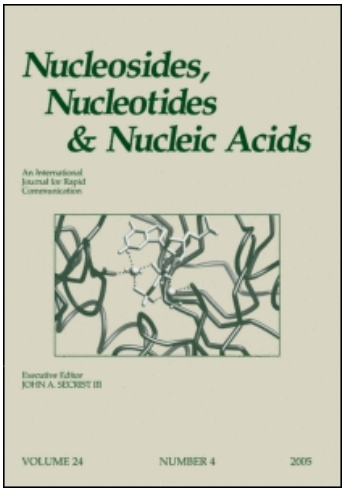

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Françeois Morvan[a]; Carole Chaix[a]; Axel Zeissler[a]; Bernard Rayner[a]; Jean-Louis Imbach[a]
[a] Laboratoire de Chimie Bio-Organique, URA 488 du CNRS, Université de Montpellier II, Montpellier cedex, France

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TRIPLE HELIX FORMING α-OLIGONUCLEOTIDES CONTAINING 5-METHYLCYTOSINE AND/OR 5-BROMOURACIL.

François Morvan*,Carole Chaix, Axel Zeissler, Bernard Rayner and Jean-Louis Imbach.

Laboratoire de Chimie Bio-Organique, URA 488 du CNRS, Université de Montpellier II, Place E. Bataillon, 34095 Montpellier cedex 5, France.

Summary: Stability of αββ triplexes constituted with an α-strand containing 5-methylcytosine and/or 5-bromouracil was studied at a wide pH range. Introduction of 5-methylcytosine increases hybridization stability of the triplexes while introduction 5-bromouracil does not change it.

Oligodeoxyribonucleotides-directed triple helix formation have been successfully used to control in vitro gene expression[1]. Binding specificity is determined from T recognition of AT base pairs (T.AT base triplets) and N3-protonated cytosine (C^+) recognition of GC base pairs (C^+.GC base triplets). Due to the necessity of C protonation the stability of triplexes is strongly pH-dependent. Thus, to decrease their destabilisation under physiological conditions (pH ≈7), introduction of modified bases was proposed[2]. Pyrimidine β-DNA binds purine Watson-Crick strand in the major groove of double helical DNA with a parallel orientation while α-DNA containing both T and C binds purine strand with an antiparallel orientation[3]. Furthermore, as α-DNA shows good nuclease resistance[4], their use as antigene agents could be attractive.

In this work we report on the stability of various αββ triplexes (Fig.1) at a wide pH range with α strands constituted with modified bases (*i.e.* 5-methylcytosine and 5-bromouracil). Triplex study was performed by UV spectroscopy (melting curve). Figure 2 shows a typical curve with the transition at lower temperature corresponding to the dissociation of the triplex and the second transition at high temperature corresponding to the dissociation of the duplex.

The stoichiometry of each triplex was established by the methode of continuous fractions (data not shown). The triplex stability as a function of pH was studied between

Y21 3'-CGATTTTTCTCTCTCTCTAGC-5'
U21 5'-GCTAAAAAGAGAGAGAGATCG-3'
Third strands :
β-Y15 5'-TTTTTCTCTCTCTCT-3'
α-Y15 3'-TTTTTCTCTCTCTCT-5'
α-mY15 3'-TTTTTmCTmCTmCTmCTmCT-5'
α-mBrY15 3'-*U*U*U*U*UmC*UmC*UmC*UmC*UmC*U-5'
*U : 5-bromouracil, mC : 5-methylcytosine.

Figure 1: Sequences of the different oligodeoxynucleotides used.

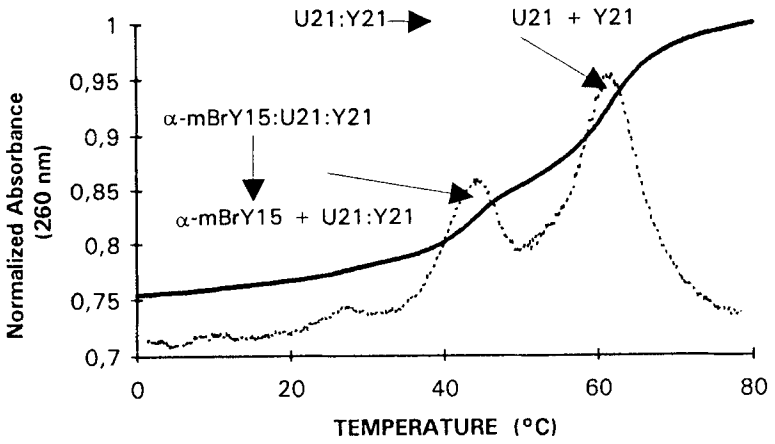

Figure 2: Melting curve of α-mBrY15:U21:Y21 (1:1:1) at pH 6. Buffer : 200 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA pH 6 .

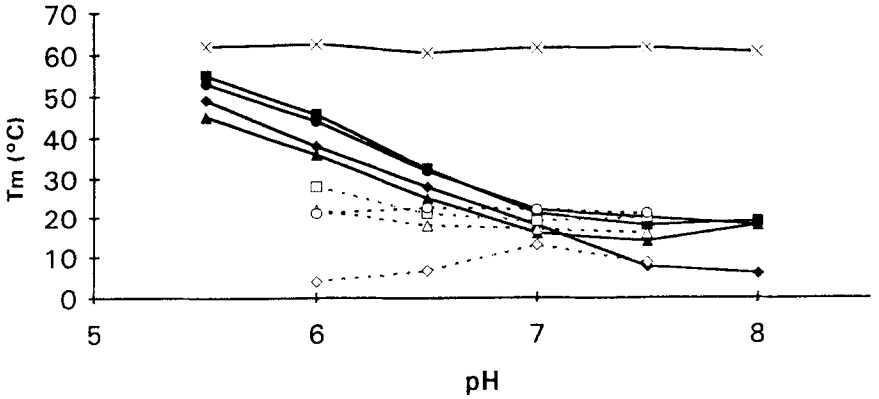

Figure 3 : Curves of triplex Tm versus pH. —×— U21:Y21; —◆— Y15:U21:Y21; ; —▲— α-Y15:U21:Y21; —●— α-mY15:U21:Y21; —■— α-mBrY15:U21:Y21; --◊-- Y15; --Δ--α-Y15; --○--α-mY15; --□--α-mBrY15

pH 5.5 to 8.0 (Fig. 3). The results lead us to different conclusions : 1) The duplex Tm is pH-independent (Tm = 62°C); 2) The introduction of 5-methylcytosine in the Hoogsteen α-strand instead of cytosine enhances triplex stability over the range of pH 5.5 to 7. In contrast, the introduction of 5-bromouracil does not change hybridization properties; 3) The α-pyrimidine strands (15-mer) present some self-association. This phenomenon occurs at each pH and is basically pH-independent (Tm ≈ 20°C). Thus starting from pH 7.0, we could assume that competition between triplex formation and self-association of the α-strands should occur and above only self-association should take place. As self-association is pH-independent we might assume that this self-association is unlikely through C^+:C base formation but more likely through T:T base formation, as previously reported for α-oligothymidylate[5]. Self-association of β-pyrimidine strand is less stable, thus competition between triplex formation and self-association occurs only around pH 7.5 and self-association should take place at higher pH.

The gain of stability brought by the introduction of 5-methylcytosine in α-series for the αββ triplexes at pH near physiological pH and the known resistance of α-oligonucleotides to nucleases allows to consider α-oligonucleotides as potential antigene agents. But the possibility of self-association of the third α-strand should be kept in mind.

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