Molecular Devices

A DNAzyme That Walks Processively and Autonomously along a One-Dimensional Track**

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Herein, we report a DNA nanodevice that autonomously and processively moves along a DNA track. The motion mechanism of this DNA device is reminiscent of that of single-headed myosins I, a family of cellular protein motors.[1] The DNA device contains a DNA enzyme (DNAzyme) that constantly extracts chemical energy from its substrate molecules (RNA) and uses this energy to fuel the motion of the DNA device.

Nanomachines have potential applications in smart materials, sensors, and optoelectronic devices.[2] Many approaches have been explored to develop such nanomachines. By taking advantage of a rich body of knowledge of DNA biophysics, various DNA nanomachines have been rationally designed and built based on conformational changes.[3] The motions include opening/closure, extension/contraction, and rotation. More complex nanomechanical devices, such as walkers and gears, have also been demonstrated.[3l,m,o] However, such devices need human intervention for each step of their motions. The DNA structures change only once when the conditions of the solution are changed or a DNA strand is added. These DNA devices are in sharp contrast with cellular protein motors and manmade machines at the macroscale, both of which can continuously work without human interference.

An autonomous DNA motor has recently been reported[3k,n]—here we refer to "autonomous" as being self-contained and not requiring human interference or other external active components such as protein enzymes. The motor performs an extremely simple motion: open and close. In a related, autonomous system, a DNA fragment could be continuously translocated along one direction by natural proteins (a combination of a DNA ligase and two restriction endonucleases).[3o] Logically, we wondered if it is possible to construct a more sophisticated, self-contained autonomous motor that could constantly walk or rotate. Herein, we demonstrate that such a machine is feasible.

The walking mechanism of the new system integrates DNAzyme activity and a strand-displacement strategy (Figure 1). The system consists of a walker and a track: The walker is a 10–23 DNAzyme, **E**,[4] which is a DNA molecule that can cleave RNA with sequence specificity (Figure 1A).

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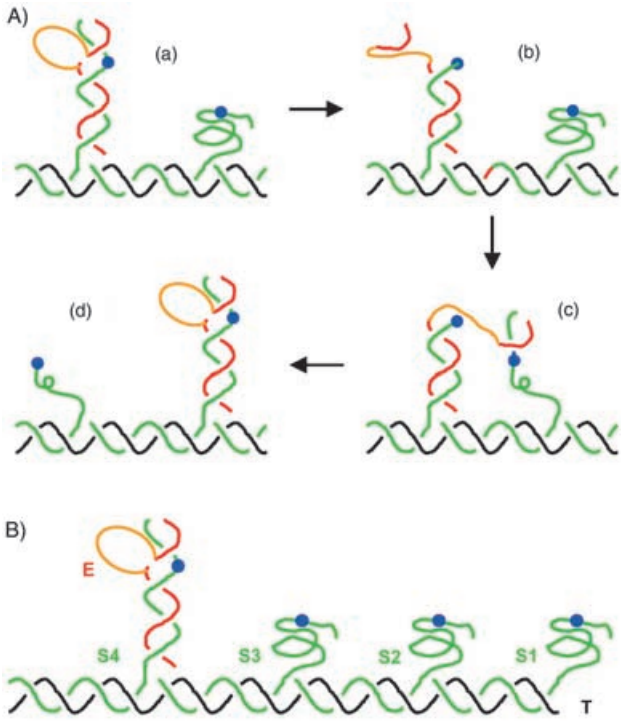

Figure 1. Scheme of a walking DNAzyme and its track. A) The walking principle. B) A construction where the walking DNAzyme is at one end of its track. Black lines: template (**T**); green lines: substrate (**S**); red/yellow lines: a 10–23 DNAzyme (red), with the catalytic core highlighted (yellow); blue dots indicate the bonds to be cleaved by the DNAzyme.

The 10–23 DNAzyme is the 23rd clone after the 10th round of selection in an experiment performed in vitro.[4] The track is a regular, linear array of RNA substrate, **S**. The 10–23 DNAzyme contains a catalytic core and two recognition arms that can bind to a RNA substrate through Watson–Crick base-pairing (Figure 1 A (a)). The two arms are asymmetrical by design: one arm is 7 bases long and the other is 15 bases long. When the RNA substrate is cleaved, the short fragment (7-base) dissociates from the DNAzyme and the long fragment (16-base), in contrast, remains stably associated with the DNAzyme under the experimental condition (b). After the short RNA fragment dissociates, the short arm of the DNAzyme becomes unpaired and can search for other complementary single strands. The RNA substrate next to the enzyme base-pairs with the short recognition arm of the DNAzyme (c). The resulting short duplex is stable as a result of intracomplex hybridization. Following this hybridization, a strand replacement occurs through branch migration, whereby the intact RNA substrate replaces the cleaved RNA fragment to result in a more stable, longer, pseudocontinuous DNA duplex (d). In this process, the DNAzyme moves from one RNA substrate to the next RNA substrate. The process can be repeated such that the DNAzyme moves continuously. Thus, the DNAzyme autonomously and processively moves along the track.

