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Photo-responsive oligonucleotides carrying azobenzene at the 2′-position of uridine

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Abstract

Azobenzene residue has been tethered to the 2′-position of uridine in oligonucleotides. On photo-irradiation, the melting temperatures between the modified oligonucleotides and their complementary DNA were significantly changed by the *cis–trans* isomerization of the azobenzene.

Keywords: azo compounds; isomerization; phosphoramidites; photochemistry.

Chemical modification of oligonucleotides has been attracting much interest,[1] various types of modified oligonucleotides have been reported so far. However, examples of photo-responsive oligonucleotides are still limited.[2–4] If the duplex and triplex formation can be controlled simply by photo-irradiation without changing pH, ionic strength and so on, various applications in vitro or in vivo are promising possibilities. Previously, azobenzene residues were incorporated into the side chain of oligonucleotides (Fig. 1a).[4] The melting temperatures (T_ms) of their duplexes with the complementary counterparts were successfully regulated by the *cis–trans* isomerization of the azobenzene on photo-irradiation.[5] This finding demonstrated that azobenzene functions as an excellent photo-device in the oligonucleotides. In these systems, however, a trimethylene chain was used instead of the deoxyribose so that the local backbone-structure was considerably different from that in natural DNA. Furthermore, these oligonucleotides inevitably lacked one nucleic acid base. From the viewpoint of applications to photo-control of gene-expression, especially in vivo, the structural change should be minimized.

In the present communication, an azobenzene residue is introduced to the 2′-position of uridine (Fig. 1b). Unlike the previously reported oligonucleotides (Fig. 1a), both the backbone and nucleic acid base remain unchanged. By using these oligonucleotides, the formation of DNA-duplex and its dissociation are regulated by irradiating either visible light or UV light.

The phosphoramidite monomer **3** was synthesized according to Scheme 1. First, the 3′- and 5′-hydroxyl groups, and the N^3-position of uridine were protected with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane and benzoyl chloride, respectively.[6] The obtained product **1** was coupled

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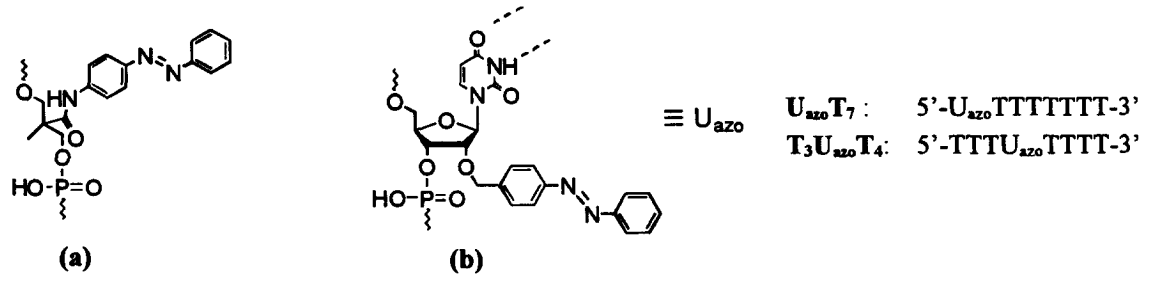
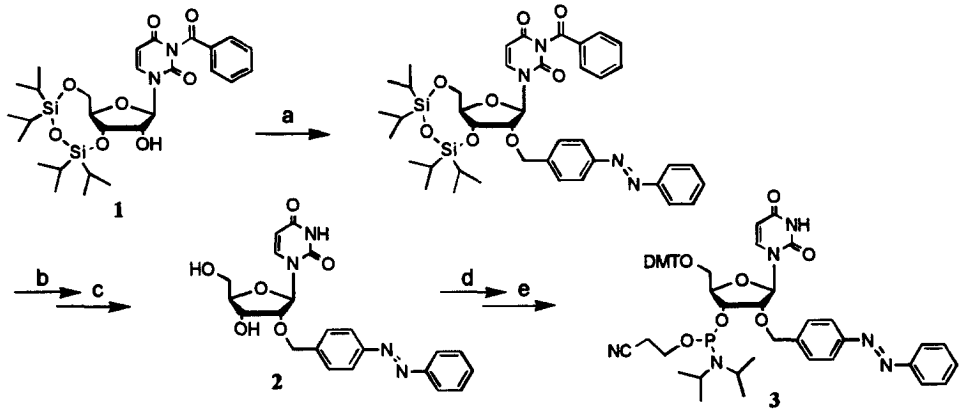


