DNA Nanomachine Switching Improved by Cationic Comb-Type Copolymer

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Summary: The unique folding and assembling properties of DNA have been elaborated to construct various nanomachines that can be reversibly switched between two or more distinct conformations. For the better applications and developments of DNA nanomachines, their responding kinetics, outputs, and sequence-selectivity need to be improved. Furthermore, the DNA nanomachines currently have several limitations in the operation conditions such as temperature, salt condition, and DNA strand concentration. In this study, we evaluated the effect of a cationic copolymer on the responses of a DNA-fueled nanomachine. We found that the copolymer increases quickness of the nanomachine under moderate conditions including physiologically relevant conditions even at very low strand concentrations (nM range).

Keywords: DNA; graft copolymers; nanomachine; nanotechnology; polycation

Introduction

Recently, the applications of DNA to nanotechnology fields have attracted increasing interests because of its sequence-specific interaction and robust physicochemical nature. Yurke et al. first demonstrated a DNA-fueled nanomachine which undergoes the structural transition from one to another in response to added DNA fuels.[1] The common operating principles of DNA-fueled nanomachines are sequence- specific hybridization and strand exchange reaction between the machine body and DNA fuels. The output and frame strength of the machines depend on the stability of DNA hybrids, while the response quickness relies on hybridization and, more specifically, strand exchange rates. It is difficult to satisfy both requirements simultaneously because of the dilemma that an increase in hybrid stability generally results in a decrease in the strand exchange rates. In order to acquire rapid and robust performance of the DNA nanomachines, the machines currently have several limitations in DNA strands and operating conditions such as temperature, salt conditions, and DNA strand concentration.[1–3]

We have previously reported that the cationic comb-type copolymers, composed of a cationic poly(L-lysine) backbone and hydrophilic dextran side chains (PLL-*g*-Dex) (Figure 1A) significantly accelerated DNA strand exchange reactions while stabilizing DNA hybrids.[4,5] Thus, these properties of the copolymer are considered to be suitable to overcome the dilemma underlying the DNA nanomachines. To assess this possibility, we investigated the effect of PLL-*g*-Dex on the actions of a DNA nanomachine ''chameleon tongue'' proposed by Mergny et al.[3] (Figure 1B). In the folded state, the machine ''Body'' forms intramolecular G-quadruplex. Upon an addition of a sequence ''Fuel 1 (F1)'', the

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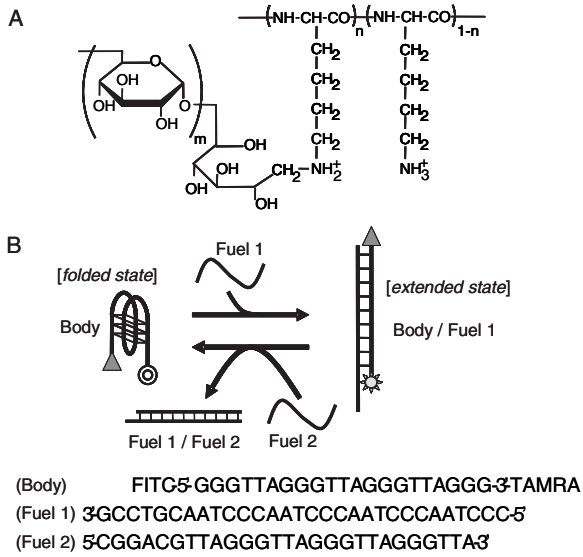

Figure 1.
(A) Structural formula of PLL-*g*-Dex copolymer. (B) Operating diagram and strand sequences of a DNA-fueled nanomachine "chameleon tongue" proposed by Mergny et al.[3]

Body is transformed into the extended state through hybridization with F1. Successive addition of "Fuel 2 (F2)" removes F1 from the Body/F1 duplex through the exchange reaction to reset the machine to the folded state. Here, we propose that a simple addition of PLL-*g*-Dex copolymer considerably improves the responses and robustness of DNA nanomachines. To our knowledge, the copolymer is the first example of an adjuvant material that improves DNA nanomachine responses.

Experimental Part

Materials

All oligonucleotides were supplied by FASMAC Co., Ltd. (Kanagawa, Japan) and purified by reverse-phase high performance liquid chromatography. Cationic comb-type copolymer (PLL-*g*-Dex) was prepared by a reductive amination reaction of PLL·HBr ($M_n = 20\,000$, BACHEM) with dextran ($M_n = 5\,900$, Dextran T-10, Amersham Pharmacia Biotech), as described previously.[6] The number average molecular weight of the resulting copolymer was 65 000 (grafting ratio$_{Dex}$: 15 mol %, dextran content: 85 wt%). PLL-*g*-Dex/DNA charge (*N/P*) ratio was kept constant at a value of 2 throughout the experiments.

