[18] M. Contel, J. Jiménez, P. G. Jones, A. Laguna, M. Laguna, *J. Chem. Soc. Dalton Trans.* **1994**, 2515.
[19] M. Contel, J. Garrido, M. C. Gimeno, P. G. Jones, A. Laguna, M. Laguna, *Organometallics* **1996**, *15*, 4939.
[20] A. N. Nesmenayov, E. G. Peralova, K. I. Grandberg, D. A. Lemenovskii, T. V. Baukova, U. B. Afanassova, *J. Organomet. Chem.* **1974**, *65*, 131.
[21] R. H. Blessing, *Acta Crystallogr. Sect. A* **1995**, *51*, 33; R. H. Blessing, *J. Appl Crystallogr.* **1997**, *30*, 467.

Photoregulation of the DNA Polymerase Reaction by Oligonucleotides Bearing an Azobenzene**

Akira Yamazawa, Xingguo Liang, Hiroyuki Asanuma, and Makoto Komiyama*

Regulation of bioreactions by external stimuli has been one of the most attractive and urgent themes of recent research.[1] The activities of enzymes have been photomodulated by attaching azobenzene and spiropyran near to their active centers.[2, 3] Furthermore, growth and differentiation of cells have been controlled by applying electric voltage.[4] However, little has been known about artificial control of biosynthesis of DNA. Here we report the first photoregulation of a template-dependent DNA polymerase reaction. By using photoresponsive oligonucleotides as modulators and irradiating with appropriate light, DNA of predetermined length is selectively produced from one template DNA.

The oligonucleotides used here are presented in Figure 1. The modulator **1b** bears an azobenzene for photoinduced *cis–trans* isomerization.[5] Its 3′ terminus is protected by a 3-hydroxypropyl residue to avoid DNA elongation from that point. The primer DNA **3** (18-mer) is labeled with fluorescein isothiocyanate (FITC) at the 5′ end. The template DNA **4** is a 54-mer. When DNA polymerization with T7 DNA polymerase was achieved at pH 7.5 and 34 °C in the dark in the presence of **1b**, only 34-mer DNA was produced (lane 3 in Figure 2). Apparently, the DNA elongation was blocked by **1b**, and stopped at its 5′ terminus. Under irradiation with UV light ($300 < \lambda < 400$ nm), however, the 34-mer DNA was not formed and instead 54-mer DNA was predominantly produced (lane 4, Figure 2). Depending on whether the light is used or not, either of these two DNAs is selectively obtained from one template.[6] The irradiation causes no significant effects on the enzymatic activity, as confirmed by careful control experiments. When unmodified oligonucleotide (**1a**) was used as the modulator, only the 34-mer DNA was produced either in the dark or under UV irradiation (lanes 1 and 2, Figure 2).

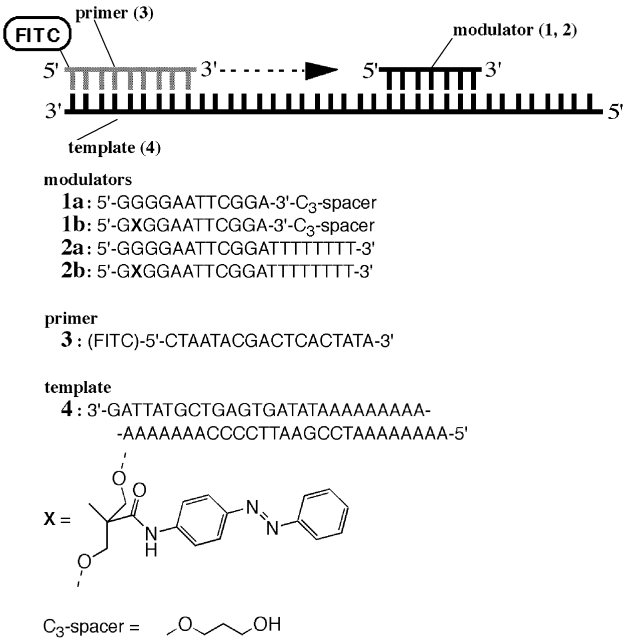

Figure 1. Strategy for the photoregulation of a T7 DNA polymerase reaction. In the modulators, **X** denotes the residue carrying an azobenzene.

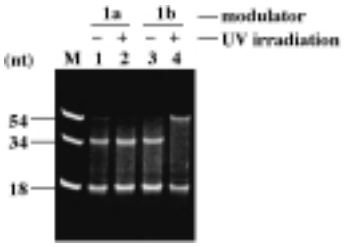

Figure 2. Polyacrylamide gel electrophoresis patterns for the T7 DNA polymerase reactions at pH 7.5 and 34 °C for 20 min. Lane 1: with the unmodified oligonucleotide **1a** in the dark; lane 2: with **1a** under UV irradiation; lane 3: with the modified oligonucleotide **1b** in the dark; lane 4: with **1b** under UV irradiation; lane M: markers (18-mer, 34-mer, and 54-mer).