Figure 1. Modified oligonucleotides carrying azobenzene in the side chain synthesized in the previous study (a) and in the present study (b). The sequences used in this study are shown on the right

with 4-bromomethylazobenzene in DMF in the presence of NaH at −20°C for 10 min, followed by quenching with aqueous NH_4Cl solution (step **a** in Scheme 1).[7] The benzoyl group was removed by NH_4OH in dioxane (89%: step **b**). Successive treatment with tetrabutylammonium fluoride (step **c**) in THF afforded the azobenzene-modified uridine (**2**: 100%). The modified uridine was converted to the 5′-protected 3′-phosphoramidite monomer **3** (78%: step **d**; 84%: step **e**) in the usual manner.[8] All the intermediates and the product were purified by silica gel column chromatography, and characterized by NMR spectroscopy.[9] According to the MALDI–TOFMS spectroscopy, molecular weights of all the modified oligonucleotides prepared from **3** fairly coincided with the calculated values.[10] The *trans*- and *cis*-isomers of the oligonucleotides, with respect to the stereochemistry of the azobenzene, were completely resolved by the reversed-phase HPLC.[4,11]



Scheme 1. Synthesis of the phosphoramidite monomer **3**. (**a**) 4-Bromomethylazobenzene, NaH, DMF; (**b**) NH_4OH, dioxane; (**c**) tetrabutylammonium fluoride, THF; (**d**) 4,4′-dimethoxytrityl (DMT) chloride, 4-(dimethylamino)pyridine, pyridine, *N*,*N*-diisopropylethylamine, CH_2Cl_2; (**e**) 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite, 1*H*-tetrazole, CH_3CN

Typical melting curves for the duplex formation between $U_{azo}T_7$ and 5′-A_8-3′ (dA_8) are depicted in Fig. 2.[12] Before photo-irradiation, the azobenzene residue in $U_{azo}T_7$ overwhelmingly takes the *trans*-form (84% as determined by the HPLC). Under these conditions, the T_m of the duplex of *trans*-$U_{azo}T_7/dA_8$ is 30.2°C (the solid line). This value is considerably higher than that of the corresponding natural duplex of 5′-T_8-3′/dA_8 (T_m=23.7°C). The *trans*-azobenzene residue stabilizes the duplex when it is tethered near the terminus. The *trans*-$U_{azo}T_7$ was promptly isomerized to the *cis*-$U_{azo}T_7$ by the irradiation of UV light (300 nm<λ<400 nm).[13] By this treatment, the *cis*:*trans* ratio became 90:10.[14] Quite significantly, the T_m of the duplex with dA_8 was lowered to 21.4°C on this isomerization (the broken line). The decrease in T_m, induced by the *trans*→*cis* isomerization, is 8.8°C. The *cis*-azobenzene was again isomerized to the *trans*-form on irradiating visible light (λ >400 nm). The melting curve of the resultant solution was virtually superimposed on that observed before the first UV irradiation. Thus, the duplex-forming activity

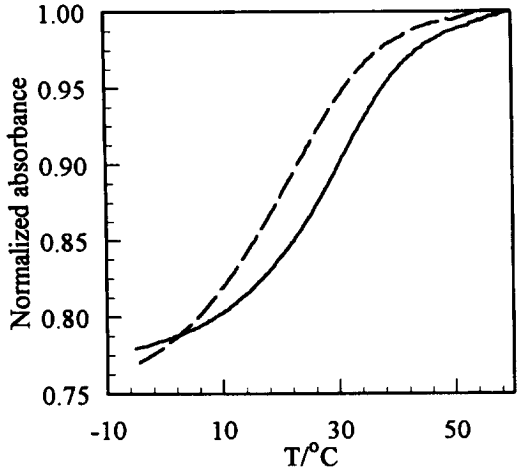


Figure 2. Melting curve of the duplex of either *trans*-$U_{azo}T_7$ (solid line) or *cis*-$U_{azo}T_7$ (broken line) with dA_8

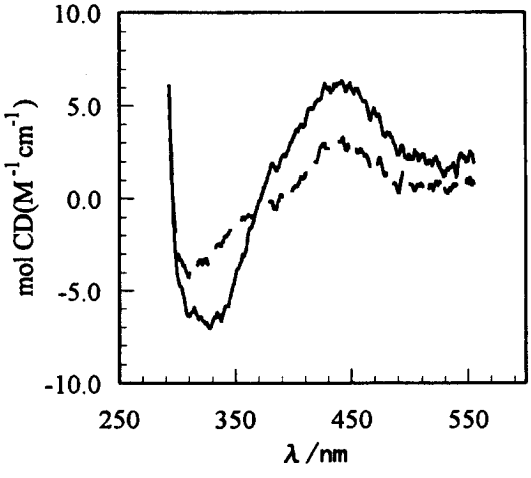


Figure 3. The CD spectra of the *trans*-$U_{azo}T_7/dA_8$ (solid line) and the *cis*-$U_{azo}T_7/dA_8$ (broken line) at 0°C

of the oligonucleotide has been satisfactorily modulated by the *cis–trans* isomerization of the azobenzene tethered to the 2′-position. Photo-regulation of duplex formation is also achieved when the modified uridine is incorporated to the inside of the oligonucleotides: the T_ms of the *trans*-$T_3U_{azo}T_4/dA_8$ duplex and the *cis*-$T_3U_{azo}T_4/dA_8$ duplex are 13.4 and 8.4°C, respectively.[15] It is noteworthy that these duplexes are more stable than the duplexes of the modified oligonucleotides of Fig. 1a prepared previously,[4] mainly because the U-A base-pair is kept intact. The T_m of the *trans*-5′-T_3XT_4-3′/dA_8 duplex (X denotes the residue bearing azobenzene in Fig. 1a) is 9.0°C.

