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Structure–activity relationships of a caged thrombin binding DNA aptamer: Insight gained from molecular dynamics simulation studies

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ABSTRACT

15-mer ssDNA aptamers play a vital role in the inhibition of α-thrombin in the blood clotting mechanism. It is of high importance to explore the structural factors controlling the inhibitory nature of the aptamer. Here we investigated the structure–function relationship of the anti-thrombin aptamer, as well as its 'caged' variant (2-(2-nitrophenyl)-propyl group (NPP)) by molecular dynamics simulations. The stability of the unmodified aptamer at different temperatures is examined in 2 ns all-atom simulations and compared to experiment. The change in structure when introducing the photo-labile caged compound is analyzed, and the regiospecificity of this modification explained on atomic level. Removal of the photo-labile group leads to the reformation of the active aptamer structure from its inactive state. The mechanism for this formation process is a concerted movement of the aptamer backbone and some highly important bases. The binding of the aptamer to thrombin with regard to the 'caged' group is studied in an explicit simulation with the aptamer–thrombin complex and the reason for the binding/unbinding nature of the aptamer shown.

1. Introduction

Quadruplex DNA or RNA molecules play a vital role in structure–activity relationships of telomeres (Blackburn, 1991a,b; Feigon et al., 1996), regulation of gene expression (Rangan et al., 2001), the activity of immunoglobulin switch regions (WeisMan-Shomer and Fry, 1993) and in several other biological processes (Wyatt et al., 1994). Quadruplex DNA has been utilized in drug design as a lead compound and hence is therapeutically important. Synthetic G-quadruplex molecules are known and have been found to interact with certain proteins like, α-thrombin (Bock et al., 1992; Paborsky et al., 1993), STAT3 protein (Jing et al., 2004), nucleolin (Dapic et al., 2003) etc., α-thrombin is a serine protease which plays a key role in the blood clotting cascade (Davie et al., 1991). Malfunctioning of α-thrombin leads to hemorrhage and thrombosis which may cause coronary heart disease and thrombotic disorders. Therefore, there is a need for a specific inhibitor to influence the action of α-thrombin. Bock et al. (1992) screened a pool of synthetic oligonucleotides and discovered that the 15-mer DNA acts as a potent inhibitor of thrombin (Thrombin Binding Aptamer—TBA) (Griffin et al., 1993; Li et al., 1994). This synthetic oligonucleotide is a single-stranded DNA with the sequence of 5′-GGTTGGTGTGGTTGG-3′ (Bock et al., 1992), and known to interact with thrombin (Fig. 1A). Based on the dG quartets three types of quadruplex DNA structures are known (Marathias and Bolton, 2000). TBA is one among these and folds into a chair-like structure in solution (Macaya et al., 1993; Wang et al., 1993a,b). In this type of structure, the residues of the quartet alternate in *syn–anti–syn–anti* fashion as shown in Fig. 1B. The glycosydic torsion and the sugar puckering in the polynucleotide are the important conformational parameters which play a vital role in the strand alignment (Macaya et al., 1993; Wang et al., 1993a,b). TBA[1] folds in such a way that it forms two guanine quartets connected by one central loop containing three residues TGT and two side loops each containing two residues TT (Macaya et al., 1993; Wang et al., 1993a,b) (Fig. 1A). The TGT loop spans the wide groove and the TT loops spans the narrow groove of TBA (Bock et al., 1992; Macaya et al., 1993; Martino et al., 2006). This aptamer binds with high affinity to α-thrombin and possesses high inhibitory potential. The three dimensional structure of the TBA–thrombin complex shows specific interactions between the exosites of thrombin and the loop region (TT loop) of the aptamer (Padmanabhan et al., 1993; Padmanabhan and Tulinsky, 1996; Kelly et al., 1996; Wang et al., 1993a,b). This is the smallest intramolecular G-quadruplex molecule known so far

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[1] *Abbreviations used:* MD, molecular dynamic; TBA, thrombin binding aptamer; cTBA, caged thrombin binding aptamer; uTBA, uncaged thrombin binding aptamer; NPP, 2-(2-nitrophenyl)-propyl group; ssDNA, single-stranded DNA.

