Photocontrol of DNA Duplex Formation by Using Azobenzene-Bearing Oligonucleotides

Hiroyuki Asanuma, Xingguo Liang, Takayuki Yoshida, and Makoto Komiyama*[a]

The duplex-forming activities of oligonucleotides can be photomodulated by incorporation of an azobenzene unit. Upon isomerizing the trans*-azobenzene to the* cis *form by irradiation with UV light, the T_m value of the duplex (with the complementary DNA) is lowered so that the duplex is dissociated. The duplex is formed again when the* cis*-azobenzene is converted to the* trans*-azobenzene by irradiation with visible light. The photoregulation is successful irrespective of the position of the azobenzene unit in the oligonucleotides. The* trans*-azobenzene in the oligonucleotides intercalates between two DNA base pairs in the duplexes and stabilizes them because of a favorable enthalpy change. The nonplanar structure of a* cis*-azobenzene is unfavorable for such an interaction. These photoresponsive oligonucleotides are promising candidates for the regulation of various bioreactions.*

KEYWORDS:

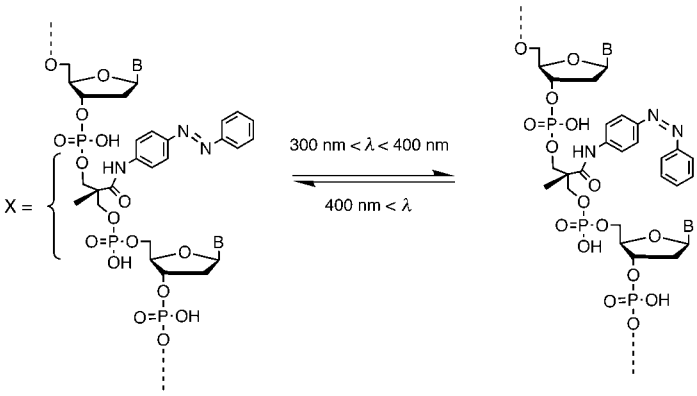
azo compounds · DNA structures · isomerizations · nucleotides · photochemistry

Introduction

Recently, much interest has been given to the artificial control of bioreactions.[1] One of the most important applications is the regulation of the expression of a specific gene by antisense and antigene strategies.[2] Various types of functionalized oligonucleotides have been prepared for these purposes.[3] However, there have been no reports on modified oligonucleotides that alter the duplex-forming activity in response to photostimuli and are applicable to the photoregulation of bioreactions. These compounds should be valuable tools for biotechnology, molecular biology, therapy, and other applications.

In a preliminary communication,[4] the authors incorporated a photoresponsive azobenzene into the side chain of oligonucleotides (see Scheme 1).[5, 6] Upon irradiation with either UV light or visible light, the azobenzene unit undergoes *cis – trans* isomerization, and accordingly the duplex-forming activities of the modified oligonucleotides (with respect to their complementary counterparts) are reversibly modulated.[7] By using these modified oligonucleotides, double-stranded DNA can be converted to two single strands (and vice versa) at a predetermined place and at a desired timing.

In the present work we have studied the photoregulation of duplex formation in more detail. By thermodynamic and spectroscopic analyses, the following points are clarified: 1) How does the position of azobenzene unit in the oligonucleotides affect the photoregulating activity?; 2) which of the thermodynamic parameters (the enthalpy change and the entropy change) governs this photoregulation?; and 3) what is the mechanism of the photo-regulation? Applications of these modified oligonucleotides to the photocontrol of enzymatic reactions are discussed.



$\mathbf{A_8}$:	5'-AAA-AAA-AA-3'	$\mathbf{T_8}$:	5'-TTT-TTT-TT-3'
$\mathbf{XA_7}$:	5'-XAA-AAA-AA-3'	$\mathbf{XT_7}$:	5'-XTT-TTT-TT-3'
$\mathbf{AXA_6}$:	5'-AXA-AAA-AA-3'	$\mathbf{TXT_6}$:	5'-TXT-TTT-TT-3'
$\mathbf{A_2XA_5}$:	5'-AAX-AAA-AA-3'	$\mathbf{T_2XT_5}$:	5'-TTX-TTT-TT-3'
$\mathbf{A_3XA_4}$:	5'-AAA-XAA-AA-3'	$\mathbf{T_3XT_4}$:	5'-TTT-XTT-TT-3'
$\mathbf{G_3XG_4}$:	5'-GGG-XGG-GG-3'	$\mathbf{C_8}$:	5'-CCC-CCC-CC-3'

