Structure and Photoinduced Electron Transfer in Exceptionally Stable Synthetic DNA Hairpins with Stilbenediether Linkers

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Received June 10, 1999

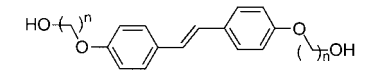
Bis(oligonucleotide) conjugates with synthetic linkers connecting short complementary oligonucleotides are known to form synthetic DNA or RNA hairpins which are, in some cases, more stable than natural hairpins which possess oligonucleotide linkers.[1,2] We report here that conjugates possessing stilbenediether (SE) linkers (Chart 1) form exceptionally stable (poly)dT−SE−(poly)dA hairpins. The crystal structure of a SE-bridged hairpin confirms that it adopts a B-form structure in which the stilbene is π-stacked with the adjacent base pair. The stilbenediether also mediates novel lattice interactions that are distinct from those normally found in DNA crystals. The singlet excited state of the stilbenediether is a strong electron donor which is rapidly quenched by either neighboring dT−dA or dC−dG base pairs which function as electron acceptors. This behavior is complementary to that of conjugates possessing a stilbenedicarboxamide linker (SA, Chart 1), which serves as an electron acceptor.[3,4]

The conjugates **1**−**7** display a long wavelength absorption band (λ_{max} = 327 nm) assigned to the stilbene π,π^* transition and a shorter wavelength band (λ_{max} = 260 nm) assigned to overlapping stilbene and nucleobase absorption bands. The 260 nm bands of conjugates **1**−**7** display an increase in absorbance (hypochromism) upon heating in aqueous buffer containing 0.1 M NaCl. The thermal dissociation profiles provide melting temperatures which are reported in Chart 1. The CD spectrum of **4** has a positive band at 283 nm and a negative band at 250 nm, consistent with formation of a B-form DNA structure in solution. The 327 nm absorption band for **4** is similar in appearance to that of SE2 in methanol (λ_{max} = 324 nm) and displays neither hypochromism nor induced circular dichroism.

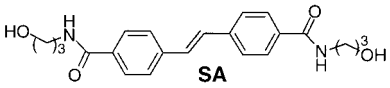
Single crystals of the bromine-labeled hairpin **7** were obtained by crystallization from solutions containing Sr^{2+} ions.[5−10] The asymmetric unit consists of four hairpins and four Sr^{2+} ions (Figure 1). All four hairpins adopt B-form DNA conformations in which the stilbenediether is π-stacked with the adjacent dG−dC base pair with a plane-to-plane separation of 3.25 Å and twist angles of between 10 and 20°. Their structures differ mainly in the sugar−phosphate backbone conformation, with the positions of the base pairs and stilbene being nearly superimposable (Figure 2). The Sr^{2+} ions are located near the SE-end of the hairpins, in the center of the minor groove, and form outer-sphere contacts to O2 and N3 acceptors of pyrimidines and purines, respectively.

Both ethylenes in the SE2 linker adopt gauche conformations, as is the case for 1,2-dimethoxyethane and poly(ethyleneoxide).[11] This results in a rather short end-to-end distance for the linker; the average distance between the outer SE2 oxygen atoms is ∼16.5 Å. The average inter-strand distance between phosphorus atoms bound to the SE2 linker is 18.1 Å and thus comparable to the average distance of 17.7 Å for phosphorus atoms from opposite strands in all other dimer steps. Evidently the SE2 linker has nearly optimal dimensions for the formation of a stable B-form DNA hairpin. Increasing the number of methylenes in the linker from two to four results in a decrease in solution T_M for the series of hairpins **3**, **5**, and **6** (Chart 1). Molecular modeling of **5** and **6** indicates that the longer linkers must form gauche-turns which are expected to be entropically unfavorable. The hairpin **6** has the same T_M as the stilbenediamide-linked hairpin $(dT)_4SA(dA)_4$, which has the same total number of atoms in the linker. Efforts to grow crystals of a SA-linked hairpin with the same base-sequence as that of **7** have been unsuccessful.