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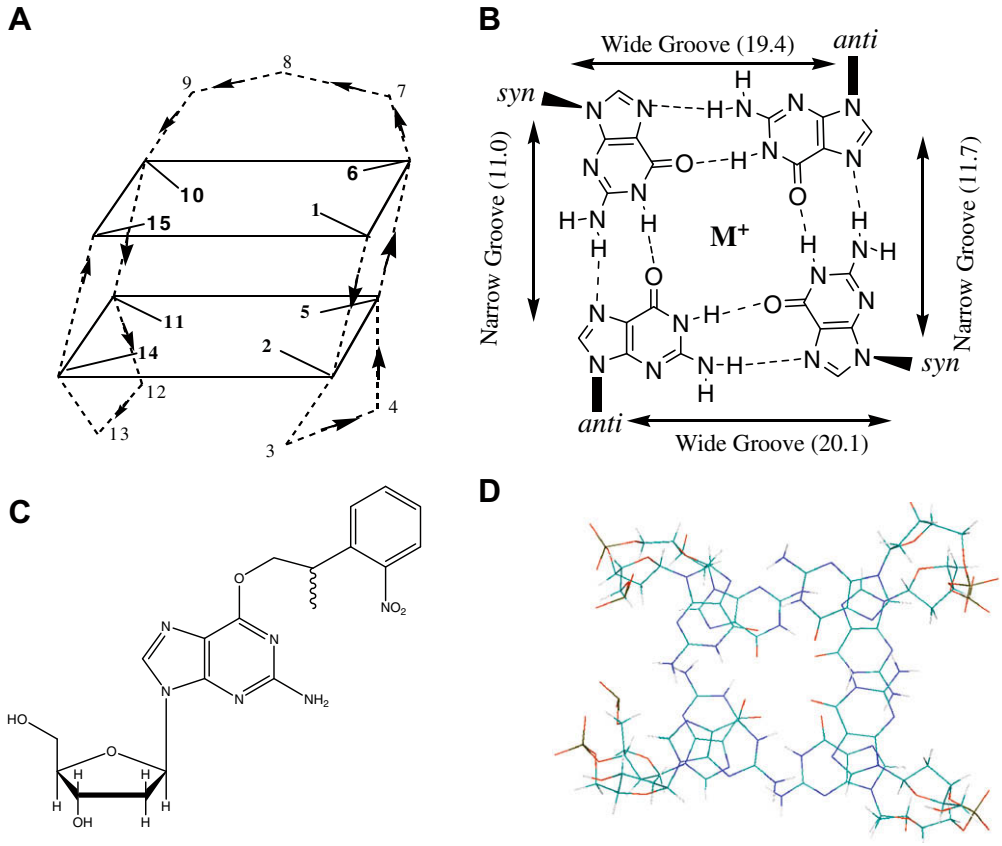

Fig. 1. Schematic diagram of TBA and it is caged guanosine variant. (A) Chair type TBA structure, (B) Hoogsteen hydrogen bonding in the guanine quartet. Groove widths are represented in Å, (C) the photo-labile caged analogue of the guanosine residue dG^{NPP}, (D) presence of G-quadruplex in TBA after 2 ns simulation.

and the quadruplex arrangement of the guanines is important for the TBA binding with thrombin. The formation and stabilization of the quadruplex is influenced by factors like Hoogsteen hydrogen bonding (Fig. 1B) (Sponer and Lankas, 2006), sequence, loop size, strand concentration, etc., The most important factor is the presence of a monovalent cation required to stabilize the negatively charged electrostatic potential created by the carbonyl oxygen of the guanines in the quadruplex core (Hud et al., 1996; Lee, 1990; Ross and Hardin, 1994). Studies were carried out to gain insight into the potassium–quadruplex DNA interaction (Marathias and Bolton, 2000). Here the 1:1 and 2:1 potassium: DNA structures were compared to indicate, how the potassium binding can determine the folding pattern of the DNA.

Several studies have been reported to examine the biophysical and chemical properties of the G-quadruplex (Di Giusto and King, 2004; Peng and Damha, 2007; Sacca et al., 2005) and also showed the sensitive nature of the quadruplex upon chemical modification (Marathias et al., 1999; Sacca et al., 2005). The control of the inhibitory function of the aptamer is a challenging aspect. In this regard, Mayer et al. (Heckel and Mayer, 2005; Mayer et al., 2005) explored a strategy to gain control over the exact temporal and spatial availability of the aptamer's inhibitory function using light as a highly orthogonal trigger signal. They added a photo-labile group in a position which is important for the aptamer's active conformation and the aptamer was temporarily inactivated. The function of the aptamer was recovered by controlled irradiation with light. This technique is referred to as caging (Mayer et al., 2005; Mayer and Heckel, 2006). The light-induced formation of highly ordered nucleic acid secondary structure (Mayer et al., 2005), has motivated us to investigate the structural characteristics and hence deactivation/reactivation mechanism of TBA using classical Molecular Dynamics (MD) simulations. Here we performed MD simulations to understand the unfolding mechanism of TBA at higher temperatures, the origin of the structural instability which leads to inactivation of TBA both in the absence and presence of thrombin and finally the structure and stability of reactivated TBA. The inactivated species contains the caging photo-labile 2-(2-nitrophenyl)-propyl group (NPP) (Mayer et al., 2005) (Fig. 1C) at the 6th guanine residue of TBA (Fig. 1A), whereas the reactivated species does not. This caged residue cannot base-pair as would a normal dG residue do. The reactivation of the caged aptamer was determined by the removal of the photo-labile group from the simulated caged aptamer. Few MD simulation studies on the TBA provide valuable information by correlating the structure of the aptamer to its dynamic function (Lopez de la Osa et al., 2006; Marathias and Bolton, 2000; Mekmaysy et al., 2008; Pagano et al., 2008; Ross and Hardin, 1994; Virno et al., 2007). These MD studies were carried out to improve the biological or biophysical properties of the TBA and none of them determined the factors responsible for the controlled inhibition of TBA in the presence and absence of the photo-labile group. Our investigation of the structural and stability factors responsible for the controlled inhibitory mechanism of TBA provide efficient insight into the regiospecific cageing (i.e. the effect of cageing was observed only when the photo-labile group was added at a specific location of TBA). The main objective of the current report is to address the experimentally unsolved structural issues responsible for the inhibitory mechanism of TBA like,

(A) The structural change of TBA upon addition of the photo-labile group and the corresponding structural behavior of TBA when the photo-labile group is removed via light radiation.
(B) To correlate the structure of caged/uncaged TBA to its inhibitory mechanism.