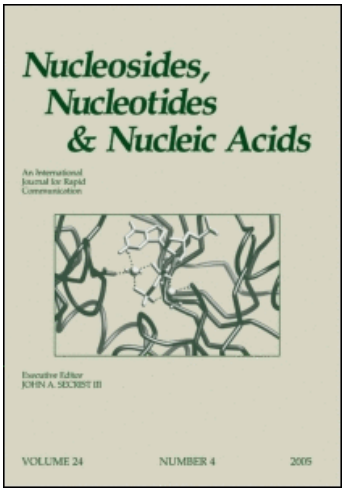

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EFFECT OF CATIONIC COMB-TYPE COPOLYMER ON QUADRUPLEX FOLDING OF HUMAN TELOMERIC DNA

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□ *Cationic comb-type copolymer (CCC) consisting of a polycationic backbone and abundant graft water-soluble chains exhibited considerable stabilization effect on DNA hybrids, such as double- and triple-stranded DNAs. Here, we describe the effect of CCC on antiparallel G-quadruplex folding of human telomeric DNA, d(GGGTTA)$_n$ in the presence of sodium ions. CCC did not significantly alter the circular dichroism (CD) spectra of d((GGGTTA)$_3$GGG) and d((GGGTTA)$_7$GGG) indicating that the CCC did not influence the antiparallel folding of the telomeric repeats. Hence, the ionic interaction of CCC with the DNA sequence did not interfere with specific interaction of the DNA with sodium ions to form G-quartets. Interestingly, CCC did not change the melting temperature of the d((GGGTTA)$_3$GGG) suggesting negligible stabilizing effect of CCC on the antiparallel quadruplex structure.*

Keywords Cationic comb-type copolymer; telomeres; DNA

INTRODUCTION

Telomeres are the nucleoprotein complexes that protect the ends of chromosomes. Among a wide variety of eukaryotic species, including humans, telomeric DNA consists of tandem repeats of a d(TTAGGG) unit, while other species have similar sequences consisting of G clusters. Mammalian telomeric DNA has 3′ single-stranded DNA overhang,

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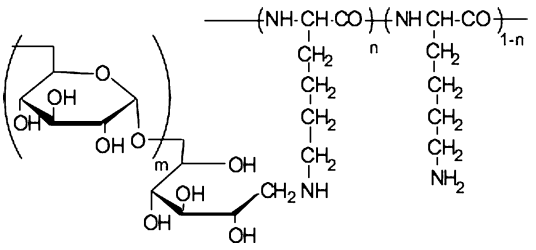

FIGURE 1 Structural formula of PLL-*g*-Dex.

d(TTAGGG)$_n$, which is known to form G-quadruplex structure in vitro, where the G-quadruplex structure is built from the vertical staking of G-quartets, hydrogen bonded complex that bind cations.[1] The G-quadruplex structures have heterogeneity in a number of constituting molecules, showing formation of monomeric, dimeric, tetrameric, and polymeric forms. The G-quadruplex structures also have diversity in strand orientation, including parallel and antiparallel conformations. For example, three and half copies, d((GGGTTA)$_3$GGG) (G3.5), of the telomeric sequence preferentially hold into intramolecular (monomeric) quadruplexes, however the strand orientations are varied depending on ionic conditions. The sodium form of the intramolecular quadruplex adopts predominantly antiparallel conformation, as judged by NMR and circular dichoroism (CD) spectroscopic analyses.[2,3] Although a crystal structure of the potassium form has a fully parallel conformation,[4] its solution structure is complicated.[5]

We have been interested in the water-soluble interpolyelectrolyte complex formed between DNA and the cationic comb-type copolymers (PLL-*g*-Dex, Figure 1) comprising of a poly(l-lysine) backbone and abundant water-soluble side chains of dextran.[6,7] The soluble complex formation allowed us to characterize DNA–polycation interaction by spectroscopic methods. We demonstrated that the copolymer significantly increased stability of DNA hybrids such as duplex[8] and triplex[6] DNAs. The copolymer accelerates hybridization rates.[9] Furthermore, the copolymer was demonstrated to stimulate the strand exchange reaction between duplexes and identical single-stranded (ss) DNAs.[10] Thus, the copolymer has strong influences on DNA hybridization and folding. On the basis of these considerations, we decided to examine effects of the copolymer also on quadruplex folding. Here, we show the results of our investigation on the influence of the copolymer on the quadruplex formation of human telomeric repeats.

EXPERIMENTS

Materials

All oligonucleotides were supplied by FASMAC Co., Ltd. (Kanagawa, Japan) and purified by reverse-phase high performance liquid chromatogra-

phy (HPLC). PLL-*g*-Dex (Figure 1) was synthesized by a reductive amination reaction as described previously.[6,8] The average molecular weights of a poly(l-lysine) main chain and dextran side chains, respectively, were 20 and 5.9 kD. The dextran content of the copolymer was 87 wt% (wt%, the corresponding weight fraction of grafted dextran in the copolymer).

