Nuclease Resistance Introduction

Like cellular DNA and RNA, synthetic oligonucleotides are prone to degradation once introduced into the cell or body fluids. Such degradation is due to the ubiquitous presence of nuclease enzymes (both exonucleases and endonucleases), as well as chemical instability (particularly for RNA). Under normal cellular conditions, this leads to fast in vivo degradation of oligos and a short half-life (1). In addition, the ease with which RNAse, being very stable, can contaminate laboratory equipment and benchtop surfaces means that RNA is susceptible to degradation under normal laboratory conditions as well. To reduce or eliminate this susceptibility, nuclease-resistant modifications (for example, phosphorothiolation or 2'-OMethyl RNA bases) can be introduced into oligonucleotides slated for in vivo and/or regular benchtop work.

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Nuclease Resistance Design Protocols

Nuclease Resistant Oligos for In vivo Applications—Design Considerations

While oligonucleotides are quickly degraded (typically within 15-30 minutes) by nucleases in both in vitro and in vivo contexts, the need to incorporate nuclease resistance into oligonucleotides is critically important for in vivo applications. Within serum or the cell, oligonucleotides can be degraded by both endo- and exonucleases. In serum, the 3'-exonucleases are of greatest concern (7), while within the cell, both 3'- and 5'-exonucleases are problematic (8). Endonucleases can also be an issue in those cases where the oligo contains a restriction site.

Based on the above, designing a nuclease-resistant oligonucleotide for in vivo applications primarily involves modifying it so as to protect it from exonucleases, while minimizing potential deleterious side-effects (such as reduced duplex stability, increased toxicity, or induction of off-target biological effects). The simplest and most cost-effective way to do this is to design the oligo as a “gapmer”, in which the linkages of the three end-most 5’- and 3’-bases are phosphorothiolated, with the remaining bases in the middle having native phosphodiester linkages. Such phosphorothiolated “gapmers” are highly resistant to both 5’- and 3’-exonuclease degradation. In addition, because phosphorothiolation lowers the binding affinity of the oligo for its target (Tm of the oligo-target duplex is lowered between 0.5C and 1.5C per linkage, depending on sequence), use of only six such linkages often yields an acceptable balance between nuclease resistance and binding affinity (if increased binding affinity is required, other modifications can also be incorporated into the oligo, such as 2’-fluoro pyrimidines, 2’-O-methyl RNA bases, or C5-propyne pyrimidines). Finally, since large numbers of phosphorothiolate linkages can be toxic (due to the presence of sulfur), using only a small number of such linkages in an oligo minimizes cellular toxicity.

If phosphorothiolation is not desired, other modifications can be used. One option is to use methylphosphonates instead of phosphorothiolation for the 5’- and 3’-end positions of the “gapmer”. Methylphosphonates lower an oligo’s binding affinity more than phosphorothiolation, however, so the use of additional modifications, such as 2’-fluoro-pyrimidines, is advisable to counteract this effect. More commonly, the substitution of 2’-O-methyl RNA bases at some or all positions of an oligo is used as an alternative to phosphorothiolation. Since the nuclease resistance conferred by 2’-O-methyl RNA lies between that of standard bases (no resistance) and phosphorothiolation (highly resistant), extensive/complete 2’-O-methylation is frequently chosen when a high level of nuclease resistance is required. 2’-O-methylation also confers higher binding affinity (that is, higher duplex Tm) to the oligo for its target, a desirable property, in many cases.
Nuclease Resistance Applications

For antisense or RNAi applications, incorporation of modifications conferring nuclease resistance is essentially indispensable and such modifications are used intensely. The most popular modification used for this purpose is phosphorothiolation, in which a phosphodiester backbone linkage is replaced by a phosphothiolate linkage. Such linkages are highly resistant to nuclease degradation, but they also lower duplex stability by about 0.5C per phosphorothioate linkage. However, judicious use of this modification (for example, placing them only at the three endmost bases of each end of the oligo to minimize exonuclease degradation) can produce excellent nuclease resistance while still maintaining reasonably good duplex stability (2). 2'-OMethyl (or other 2'-O-substituted) RNA bases also confer nuclease resistance to an oligo, and have the added benefit of increasing duplex stability as well (3,4). However, duplexes formed between oligos having 2'-OMethyl bases at all positions and RNA are incapable of activating RNase H activity (3), and this fact must be kept in mind if the user wishes to use such oligos for antisense applications. Recently, “mirror-image” (L)-nucleotide base phosphoramidites have become available as well. Oligonucleotides containing (L)-nucleotides are completely immune to nuclease attack at the incorporated positions (5). While (L)-nucleotides also do not base pair with natural (D)-nucleotides (6), they still could potentially be incorporated in, for example, the stems of molecular beacons to protect them from degradation by exonucleases.
References