Nanotechnology

An Autonomous DNA Nanomotor Powered by a DNA Enzyme**

*Yi Chen, Mingsheng Wang, and Chengde Mao**

Nanoscale mobile devices (nanomotors) have many potential applications, such as information processing, nanoelectronic devices, biosensors, and regulation of chemical reactions and molecular assembly.[1] Various artificial nanomotors have been reported in recent years. These motors include organic molecules,[2–5] engineered DNA constructs,[6–12] and inorganic/protein hybrids.[13] However, reports of autonomous synthetic nanomotors are very rare.[5,11,13] Autonomy is an important characteristic of cellular protein motors, which prompted us to ask whether it would be possible to design biomimetic nanomotors that continuously conduct mechanical motions powered by consumed chemical energy. Herein, we report the construction of a DNA nanomotor powered by an RNA-cleaving DNA enzyme.[14–17] The motor keeps operating as long as its fuel (the RNA substrate of the DNA enzyme) is available. This DNA motor is a biomimetic nanomotor that works in the same way as natural protein motors, that is, by continuously extracting chemical energy from covalent bonds and converting this energy into mechanical motions.[18–20]

Most protein motors,[18–22] which include myosin, kinesin, and F_0F_1 adenosine triphosphate (ATP) synthase, possess an ATPase activity. These motors can bind to and hydrolyze ATP to form ADP, which is then released. The energy released by the chemical process of ATP hydrolysis powers protein motors and enables them to change their conformation, which results in mechanical movement. The DNA motor presented herein works in a similar way. The catalytic domain of the DNA can bind to a DNA–RNA chimera substrate, cleave this substrate into two short fragments, and then release the pieces. This process leads the DNA motor to change its conformation, which generates nanoscale motions. We demonstrated the motion of the DNA motor by using fluorescence resonance energy transfer (FRET) techniques and monitored the autonomous cycling of the motor by observing substrate cleavage.

The design of the new autonomous DNA motor combines knowledge and experience of DNA nanotechnology[23–25] and DNA enzymes.[14–17] Figure 1 illustrates the molecular design and operation principle of the engineered DNA motor. The DNA motor contains two 15-base-pair helical domains, which are joined by a single-stranded 10-23 DNA enzyme[14] at one end and a single-base hinge at the other. The 10-23 DNA enzyme consists of a 15-base catalytic core and two flanking 7-base-long substrate-recognition arms. The DNA enzyme binds to its RNA substrate through Watson–Crick base pairing and cleaves the RNA into two short fragments. All the RNA residues of the substrate, except that flanking the phosphodiester bond to be cleaved, can be replaced with DNA residues. When the DNA enzyme is not bound to its substrate (a DNA–RNA chimera, S), divalent cations (for example, Mg^{2+}) cause the single-stranded DNA enzyme to collapse into a closed coil as a result of entropic forces (see ref. [26] and the Supporting Information). Under these conditions, the overall conformation of the DNA motor is quite compact ("closed state"). When the DNA enzyme binds to its DNA–RNA chimera substrate, the single-stranded DNA enzyme forms a bulged duplex with the substrate. This bulged duplex pushes the two helical domains of the DNA motor apart and leads the motor to adopt an "open state". Upon binding, the DNA enzyme cleaves its substrate into two short fragments (S_1 and S_2). The resulting fragments have a lower affinity for the DNA enzyme than the intact substrate and, therefore, dissociate from the DNA motor. Consequently, the DNA motor returns to the closed state and can undergo the next cycle of substrate binding (open state), cleavage, and dissociation (closed state). The DNA motor consumes one DNA–RNA chimera substrate in each open/

