Comb-Type Cationic Copolymer Expedites DNA Strand Exchange while Stabilizing DNA Duplex

Won Jong Kim, Tsutomu Ishihara, Toshihiro Akaike, and Atsushi Maruyama*[a]

Abstract: The accelerating effect of cationic substances on the DNA strand exchange reaction between a 20 bp DNA duplex and its complementary single strand was studied. A polycationic comb-type copolymer, that consists of a poly(L-lysine) backbone and a dextran graft chain (PLL-*g*-Dex) and known to stabilize triplex DNA, expedites the strand exchange reaction under physiological relevant conditions. Electrostatically a small excess of the copolymer let to a 300 – 1500-fold increase in the DNA strand exchange while large excess of spermine or cetyltrimethylammonium bromide, a cationic detergent known to promote markedly hybridization of complementary DNA strands, shows only a slight effect. The efficacy of the copolymer was not affected by a 10 mM Mg^{2+} concentration. Notably the copolymer promotes the strand exchange reaction while it stabilizes double-stranded DNA. The stabilization of strand exchange intermediates consisting of the parent duplex and the single strand by the copolymer is believed to be responsible for the observed acceleration behavior.

Keywords: DNA recognition · DNA strand exchange · graft copolymer · oligonucleotides · polycations

Introduction

The DNA strand exchange between double-stranded (ds) and single-stranded (ss) DNAs is a pivotal process in the repair of DNA damage and genetic recombination. Thus, growing interest has been placed on its mechanism. *Escherichia coli* RecA protein promotes homologous pairing by a strand exchange mechanism in vivo and in vitro.[1] The RecA protein has multivalent binding sites with DNAs and forms an intermediate complex with both dsDNA and ssDNA, in which they align homologously. Therefore a three-stranded intermediate is believed to be a kinetic transition state.[2] We have previously reported that polycationic comb-type copolymer (αPLL-*g*-Dex) having hydrophilic side chains promotes and stabilizes duplex and triplex DNA formation by reducing the counterion condensation effect accompanied by duplex or triplex formation.[3, 4] Thermodynamic and kinetic studies have shown that the αPLL-*g*-Dex copolymer increases the equilibrium association constant of the triplex formation by two orders of magnitude.[5] The copolymer-mediated triplex formation is attributed kinetically to a considerable increase in the association rate rather than a decrease in the dissociation rate.[5] These facts suggest that the copolymer stabilizes not only the matured triplex structure but also the intermediate complex consisting of dsDNA and the third DNA strand. Although the three-stranded intermediates in the strand exchange reaction are different from that in the Py·Pu:Pu or Py·Pu:Py motif triple-helix formation, both intermediates must be suffering from the electrostatic obstacle due to accumulation of phosphate anions. We hypothesized that the polycationic copolymer could also accelerate the strand exchange reaction between ssDNA and dsDNA through stabilization of the strand exchange intermediates. We have estimated the effect of αPLL-*g*-Dex copolymer on the strand exchange between DNA duplex and its complementary ssDNA. It was found that the copolymer and not polyamine (spermine) nor a cationic detergent (i.e., cetyltrimetylammonium bromide, CTAB) significantly accelerates strand exchange reaction. To our knowledge, it is the first demonstration of a synthetic polymer that shows the obvious acceleration of DNA strand exchange reaction between nonmodified DNAs.

[a] Prof. A. Maruyama, W. J. Kim, Dr. T. Ishihara, Prof. T. Akaike
Department of Biomolecular Engineering
Faculty of Bioscience and Biotechnology
Tokyo Institute of Technology, 4259 Nagatsuta
Midori, Yokohama 226-8501 (Japan)
Fax: (+81)-45-924-5122
E-mail: amaruyam@bio.titech.ac.jp

Results and Discussion

Cationic substances investigated and the ODN sequences used in this study are shown in Figure 1a and b.

