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Structural Aspects of Split G-Quadruplexes in Quadruplex-Duplex Hybrid Systems

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G-quadruplexes are secondary structures of nucleic acids increasingly employed for biological and also technological applications. Being of particular interest, a structural motif called quadruplex-duplex (QD) hybrid comprises both a G-quadruplex and duplex domain. Here, we attempted to engineer a bimolecular quadruplex-duplex hybrid through dual recognition of a receptor by a target strand with the simultaneous formation of both G-quadruplex and duplex structures. Three different constructs were designed for the G-quadruplex receptor, featuring either one, two, or three vacant sites. Addition of the target strand filled the vacancies when simultaneously hybridizing with flanking sequences to form a duplex as shown by NMR studies. Formed QD hybrid constructs either constitute an 11:1, 10:2, or 9:3 split G-quadruplex system. The 10:2 QD construct folds into a non-canonical V-loop topology. Remarkably, the latter also accommodates 5′-overhang residues of the target strand although suggested to impose a steric penalty. Formation of the duplex domain is demonstrated to be critical for the successful formation of the intact G-quadruplex domain. With the formation of a unique QD junction for detection, the constructs may constitute valuable tools for single strand capturing strategies.

Introduction

G-quadruplexes are secondary structures of nucleic acids formed by G-rich sequences. They are comprised of stacked G-tetrads with four guanines arranged in a square planar fashion, connected through eight Hoogsteen hydrogen bonds, and further stabilized by monocations in their central cavity. Most commonly observed G-quadruplexes consist of three layers of G-tetrads.[1] G-quadruplexes play significant roles in biology since their formation has been shown to control various cellular processes.[2] Various small molecules have been designed to target the G-quadruplex structure for modulating gene activities. In most cases, the ligand features an extended aromatic ring system with planar geometry to maximize π-π stacking interactions with the surface of an outer G-tetrad.[3]

The use of G-quadruplexes in nanotechnological and analytical applications was promoted by the finding that these structures can act as a DNAzyme when complexed with hemin, mimicking a peroxidase-like activity that can be detected by further reaction with a chromogen.[4] One technique applying a G-quadruplex system for detection purposes is based on splitting its structure. The split G-quadruplex is characterized as a G-quadruplex only formed by hybridization with another G-rich strand. Such a splitting approach was originally developed by Willner's group. Here, the G-quadruplex was equally split into two halves with each strand comprising two G-columns of the quadruplex core. In this strategy, a flanking sequence on one half is designed in such a way as to capture a complementary sequence of the second strand of interest. Depending on the technique or strategy, the G-quadruplex formation may be enabled but may also be disrupted upon hybridization, translating into a positive or negative signal upon reaction with hemin and a chromogen, respectively.[5,6] Subsequent variations of such bimolecular G-quadruplex architectures involve asymmetrically split G-quadruplexes with strands comprising three and one G-column of three consecutive Gs (9:3)[7,8] or even with a splitting pattern not compatible with multiples of three Gs in each of the two strands.[9,10] One study even involved an 'intramolecularly' split G-quadruplex[11] by using a bulge.[12]

In most split G-quadruplex DNAzyme designs, the recognition of the target strand mostly involves only the overhang sequences and not the guanine core in the G-quadruplex itself, thus being in close analogy to a normal Southern blot. Here, background signals even in the absence of a target[13] as well as additional signals observed from the free hemin-peroxide system itself compromise the use of the split G-quadruplex detection system.[14] Being a unique observation, the background noise can be reduced by adding a duplex-forming sequence on the overhang opposing side in an asymmetrically split G-quadruplex.[8] Nevertheless, it seems advantageous to include the G-column as part of the analytical arm for the strand of interest.

The latter concept can be derived from the ability of G-quadruplexes to carry a G-vacancy.[15] G-quadruplexes containing a G-vacancy can be designed by decreasing the number of guanines in one G-run in comparison with the other G-runs. The vacancy can subsequently be filled intramolecularly by an overhang guanine to form a snapback loop. The snapback loop motif is also known to modulate ligand binding to the G-

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