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DNA Strand Exchange Reaction Activated by Cationic Comb-type Copolymer Having Ureido Groups

Naohiko Shimada, Wei Song and Atsushi Maruyama*

Strand exchange reactions (SERs) of nucleic acids are essential for genetic recombination and often require nucleic acid chaperone activity. A variety of proteins induce strand exchange reactions in vivo. We have reported that cationic comb-type copolymers composed of a polycation backbone and hydrophilic graft chains greatly accelerate the SER in vitro and exhibit nucleic acid chaperone like activity. To elucidate structure/function relationships involved in the SER accelerating activity of the copolymer, we modified the copolymer with ureido groups. The melting temperature of dsDNA in the presence of the copolymers with ureido groups decreased with increasing ureido content, suggesting that the ureido groups have a destabilizing effect on the DNA duplex. Copolymers modified with ureido groups (about 50 mol%) accelerated a SER more strongly than unmodified copolymers. Given our previous observation that modification of the copolymer with guanidino groups resulted in enhanced chaperone-like activity, we propose that incorporation of chaotropic functional groups into the copolymer structure results in an increase in nucleic acid chaperone-like activity.

Introduction

In a strand exchange reaction (SER) a double-strand of DNA or RNA exchanges one of its strands for a homologous strand from a single-stranded (ss) or double-stranded (ds) donor. SER is a key step in genetic homologous recombination. RecA and its homologous protein Rad51 play central roles in SERs in cells. SER is also a pivotal process in folding of nucleic acids, especially RNA, into a functional conformation. Nucleic acid chaperone proteins catalyze SER from kinetically-trapped structures into the thermodynamically most favored one. HIV-1 nucleocapsid protein 7 (NCp7), the major messenger ribonucleoprotein particle protein p50, and the nucleoid associated transcriptional regulator of E. coli StpA all exhibit nucleic acid chaperone activity.

SER has been utilized in biotechnological application. There are many examples for DNA nanomachines driven by SER. Recently, DNA logic gates employing SER have been also built. Nanomachines or logic gates work properly because of sequence specific SER. However, there is an issue with response speed of the action when the DNA concentration is lower. Acceleration of SER is required for rapid response of the nanomachines or the logic gates.
We have previously demonstrated that cationic comb-type copolymers composed of a polycation backbone and abundant hydrophilic graft chains influence kinetics and thermodynamics of nucleic acid hybridization under physiologically relevant conditions. For example, poly(L-lysine)-graft-dextran (PLL-g-Dex) copolymer significantly accelerates DNA hybridization.\(^\text{13}\) Moreover, the copolymer markedly accelerates the SER and increases stability of dsDNA relative to that in buffer alone.\(^\text{14, 15}\) The copolymer was the first example of an artificially synthesized polymer that activated SER. The SER acceleration of copolymer has been applied for refining DNA detection method\(^\text{16-18}\) and DNA nanomachines.\(^\text{19, 20}\) Other artificial molecules activated SER were reported. Frykholm et al. reported that positively charged liposome enhances SER.\(^\text{21, 22}\) Chitosan, which is a natural polysaccharide with cationic groups, also activates the SER.\(^\text{25}\) The ionic interactions between the cationic copolymer and anionic DNA appear to be indispensable in the SER activation, but other interactions are also thought to be involved in the activity of the copolymer. We have prepared a variety of the cationic comb-type copolymers to estimate the effect of chemical structure of the copolymers on DNA hybridization.\(^\text{24-26}\) We previously showed that a cationic comb-type copolymer with guanidino groups accelerates SER relative to the unmodified copolymer.\(^\text{27}\) Guanidinium modification did not significantly alter cationic properties of the copolymer but did change hydrogen-bonding capacity. The guanidinium ion is also known to have chaotropic effects that destabilize dsDNA, which should accelerate SER. To further understand of the structure/function relationship of the copolymer, we have focused on non-ionic urea groups in this study. Urea, like guanidinium ion, has a large hydrogen-bonding capacity and is a strong chaotropic reagent. We prepared cationic comb-type copolymers modified with urea (ureido) groups, poly(allylamine-co-allylurea)-graft-dextran (PAU-g-Dex, Fig. 1), and evaluated its effect on SER.

