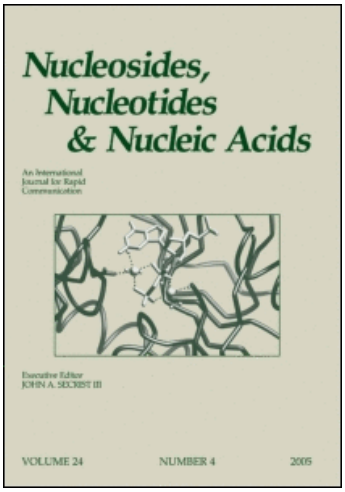

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TRIPLEX FORMATION USING OLIGONUCLEOTIDE CLAMPS CARRYING 8-AMINOPURINES

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□ *The synthesis and properties of triplex-forming DNA clamps carrying 8-aminopurines are described. The stability of triple helices is enhanced by replacing purine bases with 8-aminopurine residues. These enhanced binding properties are used for the specific capture of polypyrimidine RNA/DNA sequences of interest.*

Keywords Oligonucleotide clamps; 8-aminopurines; binding properties; triplex

INTRODUCTION

A triple helix is a structure that appears when a DNA duplex containing a polypurine track interacts with a third strand by means of specific H-bonds in the major groove of the duplex. Several biomedical (antigene strategy) and biotechnological applications based on triplex formation have been described.[1]

Depending on the orientation of the third strand with respect to the central polypurine Watson-Crick (WC) strand, triplexes are classified by two main categories: i) parallel or pyrimidine triplex motif, and ii) antiparallel or purine triplex motif.[1] Most structural studies on DNA triplexes have

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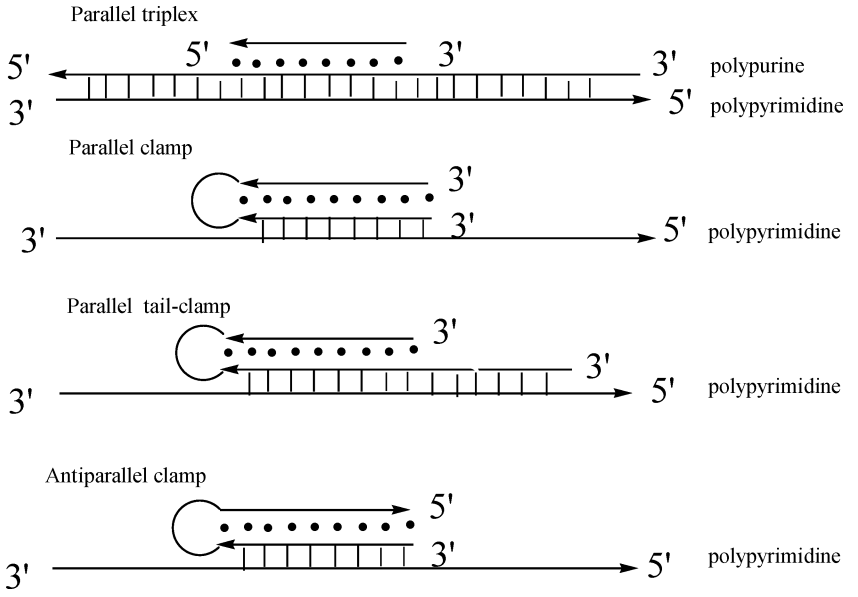
focused on parallel triplexes, which under normal laboratory conditions are more stable than the corresponding antiparallel triple structures. In order to enhance triple helix binding stability we have focused in preparing oligonucleotides containing 8-aminopurines. The introduction of an amino group at position 8 of the adenine and guanine increases stability due to the combined effect of the gain of one Hoogsteen purine-pyrimidine H-bond and the propensity of the amino group to be integrated into the "spine of hydratation" located in the minor groove of the triplex structure.[2,3]

An alternative approach to the generation of oligonucleotide-derived DNA-, or RNA-binding molecules is based on triplex formation via the linkage of one Watson-Crick strand with the third strand or triplex forming oligonucleotide (TFO). Such DNA clamps bind single-stranded nucleic acid targets by triplex formation[4] (Scheme 1). This strategy has been developed further to bind double-stranded DNA by strand displacement using PNA derivatives. In this case, the driving force is the high stability of PNA-PNA-DNA triplexes.[5]

In this communication we describe the synthesis and triplex-forming properties of DNA clamps carrying 8-aminopurines. We show that the stability of triple helices is enhanced by replacing purine bases with 8-aminopurine residues. These enhanced binding properties are used for the specific capture of polypyrimidine RNA/ DNA sequences of interest.

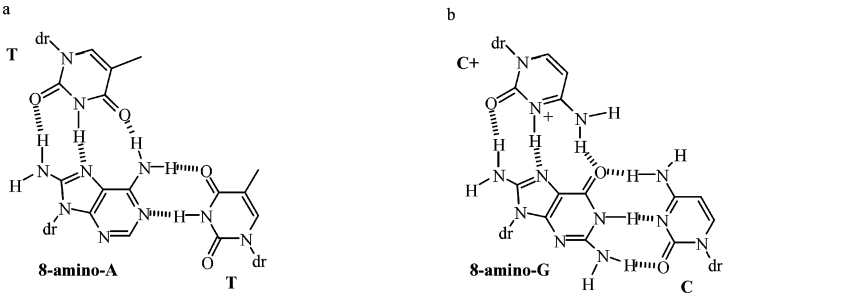


SCHEME 1 Schematic representation of triplexes formed by triplex forming oligonucleotides as well as by parallel and antiparallel clamps and tail-clamps.