Before the UV irradiation, the azobenzene in **1b** mostly takes the *trans* form.[5] Here, the melting temperature (T_m) of the duplex between **1b** and **4** is 57 °C (measured at 260 nm under the reaction conditions), and this duplex is almost completely formed at the reaction temperature (34 °C). The *trans*-azobenzene has a planar structure, and is favorably accommodated in the duplex. When the T7 DNA polymerase moves along the template **4** in the 3′→5′ direction during the polymerization, it cannot go through this double-stranded region (This enzyme requires single-stranded DNA as template) and the DNA elongation terminates at the 5′ end of **1b**. Under UV irradiation, the azobenzene in **1b** is isomerized to the *cis* form. This isomer is nonplanar so that its steric repulsion against **4** weakens the binding of **1b** to **4**. This effect

[*] Prof. Dr. M. Komiyama, Dr. A. Yamazawa, X. Liang, Dr. H. Asanuma
Research Center for Advanced Science and Technology
The University of Tokyo
4-6-1 Komaba, Meguro-ku, Tokyo, 153-8904 (Japan)
Fax: (+81) 3-5452-5209
E-mail: komiyama@mkomi.rcast.u-tokyo.ac.jp

[**] We should like to thank Professor Kazunari Taira for valuable comments. This work was partially supported by the Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan (Molecular Synchronization for Design of New Materials System). The support of the grant from the "Research for the Future" Program of the Japan Society for the Promotion of Science (JSPS-RFTF97I00301) is also acknowledged.

Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/angewandte/ or from the author.

  1433-7851/00/3913-2356 $ 17.50+.50/0

is especially notable near the 5′ end of **1b**, where the *cis*-azobenzene is covalently bound. Thus, this section is competitively excluded from **4** by the T7 DNA polymerase, when this enzyme comes to the 5′ end of **1b** in the polymerization. Accordingly, the polymerase reaction proceeds further, and provides the full-length DNA (the proposed mechanism is schematically presented in the Supporting Information).[7] Note that T7 DNA polymerase has no 5′→3′ exonuclease activity,[8] and does not cleave **1b** for the complete elongation.

These arguments are supported by the results shown in Figure 3. Here, a modified 20-mer oligonucleotide (**2b**), which covers the template **4** down to its 5′ end, was used as the modulator. This longer modulator forms a more stable duplex

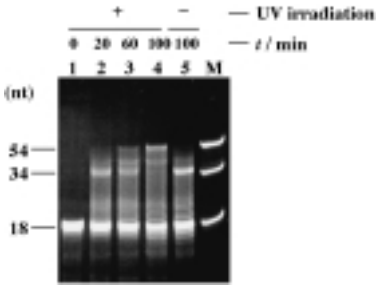

Figure 3. Time-course of the T7 DNA polymerase reaction under UV irradiation in the presence of modulator **2b**. Lane 1: $t = 0$; lane 2: $t = 20$; lane 3: $t = 60$; lane 4: $t = 100$ min. Lane 5 shows the result of the reaction in the dark after 100 min.

with **4** than **1b** does. Thus, the amount of **4** which is free from complex formation with **2b** is virtually nil, even when its azobenzene takes the *cis* form. Yet, the 54-mer DNA is efficiently formed under UV irradiation (lane 4, Figure 3). Evidently, the modulator involving the *cis*-azobenzene is excluded from the template by the enzyme during the polymerization. The breakdown of the stable **2b**–**4** duplex requires considerable energy, so that the elongation is notably retarded at the 5′ end of **2b**. As a result, the 34-mer DNA is somewhat accumulated in the early stages of the reaction (lanes 2 and 3, Figure 3). In dark conditions, however, the 34-mer DNA is the dominant product, even with long reaction times (lane 5, Figure 3).[9]

The present photoregulation is never based on the change of the amount of free **4** which is available as the template. Although the T_m of the duplex between *cis* isomer **1b** and **4** is lower than that of the *trans* isomer,[5] it is still much higher than the reaction temperature, and the duplex is dominantly formed under these conditions.[10] In conclusion, a T7 DNA polymerase reaction has been successfully photoregulated with photoresponsive oligonucleotides as modulators. The application of this finding to various purposes is currently under way.

Experimental Section

The modulators **1b** and **2b** were prepared on an automated synthesizer, with the phosphoramidite monomer carrying an azobenzene.[11] For the synthesis of **1a** and **1b**, 3′ spacer C3 CPG columns from the Glen Research Co. (Virginia) were employed. The diastereomers, with respect to the chirality of the X residue, were completely separated by reverse phase HPLC, and the fraction with the shorter retention time was used for the experiments described in the present paper. The other diastereomer gave almost the same results (see Figure 4 in the Supporting Information).

