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DNA Strand Exchange Stimulated by Spontaneous Complex Formation with Cationic Comb-Type Copolymer

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Association, dissociation, and strand exchange of nucleic acid hybrids are pivotal processes in maintenance of living systems. Various types of nucleic acid acting proteins, such as helicases and recombinases, are involved in the regulation of nucleic acid transition. Stabilization of intermediate structures in these structural transitions is thought to be one of the actions of these proteins. On the other hand, an artificial agent that is capable of manipulating nucleic acid hybridization would be beneficial to elaborate various types of DNA-based devices for DNA nanomachines,[1] nanoassembly,[2] and molecular computing.[3] These agents would be also important to refine genotyping methods and DNA medicines that target a particular gene or its transcript in order to inspect and engineer gene expression.

We have previously demonstrated that the cationic comb-type copolymers, CCCs, composed of a cationic poly(L-lysine) backbone and water-soluble side chains of dextran accelerate DNA hybridization[4] to increase stability of DNA duplexes and triplexes.[5] Furthermore, CCCs are found to stimulate the DNA strand exchange reaction (SER) between double helical DNA and its homologous single strand at a much faster rate (50 000-times at 37 °C) than spermine and *N*,*N*,*N*-trimethylhexadecylammonium bromide (cethyltrimethylammonium bromide, CTAB).[6] In this report we employed fluorescence resonance energy transfer (FRET) assay,[7] to better understand the CCC-mediated strand exchange. We considered that alleviation of counterion association during transitional intermediate formation was partly involved in CCC's acceleration mechanisms.

DNA strand exchange was monitored by FRET assay[7] using the duplexes (F1/T1 or F2/T2) labeled with fluorescein isothiocyanate (FITC) and carboxytetramethylrhodamine (TAMRA). The experimental schematic of the strand exchange detection using FRET is depicted in Figure 1A. The FITC emission that had been quenched by TAMRA was recovered by replacement of the TAMRA-labeled strand in the duplex with a homologous nonlabeled strand, enabling us to monitor the strand exchange time course. The time courses of the reaction between F1/T1 with M1, and F2/T2 with M2, in the absence or presence of CCC[8] are shown in Figure 1, B and C, respectively. Whereas no SER was detected within 5 min incubation in the absence of CCC, the reaction had reached equilibrium within 2 min in the presence of CCC. CCC increased the exchange rate 4−5 orders (Table 1). The exchange rate linearly increased with CCC/DNA charge ratio (N/P ratio) ranging from 0.1 to 1.0, suggesting that saturation of DNA/CCC interaction is not necessary for CCC to exhibit the effect. Arrhenius plots of the strand exchange of F2/T2 with M2 indicate the activation energies to be 142.7 and 118.4 kJ/mol, respectively, in the absence and presence of CCC.

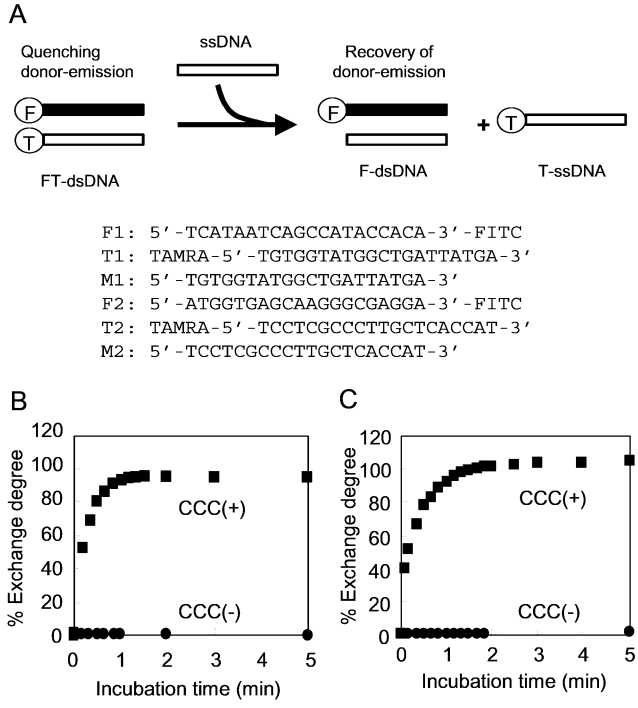

Figure 1. (A) Experimental schematic of DNA strand exchange detection by using FRET and sequences of DNAs used. (B) Time course of SER between F1/T1 ds DNA and M1 ss DNA at 37 °C. The F1/T1 ds DNA (12 nM) was incubated at 37 °C with M1 ss DNA (60 nM) in PBS buffer (10 mM sodium phosphate, 0.5 mM EDTA, 150 mM NaCl, pH 7.2) in the absence or presence of 34 nM copolymer (copolymer/DNA charge ratio = 2). The value of % exchange degree was calculated with following equation: % exchange degree = $([FI]_t - [FI]_0)/([FI]_\infty - [FI]_0) \times 100$ where $[FI]_0$ is the initial fluorescence intensity, $[FI]_t$ is that at time t, and $[FI]_\infty$ is that after the reaction reached equilibrium. The value of $[FI]_\infty$ was practically obtained by measuring the mixture that had been heat-treated (heating at 90 °C for 5 min, followed by slow cooling to 37 °C). (C) Time course of strand exchange between F2/T2 ds DNA and M2 ss DNA at 37 °C. The procedure was the same as (B).

To inspect the accelerating efficiency of the copolymer the strand exchange with a 50mer ss DNA target having intramolecular folding structures was examined. The copolymer also showed considerable accelerating effect.

