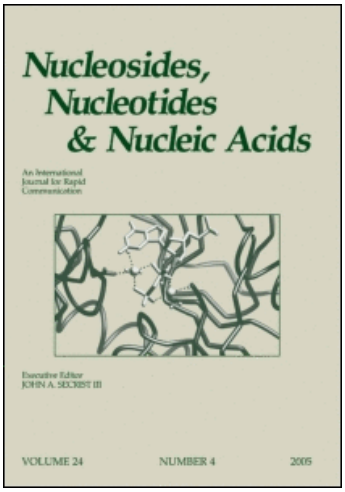

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RNase H Activation by Stereoregular Boranophosphate Oligonucleotide

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RNase H Activation by Stereoregular Boranophosphate Oligonucleotide

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ABSTRACT

A stereoregular all-(S_p)-boranophosphate oligodeoxyribonucleotide (BH_3^--ODN) 15-mer was synthesized using an enzymatic approach. The BH_3^--ODN formed a hybrid with the complementary RNA 15-mer and induced RNase H hydrolysis of the RNA strand at ODN concentrations as low as 10 nM at 37°C, but with a lower efficiency than that of its natural phosphodiester analogue.

INTRODUCTION

Boranophosphate is a class of modified internucleotide linkages, in which a borano (-BH_3) group replaces one non-bridging oxygen of the phosphodiester backbone.[1] The most remarkable property of a BH_3^--ODN is its ability to induce RNase H-mediated hydrolysis of the complementary RNA strand[2] — a property displayed by only a few oligonucleotide analogues. Previous studies in our lab

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indicated that a nonstereoregular dodecathymidine boranophosphate, $T(^{b}pT)_{11}$, mediated polyA hydrolysis by RNase H more efficiently than did its normal and phosphorothioate analogues,[2] and with relative hydrolytic rates in reverse order to the stability of the heteroduplexes. Given the widely accepted essential role of RNase H in manipulating gene expression by antisense ODNs, it is necessary to study the above relationship using other sequences, especially mixed-base sequences, and to use more physiologically relevant conditions. In this study, we developed an enzymatic method to synthesize a mixed-sequence all-boranophosphate ODN and investigated its ability to induce RNase H activity.

RESULTS AND DISCUSSION

An erbB-2 gene sequence,[3] US3 (5′-GGT GCT CAC TGC GGC-3′), was chosen to be the antisense strand of the current study. The S_p stereoregular BH_3^--ODN US3 was prepared by template-based extension of a 3′ riboU-modified DNA primer, using MMLV reverse transcriptase and the R_p isomers of 2′-deoxyribonucleoside 5′-α-[*P*-borano]-triphosphates of the four bases (dNTPαBs, N = A, T, G, C). The resulting all-(S_p)-boranophosphate ODN was cleaved from the primer by incubation in concentrated ammonium hydroxide overnight at room temperature, and then separated and purified by 20% PAGE and reversed phase HPLC. The complementary 15-mer RNA strand was 5′ labeled with hexachlorofluorescein (HEX), so that the hydrolytic products of RNase H could be separated by 20% PAGE and quantified using a Hitachi FMBIO-100 fluorescent image scanning unit. The amount of substrate cleavage showed a linear dependence on both time and the enzyme concentration under the conditions used.

Since RNase H uses DNA:RNA hybrids as substrates, the enzyme reaction at a constant RNA concentration and varied DNA concentrations was first studied to examine the extent of hybrid formation. When the RNA concentration was 10 nM (the detection limit of the fluorescence assay), the complementary BH_3^--ODN at an equal concentration was able to hybridize to the RNA strand with sufficient affinity to activate RNase H cleavage of the RNA. However, unlike its normal analogue, a five-fold greater concentration of the BH_3^--ODN was required to obtain almost complete hybridization of the RNA strand at 10 nM concentration. This observation was consistent with previous studies indicating a decreased affinity of BH_3^--ODN, relative to its natural ODN analogue, for the complementary DNA or RNA strands.[2,4,5] Moreover, under conditions where all RNA strands were hybridized, the rate of RNase H hydrolysis induced by the BH_3^--ODN was about 10-fold less than that induced by its normal analogue. Steady-state kinetic analysis at different substrate concentrations indicated that the decreased hydrolytic efficiency was a result of both a larger K_m and a smaller k_{cat} for the sequence studied, which are about 1.6-fold larger and 6-fold smaller, respectively, than those of the natural hybrid. Studies are underway to understand the cause of this decreased RNase H activity, i.e., whether it is the sequence, the stereochemistry, the stability of the hybrid substrate, and/or the changes in hybrid conformation brought about by the borano substitution.[6]

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