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DNA assembly and reassembly activated by cationic comb-type copolymer

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Abstract

Guanine-rich oligonucleotides, such as TG₄T and TG₅T, assemble into a tetramolecular quadruplexes with layers of G-quartets stabilized by coordination to monovalent cations. Association rates of the quadruplexes are extremely slow, likely owing to electrostatic repulsion among the four strands. We have shown that comb-type copolymers with a polycation backbone and abundant hydrophilic graft chains form water-soluble polyelectrolyte complexes with DNA and promote DNA hybridization. Here, we report the effect of cationic comb-type copolymers on the kinetics of tetramolecular quadruplex formation. The copolymer significantly increased the association rate of tetramolecular quadruplexes without altering kinetic effects of metal cations in quadruplex formation. Dissociation rates of the quadruplexes were also accelerated by the copolymer suggesting that the copolymer has chaperone-like activity that reduces the energy barriers associated with dissociation and re-assembly of base pairs. This hypothesis was further supported by the observation that the copolymer activated the strand exchange reaction between the quadruplex and a constituting single-stranded.

1. Introduction

Polyelectrolytes spontaneously form inter-polyelectrolyte complexes (IPECs) with oppositely charged polyelectrolytes. IPEC formation is driven by an entropic contribution from low molecular weight counterions that are released from the vicinities of each of the polyelectrolytes upon complex formation [1]. IPEC formation usually results in a pronounced change in polymer conformation from extended coil structures to highly condensed and compacted globule structures (i.e., the coil-globule transition) [1]. Due to stability and stoichiometric properties, IPECs have been exploited in assembly of a variety of nanostructures [1-3]. For example, well-organized polyelectrolyte films [4] and capsules [5] were produced using a “layer-by-layer” deposition method. Core-shell-type nanoparticles, polyion complex micelles composed of charged block copolymers having hydrophobic polymer chains and oppositely charged polyelectrolytes, have been extensively studied and are potential carriers of DNA and therapeutic agents [6-8]. In these cases, the coil-globule transition and solubility changes play pivotal roles in assembly processes, stability, and functions.

We are interested in water-soluble IPECs formed between polycations and DNA. The cationic comb-type copolymer comprised of more than 80 wt% hydrophilic graft chains and less than 20 wt% cationic backbone gives a totally soluble IPEC with DNA, and DNA condensation (the coil-globule transition) is suppressed in the IPEC [9-11]. We showed that the copolymer

promotes sequence-specific hybrid formation, such as formation of double-stranded DNA (dsDNA) and triple-stranded DNA (tsDNA) [9-11]. Salt concentration dependencies of hybrid stability are eliminated in the presence of the copolymer at least up to physiological salt concentration [12-18], suggesting that the copolymer shields electrostatic repulsion of the DNA strands to stabilize DNA assembly. Our kinetic study indicated that the copolymer significantly increased association rates of dsDNA and tsDNA [12-14]. Moreover, the copolymer also accelerates the strand exchange reaction between dsDNA and complementary single-stranded DNA (ssDNA) [15-19]. These observations indicate that the copolymer not only influences stability of DNA structures but also the association process. We hypothesize that the copolymer facilitates formation of transitional complexes, like double-stranded or triple-stranded nucleation complexes, by increasing simultaneous multi-molecular collision of negatively charged strands and stabilizing these complexes with high anionic charge densities.

G-quadruplex DNA is of interest due to recent demonstrations of its biological relevance and to applications in nano-biotechnology [20]. Well-defined nanoconstructs such as DNA nanowire [21], frayed wire [22] and synapsed wire [23] were designed using G-quadruplexes as a building block. Robust DNA-fueled DNA nanomachines were also created [24,25]. Quadruplexes were also found in several aptamers such as thrombin-binding aptamer [26] and anticancer aptamer [27], implicated that quadruplex structures could be a potential scaffold to

acquire functional DNAs. Guanine-rich sequences can fold into G-quadruplexes that are stabilized by specific coordination of certain metal ions such as Na^+ or K^+ [20]. Certain, short DNA oligomers adopt well-characterized quadruplex structures. For example, TG_4T assembles into a tetramolecular quadruplex, $[\text{TG}_4\text{T}]_4$, consisting of four parallel strands stabilized by four layers of G-quartets [28-30]. Several quadruplex formation pathways have been described, including stepwise strand assembly with dimer intermediates [31-33] and simultaneous association of four strands [34, 35]. Whatever the mechanism, the electrostatic repulsion resulting from bringing together four negatively charged strands presumably results in the extremely slow kinetics of quadruplex formation. Formation of mismatched quadruplexes that competes assembling into fully-matched canonical quadruplex was also suggested to explain the slow assembly [33]. Once the mismatched quadruplexes are formed, spontaneous re-assembly into fully matched quadruplexes must be slow because of the very slow dissociation kinetics of quadruplexes.

We hypothesized that cationic comb-type copolymers could promote the quadruplex assembly by reducing the electrostatic repulsion among the strands and/or by facilitating transformation to the canonical quadruplex from the mismatched quadruplexes through catalysis of strand exchange. We are also interested in how highly multivalent ionic interactions between the cationic copolymer and DNA influences DNA assembly that involves specific

coordination of monovalent cations, such as Na^+ and K^+ . In this study, we evaluated kinetic effects of a cationic comb-type copolymer, poly(L-lysine)-*graft*-dextran copolymer (PLL-*g*-Dex, Figure 1), on assembly and strand exchange of the quadruplex, and discussed the activities of the copolymer in relation to a nucleic acid chaperone.