The time course of strand exchange between fluorescein-labeled dsDNA (F-dsDNA) and its complementary DNA at 37 °C is shown in Figure 2a, in which the slower and faster

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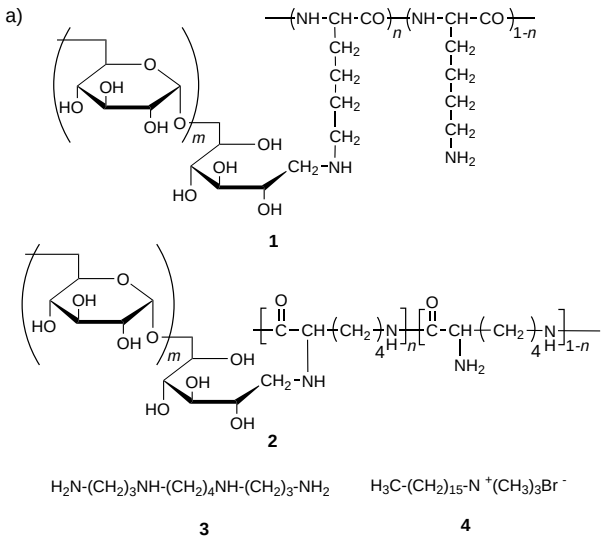
a)



b)

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ODN E1: 5'-TCATAATCAGCCATACCACA-3'
ODN E2: FITC-5'-TGTGGTATGGCTGATTATGA-3'
ODN E3: 5'-TGTGGTATGGCTGATTATGA-3'
ODN E4: 5'-ATGGTGAGCAAGGGCGAGGA-3'
ODN E5: FITC-5'-TCCTCGCCCTTGCTCACCAT-3'
ODN E6: 5'-TCCTCGCCCTTGCTCACCAT-3'
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Figure 1. a) Structural formulas of αPLL-*g*-Dex (**1**), εPLL-*g*-Dex (**2**), spermine (**3**), and cetyltrimetylammonium bromide (CTAB) (**4**). b) ODN sequences used in this study; the ODN sequences of the target duplexes (E1/E2, E4/E5) are located between 1421 bp and 1440 bp, 683 bp and 702 bp of the plasmid pEGFP-N2 (Clontech Laboratories, Inc., Palo Alto), respectively.

migration bands correspond to the original F-dsDNA and fluorescein-labeled ssDNA (F-ssDNA) dissociated from the dsDNA. The exchange reaction in the absence of the copolymer hardly occurred and less than 20% of the duplex was replaced by unlabeled ssDNA after 6 h incubation at 37 °C. In contrast, most of the F-DNA in the duplex was replaced within 5 min in the presence of the copolymer.

We then estimated formation of non-labeled dsDNA as shown in Figure 3, in which the F-dsDNA was incubated with or without ssDNA for 18 h at 37 °C in the presence or absence of the copolymer and then analyzed by gel electrophoresis. Ethidium bromide (Et-Br) staining of the gel clearly indicated the formation of dsDNA without fluorescein-label (Figure 3a versus b, lanes 5 and 6). Incubation of F-dsDNA without the complementary DNA in the absence or presence of the copolymer did not result in any dissociation of the F-ODN even after 18 h at 37 °C (Figure 3a, lanes 1 and 2). Therefore, the observed dissociation of F-ssDNA from the duplex was due to the strand exchange reaction and not to spontaneous dissociation of the duplex.

To assess the kinetic effect of the copolymer, the same exchange reaction was carried out at 15 °C (Figure 2b). We still observed the drastic effect of the copolymer. The exchange reaction in the presence of the copolymer was almost completed after 6 h incubation, while scarce exchange reaction was observed in the absence of the copolymer. The time course of strand exchange in the absence or presence of αPLL-*g*-Dex copolymer at 37 °C and 15 °C is depicted in Figure 2c, in which the degree to which exchange took place

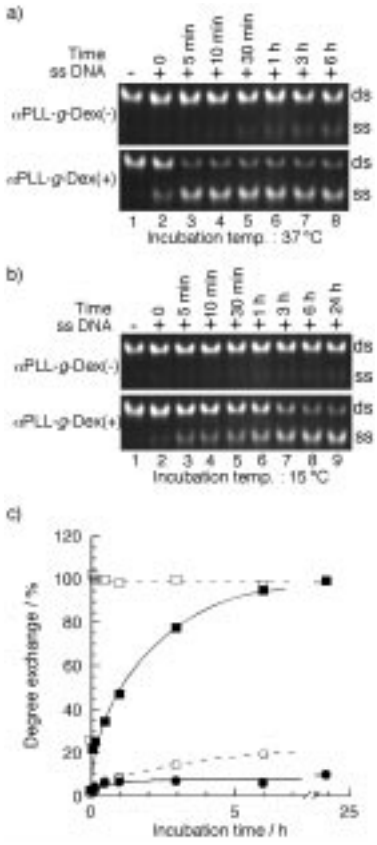

Figure 2. Strand exchange reaction in the absence or presence of the copolymer. The fluorescein-labeled duplex (E1/E2, 0.62 μM) was incubated at a) 37 °C or b) 15 °C with complementary ssDNA (E3, 3.08 μM) in PBS buffer (10 mM sodium phosphate, 0.5 mM EDTA, 150 mM NaCl, pH 7.2) in the absence or presence of 2.2 μM copolymer (polymer/DNA charge ratio = 2) for various time periods indicated above each lane. After incubation, the mixtures were separated on 13% PAAm gel and photographed. c) Time course of strand exchange reaction at 37 °C (open symbols) or 15 °C (closed symbols) in the absence (circle) or presence (square) of the copolymer.