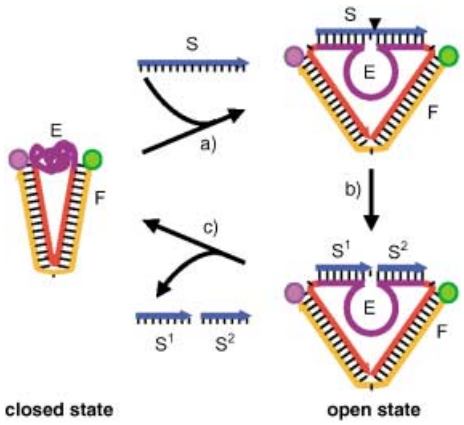

Figure 1. Structure and operation of an autonomous DNA nanomotor. The DNA motor consists of two strands (E and F). The E strand (pink-purple) contains an RNA-cleaving 10-23 DNA enzyme. The enzyme domain is colored dark purple. The F strand (gold) contains two fluorophores: fluorescein (FAM: solid green circle) and tetramethylrhodamine (TAMRA: solid pink circle). The enzyme substrate (S) is a DNA–RNA chimera (blue). a) An S strand binds to the motor and causes it to adopt an open state. b) The DNA enzyme cleaves the substrate (S). The arrowhead above the S strand indicates the cleavage site. c) The cleaved substrate fragments (S^1 and S^2) dissociate from the DNA motor, which returns to the closed state. The closed state might consist of a variety of compact conformational isomers.

[*] Y. Chen, M. Wang, Prof. C. Mao
Department of Chemistry
Purdue University
West Lafayette, IN 47907 (USA)
Fax: (+1) 765-494-0239
E-mail: mao@purdue.edu

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closed cycle. The motor autonomously cycles between the open and closed states until the fuel (the enzyme substrate) runs out.

The DNA motor was formed by slowly cooling an equimolar mixture of component DNA strands. The results of polyacrylamide gel electrophoretic analysis suggest that the DNA motor behaves as expected in both the closed and open states (Figure 2). The motors migrated as clean sharp bands, which indicates that the structures are stable. We used a substrate analogue (S′) that cannot be cleaved by the enzyme to capture DNA motors in the open state. This DNA strand has the same sequence as the DNA–RNA chimera substrate (S) except that S′ contains two DNA residues instead of two of the substrate RNA residues. S and S′ have the same specificity and nearly the same affinity for the DNA enzyme domain of the DNA motor. The electrophoresis experiment also confirmed that the cleaved short fragments have a lower affinity than the whole substrate for the DNA enzyme and do not associate with the DNA motor. Even in tenfold excess, the short fragments did not bind with the DNA motor (lane 6 in Figure 2). Instead, these fragments migrated separately from the motor.

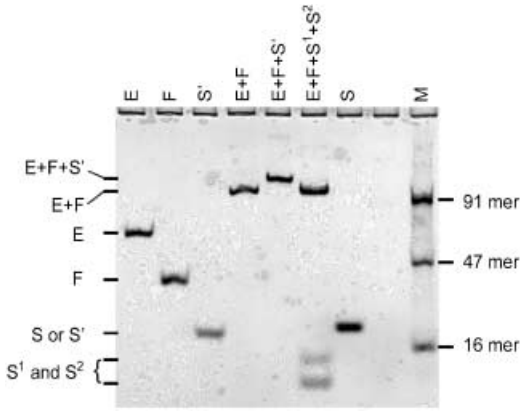

Figure 2. Native gel electrophoretic analysis of DNA motor formation. The content of each lane is indicated at the top of the gel image. A DNA motor formed in the closed state from its two component strands (E+F) was converted into the open state (E+F+S′) by the binding of an S′ strand, a DNA analogue of the substrate that cannot be cleaved by the enzyme. The mixture analyzed in lane E+F+S^1+S^2 contained a tenfold excess of (S^1+S^2) relative to (E+F). Clearly, the short fragments could not stably bind to the motors. The far right lane shows three single-stranded DNA size markers.

