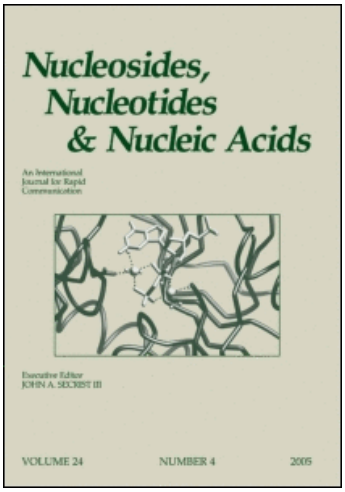

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Properties of Triple Helices Formed by Oligonucleotides Containing 8-Aminopurines

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ABSTRACT

The synthesis of parallel hairpins carrying 8-aminopurines is described. These hairpins have a high affinity for specific polypyrimidine sequences resulting in the formation of very stable triplexes.

Key Words: Oligonucleotides; Triplex; 8-Aminoadenine; 8-Aminoguanine; 2′-O-alkyl-RNA.

INTRODUCTION

Nucleic acid triplexes have wide potential applications in diagnosis, gene analysis and therapy.[1] One of the main drawbacks of the triplex technology is the low stability of triple helices especially in neutral conditions, and when the homopurine-homopyrimidine tracks have interruptions. Recently, it has been described that oligonucleotides carrying 8-aminonucleosides (8-aminoadenine,[2,3] 8-aminoguanine[4]

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and 8-aminohypoxanthine[5]) formed very stable triple helices and parallel-stranded structures.[6] The introduction of an amino group at position 8 of adenine, guanine and hypoxanthine increases the stability of the triple helix owing to the combined effect of the gain in one Hoogsteen purine-pyrimidine H-bond and to the ability of the amino group to be integrated into the "spine of hydration" located in the minor-Major groove of the triplex structure.[3–6] In this comunication we will describe the synthesis and binding properties of oligonucleotides containing 8-aminopurines. The triplex stabilizing properties of 8-aminopurines are of special interest in hairpins carrying 8-aminopurines connected head-to-head to the Hoogsteen pyrimidine strand.[7] These modified hairpins bind to the Watson-Crick pyrimidine strand via a triple helix with greater affinity than hairpins containing only natural bases, especially in neutral conditions.[7]

RESULTS AND DISCUSSION

Oligonucleotide sequences (R-22: $^{5'}$GAA GGA GGA GA$^{3'}$-$(EG)_6$-$^{3'}$TCT CCT CCT TC$^{5'}$, R-22A: $^{5'}$GAA GG**A**$^{\mathbf{N}}$ GG**A**$^{\mathbf{N}}$ GA$^{3'}$-$(EG)_6$-$^{3'}$TCT CCT CCT TC$^{5'}$, R-22G: $^{5'}$GAA G**G**$^{\mathbf{N}}$A G**G**$^{\mathbf{N}}$A GA$^{3'}$-$(EG)_6$-$^{3'}$TCT CCT CCT TC$^{5'}$ and AR-22A-RNA: $^{5'}$GAA GGA GGA GATT$^{3'}$-asym-$^{3'}$UU UCU CCU CCU UC$^{5'}$were $\mathbf{A}^{\mathbf{N}}$, $\mathbf{G}^{\mathbf{N}}$ -$(EG)_6$ and asym are 8-aminoadenine, 8-aminoguanine, hexaethyleneglycol, and asymmetric doubler; U and C are 2′-O-methyl-RNA derivatives) were prepared using phosphoramidite chemistry on an automatic DNA synthesizer. Two methods were used for the synthesis of parallel-stranded hairpins (Scheme).

In method A, the pyrimidine part was assembled using reversed C and T phosphoramidites and a reversed C-support (a support that had the nucleoside linked through the 5′ end). Then, a hexaethyleneglycol linker was added using a commercially available phosphoramidite. Finally, the purine part carrying the modified 8-aminopurines was assembled using standard phosphoramidites for the natural bases and the 8-aminopurine phosphoramidites. The phosphoramidites of 8-aminoadenine, and 8-aminoguanine were prepared as described previously.[2–5]

The second method (B) uses a special support functionalised with a diol derivative protected with two differing groups: the dimethoxytrityl (DMT) group which is removed under acidic conditions and the fluorenylmethoxycarbonyl (Fmoc) group which is removed with bases. This support allows the assembly of the parallel hairpins using standard phosphoramidites (Scheme 1). Hairpin AR22A-RNA was prepared using this method without the need of synthesizing reverse 2′-O-methyl RNA phosphoramidites.

