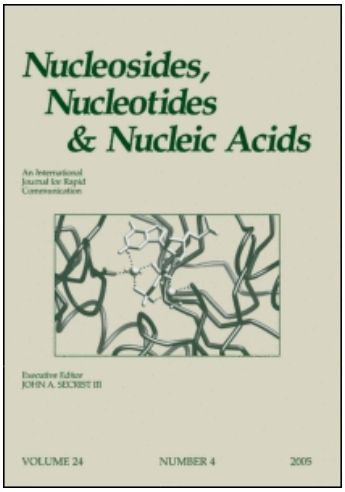


**Nucleosides, Nucleotides and Nucleic Acids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

# Fission Yeast Telomeric DNA Binding Protein Pot1 Has The Ability To Unfold Tetraplex Structure of Telomeric DNA

Hidetaka Torigoe[a]
[a] Department of Applied Chemistry, Faculty of Science, Tokyo University of Science, Tokyo, Japan

**To cite this Article** Torigoe, Hidetaka(2007) 'Fission Yeast Telomeric DNA Binding Protein Pot1 Has The Ability To Unfold Tetraplex Structure of Telomeric DNA', Nucleosides, Nucleotides and Nucleic Acids, 26: 10, 1255 — 1260
**To link to this Article: DOI:** 10.1080/15257770701528230
**URL:** http://dx.doi.org/10.1080/15257770701528230

*Nucleosides, Nucleotides, and Nucleic Acids*, 26:1255–1260, 2007

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770701528230

Taylor & Francis
Taylor & Francis Group

# FISSION YEAST TELOMERIC DNA BINDING PROTEIN POT1 HAS THE ABILITY TO UNFOLD TETRAPLEX STRUCTURE OF TELOMERIC DNA

**Hidetaka Torigoe** □ *Department of Applied Chemistry, Faculty of Science, Tokyo University of Science, Tokyo, Japan*

□ *To understand the regulation mechanism of fission yeast telomeric DNA, we analyzed the structural properties of 4Gn: d(G$_n$TTAC)$_4$ (n = 3, 4) and their interaction with the single-stranded telomeric DNA binding domain of telomere-binding protein Pot1 (Pot1DBD). 4G4 adopted only an antiparallel tetraplex in spite of a mixture of parallel and antiparallel tetraplexes of 4G3. The antiparallel tetraplex of 4G4 became unfolded upon the interaction with Pot1DBD. Considering that the antiparallel tetraplex inhibits telomerase-mediated telomere elongation, we conclude that the ability of Pot1 to unfold the antiparallel tetraplex is required for telomerase-mediated telomere regulation.*

**Keywords** Fission yeast telomeric DNA; antiparallel triplex; structure; unfolding



## INTRODUCTION

The telomere is the nucleoprotein complex located at the ends of linear eukaryotic chromosomes.[1] It is essential for maintaining chromosomal stability to inhibit DNA degradation, and achieving complete replication of the chromosomal ends.[1] In most eukaryotes, telomeric DNA consists of tandemly repeated sequences, one strand being rich in guanines. The G-rich strand terminates with a single-stranded 3′ overhang. The G-cluster 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 guanine residues aligned with each other in a square planar configuration.[2–4] Each guanine interacts with each of two adjacent guanines through two non-Watson-Crick base pair hydrogen bonds.[2–4] Successive layers of the G-quartets stack on each other

I would like to thank Ms. Ayako Furukawa for her technical assistance. This research was supported in part by Grants-in-Aid for Scientific Research (B) (16390083) and Priority Areas (17012022 and 17053027) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Address correspondence to Hidetaka Torigoe, Department of Applied Chemistry, Faculty of Science, Tokyo University of Science, 1-3 Kagurazaka, Shinjuku-ku, Tokyo 162-8601, Japan. E-mail: htorigoe@ch.kagu.tus.ac.jp

to form the tetraplex structure, which is stabilized by cations such as $Na^+$ or $K^+$.[5] Although the tetraplex structures of the perfectly repetitive telomeric DNA sequences from *Tetrahymena*,[6] *Oxytricha*,[7] and vertebrates[8] have been well-characterized, few studies have been reported on the tetraplex formation by the irregularly repetitive telomeric DNA sequences from budding[9] and fission yeasts.[10]

Tetraplex DNA binding proteins have been identified in several organisms, and some DNA-binding proteins have the ability to unfold the tetraplex structure. No fission yeast proteins have been reported to bind with or unfold the tetraplex structure. Fission yeast Pot1 is a single-stranded telomeric DNA-binding protein.[11] The N-terminal region of the fission yeast Pot1 is a single-stranded telomeric DNA-binding domain (Pot1DBD).[12] Previous studies revealed that overexpression of fission yeast Pot1 led to modest but significant telomere lengthening in vivo.[13] On the other hand, tetraplex formation is known to inhibit the telomerase-mediated telomere elongation.[14] We hypothesize that Pot1DBD may promote telomerase-mediated telomere elongation by unfolding the tetraplex, if the fission yeast telomeric DNA forms the tetraplex. In the present study, we analyzed the structural properties of fission yeast telomeric DNA sequences, and their interaction with Pot1DBD.



