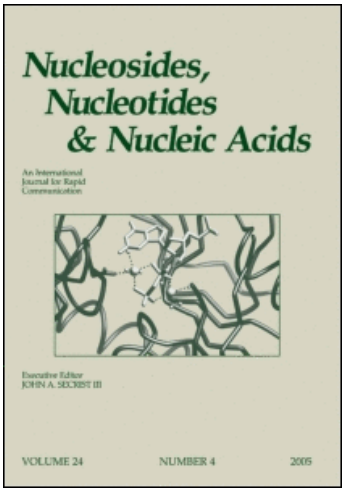

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INCORPORATION OF RIBONUCLEOSIDE 5′-(α-*P*-BORANO)TRIPHOSPHATES INTO A 20-MER RNA BY T7 RNA POLYMERASE

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▫ *The enzymatic synthesis of short boranophosphate RNA was studied by comparing the yield and pattern of abortive products of in vitro transcription at steady-state conditions with that of normal RNA. Boranophosphate short RNA can be readily synthesized by T7 RNA polymerase.*

Keywords Boranophosphate, Transcription, RNA

INTRODUCTION

Enzymatic incorporation of ribonucleoside 5′-(α-*P*-borano)triphosphate (NTPαB) by T7 RNA polymerase results in RNAs with boranophosphate modification, e.g., a backbone modification of oligonucleotides in which one of the non-bridging phosphodiester oxygens is replaced with a borane ($-BH_3$) group (Figure 1). Boranophosphate modifications increase the nuclease resistance and lipophilicity of the oligonucleotides.[1] As one example of many applications for boranophosphate oligonucleotides, we have shown that boranophosphate modifications improve the performance of small interfering RNA (20–22 bp).[2] To facilitate more studies on boranophosphate short RNAs, it is necessary to study its enzymatic synthesis.

RESULTS AND DISCUSSION

The sequence of the 20-nt RNA used in this work is 5′-GGG AGA CCA CAA CCU CUC GU-3′. Under steady-state conditions (standard transcription buffer,

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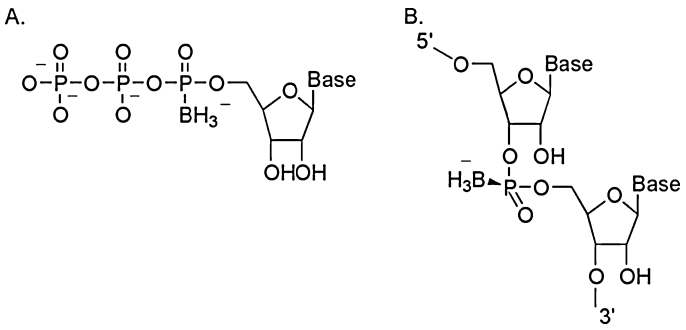

FIGURE 1 Structure of NTPαB (A) and structure of *Sp* boranophosphate linkage produced by enzymatic synthesis (B).

100 nM dsDNA template, 381 nM T7 RNA polymerase, 1 mM NTP or *Rp* isomer of NTPαB), the transcription products from T7 RNA polymerase included the full-length transcript, as well as RNAs with one or more nucleotide(s) added at the end of the full-length transcript, abortive products (3 to 8 nt), and poly(G) products, as described in the literature.[3] Specifically, 1) when GTPαB was present instead of GTP, the yield of full-length products was the same as that of normal RNA (Table 1); the abortive terminations at positions 4 to 7 were decreased by 30–40%, with the biggest decrease occurring at the G5 position (Figure 2); fewer poly(G) products were observed. 2) When ATPαB was incorporated instead of ATP (Figure 3), the yield of the full-length product decreased by ~40% (Table 1), and the percentage of abortive termination at the 4th position, where A is first incorporated,

TABLE 1 Comparison of Full-Length Products (20 and 21-nt) Yield as Copies of RNA per DNA Template at 20-Min Under Steady-State Conditions[a]

	4 NTPs	GTPαB	ATPαB	CTPαB	UTPαB
RNA/DNA	34 ± 5	34 ± 6	21 ± 2	36 ± 7	30 ± 1

[a]Shown are average values of at least three independent experiments.

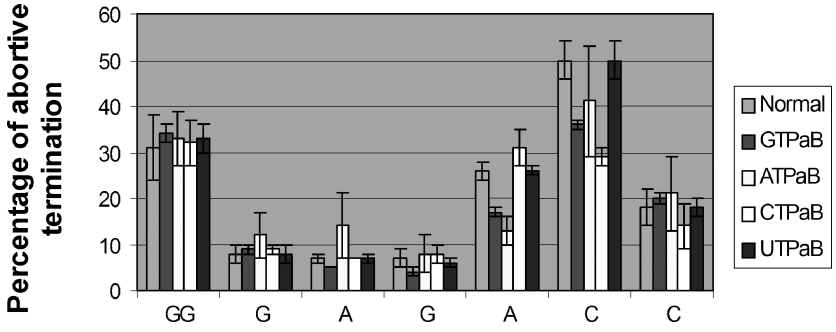

FIGURE 2 Comparison of abortive products pattern with normal or boranophosphate RNA synthesis at 20 min.

