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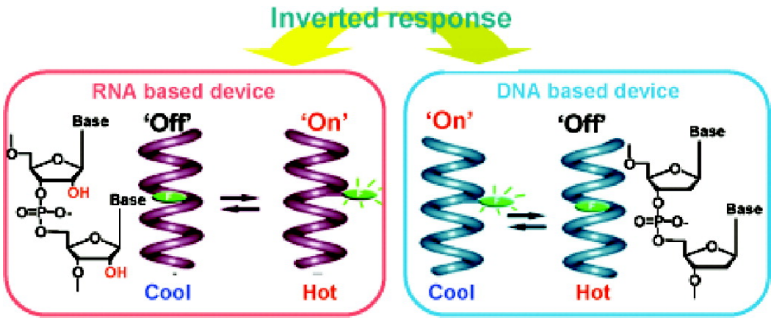

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Biomolecule-Based Switching Devices that Respond Inversely to Thermal Stimuli

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Abstract: We demonstrate a molecular switch, on the basis of the characteristic properties of DNA and RNA, which indicates a completely inverted response to thermal stimuli using the transition between right- and left-handed helices. We designed a system using aminopurine (Ap), which can convert the π-stack information of the transition from right-handed to left-handed DNA (B−Z transition) and RNA (A−Z transition) into an output giving a fluorescent signal. These two biomolecular devices together serve as "right−left" or "off−on" switches. When the temperature is changed from low to high, the RNA device changes from the off to on signal; however, the DNA device changes from on to off. The response of these RNA and DNA based devices against thermal stimulus was completely reversible.

Introduction

Responding to an external stimulus is a fundamental requirement of switchable molecular devices to achieve the conversion between two different states with on−off functions. A variety of motions of molecular devices and their directions of movement have been accomplished using different external stimuli.[1,2] In contrast, molecular devices that exhibit a completely inverted response against the same stimulus are unique and attractive prospects for new types of molecular switches. Molecular devices that use helicity and its inversion are expected to be applied to programming, sensing, and switching.[1,3−5] Recently, there has been growing interest in the use of DNA for nanomaterials, and several types of DNA based devices have been reported.[6−10] However, compared with DNA, RNA is rarely used for devices.[11,12] The different physical and chemical properties of DNA and RNA could be useful for designing nanomaterials in attempts to increase the variety of devices and responses.

Both DNA and RNA, with alternative purine−pyrimidine sequences, can convert from right-handed (B and A forms) to left-handed helices (Z form) in high-salt conditions.[13−17] The B−Z transition of DNA can also be controlled by changing the temperature; thus, left-handed Z-DNA is preferred at lower temperatures.[13] Recently, we designed a DNA thermosensing device based on the different π-stacking of B- and Z-DNA, giving a fluorescent signal of 2-aminopurine (Ap).[18] Ap is the fluorescent analogue of adenine and has been extensively used as a probe for nucleic acid structure and charge transfer in DNA.[19−22] The fluorescence of Ap is much stronger in Z-DNA than in B-DNA, because the continuous π-stacks in B-DNA cause efficient quenching of Ap, whereas the formation of discrete four-base π-stacks in Z-DNA diminishes the quenching.[18,23] This conformational change caused by external stimuli, supplying output related to such a structural change, fulfills the requirements for a molecular switching device. Furthermore, output by modulation of fluorescence is a particularly attractive feature for devices, as fluorescence offers an additional readout method.[24−26] Notably, the thermal response of RNA is inverse to that of DNA: left-handed Z-RNA, rather than right-

