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Effects of guanine bases at the central loop on stabilization of the quadruplex DNAs and their interactions with *Meso*-tetrakis(*N*-methylpyridium-4-yl)porphyrin

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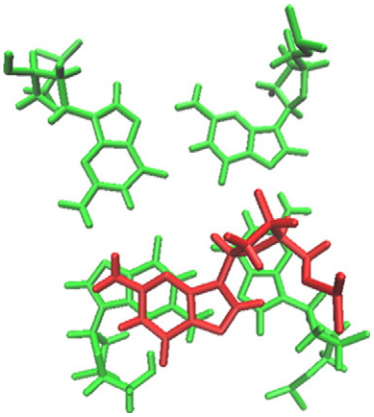
HIGHLIGHTS

- Thermal stability of a quadruplex, 5′ $G_2T_2G_2TXTG_2T_2G_2$ (X = A, I or G), was investigated.
- Thermal stability is lowest when X = A.
- Central G or I base can form hydrogen bond with G bases in upper G-quartet.
- Replacement of G base did not affect the spectral properties of TMPyP.

GRAPHICAL ABSTRACT



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ABSTRACT

The thermal stability of the G-quadruplex formed from the thrombin-binding aptamer, 5′$G_2T_2G_2TGTG_2T_2G_2$, in which the guanine (G) base at the central loop was replaced with an adenine (A) or inosine (I) base, was examined to determine the role of the central G base in stabilizing the quadruplex. Replacement of the central G base by the I base resulted in a slight decrease in thermal stability. On the other hand, the stability of the G-quadruplex decreased to a significant extent when it was replaced with the A base. The optimized structure of the G-quadruplex, which was obtained by a molecular dynamic simulation, showed that the carbonyl group of the C5 position of the central G base could form hydrogen bonds with the G1 amine group at the C7 position on the upper G-quartet. This formation of a hydrogen bond contributes to the stability of the G-quadruplex. The spectral property of *meso*-tetrakis(*N*-methylpyridium-4yl)porphyrin (TMPyP) associated with the G-quadruplex was characterized by a moderate red shift and hypochromism in the absorption spectrum, a positive CD signal, and two emission maxima in the fluorescence emission spectrum, suggesting that TMPyP binds at the exterior of the G-quadruplex. Spectral properties were slightly altered when the G base at the central loop was replaced with A or I, while the fluorescence decay times of TMPyP associated with the G-quadruplex were identical. Observed spectral properties removes the

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possibility of intercalation binding mode for TMPyP. TMPyP binds at the exterior of the quadruplex. Whether it stacks on the central loop or binds at the side of the quadruplex is unclear at this stage.

1. Introduction

Telomeres located at the end of chromosomes protect the chromosomes from deterioration and influences a range of biological processes, such as the prevention of telomerase binding, promoter activation and gene rearrangement [1]. Such biological importance has attracted considerable attention for the structure and dynamics of the G-quadruplex [2–6]. The G-quadruplex contains stretches of guanine bases (G) that are capable of forming a unique structure called the G-quadruplex, which is composed of four G-bases connected via Hoogsteen type hydrogen bonding in the same plane. The stacking interaction between the G bases as well as other electrostatic interaction also helps stabilize the G-quartet in the G-quadruplex in addition to hydrogen bonding. The presence of a monovalent cation, such as K^+ or Na^+ is essential for stabilizing the G-quadruplex [7]. These monovalent cationic ions interact with the carbonyl group of the G bases. A variety of structures depending on the number of G-quartet, nature of loops and central cation have been reported [8–15]. For example, an early NMR study of a thrombin-binding aptamer, $5'G_2T_2G_2TGTG_2T_2G_2$, showed that this DNA adopts a highly compact, high symmetrical structure that consist of tetrads of G base pairs and three loops (Fig. 1) [9]. The loops linking the G quartets on the top or bottom of the G-quadruplex can have various sequences and lengths. The nature of the loops is related to the direction of the G-quartet runs [10,15–18]. For example, a recent single molecule fluorescence technique showed that both the loop length and sequence contribute to the conformation of the G-quadruplex. The folding dynamics also depends on the loop composition [15]. On the other hand, the interaction and the role of the nucleo-bases at the central loop in the stabilization of the G-quadruplex are unclear.

