

Thermostability of Modified Oligonucleotide Duplexes

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We are often asked about the relative thermostabilities of the duplexes of DNA and RNA with various backbone and sugar modified oligonucleotides. The simple answer is shown in the following set of duplexes listed by increasing thermostability (for oligonucleotides with the same sequence). The thermostability of the duplexes is characterized by melting temperature (T_m) at which half of the DNA or RNA molecules are in a double stranded form, while the other half of the molecules are represented by single strand form.

Least Stable >> Increase of T_m >> Most Stable
 DNA:DNA < DNA:RNA < RNA:RNA < RNA:2'OMe RNA

The A-form of a duplex is characterized by 3'-endo conformation of the sugar (1). It is normally formed by two RNA molecules and is more stable than the B-form of DNA:DNA duplex which is characterized by 2'-endo conformation of sugar (1). The DNA:RNA hybrid duplexes have a conformation with both A-form and B-form characteristics with a predominance of A-form features (2). For the duplexes of DNA with DNA-RNA-DNA chimeras, the extent of A-form characteristics depends on the composition and ratio of deoxyribo- and ribonucleotides in the duplex. (A RNA stretch as few as three ribonucleotides possesses A-form conformational features sufficient for recognition and cleavage by RNase H of a DNA/RNA/DNA chimera hybridized to a DNA target (3).)

The commonly available sugar modified oligonucleotides are 2'-OMe, 2'-fluoro and 2'-amino derivatives. The presence of 2'-OMe nucleosides in DNA or RNA strands enhances thermostability of the duplexes. Modification of the 2'-deoxyribose with 2'-fluoro enhances the thermostability of the DNA-DNA duplexes by 1.3° C per insertion (4), while the 2'-amino modification destabilizes duplexes (5). A combination of 2'-fluoro and 2'-OMe nucleosides will enhance the thermostability of a duplex considerably, without resorting to more expensive nucleosides with modified bases.

Phosphorothioate oligonucleotides are commonly used in target validation or antisense experiments (6). Due to the chirality of the internucleotide phosphorothioate phosphorus (Sp and Rp), standard phosphorothioate oligonucleotides are chemically synthesized as a mixture of 2ⁿ diastereomers (where n is the number of internucleotide phosphorothioate linkages). Overall, the combination of the Sp and Rp chiral forms in the phosphorothioate oligonucleotide destabilize DNA:RNA and DNA:DNA duplexes. For example, a complex of a 15mer phosphorothioate oligonucleotide and a control phosphodiester of the same sequence with a complementary 15mer oligonucleotide RNA had T_m 's of 33.9° C and 45.1° C respectively, at described conditions. Due to the stabilization effect of the 2'-OMe group, the difference between T_m is reduced considerably for 2' OMe-RNA phosphorothioate oligonucleotides. For the duplex with DNA the control 2'-OMe-RNA strand gave a T_m of 62.8° C, whereas the 2'-OMe phosphorothioate analog gave a T_m of 57.7° C (4).

In several publications individual stereo defined diastereomers of phosphorothioate oligonucleotides were synthesized and investigated (6). Chirally pure Sp-phosphorothioate oligonucleotides have been shown to enhance the thermal stability of the duplexes with DNA and RNA compared to Rp diastereomers or mixed Sp and Rp phosphorothioates (6). However, the chirally pure phosphorothioate oligonucleotides are very expensive to

make. So far mixed Sp/Rp phosphorothioate oligonucleotides, especially 2' OMe analogs, are adequate for most applications and do not affect the thermostability adversely as to warrant the added expense required for the synthesis of chirally pure phosphorothioate oligonucleotides.

Another common backbone modification offered by TriLink is oligonucleotides containing neutral methylphosphonate linkages. These oligonucleotide analogs are also prepared as a mixture of Sp and Rp diastereomers (2ⁿ diastereomers, where n is the number of methylphosphonate internucleotide linkages). The Sp/Rp mixed version of methylphosphonate oligonucleotides, which is all that is readily available, produce complexes with complementary DNAs and RNAs. However, the thermostability of these complexes is significantly lower than similar phosphodiester DNA-DNA and DNA-RNA duplexes. The destabilizing effect of methylphosphonate oligonucleotides on the complexes with DNA or RNA is stronger than phosphorothioate oligonucleotides.

A chirally pure Rp methylphosphonate oligonucleotide analog, on the other hand only slightly destabilizes the complex with an RNA target; while the stability of the complex with a DNA target is greatly enhanced (8) compared to phosphodiester oligonucleotide. For instance, with RNA target, an Rp/Sp-mixed all-methylphosphonate oligonucleotide 15mer had a T_m of 34.3° C, whereas the phosphodiester control had a T_m of 60.8° C. An oligonucleotide with alternating Sp/Rp-mixed methylphosphonate and phosphodiester linkages had a T_m of 40.6° C, while the Rp chirally pure version of the same oligonucleotide had a T_m of 55.1° C, just slightly less than the control hybrid (7). Unfortunately, the chirally pure methylphosphonates are not available commercially, and can only be obtained through Genta, Inc. TriLink is capable of synthesizing these molecules with Genta's explicit written permission on a case by case basis.

References

1. W. Saenger, Principles of Nucleic Acid Structure, C.R. Cantor, Ed., Springer Advanced Texts in Chemistry (Springer-Verlag, New York, 1984).
2. Lesnik, et al. (1993) Biochemistry 32, 7832-7838.
3. Hogrefe, et al. (1990) J. Biol. Chem. 265, 5561-5566.
4. Kawasaki, et al. (1993) J. Med Chem. 36, 831-841.
5. Aurup, et al. (1994) Nucleic Acids Res. 22, 20-24.
6. Stec, et al. (1994) Angew. Chem. Int. Ed. Engl. 33, 709-722.
7. Reynolds, et al. (1996) Nucl. Acids Res. 24, 4584-4591.
8. Vyazovkina, E.V., Savchenko, E.V., Lokhov, S.G., Engels, J.W., Wickstrom, E., and Lebedev, A.V. (1994) Nucl.Acids Res. 22, 2401-2405.