Nanotechnology

A DNA Nanomachine Based on a Duplex–Triplex Transition**

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Herein we report the construction of a DNA nanomachine whose motion is triggered by changes in the solution pH value. Reversible formation and dissociation of a DNA triplex containing C^+G-C triplets take place when the solution pH value changes between pH 5.0 and 8.0.[1] This process constitutes the working principle of our DNA machine. We demonstrated the operation of the DNA machine by using fluorescence resonance energy transfer (FRET) techniques, and confirmed its structural integrity by native polyacrylamide gel electrophoresis (PAGE).

A great deal of effort has been devoted to the development of nanomachines because of their many potential applications in nanoelectronic devices, miniaturized biosensors, molecular computation, and smart materials.[2] DNA, in the form of versatile building blocks,[3] has been successfully used to construct DNA nanomachines.[4] The working mechanisms of these DNA machines fall into three categories: 1) conformational changes induced by environmental changes,[4a,h] 2) motion fueled by strand displacement,[4b–g,i–k] 3) autonomous motion powered by enzymatic activity.[4l] The first DNA machine was built to work by the first of these mechanisms but the majority of DNA machines operate by strand displacement. Strand-displacement-driven DNA machines are attractive because each machine can potentially be activated individually by using a sequence-specific activation technique. An enzyme-powered DNA motor has been developed more recently.[4l] It can be sequence-specifically activated and, more importantly, it operates autonomously. Herein, we report a DNA nanomachine that falls into the first category and is fuelled by H^+ and OH^- ions.

Our nanomachine contains three DNA strands (F, L, and S) and operates through the reversible formation/dissociation of a DNA triplex (Figure 1). At pH 8.0, the three strands associate to form an open complex (open state) consisting of three 15-base-pair duplexes and a single-stranded region. This single-stranded region adopts a random coil conformation in the presence of divalent cations such as Mg^{2+},[4,5] but its sequence is designed to allow it to bind to one of the duplexes

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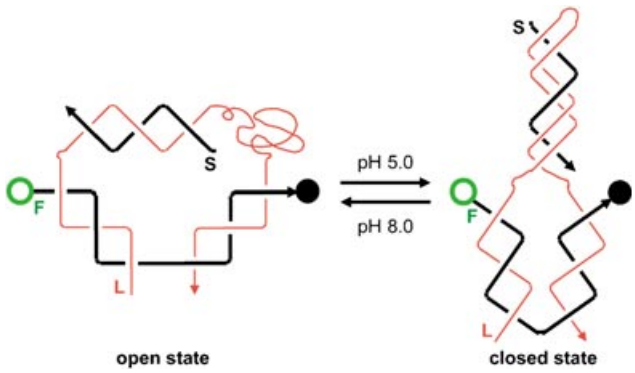

Figure 1. The construction and operation of our DNA nanomachine. The machine consists of three DNA strands: a strand with a fluorescent label (F), a long strand (L), and a short strand (S). The open and solid circles represent rhodamine green and black hole quencher-1 (BHQ-1), respectively. A DNA triplex involving the S and L strands forms and dissociates reversibly.

to form a triplex. C^+G-C triplets make up 47% of the triplex, and TA-T triplets the remaining 53%. The formation of C^+-G-C triplets depends on the protonation of cytosine residues,[1] which requires an acidic solution. A pH value of 5.0 has been reported in the literature to promote DNA triplex formation reliably.[1] Upon triplex formation, the complex as a whole becomes quite compact (closed state). When the pH value increases to pH 8.0, the C^+G-C triplets become unstable and the DNA triplex dissociates into a duplex and a single-stranded region; the DNA complex returns to its open state. The DNA machine continuously cycles between the closed state and the open state when the solution pH oscillates between 5.0 and 8.0. We intentionally designed the nanomachine with an overall structure similar to that of a previously reported autonomous DNA motor,[4l] but with a different motion actuator. We reasoned that the success of such a design would clearly demonstrate that a similar motion can be achieved by different working mechanisms and with different fuels.

The DNA machine is stable in both conformations. We synthesized the machine by combining equimolar amounts of the DNA strands F, L, and S then slowly cooling the mixture from 95 to 22°C over the course of 2 h. We first confirmed that all the strands can associate at both pH 5.0 and pH 8.0 by using native PAGE (Figure 2). At each pH value, one single, sharp band corresponding to the designed DNA complex was observed, which indicates that the DNA machine is stable under both conditions. Comparison of the designed machine with the other DNA complexes on the gels clearly showed that the DNA machine has different electrophoretic mobilities at the two different pH values. This phenomenon suggests that the DNA machine adopts different conformations at pH 5.0 and 8.0 because the electrophoretic mobility of a molecule is determined not only by its molecular weight, but also by its conformation.[1d] A closely packed molecule moves faster than a loosely packed molecule of the same weight. The observed mobility change hinted that the DNA machine adopts a more compact conformation at pH 5.0 than at pH 8.0.

