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A Contractile DNA Machine with Single-Stranded DNA as Fuel

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In the present work, one circular DNA component is generated by the hybridization of short nucleic acids with the 3' and 5' ends of single-stranded DNA chains. A contractile DNA machine has been built by means of crosslinking of circular DNAs into nanowires. The machine is powered by DNA and can be operated between contracted and extended states. This device has potentials in many applications such as targeted medicine delivery, nonviral gene delivery and in-cell cargo transportation. Furthermore, in combination with the DNA nanotechnology folding strategy, such as DNA origami, the contractile DNA machine may transduce DNA hybridization energy into controlled contraction movement in the nano- and micrometer ranges, or even in the 3D DNA nanostructures with geometric complexities.

Key Words: DNA molecular machine, Contractile action, Self-assembly, Fluorescence.

INTRODUCTION

DNA molecular machines, which can be used to produce controlled nanoscale movements, have been extensively investigated over the past years[1,2]. Taking advantages of the unique molecular recognition properties of DNA, a number of nanomechanical devices have been constructed[3,4], including DNA tweezers, scissors, molecular gears and walkers. These devices have potentials in many applications such as computing systems[5], sensor systems[6,7], nanotransporting[8], nanomedicine[9] and logic-gate operations[10]. In addition to the well-known B-type double helix, DNA is also able to fold to form a number of alternative helical forms, including triplexes and quadruplexes. Combining the distinct conformations and the natural conductive properties of DNA, Huang *et al.*[11] described a contractile DNA nanoswitch, which can toggle between a structurally extended "off" state and a contracted "on" state, with a 40-fold conductivity difference between the two. Recently, Lubrich's group reported a contractile DNA machine (CDM) constructed from a long single-stranded DNA (ssDNA) molecule synthesized by the rolling circle replication process[12].

A great deal of effort has been devoted to the development of self-assembly of 1D DNA nanowires or 2D DNA nanostructures[13,14]. The assembly of metal nanoparticles and proteins on DNA scaffolds were used to fabricate nanoscale transistor devices[15] and nanocircuitry[16]. In contrast to the previous reports[11,12], the present study applies the self-assembly of 1D DNA nanowires to built a contractile DNA machine by means of crosslinking of circular DNAs into nanowires. The machine is powered by DNA and can be operated between contracted and extended states, transducing DNA hybridization energy into controlled contraction movements.

EXPERIMENTAL

All oligonucletides used in the present study were purchased from SBS Genetech Co., Ltd. (Beijing, China) and their sequences were as follows:

S_1: 5'-CTG ACT GAC TGA ACG ATG CT -3' (20-mer)

S_2: 5'-FAM-AGT CAG ATG TAT CAG AGA AAA AAA AAA AAA ATG TAT CAG AGA TCA GTC-TAMRA-3' (48-mer)

S_3: 5'-TTT TTT TCT CTG ATA CAT GTC GAA TCA TTC ATC CTG ATA TTA GCA TCA CCA CCT TAC CAC TTC TAG CGA TAA CAC AAC T CTC TGA TAC AT TTT TTT-3' (96-mer)

S_4: 5'-AGC ATC GTT CAG TCA GTC AG-3' (20-mer)

Other chemicals employed were all of analytical grade and double distilled water (DDW) was used throughout.

Self-assembly and operation of the DNA machine: A stoichiometric amounts of S_1 and S_2 were mixed in phosphate

buffer solution (pH 7.4, 0.1 M). The solution was first heated to 90 ºC for 5 min and fast cooled to 25 ºC at which the solution was held for 0.5 h. To assemble the contractile DNA machine (CDM), the ssDNA S_3 was added to the solution containing circular DNA S_1/S_2 in a final volume of 500 µL in phosphate buffer solution (pH 7.4, 0.1 M), which consisted of 6.0 µM S_1, 6.0 µM S_2 and 6.0 µM S_3. The solution was reacted at 25 ºC for 0.5 h. The contractile DNA machine was extended by adding a stoichiometric amount of opening strand ($S4_)$ to the original sample. The extension reaction was allowed to proceed for 15 min. Recontraction was achieved by adding a stoichiometric amount of closing strand (S_1) to the extended device. The mixture was heated to 90 ºC for 5 min and fast cooled to 25 ºC and standed for 0.5 h.