## RESULTS AND DISCUSSION

To reveal the structural properties of fission yeast telomeric DNA sequences, 4Gn: $d(G_nTTAC)_4$ (n = 3 and 4), and control nontelomeric DNA sequences, Tn: $dT_n$ (n = 28 and 32), we measured CD spectra at 25°C and pH 7.5 in 20 mM Tris-HCl, 150 mM NaCl, and 1 mM DTT (Figure 1). The CD spectra of both T32 and T28 exhibit a positive peak at 276 nm and a

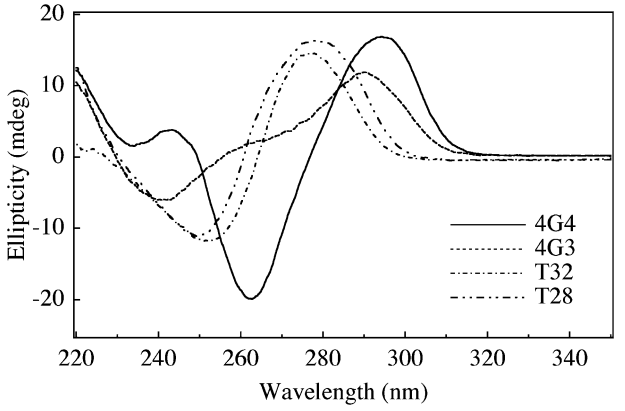


**FIGURE 1** CD spectra of 4Gn: $d(G_nTTAC)_4$ (n = 3, 4) and Tn: $dT_n$ (n = 28, 32) at 25°C and pH 7.5 in 20 mM Tris-HCl, 150 mM NaCl and 1 mM DTT. The DNA concentration was 3 $\mu$M.

negative one at 250 nm, which is typical of unstructured single-stranded DNA. This result shows that neither T32 nor T28 forms any higher-order structure under the present experimental conditions. On the other hand, the CD spectrum of 4G4 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,[15] indicating that 4G4 forms intramolecular antiparallel tetraplex DNA under the present experimental conditions with 150 mM sodium cation. In the CD spectrum of 4G3, a small positive peak around 260 nm and a negative one at 240 nm appear in addition to a large positive peak near 290 nm corresponding to an antiparallel tetraplex DNA.[15] The positive peak around 260 nm and the negative one at 240 nm are ascribed to a parallel tetraplex DNA consisting of a parallel four-stranded tetrameric structure.[15] Thus, the conformation of 4G3 is a mixture of parallel and antiparallel tetraplex DNA under the present experimental conditions with 150 mM sodium cation.

To examine the properties of the interaction between Pot1DBD and the antiparallel tetraplex of 4G4, the CD spectral change of 4G4 upon the addition of Pot1DBD was measured at 25°C and pH 7.5 in 20 mM Tris-HCl, 150 mM NaCl, and 1 mM DTT (Figure 2a). The ellipticity of the positive peak at 295 nm was decreased upon the addition of Pot1DBD in a concentration-dependent manner. The decrease in the ellipticity at 295 nm corresponds to the decrease in the amount of the antiparallel tetraplex on the interaction with Pot1DBD. This result indicates that Pot1DBD has the ability to decrease the amount of the antiparallel tetraplex of 4G4. On the other hand, the addition of Pot1DBD did not induce any significant change in the CD spectrum of T32 (Figure 2b). This result shows that Pot1DBD did not have any effect on the structure of the single-stranded DNA, which does not bind with Pot1DBD.

To reveal why the addition of Pot1DBD reduced the amount of the antiparallel tetraplex DNA (Figure 2a), the structural change of 4G4 upon the addition of Pot1DBD was examined by fluorescence resonance energy transfer (FRET) analysis[16] at 25°C and pH 7.5 in 20 mM Tris-HCl, 150 mM NaCl, and 1 mM DTT (Figure 3a). The fluorophore, 6-carboxyfluorescein (Fam), and the quencher, dabcyl, were attached to the 5′ and 3′ termini of 4G4, respectively, to give the dual-labeled F4G4D. Because the intramolecular folding of the antiparallel tetraplex structure of F4G4D in the presence of the sodium cation should bring the fluorophore and the quencher into close enough proximity for energy transfer, FRET-mediated quenching between the fluorophore and the quencher was observed. Thus, F4G4D showed low fluorescence due to FRET-mediated quenching (Figure 3a). The addition of Pot1DBD enhanced the intensity of Fam emission at 520 nm in a concentration-dependent manner (Figure 3a). The increase in the fluorescence intensity corresponds to the increase in the distance between