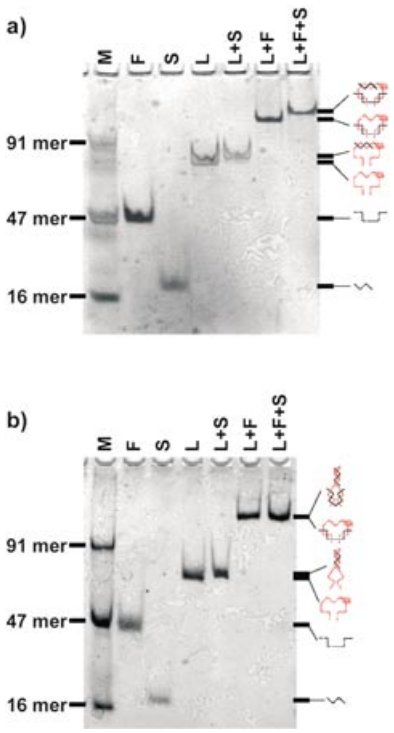

Figure 2. Native gel electrophoretic analysis of the DNA nanomachine at pH 8.0 (a) and pH 5.0 (b). The DNA strands present are indicated above each lane. Lane M contains a series of single-stranded DNA size markers. The electrophoretic mobility of the DNA nanomachine (Lane L+F+S) changes relative to those of the other DNA complexes when the pH value changes.

The conformational change was further demonstrated by the results of a FRET experiment (Figure 3). We labeled the F strand with rhodamine green and BHQ-1 molecules at its 5′ and 3′ ends, respectively. The optical properties of both rhodamine green and BHQ-1 are insensitive to any pH change between pH 4.0 and 9.0.[4h,6] In the open state of the DNA machine (pH 8.0), the fluorophore is well separated from the quencher and a strong fluorescence signal was observed. In the closed state (pH 5.0), the fluorophore is close to the quencher and the fluorescence signal decreased in

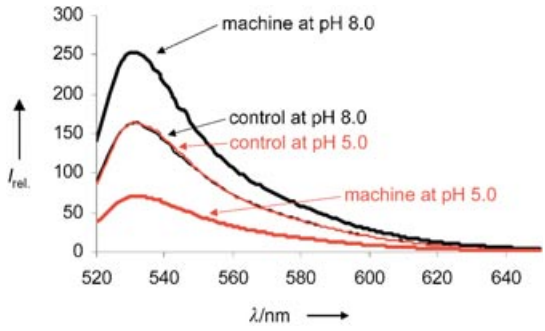

Figure 3. Fluorescence spectra of the DNA nanomachine at pH 5.0 and pH 8.0. The rhodamine green emission is more effectively quenched at pH 5.0 than at pH 8.0, which indicates that the DNA complex is more compact at pH 5.0. A control molecule that could not undergo the duplex–triplex transition was also analyzed and was observed to have the same fluorescence intensity at both pH values.

intensity. To make sure that the conformational change, and not pH change per se, was responsible for the variation of the fluorescence signal, we constructed a control molecule. This control molecule was similar to the DNA machine except that the control could not undergo the duplex–triplex transition. Fluorescence data clearly showed that the pH change could not cause the control molecule to vary its fluorescence intensity.

The DNA machine continuously cycles when the environmental pH value oscillates between 5.0 and 8.0 (Figure 4). We monitored the fluorescence signal at 530 nm, the maximum

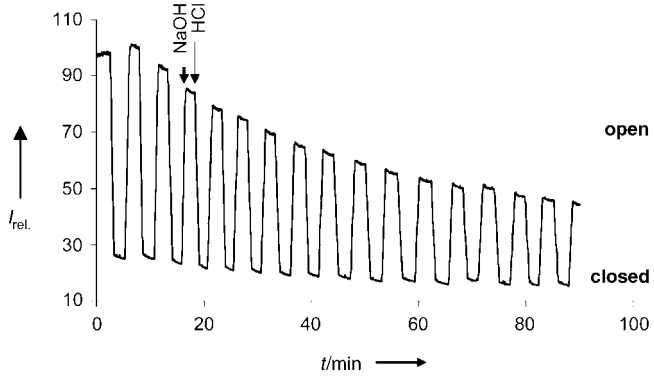

Figure 4. DNA machine cycling. Fluorescence intensity was monitored at 530 nm (λ_{em} of rhodamine green) while the solution pH value oscillated between 5.0 and 8.0.

emission wavelength of rhodamine green, while changing the solution pH value. Sixteen cycles were recorded. The overall fluorescence signal continuously decreased during the cycling experiment. We speculate that this decrease is due to photobleaching of the fluorescence dye and the inaccuracy of the pH control. Repetitive addition of NaOH and HCl diluted the sample, which also contributed to the decrease of the fluorescence signal intensity. However, this extent of dilution does not significantly affect triplex formation because this structural change is an intramolecular process.