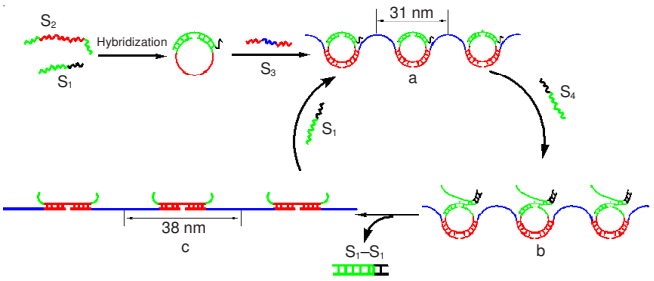
Fluorescence spectroscopy: For the fluorescence emission spectra, the excitation was performed at 470 nm and the emission data were collected either between 500 and 800 nm. All spectra were collected at 25 ºC. For cycling of the motor, the emission data were monitored at fixed wavelength of 520 nm. The maximal emission wavelengths of FAM and TAMRA are 520 and 568 nm, respectively.

Non-denaturing polyacrylamide gel electrophoresis: The DNA samples with 0.2 µM concentration were loaded onto gels contained 30 % polyacrylamide (acrylamide/N,N'-methylenebisacrylamide, 29:1) in 50 × TAE buffer (Tris-Acetate- EDTA, pH 8.5) followed by electrophoresis separation at 150 V for 1 h at 25 ºC. After staining the gel with ethidium bromide (EB), a photograph was taken with a Canon camera.

RESULTS AND DISCUSSION

Construction and operation of the contractile DNA machine: The molecular design and operation principle of the contractile DNA machine is shown in **Scheme-I**. The 3' and 5' ends of single-stranded S_2 hybridized with the short complementary nucleic acid S_1 to form the circular DNA structure, with a 8 nucleotides (nt) toe-hold. Treatment of the circular structures with S_3, which is partial complementary to S_2, results in the formation of contractile DNA machine. This molecular machine is a linear assembly of many copies of a circular unit and a single-stranded spacer segment. The circular rings perform an open-close movement and further result in the contracted-extended state of the contractile DNA machine. The 8-nt spacer strand separates the adjacent rings to prevent interactions between rings and provide sufficient flexibility. The length of a repeat unit in its contracted state (**Scheme-Ia**) is expected to be around 31 nm, 5.2 nm being contributed by the 48-nt double-stranded (ds) circular structure and 25.8 nm by the 60-nt ss spacer segment of S_3.

The circular rings are opened and closed with two fuel strands S_4 and S_1. The opening strand S_4 is fully complementary with S_1. After being added, S_4 first hybridizes with the free toe-hold of S_1, giving an average opening force of *ca.* 15 pN which is consistent with that required to pull apart double-stranded DNA. Then hybridization proceeds by strand displacement reaction until both ends of S_2 have been completely displaced and the S_1/S_4 duplex removes, reaching the extended state. The length of a repeat unit in its extended state (**Scheme-Ic**) is *ca.* 38 nm.



Scheme-I: Design and performance of the contractile DNA machine. (a) Contracted state of the contractile DNA machine (three repeat units are shown), (b) strand-replacing process to open the circular rings, (c) extended state of the contractile DNA machine. The scheme is not drawn in scale

A difference of 7 nm for one repeat unit between the contracted and extended states is observed, coming from the rigid structure of circular structure in the contracted state and double strand in the extended state. After addition of the closing strand S_1, it interacts with the both ends of one S_2 strand to form the circular ring, translating the contractile DNA machine into an overall contracted state. The rapid speed of hybridization and strand displacement provokes fast overall contraction and extension movement.

Fluorescent characterization of the contracted and extended states for the contractile DNA machine: The process of the open and close switching of the contractile DNA machine was monitored by using fluorescence resonance energy transfer (FRET) techniques. Strand S_2 is modified at its 5' and 3' ends by fluorescein (FAM) and carboxytetramethylrhodamine (TAMRA), respectively. As shown in Fig. 1, in the contracted state, the two fluorophores are close to each other and energy can be transfered efficiently from FAM to TAMRA. Therefore, the FAM signal (λ_{EM} = 520 nm) should be of low intensity and the TAMRA signal (λ_{EM} = 568 nm) should have a high intensity under these conditions. In the extended state, the two fluorophores are far away from each other and the efficiency of energy transfer is low, so FAM signal increased and the TAMRA signal decreased in intensity relative to those for the contracted state. After addition of closing strand S_1, the contractile DNA machine recovers to its contracted state. The FRET signal is very similar to that of the original contracted state with a little reduce of the fluorescence intensity, due to the photobleaching of dyes. Each addition of an equimolar amount of S_1 induces one contracted/extended cycle.

