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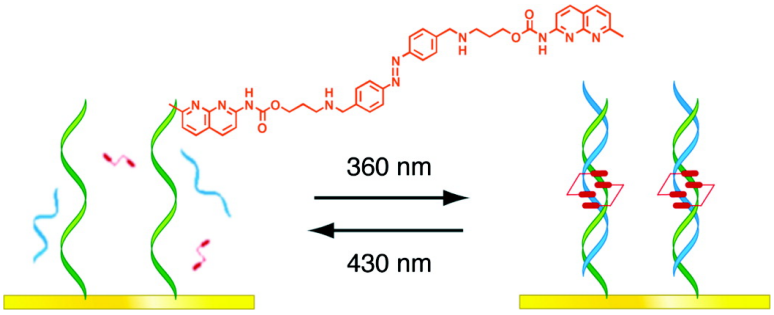

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Photoswitchable Molecular Glue for DNA

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Spontaneous hybridization between two complementary DNAs or RNAs is the unique and indispensable property of nucleic acids and triggers many biological functions. In addition, the sequence-specific hybridization of DNA is effectively used in constructing DNA-based materials and nanoarchitectures.[1] To regulate the diverse functions triggered by DNA hybridization, studies toward controlling or modulating the DNA hybridization in response to external stimuli have drawn considerable interest.[2−5] Here, we describe a photoswitchable molecular glue for DNA which accomplished the reversible control of hybridization of two natural unmodified DNAs by external light stimuli.

We have developed a series of synthetic small molecules which can specifically bind to mismatch-containing DNAs.[5−8] Since the mismatch binding ligands (MBL) increased the apparent thermodynamic stability of the mismatched DNA duplex, MBL can function as a molecular glue to adhere two single-stranded DNAs (ssDNA) that do not spontaneously hybridize with each other.[5] While the function of MBL in the first generation was the unidirectional control of DNA hybridization, pseudo-bidirectional control was achieved by thermally degradable molecular glue that can drive the DNA hybridization/dehybridization cycle by irreversible decomposition on heating followed by refilling the molecular glue.[5b]

To achieve completely reversible and bidirectional control of DNA hybridization, we have investigated the incorporation of a photoresponsive unit into MBL to drive hybridization/dehybridization cycle by external light stimuli. Naphthyridine carbamate dimer, which binds to DNA duplex containing a GG-mismatch,[5−6] was integrated into photoswitchable molecular glue (**NCDA**) by incorporating a photochromic azobenzene between two base-recognizing naphthyridine heterocycles (Figure 1). We anticipated that a reversible cis/trans isomerization of azobenzene by photoillumination changes the relative orientations and positions of the naphthyridine moieties, and therefore, results into the switching of the **NCDA** ability to adhere two ssDNAs containing the GG-mismatch (Figure 1).

NCDA was synthesized by reductive amination of 4,4′-diformylazobenzene with naphthyridine carbamate derivative. The azobenzene moiety of **NCDA** exists exclusively in the trans form under ambient light conditions. Photoirradiation of **NCDA** in sodium cacodylate buffer (pH 7.0) at a wavelength of 360 nm for 5 min produced the photostationary state consisting of a nearly equimolar mixture of *cis*- and *trans*-**NCDA**. The mixtures were reverted exclusively to the trans form upon irradiation of visible light of 430 nm (Supporting Information, Figure S1).

The stabilization of mismatch DNA duplexes by **NCDA**-binding was evaluated by the melting temperature (T_m) of an 11-mer duplex DNA 5′-(CTAA ***CGG*** AATG)-3′/5′-(CATT ***CGG*** TTAG)-3′ containing a GG mismatch in a CGG/CGG sequence (Figure 2a, DNA1·2). The CGG/CGG sequence is the most preferential binding site for the naphthyridine carbamate dimer to form a complex with

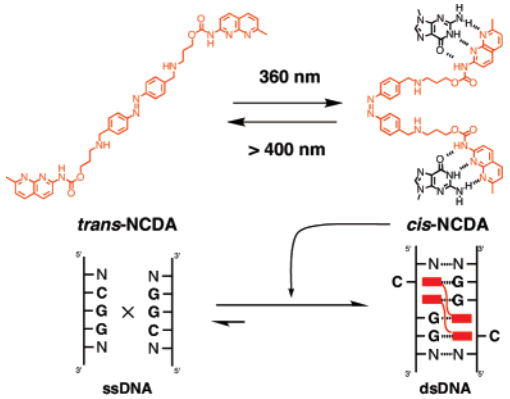

Figure 1. Illustration of the concept for photoswitching of DNA hybridization by **NCDA**. **NCDA** in the cis configuration binds selectively to a GG-mismatch site and induces formation of the DNA duplex. Formation and dissociation of the *cis*-**NCDA**-stabilized dsDNA from two unmodified DNAs can be reversibly controlled by an external light stimulus.

