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In vitro selection of a photoresponsive RNA aptamer to hemin

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ABSTRACT

A photoresponsive RNA aptamer to hemin was selected in vitro from a random sequence library of RNAs with azobenzene residues. The aptamer bound to hemin under visible light irradiation and was released by ultraviolet light.

Artificial control of dynamic molecular recognition is an important and promising research area.[1] Light has been used as a dominant external stimulus to control various processes of dynamic molecular recognition.[2,3] For example, 'photoresponsive crown ethers' and 'light-powered molecular pedal' were reported as supramolecular systems.[4] On the other hand, photoresponsive biopolymers (both DNA and peptide based) were also designed to photocontrol the interaction among biologically active molecules.[5,6] In all of the cases described above, a rigorous molecular design (commonly termed 'rational design') was performed, by which a dynamic function induced by light was appended to the molecules. However, when a structurally complicated or unknown molecule (e.g., protein) was chosen as a guest, the design of the photoresponsive host molecule was difficult. Moreover, an individual rational design method is usually required in each case.

Here, we propose another approach to achieve the photocontrol of dynamic molecular recognition. Our strategy was to introduce an 'in vitro selection' method into this research area. If a designed component with a photoswitch (e.g., azobenzene or spiropyran) can be applied to the in vitro selection process, a dynamic library of molecules that respond to light can be constructed via enzymatic polymerization. From this library of constituents, photoresponsive host molecules will be selected against various guests using the same approach. It is known that photoisomerization of photoswitches can change the structure of nucleic acids.[5,7]

The in vitro selection (SELEX) method was developed in 1990.[8] Numerous host molecules (aptamers) against various guest molecules (target molecules), such as enzymes, proteins, and small molecules, have been developed using this method.[9] Furthermore, in the last 15 years, the scope of the aptamers has been extended from natural oligonucleotides to nonnatural oligonucleotides.[10] So far, we have isolated several aptamers containing nonnatural nucleotides using the in vitro selection method.[11]

Previously, we isolated hemin-binding RNA and DNA aptamers by in vitro selection and demonstrated that these natural aptamers also exhibited peroxidase activity by forming a complex with hemin.[12] Thus, as a mimic of peroxidase, the hemin-binding aptamer can be used as a biocatalyst. On the other hand, the hemin-binding aptamer is a promising tool for regulating and understanding cell function, because heme is an essential molecule that plays critical roles in numerous biological phenomena. Therefore, in this study, we chose hemin as a target molecule for isolating a photoresponsive RNA aptamer by in vitro selection.

We synthesized a photoresponsive adenosine triphosphate (azo-ATP, Fig. 1) and applied this azo-ATP to in vitro selection. A double-stranded (ds) DNA library ($\sim 10^{11}$ molecules) was transcribed into an azobenzene-modified RNA library using azo-ATP instead of natural ATP. After visible light irradiation, the RNA

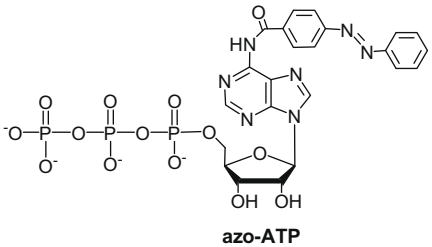

Figure 1. Chemical structure of the azo-ATP used in the transcription reaction.

