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The stability of triplex DNA is affected by the stability of the underlying duplex

David A. Rusling [a,1], Phillip A. Rachwal [a,2], Tom Brown [b], Keith R. Fox [a,*]

[a] School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, UK
[b] School of Chemistry, University of Southampton, Highfield, Southampton SO17 1BJ, UK

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ABSTRACT

We have studied the formation of DNA triple helices in different sequence contexts and show that, for the most stable triplexes, their apparent stability is affected by the stability of the underlying duplex. For a 14-mer parallel triplex-forming oligonucleotide (generating C^+.GC and T.AT triplets) at pH 5.0 the T_m is more than 10 °C lower with an intermolecular 14-mer duplex target, than it is with an intramolecular duplex, or one which is flanked by 6 GC base pairs at either end. A similar effect is seen with triplex-forming oligonucleotides that contain stabilising analogues, for which the T_m is higher for an intramolecular than an intermolecular duplex target. These results suggest that the use of simple intermolecular duplex targets may underestimate the triplex stabilisation that is produced by some nucleotide analogues.

1. Introduction

Triple helical nucleic acids are formed by binding a third oligonucleotide strand within the major groove of duplex DNA, in which bases in the third strand make specific contacts with the exposed edges of the duplex base pairs [1–5]. In the most commonly studied form the third strand is oriented parallel to the purine strand of the duplex generating T.AT and C^+.GC triplets [6–8]. The three stranded structure is usually less stable than the underlying duplex, as a result of the repulsion between the three negatively charged strands. The formation of these complexes is also limited by the requirement for low pH (required for protonation of cytosine in the C^+.GC triplet) and is usually restricted to oligopurine–oligopyrimidine tracts. A large number of nucleotide analogues have been devised in attempts to overcome these limitations [9–19].

One of the most commonly used methods for assessing the stability of these triple helical complexes is to measure their thermal stability by either UV or fluorescence melting experiments [20]. These are usually performed with short synthetic duplex targets, which are of similar length to the third strand. In some experiments the duplex has been made longer than the triplex in order to separate the duplex and triplex melting transitions [21–23], while others have employed intramolecular duplexes in order to increase the stability of the target [24–26]. In contrast in most footprinting experiments and for *in vivo* applications the duplexes in which the target sequences are located will be much longer than the third strand [19,27,28].

As a result of progress in the design and synthesis of modified nucleotides it is now possible to generate triplexes that are as stable as the underlying duplex and the entire complex melts in a single transition. We were therefore concerned that for some very stable triplexes the apparent affinity of the third strand will be limited by the stability of the underlying duplex, since melting of the duplex will probably cause the third strand to dissociate from its Hoogsteen partner. We have therefore examined how the stability of the duplex target affects triplex melting. We have done this by comparing the interaction of one oligonucleotide (TFO1, Fig. 1a) with its duplex target when this is placed in a variety of different duplex contexts. This TFO is suited to this analysis as at low pHs, and on incorporation of stabilising nucleotides, the melting of this triplex overlaps with that of the simple intermolecular duplex. For these studies we have used duplexes that are stabilised by adding GC- or AT-tails to either end, or by joining the two strands to generate an intramolecular duplex.

2. Materials and methods

2.1. Oligonucleotides

The sequences of the oligonucleotides used in these studies are shown in Fig. 1. These were prepared as previously described [23]. For the experiments with unmodified triplex-forming oligonucleotides all the duplex targets contained the oligopurine tract AGAGAGAAG-GAGGA, which was embedded within a variety of different sequence contexts. The 14-mer third strand oligonucleotide (TFO1) contained natural bases only (T and C). The oligonucleotides containing nucleotide analogues at a single central location in place of T (TFOs 2–7) were 18-mers and were targeted against the duplexes inter2 and intra2. The structures of the various nucleotide analogues are shown in Fig. 1b. In each case the purine strand of the duplex was labelled

* Corresponding author. Tel.: +44 23 8059 4374; fax: +44 23 8059 4459.
E-mail address: k.r.fox@soton.ac.uk (K.R. Fox).
[1] Present address: Department of Biochemistry, University of Bristol, University Walk, Bristol BS8 1TD, UK.
[2] Present address: DSTL, Porton Down, Salisbury SP4 0JQ, UK.