Induced circular dichromism (CD) is observed when the temperature is lower than the T_m for the corresponding duplex. For the *trans*-$U_{azo}T_7/dA_8$ duplex at 0°C, for example, CD is induced negatively at around 330 nm (corresponding to the π–π* transition of azobenzene) and positively at 440 nm (the n–π* transition): see the solid line in Fig. 3. CD is also induced for the *cis*-$U_{azo}T_7/dA_8$ duplex (the broken line in Fig. 3), although its magnitude is smaller than that of the *trans*-$U_{azo}T_7/dA_8$.[16] As expected, no CD is induced at 50°C where most of the duplex is dissociated into two single-stranded DNAs (data not shown). Apparently, the azobenzene is accommodated in the chiral environment on the duplex formation. Thus, the change in the microenvironment around the base pairs, caused by the *cis–trans* isomerization, affects the duplex stability.

In conclusion, duplex formation of the modified oligonucleotides carrying azobenzene at the 2′-position of ribonucleotides is successfully photo-regulated without changing pH and ionic strength. Applications of the present results in vivo and in vitro are currently under way.

Acknowledgements

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9. **2**: ^{1}H NMR [DMSO-d_6 (TMS), 270 MHz]: δ 11.33 (s, 1H, uracil NH), 7.91–7.54 (m, 10H, azobenzene, and uracil H_6), 5.97 (d, 1H, $J_{1',2'}$=4.62 Hz, $H_{1'}$), 5.60 (d, 1H, uracil H_5), 5.32 (d, 1H, 3′-OH), 5.16 (t, 1H, 5′-OH), 4.83 and 4.69 (d, 2H, J_{gem}=13.21 Hz, $ArCH_2$), 4.19–3.95 (m, 3H, $J_{3',4'}$=4.94 Hz, $H_{2'}$, $H_{3'}$ and $H_{4'}$), 3.65–3.61 (m, 2H, $H_{5'}$).
10. $U_{azo}T_7$: calcd: 2565.4; observed: 2565.2. $T_3U_{azo}T_4$: calcd: 2565.4; observed: 2564.8.
11. The HPLC conditions: a Merck LiChrospher 100 RP-18(e) column, 260 nm, 0.5 cm^3 min^{-1}, a linear gradient 5–25% (25 min) acetonitrile–water containing 50 mM ammonium formate. Under these conditions, *trans*- and *cis*-$U_{azo}T_7$ were eluted at 23.8 and 19.5 min, respectively.
12. The absorbance at 260 nm was monitored at pH 7.0 (10 mmol dm^{-3} phosphate buffer) on a Jasco model V-530 spectrophotometer, equipped with a programmed temperature-controller. The rate of temperature change was 1.0°C/min. The concentrations of the modified oligonucleotide and its complementary one were 50 μmol dm^{-3}, and the ionic strength was kept constant at 1 mol dm^{-3} by using NaCl. The T_m values were determined from the maximum in the first derivative of the melting curve.
13. The light from a 150 W Xenon lamp was irradiated for 10 min through an appropriate filter. Infrared light was cut off by using a water filter.
14. UV–Vis spectra were virtually unchanged before and after the T_m measurement. The *cis*-azobenzene was stable enough for the T_m measurements.
15. Unlike the *trans*-$U_{azo}T_7$ case, T_m of the *trans*-$T_3U_{azo}T_4/dA_8$ duplex was lower than that of unmodified duplex. According to Yamana et al., the pyrene- or anthraquinone-modified uridine stabilized duplexes even when it was located in the middle of the sequence: Yamana, K.; Iwase, R.; Furutani, S.; Tsuchida, H.; Zako, H.; Yamaoka, T.; Murakami, A. *Nucleic Acids Res.* **1999**, *27*, 2387, and Yamana, K.; Mitsui, T.; Yoshioka, J.; Isuno, T.; Nakano, H. *Bioconjugate Chem.* **1996**, *7*, 715.
16. The CD spectrum of the *trans*-$U_{azo}T_7/dA_8$ duplex (200–300 nm) was almost the same as that of the *cis*-$U_{azo}T_7/dA_8$.