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Molecular dynamics analysis of stabilities of the telomeric Watson-Crick duplex and the associated i-motif as a function of pH and temperature

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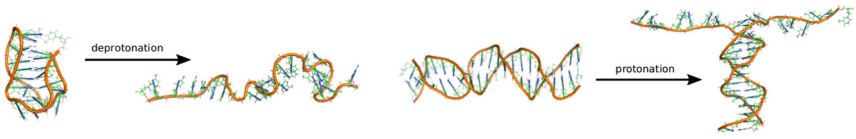
HIGHLIGHTS

- Telomeric i-motif is stabilized by strong hydrogen bonds at acidic pH.
- The Watson-Crick telomeric duplex loses hydrogen bonds between guanines and protonated cytosines at acidic pH.
- At neutral pH the telometic i-motif unfolds spontaneously within 40 ns of unbiased simulations.
- At acidic pH the telomeric Watson-Crick duplex does not spontaneously unwraps to single strands within the simulation time.

GRAPHICAL ABSTRACT



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ABSTRACT

This work deals with a molecular dynamics analysis of the protonated and deprotonated states of the natural sequence d[(CCCTAA)$_3$CCCT] of the telomeric DNA forming the intercalated i-motif or paired with the sequence d[(CCCTAA)$_3$CCCT] and forming the Watson-Crick (WC) duplex. By utilizing the amber force field for nucleic acids we built the i-motif and the WC duplex either with native cytosines or using their protonated forms. We studied, by applying molecular dynamics simulations, the role of hydrogen bonds between cytosines or in cytosine-guanine pairs in the stabilization of both structures in the physiological fluid. We found that hydrogen bonds exist in the case of protonated i-motif and in the standard form of the WC duplex. They, however, vanish in the case of the deprotonated i-motif and protonated form of the WC duplex. By determining potentials of mean force in the enforced unwrapping of these structures we found that the protonated i-motif is thermodynamically the most stable. Its deprotonation leads to spontaneous and observed directly in the unbiased calculations unfolding of the i-motif to the hairpin structure at normal temperature. The WC duplex is stable in its standard form and its slight destabilization is observed at the acidic pH. However, the protonated WC duplex unwraps very slowly at 310 K and its decomposition was not observed in the unbiased calculations. At higher temperatures (ca. 400 K or more) the WC duplex unwraps spontaneously.

1. Introduction

Telomeres are terminal parts of human chromosomes and they protect the ends of the chromosomes from deterioration or from fusion with neighboring chromosomes. Telomeres are responsible for genome integrity, cellular aging and perhaps cancer [1,2]. The telomeric DNA consists of highly repetitive short sequences (approximately 2500 times in humans) of the nucelotides (TTAGGG):(CCCTAA) [1,3,4]. Of course, most of telomeric DNA is double stranded except for the terminal part where the 3′ region (overhang) of the guanine rich strand is single-

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stranded and forms the so-called T-loop [5].

Both the guanine rich (G-rich) and cytosine rich (C-rich) strands are able to form, at least in vivo, unusual secondary structures. The G-rich strand can form various G-quadruplex structures in which four guanines form planar quartets [4,6,7]. The G-quadruplex is normally observed at neutral pH in the presence of cations (Na^+, K^+). Its large planar and aromatic architecture is well suited for binding to high affinity large polyaromatic ligands via π-π stacking [4,8,9]. The C-rich strand, as found by Gehring et al. [10], can form intercalated, quadruple-helical structures under acidic conditions. The structure (i-motif) consists of two parallel duplexes combined in an antiparallel fashion by forming intercalated hemiprotonated cytosine-cytosine base pairs. Because i-motif can reversibly fold and unfold by altering the pH it has been extensively studied as nanomachines [11] or sensors to map pH changes in living cells [12]. From the literature data it can be concluded that stable i-motif structure exists only at acidic pH; its formation at neutral pH needs either the reduced temperature (4 °C) [13] or presence of cations (Ag^+) [14]. Under mildly acidic conditions the i-motif structure has been shown to compete with duplex formation [15].

Tumor cells express telomerase, an enzyme that stabilizes chromosome ends by adding tandem telomeric DNA repeats to the 3′-overhangs, which is essential for their indefinite proliferative capacities. G-quadruplex formation can block telomerase activity [16]. Many compounds that can stabilize G-quadruplex structure and block telomerase activity have been reported and are considered as anticancer agents [4,9]. However, it remains unclear whether stabilization of i-motif structure can block telomerase activity [7]. Moreover, there are still limited published investigations into the biological function of i-motif DNA and relatively few examples of i-motif biding ligands [3,15,17–19]. Chen et al. [7]. demonstrated that stabilization of i-motif DNA by carboxylated CNT can block telomerase activity both in vitro and in vivo, and induced telomere uncapping and production of DNA-damage response and subsequent cell growth cessation. They suggested that i-motif may not directly be responsible for telomerase inhibition but its formation can make the complementary G-rich DNA to form G-quadruplexes in telomeres. However, they underlined that the precise mechanism for the carboxylated CNT induced intracellular telomerase inhibition and the consequent events remain not completely understood.