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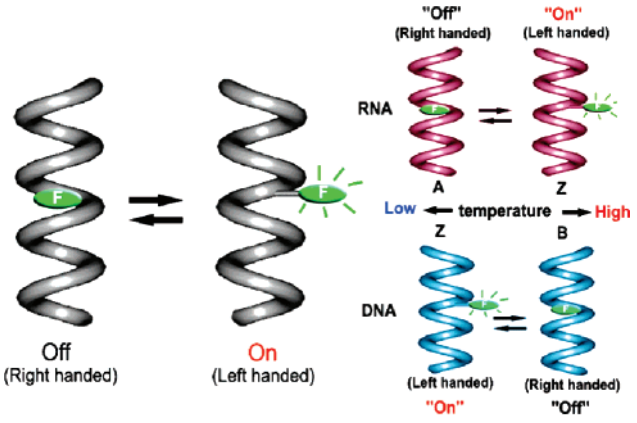

Figure 1. Schematic representation of RNA and DNA reversible molecular devices that respond inversely to thermal stimuli. When these devices have a left-handed form (Z form), intensity of fluorescence is increased; this state represents switch "on". In contrast, when these devices have a right-handed form (A and B form), intensity of fluorescence is decreased; this state represents switch "off". The green oval represents Ap.

handed A-RNA, is the stable or preferred conformation at higher temperatures.[14−17]

Here, we show molecular switching devices, on the basis of these helical transitions of DNA and RNA, that show a completely inverted response to thermal stimuli, using the transition between right- and left-handed helices. These two biomolecular devices together serve as "right−left" or "on−off" switches by fluorescent signal. When the temperature is changed from low to high, the RNA device changes from an off to an on signal, whereas the DNA device changes from on to off.

Results and Discussion

Because the structure of Z-RNA is very similar to Z-DNA[14,15] and there is continuous π-stacking in A-RNA, we expected that the fluorescence of Ap in Z-RNA would be much stronger than in A-RNA. We prepared an RNA duplex containing Ap: r(GCGCGC-Ap-CGCGCG) (RNA1)/(CGCGCGUGCGCGC) (RNA2) and examined the conformation by circular dichroism (CD) spectra. Figure 2a shows the CD spectra of RNA1/2 in different concentrations of $NaClO_4$ at 40 °C. At 1−4 M $NaClO_4$, we observed a negative Cotton effect around 285 nm and a positive Cotton effect around 266 nm (Figure 2a). With increasing $NaClO_4$ concentration from 5 to 8 M, we observed an increase in the Cotton effect around 285 nm and a decrease around 266 nm. Further addition of $NaClO_4$ did not change the CD spectrum. These responses were similar to the CD spectra of $r(CG)_3$ (Figure 2b), whose structure was confirmed as A-RNA in 1 M $NaClO_4$ and Z-RNA in 6 M $NaClO_4$ using proton nuclear magnetic resonance spectroscopy (^{1}H NMR).[14] The results indicate that RNA1/2 predominantly exists as the A conformation in 1−4 M $NaClO_4$ and as the Z conformation in 8 M $NaClO_4$. The midpoint $NaClO_4$ concentrations at the A−Z transition for RNA1/2 and $r(CG)_3$ were 5.8 and 3.5 M, respectively. Figure 2d shows the fluorescence spectra of