The relative stability of triple helices formed by the parallel-stranded hairpins and the polypyrimidine target sequence (DNA target: $^{5'}$TCT CCT CCT TC$^{3'}$) was measured spectrophotometrically at pH 6.0. In all cases, one single transition was observed with hyperchromicity of around 20–25%, which was assigned to the transition from a triplex to a random coil. Melting temperatures are shown in Table 1. Replacement of two adenines or two guanines by two 8-aminoadenines (A^N) or two 8-aminoguanines (G^N) stabilized triple helix, where an increase in the melting temperature of 9–12°C was observed (Table 1). The presence of 2′-O-methyl-RNA at the Hoogsteen strand produce a further stabilization of the triplex (ΔTm 19°C).

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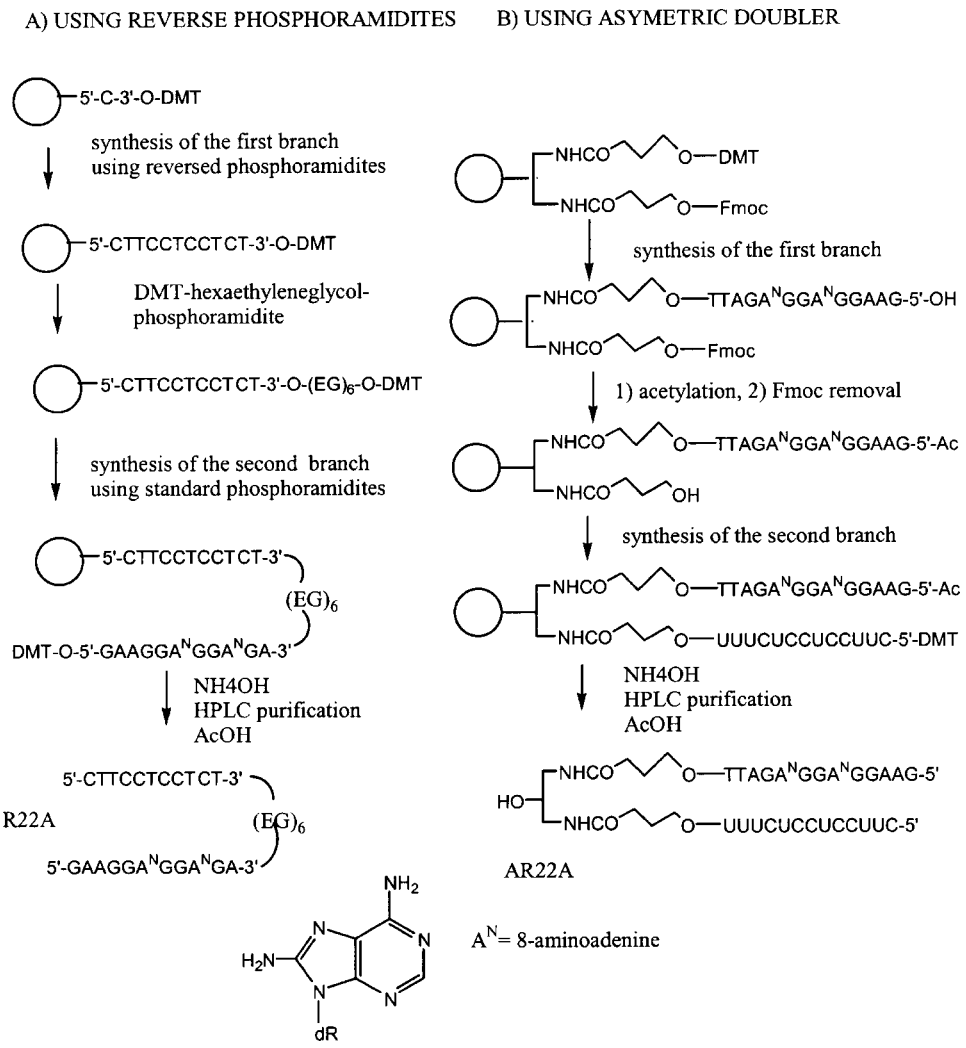

Scheme 1. Schematic representation of the two methods used for the synthesis of parallel hairpins.