Chart 1. Linker and Conjugate Structures and Melting Temperatures

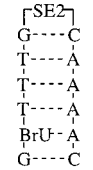


n = 2: **SE2**, n = 3: **SE3**, n = 4: **SE4**



SA

		T_M, °C
1	$(dT)_2SE2(dA)_2$	30
2	$(dT)_3SE2(dA)_3$	47
3	$(dT)_4SE2(dA)_4$	54
4	$(dT)_6SE2(dA)_6$	64
5	$(dT)_4SE3(dA)_4$	53
6	$(dT)_4SE4(dA)_4$	48



7

$T_M > 85°$

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(5) Crystals were grown from hanging droplets (0.8 mM hairpin 6, 40 mM sodium cacodylate pH 6.3, 50 mM SrCl2) that were equilibrated against 40% 2-methyl-2,4-pentanediol (MPD). Similar crystals could also be grown from solutions that were supplemented with Mg2+ instead of Sr2+. Diffraction data from a very thin plate with approximate dimensions 60 × 50 μm were collected to a resolution of 3.2 Å with a wavelength of 1.033 Å on the 19ID beamline of the Structural Biology Center at the Advanced Photon Source, Argonne, IL, using a 3 × 3 mosaic CCD detector.6 Data were integrated and merged in the HKL 2000 suite.7 The space group is monoclinic *P*21 and the unit cell dimensions are a = 27.76 Å, b = 67.89 Å, c = 61.83 Å, and β = 96.8°. The structure was solved with the Molecular Replacement method,[8] using the bromine positions (see Chart 1) to verify the solution. Refinement was performed with the program CNS,[9] setting aside 10% or the reflection data for calculating *R*-free.[10] The final *R*-factor for four hairpin molecules, four Sr^{2+} ions and 11 waters is 25.7% and the *R*-free is 26.6%, including 2864 reflections with $F > 0.5\ \sigma(F)$ within an 8−3.2 Å range. The rms deviations from standard bond lengths and angles are 0.01 Å and 1.7°, respectively.

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10.1021/ja991934u CCC: $18.00 © 1999 American Chemical Society
Published on Web 10/13/1999

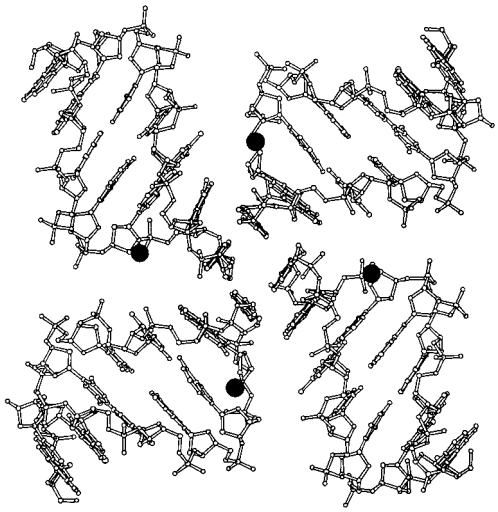

Figure 1. The four SE hairpin molecules constituting the crystallographic asymmetric unit viewed along the pseudo 4-fold axis. The crystallographic *b*-axis runs along the horizontal, the "pinwheel-like" arrangement by the stilbene moieties is visible in the center and Sr^{2+} ions are shown as filled circles.

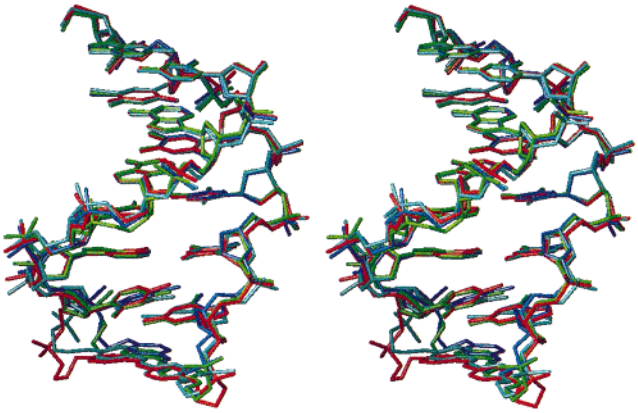

Figure 2. Stereo diagram depicting a superposition of the four crystallographically independent SE hairpin molecules. The SE units are located at the bottom.