2. Materials and methods

2.1 Materials

PLL-HCl ($M_w = 5.3 \times 10^4$) and dextran T-10 ($M_w = 8.4 \times 10^3$) were obtained from Peptide Institute, Inc. (Osaka, Japan) and Pharmacia Biotechnology (Uppsala, Sweden), respectively. Sodium hydroxide, boric acid, sodium sulfate, and acetic acid were purchased from Wako Pure Chemical Industries (Osaka, Japan). Sodium tetraborate decahydrate was obtained from Nacali Tesque, Inc. (Kyoto, Japan). An oligonucleotide with the sequence 5'-TGGGGT-3' (TG_4T) and TAMRA-labeled TG_4T (F- TG_4T) were purchased from GeneNet Co. Ltd. (Fukuoka, Japan) and FASMAC Co. Ltd. (Tokyo, Japan), respectively. Preparation of the quadruplex $[\text{TG}_4\text{T}]_4$ was performed as follows: TG_4T (1 mM) was heated to 90 °C and then slowly cooled to 4 °C in 10 mM PBS (pH 7.2), 100 mM NaCl. After the TG_4T solution was incubated for 7 days at 4 °C, the $[\text{TG}_4\text{T}]_4$ stock solution was stored at -20 °C.

2. 2 Preparation of PLL-g-Dex copolymers

PLL-g-Dex was prepared by reductive animation reaction between PLL and Dex as described in a previous report [9-11]. The obtained PLL-g-Dex was isolated using a column packed with TOYOPERAL CM-650C weakly cationic exchange resin (TOSOH, Tokyo, Japan). The column was equilibrated with 0.1 M sodium borate buffer (pH 8.5) and PLL-g-Dex was eluted with 1 M acetic acid containing 0.5 M Na₂SO₄. The polymer fraction was dialyzed against water and lyophilized. PLL-g-Dex was characterized by NMR and gel permeation chromatography (GPC) measurements.

2. 3 Thermal denaturation and renaturation in the absence or presence of copolymer

Thermal denaturation and renaturation curves of the tetramolecular quadruplex were obtained by heating/cooling experiments. Various concentrations of [TG₄T]₄ solution in 10 mM PBS (pH 7.2) containing 100 mM NaCl with (N/P =2) or without PLL-g-Dex were heated at a rate of 1 °C/min from 20 to ca. 90 °C (and vice versa). The CD signals at 263 nm of [TG₄T]₄ were recorded as a function of temperature in quartz optical cells of 0.1-mm to 10-mm path length.

2. 4 Determination of association and dissociation kinetics of quadruplex by CD measurements

TG₄T in 10 mM PBS (pH 7.2) was incubated for 10 min at 90 °C. After cooling to reaction temperatures ranging from 4 to 30 °C, PLL-g-Dex (N/P = 0 to 8) or PLL (N/P = 4) and NaCl or KCl (final concentration 100 mM) was added. The CD spectra or molar ellipticity at 263 nm of the solution (initial concentrations of TG₄T: 10-200 μM) were recorded. The quadruplex dissociation kinetics were determined as follows: The [TG₄T]₄ stock solution was diluted with 10 mM PBS (pH 7.2) containing 100 mM NaCl (final concentration of TG₄T₄ = 30 μM) with (N/P = 2) or without PLL-g-Dex at 45-60 °C. Molar ellipticity changes at 263 nm of TG₄T were collected.

2. 5 Determination of association kinetics of quadruplex by gel electrophoresis

TG₄T (final concentration: 200 μM) in 10 mM PBS (pH 7.2) without NaCl was incubated for 10 min at 90 °C. After cooling to 20 °C, a cationic polymer (PLL-g-Dex or PLL, N/P = 4) and NaCl (final concentration 100 mM) were added. After the incubation at 20 °C for the indicated time periods, DNAs were separated by 20% polyacryamide gels. The quadruplexes were stained by EtBr. A fluorescence image of the gel was obtained with a Fujifilm LAS-3000 luminescent image analyzer (Tokyo, Japan).

2. 6 Evaluation of strand exchange reaction between tetramolecular quadruplex and ssDNA

F-TG₄T (final concentration of 20 μM) in 10 mM PBS (pH 7.2) containing 100 mM NaCl with (final N/P = 2) or without PLL-g-Dex was added to [TG₄T]₄ (final concentration of 55 μM) solution in the same buffer. The mixture was incubated at 25 °C for various time periods. After the incubation, the mixture was placed in an ice bath and excess polyvinyl sulfate (PVS) was added to dissociate PLL-g-Dex from DNA. The mixtures were separated by gel electrophoresis at 100 V on a 20% polyacrylamide gel at 4 °C in 1 × TBE buffer. A fluorescence image of the gel was obtained with a Fujifilm LAS-3000 luminescent image analyzer (Tokyo, Japan). The degree of strand exchange in percent was calculated using equation (1):

$$\% \text{Exchange degree} = \frac{([FI]_t - [FI]_0)}{([FI]_t - [FI]_\infty)} \quad \text{Equation (1)}$$

Where, [FI]_t, [FI]₀, and [FI]_∞ are fluorescence intensities of strand exchanged tetramolecular quadruplex at time t, at the initial time, and after the reaction reached equilibrium, respectively. [FI]_∞ was obtained by heating the mixture at 90 °C for 5 min, followed by slow cooling and a week incubation at 4 °C.