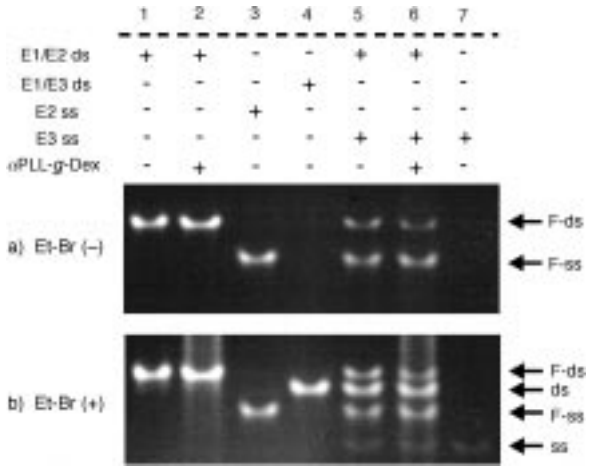

Figure 3. Gel electrophoresis images of reaction mixtures with a) or without b) ethidium bromide staining (Et-Br). Reaction mixtures indicated above the gel were incubated for 18 h at 37 °C. The mixtures were analyzed on 13% PAAm gel, and photographed with or without Et-Br staining. F-ds, F-ss, ds, and ss, respectively, denote FITC-labeled double, FITC-labeled single, double-, and single-stranded oligonucleotides. Under this condition the exchange reaction proceeded to great extent even in the absence of the copolymer, resulting in similar gel profile to that in the presence of copolymer (Figure 3a, lane 5 versus 6).

was calculated in percent using following Equation (1) to take into account the theoretical fraction of the exchanged product under equilibrium as 100%.

$$\text{Degree of exchange } (\%) = F \times ([\text{D}]+[\text{S}])/[\text{S}] \times 100 \quad (1)$$

where F, [D], and [S] are the fraction of exchanged fluorescein-labeled DNA, the initial concentrations of fluorescein-labeled duplex, and complementary ssDNA, respectively. Apparent rates of the exchange reaction at 15 °C were determined by pseudo first-order kinetic analyses (Figure 4 and Table 1). The copolymer increased the exchange rates by more than 300-fold (E1/E2 duplex with E3) and 1400-fold (E4/E5 duplex with E6). The accelerating efficiency of the copolymer was then compared with that of spermine or

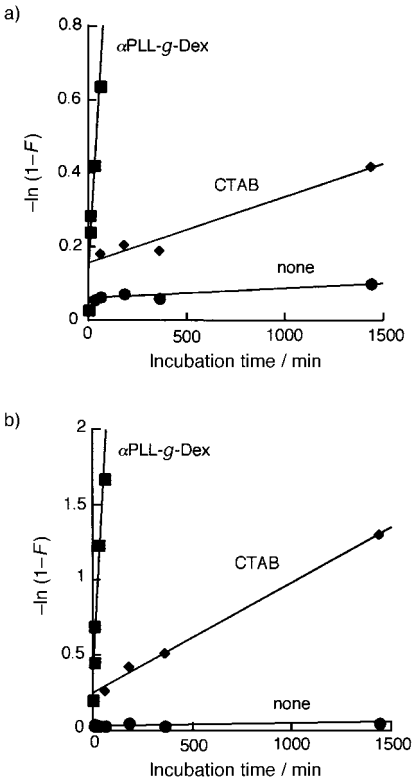

Figure 4. Pseudo first-order plots of strand exchange reactions, where F is the fraction of exchanged fluorescein-labeled DNA. a) The fluorescein-labeled duplex (E1/E2, 0.62 μM) was incubated at 15 °C with 5 molar excess of complementary ssDNA (E3, 3.08 μM) in PBS buffer in the presence of 2.2 μM PLL-*g*-Dex copolymer (polymer/DNA charge ratio = 2, ■), 3 mM CTAB (+/− charge ratio = 2.3, ◆) or in the absence of cationic substance (●). b) Pseudo first-order plots of the strand exchange between fluorescein-labeled duplex (E4/E5, 0.62 μM) and 10 molar excess of complementary ssDNA (E6, 6.2 μM). Other conditions are the same as in Figure 4a.

