Unfolding of tetraplex structure of mouse telomeric DNA by the interaction with mouse telomeric DNA binding protein Pot1

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ABSTRACT

We analyzed the structural properties of mouse telomeric DNA sequence, Tel3.5: 5'-AGGG(TTAGGG)3'-3', and nontelomeric DNA sequence, T22: 5'-T22-3', and examined the interaction with a single-stranded telomeric DNA-binding domain of mouse telomeric DNA-binding protein Pot1 (mPot1DBD). T22 did not form any higher-order structure, but Tel3.5 formed antiparallel tetraplex structure in the presence of Na+. The antiparallel tetraplex of Tel3.5 became unfolded upon the interaction with mPot1DBD. Considering that the antiparallel tetraplex is known to inhibit telomerase-mediated telomere elongation, we conclude that the ability of Pot1 to unfold the antiparallel tetraplex of the telomeric DNA is required for regulation of telomerase-mediated telomere elongation.

INTRODUCTION

Telomere, the nucleoprotein complex located at the ends of linear eukaryotic chromosomes, is essential for maintaining chromosomal stability to inhibit DNA degradation, and achieving accurate and complete replication of the chromosomal ends.1 In most eukaryotes, telomeric DNA consists of tandemly repeated sequences, one strand being rich in guanines.1 The G-rich strand terminates with a single-stranded 3′ overhang.1 The G-cluster single-stranded telomeric DNA sequences have the ability to form defined tetraplex structures.2 The fundamental structural unit of the tetraplex structure, G-quartet, is composed of four guanines aligned with each other in a square planar configuration.3 Each guanine interacts with each of the two adjacent guanines through two non-Watson-Crick GG base pair hydrogen bonds.3 Successive layers of the G-quartets stack on each other to form folded tetraplex structures, which are stabilized by cations such as Na+ and K+.2

Mouse Pot1 (mPot1) is a G-rich single-stranded telomeric DNA-binding protein.1,4 The N-terminal region of mPot1 is a single-stranded telomeric DNA-binding domain (mPot1DBD).1 A ten-base single-stranded DNA, 5′-TTAGGGTGGTAG-3′, is the minimum telomeric DNA sequence for the specific binding of mPot1DBD.

In the present study, we analyzed the structural properties of mouse telomeric DNA sequence, Tel3.5: 5′-AGGG(TTAGGG)3′-3′, and control nontelomeric DNA sequence, T22: 5′-T22-3′, by circular dichroism (CD) spectroscopy. We also examined the interaction with mPot1DBD by CD spectroscopy and fluorescence resonance energy transfer (FRET) analysis.

MATERIALS AND METHODS

We synthesized 22-mer DNA oligonucleotides, Tel3.5 and T22, on a DNA synthesizer and purified them with a reverse-phase HPLC by the standard procedure. We purchased 22-mer dye-labeled DNA oligonucleotides, FTel3.5D: 5′-Fam-AGGG(TTAGGG)3-Dabcyl-3′ and FT22D: 5′-Fam-T22-Dabcyl-3′ (where 6-carboxy-fluorescein (Fam) is a fluorophore and Dabcyl is a quencher), from Thermo Electron Corp. (Germany). The DNA solutions in the experimental buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 1 mM DTT) were heated at 95 °C for 10 min, followed by gradually cooling to room temperature, and were then kept at room temperature for at least 2 days before the experiments. The gene fragment coding for mPot1DBD was cloned into Escherichia coli expression vector, fused with the glutathione S-transferase gene. The expression plasmid was transferred into E. coli BL21 cells. The mPot1DBD protein expressed from the cells was purified to homogeneity. CD spectra were recorded on a Jasco J-725 spectropolarimeter interfaced with a microcomputer. FRET measurements were carried out on a Jasco FP-6300 spectrofluorometer interfaced with a microcomputer.

RESULTS AND DISCUSSION

Fig. 1a shows CD spectra of T22 and Tel3.5 at 25 °C in 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 1 mM DTT. The CD spectrum of T22 exhibits a positive peak at 276 nm and a negative one around 250 nm. This type of spectrum is typical of unstructured single-stranded DNA without intrastrand base pairing and base stacking.5 This result shows that T22 does not form any higher-order structure under the present experimental condition. On the other hand, the CD spectrum of Tel3.5 exhibits a positive peak at 295 nm and a negative one at 264 nm. This type of spectrum is typical of an antiparallel tetraplex DNA consisting of an intramolecular antiparallel four-stranded
structure, indicating that Tel3.5 forms antiparallel tetraplex DNA under the present experimental condition.

To examine the properties of the interaction between mPot1DBD and the antiparallel tetraplex of Tel3.5, the CD spectral change of Tel3.5 upon the addition of mPot1DBD was measured (Fig. 1b). The ellipticity of the positive peak at 295 nm was decreased upon the addition of mPot1DBD in a concentration-dependent manner. This result indicates that mPot1DBD has the ability to decrease the amount of the antiparallel tetraplex DNA.

To reveal why the addition of mPot1DBD reduced the amount of the antiparallel tetraplex DNA, the structural change of FTe3.5D upon the addition of mPot1DBD was examined by FRET analysis (Fig. 2). Because the intramolecular folding of the antiparallel tetraplex structure of FTe3.5D should bring the fluorophore (Fam) and the quencher (Dabcyl) into close enough proximity for energy transfer, FRET-mediated quenching between the fluorophore and the quencher was observed. Thus, FTe3.5D without mPot1DBD showed low fluorescence due to FRET-mediated quenching. The addition of mPot1DBD enhanced the intensity of Fam emission at 520 nm in a concentration-dependent manner. The increase in the fluorescence intensity corresponds to the increase in the distance between the fluorophore and the quencher, indicating unfolding of the antiparallel tetraplex structure of FTe3.5D. This result is consistent with the decrease in the amount of the antiparallel tetraplex of Tel3.5, observed as the CD spectral change upon the addition of mPot1DBD (Fig. 1b). This result indicates that mPot1DBD has the ability to unfold the antiparallel tetraplex DNA.

CONCLUSION

The antiparallel tetraplex structure of mouse telomeric DNA became unfolded upon the interaction with mPot1DBD. Considering that the antiparallel tetraplex is known to inhibit telomerase-mediated telomere elongation, we conclude that the ability of Pot1 to unfold the antiparallel tetraplex of the telomeric DNA is required for regulation of telomerase-mediated telomere elongation.

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