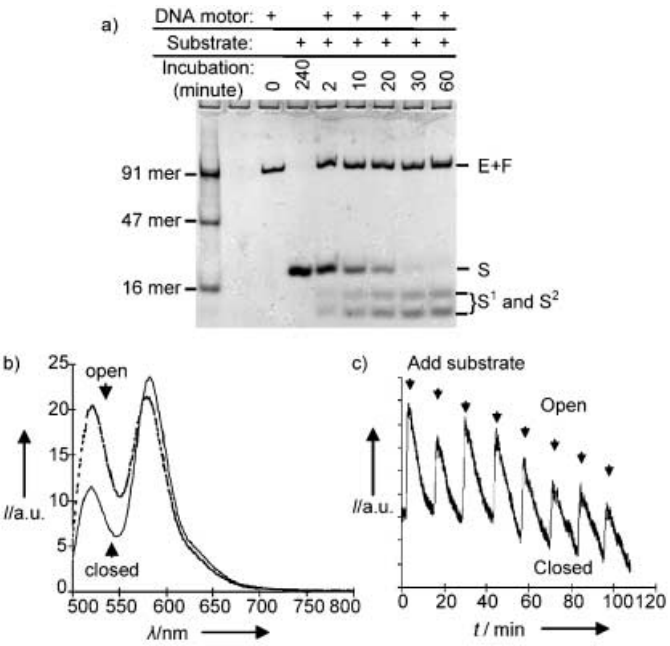

Figure 3. Motion of the DNA motor. a) The autonomous cycling of the DNA motor, as evidenced by multiple enzyme turnovers. The DNA motor (E+F) and its substrate (S) were incubated at a motor/substrate ratio of 1:20 for the indicated time periods and then resolved by polyacrylamide gel electrophoresis. The DNA motors almost completed digestion of the substrate within 30 minutes, which suggests that each motor underwent an open/closed cycle every 1.5 minutes. b) Fluorescence spectra of the DNA motor in the closed and open states. These spectra show that there was indeed a nanoscale movement upon switching between the two states. c) The DNA motor could also be cycled manually by sequential addition of equimolar amounts of enzyme substrate. Fluorescence was monitored at 520 nm, the maximum emission wavelength of FAM.

The autonomous character of the designed nanomotor is the most important achievement of this work. To demonstrate the autonomy of the DNA motor the fragmentation of the substrate was monitored while substrate molecules were consumed to power open/closed cycles of the DNA motor (Figure 3a). When the substrate and the DNA motor were incubated at room temperature, more and more substrate molecules were cleaved into two short segments with the passage of time. Under these particular experimental conditions, each DNA motor consumed an average of 20 substrate molecules in 30 minutes. In other words, each motor underwent 20 open/closed cycles in 30 minutes without any human intervention. The observed turnover speed is much greater than the cycling speeds that have been reported for human-operated systems (for example, less than 0.1 cycles per minute are reported in ref. [7]). The cycling speed could be increased to more than three cycles per minute by optimizing the working conditions.[14]

We used FRET techniques[6,7] to demonstrate that a motion does indeed accompany the chemical reaction of the DNA motor (Figure 3b). We labeled the DNA motor with two fluorophores (fluorescein (FAM) and tetramethylrhodamine (TAMRA)), one at each end of the two helical domains (Figure 1). The efficiency of the energy transfer between two fluorophores is sensitive to the distance between the fluorophores and the switching of the motor between the open and closed states could be observed readily in FRET experiments. In the closed state the two fluorophores are close to each other and energy can be transfered efficiently from FAM to TAMRA. We therefore expect the FAM signal ($\lambda_{EM} = 520$ nm) to be of low intensity and the TAMRA signal ($\lambda_{EM} = 568$ nm) to have a high intensity under these conditions. The open state should give the opposite results: the two fluorophores are far away from each other and the

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efficiency of energy transfer is low so we expect the FAM signal to increase and the TAMRA signal to decrease in intensity relative to the signals observed for the closed state. The FRET experiment confirmed this hypothesis (Figure 3b). The open state was captured by using a DNA analogue (S′ strand) of the enzyme substrate that cannot be cleaved by the enzyme. The results of this experiment show that the designed DNA motor can adopt two different conformations: closed and open. Switching between these two states requires the DNA motor to reposition its two helical domains.

The DNA motor not only operates autonomously, but can also be operated manually (Figure 3c). In the manual mode, the motor is switched between the closed and open states by sequential addition of the DNA–RNA chimera substrate. The motion of the DNA motor was observed by monitoring the fluorescence intensity at 520 nm (λ_{EM} of FAM). When the enzyme substrate was absent from the solution, the DNA motor adopted its closed state and emitted a low-intensity fluorescence signal. After addition of an equimolar amount of substrate, however, the motor bound to its substrate and changed to the open state, which resulted in an increased fluorescence signal. As time lapsed, the DNA enzyme cleaved the substrate and released the cleaved fragments. The motor returned to its closed state and the fluorescence intensity decreased as well. Each addition of an equimolar amount of substrate induced one open/closed cycle. We recorded eight open/closed cycles. The maximum fluorescence intensity decreased as the motor cycled. This decrease was presumably caused by photobleaching of the fluorescent dyes.