Herein, DNA–RNA chimeras were used as the substrate **S**. In the chimeras, only the two residues that flanked the bond to be cleaved were RNA residues, while all other residues were DNA. Such chimeras could be cleaved efficiently by the DNAzyme, but were more stable than RNA. Each **S** strand contained two segments: one for enzyme recognition and the other as a positional tag. A single template strand, **T**, could base-pair with the positional tags and organize four substrates (**S1**, **S2**, **S3**, and **S4**) into a regular, one-dimensional substrate array to constitute the track (Figure 1 B). The spacings between any two neighboring substrates was two helical turns (7 nm).

The overall DNA construct was stable. We assembled the DNA structure in a stepwise fashion and then analyzed all complexes by native polyacrylamide gel electrophoresis (PAGE, Figure 2). Each complex appeared as a single band with expected mobility which suggests that each combination of DNA strands led to the formation of a stable complex.

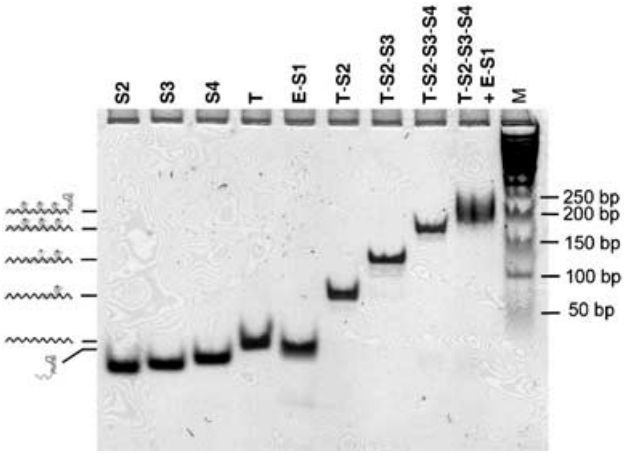

Figure 2. Native PAGE analysis of the DNA complexes. Lane M contains a series of DNA size markers (M = marker; bp = base pair).

The DNAzyme can walk in either direction, **S1**→**S4** or **S4**→**S1** (Figure 1 B). The movement of the DNAzyme was demonstrated by monitoring the differential behaviors of substrate cleavage (Figure 3). When the DNAzyme walks from **S1** to **S4**, the cleavage of the substrate would be in the time order of **S1**–**S2**–**S3**–**S4**, with **S1** cleaved initially and **S4** cleaved last of all. We performed an experiment to confirm this, with separate preparation of complexes **T**-**S2**-**S3**-**S4** and **E**-**S1** and then mixed the two complexes at 22 °C to form an intact track with the DNAzyme at **S1** (Figure 3). Upon mixing, the movement started. The DNAzyme moved from **S1** to **S2** to **S3** and finally to **S4**. This movement was accompanied by sequential cleavage of the substrate. Thus, the order of the substrate cleavage reflects the movement of the DNAzyme. Figure 3 A clearly shows that the digestion order is **S1**, **S2**, **S3**, and **S4**, which is consistent with the design. Note that a substantial population of **S1** was likely to be cleaved before or during association of complex **E**-**S1** with complex **T**-**S2**-**S3**-**S4**.

To further confirm this observation, we performed a similar experiment at 22 °C, but changed the movement to the opposite direction, **S4**→**S1**. Clearly, the order of cleavage reversed (Figure 3 B). When the walking process was performed at 37 °C in both directions, similar orders of substrate