2. 7 DNA binding to copolymer as determined by fluorescence correlation spectroscopy

Binding of DNA to the copolymer was assessed by fluorescence correlation spectroscopy [18,36,37] using an Olympus MF20 single-molecular fluorescence detection

system (Olympus, Tokyo Japan). All samples for the fluorescence correlation spectroscopy measurements were prepared in 10 mM PBS (pH 7.2) containing 100 mM NaCl and the DNA strand concentration was 120 nM (110 nM TG₄T and 10 nM F-TG₄T). PLL-g-Dex was added to the DNA sample at concentrations ranging from 0 to 200 μ M; the PLL-g-Dex concentration was determined based on the lysine residues. An aliquot (50 μ M) of each sample was transferred to a 384-well microplate. A standard solution of 1 nM RITC in the same buffer was used to derive optical parameters necessary for a proper measurement. A He-Ne laser ($\lambda_{\text{exc}} = 543$ nm) was set at 200 μ W. All measurements were carried out in at least duplicate and four scans of each sample were taken, each lasting 10 s at room temperature. The obtained data were fitted according to an autocorrelation function in the instrument software. Each data point was mean value of the measured samples. The measurements of tetramolecular quadruplex were also conducted under the same conditions.

3. Result

3. 1 Effect of PLL-g-Dex on thermal dissociation and association of tetramolecular quadruplex

We evaluated the effects of PLL-g-Dex (Mw of PLL backbone: 5.3×10^4 , Mw of dextran grafts: 8.4×10^3 , drafting degree of dextran: 16.2 mole%) on the

thermal dissociation and re-association of intermolecular quadruplexes by temperature-gradient CD measurements. As shown in Figure 2a, melting of [TG₄T]₄ quadruplex was detected as a decrease in CD signal at 263 nm as the temperature was increased. In the absence of the copolymer, considerable hysteresis between the heating and cooling curves was observed [33]. This is indicative of the slow association kinetics of the [TG₄T]₄ intermolecular quadruplex that has been previously described [31, 33]. In the presence of the copolymer, nearly reversible heating and cooling profiles were obtained, suggesting that the copolymer accelerated the association kinetics of the quadruplex. Figure 2b shows the relationship between TG₄T concentration and the midpoint of the melting transition ($T_{1/2}$) determined from the heating scans. In the absence of the copolymer, the DNA concentration dependency of $T_{1/2}$ was not significant as expected due to the slow association kinetics; this is consistent with a previous report [33]. In the presence of the copolymer, $T_{1/2}$ increased with increasing TG₄T concentration, indicating that the copolymer accelerated quadruplex assembly. Note that $T_{1/2}$ values obtained in the presence of the copolymer were lower than in the absence of the copolymer over the TG₄T concentration range of 10 to 100 μ M. This result suggests that the copolymer accelerates not only the association but also the dissociation of the tetramolecular quadruplex.

3. 2 Effect of PLL-g-Dex on association and dissociation rates of tetramolecular quadruplex

We next assessed effect of the copolymer on association of the tetramolecular quadruplex at constant temperature. Figure 3a shows the CD spectra of TG₄T (30 μ M strand) in the absence and presence of the copolymer as a function of time. In the absence of the copolymer, 50% quadruplex formation required 5800 min (ca. 4 days) in qualitative agreement with previous reports [33]. In contrast, in the presence of the copolymer, the quadruplex CD signal appeared within a few minutes and only 10 min incubation was required to achieve 50% quadruplex formation. The Fourth order rate constants, k_{on} , were estimated from changes in CD signal at 263 nm over time (Figure S1). These data are plotted as a function of the N/P ratio in Figure 3b. In the absence of the copolymer, the k_{on} at 4°C with 100 mM NaCl was $3.1 \times 10^8 \text{ M}^{-3}\text{sec}^{-1}$, consistent with a previous report [33]. In the presence of the copolymer, the k_{on} increased linearly with increasing N/P ratio and reached a plateau at N/P = 2. At N/P = 2, k_{on} was $2.8 \times 10^{11} \text{ M}^{-3} \text{ sec}^{-1}$, indicating a nearly 1000-fold acceleration of quadruplex assembly in the presence of the copolymer. Since kinetic study with CD measurements could be affected by complicated assembling processes, such as different pathways and conformations, of quadruplex formation [31-35], the association kinetics of tetramolecular quadruplexes at 200 μ M TG₄T were further estimated by gel electrophoresis assay (Figure 4). The results comparable to the CD measurement were obtained. Whereas only 40% quadruplex formation was observed after 240 min incubation in the absence of the copolymer, >80% quadruplex

formation was observed within 10 min incubation in the presence of the copolymer (Figure 4a and 4c). Note that poly(L-lysine) homopolymer hardly accelerates the quadruplex assembly (Figures 4b). It was also shown by CD study that dextran even at high concentration did not accelerate the quadruplex association (Figure S1).

The tetramolecular quadruplex assembly is faster in a potassium buffer than in a sodium buffer [33, 34]. The k_{on} value in 100 mM KCl was estimated at $1.2 \times 10^{10} \text{ M}^{-3}\text{sec}^{-1}$, implying 40-fold faster assembling than that in 100 mM NaCl. In our hands, the copolymer further increased k_{on} in 100 mM KCl (Figure 3c). At N/P = 2, the k_{on} was $5.0 \times 10^{12} \text{ M}^{-3}\text{sec}^{-1}$ in 100 mM potassium. This data is summarized in Table 1. These results suggest that the multivalent ionic interactions between the copolymer and DNA did not significantly modify the kinetic effects of monovalent ions.

We then evaluated temperature dependency of the quadruplex assembly. Consistent with previous reports [33], the k_{on} values decreased with increasing temperature in the absence of the copolymer (Figure 3d). The negative temperature dependency is consistent with a nucleation-zipper mechanism for strand association [33, 38]. The negative temperature dependency was also observed in the presence of the copolymer.

The effect of the copolymer on the quadruplex dissociation kinetics is shown in Figure 5. The copolymer moderately increased the dissociation rate in an N/P ratio dependent manner.

