Synergistic Effect of the Two Azobenzenes in the Promoter on the Photo-regulation of Transcription Reaction with SP6 RNA Polymerase

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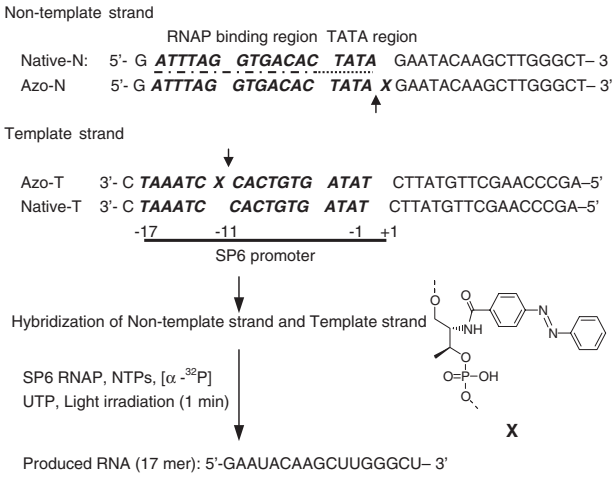
By introducing two azobenzene moieties into both RNA polymerase binding region and TATA region in the promoter of the SP6 RNA polymerase simultaneously, clear-cut photo-regulation of the transcription reaction was achieved either under dark or by UV irradiation.

Artificial regulation of gene expression is one of the current important and attractive themes for post-genome era.[1] Recently, much attention has been focused on the artificial regulation of gene-expression because of its potential applications to cell biology as well as pharmacology.[2] They can also provide new useful tools for investigating the mechanisms underlying DNA recognition and bioreactions.

Previously, we have synthesized photo-responsive DNAs carrying azobenzene moieties in the side chain for the photo-regulation of gene-expression. With these modified DNAs, formation and dissociation of its duplex and triplex were efficiently photo-regulated.[3] We also demonstrated that introduction of an azobenzene moiety into the appropriate region of the promoter is effective for the photo-regulation of transcription reaction by T7 RNA polymerase (RNAP):[4] transcription was fairly suppressed when an azobenzene took *trans*-form, whereas it proceeded efficiently on its isomerization from *trans*- to *cis*-form. This successful photo-regulation with T7 RNAP prompted us to investigate whether the present strategy for the photo-regulation, based on the modification of the promoter, is applicable to another RNAP or not. For this purpose, we use SP6 RNAP instead of T7 RNAP for the photo-regulation of transcription reaction. It is demonstrated that transcription by SP6 RNAP is also photo-regulated by the azobenzene-tethered SP6 promoter. Furthermore, still more effective photo-regulation is achieved by introducing two azobenzene moieties into the specific positions of the promoter.

SP6 RNAP belongs to a member of single subunit DNA-dependent phage RNAPs as T7 RNAP does, while both RNAPs have different promoter sequences with each other. As shown with the italic letters in Scheme 1, the SP6 promoter consists of 17 base pairs and involves two different regions: one is RNAP binding region (from −17 to −5) and the other is TATA region (from −4 to −1). All the DNAs used for the transcription reaction are listed in Scheme 1. Azobenzene-modified promoters were prepared from the corresponding phosphoramidite monomer by use of D-threoninol as a linker in a similar manner as reported previously.[3b] Transcription reaction was carried out according to the literature.[5,6]

When a native DNA duplex, **Native-N/Native-T**, was used as a promoter, full-length ^{32}P-labeled transcript (17 mer RNA) was efficiently produced either under dark or after UV irradiation for 1 min. The transcription rate on UV irradiation



Scheme 1. Sequences of SP6 promoter used in this study, and experimental procedure of transcription by SP6 RNAP.

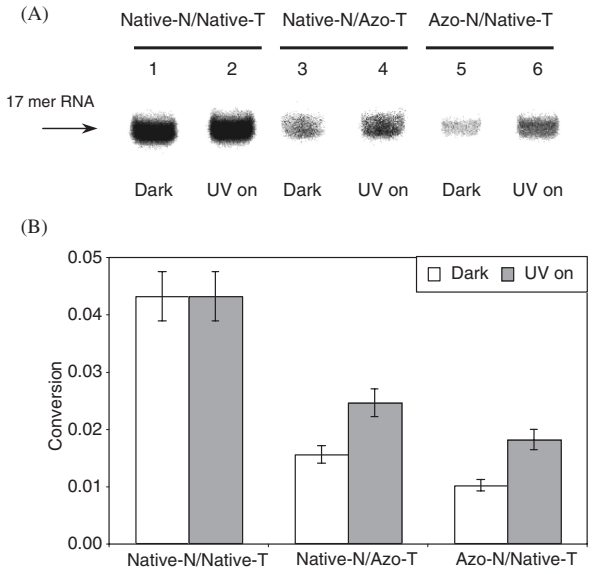


Figure 1. Photo-regulation of transcription reaction with various SP6 promoters at 37 °C under dark and after 1 min UV irradiation. Transcription reaction was carried out for 2 h. (A) PAGE patterns for the in vitro transcription products of 17 mer RNA. Lane 1, 2: **Native-N/Native-T**, lane 3, 4: **Native-N/Azo-T**, lane 5, 6: **Azo-N/Native-T**. (B) Quantitative plots of the results shown in the Figure 1A. Conversion is defined as the ratio (radioactivity of full-length product)/(total radioactivity of all the oligo- and mononucleotides in the gel). White bars: under dark conditions; gray bars: after 1 min UV irradiation.