Scheme 1. *Structures of azobenzene-modified oligonucleotides (top) and sequences of the oligonucleotides used in this study (bottom).*

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Results and Discussion

Photoinduced change of T_m values for oligonucleotide duplexes

Typical melting curves for the duplex formation between the azobenzene-modified oligonucleotide $\mathbf{A_3XA_4}$(P) and $\mathbf{T_8}$, the oligonucleotide with the complementary sequence, are shown in Figure 1. Before the photoirradiation, the azobenzene unit in $\mathbf{A_3XA_4}$(P) is overwhelmingly (about 90%) present in the *trans* form, as confirmed by HPLC analysis.[10] Consistently, the solution has a strong absorption around 350 nm (assignable to *trans*-azobenzene), and the absorbance around 440 nm (for *cis*-azobenzene) is virtually zero. Under these conditions, the melting temperature (T_m) of the $\mathbf{A_3XA_4}$(P)/$\mathbf{T_8}$ duplex is 24.8 °C (black line in Figure 1). This value is close to that (23.7 °C) of the unmodified $\mathbf{A_8}$/$\mathbf{T_8}$ duplex. Upon irradiation with UV light ($300 < \lambda < 400$ nm), the *trans*-azobenzene residue is promptly isomerized to the *cis* form (irradiation is carried out for 30 min). Concurrently, the T_m value of the duplex is lowered to 15.9 °C (gray line in Figure 1). The change in T_m (ΔT_m), induced by the *trans* → *cis* isomerization, is 8.9 °C. Still greater values of ΔT_m are obtained for other duplexes (Table 1).

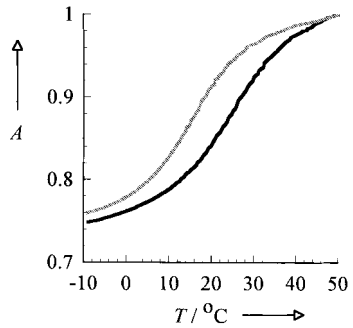


Figure 1. *Melting curves of the* trans-$\mathbf{A_3XA_4}$(P)/$\mathbf{T_8}$ *duplex (black line) and the* cis-$\mathbf{A_3XA_4}$(P)/$\mathbf{T_8}$ *duplex (gray line).*

Table 1. *Melting temperatures (T_m) of the duplexes between the modified oligonucleotides and their counterparts of complementary sequence.*[a]

Duplex	Polar fraction (P)			Less polar fraction (L)		
	T_m [°C]		ΔT_m [°C]	T_m [°C]		ΔT_m [°C]
	trans	*cis*		*trans*	*cis*	
$\mathbf{A_8}$/$\mathbf{T_8}$ (native)	23.7					
$\mathbf{XA_7}$/$\mathbf{T_8}$[b]	35.5	25.6	9.9			
$\mathbf{AXA_6}$/$\mathbf{T_8}$	33.7	21.5	12.2	31.3	23.3	8.0
$\mathbf{A_2XA_5}$/$\mathbf{T_8}$	27.2	20.4	6.8	24.8	20.1	4.7
$\mathbf{A_3XA_4}$/$\mathbf{T_8}$	24.8	15.9	8.9	19.9	14.7	5.2
$\mathbf{XT_7}$/$\mathbf{A_8}$[b]	37.2	26.9	10.3			
$\mathbf{TXT_6}$/$\mathbf{A_8}$	35.8	21.7	14.1			
$\mathbf{T_2XT_5}$/$\mathbf{A_8}$	29.6	9.0	20.6	22.4	6.6	15.8
$\mathbf{T_3XT_4}$/$\mathbf{A_8}$	20.2	−3.0	23.2	9.1	−3.0	12.1
$\mathbf{G_3XG_4}$/$\mathbf{C_8}$[b,c]	32.2	23.0	9.2			

[a] $[\mathbf{A_mXA_n}] = [\mathbf{T_pXT_q}] = 50\ \mu mol\ dm^{-3}$, $[NaCl] = 1.0\ mol\ dm^{-3}$ at pH 7.0 (10 mmol dm^{-3} phosphate buffer). [b] The diastereomers could not be separated by reversed-phase HPLC. [c] $[\mathbf{G_3XG_4}] = [\mathbf{C_8}] = 10\ \mu mol\ dm^{-3}$, $[NaCl] = 0\ mol\ dm^{-3}$.