At $N/P > 2$, the dissociation rate increased by at least 6-fold at 60°C relative to the rate in the absence of copolymer. Thus, the copolymer increased both the association and dissociation rates of quadruplex, suggesting that the copolymer has a catalytic effect of reducing the energy barriers needed for dissociation and association of nucleic acid structures, as do nucleic acid chaperones [39, 40].

3. 3 Evaluation of strand exchange reaction between tetramolecular quadruplex and ssDNA

One of unique features of nucleic acid chaperones is their ability to catalyze a strand exchange reaction. For example, a native nucleic acid chaperone, the nucleocapsid protein expressed by retroviruses, facilitates the strand exchange reaction between dsDNA and homologous single strands by reducing the energy barrier for dissociation and association of base pairing [39, 40]. To examine the ability of the copolymer to serve as a quadruplex chaperone, its influence on the strand exchange reaction between a $[TG_4T]_4$ quadruplex and a TG_4T strand was assessed (Scheme 1). In order to monitor strand exchange, fluorescently labeled TG_4T ($F\text{-}TG_4T$) was incubated at 25°C with an excess amount (a 2.5-fold molar excess based on strand concentration) of pre-formed $[TG_4T]_4$ quadruplexes. At indicated times, the reaction mixtures were analyzed by PAGE. As shown in Figure 6a, no strand exchange product, $[(TG_4T)_3 \cdot F\text{-}TG_4T]$, was detected in the reaction mixture without the copolymer. In the presence of the copolymer

(N/P = 4), increasing amounts of the strand-exchange product (upper bands in Figure 6a) appeared in a time-dependent manner. After 180 min, 65% of $[\text{TG}_4\text{T}]_4$ contained F- TG_4T (Figure 6b); our assumption was that only a single fluorescein-tagged strand was incorporated per quadruplex. The copolymer activated the strand exchange reaction in an N/P ratio dependent manner and the activation effect reached a plateau at an N/P of 4. Incubation of F- TG_4T , without $[\text{TG}_4\text{T}]_4$, in the presence of the copolymer resulted in negligible quadruplex formation (Figure S5), indicating a slight influence of $[\text{F-TG}_4\text{T}]_4$ quadruplex formation on the strand exchange reaction assay.

3. 4 Binding assay of the copolymer to ssDNA and tetramolecular quadruplex DNA

To further understand the effect of the copolymer, binding of copolymer to either ssDNA or tetramolecular quadruplex DNA was analyzed by fluorescence correlation spectroscopy [18,36,37]. Since a diffusion time calculated by the autocorrelation analysis of the fluorescence fluctuation is proportional to cubic root of molecular mass, the complex formation of oligonucleotides with the copolymer leads to an increased in the diffusion time. Figure 7 shows the fluorescence correlation spectroscopy results for the copolymer binding with either TG_4T or $[\text{TG}_4\text{T}]_4$. The diffusion times of TG_4T and $[\text{TG}_4\text{T}]_4$ increased with increasing copolymer concentration. The K_d value for the copolymer binding to TG_4T strand was

estimated as about 48 nM in strand concentration (20 μ M in lysine units); that to [TG₄T]₄ quadruplex was estimated as < 1 nM (0.5 μ M in lysine units). Though accurate estimation of K_d value for [TG₄T]₄ could not be accomplished owing to the detection limits of assay, the copolymer has at least 40-fold stronger affinity to [TG₄T]₄ quadruplex than to a TG₄T monomer. The stronger affinity of the copolymer to the quadruplex was attributed to larger number and higher charge density of phosphate anions in the quadruplex structure than in the TG₄T monomer. Our kinetic studies of [TG₄T]₄ quadruplex assembly were carried out at DNA concentration of 30 μ M; therefore, in our assays, both TG₄T and [TG₄T]₄ were associated with the copolymer to form complexes at N/P > 1.

4. Discussion

A yeast telomere binding protein, the β -subunit of *Oxytricha* telomere binding protein, and histone H1 are known all to accelerate the quadruplex formation [41-43]; for example, the β -subunit of *Oxytricha* telomere binding protein enhances the quadruplex formation of 5'-TTTTGGGGTTTTGGGG-3' by 10⁵-10⁶ folds [43]. However, the effects of the proteins on quadruplex dissociation and, more particularly, on the strand exchange reaction have not been described. Recently, quadruplex ligands that increase association rate and decrease dissociation

rate of intermolecular quadruplex formation and stabilize quadruplex structures have been reported [44].

In this study we showed that a cationic comb-type copolymer not only increased the rate of association but also dissociation rates of tetramolecular quadruplexes. The copolymer also promoted the strand exchange reaction. Thus, the copolymer has a nucleic acid chaperone-like activity. The strong electrostatic repulsion among the four strands of the quadruplex may explain the extremely slow association rates of tetramolecular quadruplexes. The copolymer likely reduces this electrostatic repulsion and stabilizes the multi-stranded transitional complex to facilitate assembly. Our previous observation that the copolymer considerably stabilizes duplex and triplex DNAs supports this hypothesis [9-11]. Formation of mismatched quadruplexes was recently suggested to be another reason for slow assembly into the fully-matched quadruplex structures [34, 35].

