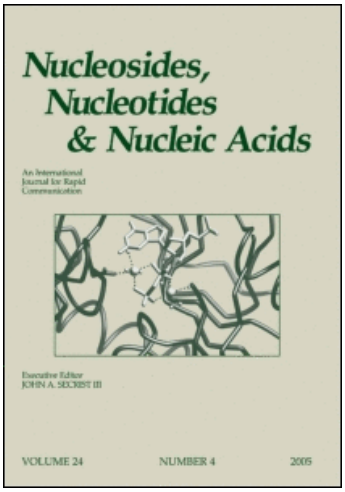

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THE EFFECT OF A SINGLE BORANOPHOSPHATE SUBSTITUTION WITH DEFINED CONFIGURATION ON THE THERMAL STABILITY AND CONFORMATION OF A DNA DUPLEX

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□ *Substitution of one non-bridging oxygen in a natural phosphodiester internucleotide linkage with a borano (-BH_3) group results in a chiral phosphorus center in boranophosphate. UV thermal melting profiles were recorded for DNA duplexes formed between a DNA 9-mer with either an Sp or a Rp boranophosphate linkage in the middle and the complementary DNA 9-mer, as well as for their unmodified parent duplex. The thermal stability of the DNA duplexes was in the order of normal > Sp borano > Rp borano. CD spectra of all three duplexes exhibited typical B-DNA profile, which closely resembled each other.*

Keywords Oligodeoxyribonucleotide Analogs, Boranophosphate, Phosphorus Chirality, Hybridization, Conformation

INTRODUCTION

Boranophosphate is one class of modified internucleotide linkages in which a borano (-BH_3) group replaces one non-bridging oxygen in a natural phosphodiester linkage.[1,2] The borano substitution introduces a new chiral center at the phosphorus atom, which assumes two configurations: *Rp* and *Sp*. Diastereomers of other phosphodiester backbone modified oligodeoxyribonucleotides (ODNs), such as methylphosphonate and phosphorothioate ODNs, have demonstrated different physicochemical and biological properties.[3,4] However, no studies have been carried out to understand the influence of phosphorus chirality on the binding affinity or conformation of boranophosphate-containing ODNs. For this purpose, three DNA duplexes comprising a DNA 9-mer with either an *Sp* or a *Rp* boranophosphate linkage in the middle, or their unmodified parent strand,

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hybridized to the complementary DNA 9-mer were prepared in our lab. Their melting temperatures (T_ms) and CD spectra were measured and compared here.

EXPERIMENTAL

The oligodeoxyribonucleotides used in this study are listed below. The unmodified ODNs were synthesized by standard phosphoramidite method on an ABI 392 DNA/RNA synthesizer. The boranophosphate-containing ODNs were prepared by block condensation of the corresponding stereo-defined dimer synthon, and the detailed synthesis and identity confirmation will be described elsewhere.[5]

9O : $5' - \text{ATGGTGCTC} - 3'$
9Sp : $5' - \text{ATGGT}_{\text{BP}(Sp)}\text{GCTC} - 3'$
where BP(*Sp*) represents *Sp*−boranophosphate
9Rp : $5' - \text{ATGGT}_{\text{BP}(Rp)}\text{GCTC} - 3'$
where BP(*Rp*) represents *Rp*−boranophosphate
a9O : $3' - \text{TACCACGAG} - 5'$

The equilibrium melting profiles were acquired using a Varian Cary 500 Bio UV-Visible spectrometer. The complementary DNA oligos were mixed in equal molar amount by diluting the corresponding stock solutions into 20 mM KH_2PO_4, 0.1 mM EDTA, 150 mM KCl, pH 7.3, and a final total strand concentration of 6 μM. Before melting, all samples were degassed by helium for about one minute, incubated at 70°C for 2 min, then cooled slowly to room temperature. UV absorbance at 260 nm was collected at 0.2°C intervals over a 20-s averaging time while the temperature was increased with the rate of 0.5°C/min. Three parallel runs were performed for each sample, and the melting temperatures were derived using Meltwin©[6] and averaged. The differences of T_m values were within 0.2°C for repetitive measurements. CD spectra were recorded on an AVIV 202 Circular Dichroism spectrometer. The samples were prepared in the same way as in UV thermal melting experiments except for a total strand concentration of 16 μM. CD signals were collected at 0.3 nm intervals with 0.5-s average time at 25°C. Three scans were performed for each sample and averaged.