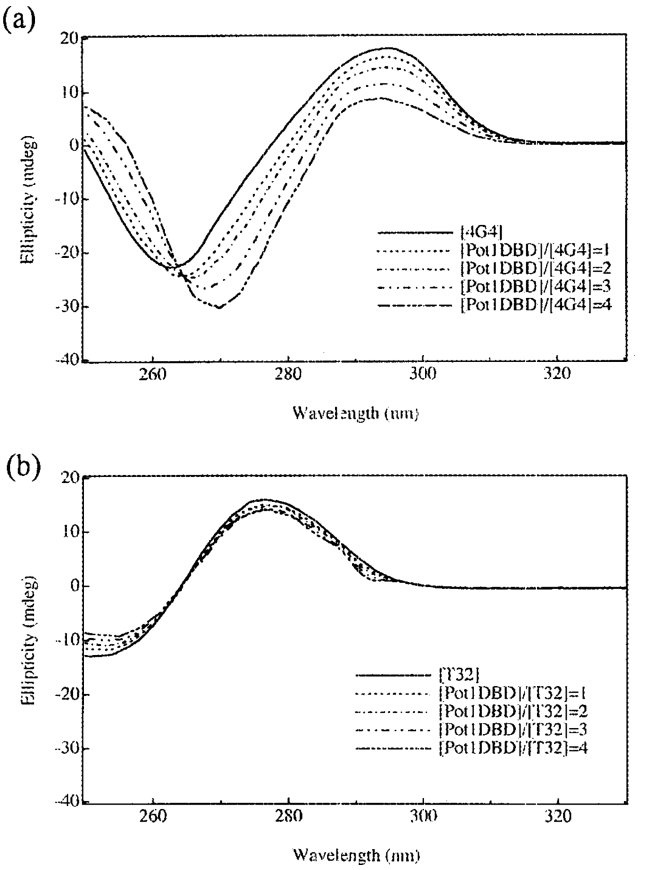


**FIGURE 2** CD spectral changes upon the interaction with Pot1DBD. 3 $\mu$M 4G4 (a) or 3 $\mu$M T32 (b) at pH 7.5 in 20 mM Tris-HCl, 150 mm NaCl and 1 mm DTT was incubated in the indicated molar ratio with Pot1DBD in the same buffer at 25°C for 60 minutes before the CD measurements.

the fluorophore and the quencher, indicating unfolding of the antiparallel tetraplex structure of F4G4D. This result is consistent with the decrease in the amount of the antiparallel tetraplex of 4G4, observed as the CD spectral change upon the addition of Pot1DBD (Figure 2a). Furthermore, dual-labeled FT32D was also prepared by attaching the fluorophore, Fam, and the quencher, dabcyl, to the 5′ and 3′ termini of T32, respectively. The addition of Pot1DBD did not induce any significant change in the fluorescence emission spectra of FT32D (Figure 3b). This result shows that Pot1DBD did not cause any significant structural change of FT32D, which does not bind with Pot1DBD. Combining these results, we conclude that Pot1DBD has the ability to unfold the antiparallel tetraplex DNA.

Considering that the antiparallel tetraplex is known to inhibit telomerase-mediated telomere elongation, we conclude that the ability of fission yeast Pot1 to unfold the antiparallel tetraplex of the telomeric DNA is required for regulation of telomerase-mediated telomere elongation.