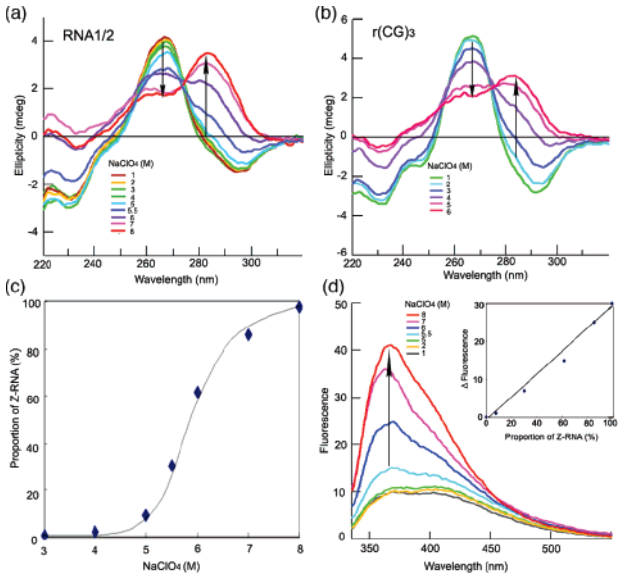

Figure 2. Circular dichroism (CD) and plot of the proportion of Z-RNA in the presence of various amounts of $NaClO_4$. (a) CD spectra of r(CGCGC-Ap-CGCGCG)/(CGCGCGUGCGCGC) (ODN1/2) at 40 °C. (b) CD spectra of $r(CG)_3$ at 25 °C. (c) Plot of proportion of Z-RNA of RNA1/2. (d) Florescence spectra of RNA1/2 in the presence of various amounts of $NaClO_4$ at 40 °C. (inset) Fluorescence intensity vs proportion of Z-RNA as a plot of the proportion of Z-RNA in RNA1/2. The samples were excited at 317 nm, and emission intensities were determined by monitoring emission at 360 nm. ΔFluorescence values were obtained by subtracting fluorescence intensity of the A-form RNA1/2 from that of RNA1/2 in each concentration of $NaClO_4$.

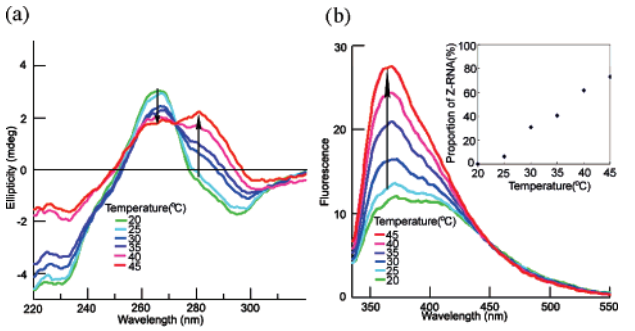

Figure 3. Circular dichroism (CD) and florescence spectra of RNA1/2 at various temperatures. (a) CD spectra of RNA1/2 in 6 M $NaClO_4$. (b) Florescence spectra of RNA1/2. (inset) Plot of the proportion of Z-RNA vs temperature.

RNA1/2 in different concentrations of $NaClO_4$ with excitation at 317 nm. The fluorescence of RNA1/2 increased with increasing $NaClO_4$ concentrations and the emission correlated linearly with the proportion of Z conformation. The results clearly demonstrate that the A−Z transition of RNA can be monitored readily from the fluorescence intensity of Ap, as reported previously for DNA.[18]

We pointed out above that Z-RNA is the more stable or "preferred" conformation at higher temperatures. Therefore, the CD and emission spectra of RNA1/2 were measured in 6 M $NaClO_4$ at different temperatures to examine the A−Z transition (Figure 3a). Because the thermal denaturation experiments indicated that the Tm value of RNA1/2 was 57 °C (Figure 1Sa), and because RNA1/2 forms a duplex (>98%) below 45 °C, the fluorescence measurements were carried out in the temperature range 20−45 °C. CD spectra indicated that at 20 °C the RNA duplex was mostly in the A form. As the temperature rose from

