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meta-Aminoazobenzene as a thermo-insensitive photo-regulator of DNA-duplex formation

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Abstract

meta-Aminoazobenzene has been introduced in the side chain of oligonucleotides as a photo-responsive molecule. Compared with the *para*-aminoazobenzene which was previously used, the thermal *cis*→*trans* isomerization was much slower: the half-lives of the *cis*-isomers of *m*- and *p*-aminoazobenzene were 13.2 h and 20 min at 50°C, respectively. By using the present oligonucleotides, duplex formation and dissociation was efficiently regulated on photo-irradiation without being disturbed by the thermal isomerization. © 2000 Elsevier Science Ltd. All rights reserved.

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Up to now, a variety of photo-responsive molecules have been synthesized for the artificial regulation of chemical and biological systems.[1] Among many photo-responsive molecules, azobenzene has been widely used due to (i) its large structural change induced by photo-irradiation and (ii) its easiness of chemical modification.[1] In almost all the cases, the azobenzene residues were introduced through *para*-substituted functional groups (such as -NH_2, -COOH, and -OH). This is mainly because these *para*-substituted azobenzenes are commercially available or easy to synthesize. We have also introduced *p*-aminoazobenzene in the side chain of oligonucleotides through the *para*-amino groups and successfully regulated the DNA duplex formation and dissociation by photo-irradiation.[2] However, one that is incurred with the use of azobenzene as a photo-regulator is the thermally induced *cis*→*trans* isomerization. The isomerization is rapid especially when functional groups are covalently attached to the *para*-position of azobenzene. In order to achieve strict photo-regulation with azobenzene, the thermal isomerization must be minimized.

According to the literature,[3] rapid thermal isomerization of *para*-substituted *cis*-azobenzene is attributed to the electronic effect of the substituent that weakens -N=N- bond. Thus, thermal stability of *cis*-azobenzene should be much improved when substituents are attached to the *meta*-position of azobenzene. However, little has been reported on a modified azobenzene with a *meta*-substituent, especially on the

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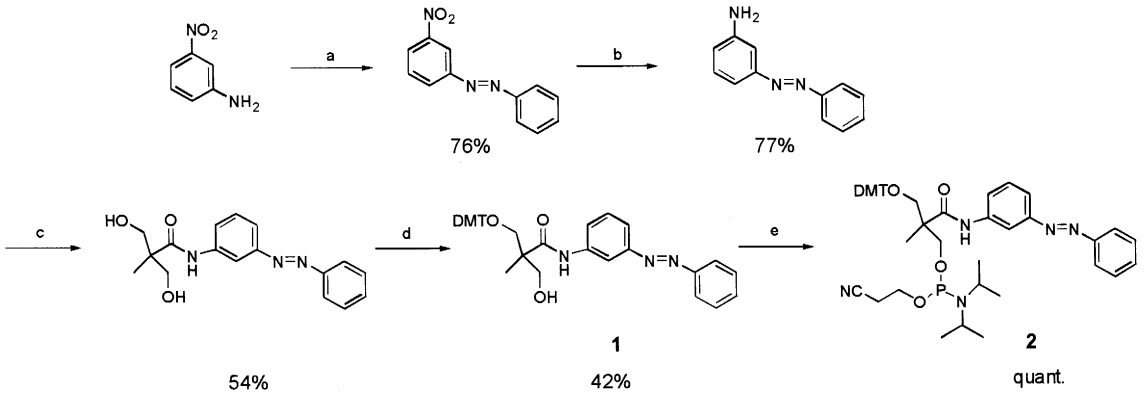
thermal durability of its *cis*-form. In the present communication, *m*-aminoazobenzene is introduced in the side chain of the oligonucleotides. This modification provides the incorporated *cis*-azobenzene residue with pronounced thermal stability. By using this oligonucleotide, the formation and dissociation of the duplex with its complementary counterpart can be sufficiently photo-regulated by the *cis–trans* isomerization of azobenzene. The *meta*-substitution effect on the thermo-insensitivity is clearly demonstrated.

The azobenzene residue was incorporated through the centered carboxyl group of trimethylene chain to minimize the perturbation of the backbone structure from the natural deoxyribose.[2] The phosphoramidite monomer **2** carrying *m*-aminoazobenzene was synthesized according to Scheme 1. First, *m*-nitroaniline was coupled with nitrosobenzene in acetic acid. The obtained *m*-nitroazobenzene was converted to *m*-aminoazobenzene through the reduction with sodium hydrosulfide in ethanol/water. Then, 2,2-bis(hydroxymethyl)propionic acid was coupled with the azobenzene in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. The obtained diol was converted to the phosphoramidite monomer according to the previous method.[2,4] All the intermediates and the product were purified by either recrystallization or silica gel column chromatography, and characterized by NMR spectroscopy.[5] The modified oligonucleotide, prepared from **2** and the conventional monomer, was 5′-AAAX^{m}AAAA-3′ (abbreviated as $\mathbf{A_3X^mA_4}$; X^m denotes the residue coming from the monomer **2**) which was purified by the reversed-phase HPLC, and characterized by MALDI-TOFMS. Two diastereomers derived from the chirality of the phosphoramidite monomer **2**, and the *trans–cis* isomers with respect to the incorporated azobenzene residue, were completely resolved by the HPLC.[6]



