L-Threoninol as a Chiral Linker of Azobenzene for the Effective Photo-regulation of DNA Triplex Formation

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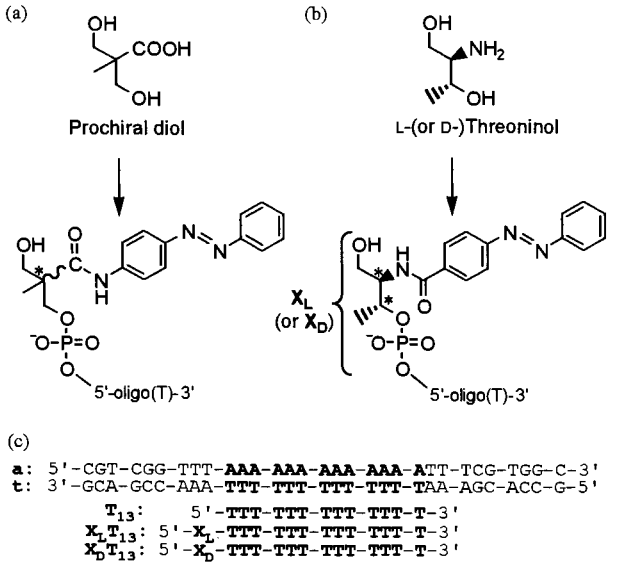
An azobenzene was enantioselectively attached to the 5'-end of oligo(T) through D- or L-threoninol. Photo-isomerization of azobenzene, tethered on L-threoninol, induced much larger change of melting temperature of triplex formation than did that on the D-form.

DNA triplex formation has been widely investigated for the potential application to the regulation of gene expression (antigene strategy). A variety of chemically modified oligodeoxyribonucleotides (ODNs) have been also synthesized for the stabilization of triplex.[1] If the stability of triplex can be controlled by external stimuli, the scope of application would be extended. We reported that an azobenzene moiety, incorporated into the ODN, can reversibly photo-regulate the formation and dissociation of DNA triplex[2] as well as DNA duplex:[3] *trans*-azobenzene stabilizes the triplex whereas *cis*-form destabilizes it. However, the modified ODN previously reported was synthesized by using the corresponding racemic phosphoramidite monomer, which was synthesized from a prochiral diol linker. Thus, two diastereomers, with respect to its chirality, were inevitably produced (Scheme 1(a)). Furthermore, these diastereomers could not be separated by reversed-phase HPLC when an azobenzene moiety was attached to 5'-end of oligo(T).[4] Therefore, measurement of melting temperature (T_m) has been carried out by applying a mixture of diastereomers and the effect of configuration of the linker on the photo-regulation activity for triplex formation remained still unclear.

Recently, we have successfully synthesized optically pure phosphoramidite monomers from D- or L-threoninol (Scheme 1(b)), and found that the modified ODN synthesized from D-threoninol has a greater photo-regulation activity for duplex formation than that from L-threoninol.[5] This result prompted us to study the effect of chirality of the linker on triplex formation. In the present paper, we report that an azobenzene tethered on the L-threoninol linker at 5'-end of oligo(T) photo-regulates the triplex formation much more effectively than does that on the D-form.

The chiral phosphoramidite monomers carrying an azobenzene moiety were synthesized from D- or L-threonine according to the previous paper.[5] The modified oligo(T)s bearing an azobenzene moiety at 5'-end were synthesized on an automated DNA synthesizer by using the above monomers and conventional ones. All the modified ODNs were purified by reversed-phase HPLC and characterized by MALDI-TOFMS.[6] Sequences of the ODNs used in this study are listed in Scheme 1(c). In the middle of the **a**/**t** duplex, the oligo(A)/oligo(T) portion is placed for the target of triplex formation.