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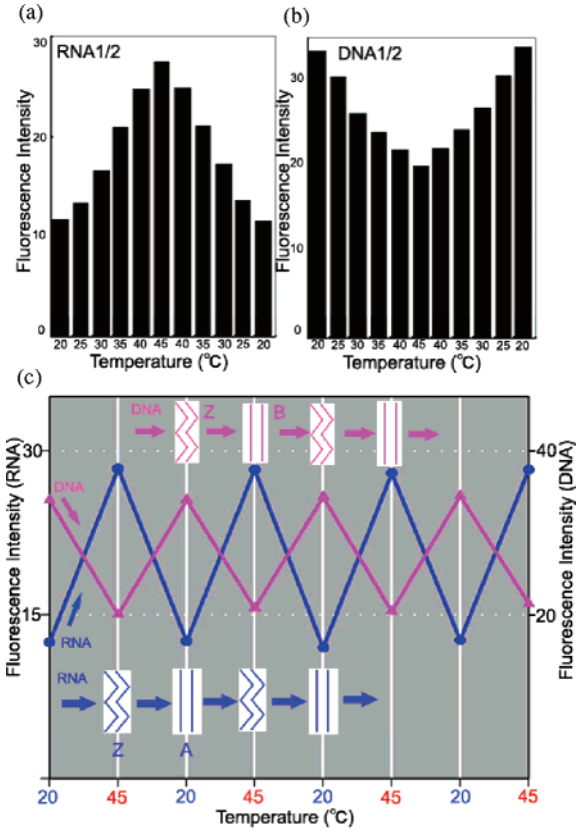

Figure 4. Reversible changes in the thermal switch comprising RNA1/2 (a) and DNA1/2 (b) showing the fluorescence intensities of RNA1/2 in 6 M $NaClO_4$ and DNA1/2 in 4 M NaCl at different temperatures. (c) Repeated experiments of fluorescence emission of RNA1/2 and DNA1/2 at 20 and 45 °C.

20 to 45 °C, the CD signal due to A-RNA decreased, and the proportion of Z-RNA increased to 73%. The proportion of Z-RNA at various temperatures is shown in Figure 3b (inset). The fluorescence spectra of RNA1/2 at 20 °C were very weak, whereas the fluorescence of the RNA1/2 dramatically increased at 45 °C (Figure 3b). The control RNA duplex, r(GCCGGC-Ap-CGGCCG) (RNA3)/r(CGGCCGUGCCGGC) (RNA4), in which the alternating purine−pyrimidine sequences were interrupted, could not form Z-RNA and did not show this increase in intensity of fluorescence at increasing temperatures under the same conditions (data not shown).

Figure 4a and b shows the fluorescence intensities of RNA1/2 and d(GCGCGC-Ap-CGCGCG) (DNA1)/(CGCGCGTGCGCGC) (DNA2) and clearly demonstrates the completely inverted responses of RNA and DNA against temperature. In RNA, the RNA1/2 was in the A form at 20 °C, and fluorescence was weak. However, at 45 °C, the conformation was in the Z form (73%), and RNA1/2 fluoresced strongly. In DNA1/2, the thermal response was opposite. At 20 °C, the proportion of DNA1/2 in the Z form was 61% and, as the temperature rose to 45 °C, the proportion of Z-DNA fell to 24%. Thus, there was a decrease in intensity of fluorescence following the B−Z transition (Figure 4b). The proportions of Z-DNA at various temperatures are shown in the Supporting Information (Figure 2S). Tm value of DNA1/2 was 72 °C, and the melting curves are shown in Figure 1Sb. The response of this RNA and DNA device to thermal stimuli was completely reversible (Figure 4c). Single-strand RNA1/2 and DNA1/2 at 90 °C showed a slightly smaller

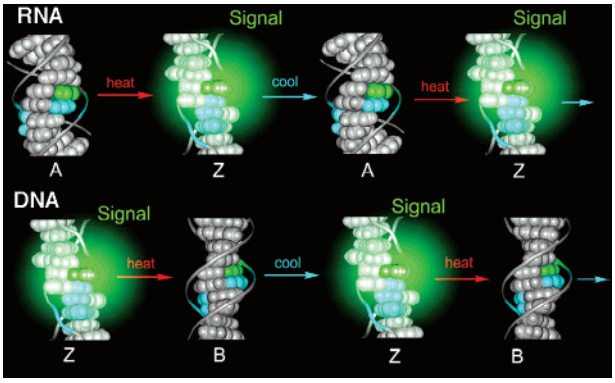

Figure 5. Concept of reversible RNA and DNA thermal switches emitting a fluorescence signal as the output.

fluorescence intensity compared with the Z form (Figure 3S), indicating that the efficient emission forms a four-base π-stack. Because both RNA1/2 and DNA1/2 show higher fluorescence in the Z form (left-handed helices) than in the A and B form (right-handed helices), these DNA and RNA devices together serve as a right−left or off−on switch.