Previously we showed that the copolymer activated the strand exchange reaction between double-stranded DNA and a homologous single strand [15, 17]. In this study, we demonstrated that the copolymers also activated strand exchange between quadruplex and single strand. Two distinct reaction pathways, a dissociative pathway and a sequential displacement pathway, have been considered for the strand exchange reaction between dsDNA and a homologous single strand [45, 46]. Although the strand exchange reaction in the quadruplex might occur through

the spontaneous and complete dissociation of quadruplex followed by re-assembly (Scheme 2a), the dissociation of quadruplex is very slow (with a half dissociation time of more than 100 days under our experimental conditions) and therefore this pathway cannot account for our observations. In contrast to the complete dissociation pathway, the sequential displacement pathway (Scheme 2c) requires only local melting to allow for the formation of a branched nucleation complex with a single-stranded counterpart. Subsequent migration of the branch point would result in formation of an exchanged quadruplex. The copolymer may stabilize more the branched nucleation complex that bears higher negative charges and local charge density than the quadruplex, leading to the observed rate acceleration compared to strand exchange in the absence of copolymer. In addition to the sequential displacement pathway, a partial dissociative pathway (Scheme 2b) may be possible; step-wise strand association into duplex and triplex intermediates has been proposed for quadruplex formation [32, 34].

DNA assembly is thermodynamically and kinetically influenced by the counterion condensation effect [47]. Valency, size, and shape of counterions influence the magnitude of the counterion condensation effect [48, 49]. The copolymer forms an inter-polyelectrolyte complex with DNA through ion exchange process with Na^+ or K^+ ions that were associated with DNA. The counterion replacement of monovalent counterions with the polycationic copolymer with larger ionic multivalency likely has a strong influence on the assembly of

polyanionic DNA and dynamics of base pairing. As others have shown, we observed differential kinetic effects of monovalent cations Na^+ vs. K^+ . The same effect was observed even in the presence of the copolymer. Regardless of the strong ionic interactions between the cationic copolymer and DNA (K_d values for the copolymer binding to TG_4T and $[\text{TG}_4\text{T}]_4$ were 48 nM and < 1 nM, respectively), coordinating interactions of metal ions to G-quartet structures are not significantly changed. These characteristics allowed additive kinetic effects of K^+ and the copolymer, leading to 10^4 -fold increased in k_{on} as shown in Table 1.

5. Conclusion

We have shown that a cationic comb-type copolymer increases the association and dissociation rates of tetramolecular quadruplexes. Furthermore, the copolymer activated the strand exchange reaction between tetramolecular quadruplex and a single strand, suggesting that the copolymer has chaperone-like activity that reduces the energy barriers associated with dissociation and re-assembly of G-quartets. Though mechanisms underlying acceleration effect of the copolymer on dissociation, association, and strand exchange of DNA assemblies are not fully understood, the copolymer may expand

the their utility, especially to the formation of devices that require rapid assembly and/or recombination platforms.

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Table 1

The association rate constants of [TG₄T]₄ quadruplex in 100 mM Na⁺ or K⁺ buffer with or without PLL-*g*-Dex (N/P = 2).

PLL- <i>g</i> -Dex	$k_{\text{on}} / \text{M}^{-3} \text{sec}^{-1}$	
	Na ⁺	K ⁺
-	3.1×10 ⁸	1.2×10 ¹⁰
+	2.8×10 ¹¹	5.0×10 ¹²

Figure legends

Scheme 1.

Assay for strand exchange reaction of tetramolecular quadruplex.

Scheme 2.

Three possible reaction routes for the strand exchange reaction of tetramolecular quadruplex.

Figure 1.

Structural formula of PLL-*g*-Dex.

Figure 2.

Temperature gradient CD profiles and concentration dependency of $T_{1/2}$ of $[\text{TG}_4\text{T}]_4$ quadruplex in absence or presence of PLL-*g*-Dex. (a) Molar ellipticity at 263 nm of $[\text{TG}_4\text{T}]_4$ quadruplex in 10 mM PBS (pH 7.2), 100 mM NaCl at a strand concentration of 500 μM TG_4T in the absence (black line) or the presence (red line) of PLL-*g*-Dex; spectra were recorded with thermal gradient with 1 $^\circ\text{C}/\text{min}$. Solid and dashed lines indicate heating and cooling profiles, respectively. (b) DNA concentration dependence of $T_{1/2}$ of $[\text{TG}_4\text{T}]_4$ in the absence (\circ) or the presence (\bullet) of PLL-*g*-Dex (obtained upon heating).

Figure 3.

Effect of PLL-*g*-Dex on TG_4T quadruplex association. The experiments were performed in 10 mM PBS (pH 7.2) containing 100 mM NaCl or KCl at 30 μM TG_4T at 4 $^\circ\text{C}$. (a) Changes in the CD spectra of TG_4T in 100 mM NaCl in the absence or the presence of PLL-*g*-Dex as a function of time. Dashed lines are CD spectra of TG_4T solution at time = 0. (b) k_{on} values at various N/P ratios in 100 mM Na^+ . (c) k_{on} values at various N/P ratios in 100 mM K^+ . (d) Arrhenius plots of TG_4T association rate constant in the absence (\circ) or the presence (\bullet) of PLL-*g*-Dex (N/P = 2) in 10 mM PBS containing 100 mM NaCl.

Figure 4.

Effect of PLL or PLL-*g*-Dex on TG_4T quadruplex association. The experiments were performed in 10 mM PBS (pH 7.2) containing 100 mM NaCl at 200 μM TG_4T at 20 $^\circ\text{C}$. DNAs were separated by 20% non-denaturing acrylamide gel electrophoresis.

Tetramolecular quadruplexes were stained by *ethidium bromide*. (a) TG₄T alone, (b) TG₄T and PLL (N/P = 4), (c) TG₄T and PLL-g-Dex (N/P = 4). (d) Time course of quadruplex molar fractions were plotted from the gel electrophoresis experiments ;(■) TG₄T, (▲) TG₄T and PLL, (●) TG₄T and PLL-g-Dex.

Figure 5.