Polyacrylamide Gel Electrophoresis to Examine the Body Conformation

DNA solutions were prepared in 10 mM sodium phosphate buffer (pH 7.2) containing 0.5 mM EDTA and 150 mM NaCl in the absence or presence of PLL-*g*-Dex. DNA samples (final conc.: 200 nM) were treated by annealing (heating to 90 °C for 3 min and gradual cooling to r. t.). Native PAGE (18%) was performed in TBE buffer at 4 °C for 1.3 h at 25 V/cm. The gel was stained with 0.01% SYBR GOLD (Molecular Probes).

Real-time Monitoring of the Operation of DNA Nanomachine by Fluorescence Resonance Energy Transfer (FRET) Assay[3]

F1 fuel DNA solution (final conc. = 9.6 nM) was added to FITC and TAMRA FRET-labeled Body strand solution (9.6 nM), dissolved in 10 mM sodium phosphate buffer (pH 7.2, 0.5 mM EDTA, and indicated concentrations of NaCl) at 37 °C.

Change in fluorescence intensity of the mixture was monitored by a JASCO FP-6500 spectrofluorometer at excitation and emission wavelengths of 490 and 520 nm, respectively. After 70 min from F1 strand injection, F2 strand was successively injected to trace the machine's operation. The measurements were carried out in the absence or presence of PLL-*g*-Dex.

UV-Melting Temperature (T_m) Measurements

DNA solutions were prepared to give a final concentration of 0.66 μM (duplexes) or 4.6 μM (G-quadruplex) in 10 mM sodium phosphate buffer (pH 7.2) containing 0.5 mM EDTA and 150 mM NaCl in the absence or presence of PLL-*g*-Dex. The mixture of DNA solution was heated at 90 °C for 3 min and gradually cooled to r.t. UV spectra and UV-melting profiles (260 nm for duplexes and 295 nm for G-quadruplexes[7]) were recorded by a Shimadzu UV-1600 PC spectrometer equipped with a TMSPC-8 temperature controller (Shimadzu, Kyoto, Japan). Melting curves were obtained at a heating rate of 0.5 °C/min. Peak temperatures in the derivative curves (d*A*/d*T*) were designated as melting points.

Results and Discussions

First, a PAGE assay was performed with annealing treatment to check the conformation of the Body strand of the machine (Figure 2). In the absence of the copolymer, the Body strand (lane 1) adopted a compact intramolecular G-quadruplex which was featured by faster electrophoretic migration than ss DNA marker (lane M). As shown in lane 2, a similar result was observed in the presence of the copolymer. This result indicates that the addition of the copolymer does not influence the intramolecular G-quadruplex conformation regardless of the annealing treatment.

We then carried out FRET assay[3] for real-time observation of the nanomachine's transformation (Figure 3). For the assay,

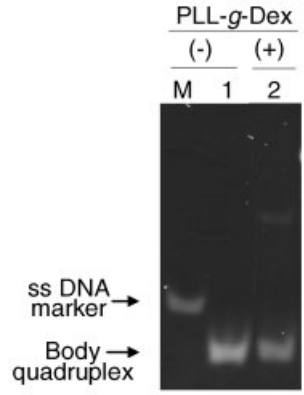

Figure 2.
The G-quadruplex folding of machine body in the absence or presence of PLL-*g*-Dex. Native-PAGE of the Body strand with annealing treatment: lane M, 20-mer ss DNA marker (5′-TAC CAC TCG TTC CCG CTC CT-3′); lane 1, a sample without PLL-*g*-Dex; lane 2, the same sample as lane 1 but with PLL-*g*-Dex. A copolymer/DNA charge (*N*/*P*) ratio was kept at a value of 2.

the Body strand labeled with both FITC and TAMRA (Figure 1B) was used. In the folded state, the fluorescence emission from FITC is quenched by TAMRA that is situated in the proximity of the FITC. The FITC emission is recovered as the machine turns to the extended state. In a previous report, increasing Body strand concentrations from 0.2 to 2 μM with a 1.25 times excess amount of Fuel strands resulted in faster transformation kinetics.[3] In this study, however, we adjusted the concentration of Body to 9.6 nM (ca. 200 times lower than that described in the previous report) in order to open the applicability of the machine in the nanomolar range. Moreover, F1 and F2 of the equimolar amount to Body were used. As shown in Figure 3, the operation of the machine was considerably slow at 50 mM NaCl, since hybridization and strand exchange rate were slow at the lower salt concentration owing to the electrostatic repulsion between DNA strands. It took more than 30 min and 70 min, respectively, to reach equilibrium state in extending and shrinking steps. Although the responses of the machine improved with increasing [NaCl] from 50 to 150 mM,

