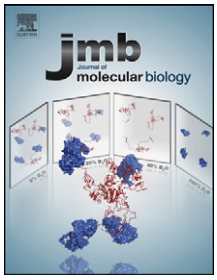

POT1–TPP1 Binding and Unfolding of Telomere DNA Discriminates against Structural Polymorphism

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Abstract

Telomeres are nucleoprotein complexes that reside at the ends of linear chromosomes and help maintain genomic integrity. Protection of telomeres 1 (POT1) and TPP1 are telomere-specific proteins that bind as a heterodimer to single-stranded telomere DNA to prevent illicit DNA damage responses and to enhance telomerase-mediated telomere extension. Telomere DNA is guanosine rich and, as such, can form highly stable secondary structures including G-quadruplexes. G-quadruplex DNA folds into different topologies that are determined by several factors including monovalent ion composition and the precise sequence and length of the DNA. Here, we explore the influence of DNA secondary structure on POT1–TPP1 binding. Equilibrium binding assays reveal that the POT1–TPP1 complex binds G-quadruplex structures formed in buffers containing Na^+ with an affinity that is fivefold higher than for G-quadruplex structures formed in the presence of K^+. However, the binding of the second heterodimer is insensitive to DNA secondary structure, presumably due to unfolding resulting from binding of the first POT1–TPP1. We further show that the rate constant for POT1–TPP1-induced unfolding of DNA secondary structure is substantially faster for G-quadruplex topologies formed in the presence of Na^+ ions. When bound to DNA, POT1–TPP1 forms complexes with similar CD spectra and enhances telomerase activity for all DNA substrates tested, regardless of the substrate secondary structure or solution monovalent ion composition. Together, these data indicate that binding of POT1–TPP1 unfolds telomere secondary structure to assist loading of additional heterodimers and to ensure efficient promotion of telomerase-mediated extension.

Introduction

Telomeres are nucleoprotein complexes that reside at the ends of linear chromosomes and are composed of repeating G-rich DNA sequences [1–3]. In mammals, telomere DNA is a repetitive, hexameric sequence of TTAGGG that extends for thousands of bases before ending in shorter single-stranded (ss) DNA (ssDNA) overhangs [1,2,4]. The G-rich telomere DNA is capable of forming stable, secondary structures that include G-quadruplexes. G-quadruplex structures are composed of stacks of G-tetrads, each of which contains four guanines that are organized in a planar arrangement stabilized by a cyclic Hoogsteen hydrogen bonding network [5–7].

Analysis of G-quadruplexes assembled *in vitro* has revealed that the central cavities formed within the structures are occupied by monovalent cations, which neutralize the electrostatic repulsion generated by the inwardly pointing keto oxygens of the guanine bases [8]. The solvent monovalent ion type is well known to influence G-quadruplex stability and folding topology (for review, see Ref. [9]). Similarly, the nucleotide (nt) sequence and length adjacent to G-quadruplex structures formed within telomere DNA sequence can also contribute to structural

polymorphism (see Ref. [8]). In all of these cases, the G-tetrad interaction is maintained, but the orientation of the intercalating nucleotides forms strands that can be oriented in parallel, antiparallel, or hybrid-type (containing both parallel and antiparallel strands) configurations. Importantly, these different G-quadruplex structures can also display functional diversity with respect to ligand binding and specificity [10,11]. In addition to a wealth of data characterizing G-quadruplex structure, stability, and specificity *in vitro* (see Ref. [12]), the existence of G-quadruplexes has been confirmed in the telomeres of human cells, within the macronuclei of ciliates, and in *Xenopus laevis* egg extract [13–17]. However, the mechanisms that proteins and enzymes associate with telomere DNA and the ways they alter its ability to form stable alternative structures are not well understood.

Telomere DNA is synthesized by a unique ribonucleoprotein complex called telomerase, an enzyme that compensates for the inability of replicative polymerases to fully extend the ends of linear chromosomes [18,19]. The ssDNA overhang of telomeres provides the substrate for telomerase; therefore, DNA secondary structures that form within this region provide obstacles to telomerase-mediated replication. For example, G-quadruplex structures may function to regulate telomerase activity, primarily by inhibiting telomerase extension [7,20–23].