When the mixture is further irradiated with visible light ($\lambda > 400$ nm), the *cis*-azobenzene unit is isomerized to the *trans* form. The melting curve of the resultant solution is virtually superimposable on that observed before the first UV irradiation. The duplex-forming activity of the oligonucleotide has been reversibly modulated by the photoirradiation.

Effect of azobenzene unit position in the oligonucleotides on the photoregulation

The T_m values for various duplexes are summarized in Table 1. From these results, the following conclusions can be drawn:

1. In both A-T and G-C duplexes, the T_m value is notably changed by the photoisomerization of the azobenzene unit. The range of ΔT_m is 5 – 23 °C for the duplexes studied here.
2. Without exception, the duplexes containing a *trans*-azobenzene unit are more stable than those containing a *cis*-azobenzene unit.
3. An azobenzene unit near the 5′ end of the oligonucleotide stabilizes the duplex (for both the *cis* form and the *trans* form). However, the stabilization is inefficient when the azobenzene unit is placed in the middle of the duplex.

These effects are especially prominent in the duplex formation by the oligo(T)s bearing an azobenzene group (see Table 1). When the position of the azobenzene group is systematically changed from the 5′ end of the oligonucleotide to its middle ($\mathbf{XT_7}$, $\mathbf{TXT_6}$, $\mathbf{T_2XT_5}$, and $\mathbf{T_3XT_4}$), the T_m values of the respective duplexes monotonically decrease. Significantly, the decrease in the T_m value for the *cis* isomers (from 26.9 to −3.0 °C) is far more

[*] *Members of the Editorial Advisory Board will be introduced to the readers with their first manuscript.*

drastic than that for the *trans* isomers (from 37.2 to 20.2 °C). As a result, the greatest ΔT_m value (23.2 °C) is observed for the **T_3XT_4/A_8** duplex.[11]

T_m differences between the diastereomers of modified oligonucleotides

The ΔT_m values for the P diastereomers are always greater than the corresponding values for the L isomers (see Table 1). For the **T_3XT_4**(P)/**A_8** duplex, $\Delta T_m = 23.2$ °C, whereas the value for the **T_3XT_4**(L)/**A_8** duplex is 12.1 °C. These differences in ΔT_m mainly stem from the fact that the P isomers containing *trans*-azobenzene units provide more stable duplexes than do the L isomers: The T_m value of the *trans*-**T_3XT_4**(P)/**A_8** duplex (20.2 °C) is by 11.1 °C higher than that of the *trans*-**T_3XT_4**(L)/**A_8** duplex (9.1 °C). With the oligonucleotides bearing *cis*-azobenzene groups, however, the difference in T_m between the P and the L isomers is only within 2.4 °C. These effects are remarkable, especially when the azobenzene unit is attached to a position in the middle of the oligonucleotide.[12]

Photocontrol of the formation/dissociation of DNA duplexes

The formation of the **A_3XA_4**(P)/**T_8** duplex and its dissociation are satisfactorily photocontrolled (Figure 2). The temperature was kept constant at 20 °C, and the absorbance at 260 nm before the photoirradiation was taken as the reference. For these measurements, neither temperature, pH, ionic strength, or other factors were changed. Under these conditions, the **A_3XA_4**(P)/**T_8** duplex is efficiently formed ($T_m = 24.8$ °C for the *trans* isomer of **A_3XA_4**(P)). Upon irradiation with UV light, the absorbance at 260 nm rapidly increases (open circle). The duplex is largely dissociated into two single-stranded oligonucleotides, and the hypochromicity due to duplex formation disappears ($T_m = 15.9$ °C for the *cis* isomer). Next, visible light is applied, and the absorbance decreases to the value before the UV irradiation (closed circle). These changes are reversibly repeated without apparent deterioration.

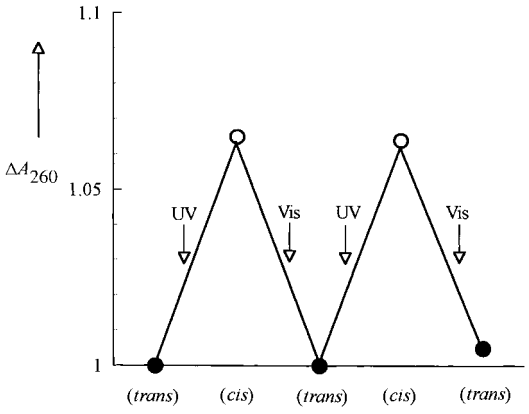


Figure 2. *Photoinduced change of the absorbance at 260 nm of a solution containing* ***A_3XA_4(P)/T_8****. Irradiation with either UV or visible light was carried out for 20 min at the positions indicated by arrows. Conditions: 20 °C, pH 7.1, 10 mmol dm^{-3} phosphate buffer, [NaCl] = 1 mol dm^{-3}, [**A_3XA_4(P)**] = [**T_8**] = 50 μmol dm^{-3}.*

