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Selectivity of Polyamines on the Stability of RNA-DNA Hybrids
Containing Phosphodiester and Phosphorothioate Oligodeoxyribonucleotides

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RNA-DNA hybrid stabilization is an important factor in efficacy of oligonucleotide-based antisense gene therapy. We studied the ability of natural polyamines, putrescine, spermidine, and spermine, and a series of their structural analogues to stabilize RNA-DNA hybrids using melting temperature (T_m) measurements, circular dichroism (CD) spectroscopy, and the ethidium bromide (EB) displacement assay. Phosphodiester (PO) and phosphorothioate (PS) oligodeoxyribonucleotides (ODNs) (21-mer) targeted to the initiation codon region of c-myc mRNA and the corresponding complementary RNA oligomer were used for this study. In the absence of polyamines, the T_m values of RNA-PODNA and RNA-PSDNA helices were 41 ± 1 and 35 ± 1 , respectively, in 10mM sodium cacodylate buffer. In the presence of a hexamine analogue of spermine at a concentration of $25 \mu\text{M}$, the hybrids were stabilized with T_m values of 80 and 78, for RNA-PODNA and RNA-PSDNA, respectively. The $d(T_m)/d(\log[\text{polyamine}])$ values, representing the concentration-dependent stabilization of hybrids helices by polyamines, increased from 10 to 24 for both the RNA-PODNA and RNA-PSDNA helices. Bisethyl substitution of the primary amino groups of the polyamines reduced the hybrid stabilizing potential of the polyamines. Among the homologues of spermine [$\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_n\text{NH}_2$, where $n=2-8$; $n=4$ for spermidine] and spermine [$\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}(\text{CH}_2)_n\text{NH}(\text{CH}_2)_3\text{NH}_2$, where $n=2-8$; $n=4$ for spermine], spermidine and spermine were the most effective agents for stabilizing the hybrid helices. At a physiologically com-

patible concentration of 150mM NaCl, the hybrid helix formed from PODNA was more stable than that formed from PSDNA in the presence of polyamines. The relative binding affinity of the polyamine homologues for the hybrid helices, as measured by the EB displacement assay, followed the same order in which they stabilized the hybrid. These result are important in the antisense context and in the general context and in the polyamine- nucleic acid interactions, and suggest that pentamine and hexamine analogues of spermine might be useful in improving the efficacy of therapeutic ODNs.