A set of six specialized proteins, collectively termed shelterin, associates with telomere DNA [24–27]. Along with telomerase, the shelterin complex contributes to maintaining the proper structure, function, and overall integrity of telomeres [27–29]. Protection of telomeres 1 (POT1) protein, along with its binding partner TPP1, binds ss telomere DNA with high affinity. DNA interactions are orchestrated through the N terminus of POT1, while its C-terminal domain is necessary for TPP1 interaction [30–33]. TPP1 is responsible for recruiting telomerase to the telomere and, together with POT1, enhances telomerase processivity by decreasing the rate constant for dissociation from telomere DNA and increasing the rate constant for telomerase extension activity [34–41].

The x-ray crystal structure of the POT1 DNA-binding domain in complex with ssDNA reveals that the protein interacts with 10 nts of telomere DNA in an extended single-strand conformation [42]. The POT1 domain is divided into two oligosaccharide-oligonucleotide binding (OB) folds with the N-terminal domain recognizing a full hexamer of telomere DNA (TTAGGG), while the C-terminal OB-fold binds to the adjacent four nts (TTAG). On longer ssDNA substrates, this recognition sequence is maintained, as multiple POT1–TPP1 proteins coat the ssDNA substrate with a protein binding to every two hexamer repeats [43]. Of the 12 nts representing two complete hexameric telomere sequences, the last two guanosines are not necessary for POT1–TPP1 binding and recognition [33,44]. These two guanosines do, however, contribute dramatically to the secondary structure of unbound DNA, as they are necessary for G-quadruplex formation.

Although physiological tracts of ss telomere DNA are ~50–200 nts in the cell [45], most *in vitro* studies of DNA binding and telomerase recruitment by POT1–TPP1 protein to date have been limited to short ss oligonucleotide substrates (<18 nts) for understanding DNA-binding and telomerase recruitment properties of POT1–TPP1 protein [34,35]. As telomere ssDNA needs at least 22 nts to form intramolecular G-quadruplex structures (see Ref. [46]), less is known regarding the influence of telomere DNA secondary structure on POT1–TPP1 binding events and on telomerase recruitment. For example, recent data demonstrate that POT1–TPP1 induces continuous unfolding of G-quadruplex structures in telomere DNA [47], yet the interaction between protein and DNA is largely dependent on the G-quadruplex topology that is formed [10].

In this report, we interrogate the impact that DNA secondary structure has on multiple POT1–TPP1 binding events. Our data reveal that secondary structure, including G-quadruplexes, impairs binding of POT1–TPP1 to telomere DNA. Furthermore, we demonstrate that POT1–TPP1 binds with variable affinities to polymorphic G-quadruplex structures formed in Na^+ *versus* K^+. After the initial loading of a single POT1–TPP1 heterodimer, however, binding of subsequent proteins occurs with similar affinity for all 24-nt substrates investigated, regardless of the secondary structure of the unbound DNA. Consistent with the differential sensitivity to G-quadruplex topology observed for equilibrium binding affinity, time-resolved circular dichroism (CD) spectroscopy demonstrates that POT1–TPP1 unfolds G-quadruplexes formed in Na^+ significantly faster than G-quadruplex structures formed in K^+. Finally, we demonstrate that the telomerase activity is impaired equally by different G-quadruplex topologies, regardless of monovalent ion composition, and the inclusion of POT1–TPP1 circumvents this obstacle. Together, our results reveal intrinsic, biochemical features of molecular recognition that govern the interaction of POT1–TPP1 with ssDNA. This information is important for understanding the *in vivo* function of POT1–TPP1 and for providing insights into its central role in telomere maintenance.

Results

An intrinsic feature of ss G-rich telomere DNA is the ability to form highly stable G-quadruplex structures. The ssDNA-binding heterodimer POT1–TPP1 must necessarily disrupt this stable structure and subsequently interact with telomerase in order to orchestrate proper telomere synthesis. To understand this essential biological function, it is important to establish how POT1–TPP1 associates with different intramolecular