

Phototriggered DNA Hairpin Formation in a Stilbenediether-Linked Bis(oligonucleotide) Conjugate

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Photochemical switches have found numerous biological applications, but have received only limited application to the control of DNA base pairing processes.¹ An isolated example is provided by the recent report of Ordoukhanian and Taylor² that photocleavage of a DNA hairpin released a oligonucleotide which then hybridized with a section of the single-stranded M13 clone CW1 with greater affinity than the intact hairpin. Among the simplest of switches is the photoisomerization of a double bond, which is known to be sensitive to environmental constraints.^{3,4} Bis-(oligonucleotide) conjugates with linkers containing photoisomerizable stilbene⁵ and azobenzene⁶ chromophores have been synthesized. However, photoisomerization has not been reported to be controlled by base pairing or vice versa.

We report here that a hairpin-forming bis(oligonucleotide) conjugate possessing a stilbenediether linker undergoes reversible trans ↔ cis photoisomerization when both the cis and trans conjugates are in the random coil conformation, but undergoes one-way cis → trans isomerization when both are in hairpin conformations.⁷ Thus base-pairing inhibits trans → cis but not cis → trans isomerization. Moreover, photoisomerization of the random coil cis conjugate under conditions where the trans conjugate forms a stable hairpin results in phototriggered base pairing. The use of base pairing to effect one-way photoisomerization and of photoisomerization to effect base pairing are without precedent.

We have recently reported the synthesis of several bis-(oligonucleotide) conjugates possessing a linker derived from the *trans*-stilbenediether diol, *t*-SE.⁸ *t*-SE has absorption and fluorescence maxima at 327 and 380 nm, respectively, in methanol solution. Its fluorescent quantum yield $\Phi_f = 0.32$ and decay time $\tau = 0.35$ ns are similar to the values reported for 4,4'-dimethoxystilbene in methanol solution.⁹ The cis isomer, *c*-SE, is nonfluorescent. Irradiation of either the trans or cis diol in nitrogen-purged DMSO solution with monochromatic 344 nm light results in conversion to a mixture consisting of 28% *t*-SE and 72% *c*-SE (eq 1). Quantum yields for photoisomerization are reported in Table 1. The value of Φ_{tc} is smaller than that for

Table 1. Photoisomerization Quantum Yields and Photoequilibria^a

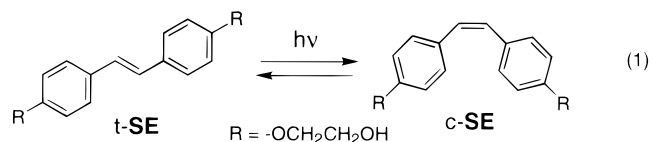
substrate	solvent	Φ_{tc}	Φ_{ct}	% trans
SE	methanol	0.37	0.36	28
SE4	50% EtOH/H ₂ O	0.19	0.10	28
SE4	1 M NaCl	<0.001	0.17	>95

^a Data for solvents deoxygenated by purging with nitrogen irradiated with monochromatic 340 nm light. Product formation monitored by HPLC. Quantum yields determined relative to photoisomerization of *trans*-stilbene at conversions of the initial isomer <20%.

Table 2. Melting Temperatures of Stilbenediether Linked Hairpins

solvent	temperature, °C	
	<i>t</i> -SE4	<i>c</i> -SE4
1.0 M NaCl	61	33
0.1 M NaCl	54	30
H ₂ O	51	
10% EtOH/H ₂ O	50	
30% EtOH/H ₂ O	38	26
50% EtOH/H ₂ O	22	

trans-stilbene, in accord with the larger fluorescence quantum yield for *t*-SE.¹⁰



Bis(oligonucleotide) conjugates with stilbenediether linkers and complementary oligonucleotide arms form stable hairpin structures in 0.1 M NaCl solutions with stems consisting of two or more dT-dA base pairs.⁸ Melting temperatures for the conjugate *trans*-(dT₄)SE(dA₄), *t*-SE4, in several aqueous and aqueous ethanol solutions are reported in Table 2. The exceptional thermal stability of these mini-hairpins is a consequence of the optimized linker design.⁸ *t*-SE4 is stable upon irradiation in water (with or without salt) under the same conditions used to convert *t*-SE to its photostationary state. However, irradiation of *t*-SE4 under denaturing conditions in 50% ethanol–water results in conversion to a mixture of *t*-SE4 and a single photoproduct formed in 72% yield.¹¹ Irradiation under denaturing conditions in water at pH > 12 resulted in more complex product mixtures. The product of irradiation in ethanol–water was isolated by HPLC and identified as the cis isomer, *c*-SE4, by comparison of its absorption spectrum with that of *c*-SE and by its photoisomerization in ethanol–water to yield the same mixture of isomers as obtained from *t*-SE4. Quantum yields for photoisomerization of *t*-SE4 and *c*-SE4 in 50% ethanol–water solution are reported in Table 1. The values are somewhat lower than for the diols *t*-SE and *c*-SE; however, the photoequilibrium is similar.

Melting temperatures for the conjugate *c*-SE4 in several solvents are reported in Table 2. The values are substantially lower than those for *t*-SE4. The crystal structure for *trans*-stilbenediether-linked hairpins with six base pairs shows that it adopts a normal B-form structure with the stilbene diol compactly stacked with the adjacent base pair.⁸ Molecular modeling of *t*-SE4 indicates that it can adopt a similar energy minimized conformation.¹²

(10) (a) Saltiel, J.; Sun, Y.-P. In *Photochromism, Molecules and Systems*; Dürr, H., Bouais-Laurent, H., Eds.; Elsevier: Amsterdam, 1990; p 64–164. (b) Waldeck, D. H. *Chem. Rev.* **1991**, *91*, 415–436.