Thermodynamic parameters for duplex formation of modified oligonucleotides

For all the **A_mXA_n/T_8** duplexes listed in Table 2, the exothermicity ($-\Delta H°$) for the modified oligonucleotides bearing *trans*-azobenzene units exceeds that for the oligonucleotides with *cis*-azobenzene units. The differences are 56 kJ mol^{-1} for the **AXA_6**(L)/**T_8** duplex and 9 kJ mol^{-1} for the **A_3XA_4**(L)/**T_8** duplex. On the contrary, the entropy change ($\Delta S°$) is favorable for duplex formation by the oligonucleotides containing *cis*-azobenzene groups, rather than by the ones containing *trans*-azobenzene groups. The exothermicity ($-\Delta H°$) increases with the increase in T_m (Figure 3 A), but $T\Delta S°$ decreases (Figure 3 B). It is concluded that the change of $\Delta H°$ is the driving force for the observed photoregulation (the formation of a DNA duplex after *cis* → *trans* isomerization and its dissociation after *trans* → *cis* isomerization). Interestingly, the plot of $\Delta H°$ versus $T\Delta S°$ provides a fairly straight line (Figure 4). The data points for both *cis*-azobenzene- and *trans*-azobenzene-modified oligonucleotides can be fitted to the same graph. The contributions of $\Delta H°$ and $\Delta S°$ are compensating each other in all these systems.

For a series of oligonucleotides containing *trans*-azobenzene units, the exothermicity ($-\Delta H°$) is greater when the residue X (see Scheme 1) is closer to the 5′ end (see the left-hand side column in Table 2). The same is true for the oligonucleotides

Table 2. *Thermodynamic parameters for DNA duplex formation by the oligonucleotides bearing either a* trans- *or a* cis-*azobenzene unit.*[a]

Duplex	$\Delta G°$ [kJ mol^{-1}]		$\Delta H°$ [kJ mol^{-1}]		$\Delta S°$ [J mol^{-1} K^{-1}]	
	trans	*cis*	*trans*	*cis*	*trans*	*cis*
XA_7/T_8	−33.6	−27.1	−198	−184	−552	−526
AXA_6(P)/**T_8**	−31.6	−24.3	−182	−141	−503	−391
AXA_6(L)/**T_8**	−30.6	−24.4	−193	−137	−544	−378
A_3XA_4(P)/**T_8**	−27.1	−23.6	−136	−128	−364	−351
A_3XA_4(L)/**T_8**	−25.3	−22.8	−139	−130	−382	−361

[a] At $T = 298$ K.

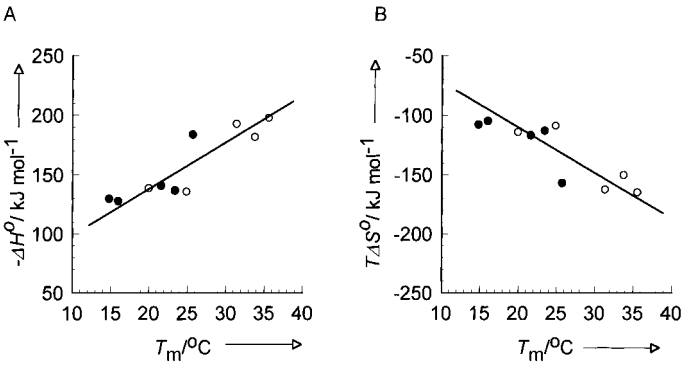


Figure 3. *The dependencies of (A) the exothermicity [$-\Delta$H°] and (B) TΔS° (T = 298 K) on T_m for the formation of* ***A_mXA_n/T_8*** *duplexes; the open circles represent data for* ***A_mXA_n*** *oligonucleotides bearing* trans*-azobenzene groups, whereas closed circles represent those containing* cis*-azobenzene groups (data taken from Table 2).*