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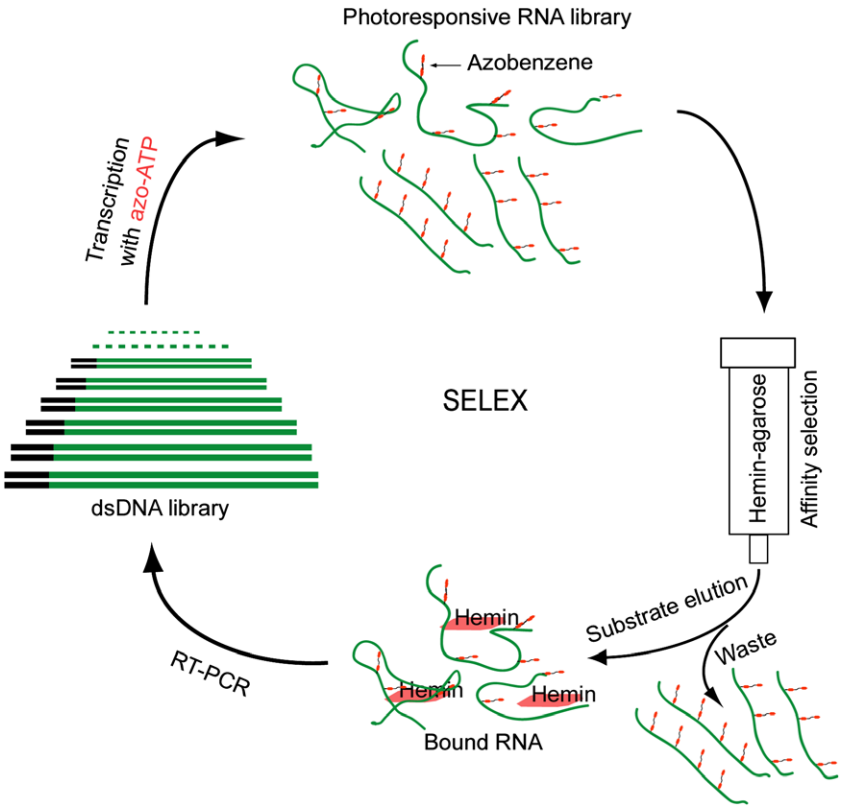

Scheme 1. In vitro selection of a photoresponsive RNA aptamer.

library was applied to a hemin-immobilized agarose column. Bound RNA was eluted using a solution containing hemin, and RT–PCR was then carried out to obtain a dsDNA library from the recovered RNA for the next round of selection (Scheme 1).

Azobenzene-modified adenosine triphosphate (azo-ATP) was synthesized as shown in Scheme S1. We confirmed that azo-ATP was photoswitchable by analyzing the change of absorption between 300 and 400 nm derived from azobenzene, either under visible light irradiation or after UV irradiation (Fig. S1a).

Before the in vitro selection, we performed the following experiments. First, isomerization from the *cis*-form to the *trans*-form was investigated. Isomerization was so slow that there was almost no spectral change over 10 h (Fig. S1b). As the *cis*-form of azobenzene conjugated with ATP was thermally stable at 37 °C, it was considered possible to isolate stable isomers during each round of the selection process. Next, we investigated whether azo-ATP can serve as a substrate for T7 RNA polymerase instead of natural ATP. As shown in Figure S2, azo-ATP can be efficiently incorporated into RNA by T7 RNA polymerase, although transcriptional efficiency was lower for azo-ATP than for natural ATP. It was also confirmed that in the absence of azo-ATP or ATP, transcription stopped at position A (see Fig. S2), resulting in a truncated RNA. In addition, it was verified that the azobenzene-tethered RNA can serve as a template for reverse-transcription.[13] We prepared an azobenzene-tethered RNA library by transcribing the dsDNA library using azo-ATP instead of natural ATP, which was followed by gel purification (Fig. 2a). The absorption band at 300–400 nm indicated that azo-ATP was incorporated into the RNA (Fig. 2b). The apparent content of azobenzene incorporated into the RNA was defined as the ratio of the absorbance at 335 nm (absorption of the *trans*-form of azobenzene) over the absorbance at 260 nm (absorption of the RNA). The content of azobenzene in the first-round RNA library was about 18%. The RNA library also exhibited photoresponsive properties, which depended on the wavelength of the light (inset of Fig. 2b). Finally, the maintenance of the sequence during transcription and reverse-transcription reactions was confirmed using azo-ATP. An oligoDNA was transcribed in the presence of azo-ATP and the transcribed moiety (RNA) containing azobenzene residues was reverse-transcribed to oligoDNA. The final sequence of oligoDNA was the same as that of the original (Fig. S3).

