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Research paper

Interaction of minor groove ligands with G-quadruplexes: Thermodynamic contributions of the number of quartets, T–U substitutions, and conformation

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ABSTRACT

In the presence of specific metal ions, DNA oligonucleotides containing guanine repeat sequences can adopt G-quadruplex structures. In this work, we used a combination of spectroscopic and calorimetric techniques to investigate the conformation and unfolding thermodynamics of the K^+-form of five G-quadruplexes with sequences: d($G_2T_2G_2TGTG_2T_2G_2$), *G2*, d($G_3T_2G_3TGTG_3T_2G_3$), *G3*, their analogs where T is replaced with U, *G2-U* and *G3-U*, and r($G_2U_2G_2UGUG_2U_2G_2$), *rG2*. These G-quadruplexes show CD spectra characteristic of the "chair" conformation (*G2* and *G2-U*), or "basket" conformation (*rG2*); or a mixture of these two conformers (*G3* and *G3-U*). Thermodynamic profiles show that the favorable folding of each G-quadruplex results from the typical compensation of a favorable enthalpy and unfavorable entropy contributions. G-quadruplex stability increase in the following order (in ΔG°_{20}): *rG2* (−1.3 kcal/mol) < G2 < G2-U <*G3-U* (chair) < *G3* (chair) <*G3-U* (basket) < *G3* (basket) (−8.6 kcal/mol), due to favorable enthalpy contribution from the stacking of G-quartets.

We used ITC to determine thermodynamic binding profiles for the interaction of the minor groove ligands, netropsin and distamycin, with each G-quadruplex. Both ligands bind with high exothermic enthalpies (∼−10.8 kcal/mol), 1:1 stoichiometries, and weak affinities (∼8×10^4 M^{-1}). The similarity of the binding thermodynamic profiles, together with the absence of induced Cotton effects, indicates a surface or outside binding mode. We speculate that the top and bottom surfaces of the G-quadruplex comprise the potential MGBL binding sites, where the ligand lies on the surface forming van der Waals interactions with the guanines of the G-quartets and loop nucleotides.

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1. Introduction

DNA is most often regarded as a duplex molecule in which the two self-complementary strands are held together by Watson–Crick base pairs. However, purine rich DNA sequences containing runs of guanines can form four-stranded structures called G-quadruplexes. Such sequences are found at the ends of chromosomes in the so-called telomeric regions and in transcriptional regulatory regions of several important oncogenes [1]. G-quartets are made up of guanine bases in DNA and RNA that associate *via* Hoogsteen hydrogen bonds to form planar G-quartets [2]. Guanine rich sequences can then form inter- or intramolecular G-quadruplexes. These G-quartets stack and form a platform connected by intermediate loops sequences of several nucleotides. Loops play a key role in the overall folding and stability of G-quadruplexes [3–7]; therefore, the length and sequence of these loops can either stabilize or destabilize a G-quadruplex. This is due to the strength of several types of molecular interactions of which the most important ones are hydrogen bonding, base–base stacking within the loops and stacking of the loops onto the G-quartets [8–10].

The conformation of a nucleic acid G-quadruplex is often investigated by circular dichroism spectroscopy, numerous publications have reported the spectral characteristics of folded G-quadruplexes in terms of the antiparallel or parallel arrangements of guanines [11–14]. However, it is the population of syn/anti geometries of guanosine glycosidic torsional angles that determines their CD spectra [11]. Since this geometry is not directly related to the strand orientation, CD spectroscopy can provide only indicative information on strand orientation [15,16]. On the other hand, the measured changes of CD peak positions and ratios of their magnitudes do reflect topological changes of the measured spectra of G-quadruplexes. The appearance of different G-quadruplex conformations and thus the ratio between CD peaks often depends highly on the nature of cations present in the solution (Na^+, K^+) [17–20].

The G-rich DNA sequences with potential to form quadruplexes have been found in a number of important biological processes.