**Experiment**

**Materials**

Poly(allylamine) hydrochloride (PA·HCl) aqueous solution (\(M_c: 1.5 \times 10^4\)), kindly supplied by Nitto Boseki, was purified by precipitation from methanol. Dextran (\(M_c: 8 \times 10^5\)) was obtained from Extrasynthese. Sodium cyanoborohydride and poly(vinylsulfonic acid), sodium salt (PVS) were purchased from Sigma-Aldrich. Oligonucleotide DNAs (Fig. 1) were purchased from FASMAC Co.. Other reagents were purchased from Wako Pure Chemical Industries.

**Preparation of graft copolymers**

Preparation of poly(allylamine)-graft-dextran (PA-g-Dex, Fig. 1) was described in a previous report.\(^\text{26}\) Briefly, the copolymer was prepared by a reductive amimation reaction of PA (\(M_c: 15 \times 10^3\)) with dextran using sodium cyanoborohydride as a reductant in sodium borate buffer (pH 8.5).\(^\text{28}\) The composition of the resulting copolymer was determined by \(^1\)H NMR in D2O, and UV-melting temperature measurements were carried at a final dsDNA concentration of 0.1 mM in 10 mM HEPS (pH 7.5) containing 10 mM, 50 mM, or 150 mM NaCl. The cationic copolymers were added to dsDNA solution at an [amino groups]copolymer/[phosphate groups]dsDNA (N/P) ratio of 5. UV melting curves at 260 nm were recorded with a V-630 spectrophotometer (JASCO) equipped with temperature controller at a heating rate of 1 °C/min. The differential absorbance (\(\Delta A = \) hyperchromicity at 260 nm) was calculated to correct for baseline shift.

dsDNA was obtained by mixing complementary strands, NF and M (Fig. 1), in equimolar amounts and annealing at 95 °C for 5 min in the presence of cationic copolymers if indicated, followed by slow cooling to 25 °C. Melting temperature (\(T_m\)) measurements were carried at a final dsDNA concentration of 1 µM in 10 mM HEPS (pH 7.5) containing 10 mM, 50 mM, or 150 mM NaCl. The cationic copolymers were added to dsDNA solution at an [amino groups]copolymer/[phosphate groups]dsDNA (N/P) ratio of 5. UV melting curves at 260 nm were recorded with a V-630 spectrophotometer (JASCO) equipped with temperature controller at a heating rate of 1 °C/min. The differential absorbance (\(\Delta A = \) hyperchromicity at 260 nm) was calculated to correct for baseline shift.

**Fig. 1** Structural formulas of (A) PA-g-Dex, (B) PAU-g-Dex, (C) PAAc-g-Dex, and (D) DNA oligonucleotide sequences used in this study.
Pseudo-first-order rate constant (k') in HEPES buffer (pH 7.5) containing 10 mM NaCl. As shown in Fig. 2A, the DNA melting temperature of dsDNA in the presence of copolymers in 10 mM HEPES buffer (pH 7.5) containing 10 mM NaCl was increased compared to that of dsDNA alone (47 °C). PAU-g-Dex having 53 mol% ureido groups (U53) stabilized dsDNA to a similar extent as that observed in the presence of PAU-g-Dex. PAU-g-Dex with higher ureido contents (U75 and U88) were less stabilizing. The Tm value in the presence of U88 was 47 °C, the same as that of dsDNA in the absence of copolymer. Similar ureido content dependencies on Tm were observed when the NaCl concentration was increased to 50 mM and to 150 mM (Fig. 2B, C). The loss of the stabilization effect of the copolymers at high ureido content was primarily caused by decrease in positive charge density. However, other contributions from the ureido modification are likely because the Tm curve in the presence of U88 had a much broader transition than that of dsDNA alone. To further assess the effect of ureido modification, we modified PAU-g-Dex with acetyl groups. PAU-g-Dex having 53 mol% ureido groups (U53; Fig. 2, solid green lines) was less stabilizing than acetylated PAU-g-Dex (PAAc-g-Dex, A54; Fig. 2, dashed green lines) although both copolymers have the same cationic density.