In summary, we have constructed an autonomous DNA nanomotor by integrating a catalytic DNA domain into a self-assembled DNA nanostructure. This work exemplifies the successful combination of structural DNA nanotechnology and catalytic DNA, which we believe could open exciting avenues to dynamic nanomaterials,[1] DNA computation,[27,28] nanorobotics, and nanodevices. The similarity between the mechanism of the DNA motor presented herein and that of cellular protein motors suggests that more complicated DNA nanomotors could conceivably be constructed by borrowing the architectures of cellular protein motors. For example, autonomous, unidirectionally translational and rotary DNA motors could be designed by mimicking myosin[20] and F_0F_1 ATP synthase,[21] respectively. It is also conceivable that a large number of different DNA motors could be designed (around 4^{14}, the pool size of the 10-23 DNA enzyme library) that are powered by different RNA substrates. Each motor could be activated individually within the same solution. Besides the potential technological applications of these motors, this work might have biological relevance. An RNA world may have existed before proteins took over most biological functions.[29] Protein motors are now involved in many essential life activities, such as DNA replication, RNA and protein synthesis, and various molecular transportation processes.[18–20] Did nucleic acids play similar functions in primitive life forms? Since the reported DNA motor could be easily translated into an RNA motor (containing a ribozyme[30]), our study might imply that nucleic acids alone could perform motor functions in primitive life forms, just as proteins do in the living systems that exist today.

Experimental Section

DNA oligonucleotides: Strand E: 5′-GGT TAG ATG GTA TGC TTC-GGC AGG CT AGC TAC AAC GAG AGT GAC TGA GCG TAA GGT CTG G-3′; strand F: 5′-FAM-GCA TAC CAT CTA ACC TCC-AGA CCT TAC GCT C-TAMRA-3′; strand S: 5′-GTC ATT CrAr-U GTC CGA-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.

Nanomotor formation: DNA strands (0.5 μM) were combined in a 1:1 molar ratio in a buffer (TAE/Mg^{2+}) composed of tris(hydroxymethyl)aminomethane (Tris) base (40 mM, pH 8.0), acetic acid (20 mM), ethylenediaminetetraacetate (EDTA; 2 mM), and $MgAc_2$ (12.5 mM). The nanomotor was formed by slowly 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).

Enzyme digestion: The DNA motor (0.5 μM) and DNA–RNA chimera substrate (10 μM) were incubated in TAE/Mg^{2+} buffer at 22 °C for various time periods and then analyzed by nondenaturing polyacrylamide gel electrophoresis.

Denaturing polyacrylamide gel electrophoresis: Gels contained 20% polyacrylamide (acrylamide/bisacrylamide, 19:1) and 8.3 M urea and were run at 55 °C. Tris-borate-EDTA (TBE) was used as the separation buffer and consisted of Tris base (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).

Nondenaturing polyacrylamide gel electrophoresis: Gels contained 12% polyacrylamide (acrylamide/bisacrylamide, 19:1). The separation buffer (TAE/Mg^{+}) consisted of Tris base (40 mM, pH 8.0), acetic acid (20 mM), EDTA (2 mM), and $(CH_3COO)_2Mg$ (12.5 mM). Gels were run on a Hoefer SE 600 electrophoresis unit at 22 °C (250 V, constant voltage). After electrophoresis, the gels were stained with Stains-all dye (Sigma) and scanned.

Fluorescence spectroscopy: DNA was dissolved in TAE/Mg^{2+} buffer. Fluorescence emission spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. All spectra were collected at 22 °C. The samples were excited at 470 nm and the emission data were collected either between 500 and 800 nm, or at a fixed wavelength of 520 nm (for cycling of the motor). The maximal emission wavelengths of FAM and TAMRA are 520 nm and 568 nm, respectively.

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