Scheme 1. Synthesis of phosphoramidite monomer **2**. (a) nitrosobenzene, acetic acid; (b) NaSH, ethanol/H_2O; (c) 2,2-bis(hydroxymethyl)propionic acid, dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, pyridine, DMF; (d) 4,4′-dimethoxytrityl (DMT) chloride, 4-dimethylaminopyridine, pyridine, CH_2Cl_2; (e) 2-cyanoethyl N,N,N′,N′-tetraisopropylphosphorodiamidite, 1*H*-tetrazole, CH_3CN

The UV–vis spectrum of *trans*-$\mathbf{A_3X^mA_4}$ (fraction (c); retention time 17.1 min) is shown as the dotted line in Fig. 1(**a**).[7] On UV-light irradiation (300 nm<λ<400 nm),[8] it was promptly isomerized to *cis*-$\mathbf{A_3X^mA_4}$ (the broken line in Fig. 1(**a**)).[9] This process is reversible so that *cis*-$\mathbf{A_3X^mA_4}$ was again isomerized to the *trans*-form on visible-light irradiation (400 nm<λ). The thermal *cis*→*trans* isomerization hardly took place. The UV–vis spectrum of *cis*-$\mathbf{A_3X^mA_4}$ after being kept at 50°C for 1 h virtually superimposed on that before the treatment (the solid line in Fig. 1(**a**)). In case of *p*-aminoazobenzene-tethered oligonucleotides (5′-AAAX^{p}AAAA-3′; $\mathbf{A_3X^pA_4}$),[10] photo-isomerization (*trans*→*cis* and *cis*→*trans*) occurred also smoothly (Fig. 1(**b**)). However, thermal *cis*→*trans* isomerization promptly occurred when the *cis*-isomer was situated at 50°C for 1 h. The UV–vis spectrum after the warming was almost the same as that of the *trans*-$\mathbf{A_3X^pA_4}$ (the solid line in Fig. 1(**b**)). These results clearly demonstrate the thermal durability of the *meta*-substituted *cis*-azobenzene: half-life of *cis*→*trans* isomerization was 13.2 h (790

min) for the *meta*-substituted aminoazobenzene, while it was only 20 min for the *para*-substituted one. The rate constants and half-lives of *cis*→*trans* isomerization at various temperatures are listed in Table 1.[11] Under physiological conditions (37°C, pH 7.0), the half-life of the *cis*-$\mathbf{A_3X^mA_4}$ was as long as 64 h. Thus, thermo-stability was greatly improved by the *meta*-substitution.[12]

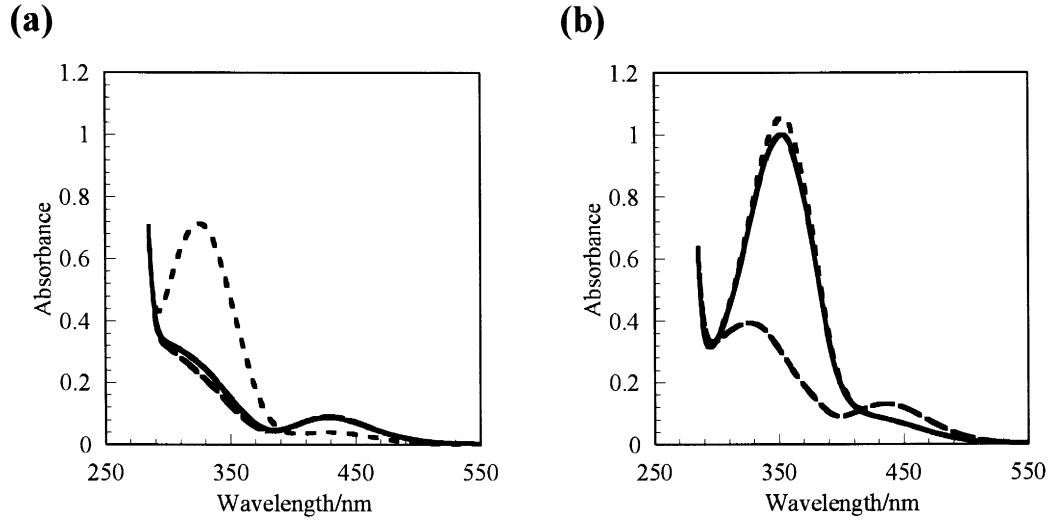


Fig. 1. UV–vis spectra of $\mathbf{A_3X^mA_4}$ (**a**) and $\mathbf{A_3X^pA_4}$; (**b**) in the *trans*-form (dotted line) and the *cis*-form (broken line). The solid lines show the spectra of *cis*-$\mathbf{A_3X^mA_4}$ and *cis*-$\mathbf{A_3X^pA_4}$ after being kept at 50°C for 1 h[11]