The four stilbenediether linkers in each asymmetric unit are aggregated in an edge-to-face or "pinwheel" tetramer geometry (Figure 1) which has previously been identified as the "unit aggregate" for extended stilbene arrays both in crystals and Langmuir−Blodgett films.[12] To our knowledge, an isolated pinwheel tetramer has not previously been reported. According to Vaday et al.[12] the packing energy of a stilbene tetramer is approximately one-third that of lattice energy of an extended layer. Thus arene−arene interactions as well as hydrophobic association may be responsible for the unusual tetramer geometry of the hairpin **7** The average dihedral angle between stilbenes is 73°, somewhat larger than the 60° dihedral angle observed for several layered stilbene crystals.[12] The shortest stilbene−stilbene edge-to-face C−C contact is 3.2 Å, similar to that for aromatic hydrocarbons which adopt a herringbone packing motif.[12,13]

The stilbenediether diol SE2 is strongly fluorescent, having an emission maximum at 380 nm, a fluorescence quantum yield $\Phi_f = 0.32$, and singlet decay time $\tau = 350$ ps in methanol solution.[14] The hairpins **4**−**7** have significantly weaker fluorescence ($\Phi_f < 0.01$) than SE2, but similar emission band shapes. Decreased fluorescence intensity in the hairpins is attributed to electron-transfer quenching in which the singlet stilbene serves as the electron donor and the neighboring base pair as the electron acceptor. The free energy of photoinduced electron transfer can be estimated from the stilbenediether diol singlet energy ($E_S = 3.45$ eV) and oxidation potential ($E_{ox} = 0.92$ V vs SCE in DMF solution) and the nucleoside reduction potentials (E_{rdn}).[15] By using the nucleoside oxidation potentials in DMF solution reported by Seidel et al.[16] reduction of dT and dC should be exergonic ($E_{rdn} = -2.26$ and -2.36 V, respectively), but reduction of dA and dG should be endergonic ($E_{rdn} = -2.67$ and < -3.0 V, respectively). The occurrence of fluorescence quenching via rapid electron transfer is supported by picosecond time-resolved transient absorption spectroscopy.[17] The singlet state of SE2 in THF solution has broad transient absorption centered at 575 nm with a decay time of 360 ps, similar to the fluorescence decay time. The hairpins **4** and **7** have a much narrower transient absorption at 530 nm which is assigned to the stilbenediether cation radical. The transients from **4** and **7** have a rise time of ~0.2 ps and decay times of 32 and 13 ps for **4** and **7**, respectively. The complete decay of the cation radical and short decay times suggest that charge recombination is more rapid than charge migration in these hairpins.

These results serve to establish that properly designed bis-(oligonucleotide) conjugates can adopt exceptionally stable B-form DNA hairpin structures in which an aromatic chromophore is positioned parallel to the adjacent base pair. The conjugates **1**−**4** have T_M values (Chart 1) which are 5−10 °C higher than those previously reported for the corresponding SA-linked hairpins[2b] and are the most stable synthetic hairpins studied to date. Factors which may influence hairpin stability include π-stacking ability of the chromophore and the total length and flexibility of the linker. In our earlier studies of arenedicarboxamide linkers, the SA[3] or naphthalenediamide[18] linker chromophore served as an electron acceptor and the nucleobases guanine or adenine as electron donors. In the present study the donor and acceptor roles are reversed, the stilbenediether serving as the electron donor and the nucleobases thymine and cytosine as electron acceptors. In addition to serving as a source of electrons for photoinjection into DNA, the stilbenediethers may function as hole traps in studies of hole hopping in DNA.

Acknowledgment. This research is supported by grants from the Division of Chemical Sciences, Office of Basic Energy Sciences, U.S. Department of Energy under contracts DE-FG02-96ER14604 (F.D.L.) and W-31-109-Eng-38 (M.R.W.) and by the Department of Energy, Office of Health and Environmental Research, Contract W-31-109-Eng-38 (to A.J.) and by the NIH Grant GM 55237 (to M.E.).

Supporting Information Available: The final coordinates and structure factors have been deposited in the Nucleic Acid Database (NDB ID code DD0019). Experimental procedures for the synthesis of linkers and hairpins are provided (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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