the NMR spectra of the bis-MTPA derivatives showed no difference between the stereoisomers.[8] The ^{2}H NMR spectrum of a statistical mixture of the three stereoisomers *R*,*R*/*S*,*S* and *R*,*S* is shown in Figure 3. Even though the line splittings

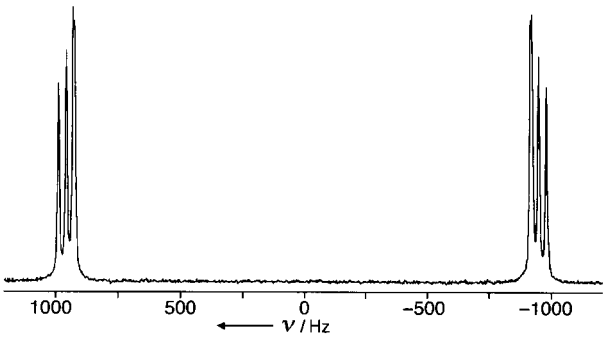

Figure 3. ^{1}H-decoupled ^{2}H NMR spectrum of diol **3** in PBLG/THF (27.5 wt% PBLG) at $T = 302$ K.

are not tremendous, the technique discriminates all the stereoisomers and would allow again a measurement of any diastereomeric and enantiomeric excess using deconvolution tools.

It has been demonstrated that ^{1}H-decoupled ^{2}H NMR spectroscopy with a chiral liquid crystalline solvent allows the NMR spectroscopic discrimination of diastereomers with remote asymmetric centers up to nine bonds apart. This discrimination originates in the difference in the molecular order parameters of the stereoisomers. To the extent where order parameters reflect the shape of a molecule, this opens up the idea of shape-recognition NMR spectroscopy. Furthermore, we have shown with a few examples that the spectral multiplicity of the ^{2}H NMR spectra leads to the unambiguous assignment of the resonances of each diastereomer.

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Photoregulation of the Formation and Dissociation of a DNA Duplex by Using the *cis*–*trans* Isomerization of Azobenzene**

Hiroyuki Asanuma, Takanori Ito, Takayuki Yoshida, Xingguo Liang, and Makoto Komiyama*

Recently, much interest has been focused on chemical modifications of oligonucleotides,[1] and various types of functionalization have been successfully accomplished. However, there has been no report on the preparation of a modified oligonucleotide that can reversibly alter the duplex-forming activity in response to an external stimulus. If one can convert double-stranded DNA into two single strands (and vice versa) at a predetermined place and time, a number of promising applications, either in vivo or in vitro, would appear.[2] Here we report the first photoregulation of the duplex-forming activity of an oligonucleotide. The melting temperature of the duplex (T_m) is notably changed when the azobenzene moiety in the side chain undergoes *cis*–*trans* isomerization. The formation of the DNA duplex and its dissociation are successfully modulated simply by irradiating with either visible or UV light.

The modified oligonucleotide 5′-AAAXAAAA-3′ (**1**, X = the residue carrying an azobenzene moiety in the side chain; see Scheme 1) was synthesized as described previously.[3, 4] The two diastereomers **1a** and **1b**, based on the chirality of the

[*] Prof. Dr. M. Komiyama, Dr. H. Asanuma, T. Ito, T. Yoshida, X. Liang
Department of Chemistry and Biotechnology
Graduate School of Engineering
The University of Tokyo
Hongo, Tokyo 113-8656 (Japan)
Fax: (+81) 3-5841-7314
E-mail: mkomi@chembio.t.u-tokyo.ac.jp

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central carbon atom of X, were completely separated from each other by reverse-phase HPLC.[5]

Figure 1a shows the typical melting curves for the duplex formed between **1a** and its complementary counterpart 5′-TTTTTTTT-3′ (**2**) at pH 7.1 (10 mmol L^{-1} of phosphate buffer). The T_m values determined from these curves are also presented. The concentration of each of the oligonucleotides is 50 μmol L^{-1}, and the ionic strength is kept constant at 1 mol L^{-1} with NaCl. Before the photoirradiation, the azobenzene residue in **1a** overwhelmingly (about 90%) adopts the *trans* form, as confirmed by HPLC and UV absorption spectroscopy.[6] Under these conditions, the T_m of the duplex of **1a** with **2** is 24.8 °C. This value is rather close to that (23.7 °C) of the duplex of the corresponding natural oligonucleotide 5′-AAAAAAAA-3′ with **2**.

