LIGHT DRIVEN NANOMACHINE COMPOSED OF AN AZOBENZENE-MODIFIED DNA

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Introduction

Recently, DNA is regarded as a functional supramolecule as well as a carrier of genetic information. Due to its excellent supramolecular properties, many machine-like DNA nano-structures such as tweezers, gears, and walkers have been constructed [1,2]. Although various excellent nano-machines have been proposed with natural DNA, their reachable performances are still limited. One of the serious problems that we should solve might be energy source for driving the nano-machines. Most of the nano-machines use natural DNAs as the fuel and free energy released during the DNA hybridization is applied to the mechanical movement. For example, the fuel DNA is hybridized with the "engine" part of the nano-machine to "move" and is removed through strand displacement by adding its complementary DNA. During repetitive operation of DNA nano-machine, however, double-stranded DNA wastes were accumulated in the solution and the working efficiency decreased gradually [2]. A new energy source is required to overcome this problem for further development of DNA-based nano-machine.

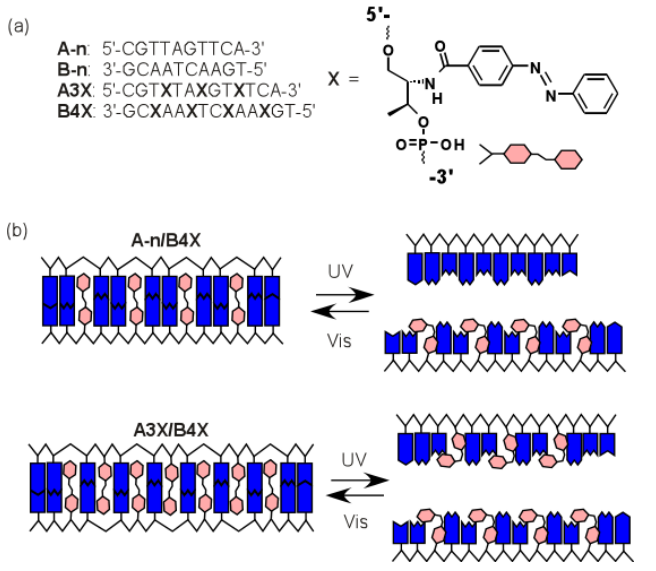

Fig.1. Sequences of the DNAs modified with threoninol nucleotide involving azobenzene (a) and schematic illustration of the photoregulation of **A-n/B4X** and **A3X/B4X** duplexes (b).

We have recently developed a new methodology for the modification of natural DNA. Threoninol nucleotide, a new base surrogate involving a functional molecule on D-threoninol as a scaffold (see X in Fig. 1), is designed to conjugate the natural DNA [3]. We found that wedge-type (bulge-type) insertion of threoninol nucleotides into natural DNA fairly stabilized the duplex even though a lot of threoninol nucleotides were inserted. In the present study, we demonstrate clear-cut photoregulation of the formation and dissociation of the duplex by artificial DNA involving azobenzene moieties, and apply it to the photon-fuelled DNA nano-machine that is 'environment-friendly' without producing DNA wastes during the operation.

Results and Discussion

Photoregulation of hybridization: The sequences of the DNA used in this study and the structure of threoninol nucleotide involving azobenzene are depicted in Fig.1. We designed two types of duplexes: a combination of natural oligonucleotide and azobenzene-modified DNA (such as **A-n/B4X** duplex), and a combination of azobenzene-modified DNAs (such as **A3X/B4X** duplex). In both cases, threoninol nucleotides involving azobenzenes are *additionally* inserted to the natural oligonucleotide. These wedge-type insertions highly stabilized the duplex in *trans*-form. The melting temperatures (T_ms) of **A-n/B4X** and **A3X/B4X** duplexes after visible light irradiation were 41.8 and 57.3 °C, respectively (see Table 1), which were much higher than

Table 1. Melting temperature of the duplexes.

Duplex	T_m / °C [a]		
	trans	*cis*	ΔT_m [b]
A-n/B-n	32.7		
A-n/B4X	41.8	~0	~42
A3X/B4X	57.3	<0	>57

a) Conditions: 4 μM DNA, 100 mM NaCl, pH 7.0 (10 mM phosphate buffer)

b) Difference in T_m between *trans* and *cis*-forms

that of the native **A-n/B-n** duplex (32.7 ^{o}C). These results demonstrate that conjugation of natural nucleotides with threoninol nucleotides in a proper manner strongly stabilizes the duplex. In contrast, isomerization from *trans* to *cis*-form by UV light irradiation significantly lowered the T_m. As listed in Table 1, T_ms of **A-n/B4X** and **A3X/B4X** duplexes in *cis*-form were 0 and below 0 ^{o}C, respectively, indicating that duplexes were dissociated to single-strands almost completely. Accordingly, clear-cut photoregulation of hybridization by light irradiation was achieved with azobenzene-tethered DNA [3].