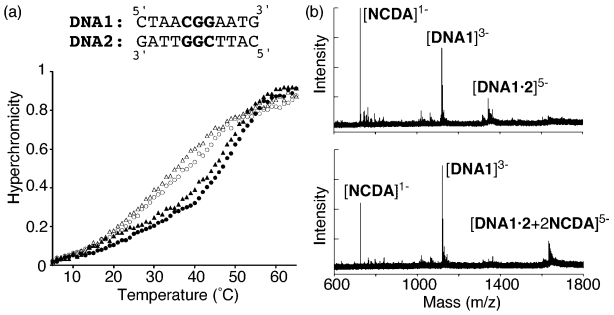

Figure 2. (a) Thermal melting profiles of DNA duplex 1·2 containing GG-mismatch (4.5 μM) in the presence of **NCDA** (18.2 μM). The absorbance at 260 nm was measured in 10 mM Na·cacodylate buffer (pH 7.0) containing 100 mM NaCl. Key: The plots before irradiation, open circles; plots after photoirradiation at 360 nm for 5 min, filled circle; subsequent photoirradiation at 430 nm for 5 min, open triangle; subsequent photoirradiation at 360 nm for 5 min, filled triangle. (b) CSI−TOF MS of duplex DNA1·2 (20 μM) in the presence of **NCDA** (40 μM) before (upper panel) and after photoirradiation at 360 nm for 5 min (lower panel).

a 2:1 stoichiometry (MBL/DNA).[5−6] The T_m of DNA1·2 was 25.6 °C, and 32.7 °C in the presence of *trans*-**NCDA** (Figure 2a, circle). After irradiation of the solution at 360 nm for 5 min, the T_m value increased by 15.2 °C (T_m = 48.0 °C, Figure 2a, filled circle). The fraction of the cis-isomer in the photoirradiated solution was 75% (Figure S2). That was higher than the 50% fraction obtained in the absence of the mismatch DNA. T_m measurements of DNA having all possible combinations of matched and mismatched base pairs showed that the GG mismatch duplex was specifically stabilized after the photoirradiation of 360 nm light (Table S1). The T_m of the GG mismatch duplex was found to reduce to the original level by the second photoirradiation of 430 nm light (Figure 2a, triangle). All the data suggested that (1) *cis*-**NCDA** stabilized the GG mismatch DNA more strongly than *trans*-**NCDA**

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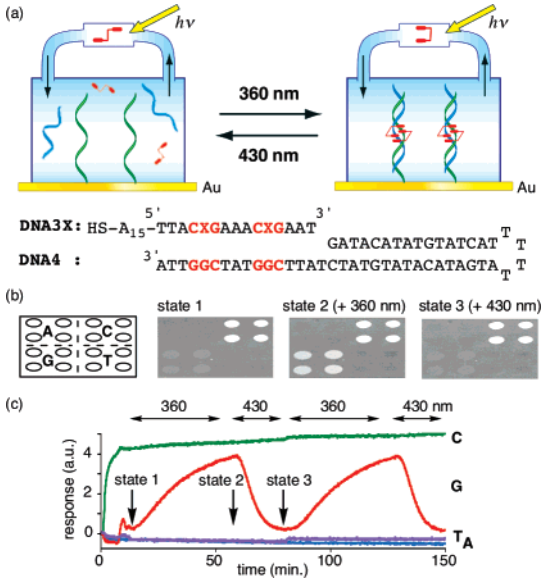

Figure 3. SPR difference imaging experiments showing photoswitching of DNA hybridization by **NCDA**: (a) Sequences of DNA oligonucleotides and schematic illustration of the experiments. The probe DNA3X possessing a terminal sulfhydryl group were immobilized on a gold chip via A_{15} spacer sequence. (b) SPR difference images of a DNA3X array. The DNA3X array (X = A, C, G, and T) was prepared on a gold chip in the pattern depicted. Key: state 1, image collected after addition of 52-mer DNA4 (1 μM) and **NCDA** (5 μM); state 2, after 360 nm photoirradiation of state 1 for 40 min; state 3, after 430 nm photoirradiation of state 2 for 20 min. (c) Time course of intensities of the spots shown in panel b. Horizontal arrows above the plots represent the irradiation periods. DNA4 and **NCDA** were injected at 0 and 6 min, respectively.

and (2) the *cis*-**NCDA**−DNA complex was disassembled upon isomerization of *cis*-**NCDA** by 430 nm photoillumination.

