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Triple helix-tetraplex equilibrium for G-rich oligonucleotide N3′→P5′ phosphoramidates: role of molecular concentration and counterions

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Abstract

The formation of a triple helix by a third N3′→P5′ phosphoramidate GT-rich strand 5′-d$(T_9G_5)_{NP}$-3′ has been studied by FTIR, CD and UV absorbance spectroscopies. We show that in presence of sodium counterions, in conditions of molecular crowding induced by high DNA concentrations, the self-association of the phosphodiester analog into a tetrameric structure is favored and prevents the formation of the triple helix. The use of a phosphoramidate third strand in the same conditions allows to minimize self-association and to form the triple helix with a 5′-d$(T_9G_5)_{NP}$-3′ third strand. Characteristic signatures of T*A·T and G*G·C base triplets have been obtained by FTIR spectroscopy. Formation of a triple helix with the unmodified GT third strand can be observed in dilute solution, in presence of lithium and divalent magnesium ions (1 M LiCl, 50 mM $MgCl_2$). In that case a biphasic UV melting profile (T_M at 40 and 60 °C) and a characteristic CD spectrum of a parallel stranded GT triple helix have been obtained.

Keywords: Oligogt phosphoramidate; Triplex; Tetraplex; FTIR

1. Introduction

DNA molecules in living systems adopt a highly packed organization. In growing bacteria and mitotic eukaryotic chromosomes DNA densities were found in the 200–400 mg/ml range [1,2]. This omnipresent molecular crowding has energetic consequences that could affect many aspects of cellular processes ([3] and ref within). Protein folding, enhanced macromolecular associations, DNA packaging, formation of tight oligomeric structures have been proposed to be stimulated by such a mechanism [4,5]. In the case of nucleic acids drastical changes of the structures have been observed. For instance the tetraplex formed by the d$(G_4T_4G_4)$ sequence can undergo an antiparallel to parallel transition [6], telomere DNA duplexes can dissociate and fold into G and C-rich quadruplexes [7]. Triple helix formation has been shown to occur under conditions that are refractory to the process in a non crowded environment [8]. Most experiments concerning gene expression control by oligonucleotides (TFO strategy, for review see [9]) have been performed in dilute solutions in which crowding effects do not occur. High DNA densities can be produced by physical confinement of large amounts of DNA within restricted volumes. In the present work we have searched to investigate the possible formation of a triple helix in high concentration conditions so as to mimic the overall DNA concentration encountered in cells, using FTIR spectroscopy, a technique that is not limited by concentration requirements.

Classically a triple helix is formed when a third strand binds to a polypurine tract in the major groove of a DNA duplex. When the third strand contains T and/or C bases the triple helix belongs to the so-called pyrimidine motif and the third strand orientation is parallel to the targeted purine strand. If the third strand contains G and/or A bases the formed triple helix belongs to the purine motif with antiparallel third strand orientation. Finally an oligonucleotide containing G and T bases can bind either parallel or

