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Direct observation of the formation of DNA triplexes by single-molecule FRET measurements

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ABSTRACT

In this report we investigated the effects of various biological and chemical factors (DNA sequence, pH, ions, and molecularity) on the formation of DNA triplexes through single-molecule FRET technique. Using this method, we determined how the third strand bound to a DNA duplex and how stable the triplex structure was under various conditions. From this study, we not only verified a variety of well-known features of DNA triplex but also discovered or experimentally supported several interesting behaviors: at neutral pH, a pyrimidine-motif triplex can be formed; the parallel arrangement was not only possible but also dominant over the antiparallel arrangement for a purine-motif triplex. We demonstrated that our method is a versatile analytical tool in studying structural aspects of nucleic acids, particularly non-classical DNA structures, and provides insights into physical mechanism of such structures.

1. Introduction

It is well-known that the genetic material of living organisms, deoxyribonucleic acids (DNA), takes a right-handed double helical structure. This canonical structure is called B-form DNA. Prior to the double helical structure of DNA proposed by Watson and Crick in 1953 [1], Pauling and Corey proposed a triple helix as the structure of DNA [2]. Although the original proposal for the basic structure of DNA turned out to be wrong, the existence of triple helix was reported and confirmed later [3].

From several decades' research, much has been learned about DNA triplex [4]. In the triplex, three oligonucleotides wind around each other and the third strand binds to a B-form DNA duplex via a Hoogsteen or a reverse Hoogsteen base pair [5] (Fig. 1). The triplex structure mainly forms on a homopurine/homopyrimidine duplex by binding either homopyrimidine or homopurine single-stranded DNA to the homopurine strand of the duplex. In the former type (pyrimidine-motif triplex), the donated single strand (dubbed triplex-forming oligonucleotide (TFO)) is aligned parallel to the homopurine target strand and thus such a triplex is called parallel pyrimidine-motif triplex. In the latter type (purine-motif triplex), the homopurine TFO is aligned antiparallel to the homopurine target strand, and thus such a triplex is called antiparallel purine-motif triplex. In each base pair plane, the base from the TFO is located at the major groove of the duplex and usually forms hydrogen bonds with the base on the homopurine target strand, which is bound to its complementary strand via Watson–Crick base pairing. In reality, a large variety of different compositions and geometries are possible for the triple helix [4,6]. The diversity and flexibility offered by the whole menagerie of DNA triplex renders the putative *in vivo* existence and biological roles of triplex ever more plausible and suggests intriguing possibilities in molecular therapeutics by enabling sequence-sensitive gene detection and targeting [7]. From the fact that the Hoogsteen hydrogen bond is sensitive to the pH of the sample (the protonation of cytosine in acidic environment is critical for the stability of the bond), the parallel duplex as well as the triplex would be a promising molecular component for *in situ* biological pH sensors [8]. The formation of DNA triplex is also very interesting from the biological view point. Friedreich ataxia, the most common inherited ataxia, is thought to be caused by a severe reduction in the levels of a protein called frataxin, which plays a crucial role in iron homeostasis in mitochondria, because the triplex structure formed in the sequence of the frataxin gene arrests its transcription and consequently suppresses the level of the protein [9].

Past several decades, numerous methods have been applied to gain structural and thermodynamic details of DNA triplex. Gel-based techniques such as chemical and enzymatic probing were

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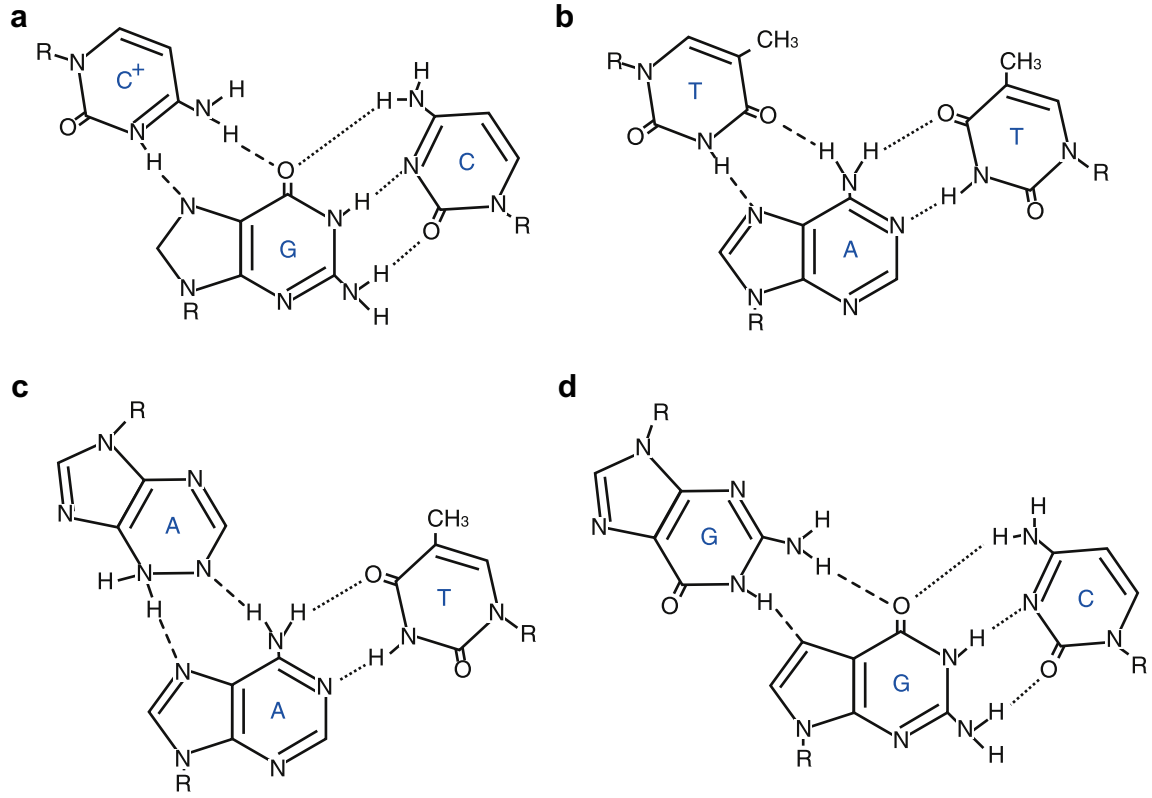