Reaction conditions for the T7 DNA polymerase reactions: The template (**4**; 3.2 μM), the primer (**3**; 3.2 μM), the modulator (**1a**, **1b**, **2a**, or **2b**; 4.0 μM), each dNTP (200 μM) and NaCl (150 mM) were incubated in Tris-HCl buffer (40 mM, pH 7.5) containing dithiothreitol and $MgCl_2$ (1 and 10 mM, respectively) at 34 °C for 30 min. The mixture was cooled in ice, and then the T7 DNA polymerase (Pharmacia; 1 unit in 25 μL), was added. At the same time, irradiation was started. The reaction was continued at 34 °C for a predetermined period of time, and stopped by addition of 80/20 formamide/water mixture (in the same volume as the reaction mixture). The mixture was denatured at 95 °C for 5 min, and subjected to electrophoresis on 20 % polyacrylamide gel containing 7 M urea. The pattern was visualized by fluorescence from the FITC on a UV illuminator.

For the *trans*→*cis* photoisomerization, UV light from 6 W UV-A fluorescence lamp (FL6BL-A, Toshiba) was shone through a UV-D36C filter (Asahi Technoglass). The intensity of UV light was 0.9 – 1.0 mW cm^{-2}. In order to avoid the thermal reverse isomerization, the irradiation was continued throughout the polymerase reactions. According to the results of reverse phase HPLC,[11] > 90 % of the azobenzene was the *trans* isomer before the UV irradiation, and > 80 % was the *cis* isomer during irradiation.

Received: January 3, 2000 [Z 14499]

[1] I. Willner, *Acc. Chem. Res.* **1997**, *30*, 347.
[2] M. Aizawa, K. Namba, S. Suzuki, *Arch. Biochem. Biophys.* **1977**, *182*, 305.
[3] a) I. Willner, S. Rubin, A. Riklin, *J. Am. Chem. Soc.* **1991**, *113*, 3321; b) T. Hohsaka, K. Kawashima, M. Sisido, *J. Am. Chem. Soc.* **1994**, *116*, 413; c) I. Hamachi, T. Hiraoka, Y. Yamada, S. Shinkai, *Chem. Lett.* **1998**, 537; d) D. G. Weston, J. Kirkham, D. C. Cullen, *Biochim. Biophys. Acta* **1999**, *1428*, 463.
[4] M. Aizawa, S. Koyama, K. Kimura, Y. Yanagida, E. Kobatake, *Denki Kagaku (Electrochemistry)* **1999**, *67*, 118, and references therein.
[5] It was recently reported that the melting temperatures (T_m) of the duplexes between these oligonucleotides and the complementary ones are notably changed on photoirradiation: H. Asanuma, T. Ito, T. Yoshida, X. Liang, M. Komiyama, *Angew. Chem.* **1999**, *111*, 2547; *Angew. Chem. Int. Ed.* **1999**, *38*, 2393. However, the present photoregulation of the polymerase reaction is based on a different chemistry (see text for details).
[6] When another 54-mer DNA was used as the template, the full-length product was produced for 20 min in almost 100 % yield (with respect to the template: see Figure 1 in the Supporting Information). The efficiency of the present photoregulated polymerization considerably depends on the sequences of components. The photoregulation was also successful, when an 89-mer DNA was used as the template.
[7] The proposed "peeling-off mechanism" has been further substantiated by the fact that the azobenzene must be placed near the 5′ end of the modulator in order to accomplish a clear-cut photoregulation. When an azobenzene was introduced near the 3′ terminus of the modulator (5′-GGGGAATTCG**X**A-3′-C_3-spacer), the dominant product was the 34-mer DNA, even with UV irradiation (see Figure 3 in the Supporting Information).
[8] K. Hori, D. F. Mark, C. C. Richardson, *J. Biol. Chem.* **1979**, *254*, 11598.
[9] Consistently, the 34-mer DNA was the dominant product, even under UV irradiation, when the unmodified 20-mer modulator **2a** was used in place of **2b**.
[10] At the reaction temperature, the amount of free **4** should be smaller than 3 %, when the azobenzene in **1b** takes the *cis* form. This value was estimated from the hypochromicity at 260 nm, measured immediately after UV irradiation. Precise determination of the T_m was unsuccessful, since thermal *cis*→*trans* isomerization was too fast at approximate T_m values (> 50 °C).
[11] H. Asanuma, T. Ito, M. Komiyama, *Tetrahedron Lett.* **1998**, *39*, 9015.

  1433-7851/00/3913-2357 $ 17.50+.50/0