Theoretical analyses of the i-motif DNA and its stabilization by various factors like presence of ligands or pH conditions are not very often in the literature. The papers by Smiatek et al. [20–22] or a few other authors [23–25] provide knowledge about unfolding/folding dynamics and themodynamics mainly as a function of pH. This contribution, based on molecular dynamics simulations, also discusses the problems related to the stability of the i-motif as a function of pH but we analyze this process in reference to the Watson-Crick (WC) duplex structure from which the i-motif can be formed in vivo. We also focus on the role of hydrogen bonds in the stabilization of the i-motif by acidic pH, which is an important starting point in the study of other ligands, particularly carbon nanotubes, stabilizing the i-motif structure.

2. Methods

The structure of the i-motif considered in this study has been derived from the 5′E sequence d[$CCCTA_2$5m$CCCTA_2CCCUA_2CCCT$] published by Phan et al. [1,14] (pdb ID 1EL2). It was utilized for an analysis of the natural sequence d[$(CCCTAA)_3CCCT$] of the vertebrate telomere. Our analysis is also focused on the d[$(CCCTAA)_3CCCT$] sequence which is obtained by simple replacement of the uracil and 5 m-cytosine in d[$CCCTA_2$5m$CCCTA_2CCCUA_2CCCT$] by thymine and cytosine, respectively. The studied here Watson-Crick (WC) duplex was build of the same sequence with the complementary d[$AGGG(TTAGGG)_3$] strand, as analyzed in [1].

All calculations were based on the natural sequence d[$(CCCTAA)_3CCCT$] and the Amber force field for nucleic acids [26] ff99 with the bsc1 modifications [27]. The constructions of the topology and of the force field were done using tleap program from the AmberTools16 package. The input scripts for tleap launched commands for using tip3p water model, suitable amounts of Na^+ and Cl^- for production of 0.145 mol L^{-1} ionic strength of solution, to balance protonation of cytosines in the case of acidic conditions and to balance the charge of phosphate backbones. All calculations were done using lammps molecular dynamics engine [28] thus the prmtop and inpcrd files from the tleap output had to be converted into lammps format. For that purpose the acpype script [29] had been utilized in order to convert the prmtop and inpcrd files into gromacs [30] files, which represent a more human readable format. Next, the self-designed procedures were applied in order to convert these data into lammps input scripts. All those conversions were verified by a direct comparison of all components of the potential energy of some probe structure obtained from amber tools/sander and lammps runs.

The calculations were carried out in NPT ensemble using 2 fs integration timestep and 12 Å cutoff distance for interatomic interactions was applied. Periodic boundary conditions were applied in all directions and the electrostatic interactions were summed by applying the particle-particle particle-mesh solver, as implemented in lammps. The pressure and temperature were controlled using the Nose − Hoover barostat. The sizes of the simulation boxes were ca. 50 × 50 × 200Å and the number of water molecules were ca. 15,000–17,000 depending on the system being studied. The water molecules were kept rigid using the shake algorithm.

The potential of mean force for each system was determined using steered molecular dynamics based on Jarzynski inequality [31,32]. Some number (from 30 to 40) of various snapshots taken from the canonical distribution were taken as the starting configurations. The O5′ atoms in each structure were immobilized using a spring force while the O3′ atom was connected to another spring moving with a constant velocity in the z direction. During the unwrapping process (steered dynamics) the instantaneous forces acting on O3′ atom were monitored and used for the numerical integration in order to obtain the effective works done against these forces, *w*. Next, the obtained dependencies of *w* vs. *d*(O5′–O3′) distance were used for averaging in order to obtain the potential of mean force. According to refs. [31]and [32] the exponential averages were used as measures of the *pmf*, i.e.

$$pmf = -kT \ln \left\langle \exp\left(-\frac{w}{kT}\right) \right\rangle \quad (1)$$

3. Results and discussion

3.1. Equilibrium structures at neutral and acidic pH

The telomeric i-motif is believed to be stable at acidic pH, i.e. when some of the cytosines become protonated [1,3,14]. Thus, the starting point of our studies was the analysis of i-motifs formed at the neutral and the acidic pH. For comparison we also studied the competitive form, i.e. the Watson-Crick duplex formed due to the presence of the guanine rich sequence which is complementary to cytosine bases strand. Also its protonated form was studied, i.e. the structure in which half of the cytosines within the strand were protonated. All these structures are schematically shown in Fig. 1 together with the schemes of bases pairings in these four cases.

The molecular dynamics simulations of these structures without any extra constraints led to the equilibrium structures shown in Fig. 2. In the cases of the i-motifs the calculations were carried out at the constant temperature 310 K starting from the spatial structures taken from the pdb file 1EL2. The constant temperature – constant pressure (1 atm) calculations lasted for 20 ns. The initial WC structure was obtained from the make-na web service (http://structure.usc.edu/make-na/) and it was subjected to equilibration for 2 ns. The final structure shown in Fig. 2 was obtained after additional 20 ns calculations at constant