**Table 1. Properties of cationic comb-type copolymers used in this study**

<table>
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<tr>
<th>polymer code</th>
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<td></td>
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<tr>
<td>U0</td>
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<td>A54</td>
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<td>33.6x10⁵</td>
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° Mol ratio of KOCN to amino groups of PA-g-Dex ([KOCN] / [PA-g-Dex] ureido group). ° Determined by 'H NMR in D₂O containing 0.1% NaOD. ° Pseudo-first-order rate constant (k') in HEPES buffer containing 10 mM NaCl.
Effect of ureido modification on strand exchange reaction

To estimate the effect of ureido modification of the cationic comb-type copolymer on a DNA SER, SERs in the presence of PAU-g-Dex copolymers with varying ureido content were explored. A PAGE assay was used to follow the SER between FITC-labeled dsDNA (F/M) and its homologous non-labeled ssDNA (NF, 30 times molar excess to the labeled dsDNA). A band corresponding to dsDNA disappeared and a new band corresponding to ssDNA appeared as a function of time (Fig. 3). Intensity of the dsDNA band slightly decreased in the absence of the copolymer after 24 hours. In the presence of PA-g-Dex (U0), a band corresponding to ssDNA was visible after a 30-min incubation and increased in intensity as a function of time. This result indicated that the cationic comb-type copolymer (U0) accelerated the SER. The SER was observed in the presence of all ureido-modified PA-g-Dex copolymers tested with the exception of U88. The reaction in the presence of copolymer U53, which is modified with 53 mol% ureido groups, was complete within 30 min. The presence of the ureido modification significantly enhanced SER relative to the unmodified copolymer; however, when the polymer was highly modified with ureido groups (U88, 88% modification), the SER was not accelerated significantly relative to the reaction in the absence of copolymer. This is likely due to a lack of ionic interactions between U88 and DNA.

In order to evaluate kinetics of SER more quantitatively, we employed a FRET assay. The extent of strand exchange over time in the absence and presence of ureido-modified copolymers are shown in Fig. 4A. No significant increase in the fluorescence intensity (which is a measure of SER) was observed in the absence of copolymer. An increase in fluorescence intensity over time was observed in the presence of all copolymers except U88. The copolymer with moderate ureido modification (U53) accelerated the SER to the greatest extent. The pseudo first order rate constants ($k'$) for the SER determined by FRET are summarized in Table 1. In the absence of copolymer, the SER was very slow ($k' < 1 \times 10^{-6}$ s$^{-1}$). The presence of U0 ($k' = 18.9 \times 10^{-5}$ s$^{-1}$) accelerated SER at least 100 times relative to the reaction in the absence of copolymer. U53 ($k' = 15.6 \times 10^{-5}$ s$^{-1}$) accelerated SER about 8 fold relative to U0. The rate of the SER in the presence of acetylated copolymer, PAAc-g-Dex (A54, $k' = 33.6 \times 10^{-5}$ s$^{-1}$) was slower than that in the presence of ureido-modified PA-g-Dex U53, even though both copolymers have the same modification degree and cationic charge density. This result strongly suggests that ureido groups facilitate SER. Fig. 4B shows the rate constants as a function of ureido content of the copolymers. In 10 mM NaCl, the rate constant increased with increasing ureido content, peaking at 53 mol%, and then decreased with further increase in ureido content. Peaks were also observed in 50 and 150 mM NaCl; the peak shifted to lower ureido content as NaCl concentration increased. This is reasonable because the ionic interaction between the copolymer and DNA is weakened with increasing NaCl concentration. Higher cationic density (i.e., lower modification degree) of the copolymer is required to maintain ionic interactions required for SER acceleration at higher NaCl concentration. The observed SER enhancement at higher NaCl concentration in the presence of copolymers was also observed in a PAGE experiment (Fig. S1).
Binding affinity of PAU-g-Dex for dsDNA and ssDNA

We assessed the binding affinity of DNA to PAU-g-Dex to understand role of the ureido group in the SER acceleration. Fluorescently labeled ssDNA or dsDNA was mixed with copolymer, and the change in fluorescence polarity was observed. Fig. 5 plots the fluorescence polarity as a function of concentration of copolymer. U0 interacted with dsDNA more strongly than with ssDNA (Fig. 5A). This was expected because cationic polymers the 200bp dsDNA has more anionic charges and a higher anionic charge density than the 200nt ssDNA. PAU-g-Dex (U29 and U53) also bound more strongly to dsDNA than to ssDNA (Fig. 5B, C). Binding profiles of A54 to dsDNA and ssDNA were not significantly different from those of U53 (Fig. 5D). Furthermore, affinities of these two copolymers for both dsDNA and ssDNA were weaker than that of U0. These results suggest that binding affinity of PAU-g-Dex copolymers to DNAs is primarily determined by electrostatic interactions.