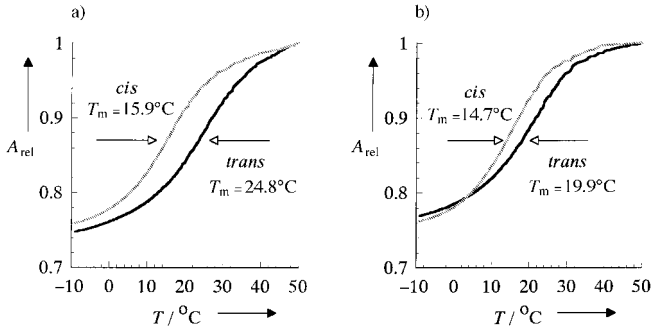

Figure 1. Melting curves of the duplexes of the diastereomers **1a** (a) and **1b** (b) with **2**. In duplicate runs, the T_m values were identical (within ±0.5 °C) with the values presented here. The T_m value of the duplex of the natural oligonucleotide (5′-AAAAAAAA-3′) with **2** is 23.7 °C.

When **1a** was irradiated with UV light ($300 < \lambda < 400$ nm), its azobenzene residue was promptly isomerized from the *trans* form to the *cis* form (Scheme 1). Significantly, the T_m value of the duplex with **2** was also lowered to 15.9 °C (Figure 1a).[7] The decrease in T_m induced by the *trans* → *cis* isomerization of the azobenzene in the side chain is as great as 8.9 °C. When the mixture was further irradiated with visible light ($\lambda > 400$ nm), the *cis*-azobenzene was isomerized back to the *trans* form as expected. The melting curve of the resultant solution was virtually superimposable on that observed before the first irradiation. Thus, the duplex-forming activity of the oligonucleotide has been satisfactorily modulated by the photoirradiation. Similarly, the T_m of the duplex of diastereomer **1b** was lowered by 5.2 °C (from 19.9 to 14.7 °C) when the azobenzene was isomerized from the *trans* form to the *cis* form by irradiation with UV light (Figure 1b).[8]

With these photoisomerizations, the formation of DNA duplexes and their dissociation can be controlled simply by irradiating with the appropriate light (UV or visible). For example, before the photoirradiation at 20 °C, **1a** mostly forms a duplex with **2** (T_m for the *trans* isomer = 24.8 °C). Upon irradiation with UV light, however, the duplex is largely dissociated into two single-stranded oligonucleotides (T_m for the *cis* isomer = 15.9 °C). The duplex is formed again after irradiation with visible light. Any change in temperature, ionic strength, and other factors is unnecessary. Consistent with this, the absorbance at $\lambda = 260$ nm reversibly changed (due to the hypochromicity) depending on what type of light was used for the irradiation at 20 °C (see the supporting information).

The change in T_m observed upon isomerization is probably associated with changes in both the polarity and the structure of the azobenzene moiety. The *trans*-azobenzene is nonpolar and planar so that it favorably stacks with the adjacent DNA bases;[9] thus, the duplex is stabilized. On the other hand, the *cis*-azobenzene is polar and nonplanar,[10] making the duplex less stable.

In conclusion, the duplex-forming activities of oligonucleotides are photoregulated by introducing azobenzene residues in their side chains. The application of the present findings to photoregulation of bioreactions is currently under way.

Experimental Section

The T_m values were measured by monitoring the absorbance at $\lambda = 260$ nm on a JASCO model V-530 spectrophotometer, equipped with a programmed temperature controller. The rate of temperature change was 1 °C min^{-1}. The photoisomerization of the azobenzene moiety in **1a** and **1b** was accomplished by irradiating with light from a 150 W Xenon lamp for 30 min through an appropriate filter. Infrared light was cut off by a water filter.

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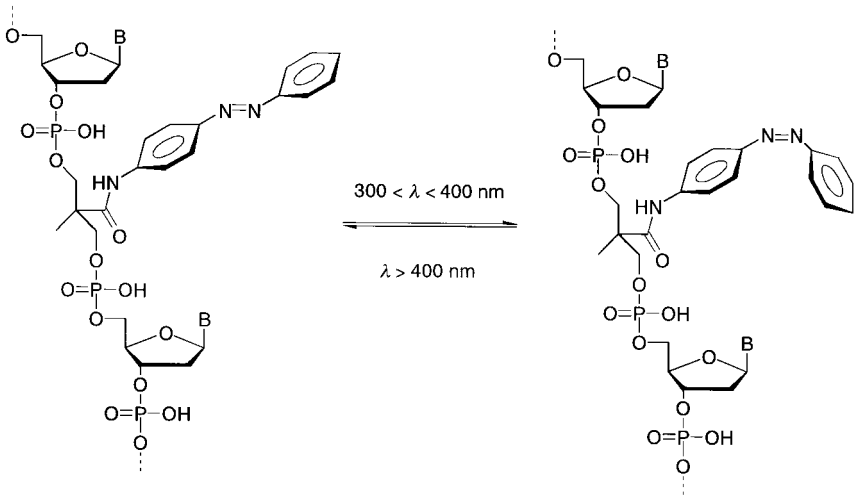

Scheme 1. Isomerization of the azobenzene moiety in the side chain of the residue X upon irradiation of oligonucleotide **1**.

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[6] The pure *trans* isomer isolated by HPLC was partially (about 10%) converted into the *cis* isomer by ambient light.