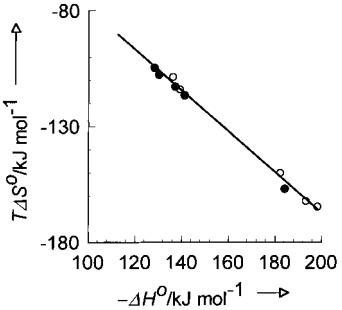


Figure 4. *Compensation of* $T\Delta S^o$ ($T = 298$ K) *and* $[-\Delta H^o]$ *for the formation of* A_mXA_n/T_8 *duplexes. Open circles represent data for* A_mXA_n *oligonucleotides bearing* trans*-azobenzene groups, whereas the closed circles represent those containing* cis*-azobenzene groups (data taken from Table 2).*

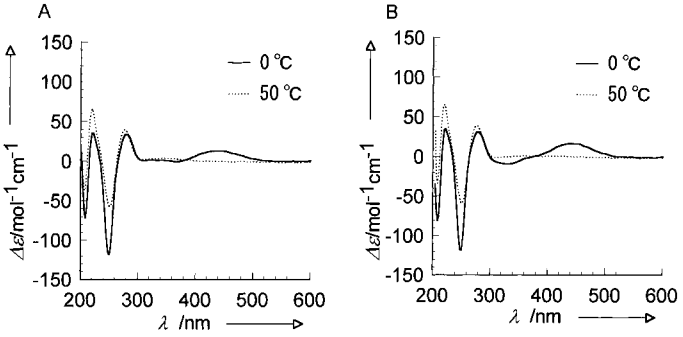


Figure 6. *CD spectra of the* trans-$A_3XA_4(P)/T_8$ *duplex (A) and the* cis-$A_3XA_4(P)/T_8$ *duplex (B) at 0 °C (solid line) and 50 °C (dotted line). Conditions: pH 7.0, 10 mmol dm^{-3} phosphate buffer, [NaCl] = 1 mol dm^{-3},* $[A_3XA_4(P)] = [T_8] = 50\ \mu$mol dm^{-3}.

containing *cis*-azobenzene units. These orders are identical with those for increasing stability (increasing T_m) of the duplexes. Apparently, the formation of duplexes is also governed by the ΔH^o term.[13]

Spectroscopic monitoring of the duplex formation by the modified oligonucleotides

The *trans*-azobenzene unit in $\mathbf{A_3XA_4}$(P) shows an absorption maximum at 352 nm. When $\mathbf{T_8}$ is added to the solution (at a temperature below the T_m of the *trans*-$\mathbf{A_3XA_4}$(P)/$\mathbf{T_8}$ duplex), the absorption band of the azobenzene shifts towards longer wavelengths. At a temperature higher than the T_m of the duplex (24.8 °C), however, the spectrum is hardly changed even after addition of $\mathbf{T_8}$. As the temperature is lowered, the concentration of the *trans*-$\mathbf{A_3XA_4}$(P)/$\mathbf{T_8}$ duplex increases and accordingly the absorption maximum of the azobenzene is changed (Figure 5).

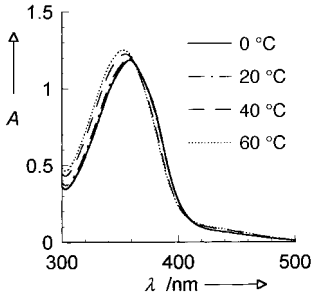


Figure 5. *UV/Vis spectra of the* trans-$A_3XA_4(P)/T_8$ *duplex at different temperatures. Conditions: pH 7.0, 10 mmol dm^{-3} phosphate buffer, [NaCl] = 1 mol dm^{-3},* $[A_3XA_4(P)] = [T_8] = 50\ \mu$mol dm^{-3}.

Consistently, the plot of λ_{max} vs. temperature has a sigmoidal shape, and the midpoint is close to the T_m of the duplex. Furthermore, on the formation of this duplex, a weak but explicit CD is induced in the 300 – 500 nm region (solid line in Figure 6 A). This induced CD corresponds to the absorption by the *trans*-azobenzene. Note that this spectrum (solid line in Figure 6 A) is obtained at 0 °C, which is below the T_m of the duplex (24.8 °C). As expected, no CD is induced at 50 °C where most of the duplex is dissociated into two single-stranded DNA oligonucleotides (dotted line in Figure 6 A). The circular dichroism in the 200 – 300 nmregion (corresponding to the absorption of nucleic acid bases) is also strengthened.

All these results indicate that the *trans*-azobenzene moiety, which has a planar structure,[14] intercalates between two base pairs of the DNA duplex.[15] Consistently, the induced CD around 350 nm is very weak (Figure 6 A). This result shows that the corresponding $\pi - \pi^*$ transition moment (which is parallel to the long axis of *trans*-azobenzene) is parallel to the plane of DNA base pairs (as expected from the intercalation mode).[16] If the azobenzene moiety were to be placed parallel to the long axis of the duplex, a strongly positive CD should be induced.