The melting curve for the triplex formation between *trans*-$\mathbf{X_LT_{13}}$ and the **a**/**t** duplex is shown in Figure 1(a) (solid line).[7] Before photo-irradiation, the azobenzene moiety in $\mathbf{X_LT_{13}}$ took



Scheme 1. Structures of the modified oligo(T)s used in the (a) previous and (b) present study. Sequences of the target DNA duplex and (un)modified oligo(T)s are also listed in (c).

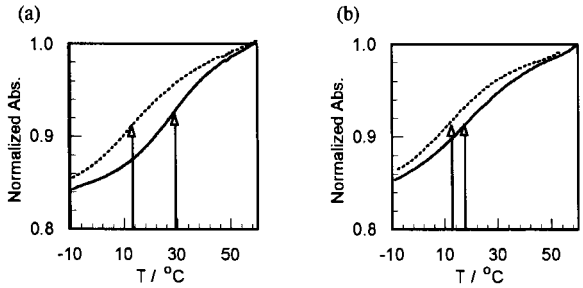


Figure 1. Melting curves for the triplexes of (a) $\mathbf{X_LT_{13}}$ and (b) $\mathbf{X_DT_{13}}$ with the **a**/**t** duplex either in the *trans*- (solid line) or *cis*-form (dotted line). The arrows in the figures indicate the T_ms. Detailed conditions for the measurement of T_m are described in Ref. 7.

the *trans*-form overwhelmingly (more than 90%), as estimated from HPLC analysis.[8] The T_m value of the *trans*-$\mathbf{X_LT_{13}}$/**a**/**t** triplex was 29.7 °C, which was higher than that (22.5 °C) of the unmodified $\mathbf{T_{13}}$/**a**/**t** triplex. The *trans*-azobenzene moiety on the L-threoninol linker significantly stabilized the triplex. Upon irradiation with UV light (300 nm < λ < 400 nm), the *trans*-azobenzene moiety promptly isomerized to the *cis*-form.[9] Concurrently, the T_m value of the triplex was lowered to 12.6 °C (dotted line in Figure 1(a)). The change of T_m (ΔT_m), induced by the *cis*→*trans* isomerization, was 17.1 °C. On the contrary, the T_m of *trans*-$\mathbf{X_DT_{13}}$/**a**/**t** triplex was as low as 17.7 °C (Figure 1(b)), which was even lower than that of $\mathbf{T_{13}}$/**a**/**t** triplex. Since T_m of the *cis*-$\mathbf{X_DT_{13}}$/**a**/**t** triplex was almost the same as that of the *cis*-$\mathbf{X_LT_{13}}$/**a**/**t** triplex (see Table 1), the ΔT_m

for the $\mathbf{X_DT_{13}}$/**a**/**t** triplex was only 5.1 °C. Thus, it is concluded that the azobenzene moiety tethered on L-threoninol induces much larger ΔT_m than does the D-form. As expected, T_ms of triplex formation for the equimolar mixture of $\mathbf{X_DT_{13}}$ and $\mathbf{X_LT_{13}}$ (1.0 μM each) with the **a**/**t** duplex took averaged values of those of the $\mathbf{X_DT_{13}}$/**a**/**t** triplex and the $\mathbf{X_LT_{13}}$/**a**/**t** triplex for both *trans*- and *cis*-isomers.

Table 1. Melting temperatures (T_ms) of the triplexes between the (un)modified oligo(T) and the **a**/**t** duplex[a]

	T_m / °C		
oligo(T)	*trans*	*cis*	ΔT_m / °C[b]
$\mathbf{X_LT_{13}}$	29.7	12.6	17.1
$\mathbf{X_DT_{13}}$	17.7	12.6	5.1
$\mathbf{X_LT_{13}}$ + $\mathbf{X_DT_{13}}$[c]	24.7	12.7	12.0
$\mathbf{T_{13}}$	22.5		

[a][oligo(T)] = [**a**] = 2.0 μM, [**t**] = 2.4 μM, [$MgCl_2$] = 0.2 M, in 10 mM HEPES buffer (pH 7.0). [b] Change of T_m induced by *cis* → *trans* isomerization. [c][$\mathbf{X_LT_{13}}$] = [$\mathbf{X_DT_{13}}$] = 1.0 μM.