(11) Formation of secondary photoproducts was observed after the photostationary state was reached in 50% ethanol–water.

(12) Hairpin structures are local minima calculated assuming B-form DNA geometry using a MM⁺ force field within Hyperchem 5.0 (Hypercube, Waterloo, Ontario).

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(6) Yamana, K.; Yoshikawa, A.; Nakano, H. *Tetrahedron Lett.* **1996**, *37*, 637–640.

(7) For examples of one-way photoisomerization see: (a) Arai, T.; Tokumaru, K. *Chem. Rev.* **1993**, *93*, 23–39. (b) Lewis, F. D.; Yang, J.-S. *J. Phys. Chem.* **1996**, *100*, 14560–14568 and references therein.

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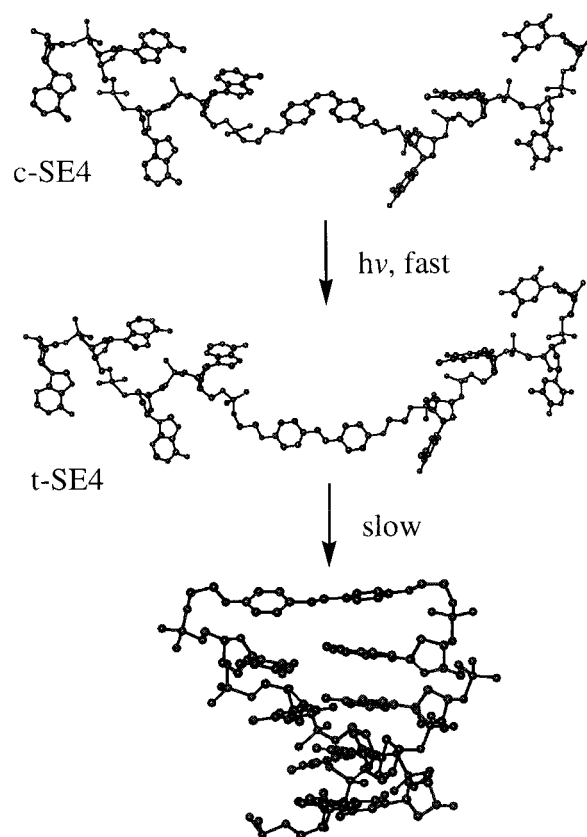
However, the *cis* linker in *c*-SE4 is too short to span the 18 Å inter-strand distance between phosphorus atoms in B-form DNA and thus disrupts the hairpin geometry. The use of longer, more flexible linkers results in a smaller difference in the melting temperatures of *trans* vs *cis* stilbenediamide-linked hairpins.⁵

Irradiation of *c*-SE4 in 0.1 or 1.0 M NaCl solution results essentially in quantitative conversion to *t*-SE4 with a moderately large quantum yield (Table 1). Under these conditions, both *c*-SE4 and *t*-SE4 are in hairpin conformations. One-way *cis* → *trans* isomerization for the stilbenediether linked hairpins might result from either the conformationally restricted base-paired geometry or selective quenching of the *t*-SE chromophore. Stilbene photoisomerization is proposed to occur via a common twisted intermediate that can decay to either *trans* or *cis* ground state.¹⁰ The large difference in hairpin loop stability for the *trans* vs *cis* isomers of SE4 could distort the "funnel" leading to the ground state, resulting in exclusive formation of the *trans* ground state.¹³ Curiously, irradiation of a stilbenedisulfonate-capped cyclodextrin results in one-way *trans* → *cis* photoisomerization.⁴

One-way *cis* → *trans* isomerization could also result from more efficient quenching of the *trans* vs *cis* singlet by an adjacent dT-dA base pair. The *trans*-stilbenediether singlet state in the hairpin dT₆-SE-dA₆ is efficiently quenched ($\Phi_f < 10^{-3}$) by the neighboring dT.⁸ Whereas quenching of the nonfluorescent singlet *c*-SE linker has not been investigated, the very short lifetime of *cis*-stilbene singlets and the presumed absence of π -stacking in the loop region of *c*-SE4 would appear to preclude efficient quenching by neighboring bases. A difference in quenching rates of the stilbenedicaboxamide (SA) linker by dA vs dG was proposed to account for the observation of photoisomerization for the SA-linked hairpin dT₆-SA-dA₆ but not for the hairpin dTTGC-SA-GCAA.^{5,14} Inhibition of stilbene isomerization has also been observed for excimer-forming duplex stilbene-linked conjugates.¹⁵

These results serve to establish that the unique structure of the base-paired DNA hairpins can be used to control photoisomerization. Conversely, photoisomerization can be used to change the structure of an oligonucleotide conjugate from single strand to hairpin. For example, irradiation of *c*-SE4 under conditions (30% ethanol/water) where it is largely in the random coil single strand conformation but *t*-SE4 is in the hairpin conformation

Scheme 1. Phototriggered Hairpin Formation



results in one-way *cis* → *trans* isomerization. A decrease in 260 nm absorbance is observed to accompany photoisomerization due to the hypochromism of the base-paired *t*-SE4 hairpin. Since photoisomerization is presumably more rapid than base pairing (Scheme 1), measurement of the changes in either absorption or circular dichroism in real time could provide direct information on the dynamics of base-pair formation.

(13) One-way *cis* → *trans* photoisomerization is also observed for 1,2-diarylethylenes in single crystals, inclusion complexes, and zeolites.³

(14) The quantum yield for *trans* → *cis* photoisomerization of the hairpin dT₆-SA-dA₆ in 1.0 M NaCl buffer is 0.042 ± 0.004 .

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