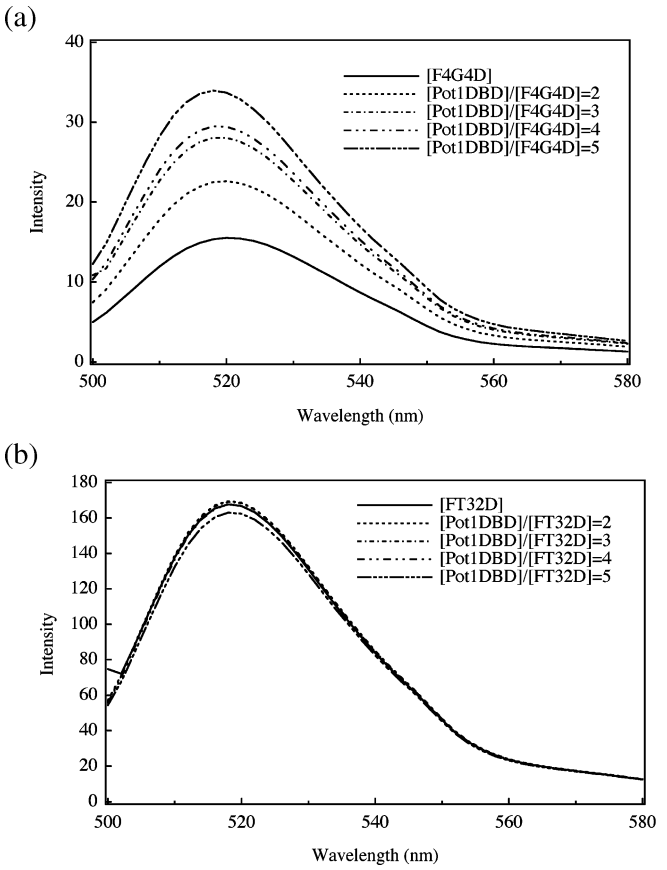


**FIGURE 3** FRET analyses of the interaction with Pot1DBD. 100 nM F4G4D (a) or 100 nM FT32D (b) at pH 7.5 in 20 mM Tris-HCl, 150 mM NaCl and 1 mM DTT was incubated in the indicated molar ratio with Pot1DBD in the same buffer at 25°C for 60 minutes before the fluorescence measurements. The excitation wavelength was 495 nm.



## REFERENCES

1. Blackburn, E.H. Telomeres and telomerase: Their mechanisms of action and the effects of altering their functions. *FEBS Lett.* **2005**, 579, 859–862.
2. Neidle, S.; Parkinson, G.N. The structure of telomeric DNA. *Curr. Opin. Struct. Biol.* **2003**, 13, 275–283.
3. Arnott, S.; Chandrasekaran, R.; Marttila, C.M. Structures for polyinosinic acid and polyguanylic acid. *Biochem. J.* **1974**, 537–543.
4. Zimmerman, S.B.; Cohen, G.H.; Davies, D.R. X-ray fiber diffraction and model-building study of polyguanylic acid and polyinosinic acid. *J. Mol. Biol.* **1975**, 92, 181–192.
5. Williamson, J.R.; Raghuraman, M.K.; Cech, T.R. Monovalent cation-induced structure of telomeric DNA: The G-quartet model. *Cell* **1989**, 59, 871–880.
6. Blackburn, E.H.; Gall, J.G. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in tetrahymena. *J. Mol. Biol.* **1978**, 120, 33–53.
7. Klobutcher, L.A.; Swanton, M.T.; Donini, P.; Prescott, D.M. All gene-sized DNA molecules in four species of hypotrichs have the same terminal sequence and an unusual 3′ terminus. *Proc. Natl. Acad. Sci. USA* **1981**, 78, 3015–3019.

8. Moyzis, R.K.; Buckingham, J.M.; Cram, L.S.; Dani, M.; Deaven, L.L.; Jones, M.D.; Meyne, J.; Ratliff, R.L.; Wu, J.R. A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. *Proc. Natl. Acad. Sci. USA* **1988**, 85, 6622–6626.
9. Shampay, J.; Szostak, J.W.; Blackburn, E.H. DNA sequences of telomeres maintained in yeast. *Nature* **1984**, 310, 154–157.
10. Sugawara, N.F. DNA Sequences at the Telomeres of the Fission Yeast *S. pombe*, Ph.D. thesis, Harvard University, Cambridge, MA, 1988.
11. Baumann, P.; Cech, T.R. Pot1, the putative telomere end-binding protein in fission yeast and humans. *Science* **2001**, 292, 1171–1175.
12. Lei, M.; Baumann, P.; Cech, T.R. Cooperative binding of single-stranded telomeric DNA by the Pot1 protein of *Schizosaccharomyces pombe*. *Biochemistry* **2002**, 41, 14560–14568.
13. Bunch, J.T.; Bae, N.S.; Leonardi, J.; Baumann, P. Distinct requirements for Pot1 in limiting telomere length and maintaining chromosome stability. *Mol. Cell. Biol.* **2005**, 25, 5567–5578.
14. Zahler, A.M.; Williamson, J.R.; Cech, T.R.; Prescott, D.M. Inhibition of telomerase by G-quartet DNA structures. *Nature* **1991**, 350, 718–720.
15. Hardin, C.C.; Henderson, E.; Watson, T.; Prosser, J.K. Monovalent cation induced structural transitions in telomeric DNAs: G-DNA folding intermediates. *Biochemistry* **1991**, 30, 4460–4472.
16. Mergny, J.L.; Maurizot, J.C. Fluorescence resonance energy transfer as a probe for G-quartet formation by a telomeric repeat. *ChemBioChem* **2001**, 2, 124–132.