Polyacrylamide gel electrophoretic analysis of the contractile DNA machine: The construction of the contractile DNA machine was investigated with native polyacrylamide gel electrophoresis (PAGE). The results of the PAGE suggest that the contractile DNA machine performs as expected in the contracted and extended states (Fig. 2). Lanes 1 and 2 in Fig. 2 are the short strand S_2 and the hybrid of S_1 and S_2, which move faster in the PAGE analysis. Lane 3 is the contracted contractile DNA machine by adding S_3 to the S_1/S_2 circular hybrids. Lane 4 is the extended contractile DNA machine with the introduction of S_4 to the ready-formed contracted mixture. In both lanes 3 and 4, there are component that moves very slowly, corresponding to the supermolecular contractile DNA machine. In both lanes 3 and 4, a band between 50 and 100-bp is

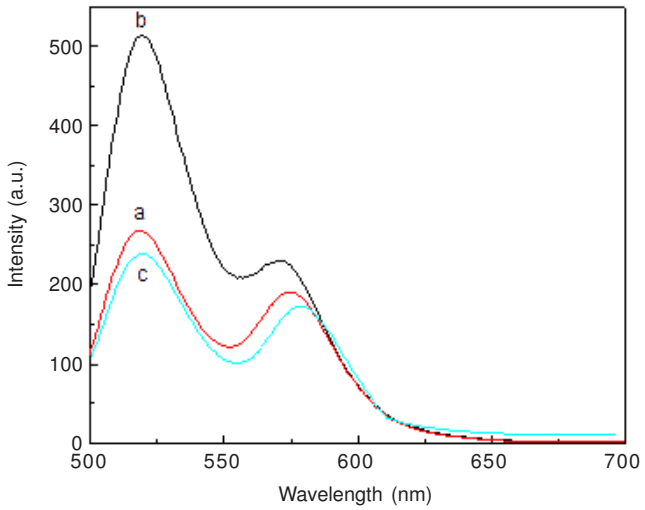


Fig. 1. Fluorescence spectra of the contractile DNA machine in the contracted (a), extended (b), and recontracted (c) states

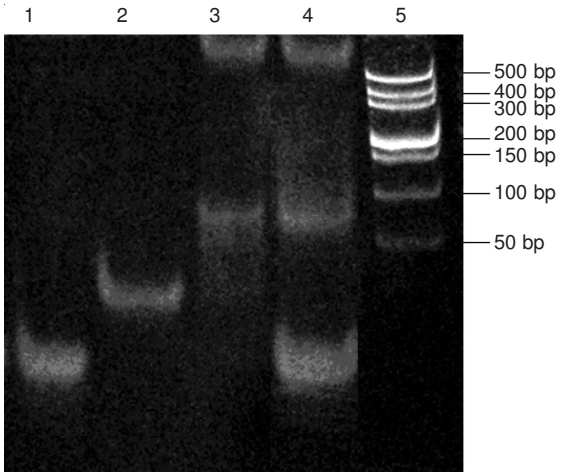


Fig. 2. PAGE analysis of the contractile DNA machine. Lanes as follows: 1, S_2; 2, S_1+S_2; 3, S_1+S_2+S_3; 4, S_1+S_2+S_3+S_4; 5, 50-bp ladder

observed. These components can be attributed to the individual $S_1/S_2/S_3$ (164-mer) hybrids and S_2/S_3 (144-mer) duplex. With further developments in hybridization conditions and a better understanding of the kinetics of the self-assembly process, we believe these undesired structures will be removed in the future. The appearance of the S_1/S_4 (40-mer) waste band upon adding of S_4 to the contracted contractile DNA machine in lane 4 confirms that it is converted into extended state.