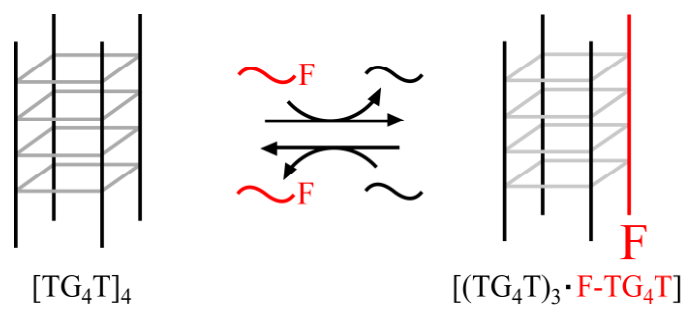
Effect of PLL-g-Dex on TG₄T quadruplex dissociation. The experiments were performed in 10 mM PBS (pH 7.2) containing 100 mM NaCl at 7.5 μ M [TG₄T]₄ at 60°C. (a) k_{off} values at various N/P ratios (b) Arrhenius plots of [TG₄T]₄ dissociation rate constant in the absence (○) or the presence (●; N/P = 2) of PLL-g-Dex.

Figure 6.

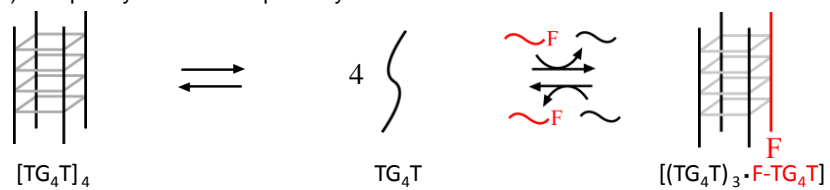
Strand exchange reaction between [TG₄T]₄ and F-TG₄T in absence or presence of PLL-g-Dex. [TG₄T]₄ (55 μ M strand concentration) was incubated at 25°C with F-TG₄T (20 μ M). a) PAGE images of the reaction mixtures (N/P = 0 or 4). b) Time courses of the strand exchange reaction at various N/P ratios. Values of % exchange were quantified from band intensities of [(TG₄T)₃·F-TG₄T].

Figure 7

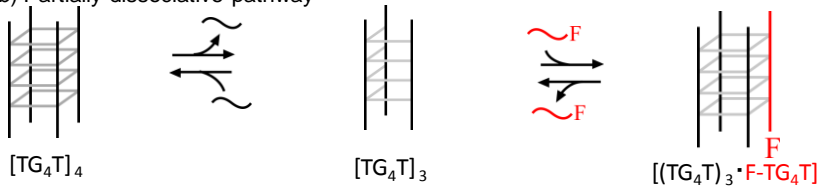
DNAs binding with the copolymer measured by fluorescence correlation spectroscopy. Either 110 nM TG₄T and 10 nM F-TG₄T (●) or 30 nM [(TG₄T)₃·F-TG₄T] (○) were mixed with various concentrations of PLL-g-Dex in 10 mM PBS buffer containing 100 mM NaCl at room temperature. Samples were analyzed by fluorescence correlation spectroscopy.



