNA2 hetero duplex. In contrast, thioDNA1 formed a thermally stable duplex with RNA2 (thioDNA1:RNA2) to give a T_m value of 64.6 ± 0.2_C, which is similar to that of RNA1:RNA2. These results made us to believe that 4'-thioDNA may behave as an RNA-like molecule despite the absence of the 2'-hydroxyl groups in the sugar moiety.

To further confirm this speculation, CD spectra of each duplex were measured. The duplex DNA1:DNA2 showed a typical B-form spectrum (having a positive band near 280 nm), while that of RNA1:RNA2 showed a typical A-form spectrum (having a positive band near 260 nm). The CD spectrum of thioDNA1:thioDNA2 had a positive band near 260 nm though a small shoulder was observed near 280 nm, and thus A-form of the duplex was suggested. Additional confirmation of the RNA-like behaviour of 4'-thioDNA

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Received: 12.11.2015 **Revised:** 13.12.2015 **Accepted:** 14.01.2016

has been done by Matsuda group. They prepared a series of oligonucleotides batch 3 (ONs3) consisting of a self-complementary Dickerson sequence (DNA3, thioDNA3 and the corresponding RNA3). The thioDNA3, as the case for RNA3, has formed more thermally stable self-complementary duplex than DNA3, and the resulting duplex showed a preference for interacting with the RNA groove binder, lividomycin. The CD spectrum of thioDNA3 has closely resembled that of RNA3 showing an A-form structure.

Katahira group has further studied the A-form structure of the 4'-thioDNA by NMR. DNA usually takes on the B-form in solution, although a structural change to the A-form occurs under dehydrating conditions [10, 11]. Structure determination by means of NMR has shown that the fully modified 4'-thioDNA unexpectedly takes on the A-form in the solution of moderate salt and neutral pH conditions. The B-form of DNA is stabilized through interactions with hydration spines located in the minor groove [10, 12]. This is due to the involvement of the O4' atoms of 2'-deoxynucleosides in these interactions through a series of hydrogen bonds, and thus contribute to the stabilization of the B-form. Because the 2'-deoxy-4'-thionucleosides S4' atoms are more hydrophobic than the 2'-deoxynucleosides O4' atoms, the hydration spines essential for stabilization of the B-form would not be formed for fully modified 4'-thioDNA, which could account for the resultant formation of the A-form in solution.

The nuclease sensitivity of 4'-thioDNA was also examined using DNase I, an endonuclease hydrolysing single- and double- stranded DNA, SVPD and 90% human serum. The DNA1 and thioDNA1 were labelled at the 50 mers end with ^{32}P and incubated under appropriate conditions in the presence of nuclease or serum. The reactions were then analysed by PAGE under denaturing conditions, and the calculation of resulting half-lives ($t_{1/2}$ s) was based on the ratio of full-length ON at each time point. The single-stranded thioDNA1 was completely intact in the presence of DNase I for up to 12 h under conditions in which DNA1 was readily hydrolysed ($t_{1/2}$ ¼ 1.5 ± 0.1 min), and these results agreed with those previously reported for nuclease S1 [8].In addition, the thioDNA1:thioDNA2 duplex was also intact in the presence of the same enzyme, while the DNA1:DNA2 duplex afforded a $t_{1/2}$ of 3.3 ± 1.2 min. For sensitivity to SVPD, the thioDNA1 was slowly hydrolysed by SVPD ($t_{1/2}$ ¼ >8 h) under conditions in which the DNA1 was readily hydrolysed ($t_{1/2}$ ¼ 2.8 ± 0.4 min). On further investigation of sensitivity in 90% human serum, the thioDNA1 was hydrolysed by the 30-exonuclease [13] and $t_{1/2}$ was 190 ± 12 min. Under the same conditions, the DNA1 afforded a $t_{1/2}$ of 40 ± 1.3 min. From these results we can conclude that the 4'-thioDNA showed significant resistance to both endonucleases and exonucleases.

CONCLUSION

On the basis of the defined structure, I can say that the remarkable properties reported for the fully modified 4'-thioDNA is supported by the following findings:

- ThioDNA exhibits a CD spectrum characteristic of the A-form [14] although DNA usually gives a CD spectrum characteristic of the B-form.
- The fully modified 4'-thioDNA unexpectedly interacts with lividomycin-A, resulting in an increase in thermal stability [1]. Lividomycin A is known to be an RNA major groove binder. In general, RNA usually takes on the A-form, and 4'-thioDNA was shown to take on the A-form.
- The fully modified 4'-thioDNA has shown resistance to cleavage by DNase I, which is a DNA-specific endonuclease [1].This result is consistent with the earlier report by Walker and collaborators [6, 8].
- The fully modified 4'-thioDNA is recognized by RNase V1 [1], which is an RNA-specific endonuclease [14].

ACKNOWLEDGEMENT

Author acknowledges the immense help received from the scholars whose articles are cited and included in references of this manuscript. The author is also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

ABBREVIATIONS

RNA= ribonucleic acid/ **DNA**= deoxyribonucleic acid/ **ONs**= oligonucleotides/ **SVPD**= snake venom phosphodiesterase/ **CD**= *Circular dichroism/* ***PAGE***= Polyacrylamide gel electrophoresis

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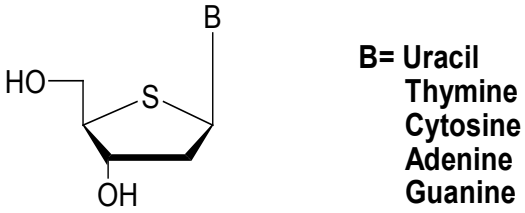

Figure 1: Structure of 2'-deoxy-4'thionucleosides.

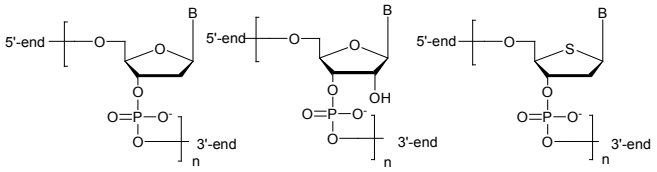

Figure 2: Comarison of the structures od DNA, RNA, and 4'-ThioDNA.

5'-d(AGTCCGAATTCACGT)-3': DNA 1 and THIODNA1
3'-d(TCAGGCTTAAGTGCA)-5': DNA2 and THIODNA2
5'-r (AGUCCGAAUUCACGU)-3': RNA 1
3'-r (UCAGGCUUAAGUGCA)-5': RNA 2

Figure 3: Sequences of DNA, 4'-thioDNA and RNA.