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Photoregulation of RNA Digestion by RNase H with Azobenzene-Tethered DNA

Daijiro Matsunaga,[‡] Hiroyuki Asanuma,*[,‡,†] and Makoto Komiyama[‡]

Research Center for Advance Science and Technology, The University of Tokyo, 4-6-1, Komaba, Meguro-ku, Tokyo 153-8904, Japan, and Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency (JST), Kawaguchi 332-0012, Japan

Received May 13, 2004; E-mail: asanuma@mkomi.rcast.u-tokyo.ac.jp

Modification of DNA is one of the growing fields in both synthetic and bio-oriented chemistries because of its potential application to gene therapy, biotechnology, and nanotechnology.[1] Previously, we have synthesized various photoresponsive DNAs tethering azobenzene or spiropyran, for the photoregulation of DNA functions.[2,3] With these modified DNAs, formation and dissociation of the duplex or triplex is efficiently photoregulated by irradiating with UV or visible light: planar *trans*-azobenzene (visible light irradiation) stabilizes the duplex while nonplanar *cis*-azobenzene (UV light irradiation) destabilizes the duplex.[2a,d]

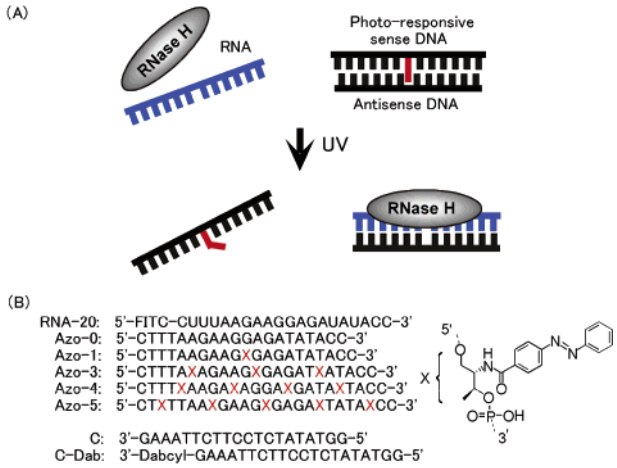
One of our purposes with the modified DNAs is artificial regulation of gene expression by photostimulation. For the regulation of gene expression, an antisense strategy based on the hybridization of target m-RNA with complementary DNA (antisense DNA) has been widely adopted.[4] According to Chiang,[5] the antisense effect involves scission of m-RNA hybridized with antisense DNA by RNase H, as well as interruption of transcription at the ribosome. Therefore, if the RNase H is efficiently photoregulated, photoregulation of gene expression based on the antisense strategy is expected. In the present paper, azobenzene-tethered DNA is applied to the photoregulation of RNase H activity by use of a model reaction system.

RNase H hydrolyzes RNA only when it is hybridized to the complementary DNA. Our strategy for the photoregulation of this enzyme is illustrated in Scheme 1A. The system is composed of RNA (substrate), antisense DNA without azobenzene, and corresponding sense DNA incorporating azobenzene moieties. When azobenzenes in the sense DNA adopt the trans form, it strongly hybridizes to the antisense DNA, and thus RNase H does not digest RNA (upper in Scheme 1A). UV light irradiation isomerizes azobenzene moieties from the trans to cis form, and sense DNA is released due to the instability of the sense/antisense duplex. The released antisense DNA is hybridized to RNA, which should be hydrolyzed by RNase H (lower in Scheme 1A). The actual model reaction system used here is composed of 20-mer RNA attached to fluorescein-4-isothiocyanate (FITC) at the 5′ terminal (**RNA-20** as substrate), 20-mer native antisense DNA (**C**), and 20-mer native (**Azo-0**) or modified DNA (**Azo-n**, **n** = 1−5) as listed in Scheme 1B.[6]

RNA digestion by RNase H did not proceed at all without antisense DNA, whereas the presence of antisense DNA **C** efficiently promoted digestion of the substrate. Although weak scission by RNase H was observed, RNA digestion was fairly suppressed when native (**Azo-0**) or azobenzene-tethered DNA (**Azo-3**) was hybridized with antisense DNA **C** in the reaction mixture in the dark (see lanes 1 and 3 in Figure 1, compare blue bars of **Azo-0** and **Azo-3** with that of **C** only in Figure 2).[7] On UV irradiation of the reaction mixture, however, RNA was efficiently digested when azobenzene-tethered DNA was used as the sense strand:[8] the amount of digested RNA after UV irradiation for the **Azo-3/C** system was more than 3-fold larger than in the dark. As expected, no acceleration by UV irradiation was observed for **Azo-0/C** without azobenzenes (compare lane 2 with 4 in Figure 1). Thus, photoregulation of RNase H reaction was attained with azobenzene-tethered DNA. With this system, off-to-on switching of RNA digestion by UV light irradiation was also attained (see Figure 1 in the Supporting Information).

