Polymerase-mediated incorporation of novel locked nucleic acids

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Introduction

- Aptamers are single-stranded nucleic acid molecules that specifically and tightly bind to targets. Aptamers are generated by a combinatorial method known as SELEX during which large populations of oligonucleotides are screened for their binding efficiency.1
- Supplementing aptamers with chemical modifications is of importance in order to improve the nuclease resistance, binding affinity, and expand the repertoire of available targets. These modifications can be included after or during the selection process, but the former often comes at the expense of a loss of binding affinity.2
- Modified nucleoside triphosphates (dN*TPs) can be included in SELEX experiments to expand the chemical space that can be explored. However, prior to their use in SELEX, knowledge on the substrate acceptance of the dN*TPs by polymerases is required.
- Herein, the synthesis and biochemical characterization of conformationally locked nucleoside triphosphate analogs is presented.

Synthesis

Figure 1. Chemical structures of the modified nucleoside triphosphates.

Analogs 3 and 4† as well as the unprotected nucleosides 6, 7, and 8 were synthesized by application of literature procedures.4 The synthesis of dN*TPs 1, 2, and 5 is shown in Scheme 1.

Scheme 1. Reagents and conditions: i) 1. 2-chloro-1,3,2-benzodioxaphosphorin-4- one, 2. (nBuNH)2H2P2O5, DMF, nBuN, rt, 45 min; 3. 1. pyridine, H2O, rt; ii) 2,2- dimethoxypropane, DMF, H2SO4; iii) 1. (MeO)2PO, POCl3, 2. H2O, 3. Dowex 50 (H+), 2.5% NH4OH; (v) 50% TFA (aq); v) 1. imidazole, Ph3P, TEA, 2.2-dipyridyl disulfide, 2. (nBuNH)2H2P2O5.

Primer extension reactions

Using a primer-template system that was used for other conformationally restricted dN*TPs,5 different DNA polymerases were screened.

Figure 2. Representative gel images (PAGE 20%) of primer extension reactions with triphosphates 5 (A) and 4 (B). P: primer; lane 1: natural control (dATP): lane 2: ladder with dATP and ddATP; lane 3: Klenow fragment; lane 4: Sequenase; lane 5: MLV RT; lane 6: HIV RT; lane 7: 9°Wu; lane 8: Therminator; lane 9: Vent (exo’); lane 10: Pwo; lane 11: Bst; lane 12: Pjor; lane 13: Taq.

All triphosphates with the exception of 5 are modest substrates (up to P+2 is observed with the Therminator DNA polymerase). Analog 5 is a better substrate: full length products are observed when Bst and Vent (exo’) are the polymerases.

Conclusions

- A comparative study of nucleoside triphosphates derived from conformationally locked nucleosides was carried out. Five different scaffolds were investigated.
- Carbocyclic locked nucleotides and bicyclonucleotides with 7-membered rings are poor substrates in primer extension reactions.
- Bicyclonucleotide with 6-membered ring can under specific circumstances give elongated primers

References