CD Measurements

CD measurements were carried out on a JASCO J-700 spectropolarimeter at 25°C. Each spectrum shown was the average of four scans that have been smoothed. Circular dichroism is expressed as molar ellipticity $[\theta] = \theta/\mathrm{cl}$, where θ is the measured ellipticity in degrees, c is the molar concentration (in repeating units of DNA) and l is the path length in quartz cell. The CD samples were prepared in 10 mM Tris buffer (pH 7.4) without NaCl or 10 mM sodium phosphate buffer (sodium buffer, pH 7.2) containing 150 mM NaCl and 0.5 mM EDTA. DNA strand concentration and polymer/DNA charge ratio ($\mathrm{N/P} = [\text{amino group}]_{\text{polymer}}/[\text{phosphate group}]_{\text{DNA}}$), respectively, were adjusted to 2.33 μM and 2.

RESULTS AND DISCUSSION

First, we evaluated by CD measurements the secondary structure of G3.5 in the absence or presence of 150 mM NaCl. As shown in Figure 2a, in the presence of NaCl, the characteristic CD profile with positive and negative CD signals at 295 nm and 260 nm, respectively, was observed for the antiparallel quadruplex structure. The results indicated that G3.5 folds into the intramolecular antiparallel quadruplex structure with sodium ion-dependent manner, as described previously.[2,3] Then we examined the effect of PLL-*g*-Dex on folding of G3.5. As shown in Figure 2b, no significant change in CD profile was observed by adding the PLL-*g*-Dex. Furthermore, the characteristic CD profile for the antiparallel quadruplex was observed in the presence of NaCl, indicating that PLL-*g*-Dex did not affect the antiparallel folding of G3.5 and its sodium ion dependency. The effect of PLL-*g*-Dex on the quadruplex folding of longer telomeric repeats was also examined with a 45 mer DNA sequence, 5′-d((GGGTTA)$_7$GGG)-3′ (G7.5). As shown in Figure 3, the CD profile characteristic for the antiparallel quadruplex was observed in the absence or presence of PLL-*g*-Dex. The cationic copolymers interact with anionic DNAs through ionic interaction with a counterion exchange process. Thus, it was supposed that PLL-*g*-Dex would inhibit interaction between DNA and monovalent cationic ions such as sodium ion and interfere with G-quadruplex folding driven by the specific interaction between DNAs and particular ions. Nevertheless, the results shown in Figure 2b and Figure 3 clearly implies that the interaction between PLL-*g*-Dex and DNA

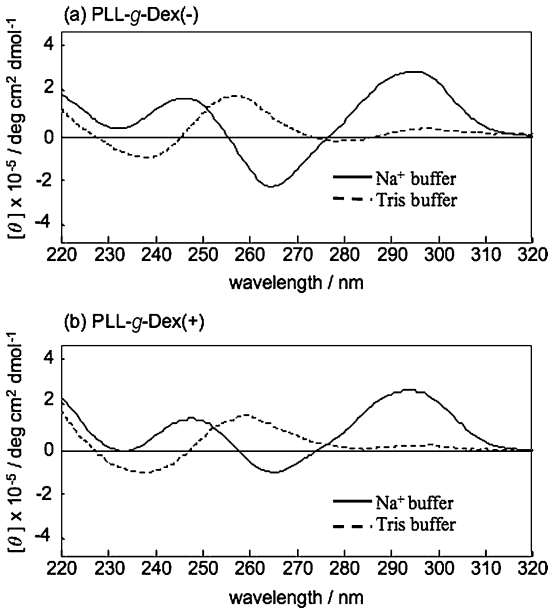

FIGURE 2 CD spectra of G3.5 in the absence or presence of 150 mM NaCl: a) without PLL-*g*-Dex, or b) with PLL-*g*-Dex.

does not significantly interfere with the interaction of the telomeric repeats with sodium ions to form G-quartet structures.

As described previously, the copolymers stabilize triplex and duplex DNAs by reducing counterion condensation effects accompanying with DNA hybridization. Like duplex and triplex DNA formation, the quadruplex folding is considered to accompany counterion condensation process. Thus, we evaluated the effect of PLL-*g*-Dex on the stability of the quadruplex. Interestingly, the copolymer did not change the melting temperature of the antiparallel quadruplex structure of G3.5. However, PLL-*g*-Dex might influence kinetics of G-quadruplex formation.[11] Further studies on the

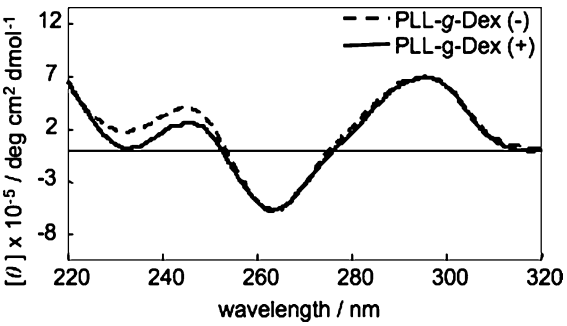

FIGURE 3 CD spectra of G7.5 in the absence or presence of PLL-*g*-Dex in 150 mM NaCl.

interaction between the copolymer and telomeric sequences are needed to better explain our observations.

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