In conclusion, by using the different properties of DNA and RNA, we have successfully demonstrated the concept of molecular devices that show a completely inverted responses to temperature (Figure 5). It was shown that the response of these devices is completely reversible. To the best our knowledge, this is the first example of reversible switching devices that show an inverted response to the same stimulus. This switching may provide a new programming and sensing system for nanodevices. Since the buffer conditions used are not physiological conditions, the device developed is a proof-of-concept device. However, by utilizing the base analogues, which can stabilize the Z conformation under low-salt concentrations,[27−29] RNA- and DNA-based switches can operate under physiological conditions. DNA is an attractive material for nanotechnology because of the feasibility of preparing various fragments with defined sequences employing chemical and enzymatical methods.[3] In addition, RNA has similar advantages in its preparation. The different properties of DNA and RNA have the potential to extend the molecular devices to construct higher order nanosystems and new types of molecular switches.

Methods

Sample Preparation. RNA and DNA oligonucleotides were purchased from Japan Bio Services Co., Ltd. (Saitama, Japan). The purity and concentrations of all oligonucleotides were determined by enzymatic digestion to complete mononucleotides. RNA1/2 and DNA1/2 in buffer solutions were incubated at 95 °C for 10 min and slowly cooled to 20 °C to produce correct duplexes before each experiment.

CD Spectroscopy and Evaluation of Conformation. CD spectra of oligonucleotide solutions (21 μM base concentration) collected in 0.5-nm steps from 320 to 220 nm were measured using an Aviv Model 62 DS/202 CD spectrometer in a 1-cm quartz cuvette in 10 mM $NaHPO_4$ (pH 7.0), 20 mM NaCl, and various concentrations of $NaClO_4$. To evaluate the proportion of Z-RNA, we slightly modified the previous

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method of evaluating the Z form of DNA.[30] Because the CD spectrum of Z-RNA shows a characteristic peak at 285 nm, we estimated the CD signal at this wavelength (295 nm was used for Z-DNA as this wavelength is characteristic).[30] To obtain the proportions of A- and Z-RNA (P_A and P_Z), we analyzed the CD spectra, taking into account the three forms (A, Z, SS) present in solution. Hence, at each temperature we made the following assumptions in eqs 1 and 2.

$$\Delta\epsilon^{285} = \Delta\epsilon_A{}^{285}P_A + \Delta\epsilon_Z{}^{285}P_Z + \Delta\epsilon_{SS}{}^{285}P_{SS} \quad (1)$$

$$P_A + P_Z + P_{SS} = 1 \quad (2)$$

P_A, P_Z, and P_{SS} are the molar fractions of the A, Z, and single-strand components, respectively. The relative $\Delta\epsilon$ values have been estimated from the CD signals at 285 nm and were considered independent of the temperature and salt concentrations. A UV melting experiment indicated that P_{SS} is negligible relative to each CD spectrum at a given temperature. The equations were solved to provide estimates of P_A and P_Z.

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Fluorescence Measurement. Steady-state fluorescence measurements of Ap-containing RNA and DNA were conducted using a JASCO FP-6300 spectrofluorometer. Measurements were performed using fluorescence cells with a 0.5-cm path length. The buffer and concentrations of $NaClO_4$ were the same as for CD measurement. The duplex concentration for emission measurements was 130 μM (base concentration).

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Supporting Information Available: Melting curves of RNA1/2 and DNA1/2, plot of the proportions of Z-DNA1/2, and fluorescence spectra of RNA1/2 and DNA1/2 under different temperatures. This material is available free of charge via the Internet at http://pubs.acs.org.

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