When the *trans*-azobenzene moiety in $\mathbf{A_3XA_4}$(P) is isomerized to the *cis* form by UV irradiation, a notable CD is also induced in the 200 – 500 nm region (Figure 6 B). Probably, the *cis*-azobenzene is not directly interacting with the DNA bases. Since the nonplanar structure of *cis*-azobenzene[17] is unfavorable for intercalation into DNA duplexes, it would destabilize the duplex by steric repulsion.

As shown by the thermodynamic analysis (Table 2), the *cis* → *trans* isomerization of the azobenzene moiety facilitates duplex formation because of a favorable ΔH^o term. The increased exothermicity for the *trans*-azobenzene-modified oligonucleotides is ascribed to the stacking interactions of the azobenzene with the adjacent base pairs. These interactions are stronger than the hydrogen bonds in Watson – Crick A/T base pairs so that the duplexes of modified oligonucleotides (containing *trans*-azobenzene units) are even more stable than the native duplexes (e.g., the $\mathbf{A_8}/\mathbf{T_8}$ duplex). When an azobenzene unit is placed in the middle of an oligonucleotide, the resulting duplexes are destabilized. This finding is consistent with the previous results that internal mismatches destabilize duplexes to a greater extent than terminal mismatches.[18]

Conclusions

By introducing a photoresponsive azobenzene group into oligonucleotides, the formation/dissociation of DNA duplexes has been successfully photoregulated (Figure 7). The T_m values are significantly changed, irrespective of the position of the azobenzene unit in the oligonucleotides. In these photoregula-

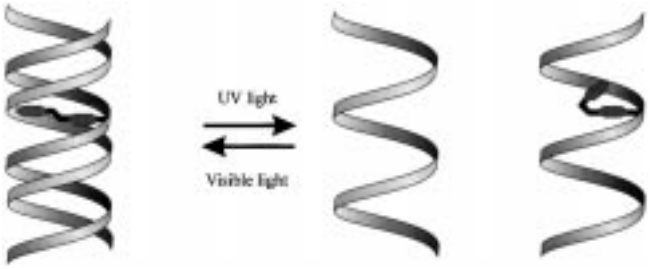
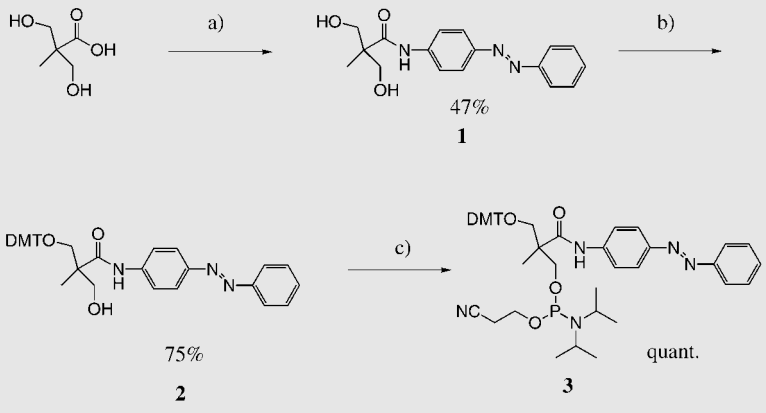


Figure 7. *Schematic illustration of the photoregulation of duplex formation by using photoisomerization of azobenzene units.*

tions, the *trans*-azobenzene stabilizes the duplexes by intercalation, whereas the *cis*-azobenzene destabilizes them. The present modified oligonucleotides are suitable for the photoregulation of gene expression (and of various enzymatic reactions), since 1) the structural modification of the oligonucleotides is minimized,[5] 2) the photoregulation is reversible and involves no side reaction, and 3) light is a clean and easily controllable stimulus. According to our recent experiments, these photoresponsive oligonucleotides can be successfully used as modulators of DNA elongation by T7 polymerase.[19] Whether DNA elongation is terminated at a desired site or proceeds down to the end of the template DNA is clearly dictated by photoinduced *cis*–*trans* isomerization of the azobenzene.