After confirmation of these fundamental conditions, in vitro selection was performed using azo-ATP according to Scheme 1. An enhancement of the binding percentage was observed after five rounds of selection, and the azobenzene content of the RNA library became stable (Table S1). Therefore, the bound RNAs in the sixth and eighth rounds were amplified by RT–PCR, and the resulting dsDNA libraries were cloned and sequenced, respectively. In contrast to the sixth round, only two kinds of full-length sequence (termed A8-6 and A8-10 RNA, see Table S2) were obtained after eight rounds of selection.[14]

Therefore, we investigated the binding properties and photoresponsiveness of the A8-6 and A8-10 RNA molecules via the analysis of spectral changes of the Soret band of hemin (Fig. 3).[15] The black lines show the Soret absorption spectra induced by hemin alone. The green lines show the Soret absorption spectra when hemin was added to the solution of RNA after visible light irradiation. Subsequently, we irradiated the solutions containing RNA and hemin with UV light. The Soret absorption spectra after UV irradiation are shown as purple lines. In the presence of RNA, the absorption of the Soret bands increased after visible light irradiation compared with the case of hemin alone. This hyperchromicity suggests that both the A8-6 and A8-10 RNA molecules bound hemin,[12,16] as hyperchromicity of the Soret band is an indicator of the hydrophobicity of the hemin environment.[17] Interestingly, UV irradiation of the A8-6 RNA led to a decrease in absorption (purple line in Fig. 3a). This suggested that hemin was released from the A8-6 RNA. However, in the case of A8-10 RNA, no significant decrease in absorption was observed after UV irradiation

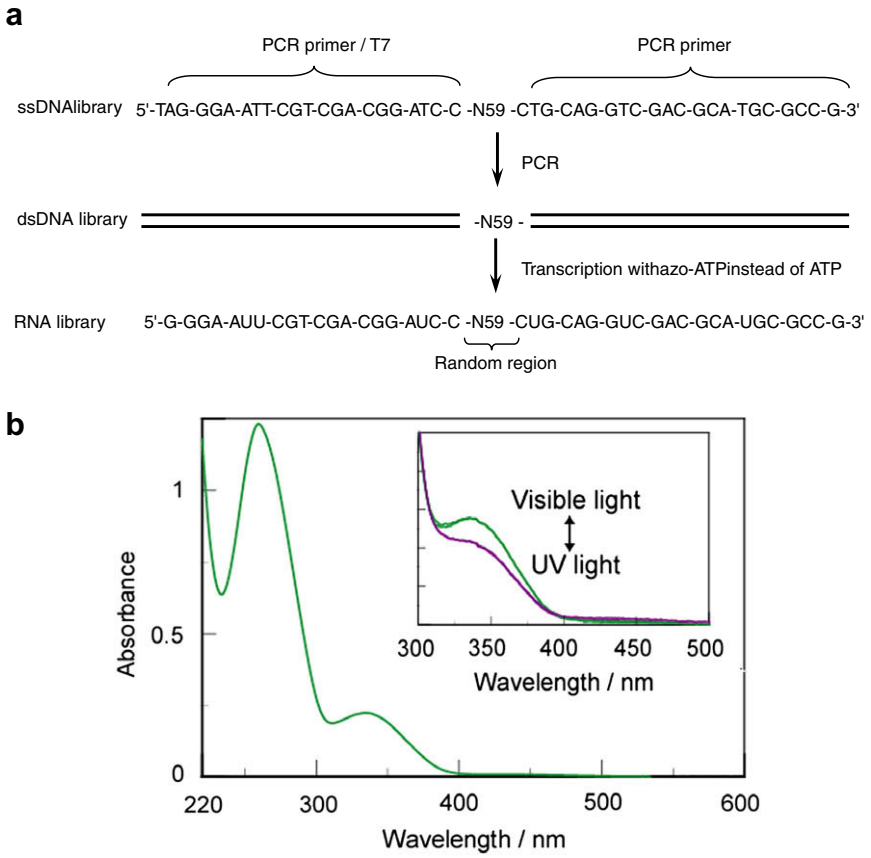

Figure 2. (a) Schematic illustration of the preparation of the azobenzene-tethered RNA library. (b) UV–vis spectrum of the first-round RNA library after visible light irradiation. Inset, spectral changes of the RNA library after visible light or UV irradiation.