[7] In the determination of the T_m value of the *cis* isomer, UV light was irradiated in the middle of the measurement to minimize the effect of the thermal isomerization to the *trans* form. By this treatment, the fraction of the *cis* isomer was kept almost constant at 70% throughout the measurement, as confirmed by HPLC and UV/Vis spectroscopy.

[8] For the duplex between 5′-GGGXGGGG-3′ and 5′-CCCCCCCC-3′, the *trans*→*cis* isomerization of the incorporated azobenzene moiety resulted in a decrase in T_m from 32.2 to 23.0 °C ([oligonucleotide]$_0$ = 10 μmol L^{-1} at pH 7.1 (without NaCl)). Here the modified oligonucleotide was used as a mixture of the two diastereomers since they could not be separated by HPLC.

[9] The argument is substantiated by the fact that the *trans*-azobenzene moiety of **1a** exhibits a bathochromic shift upon formation of the **1a**–**2** duplex. In aqueous solutions, the absorption maximum of the azobenzene appears at 353 nm. When **2** is added to the solutions (at a temperature below the T_m of the duplex), however, the absorption band shifts towards longer wavelength (e.g., the absorption maximum is located at 359 nm under the conditions presented in the text). As expected, the absorption spectrum is hardly affected by **2** when the temperature is higher than the T_m.

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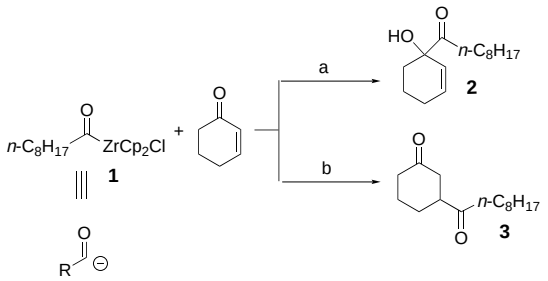
Acylzirconocene Chloride as an "Unmasked" Acyl Anion: Enantioselective 1,2-Addition to α,β-Unsaturated Ketone Derivatives**

Yuji Hanzawa,* Nobuhito Tabuchi, Kosuke Saito, Satoshi Noguchi, and Takeo Taguchi*

Recently, we reported palladium-catalyzed regioselective acylation reactions[1] of α,β-unsaturated ketone derivatives with acylzirconocene chloride.[2] The acyl group of acylzirconocene chloride reacted as a formal "unmasked" acyl anion.[3] In particular, the regioselectivity of the reaction can be controlled by modifying the palladium catalyst system. For example, in the reaction of nonanoylzirconocene chloride (**1**) with cyclohexenone (Scheme 1), the use of [$PdCl_2(PPh_3)_2$] (5 mol%) as catalyst in toluene gave the 1,2-addition product **2**, whereas the use of 10 mol% [$Pd(OAc)_2$]/$BF_3 \cdot OEt_2$ in THF/diethyl ether gave the 1,4-addition product **3** (Scheme 1).[4] A 5 mol% $Pd(OAc)_2$/PPh_3 (Pd/P = 1/2) system was also found to be an effective catalyst for regioselective formation of the 1,2-acylation product **2**. Bidentate diphosphane ligands such as 1,2-bis(diphenylphosphanyl)ethane (dppe) and 1,3-bis(diphenylphosphanyl)propane (dppp) gave lower regioselectivities.



Scheme 1. Regioselective acylation of cyclohexenone with nonanoylzirconocene chloride (**1**): a) 1,2-acylation, 5 mol% [$PdCl_2(PPh_3)_2$] or 5 mol% $Pd(OAc)_2$/PPh_3 (Pd/P = 1/2); b) 1,4-acylation, 10 mol% $Pd(OAc)_2$/$BF_3 \cdot OEt_2$.

In the palladium-catalyzed reactions of **1** with α,β-unsaturated ketones, concomitant formation of diketone **4** (<10%) was observed.[5] This suggests a transmetalation of **1** with Pd^{II} and subsequent reductive elimination of Pd^0 from the resulting bis-acylpalladium complex to give **4** (Scheme 2). Thus, electron transfer from Pd^0 to cyclohexenone, formation of the acylpalladium π-allylic complex **5**, and reductive elimination of Pd^0 would give the 1,2- or 1,4-acylation product (**2** or **3**) (Scheme 2).[6] The role of the triphenylphosphane ligand in the regioselective formation of **2** could be explained by preferred formation of the stereochemically less crowded intermediate complex **5A** rather than **5B** and subsequent reductive elimination of Pd^0 from **5A**.

[*] Prof. Dr. Y. Hanzawa, Prof. Dr. T. Taguchi, N. Tabuchi, K. Saito, S. Noguchi
School of Pharmacy, Tokyo University of Pharmacy & Life Science
1432-1 Horinouchi, Hachioji, Tokyo 192-0392 (Japan)
Fax: (+81) 426-76-3257
E-mail: hanzaway@ps.toyaku.ac.jp

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