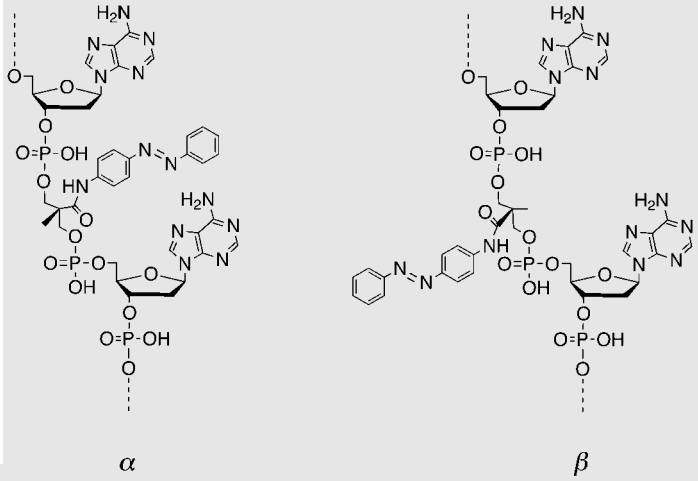
Experimental Section

Materials: The phosphoramidite monomer **3** bearing an azobenzene group was synthesized according to Scheme 2 (details of the synthesis are described in the Supporting Information).[8] 2,2-Bis(hydroxymethyl)propionic acid, *N*,*N*′-dicyclohexylcarbodiimide, *N*-hydroxybenzotriazole, 4-aminoazobenzene, 4,4′-dimethoxytrityl chloride (DMT-Cl), 4-(dimethylamino)pyridine, and 1*H*-tetrazole (Tokyo Kasei), as well as 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphordiamidite (Aldrich), were commercially obtained and used without further purification. Pyridine and dimethylformamide (DMF) were distilled after being dried over CaH_2. The conventional phosphoramidite monomers, controlled-pore glass (CPG) columns, and other reagents for DNA synthesis were purchased from Glen Research Co.



Scheme 2. *Synthesis of the phosphoramidite monomer* ***3****. a) 4-aminoazobenzene, dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, DMF; b) 4,4′-dimethoxytrityl chloride (DMT-Cl), 4-dimethylaminopyridine, pyridine, CH_2Cl_2; c) 2-cyanoethyl* N,N,N′,N′*-tetraisopropylphosphordiamidite, 1*H*-tetrazole, CH_3CN.*

Synthesis of azobenzene-modified oligonucleotides and separation of the isomers: All the modified oligonucleotides shown in Scheme 1 were synthesized on an automated DNA synthesizer by using the phosphoramidite monomer **3** and conventional monomers. After the recommended workup, they were purified by reversed-phase HPLC (Merck LiChrospher 100 RP-18(e) column, flow rate 0.5 mL min^{-1}, linear gradient 5 → 25 % (25 min) acetonitrile in water containing 50 mmol dm^{-3} ammonium formate, detection at 260 nm). These modified oligonucleotides occur in two diastereomeric forms (α and β, Scheme 3) due to the chirality of building block X (see Scheme 1). Furthermore, the azobenzene residue in each of them is either in the *cis* form or the *trans* form. In the present study, all of these four isomers were completely separated by the reversed-phase HPLC described above. The first and the third fractions (in the order of increasing retention time) were the *cis* and the *trans* isomers of one diastereomer, whereas the second and the fourth fractions are the *cis* and the *trans* isomers of the other diastereomer.[8] Hereafter, the *cis* and the *trans* isomers of the former (more polar) diastereomer is designated as P, whereas the corresponding stereoisomers of the latter (less polar) diastereomer is designated as L (e.g., *trans*-$\mathbf{A_3XA_4}$(P) and *cis*-$\mathbf{A_3XA_4}$(L)). With three oligonucleotides ($\mathbf{XA_7}$, $\mathbf{XT_7}$, and $\mathbf{G_3XG_4}$), the α and β diastereomers were not sufficiently separated by HPLC, and thus they were used as the mixtures (note that the *cis* and the *trans* isomers were completely separated even in these cases).