Understanding the binding mode and interaction of metal complexes including metallo-porphyrin derivatives to G quadruplexes is important for identifying rational biological applications of these molecules, such as the development of anticancer drugs [19]. Various binding modes of the free base and metallo-porphyrin have been reported, including the intercalation of planar porphyrin between two adjacent G-quartets [20–26], weak external binding via electrostatic interactions [27–31], and stacking on the external G-tetrads [32–39]. A recent absorption spectrum, circular dichroism (CD) and fluorescence study reported that a representative of the cationic porphyrin family, *meso*-tetrakis(*N*-methylpyridium-4yl)porphyrin (TMPyP, Fig. 1), exhibited an external binding mode to the $5'G_2T_2G_2TGTG_2T_2G_2$ quadruplex [27].

In this study, the role of the G_8 base at the central loop in the stability of the G-quadruplex was investigated by comparing the thermal stability of various G-quadruplexes at which G_8 base was replaced with adenine (A) or inosine base (I) (Fig. 1). The binding mode of TMPyP to G-quadruplexes having different bases at the central loop was also examined by CD and fluorescence decay profiles.

2. Experimental

2.1. Materials and methods

The thrombin-binding aptamer, $5'G_2T_2G_2TXTG_2T_2G_2$ (X = A, I or G), was purchased from SBS Genetech Co., Ltd (China) and TMPyP from Frontier Scientific Inc. (Logan, Utah). They were dissolved in 5 mM cacodylate buffer, pH 7.0, and used for the measurements without further purification. The concentrations of the oligonucleotide and TMPyP were determined spectrophotometrically using the extinction coefficients of $\varepsilon_{421nm} = 2.26 \times 10^5\ M^{-1}\ cm^{-1}$ and $\varepsilon_{260\ nm} = 1.43 \times 10^5\ M^{-1}\ cm^{-1}$ for TMPyP and oligonucleotide, respectively. The quadruplex was formed by the addition of 100 mM KCl followed by heating at 80 °C for 10 min and annealing overnight at room temperature. The formation of the quadruplex was confirmed by its characteristic CD spectrum. The CD spectra were recorded on a Jasco J-810 spectropolarimeter (Tokyo, Japan) and the absorption spectra were obtained on a Cary 100 spectrophotometer (Palo Alto, CA). The temperature was increased by 0.2 °C every 2 min using a built-in peltier at the temperature-dependent CD intensity that reflects the unfolding of the quadruplex. Fluorescence emission spectrum was recorded on an FS-2 fluorimeter at 20 °C (Sinco, Co. Seoul, Korea) with excitation at 421 nm. The silt widths for both excitation and emission were 10/10 nm. The fluorescence decay profiles were measured on an iHR320 TSCPC system constructed at the Center for Research Facilities, Kongju National University. The sample was excited at 405 nm and the emission was detected at 650 nm.

2.2. Computation

All molecular dynamic (MD) simulations were carried out using the CHARMM program with the provisions for calculating multipolar

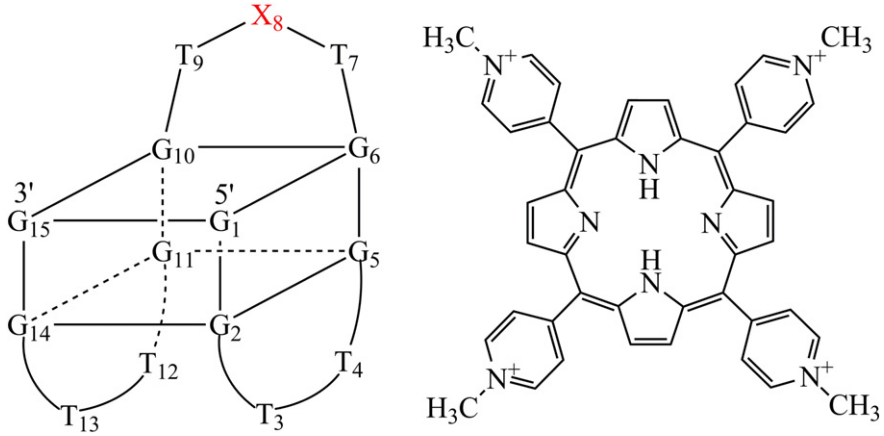

Fig. 1. Schematic diagram of the $5'G_2T_2G_2TXTG_2T_2G_2$ quadruplex (where X = G, A, or I) and the chemical structure of TMPyP.