doubled with ATPαB compared to that with ATP (Figure 2). According to the crystal structure[4] and other biochemical data,[5] some conformational change occurs with the enzyme when the transcript length increases from 3 to 4 nt. Thus, the effect of modification at the 4th position on transcription was not unexpected. 3) When CTPαB substituted for CTP, the yield of the full-length products was unchanged (Table 1). As shown (Figure 3 and other gels not presented), the abortive cycling stage for this sequence is 3 to 7 nt. Interestingly, the amount of the

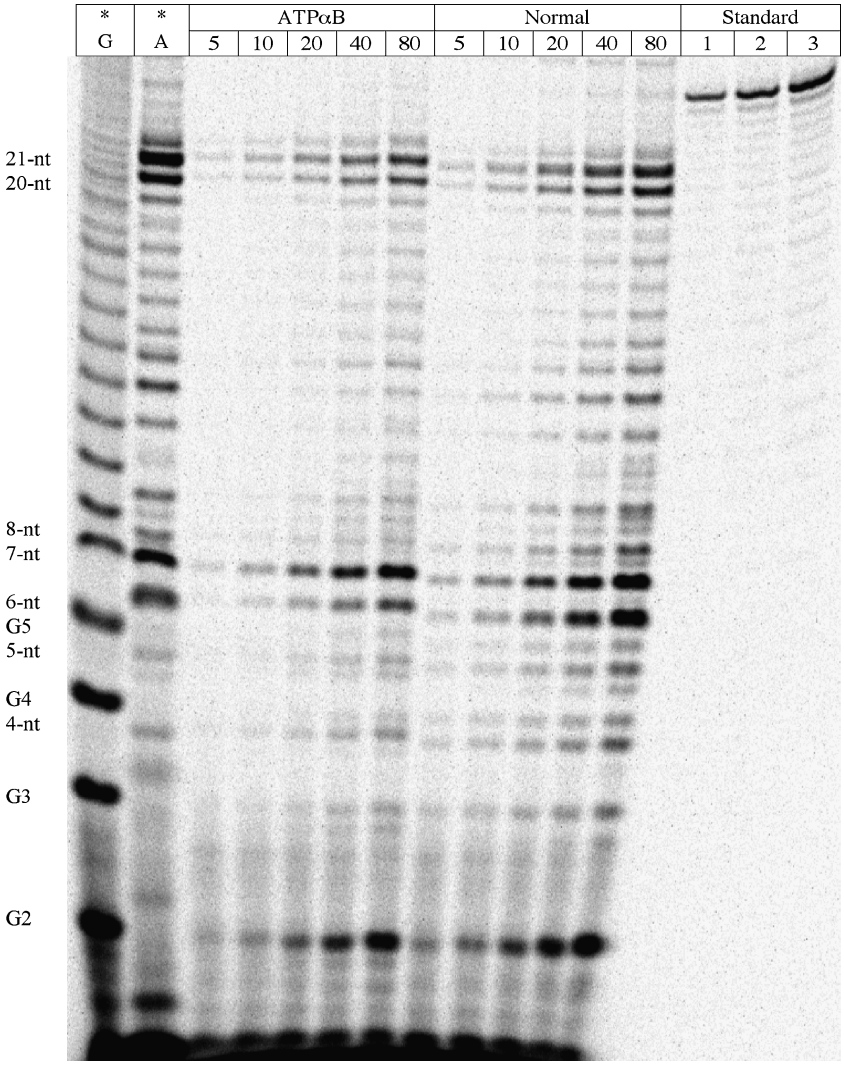

FIGURE 3 In vitro transcription with ATPαB substituting for ATP. Transcripts were labeled with γ-^{33}P-GTP. Lane *A is reaction mixture labeled with α-^{33}P-ATP. Lane *G is reaction mixture with γ-^{33}P-GTP as the only ribonucleoside triphosphate present. The banding patterns of gels for other NTPαB are nearly the same as this one. Lane Standard is RNA with known amount of radioactivity loaded for the purpose of quantification.

abortive termination at positions C7, where C is first incorporated, decreased by ~40% (Figure 2). 4) There are only three U residues in this sequence. With UTPαB substituting for UTP, neither the yield of the full-length nor the abortive termination pattern changed (Figure 2, Table 1).

In conclusion, boranophosphate short RNAs (here 20-nt) can be readily synthesized with T7 RNA polymerase. The yield of the full-length boranophosphate RNA with the sequence used in this study is most sensitive to whether the nucleotide at the fourth position is modified or not, which supports the critical role of the 4th position in the conformational change in the early stage of transcription. Results of this work should help in designing enzymatically synthesized boranophosphate modified siRNA.

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