Scheme 3. *The two diastereomers of the modified oligonucleotides (α and β) with respect to the configuration of building block X (see Scheme 1).*

Characterization of the modified oligonucleotides: The matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra (negative mode) were in satisfactory agreement (within experimental error) with the expected structures. Compounds $\mathbf{A_nXA_m}$: *m*/*z*: $\mathbf{XA_7}$, 2507; $\mathbf{AXA_6}$(P), 2507; $\mathbf{AXA_6}$(L), 2507; $\mathbf{A_2XA_5}$(P), 2507; $\mathbf{A_2XA_5}$(L), 2509; $\mathbf{A_3XA_4}$(P), 2505; $\mathbf{A_3XA_4}$(L), 2504 (calcd for $[\mathbf{A_nXA_m} - H^+]$: 2504); compounds $\mathbf{T_nXT_m}$: *m*/*z*: $\mathbf{XT_7}$, 2440; $\mathbf{TXT_6}$(P), 2443; $\mathbf{TXT_6}$(L), 2441; $\mathbf{T_2XT_5}$(P), 2445; $\mathbf{T_2XT_5}$(L), 2446; $\mathbf{T_3XT_4}$(P), 2444; $\mathbf{T_3XT_4}$(L), 2444 (calcd for $[\mathbf{T_nXT_m} - H^+]$: 2441); $\mathbf{G_3XG_4}$: *m*/*z*: 2616 (calcd. for $[\mathbf{G_3XG_4} - H^+]$: 2616).

Photoisomerization of the azobenzene-modified oligonucleotides: In order to isomerize the azobenzene unit from the *trans* form to the *cis* form, the oligonucleotide solutions were irradiated with light from a 150-W xenon lamp through a UV-D36C filter (Asahi Technoglass Corporation). Infrared light was cut off by using a water filter. The intensity of light at the specimens was 5.3 mW cm^{-2}. For the *cis* → *trans* isomerization, an L-42 filter was used (the light intensity was 230 mW cm^{-2}). Under these conditions, the isomerization (either *trans* → *cis* or *cis* → *trans*) was usually equilibrated within 1 min. To ensure the completion of isomerization, however, irradiation was continued for 20 – 30 min.

Measurement of the melting temperatures of the duplexes: The absorbance at 260 nm was monitored at pH 7.0 (10 mmol dm^{-3} phosphate buffer). The T_m value was determined from the maximum in the first derivative of the melting curve. The temperature ramp was 1.0 °C min^{-1}. The concentration of each of the DNA oligomers was 50 μmol dm^{-3}, the salt concentration was 1 mol dm^{-3} NaCl (unless otherwise stated). The melting curves for cooling and heating were virtually identical to each other. In the determination of the T_m value of the *cis* isomer duplexes, the UV light was applied in the middle of the measurement in order to minimize the effect of thermal *cis* → *trans* isomerization. By these procedures, the fraction of the *cis* isomer in the specimens was kept almost constant at 70% throughout the measurement.

Spectroscopic measurements: The UV-visible spectra were measured on a JASCO model V-530 spectrophotometer, while the circular dichroism (CD) spectra were recorded on a JASCO model J-725 spectropolarimeter. Both spectrometers were equipped with programmed temperature controllers.

Determination of the thermodynamic parameters of duplex formation: The enthalpy change (ΔH°) and the entropy change (ΔS°) for the duplex formation were determined by using Equation (1),[9]

$$T_m^{-1} = (2.30R/\Delta H^\circ)\log(c_t/4) + (\Delta S^\circ/\Delta H^\circ) \quad (1)$$

where c_t is the total concentration of oligonucleotides comprising both the modified oligonucleotide and its counterpart (R is the gas constant). The c_t values were varied from 2 to 100 μmol dm^{-3}. The changes in Gibbs free energy at 25 °C (ΔG°(298 K)) were calculated from the ΔH° and ΔS° values.

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[11] Efficient photoregulation by the *trans* – *cis* isomerization is also possible for a DNA of random sequence. For instance, 5′-CGAXGTC-3′ (more polar fraction) was hybridized with its complementary sequence (3′-GCTTCAG-5′). The T_m of the duplex in the *trans* form was 25.4 °C, whereas that of the *cis* form was below 5 °C (pH 7.0, [DNA] = 50 μmol dm^{-3}, [NaCl] = 1 mol dm^{-3}).

[12] In the α diastereomer in Scheme 3, both the nucleic acid bases and the azobenzene residue protrude in the same direction. In water, the hydrophobic *trans*-azobenzene preferably stacks onto the adjacent DNA bases and is efficiently shielded from water molecules. Thus, this diastereomer is expected to be more polar than the β isomer. The P (more polar) fraction is tentatively assigned to the α isomer, whereas the L (less polar) fraction is the β isomer. The *trans*-azobenzene in the α isomer feasibly intercalates into the duplex. This is also consistent with the fact that the P isomers (with *trans*-azobenzene) form more stable duplexes than do the L isomers (see Table 1). In order to elucidate these configurations unambiguously, a ^{1}H NMR study is currently under way.

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