Control of Excimer Emission and Photochemistry of Stilbene Units by Oligonucleotide Hybridization

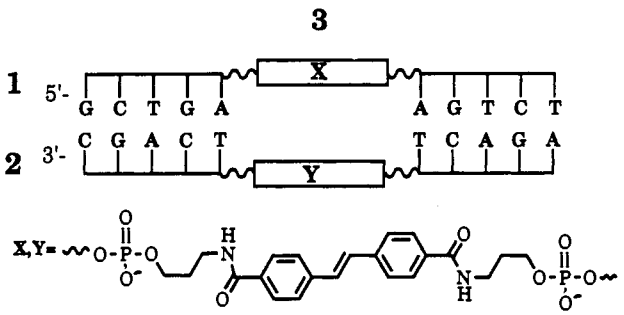
Robert L. Letsinger* and Taifeng Wu

Department of Chemistry
Northwestern University
Evanston, Illinois 60208

Received November 8, 1993
Revised Manuscript Received December 13, 1993

We describe a novel oligonucleotide construct that should prove generally useful in designing systems to align nonbonded organic fragments in an aqueous environment. As illustrated in Chart 1, short oligonucleotides are covalently attached at two positions in each fragment to be studied. Nucleotide sequences are selected such that oligomers in one strand are complementary to those in the other. Hybridization then serves to bring the two fragments (X,Y) into a defined position as bridges suspended between associated oligonucleotide segments. The stilbene chromophore was chosen as a reporter group (X = Y) to test the application of this concept. We find that this system indeed provides a unique opportunity to observe spectral and photochemical properties of adjacent nonbonded stilbene groups.

Chart 1



Oligomers **1** and **2** were prepared from nucleoside phosphoramidite reagents and *trans*-DMT-O$(CH_2)_3$NHC(O)C_6H_4-CH=CHC_6H_4C(O)NH$(CH_2)_3$OP(OCH_2CH_2CN)(N*i*-Pr_2) (**5**).[1] Each oligomer exhibited maxima in the UV spectrum (aqueous 0.1 M NaCl, pH 7.0, 23 °C) at 258 nm (nucleosides) and 336 nm (stilbene unit). On mixing, (final solution 5 μM in each oligomer), hypochromicity was observed in the region of the stilbene absorbance as well as at 260 nm, and λ_{max} for the longer wavelength band shifted to 330 nm. This behavior indicates that the stilbene units in **1** and **2** had been brought into proximity. Thermal dissociation experiments[2] showed that the oligomers form a relatively stable complex (**3**). The melting curves for **3** and the corresponding duplex lacking the bridging elements (5'GCTGAAGTCT/3'CGACTTCAGA) are similar in shape and degree of hyperchromicity, and the T_m value for **3**, 37 °C, compares favorably with that for the unmodified duplex, T_m = 41°C. The high T_m value for **3** suggests that the two linked five-mer segments in **1** and **2** act cooperatively in the association.[3]

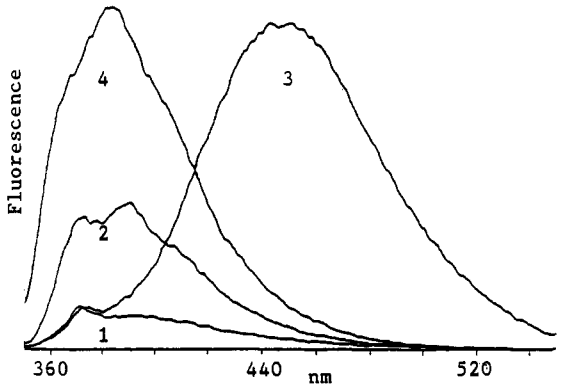


Figure 1. Fluorescence spectra for: (1) oligomer **1**; (2) oligomer **2**; (3) mixture of **1** + **2**; (4) model stilbene derivative (**4**). Spectra were run in a Perkin-Elmer LS 50B instrument at 0 °C in aqueous 0.1 M NaCl, pH 7.0 (10 mM Tris-HCl) with each stilbene derivative 0.1 μM; λ_{ex} = 330 nm.

The rich photochemistry of stilbene and its derivatives has stimulated extensive work on the relevant photoexcited states;[4] nevertheless, experimental information on stilbene excimers, which have been proposed as intermediates in photodimerization,[4a] is quite limited.[5] Conventional fluorescence techniques have not proved generally helpful in studying these intermediates, presumably because of the short lifetime of the precursor excited singlet states and the rapid collapse of the excimer to a photodimer.