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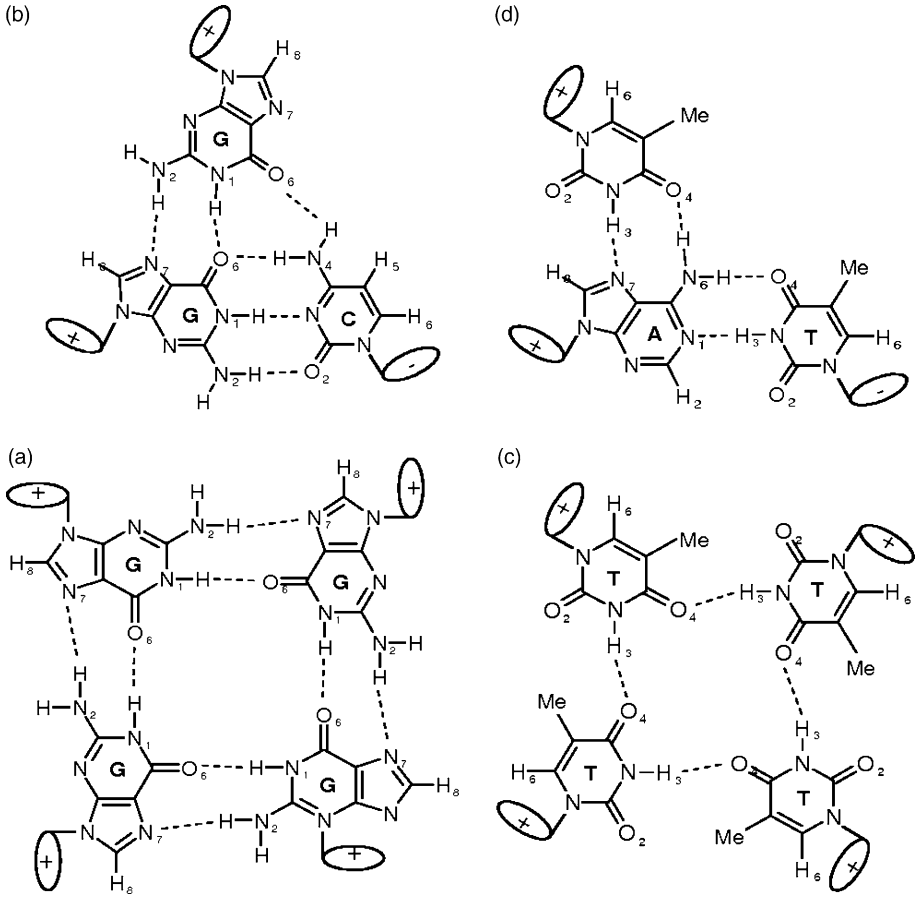

Scheme 1. Models for (a) guanine tetrads; (b) Hoogsteen G*G·C base triplets; (c) T tetrads and (d) Hoogsteen T*A·T base triplets.

antiparallel to the purine strand of the duplex depending on the number of GpT steps present in the sequence. G*G·C and T*A·T base triplets are formed with either Hoogsteen type third strand base pairing in parallel orientation (Scheme 1(b) and (d)) or reverse Hoogsteen type base pairing in antiparallel orientation (for review see [10]).

We have studied the formation of a triple helix aimed at the target sequence 5′-AAAAAAAAAGGGGG-3′ (close to the HIV-1 polypurine tract 5′-AAAAGAAAAGGGGGGA-3′ [11]). The third strand chosen was a GT rich strand 5′d-TTTTTTTTTGGGGG-3′ containing all N3′→P5′ phosphoramidate linkages which will be designated as $d(T_9G_5)_{NP}$ (while its natural phosphodiester analog will be designated as $d(T_9G_5)_{PO}$). To our knowledge no structural data on triple helices formed with a third GT phosphoramidate strand have yet been published. This modification of the DNA backbone has proved to be extremely efficient to help in the stabilization of DNA triple helices formed with a third TC rich sequence [12,13]. Moreover inhibition of transcription elongation has been found for oligo GT phosphoramidate third strands in vitro [14] and in cells [15].

The high DNA concentrations used to mimic the molecular crowding encountered in vivo may lead to a competition between the formation of a desired triple helix and the third strand self-association into a tetraplex structure. Thus we have first studied the third phosphoramidate GT strand $d(T_9G_5)_{NP}$ alone, in presence of either Na^+ or K^+ ions, as well as its phosphodiester $d(T_9G_5)_{PO}$ analog. At low temperature tetraplex structures are found, stabilized by G and T quartets (Scheme 1(a) and (c)). However conditions are obtained in which no such tetrameric structure is formed by the phosphoramidate GT strand $d(T_9G_5)_{NP}$ in presence of sodium counterions. We present in a second part the results concerning the formation of the triple helices. We find that in presence of sodium counterions and in concentrated conditions the triple helix with a third phosphoramidate $d(T_9G_5)_{NP}$ strand is formed, whereas in the same conditions, the phosphodiester oligonucleotide $d(T_9G_5)_{PO}$ fails to bind to the duplex because of self-association of the third strand. Base pairing, sugar geometries and stabilities have been studied by FTIR spectroscopy. In dilute solutions UV and CD experiments show that in presence of lithium ions, conditions in which the third phosphodiester $d(T_9G_5)_{PO}$ strand remains unassociated, it is possible to observe the formation of the triple helix with the unmodified third strand.