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A

Triplex-forming oligonucleotides

TFO1 5′-Q-TCTCTCTTCCTCCT

5′-Q-TCTCTCTT**N**TCCTCCTCC
TFO2 N = T
TFO3 N = DMAPdU
TFO4 N = APdU
TFO5 N = GPdU
TFO6 N = BAU
TFO7 N = BGU

Target duplexes

Inter1
5′-F-AGAGAGAAGGAGGA
3′-TCTCTCTTCCTCCT

Intra1
5′-F-AGAGAGAAGGAGGA$_H$
3′-TCTCTCTTCCTCCT

3′-ATATAT
5′-F-AGAGAGAAGGAGGAATATAT
3′-TCTCTCTTCCTCCTTATATA

5′-ATATAT
5′-TATATA**U**AGAGAGAAGGAGGA
3′-ATATATATCTCTCTTCCTCCT

5′& 3′-GCGCGC
5′-GCGCGC**U**AGAGAGAAGGAGGAGCGCGC
3′-CGCGCGATCTCTCTTCCTCCTCGCGCG

3′-GCGCGC
5′-F-AGAGAGAAGGAGGAGCGCGC
3′-TCTCTCTTCCTCCTCGCGCG

5′-GCGCGC
5′-GCGCGC**U**AGAGAGAAGGAGGA
3′-CGCGCGATCTCTCTTCCTCCT

Inter2
5′-F-AGAGAGAAAAGGAGGAGG
3′-TCTCTCTTTTCCTCCTCC

Intra2
5′-F-AGAGAGAAAAGGAGGAGG$_H$
3′-TCTCTCTTTTCCTCCTCC

B

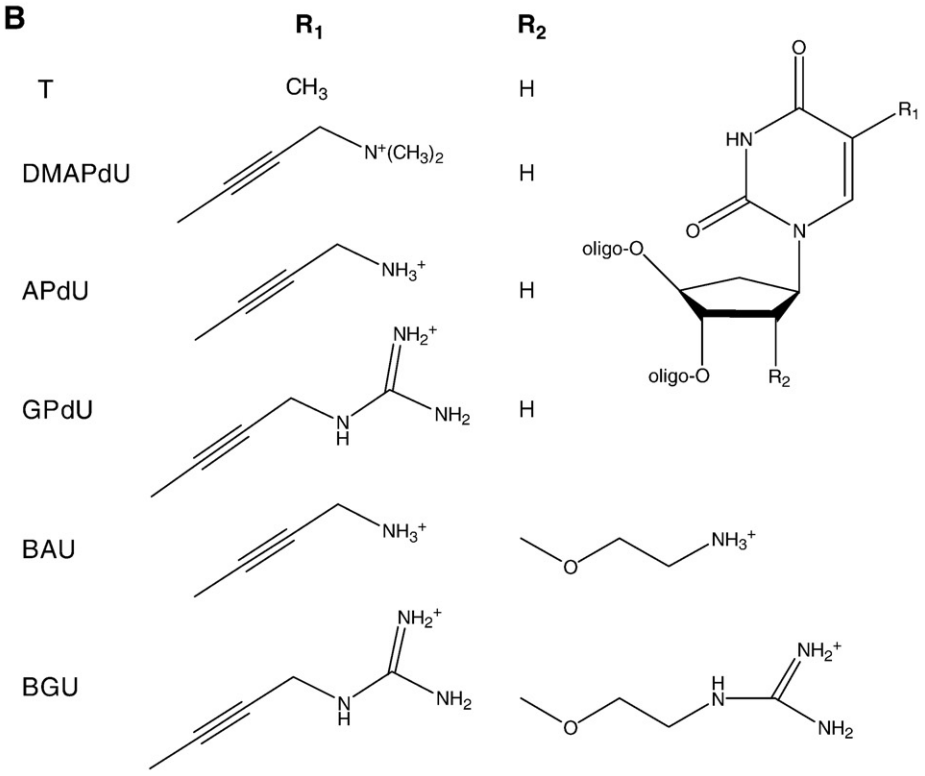

Fig. 1. A) Sequences of the triplex-forming oligonucleotides TFO1-7 and the various duplexes used in this work. H = hexaethyelene glycol; U = FAM-dU. B) Structures of the nucleotides analogues used for TFOs 2–7.