Fluorescent spectra (λ_{ex} = 330 nm) for oligomers **1**, **2**, and complex **3** (i.e., **1** + **2**) are shown in Figure 1, along with data for a nonnucleotide model (**4**, a diol precursor to phosphoramidite **5** in which H replaces DMT and P(OCH_2CH_2CN)N*i*-Pr_2). Compounds **1** and **2** fluoresce in the region characteristic for stilbene, but the intensity of fluorescence is substantially less than that from **4**. This reduction may be attributed to quenching of fluorescence by the nucleotides in the single stranded oligomers. Quenching is greater in **1** than in **2**, as expected since the guanine base, which is the most effective quenching unit in the oligomers, lies nearer the stilbene group in this oligomer. When equivalent amounts of **1** and **2** are mixed, however, the stilbene fluorescence is greatly reduced and a strong new band with λ_{max} near 450 nm appears. Titration of **1** with aliquots of **2** showed that this band stems from a 1:1 complex of **1** and **2**. The most plausible explanation is that the new band represents emission from an excimer generated on photoexcitation of the aligned stilbene groups in **3**.[6] An interesting feature of the system is that alignment of the stilbene groups depends on a reversible reaction that can be readily controlled. As shown in Figure 2, excimer emission

(1) Diol **4** and phosphoramidite reagent **5** were prepared from *trans*-stilbene-4,4'-dicarboxylic acid by the procedures used in making similar compounds from terephthalic acid, see: Salunkhe, M.; Wu, T.; Letsinger, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 8768–8772. Oligomers **1** and **2** were synthesized using a Cyclone DNA synthesizer using standard phosphoramidite chemistry and were purified by RP HPLC.

(2) The heating experiments demonstrating dissociation of the oligomers were carried out at pH 7.0 in water containing NaCl (0.10 M), Tris-HCl buffer (10 mM), and oligonucleotides (5 μM each). The apparatus and procedure were as described by Gryaznov and Letsinger: Gryaznov, S. M.; Letsinger, R. L. *Nucleic Acids Res.* **1993**, *21*, 1403–1408.

(3) Cooperative binding has been reported for the interaction of RNA with oligonucleotides joined by a polyethylene glycol linker: Cload, S. T.; Schepartz, A. *J. Am. Chem. Soc.* **1991**, *113*, 6324–6326.

(4) For reviews, see: (a) Lewis, F. D. *Adv. Photochem.* **1986**, *13*, 165–235. (b) Waldeck, D. H. *Chem. Rev.* **1991**, *91*, 415–436. (c) Whitten, D. G. *Acc. Chem. Res.* **1993**, *26*, 502–509.

(5) Excimer fluorescence has been reported for thermoluminescence of solutions of stilbene in squalene exposed to ^{60}Co gamma rays at 77 K: Brocklehurst, B.; Bull, D. C.; Evans, M.; Scott, P. M.; Stanney, G. *J. Am. Chem. Soc.* **1975**, *97*, 2977–2978. A similar bathochromic shift has also been reported for fluorescence emission from pendant stilbene groups attached to polyglutamic acid (Fissi, A.; Houben, J. L.; Rosato, N.; Lopes, S.; Pieroni, O. *Makromol. Chem., Rapid Commun.* **1982**, *3*, 29–33) and to copolymers containing poly(menthyl acrylate) (Altomare, A.; Carlini, C; Ciardelli, F.; Solaro, R.; Houben, J. L.; Rosato, N. *Polymer* **1983**, *24*, 95–100). A stilbene excimer has been proposed as a possible intermediate observed by picosecond absorption spectroscopy of stilbene irradiated in benzene: Peters, K. S.; Freilich, S. C.; Lee, J. *J. Phys. Chem.* **1993**, *97*, 5482–5485. Fluorescence in this excimer range has also been reported for alkylated stilbene derivatives aggregated in Langmuir–Blodgett films (ref 4c).

(6) Although oligomers **1** and **2** differ in nucleotide sequence, we believe the term "excimer" is more descriptive than "exciplex" for the species formed by the photoexcited stilbene moieties since the relevant stilbene chromophore is the same in the two oligomers (the UV spectra for the individual oligomers in the 300–380-nm range are the same within the accuracy of the measurements).

(7) (a) Syamala, M. S.; Ramamurthy, V. *J. Org. Chem.* **1986**, *51*, 3712–3715. (b) Ito, Y.; Kajita, T; Kunimoto, K.; Matsuura, T. *J. Org. Chem.* **1988**, *54*, 587–591.

0002-7863/94/1516-0811$04.50/0

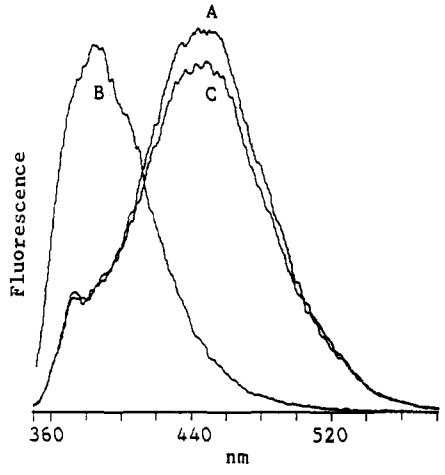


Figure 2. Fluorescence spectra for oligomers **1** + **2**, each 0.1 μM, determined at room temperature at (A) pH 7.0 as in Figure 1; (B) pH 12 (10 mM in NaOH); and (C) pH 7.0, after neutralization of the solution in B by addition of HCl; λ_{ex} = 330 nm.

is replaced by the stilbene emission when complex **3** is dissociated by increasing the pH from 7 to 12. On subsequent neutralization of the solution, the excimer band returns. A similar reversal can be effected by heating and then cooling the solution at pH 7.