Discussion

The strand exchange reaction between short dsDNA and a homologous ssDNA likely proceeds through nucleation and branch migration as depicted in Fig. 6. The terminal ends of dsDNA have breathing dynamics as base pairs associate and dissociate. The SER is initiated by nucleation step where the ssDNA partially binds to the breathing dsDNA terminus to form a three-stranded intermediate. The nucleation step is followed by a rapid branched migration step. The three-stranded intermediate must be thermodynamically unstable because of limited number of base-pairs and local accumulation of anionic charges due to the phosphate ions. This nucleation step is considered to be the rate determining step. We speculate that cationic comb-type copolymers accelerate SER by stabilizing the three-stranded intermediate by reducing electrostatic repulsion. When the three-stranded intermediate is stabilized by the presence of copolymer, the rate-determining step of SER should be the breathing step. Since the cationic comb-type copolymers increased the thermal stability of dsDNA, breathing frequency must be decreased in the presence of the copolymer, and this may cause a bottleneck in SER. Hence the SER accelerating activity of the copolymers might be enhanced by reducing the stabilizing effect of the copolymer for dsDNA.

In this paper, we focused on modification of the copolymer with ureido groups. Due to chaotropic activity, urea denatures highly ordered structures of proteins and nucleic acids, and,
therefore, we assumed that incorporation of the chaotropic activity in the copolymers would result in less stabilization of dsDNA. As expected, in the presence of PA-g-Dex modified with ureido groups the thermal stability of dsDNA was decreased relative to stability in the presence of unmodified copolymer (Fig. 2). Since the ureido modification of the copolymer resulted in decrease in cationic charge density of the copolymer, both decrease in cationic charge density and incorporation of the ureido activity are seemingly involved in the destabilizing effect. Comparison of ureid and acetyl modified copolymers, however, strongly indicated a substantial role of the ureido groups in the destabilization activity (U53 vs. A54, Fig. 2B, C). A 54 mol % level of acetyl modification of PA-g-Dex did not alter the $T_m$ of dsDNA relative to that in the presence of unmodified copolymer, however, the same level of ureido modification decreased the $T_m$ significantly.

As summarized in Fig. 4, the SER acceleration effect due to copolymer was enhanced by ureido modification. The ureido modification likely facilitates nucleation frequency by increasing dsDNA breathing. Although there were no significant difference in $T_m$ of dsDNA in the presence of U53 or U0 under 10 mM NaCl condition (Fig. 2A), U53 showed higher acceleration activity than U0 (Fig. 3 and Fig. 4). This may be due to local destabilization by U53. As clearly seen in the data shown in Fig. 4, there is an optimum degree of modification. This suggests that ionic interactions are prerequisite for copolymer facilitation of SER. A shift in the peak position to lower ureido content with increasing NaCl concentration supports this idea. We previously analyzed the effect of a copolymer modified with guanidium groups, another chaotropic agent. The guanidino modification also resulted in a loss in stabilizing effect and an increase in SER acceleration relative to the unmodified copolymer. Taking into account these observations, DNA denaturing activity of these functional groups was maintained when these groups were conjugated with polymer chains. This destabilization effect on dsDNA may enhance breathing dynamics of base pair association/dissociation, leading to the enhancement of the strand exchange reaction.

Conclusions

In this study, we explored effect of cationic copolymers modified with chaotropic ureido groups on a DNA strand exchange reaction. The copolymers modified to about 50 mol % ureido content accelerated the SER 8 fold relative to the unmodified copolymer. Ureido groups in the copolymer likely increased breathing dynamics of dsDNA to accelerate SER. We propose that incorporation of chaotropic functional groups such as ureido and guanidinium groups into the copolymer structure can be used in design of artificial nucleic acid chaperones.

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Notes and References

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†Electronic Supplementary Information (ESI) available: PAGE experiment of SER at higher NaCl concentration in the presence of copolymers. See DOI: 10.1039/c000000x/

Ureido modification of cationic graft copolymers accelerated DNA strand exchange reaction relative to unmodified copolymers.