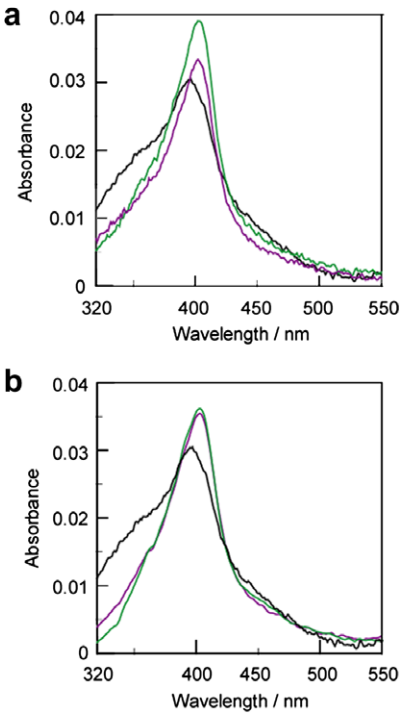

Figure 3. Evaluation of the photoresponsiveness of the A8-6 (a) and A8-10 (b) RNA aptamers via the analysis of spectral changes at the Soret band of hemin. After visible light irradiation, hemin was added to the solution of the RNA aptamer and incubated for 30 min at room temperature (green lines). Subsequently, the solution was UV irradiated for 3 min (purple lines). Black lines, hemin alone.[15]

(purple line in Fig. 3b). Therefore, the A8-6 RNA aptamer was photoresponsive against hemin. The A8-6 RNA aptamer binds hemin after visible light irradiation and releases hemin after UV irradiation.

We investigated the photoresponsiveness of the A8-6 RNA aptamer in another experiment. The peroxidase activity of the A8-6 RNA aptamer–hemin complex was investigated after visible and UV light irradiation (Fig. 4). Peroxidase activity was evaluated according to the rate of ABTS oxidation as reported previously.[12b]

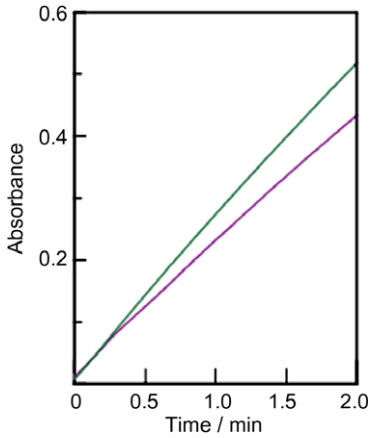

Figure 4. Peroxidase activity of the A8-6 RNA aptamer–hemin complex under visible light (green line) and UV light irradiation (purple line) conditions. H_2O_2 and ABTS were added to the solution containing the A8-6 RNA aptamer and hemin. RNA, 0.25 μM; hemin, 0.25 μM; ABTS, 2.5 mM; H_2O_2, 0.75 mM.

As shown in Figure 4, the A8-6 RNA aptamer–hemin complex exhibited peroxidase activity after visible and UV light irradiation. However, the rate of ABTS oxidation after visible light irradiation (green line in Fig. 4) was faster than that after UV light irradiation (purple line in Fig. 4). Because the aptamer–hemin complex is the active catalyst in the peroxidase reaction, this difference in enzyme activity indicates that the amount of aptamer–hemin complex present was greater after visible light irradiation than after UV light irradiation. The result demonstrated that the A8-6 RNA aptamer exhibited photoresponsiveness to hemin.

In conclusion, we successfully constructed a photoresponsive RNA library that tethered azobenzene moieties randomly. A photoresponsive RNA aptamer against hemin was isolated using in vitro selection combined with photomanipulation. This is the first example of the introduction of an aptamer into the photocontrol of dynamic molecular recognition. The application of the strategy proposed in this study renders in vitro selection of photoresponsive host molecules against various guests promising.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.109.

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13. When RT–PCR was performed with azobenzene-tethered RNA, full-length dsDNA was produced (also see Fig. S3).
14. Truncated fragments were observed in other clones.
15. In Figure 3, the absorption derived from azobenzene, which was tethered to the RNA aptamer, was subtracted from the azobenzene-tethered RNA aptamer in the absence of hemin (see Supplementary data: interaction between hemin and RNA aptamer).
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