Irradiation of organic or aqueous solutions of stilbene and its derivatives yields mixtures of cis–trans isomers, unimolecular cyclization products, and stereoisomeric cyclobutane derivatives (stilbene dimers).[4] Low yields, at best, have been reported for the dimerization reaction.[7] In marked contrast, irradiation of a dilute solution of complex **3** for 1 min converts the two strands to a photoproduct in high yield (Figures 3 and 4). Several lines of evidence, taken in conjunction with known stilbene photochemistry, point strongly to formation of an adduct in which **1** and **2** are linked via a central cyclobutane ring. Thus, after brief irradiation of **3**, UV absorption is very weak above 300 nm (Figure 3, spectrum 3); the elution time on anion-exchange HPLC is significantly greater for the photoproduct (23.6 min) than for oligo-**1** (18.4 min) or oligo-**2** (16 min), indicative of formation of a higher molecular weight polyanion (Figure 4), and the T_m value for dissociation of the oligonucleotide chains is 67 °C when determined after irradiation of a solution **3**, as compared to 37 °C when measured before irradiation. This increase in T_m is characteristic of the enhancement observed when short complementary oligonucleotides are linked by a covalent bridge.[1] As controls for the photolysis experiment, oligomers **1** and **2** were irradiated separately under the same conditions employed for **3** (for UV changes, see Figure 3). Some isomerization occurred, though at a slower rate than photoaddition for **3** (Figure 3), but no products eluting on ion-exchange HPLC later than the initial oligomers (i.e., no photodimers) were found.

These experiments with the stilbene chromophore demonstrate the feasibility of using bridged, complementary oligonucleotides to align organic groups reversibly in aqueous solution. Time-resolved fluorescence studies and work on related self-organizing systems are in progress.

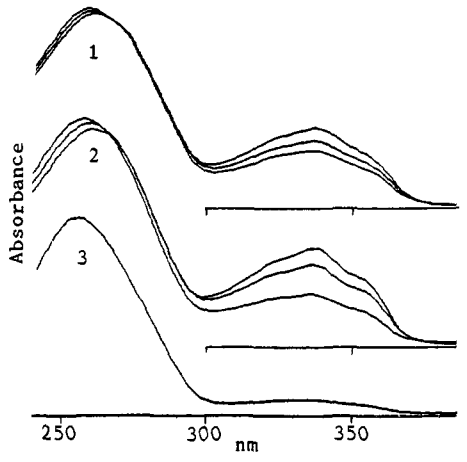


Figure 3. UV spectra of oligomers (5 μM each, in aqueous 0.10 M NaCl, pH 7.0, 10 mM Tris.HCl) after irradiation in a UV cuvette for the indicated time in a Rayonet reactor equipped with two RPR 3500-Å 24-W lamps: (1) **1** after 0, 4, and 10 min (in order, top down); (2) **2** after 0, 1, and 4 min; and (3) **1** + **2** after 1 min.

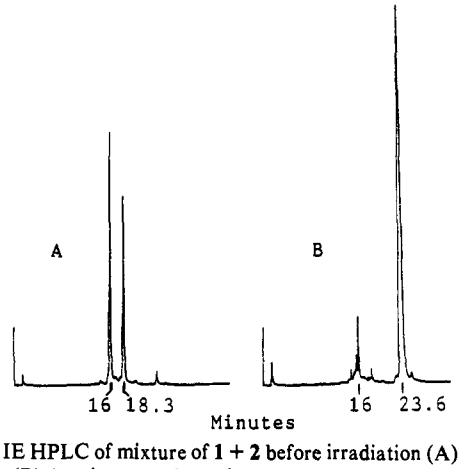


Figure 4. IE HPLC of mixture of **1** + **2** before irradiation (A) and after irradiation (B) (products are from the experiment in Figure 3, curve 3). Peaks at 16, 18.3, and 23.6 min correspond to **2**, **1**, and photoadduct, respectively. Note that most of **1** has been consumed; small peaks near 16 min probably are due to **2**, present in slight excess relative to **1**, and its cis isomer. HPLC was carried out on a Dionex Omni Pak Na1004 column at pH 12 (10 mM NaOH) with a 2% gradient of 1.0 M NaCl in 10 mM NaOH.

Acknowledgment. This research was supported by a research grant (GM 10265) from the National Institute of General Medical Sciences. We are grateful to Professor Frederick D. Lewis and Dr. Dario M. Basani for helpful discussions pertaining to the photochemistry.