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For instance the defects in proteins that have high affinity for quadruplex DNA can lead to errors in replication, transcription, and recombination as well as to increase in the rates of aging and tumor formation [21]. Telomeric G-quadruplex structures occurring in the single-stranded overhangs at the ends of chromosomes seem to inhibit telomerase [22–25]. There are also reports on G-quadruplexes forming oligonucleotides that act as inhibitors of HIV integrase [26] and thrombin [27]. Molecules have also been discovered that can bind to quadruplex DNA and inhibit proliferation in cancer cells presumably by means of telomerase inhibition [28–30]. These observations have attracted a lot of scientists to study the use of DNA quadruplexes as drugs and as therapeutic targets [1,31]. To increase the efficiency of existing drugs and to develop new ones, a better understanding of interactions between ligands and DNA at the molecular level is needed. It is now well established that this cannot be accomplished by structural or computational studies alone but an insight into the binding thermodynamic properties of these systems is a suitable alternative approach [32–39]. In the absence of high resolution structural studies, thermodynamics can help to determine the type of binding mode by which ligands bind non-covalently to DNA. There are four different ways that ligands bind to DNA: intercalation, minor groove binding, partial insertion and outside or surface binding [33,38]. The binding of the so-called "minor groove binding ligands" (MGBL) to B-DNA is of great interest to researchers and has been extensively studied for several decades now [39–44]. Whereas intercalation between adjacent base pairs is a common phenomenon in duplex DNA, in the case of quadruplexes there seems to be no binding of chromophores between G-quartets [45]. Early NMR and X-ray studies on structurally simple tetramolecular quadruplex–ligand complexes concurred in establishing that the chromophores stack onto the terminal G-quartets of quadruplexes [46]. One example is the crystal structure of the 1:1 complex of a disubstituted acridine ligand with the dimeric Oxytricha nova telomeric quadruplex showing this G-quartet end-stacking binding mode [47,48]. However, this may not be the only way ligands can interact with G-quadruplexes, as indicated below. G-quadruplexes have already been extensively studied by our laboratory. One such investigation is of the thrombin aptamer d($G_2T_2G_2TGTG_2T_2G_2$), that inhibits thrombin-catalyzed fibrin clot formation [9,49–54]. One focus of the studies was to modify the sequence of the loops to determine their energetic, ion and water binding contributions. Very recent investigations shows that distamycin and its derivatives interacts with the grooves of the $[d(TG_4T)]_4$ quadruplex [55,56]. In light of these results, we have decided to investigate whether the MGBL netropsin and distamycin (Scheme 1) can bind to the thrombin aptamer and what is the nature of their binding. We are also interested on how additional G-quartets, the substitutions of thymine with uracil, and conformational changes are influencing the binding of MGBL.

2. Materials and methods

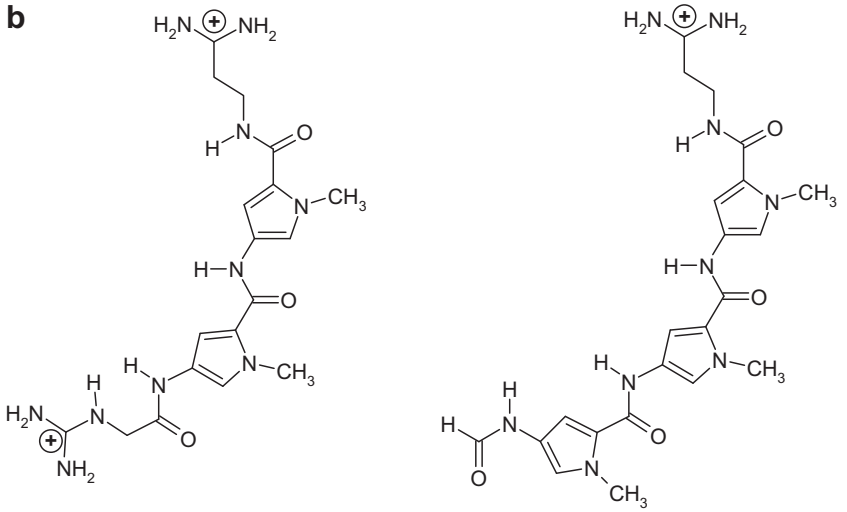
2.1. Materials

The oligonucleotides (ODNs) and their designations: d($G_2T_2G_2TGTG_2T_2G_2$), *G2*; d($G_2U_2G_2UGUG_2U_2G_2$), *G2-U*; d($G_3T_2G_3TGTG_3T_2G_3$), *G3*; d($G_3U_2G_3UGUG_3U_2G_3$), *G3-U*; r($G_2U_2G_2UGUG_2U_2G_2$),

a

d(GGTTGGTGTGGTTGG)	"*G2*"
d(GGUUGGUGUGGUUGG)	"*G2-U*"
r(GGUUGGUGUGGUUGG)	"*rG2*"
d(GGGTTGGGTGTGGGTTGGG)	"*G3*"
d(GGGUUGGGUGUGGGUUGGG)	"*G3-U*"

b



Netropsin (*Net*) Distamycin (*Dst*)

Scheme 1. (a) The 5′–3′ sequences and labels of G-quadruplexes used in these studies. (b) Two dimensional structures of MGBL ligands.

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