RESULTS AND DISCUSSION

Parallel Clamps

Sequence-specific triple-helix structures can be formed by DNA clamps. Parallel-stranded DNA clamps [6] consist of purine residues linked to a homopyrimidine chain of inverted polarity by 3′-3′ or 5′-5′ internucleotide junctions (Scheme 1), which interact with single-stranded homopyrimidine nucleic acid targets.[7] In this type of triplex, the homopurine strand binds the homopyrimidine target through Watson-Crick bonds; and the clamp homopyrimidine strand, which binds via Hoogsteen bonding, is stabilized under acidic conditions. The parallel-stranded oligomers were prepared following previously described protocols.[6–8] For example, the preparation of 3′-3′ hairpins was performed in three parts. First, the purine part, using G and A phosphoramidites and 8-aminopurine phosphoramidites (2, 3) was prepared. After the assembly of the purine part, an hexaethyleneglycol linker or a short oligonucleotide sequence was added to form a loop. And, finally, the pyrimidine part was assembled using reversed C and T phosphoramidites.[6–8] The relative stability of triple helices formed by oligonucleotide clamps and the polypyrimidine target sequence were measured spectrophotometrically at different pHs (pH 5.5–7.0). The target sequence was a polypyrimidine sequence of 11 bases. [2,3] In all cases one single transition was observed with a hyperchromicity around 25% at acidic pH and 20% at neutral pH. The melting curve was assigned to the transition from triple helix to random coil. Replacement of A and G by 8-aminoadenine and 8-aminoguanine in the triple helix, results in a high stabilization (2–10°C per substitution in the range from pH 5.5 to pH 7.0; Scheme 2).[8] The strongest stabilizing effect was found on 8-aminoguanine and at neutral pH. Furthermore, 8-aminoguanine and 8-aminoadenine are also stabilizing Hoogsteen parallel-stranded duplex structures.[9]



SCHEME 2 Schematic representation of parallel triplex triads involving a) 8-aminoadenine and b) 8-aminoguanine. Increased on melting temperatures (ΔTm) observed by the presence of 8-aminopurines in the corresponding parallel triplex.

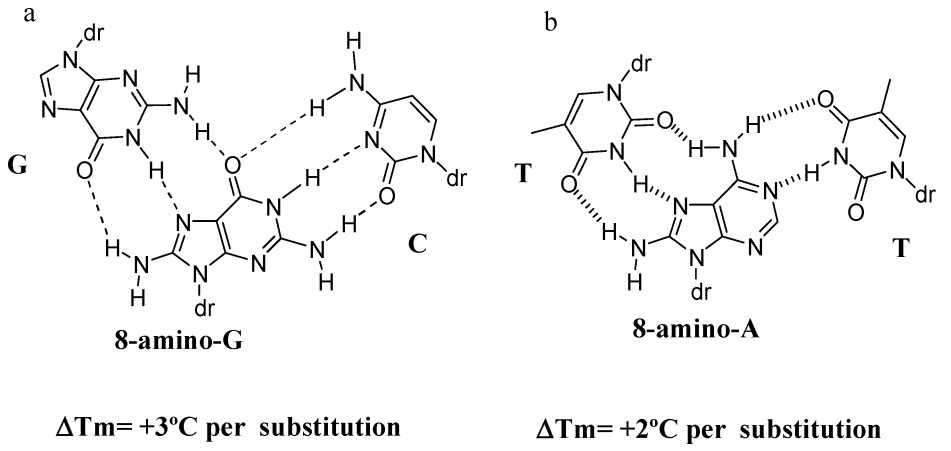
Antiparallel Clamps

This same strategy has been proven to generate stable antiparallel triplexes using purine-rich clamps as templates (i.e., antiparallel-clamps). Antiparallel clamps formed by a G,A- or G,T-Hoogsteen sequence linked to a polypurine sequence carrying 8-aminopurines also have been prepared (Scheme 1)[10] and shown to form more stable triplexes if 8-aminoguanine is present in the purine Watson-Crick position (Scheme 3). The presence of 8-aminoadenine stabilizes antiparallel clamps carrying G,T-Hoogsteen strand (Scheme 3b).[10]

Applications Based on Triplex Formation

The increased binding properties of clamps carrying 8-aminopurines may be of special interest for the development of applications based on triple-helix formations. One of the first applications involved capture of the cauliflower mosaic virus 35S promoter sequence by 8-aminopurine oligonucleotide clamps carrying biotin .[11] The presence of this sequence serves as a marker for genetically modified organisms (GMO) in food. The cauliflower mosaic virus 35S promoter sequence features a 12-base polypyrimidine target. Binding of oligonucleotide clamps was prevented by the target's secondary structure. Moreover we have demonstrated that adding a tail sequence to the Watson-Crick strand in such modified clamps (tail-clamps, Scheme 1) overcomes structural interferences, while simultaneously increasing the stability of triplex formation to a high degree.

A similar result was found during the development of capture probes for the detection of bacteria on food. We synthesized parallel tail-clamps



SCHEME 3 Schematic representation of antiparallel triplex triads involving a) 8-aminoguanine and b) 8-aminoadenine. Increased on melting temperatures (ΔTm) observed by the presence of 8-aminopurines in the corresponding antiparallel triplex

(Scheme 1) designed to bind with *Listeria innocua iap* mRNA sequences containing a polypyrimidine track.[12] Our aim was to obtain optimum conditions for the triplex affinity capture of *Listeria innocua iap* mRNA sequences in order to develop new detection methods for pathogens based on the specific identification of their nucleic acids. In our study, we explored the effects of pH on the interaction of parallel tail-clamps with their target by UV thermal melting analysis. Optimal results were obtained with tail-clamps carrying 8-aminoadenine moieties under neutral pH conditions: 45% of the *iap* mRNA molecules from a total RNA solution were captured.[12]

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