Repetitive operation of the contractile DNA machine: The repetition between contracted and extended states of the designed nanomachine can be achieved by operating manually with adding of opening strand S_4 and closing strand S_1 (Fig. 3). The motion of the DNA motor is observed by monitoring the fluorescence intensity at 520 nm for FAM. The DNA machine adopts its contracted state with low-intensity fluorescence signal. With the introduction of S_4, the contractile DNA machine changes to the extended state, resulting in increased fluorescence signal. With further addition of S_1, the contractile DNA machine reaches its recontracted state. Four cycles are recorded in our experimental and the maximum fluorescence intensity decreased as the machine cycled, due to the photobleaching of the fluorescent dyes.

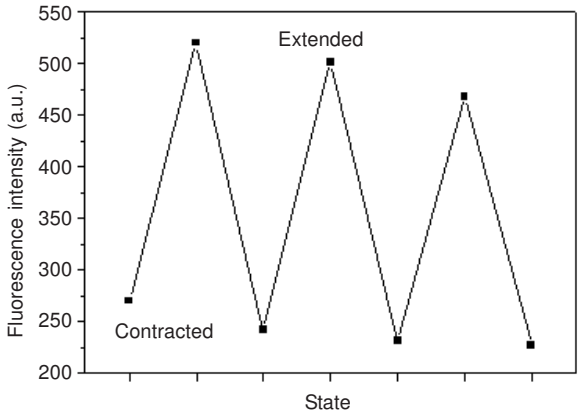


Fig. 3. Cycling motion of the contractile DNA machine. Fluorescence was monitored at 520 nm

Conclusion

In summary, we have built a contractile DNA machine by means of crosslinking of circular DNAs into nanowires. The machine is powered by DNA and can be operated between contracted and extended states. In contrast to the previous report[12], that constructed a contractile DNA machine from a long ssDNA obtained with rolling circle amplification (RCA), the present study demonstrates the use of self-assembly of circular DNA. Furthermore, in combination with the DNA nanotechnology folding strategy, such as DNA origami, the contractile DNA machine may transduce DNA hybridization energy into controlled contraction movement in the nano- and micrometer ranges, or even in the 3D DNA nanostructures with geometric complexities.

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REFERENCES

1. B. Yurke, A.J. Turberfield, A.P. Mills Jr., F.C. Simmel and J.L. Neumann, *Nature*, **406**, 605 (2000).
2. E.C. Garner, *Science*, **322**, 1486 (2008).
3. B.Q. Ding and N.C. Seeman, *Science*, **314**, 1583 (2006).
4. X.M. Li, W. Li, A.Q. Ge and H. Y. Chen, *J. Phys. Chem. C*, **114**, 21948 (2010).
5. J. Elbaz, O. Lioubashevski, F. Wang, F. Remacle, R.D. Levine and I. Willner, *Nat. Nanotechnol.*, **5**, 417 (2010).
6. H. Zhang, C. Fang and S. Zhang, *Chem.-Eur. J.*, **16**, 12434 (2010).
7. T. Li, S. Dong and E. Wang, *J. Am. Chem. Soc.*, **132**, 13156 (2010).
8. J. Bath, S.J. Green and A.J. Turberfield, *Angew. Chem. Int. Ed.*, **44**, 4358 (2005).
9. M.N. Win and C.D. Smolke, *Science*, **322**, 456 (2008).
10. X.W. Liu, Y.D. Chen, L. Li, J.L. Lu and D.S. Zhang, *Spectrochim. Acta A*, **86**, 554 (2012).
11. Y.C. Huang and D. Sen, *J. Am. Chem. Soc.*, **132**, 2663 (2010).
12. D. Lubrich, J. Lin and J. Yan, *Angew. Chem. Int. Ed.*, **47**, 7026 (2008).
13. N.C. Seem, *Annu. Rev. Biophys. Biomol. Struct.*, **27**, 225 (1998).
14. F.A. Aldaye, A.L. Palmer and H.F. Sleiman, *Science*, **321**, 1795 (2008).
15. K. Keren, R.S. Berman, E. Buchstab, U. Sivan and E. Braun, *Science*, **302**, 1380 (2003).
16. B. Basnar, Y. Weizmann, Z. Cheglakov and I. Willner, *Adv. Mater.*, **18**, 713 (2006).