Table 1. Melting temperatures (°C) for the triplex formed by hairpin derivatives and their target. Data obtained at 1 M NaCl, 100 mM sodium phosphate/citric acid pH 6.0.

Hairpin	Tm (DNA target)[1]	ΔTm[2]	Tm (RNA target)[1]	ΔTm
R22	47	0	40	0
R22A	56	9	55	15
R22G	59	12	Not determined	—
AR-22A-RNA	66	19	71	31

[1]DNA target: $^{5'}$TCT CCT CCT TC$^{3'}$and 2′-O-methyl-RNA target: $^{5'}$UCU CCU CCU UC$^{3'}$.
[2]ΔTm = Tm-Tm of R-22 in the same conditions.

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Binding of these hairpins to a 2′-O-methyl-RNA target ($^{5'}$UCU CCU CCU UC$^{3'}$) gave unexpected results. Unmodified hairpin (R-22) had a less efficient binding to RNA target while hairpin having two 8-aminoadenines had a similar affinity. Triplex formed by the hairpin carrying 2′-O-methyl-RNA at the Hoogsteen strand (AR22A-RNA) with its RNA target had the highest Tm. This result is in agreement with previous data[8] and indicates that parallel hairpins carrying 8-aminopurines and 2′-O-methyl-RNA may be powerful tools for blocking RNA translation.

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REFERENCES

1. Chan, P.P.; Glazer, P.M. Triplex DNA: Fundamentals, advances and potential applications for gene therapy. J. Mol. Med. **1997**, *75*, 267–282.
2. Kawai, K.; Saito, I.; Sugiyama, H. Stabilization of Hoogsteen base pairing by introduction of amino group at the C8 position of adenine. Tetrahedron Lett. **1998**, *39*, 5221–5224.
3. Güimil García, R.; Ferrer, E.; Macias, M.J.; Eritja, R.; Orozco, M. Theoretical calculations, synthesis and base-pairing properties of oligonucleotides containing 8-amino-2′-deoxyadenosine. Nucleic Acids Res. **1999**, *27*, 1991–1998.
4. Soliva, R.; Güimil García , R.; Blas, J.R.; Eritja, R.; Asensio, J.L.; González, C.; Luque, F.J.; Orozco, M. DNA-triplex stabilizing properties of 8-aminoguanine. Nucleic Acids Res. **2000**, *28*, 4531–4539.
5. Cubero, E.; Güimil García, R.; Luque, F.J.; Eritja, R.; Orozco, M. The effect of amino groups on the stability of DNA duplexes and triplexes based on purines derived from inosine. Nucleic Acids Res. **2001**, *29*, 2522–2534.
6. Cubero, E.; Aviñó, A.; de la Torre, B.G.; Frieden, M.; Eritja, R.; Luque, F.J.; Gonzalez, C.; Orozco, M. Hoogsteen-based parallel-stranded duplexes of DNA. The effect of 8-amino derivatives. J. Am. Chem. Soc. **2002**, *124* 3133–3142.
7. Aviñó, A.; Frieden, M.; Morales, J.C.; de la Torre, B.G.; Güimil García, R.; Azorín, F.; Gelpí, J.L.; Orozco, M.; González, C.; Eritja, R. Properties of triple helices formed by parallel-stranded hairpins containing 8-aminopurines. Nucleic Acids Res. **2002**, *30*, 2609–2619.
8. Morvan, F.; Imbach, J.L.; Rayner, B. Comparative stability of eight different triple helices formed by differently modified DNA and RNA pyrimidine strands and a DNA hairpin. Antisense & Nucleic Acid Drug Dev. **1997**, *7*, 327.