As summarized in Table 1, large ΔT_m of the $\mathbf{X_LT_{13}}$/**a**/**t** triplex is primarily attributed to the effective stabilization by *trans*-azobenzene on L-threoninol. *trans*-Azobenzene on each threoninol linker intercalates between the A–T pairs as evidenced by the induced circular dichroism (ICD) at around 360 nm (π–π* transition of azobenzene) at 0 °C (solid lines in Figure 2). Weak but explicit CDs were negatively induced for both *trans*-$\mathbf{X_LT_{13}}$/**a**/**t** and *trans*-$\mathbf{X_DT_{13}}$/**a**/**t**, which are characteristic to the intercalation.[10] Larger ICD for the *trans*-$\mathbf{X_LT_{13}}$/**a**/**t** triplex (compare Figure 2(a) with (b)) below T_m indicates the stronger intercalation of *trans*-azobenzene on the L-form. Assumedly, *trans*-azobenzene on L-form would intercalate between the A–T base pairs much more favorably than that on D-form.

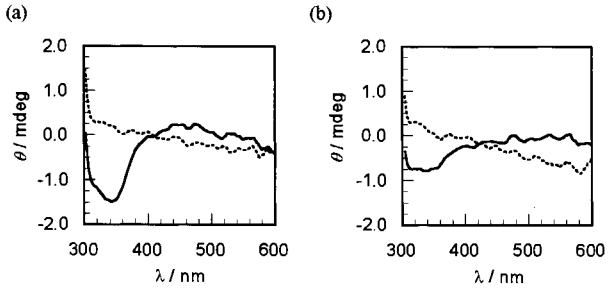


Figure 2. CD spectra for the triplexes of (a) *trans*-$\mathbf{X_LT_{13}}$ and (b) *trans*-$\mathbf{X_DT_{13}}$ at 0 °C (solid line) and 60 °C (dotted line) with the **a**/**t** duplex. [oligo(T)] = [**a**] = 5.0 μM, [**t**] = 6.0 μM, [$MgCl_2$] = 0.2 M, in 10 mM HEPES buffer (pH 7.0).

In conclusion, *cis*–*trans* isomerization of azobenzene, tethered on L-threoninol, induces much larger ΔT_m of triplex formation than does that on the D-form. The larger ΔT_m of the $\mathbf{X_LT_{13}}$/**a**/**t** triplex is primarily attributed to the effective stabilization of triplex by *trans*-azobenzene on L-threoninol. Application of various intercalators conjugated on the L-threoninol linker for stabilizing the DNA triplex is currently under way.

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References and Notes

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4 Incorporation of azobenzene moiety at the 5'-end of oligo(T) is especially effective for the photo-regulation of triplex formation: see ref 2.

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6 MALDI-TOFMS for $\mathbf{X_LT_{13}}$: obsd 4264, $\mathbf{X_DT_{13}}$: obsd 4265 (calcd for [$\mathbf{X_LT_{13}}$–H^+]: 4265).

7 The concentrations of **a**, **t**, and oligo(T) were 2.0, 2.4, and 2.0 μM (M = mol dm^{-3}), respectively, in pH 7.0 HEPES buffer (10 mM) in the presence of 0.2 M $MgCl_2$. The **a**:**t** ratio was kept at 1:1.2 in order to ensure the absence of free **a**, which should form a duplex with the modified oligo(T) and perturb the T_m measurement. The T_ms were determined from the maximum in the first derivative of the melting curves, which were obtained by measuring the absorbance at 280 nm as a function of temperature. The temperature ramp was 1.0 °C/min. Throughout the T_m measurement, the fraction of *cis*- and *trans*-isomers was kept almost constant as evidenced by the UV–vis spectroscopy.

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