Since CCCs spontaneously interact with DNA to form interpolyelectrolyte complexes, we assessed influence of ionic strength on the CCC-mediated strand exchange to elucidate a role of the ionic interaction. As shown in Figure 2A, the acceleration effect of CCC is significantly reduced as ionic strength of the medium increased, implying that the ionic interaction between CCC and DNA plays a pivotal role in the strand exchange stimulation. At

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Table 1. Melting Temperatures of Duplexes and Kinetic Parameters for Strand Exchange Reaction

	T_m [°C][a]		strand exchange rate constant [$s^{-1} M^{-1}$][b]		
	CCC (–)	CCC (+)	$k'(-)\times10^6$	$k'(+)\times10^2$	$k'(+)/k'(-)$
F1/T1	55	76	<0.45[c]	3.7	$>5 \times 10^4$ [c]
F2/T2	64	82	1.5	4.5	2.9×10^4

[a] Values of T_m were determined by fluorescence melting analysis using SYBR Green I dye at duplex concentration of 12 nM. Reproducibility of the reported T_m values is ±1 °C. [b] Values of $k'(-)$ and $k'(+)$ represent the pseudo-first-order rate constants for the strand exchange between F1/T1 ds DNA and M1 ss DNA or between F2/T2 ds DNA and M2 ss DNA in the absence and presence (N/P = 2) of PLL-*g*-Dex, respectively. Regression parameters, r^2, in pseudo-first-order analyses were over 0.95 for all rate constants determination. [c] The values could not be estimated owing to absolutely small $k'(-)$ value.

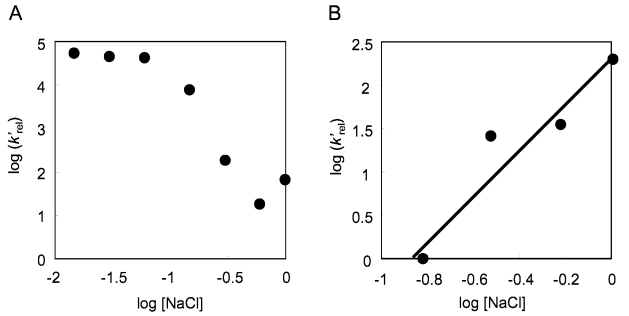

Figure 2. Salt dependency of SER in the presence (A) or absence (B) of CCC at 37 °C. The fitted line of log $k'_{rel} = 2.6$ log [NaCl] + 2.3 [$r^2 = 0.91$) is presented. The values of k'_{rel} represent the pseudo-first-order rate constants relative to that under 150 mM NaCl without CCC.

[NaCl] = 15 mM more than 5×10^4-fold acceleration was observed.

SER of short duplexes has been explained with two distinct pathways, a dissociative pathway and a sequential displacement pathway.[9,10] The dissociative pathway occurs by the spontaneous and complete dissociation of the initial duplex followed by association of the target strand. The sequential displacement pathway requires only the partial melting of the initial duplex to allow for the formation of a branched nucleation complex with the homologous strand. Subsequent migration of the branched point results in formation of a final duplex. The former pathway is dominant for the strand exchange at reaction temperatures near T_m of the duplex, whereas the later pathway is predominant at reaction temperature far lower than the T_m.[9] At physiological condition, the strand exchange of duplexes longer than 14 bp could take place dominantly via sequential displacement pathway.[9] Melting temperatures of the duplexes used in this study were 55 °C (F1/T1) and 64 °C (F2/T2) and much higher than the experimental temperature, 37 °C. Hence, the sequential displacement pathway is probably a major route of the SER under our experimental condition. The magnitude of the activation energy also supported this consideration. The activation energy estimated for the strand exchange between F2/T2 vs M2 was 142.7 kJ/mol, being consistent with the value previously reported for the displacement pathway.[9,11] Furthermore, as CCC increased T_m of duplexes[4] (Table 1) by about 15 °C, we can assume the sequential displacement pathway in the strand exchange.

The sequential displacement pathway involves three-stranded intermediates of the branched nucleation whose formation is seemingly impeded by counterions accumulation process. The accumulation of counterions is thermodynamically unfavorable and is well documented by counterion condensation theories for ds DNA association.[12,13] However, its relation to SER has not been settled. Figure 2B shows the ionic strength dependency of the SER in the absence of CCC. The strand exchange rate increased with an increase in the ionic strength, showing that the SER involves the transitional intermediate whose formation is hampered by counterion accumulation process. The result implies that the three-stranded intermediate formation and not partial dissociation of the initial duplex is the rate-limiting process of SER. Plots of log k' vs log [NaCl] are linear with a slope of 2.6 (Figure 2B), indicating 2.6 sodium ions associate with the DNA during the rate-limiting step of the SER. Importantly, the extent of counterions associated during the rate-limiting step of the SER is almost twice larger than that (1.4 sodium ions) described for duplex formation.[13] To our knowledge, there has not been a previous report describing the extent of counterion association in the rate-limiting step of SER. Similar to the salt effect, the copolymer accelerated SER by promoting the intermediate formation through interpolyelectrolyte complex formation that alleviates the counterion condensation effect.

In conclusion, manipulation of DNA's counterions with CCC results in stabilization of not only matured hybrids but also the nucleation complexes, leading to the accelerated hybridization.

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Supporting Information Available: Arrhenius plot for the DNA SER with or without copolymer, N/P ratio dependency of SER, and time course of strand exchange between F2/T2 ds DNA and 50mer ss DNA (PDF). The material is available free of charge via the Internet at http://pubs.acs.org.

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