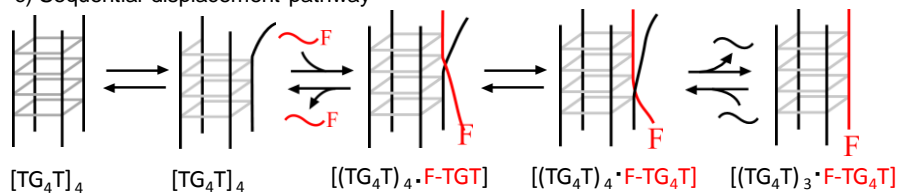
a) Completely dissociative pathway

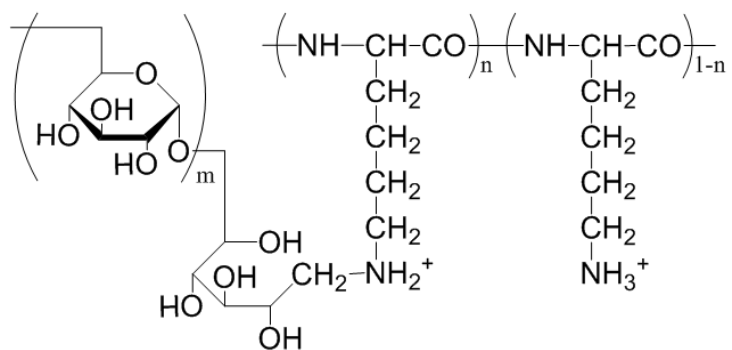


b) Partially dissociative pathway

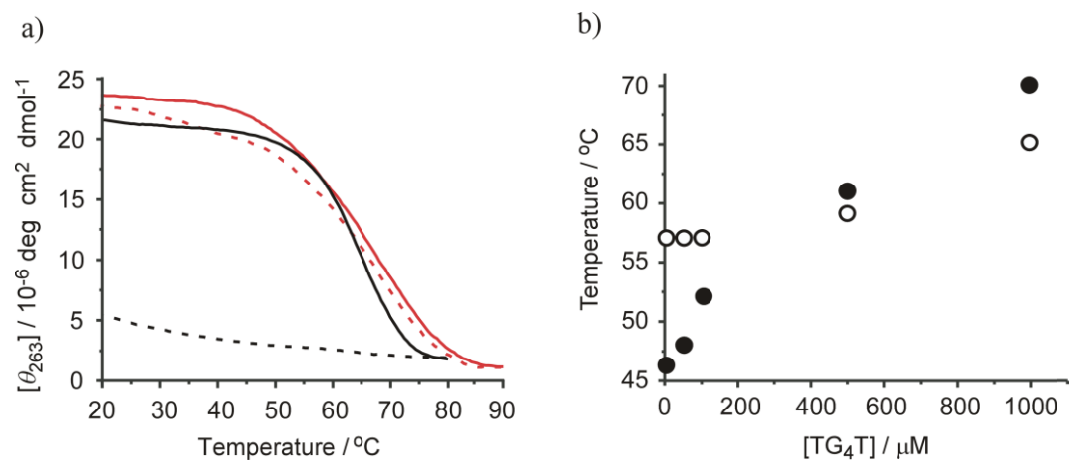


c) Sequential displacement pathway

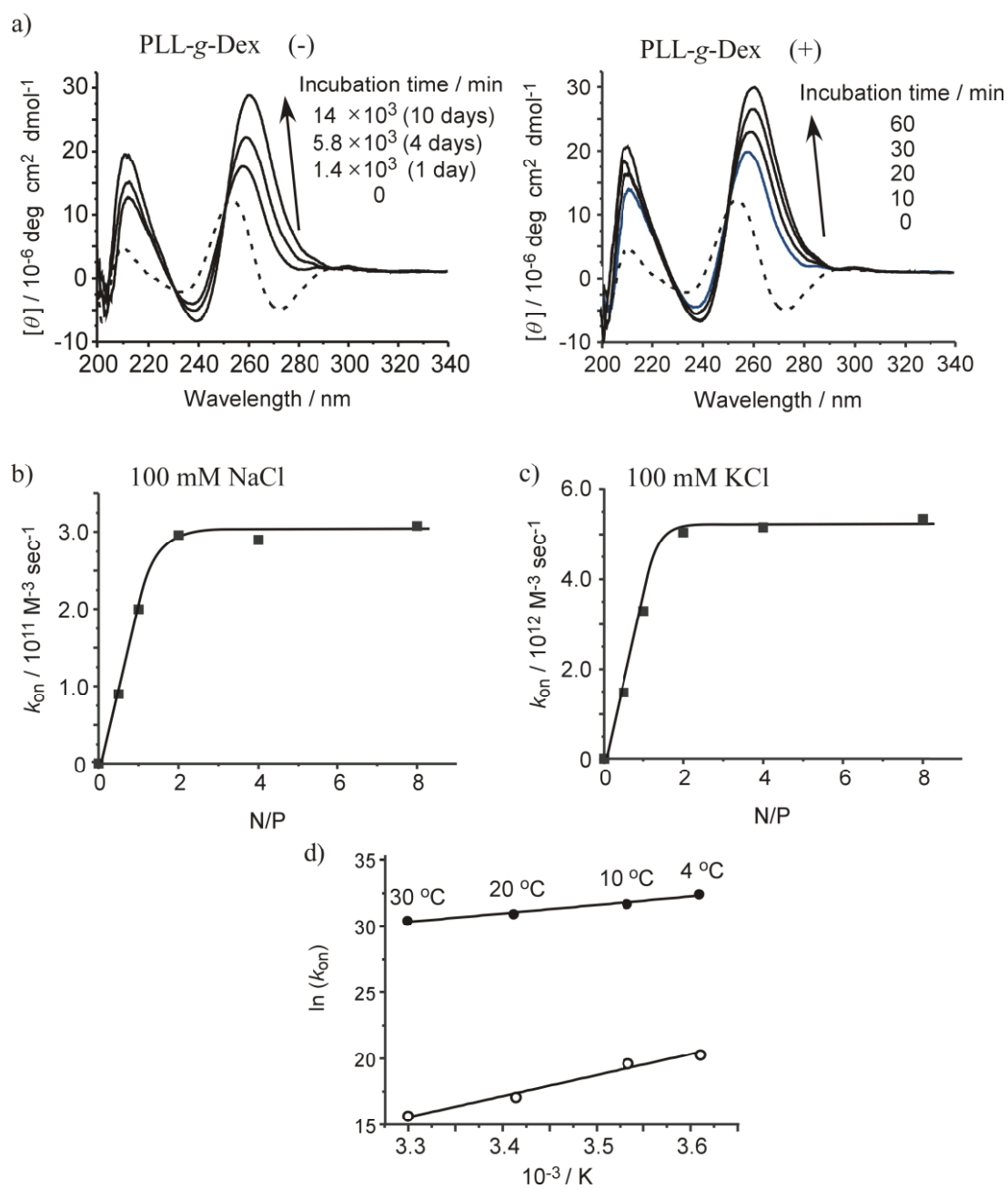




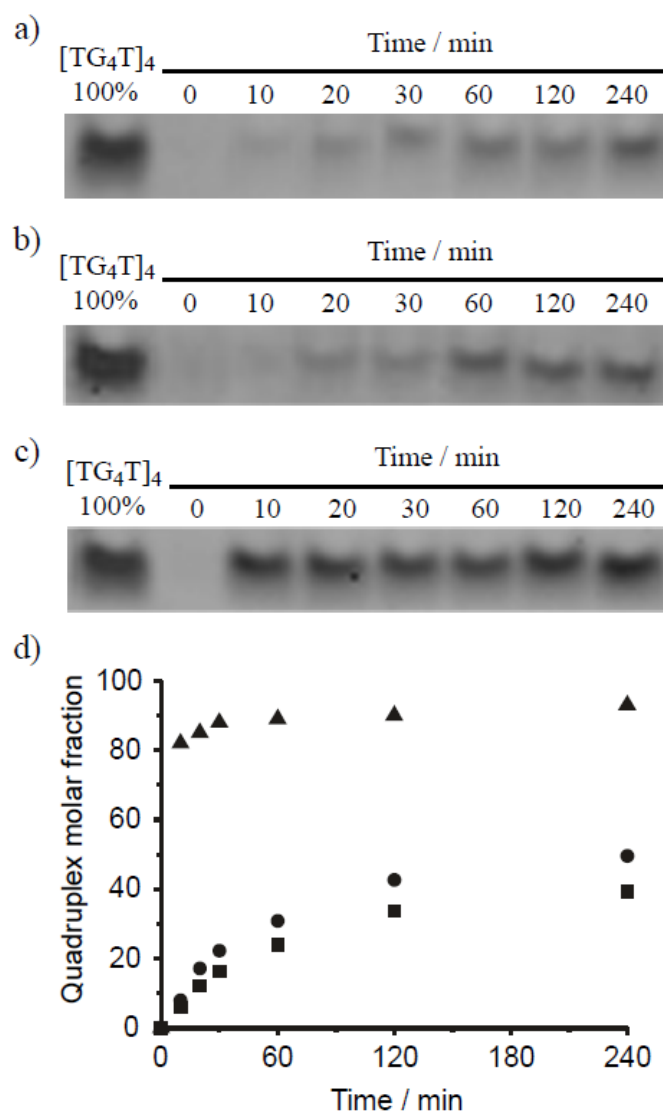