CTAB. As summarized in Table 1, the detectable acceleration effect on the strand exchange reaction is observed for CTAB and not for spermine while the copolymer shows the pronounced effect. Spermine is known to stabilize Py·Pu:Py motif triplex moderately under physiological ionic environment[6] but, it scarcely accelerates the strand exchange reaction. Similarly, CTAB which was reported to expedite significantly the renaturation of complementary DNA strands [7] is not effective in accelerating the strand exchange. Note

Table 1. The strand exchange reactions in the absence or presence of the cationic substances.[a]

ODNs	Substance	Degree of exchange [%][b]	k'[$M^{-1}s^{-1}$]	k'(rel.)
E1/E2+E3[c]	none	2.8	0.14	1
	1 mM spermine	5.0	n.d.[d]	n.d.
	10 mM spermine	3.2	n.d.	n.d.
	3 mM CTAB	15.2	0.96	6.7
	30 mM CTAB	9.6	n.d.	n.d.
	14.3 μM εPLL-*g*-Dex[e]	10.4	n.d.	n.d.
	2.2 μM αPLL-*g*-Dex[e]	78.7	47.3	330
	2.2 μM αPLL-*g*-Dex[e,f]	70.6	40.2	281
E4/E5+E6[g]	none	4.8	0.043	1
	3 mM CTAB	34.1	2.05	47.1
	3.8 μM αPLL-*g*-Dex[e]	87.1	63.6	1460

[a] All reactions were carried out at 15 °C in 10 mM sodium phosphate buffer (pH 7.2) containing 150 mM NaCl and 0.5 mM EDTA. [b] % Exchange degree obtained with 3 h incubation. [c] Exchange reaction of E1/E2 duplex with 5 molar excess of E3. [d] Not determined. [e] Polymer/DNA charge ratio = 2. [f] Strand exchange reaction in the presence of 10 mM $MgCl_2$. [g] Exchange reaction of E4/E5 duplex with 10 molar excess of E6.

that comb-type copolymers having ε-polylysine backbone failed to accelerate the exchange reaction (Table 1). Cationic charge densities along the polymer backbone are considered to be a key factor in the acceleration behavior. In addition, the accelerating effect of αPLL-*g*-Dex was not significantly influenced by 10 mM $MgCl_2$ (Table 1) which stabilizes the dsDNA. A similar tendency was obtained when a strand exchange study using a set of ODNs with different sequences was examined (Table 1).

Using modified ODNs accelerated strand displacement within dsDNA has previously been demonstrated.[8, 9] Peptide nucleic acids (PNAs) is known to hybridize to dsDNA with a homopyrimidine–homopurine stretch through triple helical intermediates.[8] Accelerated hybridization of PNA or ODNs conjugated with a cationic peptide or protein to non-homopyrimidine–homopurine dsDNA within inverted repeat and AT-rich sequences was also described.[9] In either cases, dsDNA with the modified ODN has higher stability than the native DNA duplex. Therefore, the exchange reaction is partly driven by the negative change in free energy owing to the stable duplex formation. This fact makes it difficult to understand strand exchange mechanisms because the modification affects thermodynamics of not only the exchanged products but also intermediate complexes. In contrast to the above cases, unmodified ODNs are employed in this study, so that the dsDNA product is the same after the reaction as the parent dsDNA before the reaction. The exchange reaction proceeded under equilibrium conditions and thereby was accompanied by no change in free energy. Hence, stabilization of the intermediate complexes to reduce activation energy would be a major role of the copolymer.

The strand exchange reaction promoted by the copolymer may be explained by two distinct reaction models that differ in the intermediate complexes; i) spontaneous and partial unwinding (breathing) of the parent duplex, followed by the heteroduplex formation with third ssDNA, branch migration, and elongation of the exchanged duplex, or ii) formation of triple-stranded intermediates by the use of non-Watson–Crick interactions[2, 10] followed by dissociation of ssDNA

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strand. The former model appeared to be reasonable for the strand exchange between short DNAs because of frequent breathing of the duplex ends. However, this interpretation was challenged by the fact that the copolymer stabilizes DNA duplex.[4] The T_m of the E1/E2 duplex was increased by 12 °C in the presence of the copolymer (Figure 5), which implies that the copolymer considerably reduces the spontaneous breathing of the duplex. Alternatively, it may be possible that the copolymer expedites the strand exchange through stabilization of the triple-stranded intermediates described in the latter model. Further studies including spectroscopic observation and strand exchange reaction using circular (or longer) DNA are needed to identify the mechanism involved in the copolymer-mediated strand exchange.