The formation of the *cis*-**NCDA**−DNA complex was confirmed by the cold-spray ionization time-of-flight mass spectrometry (CSI−TOF MS). With *trans*-**NCDA**, ions corresponding to the DNA were the only detectable peaks (Figure 2b, upper panel). Upon photoirradiation at 360 nm, the ion peak at *m*/*z* 1633.28 corresponding to $[2\mathbf{NCDA}+\mathrm{DNA1}\cdot 2]^{5-}$ (calcd: 1632.0) was clearly detected (Figure 2b, lower panel). The 2:1 stoichiometry between *cis*-**NCDA** and the duplex is in good agreement with the previously characterized complexes of the naphthyridine carbamate dimer binding to the CGG/CGG sequence.[5−7] On the other hand, complexes of *trans*-**NCDA** and DNA were not detected under the conditions. This is most likely because the folded *cis*-azobenzene linkage in **NCDA** allows two naphthyridine moieties to be placed in the appropriate positions for the binding, whereas the extended *trans*-azobenzene does not.

The reversible control from ssDNA to dsDNA, and vice versa, by **NCDA** was further investigated by the surface plasmon resonance (SPR) imaging with the DNA immobilized on the gold surface.[9] A large T_m difference before and after photoirradiation that is necessary for spontaneous DNA hybridization/dehybridization was attained by incorporating two CGG/CGG sites in 15 mer DNA (Figure S6). Probe DNA3X 5′-(TAA ***CXG*** AAA ***CXG*** AAT)-3′, where X is A, C, G, and T, were immobilized through A_{15} spacer on a gold surface for the SPR imaging measurement. The hybridization of the hairpin DNA4 having an overhang sequence of 5′-(ATT ***CGG*** TAT ***CGG*** TTA)-3′ was monitored by the SPR difference image before and after photoirradiation (Figure 3a). The DNA4 and *trans*-**NCDA** were added in the bulk solution and circulated between a photoreaction cell and a SPR cell equipped with the DNA3X-immobilized gold surface. Strong SPR signals due to the hybridization of DNA4 were observed selectively at the spots of DNA3C having the fully complementary sequence to the overhang sequence (Figure 3b, state 1). The circulating solution was irradiated in the photoreaction cell with 360 nm light to isomerize **NCDA** from its trans to cis form. Immediately after photoirradiation, SPR signals started to emerge at the spots of DNA3G with the intensity of SPR signal being increased as the irradiation of 360 nm light continued (Figure 3b,c, state 2, X = G). SPR signals were not detected at the spots of DNA3A and DNA3T, indicating that hybridization with DNA4 selectively occurred on DNA3G with the assistance of *cis*-**NCDA**. Subsequent irradiation of the circulating solution with 430 nm light resulted in the disappearance of SPR signals at the DNA3G spots (state 3). The decrease of the concentration of *cis*-**NCDA** in the bulk solution by photoisomerization caused the dissociation of *cis*-**NCDA** bound to the GG mismatch, eventually leading to the dehybridization of DNA4 from DNA3G. These results clearly showed that the photoisomerization of **NCDA** in fact controlled the hybridiation and dehybridization of the DNA duplex containing GG-mismatch in response to external light stimuli.

In conclusion, incorporation of the photochromic azobenzene linkage into MBL permits reversible control of DNA hybridization by external light stimulus both in homogeneous solution and on the gold surface. **NCDA** is a new class of molecules that functions as a photoswitchable molecular glue for DNA and will be useful for controlling the biological functions triggered by DNA hybridization and reversible construction of DNA-based nanoarchitectures.

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Supporting Information Available: Synthetic details of **NCDA**. HPLC profiles for isomerization, UV, and CSI−TOF MS analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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