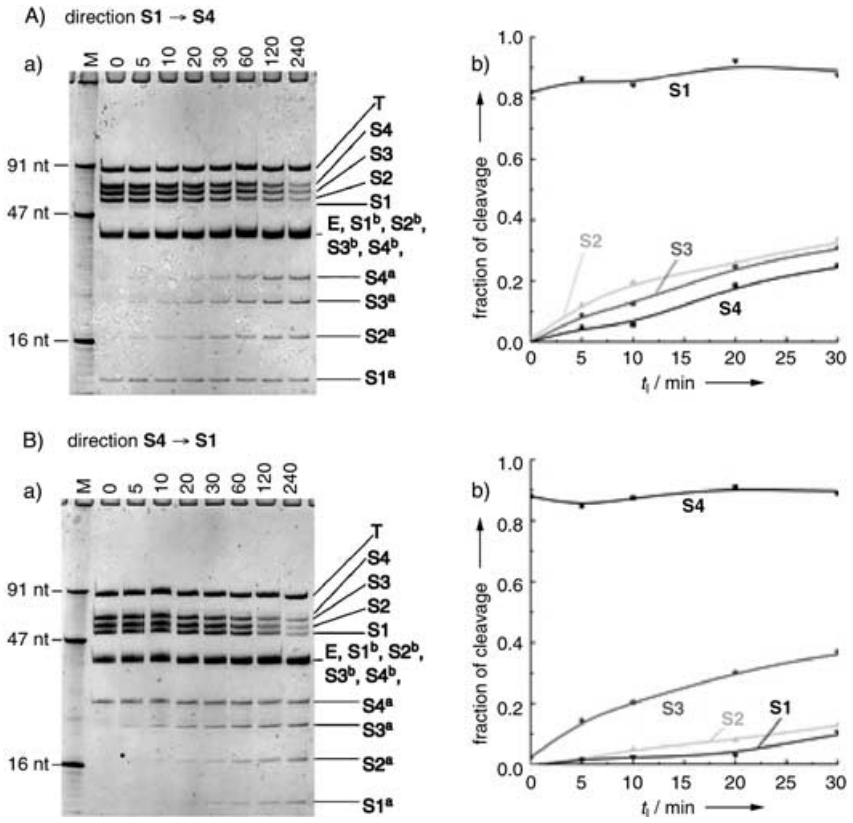

Figure 3. Denaturing PAGE monitoring the movement of the DNAzyme at 22 °C in the directions **S1**→**S4** (A) and **S4**→**S1** (B): a) gel images as a function of incubation time, t_i (lanes M contain three single-stranded DNA size markers; nt = nucleotide (bases); superscripts a and b denote short and long cleaved substrate fragments, respectively); b) quantification of the differential substrate cleavage.

cleavage were observed to those seen at 22 °C, but the process occurred faster at the higher temperature (see Supporting Information). When these walking experiments were repeated (at both temperatures and in both directions), exactly the same orders of substrate cleavage were observed (see Supporting Information).

To exclude the possibility that DNAzyme dissociates from the track after cleaving each substrate, we performed a control experiment (Figure 4). The DNAzyme and substrate (or the long cleaved substrate fragment) formed stable complexes under a native condition. The complexes migrated much slower than either the DNAzyme or the substrate alone by native PAGE, and no dissociation was observed. Thus, we confirmed that the DNAzyme did not dissociate from the track and that the movement was processive. In another control experiment, one substrate (**S4**) was incubated with the DNAzyme for two hours, and then another substrate (**S1**) was added to the solution. Upon addition of **S1**, aliquots of the mixture were withdrawn at various time intervals and the enzyme activity was quenched by addition of an excess of EDTA to remove free Mg^{2+}, an essential cofactor of the DNAzyme. The samples were then analyzed by both denaturing and native PAGE, which showed that **S4** was almost completely cleaved, while **S1** underwent almost no cleavage (see Supporting Information). This experiment confirmed that the enzyme could migrate from one substrate to another much more efficiently when both substrates were located along one track than when the substrates were in different complexes.

In conclusion, we have developed a DNA nanodevice that can autonomously move along an engineered track. In similar fashion to a cellular protein motor, it consumes chemical energy for autonomous motion. However, the current DNA device destroys its track when it walks, so the track is not reuseable. Relative to protein motors, the current DNA device moves extremely slowly. On the other hand, cellular protein motors move along straight protein tracks, whereas our engineered DNA system is much more versatile. It is conceivable to engineer tracks to allow a DNA device to move in a more complicated geometry—for example, smooth curves and sharp turns. Other questions that remain include: Can we develop a system with a reuseable track? Can we directly visualize the movement? Can the DNA device transport any cargo? Our next objective is to address these challenges.