Application to light-driven nano-tweezers: We next applied the azobenzene-tethered DNA to the light-driven nano-tweezers. The photoresponsive tweezers were designed on the basis of DNA-fuelled tweezers reported by Yurke et al [4]. As illustrated in Fig.2, tweezers are composed of three strands **A**, **B**, and **C**. Strand **A** is hybridized with strands **B** and **C** to form two stiff double-stranded arms that are 22 base pairs long and sufficiently stable. Tetrachlorofluorescein (**TET**) and carboxytetramethylrhodamine (**TAMRA**) are attached respectively to the 5' and 3' ends of strand **A**. Opening and closing of the tweezers are controlled by azobenzene-tethered $\mathbf{F_{12X}}$ strand that is complementary to the overhangs of **B** and **C** strands and is composed of 12 threoninol nucleotides involving azobenzenes and 20 natural nucleotides [5]. When the strand $\mathbf{F_{12X}}$ is hybridized, the tweezers are closed and fluorescence from TET (540 nm) is quenched by resonant energy transfer to **TAMRA**. However, when the strand $\mathbf{F_{12X}}$ is dissociated, the tweezers are open and fluorescence from **TET** is recovered. Figure 3 shows the fluorescence emission spectra of the open and closed tweezers. When strands **A**, **B**, **C**, and $\mathbf{F_{12X}}$ were mixed and visible light was irradiated to this solution at 50 ^{o}C, fluorescence from TET became rather weak (see dotted line in Fig.3) because the tweezers were closed in *trans*-form. As exactly designed, fluorescence recovered in *cis*-form (after UV irradiation, see solid line in Fig.3) due to the opening of the tweezers. Since this photon-fuelled nano-machine does not produce any waste duplex during the operation, successive photo-irradiation to the solution did not lower the cycling efficiency at all [5], while the efficiency of original DNA-fuelled tweezers decreased by about 40% after 7 cycles [4].

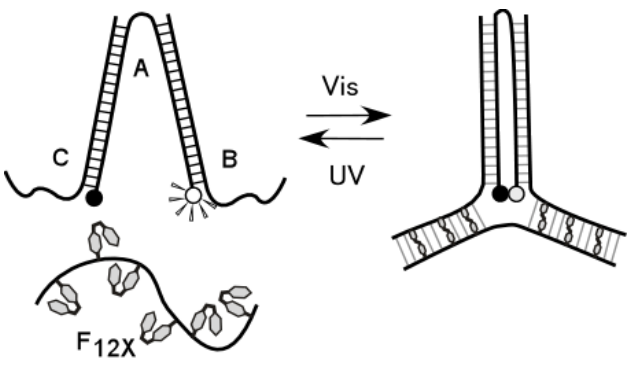

Fig.2. Schematic illustration of the nano-tweezers by light irradiation.

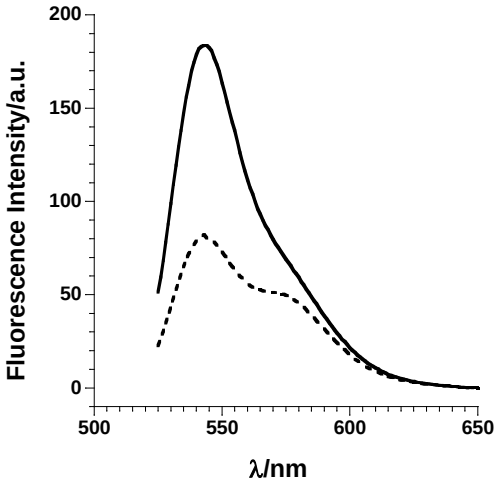

Fig. 3. Close and open of the nano-tweezers by UV (solid line) and visible light (dotted line) irradiation at 50 ^{o}C.

In conclusion, formation and dissociation of the duplex was efficiently photoregulated by azobenzene-tethered DNA on D-threoninol (threoninol nucleotide). Light-driven nano-tweezers were also constructed with this photoresponsive DNA.

References

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