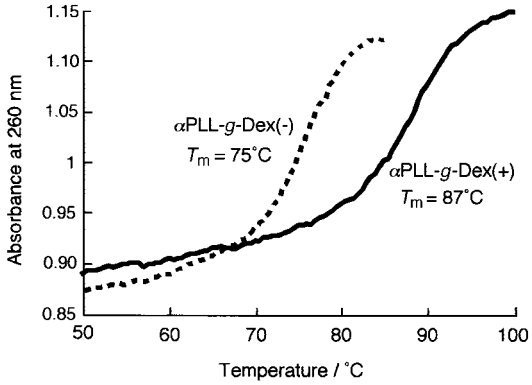

Figure 5. UV/T_m profile of duplex (E4 and E6) in the presence or absence of αPLL-*g*-Dex copolymer. ODN E4 and E6 were dissolved in 10 mM sodium phosphate buffer containing 150 mM NaCl (pH 7.2). The UV/T_m curves were recorded at heating rate of 0.5 K per min with a DU-640 spectrometer (Beckman) equipped with a micro-T_m apparatus.

Spermine is known to stabilize both a DNA duplex and triplex in solutions with low ionic strength. However, the stabilizing efficacy of spermine is considerably reduced with increasing ionic strength. Conversely, the copolymer with much lower concentration than spermine maintains its strong stabilization efficacy over the physiological ionic strength; this indicates a considerably stronger interaction of the copolymer with DNA due to its higher multivalency of cations. Although there is such a strong interaction, the copolymer, unlike cationic homopolymers including polylysine, does not induce a change in the highly ordered structure of a DNA duplex and triplex.[3a,d] These unique properties of the copolymer may play an important role in the observed acceleration of the DNA strand exchange.

Experimental Section

Oligonucleotide purification and duplex preparation: Oligodeoxynucleotides (ODNs) and 5′-fluorescein-labeled ODNs (F-ODNs) were purchased from Nippon Fluor Mills Co. (Tokyo, Japan). ODNs were purified by reverse-phase high performance liquid chromatography (HPLC) and analyzed by matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry. A fluorescently labeled DNA duplex was obtained by mixing F-ODN (E2 or E5) and its complementary ODN (E1 or E4) in equimolar amounts and annealing at 95° C for 5 min, followed by slow cooling to room temperature over 12 h. The formation of the duplex was checked by a polyacrylamide gel (PAGE) or HPLC and isolated from free ssDNA by HPLC if needed.

Synthesis of polycationic comb-type copolymers: Comb-type copolymers (αPLL-*g*-Dex and εPLL-*g*-Dex) were synthesized by reductive amination reaction of αPLL·HBr (M_n: 20000, BACHEM California Inc., Torrance, USA) or εPLL·HCl (M_w: 5100, Chisso Co., Tokyo Japan) with dextran (M_n: 5900, Dextran T-10, Phamacia Biotech, Uppsala, Sweden), as described previously.[3, 11] The resulting copolymers were isolated by ultrafiltration to remove unreacted Dex. Finally, the copolymers were lyophilized. ^{1}H NMR spectroscopy and gel permeation chromatography (GPC) were employed to characterize the resulting copolymers.

DNA strand exchange reaction: Phosphate-buffered solutions (PBS) (5 μL) with or without a cationic reagent (either copolymer, spermine, or CTAB) was added to PBS buffer (10 μL, 10 mM sodium phosphate, 0.5 mM EDTA, 150 mM NaCl, pH 7.2) on ice, containing dsDNA (15.4 pmol). Strand exchange reaction was initiated by adding PBS (5 μL) with the complementary ODN (77 pmol) which had the same sequence as the F-ODN of the duplex. The polymer/DNA ([amino groups]$_{copolymer}$/[phosphate groups]$_{DNA}$) charge ratio was kept constant at a value of two throughout this study. After incubation for various periods of time at 37 °C or 15 °C, the reaction was stopped by cooling the samples on ice and analyzed on 13% PAGE. If cationic substance was added to the reactions during incubation, the respective buffer (5 μL) with salmon sperm DNA (or SDS for CTAB) was also added to dissociate the cationic substance from DNA before electrophoresis.[3b] The amount of F-ODN in each band was quantified with Biofil Bioimage Analyser (Vilber Lourmat, France).

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