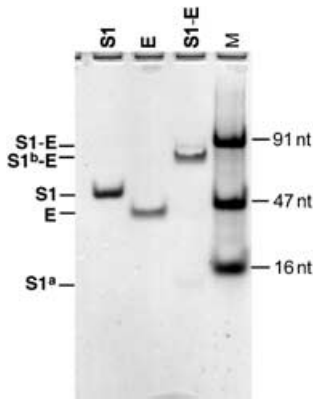

Figure 4. Native PAGE shows that complex **E-S**^{b} is stable, and no dissociation can be observed. Lane M contains three single-stranded DNA size markers.

Experimental Section

Oligonucleotides: All oligonucleotides were designed with a computer program SEQUIN[5] and purchased from Integrated DNA Technologies (IDT), Inc. Strand **E**: 5′-AGT GCT GAT TCG GAC AGG CTA GCT ACA ACG AGA GTG AC-3′; Strand **T**: 5′-ACC ATC TGT GGC ATA GCA GCG AGT ATC TAA CGC ATG GAA GCG TCG ATC TTG AGC ATT GGA GCG GCA ATC TCC TGC ATC ATC GCG-3′; Strand **S1**: 5′-TTT TTG TCA CTC rArUG TCC GAA TCA GCA CTT TCG CGA TGA TGC AGG AGA TTG C-3′; Strand **S2**: 5′-TTT TTT TTT TGT CAC TCrA rUGT CCG AAT CAG CAC TTT CGC GAT GAT GCA GGA GAT TGC-3′; Strand **S3**: 5′-TTT TTT TTT TTT TTT GTC ACT CrArU GTC

CGA ATC AGC ACT TTC GCT TCC ATG CGT TAG ATA CT-3′; Strand **S4**: 5′-TTT TTT TTT TTT TTT TTT TTG TCA CTC rArUG TCC GAA TCA GCA CTT TCG CTG CTA TGC CAC AGA TGG T-3′.

Formation of the Walking System: 1) For the system in which the DNAzyme moved from **S1** to **S4**, the two parts (strands **E** and **S1** (part A); strands **T**, **S2**, **S3**, and **S4** (part B)) were prepared separately. For each part, 1.0 μM DNA strands were combined in an equimolar ratio in TAE/Mg^{2+} (TAE = Tris–acetate–EDTA; EDTA = ethylenediamine tetraacetic acid) buffer, which contained Tris buffer (40 mM, pH 8.0), acetic acid (20 mM), EDTA (2 mM), and $Mg(OAc)_2$ (12.5 mM). Parts A and B were formed by quickly cooling the DNA solution from 95 °C to 22 °C over 10 min. 2) For the system in which the DNAzyme moved from **S4** to **S1**, part A consisted of strands **E** and **S4** while part B consisted of strands **T**, **S1**, **S2**, and **S3**.

DNAzyme Walking: An equimolar ratio of parts A and B was mixed and then incubated at 22 °C or 37 °C to start the movement. At various time intervals during the experiment, a 10-μL aliquot was taken out and the reaction was quenched by the addition of a solution of EDTA (2 μL, 0.1 M) and formamide (6 μL), then the mixture was left on dry ice. All samples were then analyzed by denaturing PAGE.

Denaturing PAGE: Gels contained 20% polyacrylamide (acrylamide/bisacrylamide, 19:1) and urea (8.3 M) and were run at 55 °C. Tris–borate–EDTA (TBE) buffer, which comprised Tris (89 mm, pH 8.0), boric acid (89 mm), and EDTA (2 mm), was used as the separation buffer. Gels were run on a Hoefer SE 600 electrophoresis unit at 600 V (constant voltage).

Native PAGE: Gels contained 6–12% acrylamide (19:1, acrylamide:bisacrylamide) and TAE/Mg^{2+} buffer. 1 μL of a solution of tracking dye, which contained TAE/Mg^{2+}, 50% glycerol, and 0.2% each of Bromophenol Blue and Xylene Cyanol FF, was added to each sample (0.3 μg DNA in 10 μL of TAE/Mg^{-2+}). Gels were run on an FB VE-10–1 vertical electrophoresis system at 120 V (constant voltage) at 4 °C. Gels were stained with Stains-All (0.01% Stains-All from Sigma, 45% formamide) and then scanned.

Quantification of Reaction: Upon completion of electrophoresis, the gels were stained with Stains-All dye (Sigma) and the images were scanned into a computer as jpeg files. With the image files, each band was estimated using OptiQuant (Packard), an image analysis and processing software.

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[6] While attending the workshop "*Engineering a DNA World*" at Caltech in January 2005, we became aware that Prof. M. N. Stojanovic at Columbia University was working on a related system.