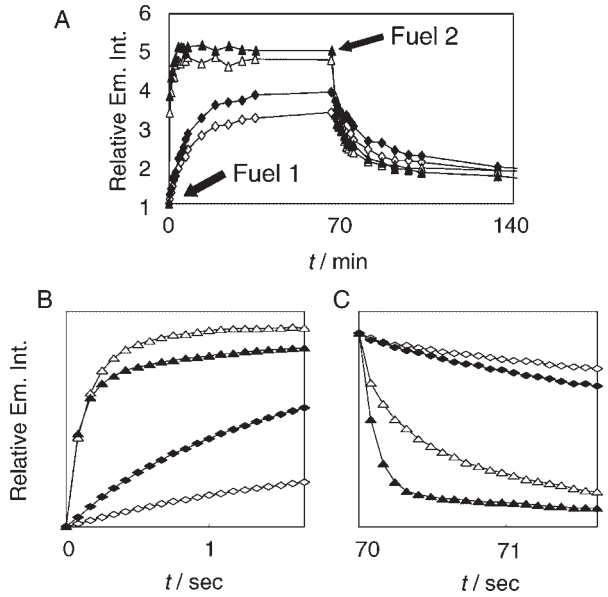

Figure 3.
Operation of a DNA nanomachine "chameleon tongue" in the absence (diamond) or presence (triangle) of PLL-*g*-Dex (*N/P* = 2). The reaction was performed at 37 °C in 10 mM sodium phosphate buffer (pH 7.2) containing 0.5 mM EDTA with either 50 mM (open) or 150 mM (closed) NaCl. Detailed time courses of the extending (B) and shrinking (C) steps.

the extending and shrinking steps, respectively, were considerable slower (< ca. 1/70) (Figure 3B and C) than those shown in the previous paper.[3] Although the machine operation slightly improved at 150 mM NaCl, it still took about 30 min or more to reach equilibrium state in either extending or shrinking step.

On the other hand, when PLL-*g*-Dex being kept a copolymer/DNA charge (*N/P*) ratio at a value of 2 (final conc. = 1.3 μg/ml) was present, the fluorescence intensity sharply changed in response to the injected fuel. The initial transformation rates of both extending and shrinking steps considerably increased. Furthermore, larger changes in the fluorescence intensity were observed, suggesting more robust actions of the machines. It is conceivable that the copolymer accelerated the machine responses by reducing electrostatic repulsion between DNA strands. The initial reaction rates of both extending and shrinking steps at 150 mM NaCl increased more than 20-fold by adding PLL-*g*-Dex (Figure 3B and C).

To inspect the effect of the copolymer, we assessed the melting temperatures (T_m) of the Body G-quadruplex and Body/F1 and F1/F2 duplexes according to the method previously reported (data not shown).[7] The T_m values of both the quadruplex and the duplexes became higher with increasing salt concentrations in the absence of the copolymer, suggesting that these structures were similarly stabilized by reducing electrostatic repulsion among phosphate ions, or in other words, by alleviating counterion condensation effect. In contrast, PLL-*g*-Dex selectively increased the melting temperatures only of the duplexes and not of the quadruplex. The copolymer increased the melting temperatures by approximately 15 °C and 10 °C, respectively, at 50 mM and 150 mM NaCl. Thus, the extending rate was increased by the copolymer through ds DNA (Body/F1) stabilization and SER acceleration.

Similarly the shrinking rate was likely increased by the copolymer through SER acceleration. The activity of PLL-*g*-Dex to stabilize selectively the ds DNA and not the quadruplex seems to play a role in the observed acceleration. The reason why the copolymer is inert to the quadruplex stability is remained to be solved; however, the difference in the total amount of negative charges and their density between the quadruplex and double stranded structures might be involved. Probably, the copolymer accelerated the machine responses by reducing electrostatic repulsion between DNA strands.

Conclusions

Strict optimizations of operating conditions and DNA sequences have been prerequisite for achieving the quick and robust responses of DNA-fueled nanomachines. Efficient hybridization and strand exchange reaction have generally required high salt or DNA concentrations. These restrictions in the operating conditions severely limit the potential applications of the machines. Here a simple addition of a trace amount of the copolymer is demonstrated to be useful to boost the performance of the DNA nanomachines at a low strand concentration (nM range) under physiologically relevant conditions. Further study to assess the effect of the copolymer on the cycling efficacy of the machine is now on going in our laboratory. We expect that adjuvant materials like the cationic copolymer might extend the applicability of DNA nanomachines in biotechnology as well as nanotechnology fields.

Acknowledgements: We gratefully acknowledge the Grant-in-Aid for Scientific Research (No. 16200034) from the Japan Society for the Promotion of Science, the Joint Project for Materials Chemistry, and the 21st Century COE Program, ''Functional Innovation of Molecular Informatics'', from the Ministry of Education, Culture, Science, Sports and Technology of Japan. S.W.C was supported by the JSPS postdoctoral fellowship.

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