Table 1
The rate constants and half-lives of thermal *cis*→*trans* isomerization[11]

Temp./°C	$\mathbf{A_3X^pA_4}$		$\mathbf{A_3X^mA_4}$	
	$k/10^{-3}\text{min}^{-1}$	$\tau_{1/2}$/min	$k/10^{-3}\text{min}^{-1}$	$\tau_{1/2}$/h
50	34	20	0.87	13.2
37	12	56	0.18	64
25	4.5	150	-[a)]	-

a) Thermal isomerization was too slow to measure.

The melting temperature of the duplex between the present modified oligonucleotide and its counterpart ($\mathbf{T_8}$: 5′-TTTTTTTT-3′) was reversibly altered by the *cis–trans* isomerization. The T_ms of *trans*-$\mathbf{A_3X^mA_4/T_8}$ and *cis*-$\mathbf{A_3X^mA_4/T_8}$ were 24.3°C and 16.7°C, respectively.[13] These values were comparable to those of the *p*-aminoazobenzene-tethered oligonucleotides: T_ms of *trans*-$\mathbf{A_3X^pA_4/T_8}$ and *cis*-$\mathbf{A_3X^pA_4/T_8}$ were 24.8°C and 15.9°C, respectively. Thus, formation and dissociation of the DNA duplex could be sufficiently regulated even when the amino group was attached to the *meta*-position.

In conclusion, a thermo-insensitive and photo-responsive oligonucleotide was successfully synthesized from *m*-aminoazobenzene. By using this oligonucleotide, regulation of the duplex formation and dissociation was achieved only by photo-irradiation.

Acknowledgements

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5. Compound **1**: ^{1}H NMR [$CDCl_3$ (TMS), 270 MHz] δ 9.43(s, 1H, -NHCO-), 7.94–6.83 (m, 22H, aromatic protons of DMT and azobenzene), 3.76 (s, 6H, -OCH_3), 3.68 and 3.64 (d, 2H, J_{gem}=11.2 Hz -CH_2OH), 3.42 and 3.40 (d, 2H, J_{gem}=6.6 Hz, DMT–OCH_2-), 1.34 (s, 3H, -CH_3).
6. 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, four isomers were eluted at (a) 15.9 min, (b) 16.2 min, (c) 17.1 min, (d) 17.7min, respectively. The fractions (a) and (c) are *cis*- and *trans*-isomers of $\mathbf{A_3X^mA_4}$ with the same configuration, and (b) and (d) are *cis*- and *trans*-isomers of another configuration, respectively. MALDI TOFMS analysis: (c) fraction: obsd (negative mode) 2508, calcd 2504, (d) fraction; obsd 2508, calcd 2504.
7. Before UV irradiation, 75% of $\mathbf{A_3X^mA_4}$ existed as the *trans*-form.
8. The light from a 150 W Xenon lamp was irradiated for 20 min through an appropriate filter. Infrared light was cut off by using a water filter.
9. 85% of *cis*-form was generated by this treatment as determined by HPLC.
10. This oligonucleotide was synthesized according to Ref. 2. Four isomers of $\mathbf{A_3X^pA_4}$ were completely resolved by the HPLC, and the relationship between these isomers were identical with that for $\mathbf{A_3X^mA_4}$. In this paper, the fraction (c) of $\mathbf{A_3X^pA_4}$ was used.
11. The specimen was as follows: $[\mathbf{A_3X^mA_4}]([\mathbf{A_3X^pA_4}])$=50 μmol dm^{-3}, [NaCl]=1 mol dm^{-3}, pH 7.0 (10 mmol dm^{-3} phosphate buffer). The rate constants of the thermal *cis*→*trans* isomerization were determined by the UV–vis spectroscopy from the change of absorbance at 325 nm (for $\mathbf{A_3X^mA_4}$) or 350 nm (for $\mathbf{A_3X^pA_4}$).
12. The present results indicate that thermal stability will be also improved by *meta*-substitution with other functional groups such as -OH.
13. The absorbance at 260 nm was monitored 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 oligonucleotides and 5′-TTTTTTTT-3′ were 50 μmol dm^{-3}, and the ionic strength was kept constant at 1 mol dm^{-3} by using NaCl at pH 7.0 (10 mmol dm^{-3} phosphate buffer). The T_m values were determined from the maximum in the first derivative of the melting curve.