The photoregulating ability depended on the number of azobenzene moieties in the sense DNA. With any **Azo-n**/**C** combination,

Scheme 1. Illustration of the Strategy for the Photoregulation of RNase H (A), and Sequences of DNA and RNA Used in This Study (B)



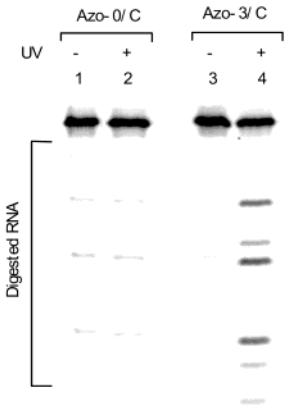


Figure 1. PAGE analysis of the digestion of **RNA-20** by RNase H in the presence of **Azo-n/C** at 37 °C for 15 min. Lane 1, **Azo-0/C** in the dark; lane 2, **Azo-0/C** after UV irradiation; lane 3, **Azo-3/C** in the dark; lane 4, **Azo-3/C** after UV irradiation.

[‡] The University of Tokyo.
[†] PRESTO, JST.

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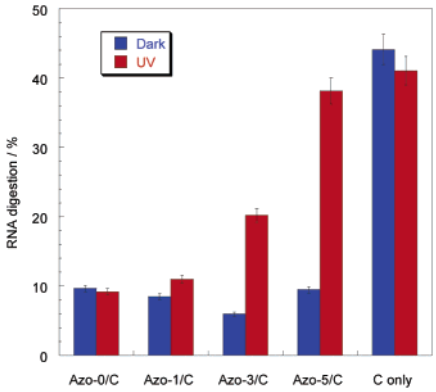

Figure 2. Effect of the number of azobenzene moieties on the RNA digestion at 37 °C for 15 min in the dark (blue bars) or after UV light irradiation (red bars) in the presence of **Azo-n/C** duplex or antisense **C** strand only.

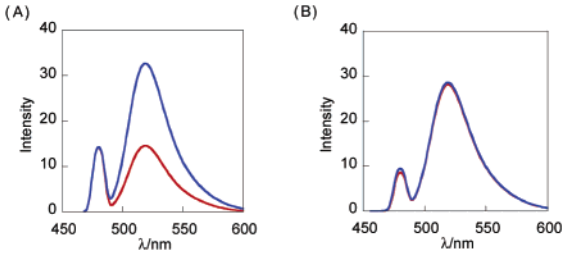

Figure 3. Change of the intensity of fluorescence at 37 °C from FITC on RNA-20 before (blue lines) and after (red lines) 20 min UV light irradiation for **Azo-4/C-Dab** (A) and **Azo-0/C-Dab** (B) systems.

digestion of RNA was suppressed in the dark, as shown by the blue bars in Figure 2. This is due to the strong hybridization of **Azo-n** with **C** when azobenzenes take the trans form: the melting temperatures of **Azo-1/C**, **Azo-3/C**, and **Azo-5/C** duplexes in the trans form were 58.5, 61.1, and 60.8 °C, respectively, with the same buffer as for RNA digestion experiments.[9] Introduction of *trans*-azobenzenes did not interfere with duplex formation.[10] In contrast, the RNA digestion rate increased with the number of azobenzenes when UV light was irradiated, as shown by the red bars in Figure 2.[11,12] This result is based on the fact that the duplex became unstable in the cis form. Melting temperature uniformly decreased with the number of azobenzenes when they took cis form: T_ms of **Azo-1/C**, **Azo-3/C**, and **Azo-5/C** duplexes were 54.6, 49.2, and 42.6 °C, respectively. Thus, the RNA digestion rate strongly correlated with the melting temperatures, indicating that **C** strand released from **Azo-n** by UV irradiation hybridizes with **RNA-20**, which is thus digested by RNase H. Hybridization of **C** with **RNA-20** by UV light irradiation was also substantiated from the following fluorescent experiment with **RNA-20**, **Azo-4**, and **C-Dab** involving 4-[4-dimethylaminophenylazo]benzoic acid (Dabcyl) chromophore as a fluorescence quencher at the 3′ terminal.[13] Before UV irradiation, strong fluorescence from FITC was observed at 37 °C where **RNA-20** was isolated due to the hybridization of **C-Dab** with **Azo-4**. UV irradiation of this solution lowered the fluorescence (compare red and blue lines in Figure 3A) because **C-Dab** released by trans-to-cis isomerization hybridized with **RNA-20**, and the Dabcyl on **C-Dab** quenched the fluorescence. As expected, no quenching by UV irradiation was observed when **Azo-0** without azobenzenes was used in place of **Azo-4** (Figure 3B).

In conclusion, photoregulation of RNase H activity was achieved with azobenzene-tethered DNA. Photoregulating efficiency increased with the number of incorporated azobenzenes. By use of this system, photoregulation of gene expression based on the antisense strategy is predicted.