(300 nm $< \lambda <$ 400 nm) was the same as that under dark (compare lane 1 with lane 2 in Figure 1A). On the contrary, when **Native-N/Azo-T** duplex involving one azobenzene moiety at RNAP binding region was used as a promoter, photo-irradiation affected the transcription rate. Transcription was fairly suppressed under dark and was accelerated by UV irradiation (compare lane 3 with lane 4 in Figure 1A).[7] As a result, the ratio of the amount of transcript prepared after UV irradiation to that obtained under dark was 1.5. Similar results were also obtained when an azobenzene was introduced into another RNAP binding region (data not shown).[8] These results coincide with the T7 RNAP case,[4] indicating that *trans*-azobenzene tends to suppress the transcription reaction by RNAP compared with *cis*-azobenzene. Suppression of the transcription by *trans*-azobenzene was also observed when an azobenzene was introduced to TATA region (compare lane 5 with lane 6 in Figure 1A). With **Azo-N/Native-T** combination, the ratio of transcript after UV irradiation to that under dark was 1.8.

Although photo-regulation of transcription was possible with SP6 RNAP, difference of the transcription rate between *trans*- and *cis*-form was below 2.0 when only one azobenzene moiety was introduced to the promoter. In order to achieve effective on-off switching by light irradiation, still larger change of transcription rate by light irradiation should be desirable. For this purpose, two azobenzene moieties are introduced into the SP6 promoter. When **Azo-N** and **Azo-T** (each of them involves one azobenzene) were combined, distinct synergistic effect of photo-regulation was observed. As shown in Figure 2, transcription was strongly interfered under dark (lanes 1–4 in Figure 2A). In contrast, transcription efficiently proceeded when UV light was irradiated to this reaction mixture for 1 min prior to the incubation (lanes 5–8 in Figure 2A). The ratio of transcript after UV irradiation to that under dark was 3.0 at 2 h and it became much larger when the incubation was continued (see Figure 2B).[9] It should be noted that the difference of transcription between UV irradiation and under dark condition with **Azo-N/Native-T** or **Native-N/Azo-T** combination was below 2.0 (see Figure 1). This fact indicates that incorporation of multiple azobenzenes is one of the promising strategies to raise the difference.

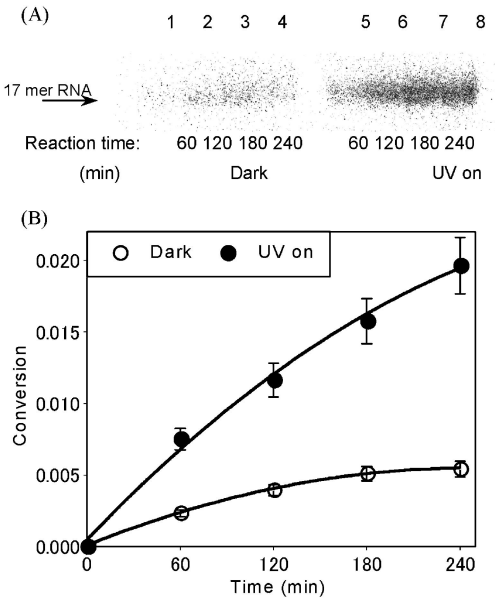


Figure 2. Time course of transcription reaction at 37 °C under dark and after UV irradiation with SP6 promoter by the combination of **Azo-N** and **Azo-T**. (A) PAGE patterns of the full-length product. Lanes 1–4: t = 60, 120, 180, 240 min under dark. Lanes 5–8: t = 60, 120, 180, 240 min after UV irradiation. (B) Quantitative plots of the results shown in the Figure 2a. Closed circles: after 1 min UV irradiation; open circles: under dark conditions.

In conclusion, transcription reaction by SP6 RNAP is photo-regulated especially with modified promoter involving two azobenzenes at both TATA and RNAP binding regions. With this photo-responsive promoter, photo-regulation of *in vitro* translation is also expected.

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6 Promoters (non-template DNA and template DNA as shown in Scheme 1) were annealed in 10 mM Tris-HCl buffer (pH 7.5) containing 10 mM NaCl by heating at 95 °C for 3 min and cooling to 37 °C for 30 min. Transcription was carried out in 20 μL of 40 mM Tris-HCl buffer (pH 7.5) with 22 mM $MgCl_2$, 2 mM spermidine, 5 mM DTT, 0.01% BSA, 0.01% Triton X-100, 0.5 mM each NTP, 2 μCi of [α-^{32}P] UTP, 1 μM promoter DNA, and 60 units of SP6 RNAP (Takara Shuzo, Kyoto). After incubation at 37 °C, the reaction was quenched by addition of 20 μL bromophenol blue solution, and the reaction products were identified by 20% polyacrylamide/7 M urea gel electrophoresis (PAGE). All the experiments were carried out at least three times, and the error was within 10%.

7 UV light from 6-W UV-A fluorescent lamp (FL6BL-A, Toshiba) was irradiated through a UV-D36C filter (from Asahi Technoglass) for 1 min to isomerize *trans*-azobenzene into *cis*-form just before starting the incubation. By this treatment, about 20–30% of the *trans*-azobenzene was isomerized. DNA was not damaged under the present conditions of UV irradiation on the basis of the HPLC analysis.

8 Transcription rate significantly depends on the position of X residue in the promoter.

9 Amount of transcript at 120 min after 1 min UV irradiation was about 40% with respect to that with **Native-N/Native-T** system.

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