RESULTS AND DISCUSSION

All three DNA duplexes denatured upon temperature increase in a cooperative manner and with essentially the same percent hypochromicity (Figure 1). The average melting temperatures of 9O:a9O, 9Sp:a9O, and 9Rp:a9O were 40.1°C, 39.1°C, and 38.2°C respectively. The 1°C difference between the

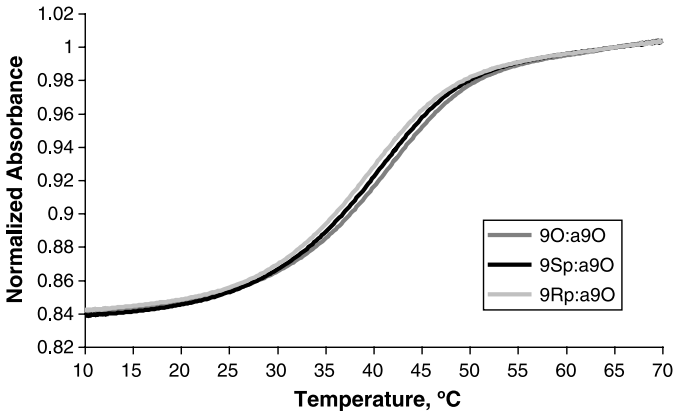

FIGURE 1 Representative melting curves of 9O:a9O, 9Sp:a9O, and 9Rp:a9O at 6 μM total strand concentration. The absorbance was normalized to 1 at 65°C.

melting temperatures of 9O:a9O and 9Sp:a9O was close to a previous observation by our lab, where a 0.7°C lower T_m was noted for the DNA duplex containing a 14-mer DNA with single *Sp*-boranophosphate substitution three nucleotides from the 3′ end relative to its unmodified counterpart.[7] The binding affinity order, *Sp* borano > *Rp* borano, was contrary to that observed for methylphosphonate substitution, where the *Rp* diastereomers always formed more stable complexes with the complementary DNA than their *Sp* counterparts.[8,9] For the phosphorothioate modification, the situation is more complicated—the *Rp* diastereomers could form DNA duplexes that were more stable, less stable, or with the same stability compared to the *Sp* analogues.[4,10–13] A recent thermodynamic study with DNA duplexes containing stereoregular all-*Rp*, all-*Sp*, and nonstereoregular "mix" phosphorothioate ODNs of different sequences concluded that the relative stability depended on the sequence rather than the phosphorus configuration.[14] We do not

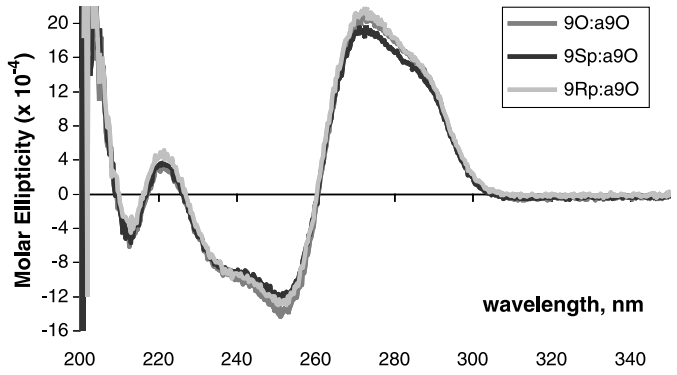

FIGURE 2 CD spectra of 9O:a9O, 9Sp:a9O, and 9Rp:a9O at 16 μM total strand concentration and 25°C.

know whether the relative stability observed in our study for boranophosphate containing DNA duplexes (*Sp* borano > *Rp* borano) is also sequence dependent.

CD spectra of 9O:a9O, 9Sp:a9O, and 9Rp:a9O all exhibited a typical B-DNA profile within the UV absorption range of 200 to 350 nm, which had a positive maximum near 270 nm and a negative minimum near 250 nm, with the crossover point at about 260 nm (Figure 2). All three CD spectra closely resembled each other, and no observable global conformation change was introduced by the single boranophosphate substitution.

This is the first report on the effect of stereo-defined boranophosphate substitution on duplex stability and conformation. Studies with different sequences, RNA targets, and more than one stereospecific boranophosphate substitution are under way.

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