Moriyama et al. Figure 1



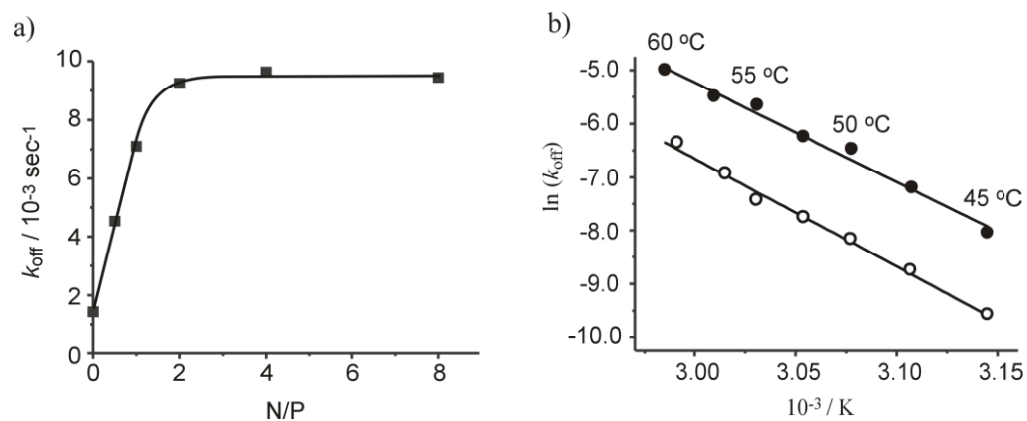
Moriyama et al Figure 2



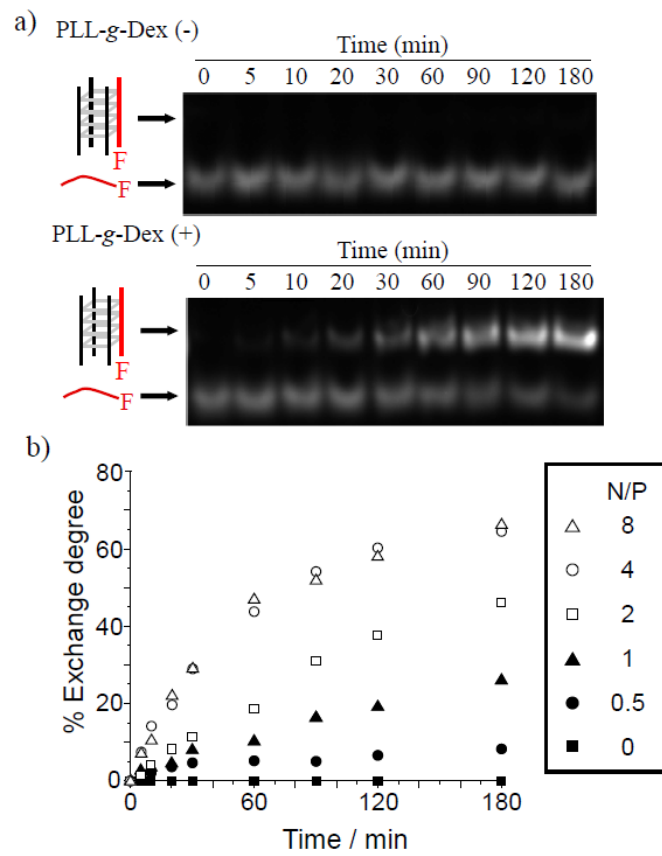
Moriyama et al Figure 3



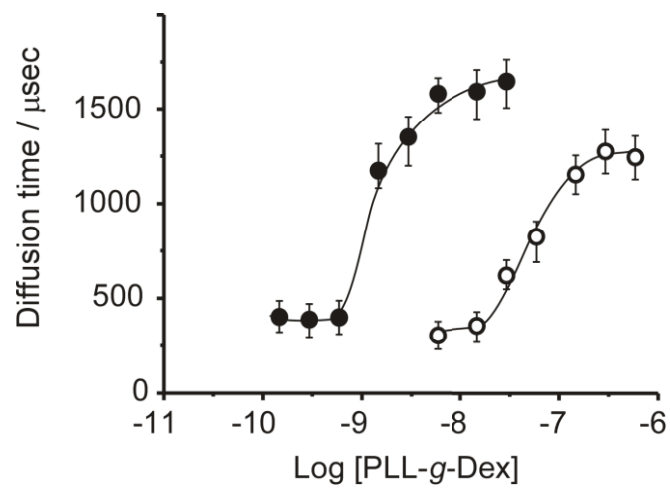
Moriyama et al Figure 4



Moriyama et al Figure 5



Moriyama et al Figure 6



Moriyama et al Figure 7