In summary, we have constructed a DNA nanomachine whose mechanism is based on the formation and dissociation of a DNA triplex. This machine uses H^+ and OH^- as fuels, which gives it four unique characteristics: 1) The fuels are among the cheapest and most common chemicals. If a massive amount of DNA machines were needed, the cost of the fuels would remain low. 2) The only waste products of the DNA machine are water and NaCl, which are not damaging to the motor or the environment. 3) The fuels consist of small particles that can easily diffuse through small pores or channels. This property is potentially important for many applications. For example, if a DNA machine is placed in a protein channel or a nanofluidic channel to control the permeability of that channel, the ability of the machine to function depends critically upon the accessibility of the fuels. In such a situation, H^+ and OH^- ions are clearly superior to large biomacromolecules such as DNA and RNA. 4) One disadvantage of the reported DNA machine is that all the machines in a given sample move in response to the same stimulus. We cannot control each molecular machine individually without spatial segregation of the nanomachines. For a nanomachine, it is interesting to ask 1) will the machine work under any load? and 2) what force can the machine generate? Unfortunately, it is difficult to answer such questions at the present stage because our design is quite complicated. We expect our machine to generate a weaker force than DNA-hybridization-fueled machines[4b–k] since a DNA triplex is generally less stable than a DNA duplex. Other challenges in the field of DNA nanomachine design include: 1) What complicated motions can DNA machines perform? 2) What kind of work can DNA machines do? 3) Could DNA machines communicate with each other and work cooperatively? We wish to address these questions in the near future.

Experimental Section

DNA oligonucleotides: a) DNA sequences for the nanomachine: Strand F, 5′-rhodamine green-CCA TAC CAT CTA ACC TCC AgA CCT TAC gCT C-black hole quencher-1-3′; Strand L, 5′-ggT TAg ATg gTA Tgg TTC TTC TCT CCT TTC CTT TT**C CTT TCC TCT CTT CC**T gAg CgT AAg gTC Tgg-3′ (the triplex-forming segment is indicated by bold letters); Strand S, 5′-ggA AAg gAg AgA AgA-3′. b) DNA sequences for the control molecule: Strand F, 5′-rhodamine green-CCA TAC CAT CTA ACC TCC AgA CCT TAC GCT C-black hole quencher-1-3′; Strand L′, 5′-ggT TAg ATg gTA TgC TTC ggA CAg gCT AgC TAC AAC gAg AgT gAC TgA gCg TAA ggT CTg g-3′; Strand S′, 5′-gTC ACT CAT gTC CgA-3′. All oligonucleotides were purchased from Integrated DNA Technologies, Inc. and purified by denaturing polyacrylamide gel electrophoresis.

Formation of DNA complexes: The component DNA strands (0.5 μM) were combined in equimolar quantities in a buffer. At pH 8.0, we used tris(hydroxymethyl)aminomethane Tris-Mg^{2+} buffer, which contained Tris buffer (50 mM, pH 8.0), NaCl (100 mM), and $Mg(CH_3COO)_2$ (10 mM). At pH 5.0, we used 2-morpholinoethanesulfonate MES-Mg^{2+} buffer, which contained MES (50 mM, pH 5.0), NaCl (100 mM), and $Mg(CH_3COO)_2$ (10 mM). The nanomachine was formed by cooling the DNA solution as follows: 95 °C (3 min), 65 °C (30 min), 50 °C (30 min), 37 °C (30 min), and 22 °C (30 min).

Denaturing polyacrylamide gel electrophoresis: Gels contained 20 % polyacrylamide (acrylamide/bisacrylamide, 19:1) and 8.3 M urea and were run at 55 °C. The running buffer was Tris-borate-EDTA (TBE; EDTA, ethylenediaminetetraacetate), which consisted of Tris buffer (89 mM, pH 8.0), boric acid (89 mM), and EDTA (2 mM). Gels were run on a Hoefer SE 600 electrophoresis unit at 600 V (constant voltage).

Native polyacrylamide gel electrophoresis: Gels contained 12 % polyacrylamide (acrylamide/bisacrylamide, 19:1) and were run on a FB-VE10-1 electrophoresis unit (Fisher Biotech) at 22 °C (80 V, constant voltage). After electrophoresis, the gels were stained with Stains-all dye (Sigma) and scanned. Tris-Mg^{2+} buffer and MES-Mg^{2+} buffer were used for electrophoresis at pH 8.0 and pH 5.0, respectively.

Fluorescence spectroscopy: DNA (0.2 μM) was dissolved in Tris-acetate-EDTA-Mg^{2+} buffer (500 μL), which contained Tris base (40 mM), acetic acid (20 mM), EDTA (2 mM), and $Mg(CH_3COO)_2$ (12.5 mM). The buffer pH was adjusted to either pH 5.0 or 8.0 with 1M NaOH or 1M HCl. Fluorescence emission spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. All spectra were collected at 22 °C. The samples were excited at 504 nm and the emission data were collected either from 520 to 650 nm, or at 530 nm (for motor cycling). The maximal emission wavelength of rhodamine green is 535 nm.

Cycling the DNA machine: DNA (0.2 μM) was dissolved in Tris-acetate-EDTA-Mg^{2+} (TAE/Mg^{2+}) buffer (500 μL). We changed the

buffer pH between 5.0 and 8.0 by alternately adding 1M HCl (11.1 μL) and 1M NaOH (11.1 μL). The pH value of the TAE/Mg^{2+} buffer was stable at both pH 5.0 (pK_a of acetic acid is 4.75) and pH 8.0 (pK_a of Tris is 8.3).

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