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Supporting Information Available: UV light-triggered acceleration of RNA digestion with **Azo-3/C** system. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Charache, S. C.; Terrin, M. L.; Moore, R. D.; Dover, G. J.; Barton, F. B.; Eckert, S. V.; McMahon, R. P.; Bonds, D. R. *New Engl. J. Med.* **1995**, *332*, 1317. (b) Milligan, J.; Matteucci, M. D.; Matin, J. C. *J. Med. Chem.* ***1993***, *36*, 1923. (c) Matteucci, M. D.; Wagner, R. W. *Nature* **1996**, *384*, 2103. (d) Glazer, V. *Genet. Eng. News* **1996**, *16*, 16, 27. (e) Ando, H.; Furuta, T.; Tsien, R. Y.; Okamoto, H. *Nature Genet.* **2001**, *28*, 317. (f) Murakami, A.; Yamamoto, Y.; Namba, M.; Iwase, R.; Yamaoka, T. *Bioorg. Chem.* **2001**, *29*, 223.

(2) (a) Asanuma, H.; Takarada, T.; Yoshida, T.; Tamaru, D.; Liang, X.; Komiyama, M. *Angew. Chem., Int. Ed.* **2001**, *40*, 2671−2673. (b) Asanuma, H.; Tamaru D.; Yamazawa, A.; Liu, M. Z.; Komiyama, M. *ChemBioChem.* **2002**, *3*, 786−789. (c) Liang, X.; Asanuma, H.; Komiyama, M. *J. Am. Chem. Soc.* **2002**, *124*, 1877−1883. (d) Liang, X.; Asanuma, H.; Kashida, H.; Takasu, A.; Sakamoto, T.; Kawai, G.; Komiyama, M. *J. Am. Chem. Soc.* **2003**, *125*, 16408−16415.

(3) Asanuma, H.; Shirasuka, K.; Yoshida, T.; Takarada, T.; Liang, X.; Komiyama, M. *Chem. Lett.* **2001**, 108−109.

(4) Ittig, D.; Liu, S. K.; Renneberg, D.; Schumperli, D.; Leumann, C. J. *Nucleic Acids Res.* **2004**, *32*, 346−353.

(5) Chiang, M. Y.; Chan, H.; Zounes, M. A.; Freier, S. M.; Lima, W. F.; Bennett, C. F. *J. Biol. Chem.* **1991**, *266*, 18162−18171.

(6) The actual procedure of the reaction is as follows: first, a mixture of sense and antisense DNAs was annealed in buffer solution (10 mM Tris-HCl buffer (pH 8.0), 10 mM $MgCl_2$, 50 mM NaCl, 1 mM 2-mercaptoethanol, 50 μg/mL BSA) by heating at 90 °C for 5 min and cooling to 37 °C for 30 min. After this annealing procedure, UV light was irradiated at 37 °C for 30 min from a 6-W UV-A fluorescent lamp (FL6BL-A, Toshiba) through a UV-D36C filter (from Asahi Technoglass). The intensity of UV light was below 100 μW cm^{-2}. FITC-labeled substrate RNA (**RNA-20**) and 3 U of RNase H1 (Takara Bio Inc.; one U of RNase H in 15 μL corresponds to 16 nM) were then added to this solution, and the resulting reaction mixture was incubated at 37 °C. The total volume of the reaction mixture was 15 μL, and final concentrations of [**Azo-n**], [**C**], and [**RNA-20**] were 0.1, 0.01, and 5 μM, respectively. After 15 min, 5 μL of the reaction mixture was sampled and added to 5 μL of loading buffer containing EDTA (50 mM EDTA, 45 mM Tris-HCl, 45 mM borate, 7 M urea) to terminate the reaction. Finally, 5 μL of the resulting mixture was subjected to electrophoresis on 20% polyacrylamide gel containing 7 M urea. Imaging and quantification of the digested RNA was carried out on a Fuji film FLA-3000G fluorescent analyzer.

(7) Weak scission of RNA was probably based on the gradual strand-exchange of substrate RNA with sense DNA that was on antisense DNA.

(8) More than 80% of the substrate was digested after 60 min of incubation.

(9) For the T_m measurements, the concentration of each DNA was 2 μM. The same buffer solution as that for RNA digestion (except for the absence of BSA) was applied to the T_m measurement.

(10) Note that T_m of **Azo-0/C** duplex was 57.1 °C under the conditions employed.

(11) Amount of digested RNA for **Azo-5/C** after UV irradiation was almost the same as that of **C** only, indicating that acceleration of digestion with *cis*-azobenzenes became saturated under the reaction conditions employed.

(12) Kinetic parameters, K_m and k_{cat}, of the present reaction system under the conditions described in ref 6 were determined as 9.4 nM and 0.13 s^{-1}, respectively, from Lineweaver−Burk plots.

(13) A mixture of **Azo-4** and **C-Dab** was annealed in the buffer solution (10 mM Tris-HCl buffer (pH 8.0), 10 mM $MgCl_2$, 50 mM, NaCl) by heating to 90 °C for 5 min and cooling to 37 °C for 30 min. The resulting duplex solution was then added to the **RNA-20** solution (same buffer solution was used) in the UV cell. Final concentrations of **Azo-4**, **C-Dab**, and **RNA-20** were 113, 75, and 75 nM, respectively. Fluorescence measurements were carried out at 37 °C before and after UV irradiation for 20 min at 37 °C.

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