Fig. 1. Base arrangements in DNA triplexes. In each base triad, the bases in the middle and in the right are from the duplex and paired by Watson–Crick base pairing (dotted) and the base in the left is from the third strand (TFO). A pyrimidine-motif triplex uses the triads shown in the upper panels ((a) C^+*G.C and (b) T*A.T) and the bases on the third strand bind to the purine-rich sequence (target strand) by Hoogsteen base pairing (dashed). Here C^+ indicates a protonated cytosine. A purine-motif triplex uses the triads shown in the lower panels ((c) A*A.T and (d) G*G.C) and the bases on the third strand bind to the purine-rich sequence by reverse Hoogsteen base pairing (dashed). Asterisks and dots in the triad notations indicate (reverse) Hoogsteen and Watson–Crick pairing, respectively. R represents the deoxyribose residue connected to the base.

commonly used to characterize the formation of DNA triplex. Although the methods have been well established, structural information is only acquired after multi-step, destructive processes such as chemical or enzymatic treatments [10,11]. The gel methods also provide indirect information that requires interpretation or model-based analysis. Traditional optical and thermal techniques have similar limitations [12]. Being more straightforward, the fluorescence resonance energy transfer (FRET) technique, which detects the change in the distance between two positions at the nanometer scale, was used to investigate the strand arrangements of a triplex [13]. Unfortunately, such an ensemble technique is seriously limited in the case of heterogeneous population and dynamic conformational changes.

Here, we investigated the formation of DNA triplex by single-molecule FRET measurements. Taking advantage of the technique, we were able to clearly distinguish two different orientations of the third strand. We also observed that divalent cations stabilize the triplex structure and the acidic condition facilitates the triplex formation in the use of a homopyrimidine TFO. We found that a homopyrimidine TFO can yield a triplex even at neutral pH and this triplex undergoes dynamic transitions between a folded and an unfolded state. Intriguingly, when the third homopurine strand was provided as a separate molecule, the orientation of the TFO was opposite to the well-known antiparallel orientation: in the absence of geometrical restriction, the parallel arrangement thought to be less stable in fact appears more stable than the antiparallel counterpart. This work demonstrates that our single-molecule approach is simple and still powerful as it can characterize physical aspects of biological problems unambiguously and quantitatively.

2. Methods and materials

2.1. Samples

We purchased all the oligonucleotide samples from Integrated DNA Technologies, Inc (Coralville, Iowa, USA). For the intramolecular pyrimidine-motif triplex (intraY), the following strands were used: 5′ AAG AAG AAG AAG AAG (Cy5) TGG CGA CGG CAG CGA (Bio) 3′ and 5′ TCG CTG CCG TCG CCA CTT CTT CTT CTT CTT TTT TCT TCT TCT TCT TCT TC (Cy3) 3′ where Bio is a biotin label for surface immobilization, and Cy3 and Cy5 are a donor and an acceptor dye for FRET measurement, respectively. For the intermolecular pyrimidine-motif triplex (interY), the following strands were used: 5′ AAG AAG AAG AAG AAG (Cy5) TGG CGA CGG CAG CGA 3′, 5′ (Bio) TCG CTG CCG TCG CCA CTT CTT CTT CTT CTT 3′, and 5′ TTC TTC TTC TTC TTC (Cy3) 3′. The first two oligonucleotides were hybridized first and then the third strand bound as a TFO. The sequence involved in triplex formation was identical to the one in the intramolecular triplex. For the intramolecular purine-motif triplex (intraR), the following strands were used: 5′ (Cy3) GAA GAA GAA GAA GAA CTT TTA AGA AGA AGA AGA AGT GGC GAC GGC AGC GA 3′ and 5′ (Bio) TCG CTG CCG TCG CCA (Cy5) CTT CTT CTT CTT CTT 3′. For the intermolecular purine-motif triplex (interR), the first two strands for interY were used again and the third strand was replaced with 5′ GAA GAA GAA GAA GAA (Cy3) 3′. We also tested the same third oligonucleotide with a Cy3 dye labeled in the opposite position: 5′ (Cy3) GAA GAA GAA GAA GAA 3′. Again, the sequence involved in triplex formation was identical to the one in the intramolecular triplex.